Table 3 Univariate analysis: factors predictive of sustained virological response in cirrhotic patients with genotype 1b

Factor	Value (range or number)		*P* value
	SVR $n = 11$	Non-SVR $n = 64$	
Sex (male/female) (cases)	5/6	28/36	0.916
Mean age/range (years)	62.5 ± 9.2	61.6 ± 8.6	0.664
White blood cells (/mm^3)	4,042.7 ± 1,377.4	3,930.0 ± 1,154.8	0.811
Hemoglobin (g/dl)	13.1 ± 1.4	12.9 ± 1.8	0.632
Platelet count (×10^4/mm^3)	10.6 ± 2.8	9.4 ± 3.7	0.185
AST (IU/l)	86.8 ± 4.3	63.0 ± 33.0	0.119
ALT (IU/l)	112.6 ± 92.9	66.5 ± 38.0	0.027
AST/ALT	0.85 ± 0.17	1.04 ± 0.27	0.022
Total bilirubin (mg/dl)	0.9 ± 0.4	1.0 ± 0.4	0.306
Albumin (g/dl)	4.0 ± 0.4	3.8 ± 0.4	0.382
PT percentage activity (%)	88.4 ± 11.6	82.1 ± 9.3	0.137
Associated esophageal varices (cases) (present/absent)	3/8	44/20	0.009
History of treatment for HCC (cases) (with or without)	2/9	19/45	0.432
Previous treatment of IFN (cases) (with or without)	6/5	38/26	0.764
HCV-RNA (KIU/ml)			
Low level (<1,000 KIU/ml) (cases)	2	20	
High level (≥1,000 KIU/ml) (cases)	9	44	0.379
ISDR (wild type/non-wild type)/ND	7/3/1	53/9/2	0.223
Core aa 70 [arginine/glutamine (histidine)]/ND	5/5/1	33/30/1	0.889
Core aa 91 (leucine/methionine)/ND	8/2/1	46/17/1	0.640
IL28B (rs 8099917) TT/TG or GG/ND	10/1/0	35/26/3	0.035

AST Aspartate aminotransferase, *ALT* alanine aminotransferase, *AST/ALT* aspartate aminotransferase/alanine aminotransferase ratio, *ND* not determined

Prognostic factors for SVR in HCV-related cirrhotic patients with genotype 1b

In patients with genotype 1b and a high viral load in the cirrhosis group, patient characteristics were compared between those who did (SVR group) and those who did not achieve an SVR (non-SVR group) (Table 3).

Comparing the numbers of patients with esophageal and gastric varices, there were significantly fewer patients with these varices in the SVR group compared to the non-SVR group (3/11 vs. 44/64, $P = 0.009$). With regard to liver enzymes, serum alanine aminotransferase was significantly higher in the SVR group compared to that of the non-SVR group (112.6 ± 92.9 IU/l vs. 66.5 ± 38.0 IU/l, $P = 0.027$). Furthermore, the AST/ALT ratio was significantly lower in the SVR group compared to the non-SVR group (0.85 ± 0.17 vs. 1.04 ± 0.27, $P = 0.022$).

The SVR group was significantly more likely to carry the TT allele of the IL28B genetic polymorphism compared to the non-SVR group (10/11 vs. 35/61, $P = 0.035$).

In patients with genotype 1b and a high viral load, the IL28B genetic polymorphism and therapeutic effect rate were examined according to the chronic hepatitis group, the untreated cirrhosis group, and the splenectomy or PSE group (Fig. 3b).

For patients in the chronic hepatitis group with a platelet count of at least 80,000, the SVR rate in those with the major homo(TT) allele of the IL28B genetic polymorphism was 55 % (44/80 patients) compared to 26.1 % (12/46 patients) in those with the hetero/minor(TG/GG) allele. For patients in the cirrhosis group with a platelet count of at least 80,000, the SVR rate in patients with the TT allele of the IL28B genetic polymorphism was 29.2 % (7/24 patients) compared to 7.7 % (1/13 patients) in those with the TG/GG allele. Patients with a platelet count of less than 80,000 did not achieve an SVR, independent of whether they had the TT allele of the IL28B genetic polymorphism or the TG/GG allele.

In contrast, in patients who underwent splenectomy or PSE, the SVR rate was 18.8 % (3/16 patients) in patients with the TT allele of the IL28B genetic polymorphism, although 0 % (0/9 patients) in patients with the TG/GG allele.

Patients who discontinued IFN therapy

In the chronic hepatitis group, 169 patients completed IFN therapy while 40 patients discontinued it. In the cirrhosis group, 49 patients completed IFN therapy while 41 patients discontinued it.

Reasons for withdrawal from treatment are shown in Table 4. In the cirrhosis group, treatment was discontinued in ten patients because they did not have virus-negative

Table 4 Main causes of treatment discontinuation

Cause of discontinuation	No. of patient	
	Chronic hepatitis ($n = 209$)	Cirrhosis ($n = 90$)
Fatigue	6	5
Neutropenia	2	0
Thrombocytopenia	0	3
Anemia	0	1
Fundal hemorrhage	1	2
Auditory disturbance	1	1
Suspicion of interstitial pneumonia	2	2
Dizziness	3	0
Pruriris	5	1
Depression	2	1
Presyncope	1	1
Liver function flare	1	1
Infection	0	1
No virological response	13	10
HCC occurrence	2	11
Death of accident	0	1
Patient's reasons	1	0

results by 24 weeks after starting IFN/RBV therapy. Another 11 patients developed liver cancer during treatment, and 1 patient died due to other factors. Nineteen patients ceased treatment because of side effects, which accounted for 21.1 % of 90 patients with cirrhosis who underwent the treatment. There were no serious adverse side effects reported.

Discussion

In PEG-IFN/RBV therapy for chronic hepatitis C, the SVR rate decreased in the setting of progressive fibrosis. Treatment outcomes for HCV-related cirrhosis describe low SVR rates [10–12]. In this study, in the chronic hepatitis group, the SVR rates of PEG-IFN/RBV therapy in patients with genotype 1b and a high viral load and patients with genotype 2a/2b were 46.4 and 80.4 %, respectively. In comparison, in the cirrhosis group, the SVR rates in patients with genotype 1b and patients with genotype 2a/2b were 14.7 and 46.7 %, respectively, indicating a significantly poorer therapeutic effect.

Adherence to IFN therapy is important to achieve a therapeutic effect. It has been suggested that 80 % or more of the basic dose should be administered [13, 14]. However, patients with cirrhosis complicated by hypersplenism have thrombocytopenia, and it becomes exceedingly difficult to initiate IFN therapy and achieve an adequate therapeutic adherence. Due to ongoing thrombocytopenia, which may fall below the recommended level for therapy cessation, the therapeutic effect will therefore not be obtained.

In this study, in the chronic hepatitis group, we were able to maintain 80 % or more of the IFN dose in 76.6 % of patients with a platelet count of 80,000 or more. In this group, the SVR rates were 46.7 and 80.0 % in patients with genotype 1b and a high viral load and patients with genotype 2a/2b, respectively, which were relatively better results.

However, the cirrhosis group showed low adherence; 35.4 % of the patients with a platelet count of 80,000 or more were maintained on 80 % or more of the IFN dose. The SVR rates were 20.5 and 33.3 % in patients with genotype 1b and high viral load and patients with genotype 2a/2b, demonstrating a low therapeutic effect rate. In other words, in the cirrhosis group, there was a decreased adherence and therapeutic effect rate despite sufficient levels of platelets prior to IFN therapy.

Furthermore, in the cirrhosis group, the administration rate was ever lower in patients with a platelet count of less than 80,000. Only 8.3 % of these patients maintained 80 % of the IFN dose. No patients achieved an SVR among the patients with genotype 1b and a high viral load. We found that the platelet count was an important factor for maintaining the IFN dose maintenance regimen and influenced the therapeutic effect rate.

In addition, cytopenia including thrombocytopenia greatly prevents the induction of IFN therapy [16]. Although our study did not examine this specifically, we hypothesize that many patients likely found it difficult to initiate IFN therapy because of thrombocytopenia.

Our study included eight patients with a platelet count of less than 50,000, and splenectomy or PSE allowed all of them to initiate IFN therapy. These procedures also improved the platelet count and allowed 18 of the total 30 (60.0 %) who underwent these procedures to maintain 80 % or more of the IFN dose for 24 weeks or more. The IFN dose maintenance regimen was very acceptable in the treated group compared to the untreated group. However, in patients with genotype 1b in the treated group, the SVR rate was low at 12.0 %, and the therapeutic effect rate failed to improve. Although we cannot completely clarify why the improvement of drug adherence did not result in a favorable outcome, the presence of advanced liver fibrosis and its related portal hypertension could also influence the SVR rate, as well as the adherence to IFN therapy.

It was difficult to evaluate patients with genotype 2a/2b because they were few in number. Nevertheless, the SVR rate slightly improved by 60 % (3/5 patients) in the treated group when compared to the untreated group.

Recently, ISDR and amino acid substitutions of core 70 and core 91 in the core region of HCV have been reported as factors associated with the therapeutic effect of PEG-IFN/RBV combination therapy for patients with genotype 1b and a high viral load [24, 25]. In the human genome, a single nucleotide polymorphism (SNPs) of IL28B is an important factor [26–28]. Therefore, these factors were included in our analysis of prognostic factors for a therapeutic effect leading to SVR in patients with genotype 1b and a high viral load. In our study, this group had experienced a poor therapeutic effect, especially in comparison to patients with genotype 2a/2b.

Univariate analysis revealed that there were significantly more patients with esophageal and gastric varices in the non-SVR group than in the SVR group. Furthermore, patients with a higher serum aspartate aminotransferase and lower AST/ALT ratio were more likely to achieve an SVR. Hence, we considered that in the cirrhosis group, the therapeutic effect rate was decreased in patients who develop fibrosis of the liver with portal hypertension. In addition, significantly more patients who achieved an SVR had the TT allele of the IL28B genetic polymorphism than the TG/GG allele.

In the multivariate analysis, SVR was associated with patients without esophageal varices [odds ratio, 11.02; 95 % confidence interval (CI), 2.058–99.979; $P = 0.0038$] and patients with the TT allele of the IL28B genetic polymorphism (odds ratio, 16.4; 95 % CI, 1.870 to −426.906; $P = 0.0085$; results not shown in this study because the 95 % confidence interval was too large).

In patients with genotype 1b and a high viral load, the therapeutic effect rate was low even in those with the TT allele of the IL28B genetic polymorphism. This therapeutic effect rate did not improve despite splenectomy or PSE performed to improve adherence to IFN therapy. In patients with the TG/GG allele of the IL28B genetic polymorphism, treatment outcomes were even worse. In the cirrhosis group, low adherence contributed to a poor therapeutic effect of IFN. However, improving adherence alone will not directly lead to an improved SVR rate.

Splenectomy or PSE does carry procedural-related risks, and there have been case reports of death caused by infection during IFN therapy. Hence, splenectomy or PSE appears to be indicated for the purpose of IFN therapy in patients with genotype 2a/2b who are more likely to achieve an SVR by dose maintenance. In this study, we investigated the patients who received PEG-IFN/RBV combination therapy. There were five chronic hepatitis patients with genotype 1b and a low viral load, while there were no patients with genotype 1b and a low viral load in the cirrhosis groups (groups C, D, and E). We therefore could not compare the data of the patients with genotype 1b and a low viral load between the chronic hepatitis groups and the cirrhosis groups. However, splenectomy or PSE may also be indicated in patients with genotype 1b and a low viral load. For patients with genotype 1b and a high viral load, these procedures may be indicated in patients with the TT allele of the IL28B genetic polymorphism.

In general, there are several differences in the pre- and post-hematologic parameters between PSE and splenectomy. However, we were not able to evaluate the effect of splenectomy separately from that of PSE because there were only two patients who received PSE in this study. Additionally, medications to treat thrombocytosis include Eltrombopag, a thrombopoietin receptor agonist used for idiopathic thrombocytopenic purpura. A case has been reported detailing the benefit of Eltrombopag on the therapeutic effect of IFN in patients with HCV-related cirrhosis found to be thrombocytopenic [31]. Further studies are required to examine which procedure or medication, be it splenectomy, PSE, or Eltrombopag, best ameliorates thrombocytopenia prior to IFN therapy.

In recent years, direct-acting antiviral agents (DAAs) have been used as novel anti-HCV drugs, greatly altering the therapeutic strategy for chronic hepatitis C. In Japan, telaprevir (TVR) has been used in patients with chronic hepatitis C (patients with genotype 1 and a high viral load), and three agents (TVR/PEG-IFNα-2b/RBV) in combination therapy have been used since November 2011. About 90 % of patients who experience a relapse following pretreatment or patients with the TT allele of IL28B in particular achieved an SVR, indicating dramatically improved effectiveness in treatment [32]. However, there are various issues with TVR. Currently, it is not indicated in patients with cirrhosis, and its therapeutic effect is suboptimal for those who did not respond to pretreatment and patients with the TG/GG allele of the IL28B genetic polymorphism. Furthermore, more patients choose to discontinue treatment because of side effects compared to those treated with PEG-IFN/RBV combination therapy. A clinical trial of the second-generation protease inhibitor is ongoing. It is expected that the therapeutic effect and tolerability of this medication will be improved.

IFN can be difficult to initiate in patients with cirrhosis, which may be the initial cause for cytopenia. Cytopenia can occur as a side effect of IFN treatment, but the blood cells are only slightly decreased because of PEG-IFN lambda/RBV combination therapy since the receptor for IFN-λ is not found in hematopoietic cells [33]. Therefore, we expect that it will be indicated in patients with low platelet counts. Studies of multiple combination DAA therapy of BMS-790052 and BMS-650032 are ongoing. These studies involve patients who did not respond to pretreatment, and all of them achieved an SVR, although many have the TG/GG allele of the IL28B genetic polymorphism. It is also expected that the adverse effects will

be mild, and we are optimistic that this may be suitable treatment for cirrhosis [34].

The treatment outcomes of PEG-IFN/RBV combination therapy for cirrhosis remain suboptimal. However, some patients can achieve an SVR if the treatment is completed. Furthermore, following splenectomy or PSE in thrombocytopenic patients, IFN can be initiated with subsequent improved adherence to IFN. Unfortunately, our study found a low therapeutic effect rate. Given the high number of patients who discontinued IFN therapy because of side effects or oncogenesis and considering the complications and potential death associated with splenectomy or PSE, prognostic factors such as the HCV genotype and IL28B genetic polymorphism can assist in the decision to proceed with splenectomy or PSE in patients where a good therapeutic effect can be expected.

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ORIGINAL ARTICLE

Regional Hepatic Regeneration After Liver Resection Correlates Well with Preceding Changes in the Regional Portal Circulation in Humans

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Abstract
Background and Aims While portal hemodynamics largely affects the liver regeneration after partial hepatectomy, whether the remnant liver homogeneously regenerates is unclear, especially in humans. We hypothesized that change in flow distribution varies in each remnant portal branch after liver resection in humans and the liver consequently regenerates heterogeneously.
Methods Twenty-two patients who underwent anatomical hepatic resection preserving intact drainage veins were analyzed. Based on perioperative contrast-enhanced computed tomography, the regional hepatic regeneration in each segment was analyzed using a region growing software. The perioperative change in the distribution of blood flow in each portal branch was assessed using the computational flow dynamics technique. The correlation between the change in the portal flow distribution and the later regional hepatic regeneration was investigated.
Results The distribution of portal blood flow in each remnant branch largely changed at 2 weeks (71–389 %). Each remnant segment also heterogeneously regenerated at 3 months (85–204 %). Meanwhile, a good correlation between the regional regeneration rate at 3 months and the relative change in the flow distribution in each circulating portal branch at 2 weeks was detected in each patient ($r = 0.74$–0.99).
Conclusions After partial hepatectomy, the change in blood flow varies in each remnant portal branch and the liver heterogeneously regenerates in humans. The good correlation between the earlier change in the portal flow distribution and the later regional hepatic regeneration strongly suggests that the portal venous flow most likely regulates the non-uniform liver regeneration after hepatic resection in humans.

Keywords Computational flow dynamics · Portal hemodynamics · Anatomical hepatic resection · Liver regeneration

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Introduction

The capacity of the liver to regenerate after partial hepatectomy (PH) has been well studied in experimental animal models and in humans [1, 2]. According to these analyses, a variety of factors, including relatively increased portal vein pressure, subsequent increase in the shear stress against the portal venous wall, and several types of cytokines and growth factors, produced in the liver or other organs, have been considered to cooperatively play significant roles in the regenerative process. Moreover, a possible molecular mechanism by which sinusoidal endothelial cells regulate hepatocyte proliferation after PH has been recently elucidated [3]. The pressure in the portal venous system is much lower than that in the hepatic artery, and the portal blood flow is, therefore, easily affected by surgical procedures. Indeed, the relationship between the portal venous flow or pressure and liver regeneration in humans has been analyzed in patients who underwent living-donor liver transplantation [4–6]. Meanwhile, detailed regenerative analyses after PH in relation to

portal blood flow have been limited in humans. Namely, whether the blood flow in each remnant portal branch equally changes or if each remnant hepatic segment homogeneously regenerates after PH is obscure in humans. Meanwhile, we often observe different regenerative patterns in the remnant livers in clinical situations even after the same resection procedures. Thus, we hypothesized that change in blood flow varies in each remnant portal branch after PH and that regional hepatic volume also heterogeneously regenerates. Moreover, we also hypothesized that earlier heterogeneous hemodynamic change in each portal branch possibly regulates later regional regeneration in each area that the portal branch circulates.

Doppler ultrasound has been typically employed since the early 1990s to evaluate portal blood flow in humans [7]. The measurement of blood flow in the main portal trunk or the first major branches can be relatively accurate but may become less reliable when more peripheral branches are analyzed. To analyze changes in the portal blood flow in each remnant branch after PH, we need a more objective and reliable method to evaluate portal hemodynamics. Recent advances in the medical imaging technology have made obtaining three-dimensional (3D) image data of human organs easy. In the liver, 3D images of the hepatic vessels, including hepatic arteries, portal veins, and hepatic veins, and the hepatic parenchyma can be clearly extracted from computed tomography (CT) or magnetic resonance imaging data [8]. Using the 3D CT image of the liver and a region-growing method software, we previously reported that the accurate simulation of regional hepatic volume is possible based on the portal vein circulation [9]. Meanwhile, several engineering studies have reported the usefulness of computational fluid dynamics (CFD) to analyze hemodynamics in the human aortic arch, abdominal aorta, or cerebral arteries [10–12]. However, the hemodynamic analysis of portal blood flow employing the CFD techniques has been reported rarely in humans.

In the present study, we employed CFD techniques to evaluate the hemodynamics of the portal veins in humans and to simulate the perioperative changes in each portal branch, and verified our hypothesis that change in blood flow varies in each remnant portal branch after PH. Moreover, we analyzed regional hepatic regeneration to evaluate whether regeneration occurs homogeneously or not in the remnant liver. Then, we compared the earlier changes in the portal hemodynamics with the later regional hepatic regeneration. We report here for the first time that regional hepatic regeneration or the change in portal flow distribution does not homogeneously occur after PH in the remnant human liver and that the regional regeneration correlates well with preceding change in the blood flow distribution in each circulating portal branch.

Patients and Methods

Patients

A total of 22 patients with primary hepatic cancer (20 with hepatocellular carcinoma [HCC] and two with intrahepatic cholangiocarcinoma [ICC]) who met the following conditions were selected among the patients who received hepatic resection at Hyogo College of Medicine between 2006 and 2012 ($n = 377$). Namely, the patients who underwent anatomical hepatic resection preserving all intact major hepatic drainage veins, right, middle, and left hepatic veins, in the remnant liver ($n = 66$ out of 377) and also received thin-sliced contrast-enhanced CT scanning, which is usable for the computational flow dynamic simulation, preoperatively, at 2 weeks, and at 3 months after the hepatic resections ($n = 22$ out of 66), were enrolled in the present study. Informed consent for the pre- and postoperative examinations, such as CT and Doppler ultrasound, and for the use of all patient data in the entire study was obtained from each patient before all analyses. The study protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki and was approved by the institution's Ethics Committee.

Computed Tomography (CT)

All the patients preoperatively underwent contrast-enhanced CT scanning with 2 ml/kg (maximum, 100 mL) of contrast medium injected intravenously within 33 s. All patients were fasted at least 5 h before the CT scanning, and the same scanning protocol was used in all patients. The arterial phase, portal venous phase, and hepatic venous phase images were obtained as 2-mm thick slices by scanning at 35, 65, and 90 s, respectively, after the bolus infusion of the contrast agent using a multi-detector (MD) CT scanner (SOMATOM Definition AS+, Siemens AG, Munich, Germany). The same procedure was performed 2 weeks after the hepatic resection, when the hemodynamics of patients stabilizes, and 3 months after the operation, when the liver regeneration in most of patients was considered to reach a plateau [13].

Evaluation of Regional Hepatic Volume

The region-growing method software (Organs Volume Analysis; Hitachi Medico, Chiba, Japan) was used preoperatively to evaluate the regional hepatic volume [9]. Volumetric measurements were performed based on an algorithm in which the liver parenchyma was divided in proportion to the diameters and distances between the vessels. The regional hepatic volume was also simulated in the remnant liver in the same way 3 months after the

operation to calculate the regional hepatic regeneration rate during the period. The regeneration rate in each segment was calculated as follows: segment volume at 3 months/preoperative segment volume × 100 (%).

Construction of 3D Models of Portal Branches for Flow Simulation

The 3D images obtained from the MD-CT data (Fig. 1a, b) were segmented with the INTAGE Volume Editor (CYBERNET, Tokyo, Japan) to produce a 3D computer model of the portal vein starting just downstream of the level of the splenic vein. The scanned geometry contained multiple small irregularities, which were trimmed and surface smoothed using 3-matic software (Materialise, Leuven, Belgium). To perform the flow simulations, each model was meshed or subdivided into a finite number of small volume elements. Such a volume mesh was created using STAR CCM+ version6 (CD-adapco, Melville, NY, USA) (Fig. 1c). The mesh density was between approximately 2×10^5 and 10×10^5 elements, depending on the size and number of portal branches.

Numerical Simulations of Blood Flow Distribution in the Portal Veins

After constructing a 3D volume mesh model, the CFD software ANSYS FLUENT version13 (ANSYS Inc., Canonsburg, PA, USA) was used to numerically solve the Navier–Stokes equations, assuming rigid wall and Newtonian flow with a density of 1.055 g/cm^3 and viscosity of 0.0049 Pa s. For a realistic flow field simulation, boundary conditions are required to provide information on the flow at the inlet and outlets of the model. These boundary conditions should ideally be obtained via in vivo measurement. However, obtaining in vivo pre- and postoperative flow and pressure in each patient is difficult. Therefore, we focused only on simulating the distribution of flow in each portal branch in the present study. In the preliminary study, we simulated the flow distribution in each branch under the flow volumes of 600, 800, and 1,200 ml/min at the inlet and obtained similar results under all conditions. In the same manner, we simulated the flow distribution under the pressure of 0, 10, and 20 Pa at the outlets, and the resulting flow distribution in each branch did not significantly change under any condition. Therefore, we set the flow volume of the inlet at 1,200 ml/min and the pressure of the outlets at 0 Pa for all simulations and simulated the flow distribution in each portal branch outlet (Fig. 1d, 2a).

Doppler Ultrasound for Measurement of Portal Blood Flow

The measurement of portal blood flow using Doppler ultrasound is relatively reliable at the main portal trunk and the first portal branches [7]. Therefore, we compared the

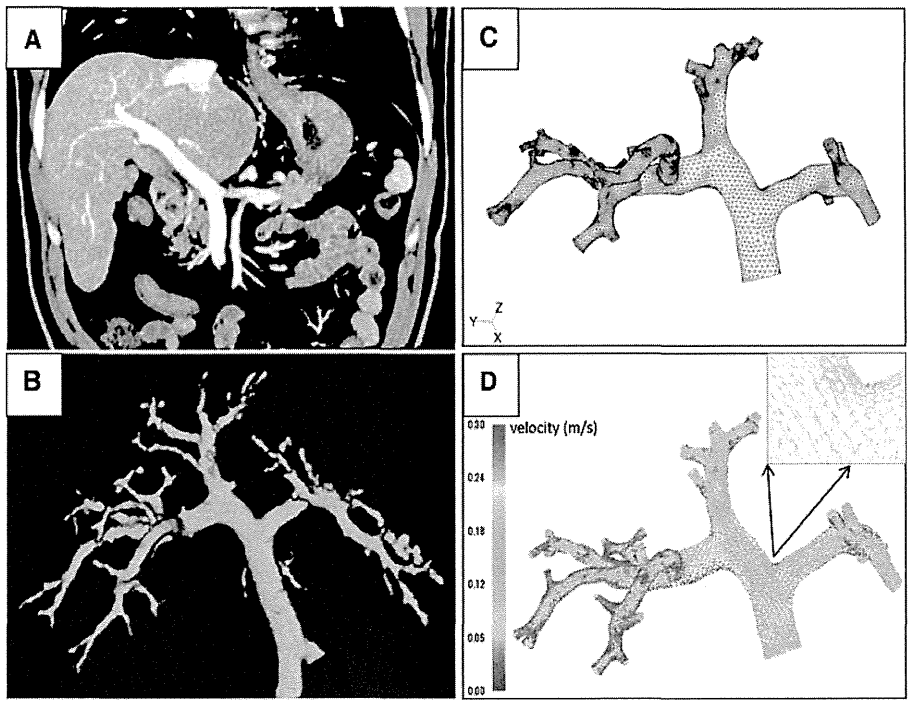

Fig. 1 Construction of 3D portal mesh model and flow simulation. Based on the data from contrast-enhanced computed tomography (CE-CT) (**a**), 3D images of the portal venous system were obtained (**b**). Then, a 3D computer mesh model of the portal vein was constructed (**c**), and the flow simulation was performed using CFD software (**d**). Insert (**d**) is an enlarged view of simulated velocity vectors

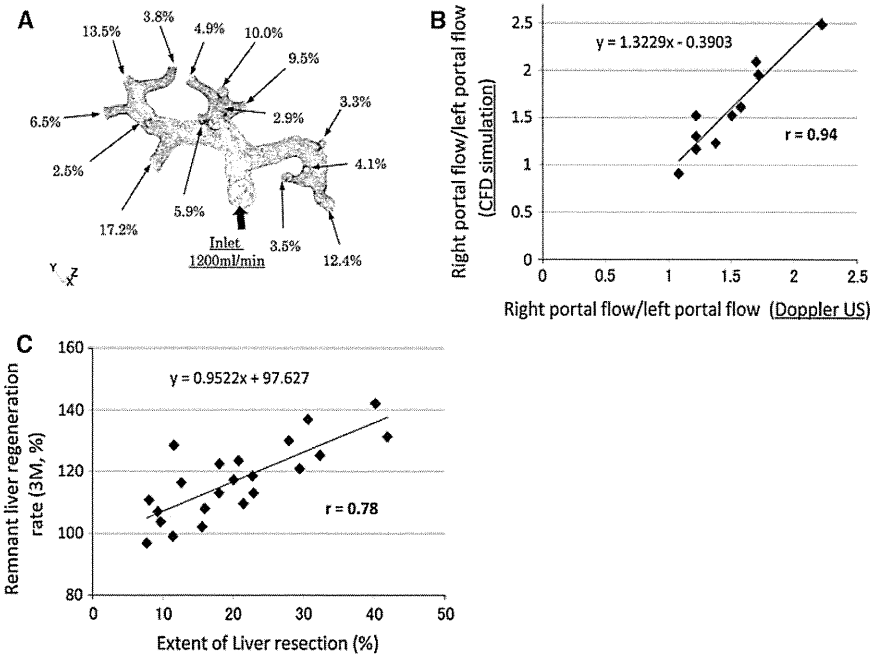

Fig. 2 Portal flow distribution and whole liver regeneration over 3 months. The flow distribution in each outlet of the 3D portal mesh model was calculated (**a**). The portal flow at the inlet was set at 1,200 ml/min, and the pressure at the each outlet was set at 0 Pa. A strong correlation between the ratio of right portal blood flow and left portal blood flow assessed by the Doppler ultrasonography and that assessed by the CFD simulation was detected (**b**). A good positive correlation between the regeneration rate of the remnant whole liver and the extent of liver resection was detected (**c**). *r* correlation coefficient

flow ratio in the right and left portal veins obtained from Doppler ultrasound and the ratio simulated by the CFD method in patients who underwent Doppler US before the operation ($n = 10$ out of 22) to validate the accuracy of the CFD simulation of the flow distribution in the portal branches. A relatively good correlation ($r = 0.94$, $p < 0.01$) between the ratio of right portal flow/left portal flow evaluated by Doppler US and the ratio simulated by CFD was observed (Fig. 2b), implying that this CFD simulation is feasible for the portal hemodynamic analysis.

Histological Analysis of the Liver Fibrosis

The degree of liver fibrosis was evaluated in each patient using the resected liver specimens. Knodell's histological liver fibrosis score system was employed to evaluate the degree of liver fibrosis [14].

Statistical Analysis

The data are presented as the mean ± SD (range, median). Statistical analyses were performed using Statview version 5.0.1 (SAS Institute Inc., Cary, NC). The correlations between two variables were analyzed using standard Pearson's correlation analysis. Scheffe's multiple comparison test was used to analyze the relation between the degree of liver fibrosis and the slope of the regression lines between the changes in portal flow distribution (% at 2 weeks) and the regional regeneration rate (% at 3 months).

Results

Patient Characteristics

The clinical features of the patients are shown in Table 1. According to the liver function tests, Child-Pugh scores, and MELD scores, the analyzed patients had relatively good liver functional reserve, while a significant degree of liver fibrosis was detected in some of the patients according to the histological liver fibrosis score.

Extent of Liver Resection and Regeneration Rate

The whole remnant liver regeneration during the 3 months after the operation (whole liver volume at 3 months/[preoperative whole liver volume − resected liver volume] × 100 [%]) was compared with the extent of liver resection (resected non-tumorous liver volume/preoperative whole non-tumorous liver volume × 100 [%]) in each patient. As expected, a good positive correlation ($r = 0.78$, $p < 0.001$) was detected between these parameters (Fig. 2c), supporting the previous observation that the regeneration rate after hepatic resection was proportional to the extent of liver resection [13]. The pattern of hepatic

Table 1 The clinical features of patients ($n = 22$)

Characteristic	Value
Age (years)	69 ± 9.3 (44–83, 71)
Sex (male/female)	15/7
Tumors (HCC/ICC)	20/2
Preoperative serum albumin (mg/dl)	4.0 ± 0.45 (2.9–4.9, 3.9)
Preoperative serum T.Bil (mg/dl)	0.87 ± 0.25 (0.4–1.2, 0.9)
Preoperative prothrombin activity (%)	88.7 ± 11.5 (65.4–107.4, 89.1)
Child-Pugh score	5.3 ± 0.72 (5–7, 5)
MELD score	7.6 ± 1.2 (6–11, 7)
Platelet counts ($\times 10^3/\mu l$)	157 ± 66 (75–346, 143)
Extent of liver resection (%)	20.4 ± 10.0 (9.3–41.9, 19.1)
Knodell's liver fibrosis score	2.5 ± 1.1 (1–4, 3)

Mean ± SD (range, median)

HCC hepatocellular carcinoma, *ICC* intrahepatic cholangiocellular carcinoma, *T.Bil* total bilirubin, *SD* standard deviation

resection (resected area) in each patient is shown in Table 2.

Changes in Portal Flow Distribution at 2 Weeks and Regional Hepatic Regeneration at 3 Months

Figure 3b shows the portal flow velocity and flow distribution in the portal branches in a patient who underwent anterior sectionectomy (case1: resection of segments 5 and 8) for HCC. Notably, changes in the flow distribution at 2 weeks were greatly different between each branch (71–208 %) even though all hepatic drainage veins in each remnant region were preserved intact. Next, the regional hepatic regeneration in each uninjured remnant segment was analyzed 3 months after the operation (Fig. 3a). Interestingly, the hepatic regeneration rate in each analyzed segment was not uniform during the period (104–122 %), implying that hepatic regeneration after PH does not occur homogeneously throughout the remnant liver in humans. Then, the regional hepatic regeneration rate in each segment at 3 months was compared with the preceding change in the flow distribution in each portal branch circulating the area at 2 weeks (Fig. 3c). A strong correlation ($r = 0.98$, $p < 0.05$) between these two parameters was observed, suggesting that the preceding change in the portal hemodynamics at 2 weeks possibly regulates the later regional hepatic regeneration.

Similarly, this correlation was analyzed in various resection patterns. Figure 4 shows the data in a patient who underwent hepatic resection of segment 7 (case 10). The flow distribution dramatically changed in each portal branch at 2 weeks (98–219 %, Fig. 4b), and the regional hepatic regeneration rate at 3 months varied in the remnant liver (105–129 %, Fig. 4a). Meanwhile, a strong correlation between these two parameters in each segment was again detected ($r = 0.97$, $p < 0.05$), even though the resection pattern was different (Fig. 4c).

Table 2 Regression line between the regional regeneration rate at 3 months (Y) and the changes in portal flow distribution at 2 weeks (X) in each case; $Y = aX + b$

Case	Resected area	a (slope)	b (Y-intercept)	r (correlation coefficient)
1	S5 + S8	0.1307	94.00	0.98
2	S8	0.373	74.98	0.98
3	S5 + S8	0.3751	74.73	0.97
4	S5 + S8	0.3715	65.19	0.96
5	S8	0.4865	66.16	0.86
6	S5 + S8	0.7775	22.99	0.79
7	S5 + S8	0.881	78.63	0.77
8	S8	0.4483	58.39	0.75
9	S8	0.445	85.31	0.74
10	S7	0.2244	81.13	0.97
11	S7	0.6504	37.47	0.99
12	S5	0.041	101.6	0.93
13	S4	1.0703	−4.698	0.93
14	S8	0.271	93.40	0.90
15	S6 + S7	0.2306	79.59	0.85
16	S4 + S5 + S8	0.3106	101.3	0.84
17	S6 + S7	1.7053	−50.48	0.81
18	S6	0.548	51.40	0.77
19	S8	0.379	59.73	0.97
20	S5 + S6	0.905	17.35	0.92
21	S4 + S5 + S8	1.264	−21.33	0.96
22	S5 + S8	0.656	17.37	0.86

The correlation analyses in all 22 patients are summarized in Table 2. The detected correlation coefficient was 0.99–0.74, suggesting that good or strong correlations exist between these two parameters, while the slope value of the regression line was largely different among the patients (0.1307–1.7053). Interestingly, the segment in which the regional regeneration rate was the greatest among the remnant liver was not identical even for the same resection pattern (Fig. 5a, b). Moreover, no obvious correlation was detected between the preoperative portal branching patterns and the postoperative regenerative patterns.

Regenerative Response to Change in Portal Hemodynamic and Liver Fibrosis

To speculate the mechanism underlying the various slope values of the regression lines (Table 2, a), the slope value, which was obtained when correlation analysis was performed between the changes in portal flow distribution

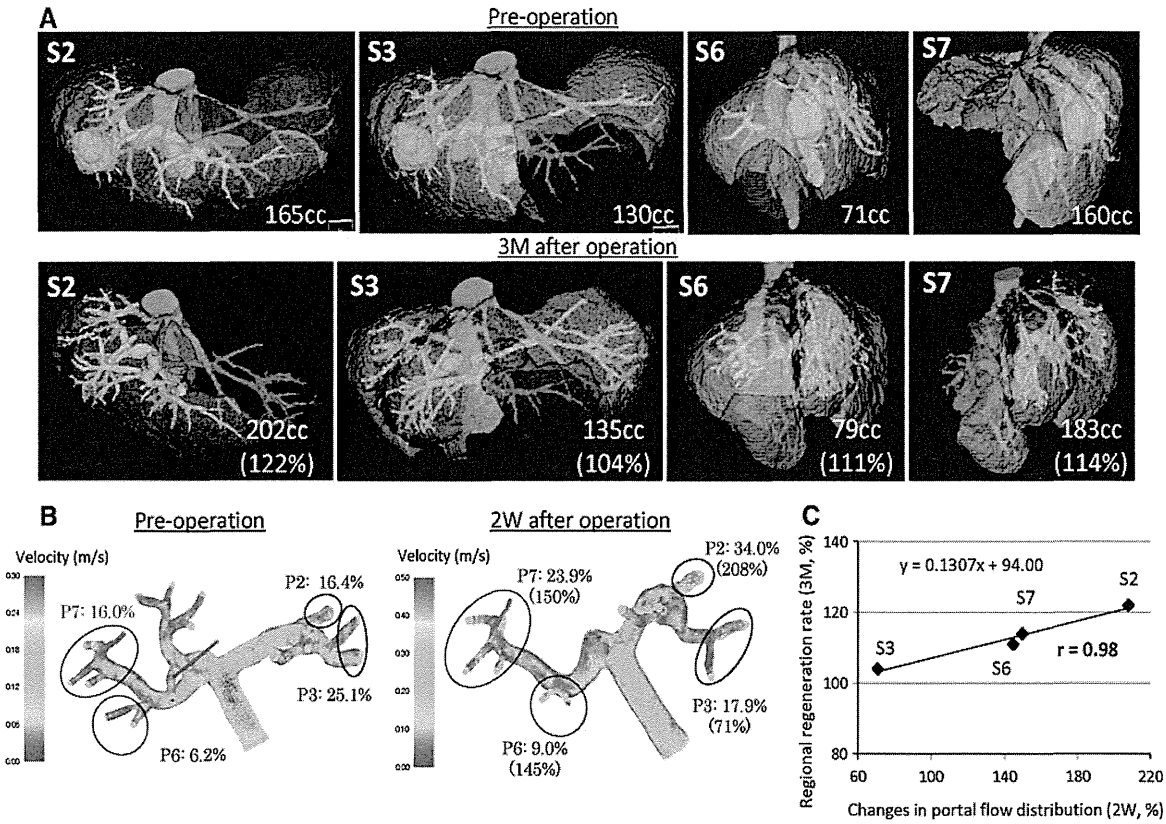

Fig. 3 Regional hepatic regeneration and change in portal flow distribution. The hepatic volume at each uninjured segment was calculated preoperatively and 3 months after the operation, and the regional hepatic regeneration rates were analyzed after the anterior sectionectomy (case 1, **a**). Percentages in the parentheses represent regeneration rate in each segment. The flow distribution was assessed preoperatively and 2 weeks after the operation in each portal branch (**b**). Percentages in the *parentheses* represent relative change in flow distribution compared with the preoperative data in each branch. A strong correlation between the regional hepatic regeneration rate and the change in the flow distribution in each analyzed segment was detected ($r = 0.98$, **c**)

(% at 2 weeks) and the regional regeneration rate (% at 3 months), was compared with the liver fibrosis score in each case (Fig. 5c). The slope value gradually decreased with liver fibrosis progression, implying that fibrotic livers are less responsive to changes in portal flow distribution during the period.

Discussion

Liver regeneration after partial hepatectomy has been well documented in humans and in animal models [1, 2]. However, whether the regeneration occurs homogeneously in the whole remnant liver was unclear. In the present study, we clearly demonstrated for the first time that regional hepatic regeneration in the remnant liver 3 months after operation does not occur homogeneously in humans. For the accurate evaluation of the regional regeneration rate, we analyzed the volumes of only uninjured segments after the operation. The regional outflow block of hepatic veins in the remnant liver is well known to have the potential to lead to atrophy of the congested area. To rule out this possibility, we enrolled only patients who underwent anatomical hepatic resection that preserved all major drainage hepatic veins, and this was one of the reasons why the number of subjects was relatively small in this study. Interestingly, the degree of regeneration in each preserved segment greatly varied among the patients, even though the same pattern of hepatic resection was performed, indicating that the regional regeneration in the remnant liver does not depend on the pattern of the hepatic resection. Compared with the high pressure of the arterial system, portal venous pressure is relatively low, and its hemodynamics may be easily affected by surgical procedures. Therefore, we speculated that the relative change in portal flow distribution after liver resection potentially regulates this non-uniform regional hepatic regeneration.

To simulate the change in the portal flow distribution after hepatic resection in humans, we employed the CFD technique. The advantage of the CFD simulation is that the

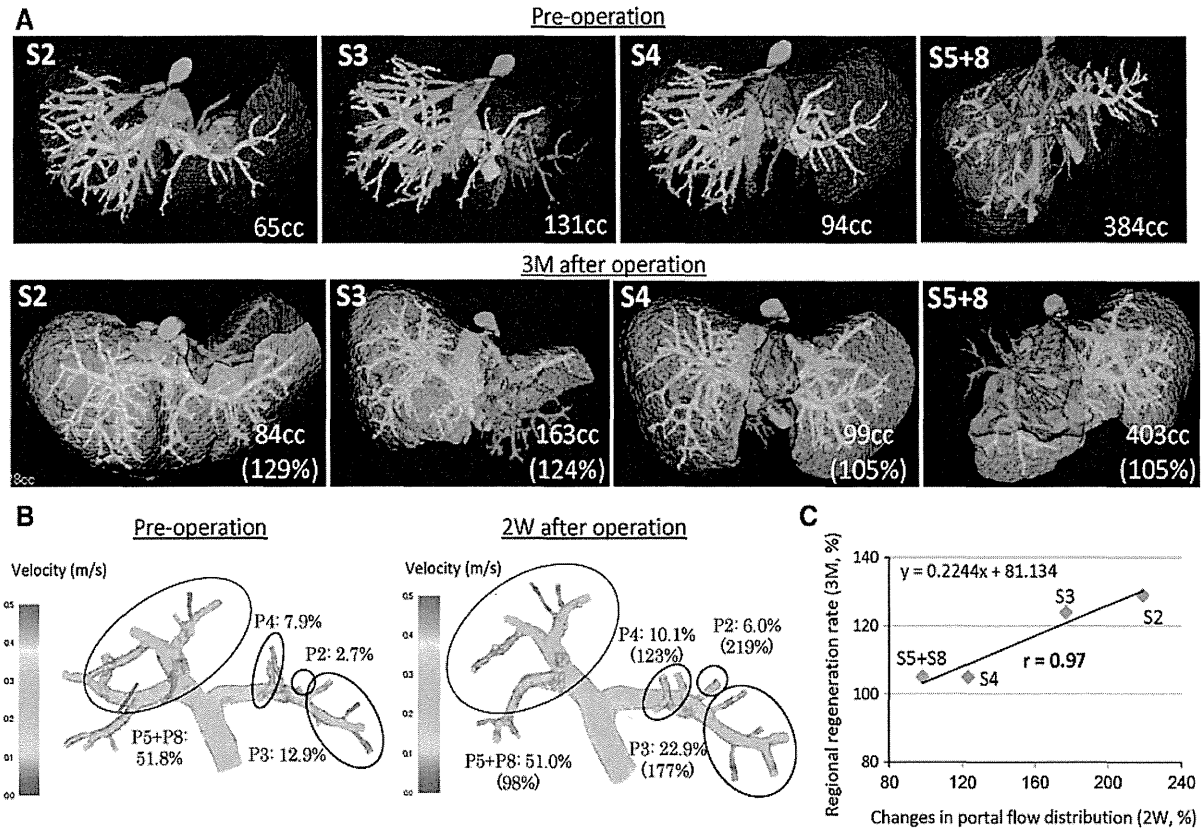

Fig. 4 Regional hepatic regeneration and change in portal flow distribution. The hepatic volume at each uninjured segment was calculated preoperatively and 3 months after the operation, and the regional hepatic regeneration rates were analyzed after the resection of segment 7 (case 10, **a**). Percentages in the *parentheses* represent the regeneration rate in each segment. The flow distribution was assessed preoperatively and 2 weeks after the operation in each portal branch (**b**). Percentages in the parentheses represent relative change in the flow distribution compared with the preoperative data in each branch. A strong correlation between the regional hepatic regeneration rate and the relative change in flow distribution in each analyzed segment was detected ($r = 0.97$, **c**)

hemodynamics can be estimated even in the peripheral branches if an accurate 3D mesh model can be obtained. However, boundary conditions at the inlet and outlets can largely affect the resulting hemodynamics. Therefore, we focused only on the flow distribution in the present study because we observed no significant change in the flow distribution when the flow at the inlet was changed from 600 to 1,200 ml/min or when the pressure at the outlets was changed from 0 to 20 Pa. Compared with the conventional portal flow measurement by Doppler US, the flow simulation by the CFD technique showed good feasibility in the analyzed patients. According to the CFD simulation, the distribution of the blood flow in each remnant portal branch was largely changed 2 weeks after the hepatic resection. Even with the same pattern of hepatic resection, the changes in the flow distribution largely varied, suggesting that the morphological change of the remnant portal branches itself rather than the resection pattern may regulate this change in hemodynamics.

Then, we compared the regional regeneration rate at 3 months with the change in the flow distribution in the portal branch circulating in each segment at 2 weeks. The region growing software employed in this study uses the diameter of portal branch as an important fundamental factor to calculate the regional volume as we previously reported [9]. The flow dynamic simulation also uses the same factor, the diameter of portal branch, as a one of the calculating factors. Thus, strong correlation between the regional hepatic volume and the simulated portal flow could be theoretically achieved if both the simulations were performed using the same 3D mesh model obtained at the same time point, and the comparison has less meaning. Meanwhile, we compared those two parameters at different time points, i.e. at 2 weeks when the hemodynamics of patients stabilizes and at 3 months when the liver regeneration in most of the patients was considered to reach a plateau. We assumed that a significant relation as "cause and effect" between these two parameters possibly exists if a good correlation can be detected when the simulations are performed at different time points. According to this analysis, a good or strong correlation was observed between these two parameters in each patient, even though

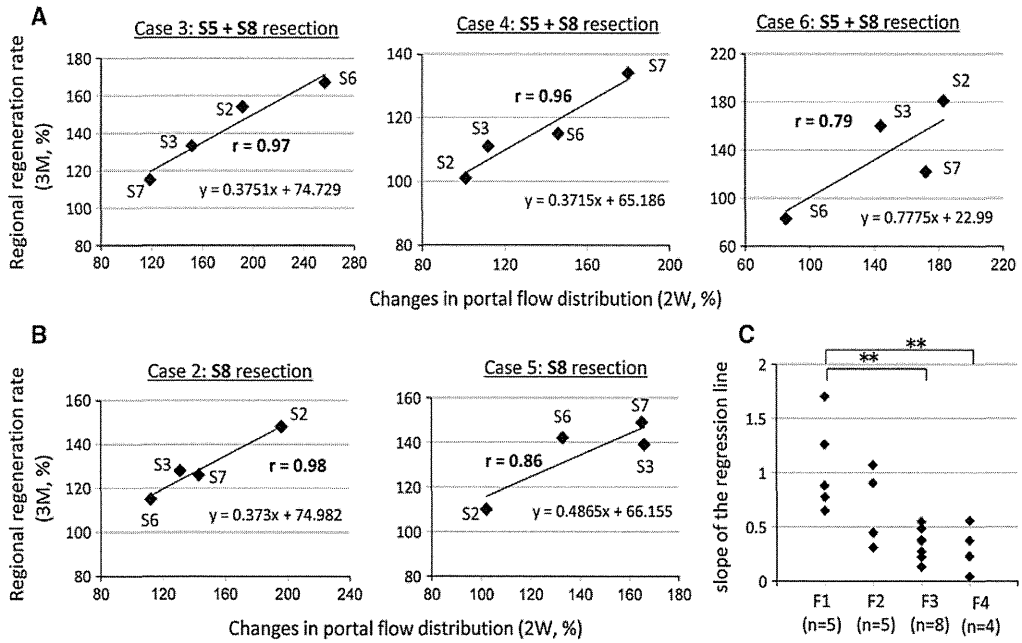

Fig. 5 Comparison of the regenerative patterns after the same resection procedures and relation between the degree of liver fibrosis and regenerative response. Correlations between the regional hepatic regeneration rates and the relative changes in flow distribution in each analyzed segment after the same resection procedures. **a** Resection of segments 5 and 8. **b** Resection of segment 8. The segment in which the regenerative response was greatest varied in each patient even after the same resection procedures. **c** The slope of the regression line between the regional hepatic regeneration rate at 3 months and the relative change in portal flow distribution at 2 weeks was compared with the degree of liver fibrosis score in each patient (F1-4). Scheffe's multiple comparison test was used to analyze the relation. ** $p < 0.01$

the regeneration pattern and change in the flow distribution largely varied among the patients. Even though we cannot definitely conclude from our results that the preceding change in portal flow distribution after PH regulates the later heterogeneous liver regeneration, we clearly demonstrated here, for the first time, that the preceding changes in the blood flow distribution in the portal branches correlates well with the later non-uniform regional regeneration in each segment in humans.

The theory that portal blood flow regulates liver regeneration is not totally new, and the role of the portal circulation in the initiation and promotion of liver regeneration after PH has been well discussed [1]. However, heterogeneous change in portal flow distribution and non-uniform regenerative response in each remnant segment after PH has not been systematically analyzed in humans. Therefore, our data again confirmed fundamental importance of portal flow in liver regeneration after PH in humans.

The CFD technique has been utilized mainly in the arterial system in human organs [10–12], and its usefulness has recently become recognized. Meanwhile, the CFD analysis in the portal tract, a lower pressure system, has been rarely reported, especially in humans. In the present study, we only analyzed the flow distribution in the portal branches because obtaining the actual in vivo data concerning the flow and pressure at the inlet and outlets of the 3D portal mesh model was difficult in humans. If the direct measurement of the boundary conditions at the inlet and outlets is possible, the CFD technique theoretically makes analyzing the flow volume, velocity, pressure, and wall shear stress possible in the 3D mesh model. Meanwhile, the CFD technique in the present form is still a time- and cost-consuming process, and further improvement in technology will be required to easily obtain more CFD data for use in general clinical situations. Nevertheless, the CFD technique has appeared to possess the potential for becoming a very useful strategy to analyze hepatic hemodynamics in the near future. Such an advanced simulation system would elucidate more clearly the mechanism of unique liver regeneration in humans.

Finally, clinical implication of our results should be considered. Heterogeneous liver regeneration after liver resection itself may possess less meaning in clinical practice, while its prediction would be of more importance. However, preoperative prediction of changes in portal hemodynamics after PH is still difficult in the present form of simulation system because the morphological change of remnant portal branches is largely affected by the change in the shape of whole remnant liver and the stiffness of each

portal branch. Incorporation of additional data concerning vascular stiffness or tissue deformation after PH into the portal hemodynamic simulation system would be useful for the development of a prediction system in the future. Using such an advanced system, prediction of regional hepatic regeneration after PH would possess significant meaning for safe liver surgery. In surgical treatment of multiple metastatic liver tumors from colorectal cancers, multi-step liver resections are sometimes employed. In such situations, it would be very useful for planning the second or third hepatic resection if we will be able to predict which remnant segment will predominantly regenerate after the first operation.

In summary, the non-uniform regional hepatic regeneration after PH correlated well with the preceding heterogeneous changes in portal venous flow distribution in humans, strongly suggesting the important role of the portal circulation in the regenerative process.

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Conflict of interest None.

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Original Article

Survey of non-B, non-C liver cirrhosis in Japan

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Aim: The aim of this survey was to reveal clinical features for each etiology of non-B, non-C liver cirrhosis (NBNC LC) in Japan.

Methods: In a nationwide survey of NBNC LC in Japan at the 15th General Meeting of the Japan Society of Hepatology, 6999 NBNC LC patients were registered at 48 medical institutions. Epidemiological and clinical factors were investigated.

Results: The percentage of NBNC LC among LC patients was 26%. NBNC LC patients were categorized into 11 types according to etiological agents: non-alcoholic steatohepatitis (NASH), 14.5%; alcoholic liver disease (ALD), 55.1%; fatty liver disease (FLD), except NASH, ALD, and other known etiology, 2.5%; primary biliary cirrhosis, 8.0%; other biliary cirrhosis, 0.8%; autoimmune hepatitis, 6.8%; metabolic disease, 0.6%; congestive disease, 0.8%; parasitic disease, 0.2%; other known etiology, 0.2%; and unknown etiology, 10.5%. Compared with previous surveys, the percentage of ALD remained unchanged, whereas that of NASH increased. The mean age and percentage of females were significantly higher in NASH patients than in ALD and FLD patients. Prevalence of diabetes mellitus was significantly higher in NASH and FLD patients than in ALD ones. Prevalence of hepatocellular carcinoma (HCC) in NBNC LC patients was 35.9%. Among NASH, ALD and FLD patients, 50.9%, 34.3% and 54.5% had HCC, respectively. Positivity of hepatitis B core antibody was significantly higher in HCC patients than in those without HCC (41.1% vs 24.8%).

Conclusion: This survey determined the etiology of NBNC LC in Japan. These results should contribute new ideas toward understanding NBNC LC and NBNC HCC.

Key words: alcoholic liver disease, hepatocellular carcinoma, non-alcoholic steatohepatitis, non-B, non-C liver cirrhosis

INTRODUCTION

A NATIONWIDE SURVEY of liver cirrhosis (LC) for each etiology has been conducted as the main theme on four occasions at the national academic conference in Japan. Therefore, many registered patients have been surveyed on uniform diagnostic criteria.[1] The 15th General Meeting of the Japan Society of Hepatology was held in October 2011. In a featured session in this meeting, we conducted a nationwide survey of non-B, non-C LC (NBNC LC) in patients at medical institutions in Japan. NBNC LC was the main theme of the featured session in this meeting for two reasons. First, the prevalence of non-alcoholic fatty liver disease (NAFLD) is increasing and has been recently reported to be approximately 20% in adults in Japan. Approximately 1% of adults in Japan are estimated to have non-alcoholic steatohepatitis (NASH).[2,3] Thus, NASH is the most common chronic liver disease not only in Western countries but also in Japan. NASH patients can develop LC and even hepatocellular carcinoma (HCC), although there have been few investigations concerning the incidence of LC associated with NASH (NASH LC)

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in Japan. Second, the number of NBNC HCC patients has been rapidly increasing, and it has been recently reported to account for approximately 15% of all HCC patients in Japan.[4] Most NBNC HCC patients seem to have LC with alcoholic liver disease (ALD LC); however, NASH LC has been noted as a high-risk group of NBNC HCC. Nevertheless, HCC complicated with NBNC LC of an unknown cause has been occasionally reported. Therefore, it is important to investigate the clinical features of NBNC LC, which will lead to the development NBNC HCC. Based on these backgrounds, we report the characteristics of NBNC LC in Japan. This was one of the programs of the 15th General Meeting of the Japan Society of Hepatology in 2011.

METHODS

Patient database

AT 48 MEDICAL institutions (all investigators are listed in Appendix I) (Table 1), 6999 subjects were diagnosed with NBNC LC based on the negative results for serum hepatitis B surface antigen (HBsAg), anti-hepatitis C antibody and hepatitis C virus (HCV) RNA. The patients registered in this study were clinically (laboratory examinations and imaging studies) and histologically diagnosed with LC based on the criteria proposed by a previous nationwide survey (the 44th Annual Meeting of the Japan Society of Hepatology in 2008).[1] The NBNC LC patients were categorized into 11 types according to etiology: (i) NASH; (ii) ALD; (iii) fatty liver disease (FLD); (iv) primary biliary cirrhosis (PBC); (v) other biliary cirrhosis (such as primary sclerosing cholangitis [PSC] and secondary biliary cirrhosis); (vi) autoimmune hepatitis (AIH) (including AIH-PBC overlap syndrome); (vii) metabolic disease (such as Wilson's disease, hemochromatosis and glycogen storage disease); (viii) congestive disease (including Budd–Chiari syndrome); (ix) parasitic disease (such as Japanese schistosomiasis); (x) other known etiology (such as sarcoidosis and drug-induced liver injury); and (xi) unknown etiology. The diagnosis of NASH was based on the following criteria: (i) absence of clinically significant alcohol consumption (intake of ≤20 g ethanol/day); (ii) appropriate exclusion of other liver diseases; (iii) complications with risk factors of steatosis such as obesity (in particular, visceral obesity), metabolic syndrome and diabetes mellitus; and (iv) the presence of steatosis on liver histology (histological

Table 1 Forty-eight medical institutions registered at the 15th General Meeting of the Japan Society of Hepatology on 2011

Akita University Graduate School of Medicine
Asahikawa-Kosei General Hospital
Asahikawa Medical University
(Division of Gastroenterology and Hematology/Oncology)
(Division of Metabolism and Biosystemic Science)
Asahikawa Red Cross Hospital
Chiba University
Dokkyo Medical University
Ehime Prefectural Central Hospital
Ehime University Graduate School of Medicine
Fukushima Medical University School of Medicine
Gunma University Graduate School of Medicine
Hyogo College of Medicine
Iwate Medical University
Jikei University School of Medicine, Katsushika Medical Center
Juntendo University School of Medicine
Kagawa University
Kanazawa Medical University
Keio University School of Medicine
Kumamoto University
Kurume University School of Medicine
Kyoto Second Red Cross Hospital
Mie University Graduate School of Medicine
Musashino Red Cross Hospital
Nagano Red Cross Hospital
Nara Medical University
National Center for Global Health and Medicine
Nihon University School of Medicine
Niigata Prefectural Central Hospital
Niigata University Medical and Dental Hospital
Oji General Hospital
Osaka City University
Osaka Police Hospital
Osaka Red Cross Hospital
Saiseikai Suita Hospital
Saitama Medical University
Sapporo City General Hospital
Sapporo-Kosei General Hospital
Shinshu University School of Medicine
Teikyo University School of Medicine
Teine-Keijinkai Hospital
Tokyo Medical and Dental University
Tokyo Medical University Ibaraki Medical Center
Tokyo Women's Medical University
Tottori University School of Medicine
University of Tokyo
University of Yamanashi
(First Department of Internal Medicine)
(First Department of Surgery)
Yamagata University Faculty of Medicine

diagnosis) or imaging studies (imaging diagnosis). The diagnosis of ALD was based on the proposed Diagnostic Criteria for Alcoholic Liver Disease by a Japanese study group for ALD (the Takada group).[5] The diagnosis of FLD was based on the following criteria: (i) alcohol consumption between that for NASH and ALD (i.e. intake of >20 g and <70 g ethanol/day); (ii) appropriate exclusion of other liver diseases; and (iii) the presence of steatosis on liver histology or imaging studies.

The following variables were used to investigate the clinical features of NBNC LC: age; sex; body mass index (BMI); prevalence of diabetes mellitus (DM), impaired glucose tolerance, hypertension and dyslipidemia; Child–Pugh classification; prevalence of gastroesophageal varices and HCC; and presence of hepatitis B core antibody (anti-HBc). In addition, the percentage of NBNC LC was investigated among all LC patients at each institution and was compared with previous reports. The ethics committees of the appropriate institutional review boards approved this study in accordance with the Declaration of Helsinki (2000).

Statistical analyses

Statistical tests were performed using the IBM SPSS Statistics ver. 21. The statistical significance of difference was determined using the χ^2-test, Mann–Whitney *U*-test and multivariate Cox's proportional hazard model as appropriate. $P < 0.05$ was considered statistically significant.

RESULTS

Percentage of NBNC LC among all LC patients

WE CALCULATED THE percentage of NBNC LC among all 25 020 LC patients at 37 registered institutions. The percentages of NBNC LC, hepatitis B virus (HBV)-related cirrhosis, HCV-related cirrhosis, and both HBV- and HCV-related cirrhosis were 26%, 12%, 60.9% and 1.1%, respectively. Compared with a previous nationwide survey (the 44th Annual Meeting of the Japan Society of Hepatology in 2008),[1] there was no significant difference between them (Table 2).

Frequency of each etiology among NBNC LC patients

We determined the frequency and percentage of each etiology among all 6999 NBNC LC patients at 48 registered institutions. The percentages of each etiology were as follows: NASH, 14.5%; ALD, 55.1%; FLD, 2.5%; PBC, 8.0%; other biliary cirrhosis, 0.8%; AIH, 6.8%; metabolic disease, 0.6%; congestive disease, 0.8%; parasitic disease, 0.2%; other known etiology, 0.2%; and unknown etiology, 10.5% (Table 3). Among 1015 NASH patients, 309 (30.4%) were diagnosed histologically, 402 (39.6%) were diagnosed by imaging studies and the method of diagnosis of 304 patients (30%) was not described in detail. Among 60 patients with other biliary cirrhosis, 71.7% had PSC and the rest had cholestatic diseases, except PBC and PSC (such as congenital biliary atresia and secondary biliary cirrhosis). Among 39 metabolic disease patients, 66.7% had Wilson's disease, 25.6% had hemochromatosis (glycogen storage disease, amyloidosis and citrullinemia in one patient each). All 12 cases of parasitic disease were Japanese schistosomiasis. Of 11 patients with other known etiology, two patients sarcoidosis, two post-liver transplantation, two post-hepatectomy, one drug-induced liver injury, one systemic lupus erythematosus-related liver injury and the diagnosis of the remaining patients was not described in detail.

Compared with the survey at the 44th Annual Meeting of the Japan Society of Hepatology in 2008,[1] the percentage of ALD among all NBNC LC patients did

Table 2 Percentage of NBNC LC among all patients with liver cirrhosis compared with the 44th Annual Meeting of the Japan Society of Hepatology on 2008[1]

	The 15th General Meeting of the Japan Society of Hepatology on 2011 ($n = 25\,020$)	The 44th Annual Meeting of the Japan Society of Hepatology on 2008 ($n = 33\,379$)	*P*-value
NBNC LC	26.0%	24.0%	N.S.
HBV-related cirrhosis	12.0%	13.9%	N.S.
HCV-related cirrhosis	60.9%	60.9%	N.S.
both HBV- and HCV-related cirrhosis	1.1%	1.2%	N.S.

P-values were analyzed by χ^2-test.
HBV, hepatitis B virus; HCV, hepatitis C virus; NBNC LC, non-B, non-C liver cirrhosis; N.S., not significant.

Table 3 Frequency of each etiology among patients with NBNC LC compared with the 44th Annual Meeting of the Japan Society of Hepatology on 2008[1]

	The 15th General Meeting of the Japan Society of Hepatology on 2011 ($n = 6999$)	The 44th Annual Meeting of the Japan Society of Hepatology on 2008 ($n = 8011$)	*P*-value
NASH	14.5%	8.7%	$P < 0.001$
ALD	55.1%	56.3%	N.S.
FLD	2.5%	–	–
PBC	8.0%	9.9%	$P < 0.001$
Other biliary cirrhosis	0.8%	1.2%	$P < 0.001$
AIH	6.8%	7.9%	$P = 0.018$
Metabolic disease	0.6%	1.2%	$P < 0.001$
Congestive disease	0.8%	1.2%	$P = 0.013$
Parasites	0.2%	0.4%	$P = 0.011$
Other known etiology	0.2%	0.8%	$P < 0.001$
Unknown etiology	10.5%	12.4%	$P < 0.001$

P-values were analyzed by χ^2-test.
AIH, autoimmune hepatitis; ALD, alcoholic liver disease; FLD, fatty liver disease; NASH, non-alcoholic steatohepatitis; NBNC LC, non-B, non-C liver cirrhosis; N.S., not significant; PBC, primary biliary cirrhosis.

not change (55.1% vs 56.3%), whereas that of NASH increased (14.5% vs 8.7%; $P < 0.001$) (Table 3).

Clinical features of NBNC LC patients

The male : female ratio for the NBNC LC patients was 1.93. The percentages of each etiology among 4608 male and 2391 female patients were as follows: NASH (9.5% and 24%), ALD (73.4% and 19.8%), FLD (3.4% and 0.9%), PBC (1.9% and 20%), other biliary cirrhosis (0.8% and 0.9%), AIH (1.5% and 17.1%), metabolic disease (0.5% and 0.8%), congestive disease (0.8% and 0.8%), parasitic disease (0.2% and 0.1%), other known etiology (0.1% and 0.2%) and unknown etiology (7.9% and 15.4%), respectively (Fig. 1). The male : female ratio for each etiology among the NBNC LC patients was as follows: NASH, 0.77; ALD, 7.12; FLD, 6.86; PBC, 0.18; other biliary cirrhosis, 1.73; AIH, 0.17; metabolic disease, 1.29; congestive disease, 2.17; parasitic disease, 5; other known etiology, 0.83; and unknown etiology, 0.99 (Table 4). Thus, the NASH patients were predominantly female as opposed to the ALD and FLD patients who were predominantly male.

The mean age at clinical diagnosis in the NBNC LC patients for NASH, ALD, FLD, PBC, other biliary cirrhosis, AIH, metabolic disease, congestive disease, parasitic disease, other known etiology and unknown etiology was 66.9, 60.3, 64.2, 63.6, 51.3, 64.5, 42.6, 52.7, 77.4, 56.1 and 68.8 years, respectively. In the patients with NASH, AIH, congestive disease and unknown etiology, the mean ages at clinical diagnosis of the male patients were lower than those of the female patients ($P < 0.001$). In contrast, in the ALD, FLD, PBC and metabolic disease patients, the mean ages at clinical diagnosis of the female patients were lower than those of the male patients ($P < 0.001$) (Table 5).

Regarding the risk factors of NASH, the following variables were investigated in the NASH, ALD and FLD patients: BMI and the prevalence of DM, impaired glucose tolerance (IGT), hypertension and dyslipidemia. BMI in the NASH, ALD and FLD patients was 27, 23.4 and 25 kg/m^2, respectively, and the differences among them were statistically significant. The prevalence of DM and IGT in the NASH and FLD patients (63% and 57%, respectively) was significantly higher compared with that in the ALD patients (31%) ($P < 0.001$). The prevalence of dyslipidemia in the NASH and FLD patients (25% and 29%, respectively) was significantly higher compared with that in the ALD patients (14%) ($P < 0.001$). The prevalence of hypertension in the NASH patients (52%) was significantly higher compared with that in the ALD and FLD patients (28% and 35%, respectively) ($P < 0.001$) (Table 6).

The levels of hepatic functional reserve based on the Child–Pugh classification for each etiology are summarized in Table 7. The percentages of moderate-to-low hepatic reserve (Child–Pugh class B and C) in the ALD and AIH patients (52.9% in both) were significantly higher compared with those in the NASH and FLD patients (35.8% and 27%, respectively) ($P < 0.001$).

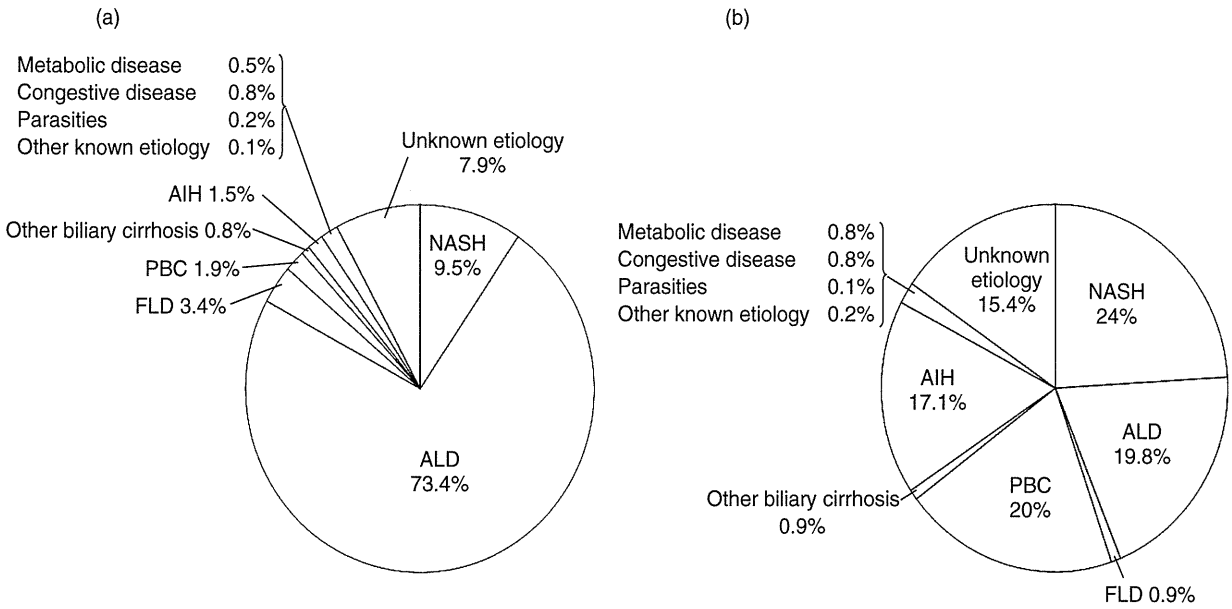

Figure 1 Frequency of each etiology among male or female patients with NBNC LC. (a) Male, (b) female. AIH, autoimmune hepatitis; ALD, alcoholic liver disease; FLD, fatty liver disease; NASH, non-alcoholic steatohepatitis; NBNC LC, non-B, non-C liver cirrhosis; PBC, primary biliary cirrhosis.

To determine the frequency of complicated portal hypertension patients, the prevalence of gastroesophageal varices was calculated. The prevalence in the ALD and PBC patients (54.5% and 61.9%, respectively) was significantly higher compared with that in the patients with NASH, FLD, AIH and unknown etiology (40.8%, 40.7%, 48.2% and 45.9%, respectively) ($P < 0.05$). Considering only patients with Child–Pugh class A, the prevalence of gastroesophageal varices in PBC patients was highest among all etiologies. ALD had significantly higher prevalence than NASH, the histology of which was very similar (Table 8).

Table 4 Male : female ratio of each etiology

	Male ($n = 4608$)	Female ($n = 2391$)	Male : female ratio
NASH	440	575	0.77
ALD	3381	475	7.12
FLD	151	22	6.86
PBC	87	477	0.18
Other biliary cirrhosis	38	22	1.73
AIH	69	409	0.17
Metabolic disease	22	19	1.29
Congestive disease	39	18	2.17
Parasites	10	2	5.00
Other known etiology	5	4	0.83

AIH, autoimmune hepatitis; ALD, alcoholic liver disease; FLD, fatty liver disease; NASH, non-alcoholic steatohepatitis; NBNC LC, non-B, non-C liver cirrhosis; N.S., not significant; PBC, primary biliary cirrhosis.

The prevalence of HCC in the NBNC LC patients was 35.9%. Among 2438 NBNC HCC patients, 51.9% were diagnosed with HCC simultaneously with the diagnosis of NBNC LC, 25.6% were diagnosed after, 1.4% were diagnosed before the diagnosis of NBNC LC and the diagnosis of the remaining patients was not described in detail. The male : female ratio for the NBNC HCC patients was 3.06. The percentage of each etiology among the HCC patients was as follows: NASH, 19.9%; ALD, 53.4%; FLD, 3.7%; PBC, 3.2%; other biliary cirrhosis, 0.2%; AIH, 4.9%; metabolic disease, 0.1%; congestive disease, 0.7%; parasitic disease, 0.1%; other known etiology, 0%; and unknown etiology, 13.8%. The percentage of NASH among the NBNC HCC patients was significantly higher than that among the NBNC LC patients (19.9% vs 14.5%, $P < 0.001$). The clinical diagnosis of HCC was made at a mean age of 67.2 years in all patients. The mean age of onset of HCC was 70.8, 64.8 and 68.4 years in the NASH, ALD and FLD patients, respectively, and the differences among them were significant ($P < 0.001$). The prevalence of

Table 5 The mean ages at clinical diagnosis in the patients with NBNC LC

	Total ($n = 6999$)	Male ($n = 4608$)	Female ($n = 2391$)	*P*-value (M vs F)
NASH	66.9 ± 11.6	64.8 ± 13.2	68.5 ± 9.8	$P < 0.001$
ALD	60.3 ± 11.0	60.9 ± 10.7	55.7 ± 12.1	$P < 0.001$
FLD	64.2 ± 11.8	64.7 ± 11.3	61.2 ± 15.0	$P < 0.001$
PBC	63.6 ± 12.1	66.0 ± 11.3	63.2 ± 12.0	$P < 0.001$
Other biliary cirrhosis	51.3 ± 20.7	52.0 ± 22.0	50.0 ± 19.0	$P < 0.001$
AIH	64.5 ± 12.2	63.3 ± 14.2	66.0 ± 11.7	$P < 0.001$
Metabolic disease	42.6 ± 18.2	44.0 ± 18.0	40.7 ± 19.0	$P < 0.001$
Congestive disease	52.7 ± 20.4	50.5 ± 20.7	57.4 ± 19.6	$P < 0.001$
Parasites	77.4 ± 5.9	76.5 ± 6.1	81.5 ± 2.1	$P < 0.001$
Other known etiology	56.1 ± 19.1	53.0 ± 18.7	58.7 ± 20.8	$P < 0.001$
Unknown etiology	68.8 ± 11.9	67.9 ± 13.0	69.8 ± 10.7	$P < 0.001$

All results are expressed as mean ± standard deviation. *P*-values were analyzed by Mann–Whitney *U*-test.
AIH, autoimmune hepatitis; ALD, alcoholic liver disease; FLD, fatty liver disease; NASH, non-alcoholic steatohepatitis; NBNC LC, non-B, non-C liver cirrhosis; PBC, primary biliary cirrhosis.

HCC in the patients with NASH, FLD and unknown etiology (50.9%, 54.5% and 47.5%, respectively) were significantly higher compared with that in the ALD, PBC and AIH patients (34.3%, 14.4% and 26.0%) ($P < 0.0001$). The percentage of moderate-to-low hepatic reserve (Child–Pugh class B and C) in HCC in AIH patients was significantly higher than those in the patients with NASH, FLD and unknown etiology

Table 6 Risk factors of NASH in the patients with NASH, ALD and FLD

Variable	NASH ($n = 1015$)	ALD ($n = 3856$)	FLD ($n = 173$)	*P*-value
Body mass index (kg/m^2)	27.0 ± 4.3	23.4 ± 6.4	25.0 ± 3.7	$P < 0.001$*,**
Diabetes mellitus or Impaired glucose tolerance	62.5%	37.5%	56.5%	$P < 0.001$*
Dyslipidemia	25.0%	13.5%	29.4%	$P < 0.001$* $P = 0.01$**
Hypertension	52.0%	28.2%	34.7%	$P < 0.001$*,**

ALD, alcoholic liver disease; FLD, fatty liver disease; NASH, non-alcoholic steatohepatitis.
Results of body mass index are expressed as mean ± standard deviation. *P*-values were analyzed by Mann–Whitney *U*-test and χ^2-test.
*NASH vs ALD, **NASH vs FLD.

Table 7 Levels of hepatic functional reserve based on the Child–Pugh classification

Child–Pugh classification	Class A	Class B	Class C	Percentages of both class B and C	*P*-value
NASH ($n = 783$)	503	222	58	35.8%	
ALD ($n = 2710$)	1276	867	567	52.9%	$P < 0.001$*
FLD ($n = 89$)	65	18	6	27.0%	
PBC ($n = 355$)	204	105	46	42.5%	
AIH ($n = 295$)	139	106	50	52.9%	$P < 0.001$** $P = 0.01$***
Unknown etiology ($n = 515$)	300	150	65	41.7%	

AIH, autoimmune hepatitis; ALD, alcoholic liver disease; FLD, fatty liver disease; NASH, non-alcoholic steatohepatitis; NBNC LC, non-B, non-C liver cirrhosis; PBC, primary biliary cirrhosis.
P-values were analyzed by χ^2-test.
*vs NASH, FLD, PBC and unknown etiology; **vs NASH and FLD; ***vs PBC and unknown etiology.