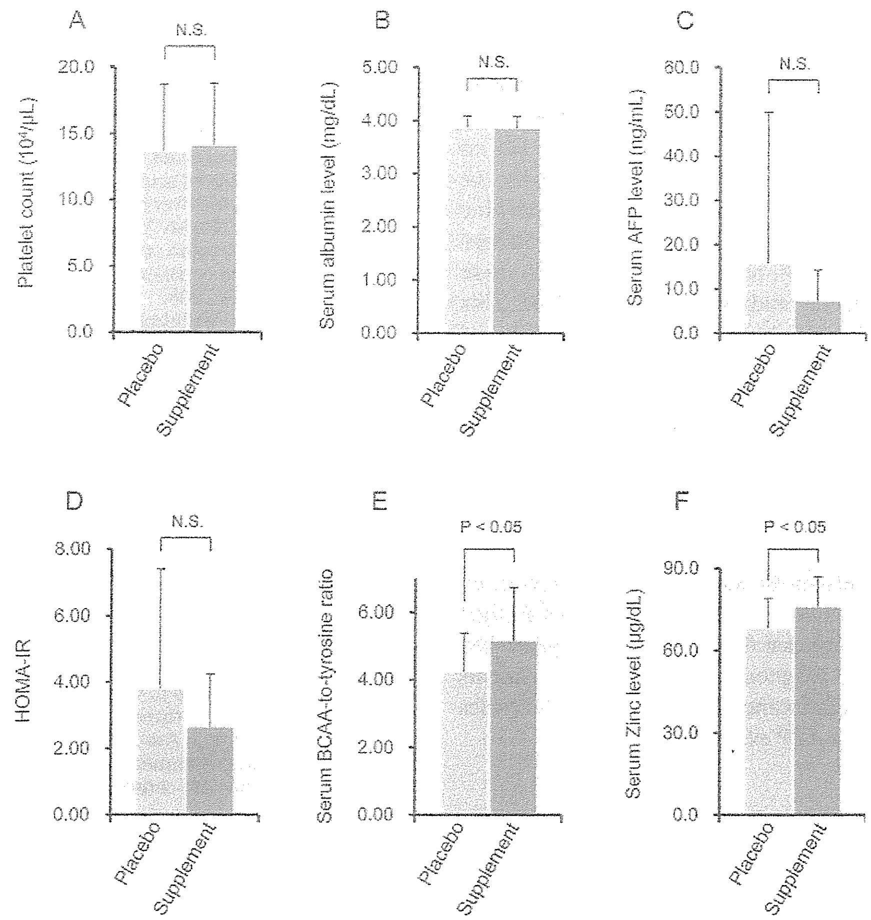

Figure 3. Effects of the BCAA and zinc-enriched supplement on prognostic factors: (A) Platelet count, (B) serum albumin level, (C) serum AFP level, (D) HOMA-IR value, (E) serum BCAA-to-tyrosine ratio and (F) serum zinc level. The data are expressed as the mean ± SD. The gray area is within the reference values of each parameter. Differences between the placebo and supplement groups were analyzed using the Mann-Whitney U test. P<0.05 was considered to indicate a statistically significant difference. AFP, α-fetoprotein; HOMA-IR, homeostasis model assessment for insulin resistance; BCAA, branched-chain amino acids.

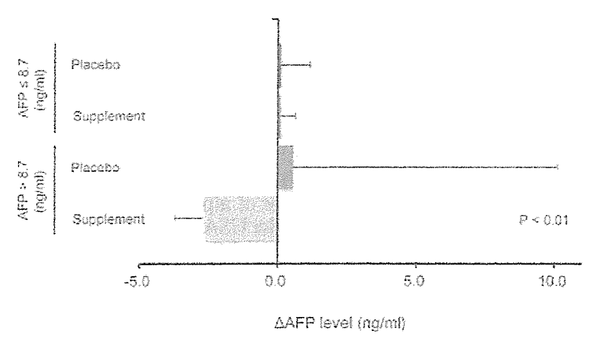

Figure 4. Stratification analysis according to the serum α-fetoprotein (AFP) level at baseline. The patients in the placebo and supplement group were further classified into two groups based on the serum AFP level at baseline: One group with AFP levels within the reference value (≤8.7 ng/ml) and another group with elevated serum AFP levels (>8.7 ng/ml). Changes in the serum AFP levels were expressed as ΔAFP (day 60 AFP level vs. day 0 AFP level) and compared among the groups. Statistical comparisons between multiple groups were performed using the Kruskal-Wallis test. P<0.05 was considered to indicate a statistically significant difference.

the BCAA and zinc-enriched supplement. However, the stratification analysis revealed a significant reduction in the ΔAFP levels in the supplement group with elevated AFP levels at baseline compared with the other groups. Although the reasons for the supplement-induced reductions in serum AFP levels are unclear, our findings are supported by previously published studies. First, Hagiwara *et al* (22) reported that BCAAs induce apoptosis in HCC cell lines by promoting a negative feedback loop from the mammalian target of rapamycin complex 1/S6K1 to the PI3K/Akt pathway and by suppressing the mammalian target of rapamycin complex 2 kinase activity towards Akt. Second, zinc stabilizes zinc finger proteins, which bind to DNA, and Nakao *et al*, as well as Xie *et al*, reported that zinc fingers and homeoboxes 2 and zinc finger and BTB domain-containing protein 20 repress the postnatal expression of AFP by interacting with the AFP gene promoter regions (23,24). Thus, BCAAs and zinc may independently contribute to a reduction in serum AFP levels by causing apoptosis of hepatoma cells and repressing AFP expression.

In this study, the BCAA and zinc-enriched supplement did not affect the platelet count, HOMA-IR value or HCV RNA levels. Conversely, previous basic studies demonstrated that valine, a BCAA, increased blood platelet counts in carbon tetrachloride-treated cirrhotic rats (25). Leucine and isoleucine have been shown to improve insulin resistance in mice fed a high-fat diet (26,27). Valine has been shown to suppress HCV genome replication in a dose-dependent manner (28). Although the reason for the discrepancy between these previous studies and our study remains unknown, BCAAs may exert beneficial effects on the platelet count, HOMA-IR value and serum HCV RNA levels only under specific conditions. We also demonstrated that no subjective symptoms were significantly improved by the BCAA and zinc-enriched supplementation. BCAAs and zinc have been previously reported to improve muscle cramps and taste disorders (16,29,30), respectively. However, these symptoms were mild in the study subjects at baseline. This may explain why significant changes in muscle cramps and taste disorders were not evident in this study.

In conclusion, we examined the effects of a BCAA and zinc-enriched supplement on prognostic factors in HCV-infected patients. There were no significant changes in platelet count, serum albumin levels or HOMA-IR values. However, serum BTR and zinc levels were significantly improved by the supplementation. In addition, a stratification analysis revealed a significant reduction in ΔAFP levels in the supplement group, with an increase in AFP levels compared with the other groups. In light of these results, we conclude that the BCAA and zinc-enriched supplement may improve prognosis in HCV-infected patients by improving amino acid imbalance, reducing zinc deficiencies and partly downregulating AFP expression.

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ORIGINAL ARTICLE—LIVER, PANCREAS, AND BILIARY TRACT

The morbidity and associated risk factors of cancer in chronic liver disease patients with diabetes mellitus: a multicenter field survey

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Abstract
Background and aims Diabetes mellitus is associated with various cancers; however, little is known of the relationship between cancer and diabetes in chronic liver disease (CLD) patients. The aim of this study is to investigate the morbidity and associated factors of cancer, including the use of anti-diabetics, in CLD patients with diabetes.
Patients and methods We performed a multicenter survey in 2012 and 478 CLD patients with diabetes were enrolled (age 64.3 ± 12.1 years, female/male 187/291). A frequency analysis of cancer and antidiabetic use was performed. Independent factors for cancer were analyzed using logistic regression and decision-tree analysis.
Results The morbidity of cancer was 33.3 %. Hepatocellular carcinoma (HCC) and extra-hepatic cancer were diagnosed in 24.7 and 11.3 % of enrolled patients, respectively. The frequency of antidiabetic use was 66.5 %. Of prescribed antidiabetics, 39 % were dipeptidyl-peptidase 4 inhibitors; however, their use was not significantly associated with cancer. In contrast, the use of exogenous insulin (OR 2.21; 95 % CI 1.16–4.21, $P = 0.0165$) and sulfonylurea (OR 2.08; 95 % CI 1.05–3.97, $P = 0.0353$) were independently associated with HCC and extra-hepatic cancer, respectively. In decision-tree analysis, exogenous insulin and sulfonylurea were also identified as a divergence factor for HCC and extra-hepatic cancer, respectively.
Conclusions We found a high morbidity of not only HCC, but also extra-hepatic cancer in CLD patients with diabetes. We also showed a possible association between the use of antidiabetics and the morbidity of cancer. Thus, a large-scale cohort study is needed to establish a therapeutic strategy for diabetes to suppress carcinogenesis in CLD patients.

Keywords Diabetes mellitus · Chronic liver disease · Morbidity · Risk factor

Abbreviations

HCC	Hepatocellular carcinoma
CLD	Chronic liver disease
DPP-4	Dipeptidyl peptidase-4
AST	Aspartate aminotransferase
APRI	AST to platelet ratio index
ALT	Alanine aminotransferase
GGT	Gamma-glutamyl transpeptidase
HbA1c	Hemoglobin A1c

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HCV	Hepatitis C virus
HBV	Hepatitis B virus
MAPK	Mitogen-activated protein kinase
IGF	Insulin-like growth factor

Introduction

Diabetes mellitus is a known independent risk factor for a number of different cancers [1]. Recently, population-based studies and meta-analyses demonstrated that diabetes mellitus is a potent risk factor for hepatocellular carcinoma (HCC) [2, 3]. In addition, diabetes mellitus is a risk factor for extra-hepatic cancers including pancreatic cancer, bile duct cancer, and colon cancer [4–6], and is also known to increase the risk of other extra-hepatic cancers, including gynecologic cancers, respiratory tumors, and hematological malignancies [7–9].

Diabetes mellitus consists of a number of diverse diseases, including impaired insulin secretion and insulin resistance. Patients with chronic liver disease (CLD) often develop increased insulin resistance and pancreatic β cells consequently secrete excess insulin in order to maintain glucose homeostasis [10, 11]. Thus, hyperinsulinemia is a feature of CLD patients with diabetes. Insulin is a potent mitogen and promotes cell proliferation [12], and hyperinsulinemia is a risk factor for the development of cancer in patients with diabetes mellitus [1, 13]. These previous findings suggest a possible association between cancer and diabetes in CLD patients; however, no practical data are available for the morbidity of cancer in CLD patients with diabetes.

Established risk factors for carcinogenesis include age, sex, smoking, excessive alcohol intake, and chronic viral infection [14]. In addition, we, along with others, have reported a possible association between the use of anti-diabetic agents and carcinogenesis [15, 16]. The use of sulfonylurea, an insulin secretagogue, and exogenous insulin are associated with HCC and extra-hepatic cancers including pancreatic cancer, colon cancer, and breast cancer [15–18]. Recently, dipeptidyl peptidase-4 (DPP-4) inhibitor has become widely used to treat diabetes mellitus because of its ability to lower glucose levels with a low risk of hypoglycemia; however, a possible association between the use of DPP-4 inhibitors and cancer has never been investigated in CLD patients with diabetes.

The aims of this study were to investigate the morbidity of cancer and cancer-associated factors, including the use of anti-diabetics, in CLD patients with diabetes.

Subjects and methods

Ethics

The study protocol conformed to the ethical guidelines of the Declaration of Helsinki, as reflected in the prior approval given by each institutional review board. None of the subjects were institutionalized.

Study design

In 2012, we performed a multicenter cross-sectional study to investigate the morbidity of cancer and cancer-associated factors, including the use of anti-diabetics, in CLD patients with diabetes.

Subjects

Inclusion criteria were patients with (1) 20 years of age or more, (2) CLD complicated with diabetes mellitus, and (3) regular medical consultations with a hepatologist. Exclusion criteria were (1) type 1 diabetes mellitus, juvenile diabetes mellitus, or gestational diabetes mellitus, (2) severe pancreatitis, (3) adrenal gland disease, (4) pituitary disease, and (5) a gonadal disorder. We enrolled 478 CLD patients with diabetes in this study from five medical institutions in Japan.

Definition of CLD and its etiology

Regardless of the etiology of liver disease, chronic liver disease was diagnosed on the basis of hepatic inflammation that had lasted for more than 6 months, and findings of histopathology, ultrasonography, computed tomography, or magnetic resonance imaging.

The etiology of CLD was examined by biochemical tests, imaging examinations, and/or liver biopsy as previously described [19–23]. Briefly, chronic hepatitis C was diagnosed by positive results of anti-hepatitis C virus (HCV) and/or HCV RNA [20]. Chronic hepatitis B was diagnosed by positive results of hepatitis B surface antigen and/or hepatitis B virus (HBV) DNA [20]. Autoimmune hepatitis was diagnosed by the Diagnostic Criteria of the International Autoimmune Hepatitis Group [21]. Primary biliary cirrhosis was diagnosed based on the Clinical Guideline of Primary Biliary Cirrhosis by the Intractable Hepato-Biliary Disease Study Group [22]. Non-alcoholic fatty liver disease was diagnosed based on the Practice Guideline by the American Gastroenterological Association, American Association for the Study of Liver Diseases, and American College of Gastroenterology [19]. Alcoholic liver disease was diagnosed according to the Diagnostic

Criteria of Alcoholic Liver Disease by the Japanese Society for Biomedical Research on Alcohol [23].

Definition of liver cirrhosis

Liver cirrhosis was diagnosed by aspartate aminotransferase (AST) to platelet ratio index (APRI); serum AST level (U/L)/upper limit of normal AST (33 U/L) $\times$ 100/platelet count ($\times 10^6$/mL). APRI is a noninvasive index and can predict liver cirrhosis. Patients with APRI values above 2 were diagnosed as with liver cirrhosis as previously described [24].

Definition of diabetes mellitus

Diabetes mellitus was diagnosed on the basis of fasting blood glucose levels >126 mg/dL or HbA1c levels >6.5 % according to the Diagnostic Criteria for Diabetes Mellitus [25], or by the use of anti-diabetic agents.

Definition of cancer

Cancer was defined as any type of malignant neoplasm including epithelial and non-epithelial tumors. The diagnosis of cancer was based on finding(s) of histopathology and/or by a combination of serum tumor makers and imaging procedures such as ultrasonography, computed tomography, magnetic resonance imaging, endoscopy, and/or angiography.

Diagnosis of HCC

HCC was diagnosed by a combination of tests for serum tumor makers such as alpha-fetoprotein and des-gamma-carboxy prothrombin, and imaging procedures such as ultrasonography, computed tomography, magnetic resonance imaging, and/or angiography.

Definition of extra-hepatic cancer, digestive cancer, and non-digestive cancer

Extra-hepatic cancer was defined as cancer in any organ except for the liver, and was further classified as either digestive cancer or non-digestive cancer. Digestive cancer was defined as cancer in the oral cavity, esophagus, stomach, colon, gallbladder, or pancreas. Cancer other than digestive cancer was defined as non-digestive cancer. The diagnosis of each cancer was based on finding(s) of histopathology and/or by a combination of serum tumor makers and imaging procedures such as ultrasonography, computed tomography, magnetic resonance imaging, endoscopy, and/or angiography.

Definition of cardiovascular event

A cardiovascular event was defined as acute myocardial infarction or stroke, the diagnosis of which was based on clinical symptoms and findings of electrocardiogram recordings, biochemical tests, echocardiography, coronary angiography, computed tomography, or magnetic resonance imaging as previously reported [26].

Diagnosis of diabetic retinopathy, diabetic nephropathy, and diabetic neuropathy

Diagnosis of diabetic retinopathy, diabetic nephropathy, and diabetic neuropathy was based on findings of urine and biochemical tests, ophthalmoscopy, tendon reflex tests, and vibration sense tests as previously described [27–29].

Database

Using on medical records, a database of 478 CLD patients with diabetes was created on the basis of the following six categories:

Category 1: age, sex, body mass index, and blood pressure.
Category 2: any type of cancer, HCC, extra-hepatic cancer, digestive cancer, and non-digestive cancer.
Category 3: cardiovascular disease, diabetic retinopathy, diabetic nephropathy, and diabetic neuropathy.
Category 4: chronic hepatitis C, chronic hepatitis B, non-alcoholic fatty liver disease, alcoholic liver disease, autoimmune hepatitis, and primary biliary cirrhosis.
Category 5: platelet count, serum AST level, serum alanine aminotransferase (ALT) level, serum gamma-glutamyl transpeptidase (GGT) level, serum albumin level, serum total bilirubin level, prothrombin activity, serum total cholesterol level, and serum triglyceride level.
Category 6: disease duration of diabetes mellitus, fasting blood glucose level, blood hemoglobin A1c (HbA1c; National Glycohemoglobin Standardization Program; NGSP), and use of a DPP-4 inhibitor, sulfonylurea, exogenous insulin, α-glucosidase inhibitor, biguanide, glinide, thiazolidine, and glucagon-like peptide 1 agonist.

Statistical analysis

Data are expressed as the number or mean $\pm$ standard deviation (SD). Nonparametric comparisons were made using the Wilcoxon signed-rank test, and categorical comparisons were made using Fisher's exact test. Independent factors for cancer were analyzed using logistic regression and decision-tree analysis as described previously [30, 31]. The level of statistical significance was set at $P < 0.05$.

Results

Patient characteristics

The patient characteristics are summarized in Table 1. The mean age was 64.3 years and the ratio of women to men was 1:1.56. Chronic hepatitis C and non-alcoholic fatty liver disease were the major etiologies of chronic liver disease. Liver cirrhosis was seen in 14.9 % of enrolled patients. APRI values were significantly higher in patients with chronic hepatitis C, chronic hepatitis B, and alcoholic liver disease (Supplementary Table 1).

Table 1 Patient characteristics

	Subjects
N	478
Age (years)	64.3 ± 12.1
Sex (female/male)	187/291
Body mass index (kg/m^2)	24.5 ± 4.2
Systolic/diastolic blood pressure (mmHg)	128.9 ± 12.4/ 74.6 ± 12.3
Etiology of chronic liver disease	
Chronic hepatitis C	38.1 % (182/478)
Chronic hepatitis C with sustained virologic response by interferon therapy	8.6 % (41/478)
Chronic hepatitis B	7.3 % (35/478)
Non-alcoholic fatty liver disease	29.5 % (141/478)
Alcoholic liver disease	6.9 % (33/478)
Autoimmune hepatitis	5.4 % (26/478)
Primary biliary cirrhosis	2.5 % (12/478)
Others	1.7 % (8/478)
Biochemical examinations	
Platelet count (×10^3/mm^3)	16.1 ± 7.4
AST (IU/L)	43.0 ± 30.6
ALT (IU/L)	42.2 ± 36.1
GGT (IU/L)	77.4 ± 122.8
Albumin (g/dL)	3.93 ± 0.58
Prothrombin time (%)	91.5 ± 20.2
Total bilirubin (mg/dL)	0.88 ± 0.42
Total cholesterol (mg/dL)	172.4 ± 38.4
Triglyceride (mg/dL)	129.2 ± 94.8
Presence of liver cirrhosis	14.9 % (71/407)
APRI	1.11 ± 1.18

Data are expressed as number or mean ± SD

AST aspartate aminotransferase, *ALT* alanine aminotransferase, *GGT* gamma-glutamyl transpeptidase, *APRI* AST to platelet ratio index

The variables associated with diabetes mellitus are summarized in Table 2. The mean HbA1c level was 6.5 %. The morbidity of diabetic retinopathy, diabetic nephropathy, and diabetic neuropathy were 10.4, 12.1, and 5.8 %, respectively.

Overall, 66.5 % (318/478) patients were treated with an antidiabetic agent. DPP-4 inhibitor was the most frequently prescribed (39.0 %), followed by sulfonylurea (25.5 %) and exogenous insulin (25.5 %) (Table 2).

The morbidity of cardiovascular disease

The morbidity of cardiovascular disease was 6.1 % (29/478) and there were no etiological differences in the morbidity of cardiovascular disease (Supplementary Table 2).

The morbidity of cancer

The morbidity of cancer was 33.3 % (159/478). Among the patients with cancer, multiple primary tumors were found in 10.0 % of cases (9.4 and 0.6 % for double and triple cancer, respectively). The overall morbidity of HCC was 24.7 % (118/478) (Fig. 1a) and patients with chronic hepatitis C, chronic hepatitis B, and alcoholic liver disease showed significantly higher morbidity of HCC (Supplementary Table 2).

The morbidity of extra-hepatic cancer was 11.3 % (54/478) (Fig. 1a). Amongst the patients with extra-hepatic cancer, digestive cancer and non-digestive cancer

Table 2 Glucose metabolism, complications of diabetes, and use of anti-diabetic medication

	Subjects (n = 478)
Disease duration of diabetes mellitus (year)	5.4 ± 5.6
Fasting blood glucose (mg/dL)	135.7 ± 44.4
HbA1c (%)	6.5 ± 0.9
Diabetic retinopathy	10.4 % (48/478)
Diabetic nephropathy	12.1 % (56/478)
Diabetic neuropathy	5.8 % (27/478)
Use of anti-diabetic agent	66.5 % (318/478)
DPP-4 inhibitor	39.0 % (124/318)
Sulfonylurea	25.5 % (81/318)
Exogenous insulin	25.5 % (81/318)
α-Glucosidase inhibitors	23.9 % (76/318)
Metformin	16.7 % (53/318)
Glinides	8.8 % (28/318)
Pioglitazone	6.6 % (21/318)
GLP-1 agonists	5.3 % (17/318)

Data are expressed as number or mean ± SD

HbA1c hemoglobin A1c, *DPP-4* dipeptidyl-peptidase 4, *GLP* glucagon-like peptide

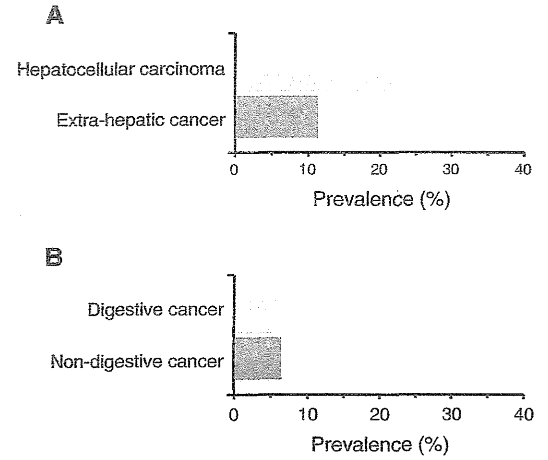

Fig. 1 **a** The morbidity of hepatocellular carcinoma and extra-hepatic cancer. **b** The morbidity of digestive cancer and non-digestive cancer

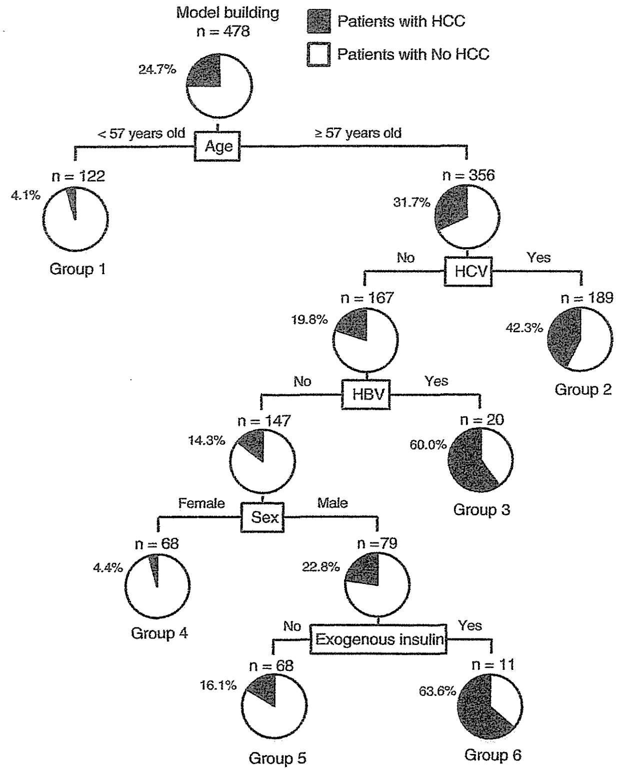

Fig. 2 Decision-tree algorithm for hepatocellular carcinoma. The subjects were classified according to the indicated cutoff value for each variable. The *pie graphs* indicate the proportion of patients with no hepatocellular carcinoma (*white*) and patients with hepatocellular carcinoma (*black*) in each group. *HCC* hepatocellular carcinoma, *HCV* hepatitis C virus, *HBV* hepatitis B virus

accounted for 5.6 % (27/478) and 6.5 % (31/478) of cases, respectively (Fig. 2b). There were no etiological differences in the morbidity of extra-hepatic cancer, digestive cancer, and non-digestive cancer (Supplementary Table 2).

Logistic regression analysis for cancer

In this analysis, non-alcoholic fatty liver disease, alcoholic liver disease, and APRI were not identified as independent factors associated with HCC. Age, chronic hepatitis C, chronic hepatitis B, and male gender were found to be independent risk factors for HCC (Table 3). Although HbA1c was not an independent risk factor, use of exogenous insulin was identified as an independent risk factor for the incidence of HCC (OR 2.21; 95 % CI 1.16–4.21; $P = 0.0165$) (Table 3). The use of sulfonylurea was identified as an independent risk factor for extra-hepatic cancer (OR 2.08; 95 % CI 1.05–3.97; $P = 0.0353$) (Table 3).

Even when patients with chronic hepatitis C and chronic hepatitis B were excluded from the analysis subjects, use of exogenous insulin or sulfonylurea was also identified as an independent risk factor for the incidence of HCC or extra-hepatic cancer, respectively (Supplementary Table 3 and 4).

Decision-tree algorithm for HCC

In order to clarify the profile of HCC patients, a decision-tree algorithm was created using five divergence variables to classify six groups of patients (Fig. 2). An age of 57 years was the cutoff value for the initial classification. Among those patients aged ≥57 years, diagnosis of chronic hepatitis C was the variable for the second division. Among the patients with no hepatitis C virus (HCV) infection, diagnosis of chronic hepatitis B was the third division, and

Table 3 Logistic regression analysis for the incidence of HCC and extra-hepatic cancer

Event	Factors	Unit	Logistic regression analysis		
			Odds ratio	95 % confidence interval	*P* value
HCC	Age	1	1.12	1.08–1.15	<0.0001
	Chronic hepatitis C	N/A	5.20	2.88–9.81	<0.0001
	Chronic hepatitis B	N/A	10.26	3.98–27.6	<0.0001
	Male	N/A	2.50	1.44–4.45	0.0010
	Use of exogenous insulin	N/A	2.21	1.16–4.21	0.0165
	HbA1c	1	0.82	0.60–1.11	0.2046
	GGT	1	1.00	0.999–1.002	0.2009
Extra-hepatic cancer	Age	1	1.04	1.02–1.07	0.0008
	Sulfonylurea	N/A	2.08	1.05–3.97	0.0353
	Chronic hepatitis C	N/A	0.56	0.30–1.00	0.0532

GGT gamma-glutamyl transpeptidase, *HbA1c* hemoglobin A1c among the patients with no hepatitis B virus (HBV) infection, gender was the fourth division. Then, among male patients, the use of exogenous insulin was the fifth division. Thus, 63.6 % of patients had HCC from among those who were aged ≥57 years, had no HCV or HBV infection, were male, and used exogenous insulin (Group 6; Fig. 2). On the other hand, 16.1 % of the patients not treated using exogenous insulin had HCC (Group 5; Fig. 2). In this analysis, non-alcoholic fatty liver disease or alcoholic liver disease was not identified a divergence variable for HCC.

Even when patients with chronic hepatitis C and chronic hepatitis B were excluded from the analysis subjects, use of exogenous insulin was also a divergence variable for the incidence of HCC (Supplementary Figure 1A and B).

Decision-tree algorithm for extra-hepatic cancer

In order to clarify the profile of extra-hepatic cancer patients, a decision-tree algorithm was created using two divergence variables to classify three groups of patients (Fig. 3). An age of 78 years was the cutoff value for the initial classification. Among the patients who were aged ≥78 years, the use of sulfonylurea was the second division. Thus, 56.2 % of the patients aged ≥78 years and treated with sulfonylurea had extra-hepatic cancer (Group 3; Fig. 3). On the other hand, 21.3 % of the patients who were not treated with sulfonylurea had extra-hepatic cancer (Group 2; Fig. 3). Although it was not statistically significant, a tendency of high incidence of digestive cancer was seen in the sulfonylurea group compared to the non-sulfonylurea group in patients with extra-hepatic cancer (68.8 vs. 42.1 % $P = 0.0738$).

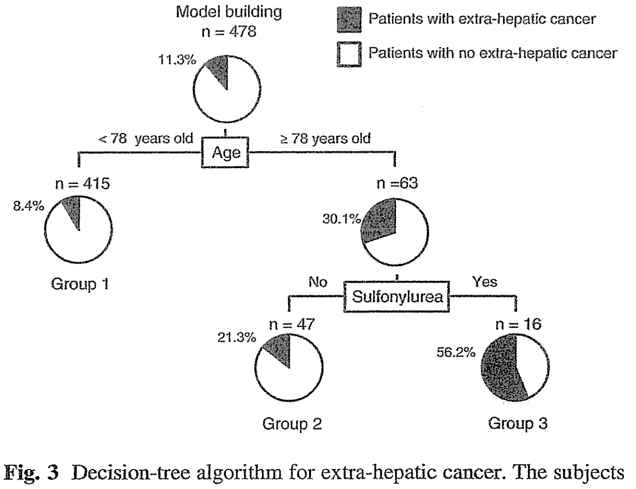

Fig. 3 Decision-tree algorithm for extra-hepatic cancer. The subjects were classified according to the indicated cutoff value for each variable. The *pie graphs* indicate the proportion of patients with no extra-hepatic cancer (*white*) or patients with extra-hepatic cancer (*black*) in each group

Even when patients with chronic hepatitis C and chronic hepatitis B were excluded from the analysis subjects, use of sulfonylurea was also a divergence variable for the incidence of extra-hepatic cancer (Supplementary Figure 2A and B).

Discussion

In this study, we found that there was a high morbidity of both HCC and extra-hepatic cancer in CLD patients with diabetes. Moreover, the use of sulfonylurea and exogenous insulin was independently associated with the risk of HCC and extra-hepatic cancer, respectively.

The morbidity of cardiovascular disease was 6.1 % in this study. In contrast, Limori et al. reported that the morbidity of cardiovascular disease was 26.8 % in diabetic patients [32]. It is unclear why there is a difference in the morbidity for cardiovascular disease between this previous study and our study; however, a possible explanation is the difference in etiology of diabetes mellitus. In this study, we enrolled CLD patients with diabetes. Serum cholesterol level is associated with atherosclerosis and subsequent microvascular and macrovascular events [33]. Since cholesterol synthesis is impaired in patients with chronic liver disease, the morbidity of cardiovascular disease may be relatively low in CLD patients with diabetes. In fact, the average level of cholesterol was in the normal range in this study and the morbidities of microvascular complications such as diabetic retinopathy, nephropathy, and neuropathy were lower than previously reported [34–36]. These findings support our hypothesis and suggest that a low morbidity of cardiovascular disease may be a feature of CLD patients with diabetes.

In this study, the morbidity of HCC was 24.7 %. This study was conducted in center hospitals for liver disease, and therefore, institutional bias may partly explain this finding. We also note that there was a high morbidity of extra-hepatic cancer. There are generally more opportunities to coincidentally detect digestive cancer in patients with chronic liver disease, as they are frequently examined by abdominal computed tomography and upper gastrointestinal endoscopy. In addition, we revealed that the morbidity of digestive cancer was similar to that of non-digestive cancer, indicating that carcinogenic potential may be higher in CLD patients with diabetes. An increased insulin resistance and subsequent hyperinsulinemia is a hallmark of CLD patients with diabetes [10, 11]. Insulin binds to the insulin receptor and activates the insulin receptor substrate/mitogen-activated protein kinase (MAPK)/extracellular signal-regulated kinase cascade [13]. Insulin can also bind to the insulin-like growth factor (IGF)-1 receptor and subsequently activate the Raf/MAPK kinase/MAPK pathway [37]. Moreover, excess insulin competes with IGF-1 binding to the IGF-binding protein, resulting in an increase in the serum level of IGF-1, which is a potent stimulator of carcinogenesis [13]. Taken together, hyperinsulinemia may be one of the causes of the high morbidity of cancer in CLD patients with diabetes.

The possible relationship between the use of antidiabetic agents and carcinogenesis remains a controversial issue. In this study, we found that DPP-4 inhibitor was the most frequently prescribed agent, accounting for 39.0 % of all prescribed antidiabetic agents. Although we could not evaluate the period of antidiabetic mediation and this study was not designed to investigate a relationship between use of anti-diabetic agents and carcinogenesis, Kissow et al. [38] reported that DPP-4 inhibition did not accelerate neoplasia in carcinogen-treated mice, and White et al. [39] reported that the incidence of cancer was similar with DPP-4 inhibitor and placebo in diabetic patients with acute coronary syndrome. Likewise, in this study, we found that the use of DPP-4 inhibitor was not an independent risk factor for cancer in CLD patients with diabetes. However, logistic regression analysis revealed that the use of exogenous insulin and sulfonylurea was an independent risk factor for HCC and extra-hepatic cancer, respectively. Similar findings were also seen, even when patients with chronic hepatitis C and chronic hepatitis B were excluded from the analysis subjects. Both exogenous insulin and sulfonylurea increase the serum insulin level, which in turn could up-regulate mitosis and cell growth [12]. We and other groups have previously demonstrated that the use of exogenous insulin is a risk factor for HCC [15, 40], and sulfonylurea has been shown to be a risk factor for extra-hepatic cancers such as colon cancer [41] and pancreatic cancer [17]. Thus, the results of this study concur with those of previous reports.

Finally, we performed a decision-tree analysis, revealing that age was the first divergence factor for the incidence of both HCC and non-hepatic cancer. These findings indicate that aging is the most significant carcinogenic factor for cancer. In the decision-tree analysis, liver function tests and APRI were not divergence variables for the incidence of HCC. Since only 14.9 % of the enrolled patients were liver cirrhosis in this study, liver cirrhosis might not be selected as a factor associated with the incidence of HCC because of insufficient number of cirrhotic patients. In the algorithm for HCC, the use of exogenous insulin was not a significant risk factor in HCV-infected or HBV-infected patients, suggesting that HCV or HBV infection may dilute the impact of exogenous insulin on HCC. However, the use of exogenous insulin was a significant risk factor for male patients. Similarly, the use of exogenous insulin was a divergence variable for male patients, even when patients with chronic hepatitis C and chronic hepatitis B were excluded from the analysis subjects. IGF-1 is known to stimulate androgen receptor activity, through a β integrin-dependent mechanism, which also plays an important role in cancer progression [42] and might explain this gender-specific component of cancer risk.

For extra-hepatic cancer, sulfonylurea rather than exogenous insulin was found to be the second most significant risk factor. Similarly, the use of sulfonylurea was the second most significant risk factor for extra-hepatic cancer, even when patients with chronic hepatitis C and chronic hepatitis B were excluded from the analysis subjects. Sulfonylurea administration results in the increased expression of ATP-sensitive potassium channels, which in turn promotes insulin secretion from pancreatic beta cells as well as proliferation of various types of cancer cells in culture [43, 44]. Thus, in addition to a hyperinsulinemia-dependent mechanism, sulfonylurea may also directly increase the carcinogenic potential in very elderly patients. The association of anti-diabetic agents with cancer may therefore differ with the presence of other carcinogenic factor(s).

A limitation of this study is that we could not evaluate the precise duration of anti-diabetic medication. To investigate causal relationship between the use of anti-diabetic agents and carcinogenesis, further study will be focused on the duration of anti-diabetic medication.

In conclusion, in this study, we found that there is a high morbidity of both HCC and extra-hepatic cancer in CLD patients with diabetes. This study also revealed that the use of sulfonylurea and exogenous insulin were risk factors for HCC and extra-hepatic cancer, respectively, in CLD patients with diabetes. Thus, a large-scale cohort study is needed to identify therapeutic strategies for diabetes to suppress carcinogenesis in CLD patients.

Conflict of interest The authors declare that they have no conflict of interest.

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Original Article

Interleukin 28B polymorphism predicts interferon plus ribavirin treatment outcome in patients with hepatitis C virus-related liver cirrhosis: A multicenter retrospective study in Japan

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Aim: This study evaluated the efficacy of interferon plus ribavirin and examined whether interleukin 28B (IL28B) polymorphism influenced treatment outcome in Japanese patients with hepatitis C virus (HCV)-related liver cirrhosis (LC).

Methods: Fourteen collaborating centers provided details of 261 patients with HCV-related LC undergoing treatment with interferon plus ribavirin. Univariate and multivariate analyses were used to establish which factors predicted treatment outcome.

Results: Eighty-four patients (32.2%) achieved a sustained virological response (SVR). SVR rates were 21.6% (41/190) in patients with HCV genotype 1 with high viral load (G1H) and 60.6% (43/71) in patients with non-G1H. In patients with non-G1H, treatment outcome was effective irrespective of IL28B polymorphism. In those with G1H, SVR was achieved in 27.1% of patients with the IL28B rs8099917 TT allele compared with 8.8% of those with the TG/GG alleles ($P = 0.004$). In patients with G1H having TT allele, treatments longer than 48 weeks achieved significantly higher SVR rates than treatments less than 48 weeks (34.6% vs 16.4%, $P = 0.042$). In patients with G1H having TG/GG alleles, treatments longer than 72 weeks achieved significantly higher SVR rates than treatments less than 72 weeks (37.5% vs 4.1%, $P = 0.010$).

Conclusion: Interferon plus ribavirin treatment in Japanese patients with non-G1H HCV-related LC was more effective than those with G1H and not influenced by IL28B polymorphism. In those with G1H, IL28B polymorphism may predict SVR and guide treatment duration: SVR rates were higher in those with the TT allele treated for more than 48 weeks and those with the TG/GG alleles treated for more than 72 weeks.

Key words: cirrhosis, hepatitis C virus, interferon, interleukin 28B, ribavirin

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INTRODUCTION

CHRONIC HEPATITIS C virus (HCV) infection is a leading cause of liver cirrhosis worldwide.[1] Patients with HCV-related liver cirrhosis (LC) are at increased risk of hepatic decompensation and hepatocellular carcinoma (HCC).[2–4] The therapeutic goal in these patients should be the prevention of liver-related mortality. A randomized trial conducted in Japan was the first to suggest that interferon (IFN) may reduce the risk of HCC in patients with HCV-related LC.[5] Recent studies have shown that patients with HCV-related LC who achieved a sustained virological response (SVR) with antiviral therapy had a significant reduction in liver-related mortality.[6,7] However, patients with HCV-related LC show a lower SVR rate than non-cirrhotic patients, as well as a reduced tolerance to the therapy.[8,9] A previous meta-analysis revealed that the overall SVR rate in patients with cirrhosis was 33.3%, and was significantly higher in patients with HCV genotypes 2 and 3 (55.4%) than in those with HCV genotypes 1 and 4 (21.7%).[10]

Genome-wide association studies have recently shown that single nucleotide polymorphisms (SNP) near the interleukin 28B (IL28B) region (rs8099917, rs12979860) are the most powerful predictors of SVR to pegylated (PEG) IFN plus ribavirin in patients with HCV genotype 1 infection.[11–13] However, it is not clear whether IL28B polymorphism can be used to predict the virological response to treatment of HCV-related LC. This study evaluated the efficacy of IFN plus ribavirin, and the association between IL28B polymorphism and the treatment efficacy in Japanese patients with HCV-related LC.

METHODS

THIS WAS A multicenter retrospective study of patients with HCV-related LC who had received treatment with IFN plus ribavirin in 14 hospitals in Japan.

Patient selection

Data were collected from 290 patients with HCV-related LC receiving treatment with IFN plus ribavirin in 14 academic and community hospitals. All patients had compensated HCV-related LC with clinical or histological data available. The diagnosis of cirrhosis met at least one of the following criteria: liver biopsy specimens with cirrhosis, diffuse formation of the nodules on the liver surface in peritoneoscopy, over 12.5 kPa in liver stiffness values on transient elastography, signs of portal hypertension on ultrasound scan (splenomegaly, portal vein enlargement, re-permeabilization of the umbilical vein, or presence of portal-systemic shunts), presence of esophageal varices on endoscopy or positive values using the following discriminant by Ikeda and colleagues: $z = 0.124 \times (\gamma\text{-globulin [\%]}) + 0.001 \times (\text{hyaluronate}) (\mu g\ L^{-1}) - 0.075 \times (\text{platelet count } [\times 10^4 \text{ counts/mm}^3]) - 0.413 \times \text{sex (male, 1; female, 2)} - 2.005$.[14–16] Principal investigators in 14 hospitals identified eligible patients and entered data in a predefined database.

Combination therapy

Of the 290 patients identified, 29 were not genotyped for IL28B SNP, thus the data of 261 patients were analyzed. A total of 190 patients were infected with HCV genotype 1 with high viral load (>100 KIU/mL) (G1H) (72.8%) and the remaining 71 (27.2%) were classified as non-G1H. Twenty-two patients were HCV genotype 1 with low viral load, 46 were genotype 2a or 2b, and three were of unknown genotype. Two hundred and twenty-four (85.8%) patients were treated with PEG IFN-α-2b (1.5–1.0 μg/kg bodyweight per week), 20 (7.7%) patients were treated with PEG IFN-α-2a (45–180 μg/week) and the remaining 17 (6.5%) patients were treated with IFN-α-2b or IFN-β. IFN-α-2b and IFN-β were administrated at a median dose of 6 million units each day (seven times per week for the initial 2 or 4 weeks, followed by three times per week thereafter). All patients also received oral ribavirin (600–1000 mg/day). Median treatment duration was 48 and 28 weeks in G1H and non-G1H, respectively. The individual attending physician determined the treatment regimes and their duration.

Virological response during therapy and definitions

The efficacy end-point was SVR, defined as undetectable serum HCV RNA 24 weeks after treatment. Relapse was defined as undetectable serum HCV RNA at the last treatment visit but detectable serum HCV RNA again at the last follow-up visit. Breakthrough was defined as reappearance of serum HCV RNA during treatment. A non-responder was defined as serum HCV RNA never undetectable during treatment. A rapid virological response (RVR) was defined as undetectable serum HCV RNA at treatment week 4, and a complete early virological response (cEVR) was defined as undetectable serum HCV RNA at treatment week 12. A late virological response (LVR) was defined as detectable serum HCV RNA at 12 weeks that became undetectable within 36 weeks of the start of treatment.

Determination of IL28B genotype

Interleukin 28B (rs8099917) was genotyped in each of the 14 hospitals by Invader assay, TaqMan assay or by direct sequencing, as previously described.[17,18]

Statistical analysis

Results were analyzed on the intention-to-treat principle. Mean differences were tested using Student's *t*-test. The difference in the frequency distribution was analyzed with Fisher's exact test. Univariate and multivariate logistic regression analyses were used to identify factors independently associated with SVR. The odds ratios (OR) and 95% confidence intervals (95% CI) were also calculated. The parameters that achieved statistical significance on univariate analysis were entered into multivariate logistic regression analysis to identify significant independent factors. Data were analyzed with JMP version 9.0 for Macintosh (SAS Institute, Cary, NC, USA). All statistical analyses were two sided, and $P < 0.05$ was considered significant.

RESULTS

OF THE 261 patients included in our analysis, 84 patients (32.2%) achieved SVR (Fig. 1). The rate of relapse and breakthrough was 24.9% and the non-responder rate was 33.3%. There were 25 patients (9.6%) who required early discontinuation of treatment because of adverse events. Baseline demographic and clinical features are summarized in Table 1. The age of the patients was 60.7 ± 8.9 years and 50.6% were male. Of the patients studied, 125 patients (47.9%) had been treated with IFN previously, and 75 (28.7%) had not responded to previous treatment. One hundred and six patients (40.6%) had been treated for HCC before. There were 85 patients with esophageal varices (32.6%). There were 190 patients with G1H and 133 (70%) of these had the TT allele at IL28B rs8099917. There were 71 patients in the non-G1H group, 51 (71.8%) of whom were found to have the TT allele at IL28B rs8099917.

Virological response rates in patients with G1H and non-G1H HCV-related LC

The SVR rates were 21.6% (41/190) in patients with G1H and 60.6% (43/71) in patients with non-G1H (Table 2). There were no statistically significant differences between the G1H and non-G1H groups with regard to dose reduction rates of IFN or ribavirin. Dose reduction of IFN was required in 51.3% of patients and dose reduction of ribavirin in 53.6% of patients. Treatment duration in patients in the G1H group was significantly longer than those in the non-G1H group ($P = 0.010$).

Association between IL28B rs8099917 genotype and treatment response

Sustained virological response was achieved in 37.0% of patients with the rs8099917 TT allele and 20.8% in those with the TG or GG allele. Virological responses, including SVR, relapse and breakthrough, in patients with the rs8099917 TT allele were significantly higher than in those with rs8099917 TG or GG allele ($P = 0.013$ and 0.012, respectively; Table 3). The proportion of non-responders among patients with the rs8099917 TG or GG allele was significantly higher than in those with the TT allele ($P = 0.002$). There was no

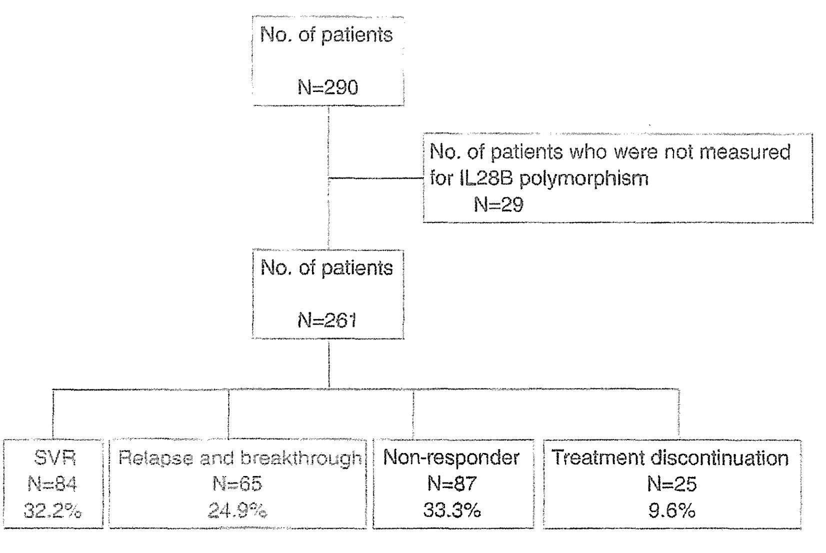

Figure 1 Flowchart showing the characteristics of the study cohort. IL28B, interleukin 28B; SVR, sustained virological response.

Table 1 Summary of demographic and baseline characteristics ($n = 261$)

	G1H, $n = 190$	Other than G1H, $n = 71$	All patients, $n = 261$
Sex (M : F)	95:95	37:34	132:129
Age (years)	60.5 ± 9.3	61.2 ± 7.8	60.7 ± 8.9
BMI (kg/m^2)	23.8 ± 3.5	23.4 ± 3.2	23.7 ± 3.4
IFN treatment history	91 (47.9%)	34 (47.9%)	125 (47.9%)
HCC treatment history	75 (39.5%)	31 (43.7%)	106 (40.6%)
Presence of EV	60 (31.6%)	25 (35.2%)	85 (32.6%)
Total bilirubin (mg/dl)	1.1 ± 0.9	1.1 ± 1.4	1.1 ± 1.2
AST (IU/L)	79.1 ± 44.2	75.8 ± 57.7	79.9 ± 52.7
ALT (IU/L)	82.4 ± 56.4	81.9 ± 75.4	83.3 ± 66.2
GGT (IU/L)	83.8 ± 107.8	87.0 ± 140.1	84.6 ± 115.8
Albumin (g/dL)	3.7 ± 0.5	3.8 ± 0.4	3.7 ± 0.5
Prothrombin (%)	86.2 ± 14.4	83.7 ± 16.7	85.5 ± 15.1
WBC (/μL)	4407 ± 1592	4190 ± 1930	4348 ± 1667
Hemoglobin (g/dL)	13.2 ± 1.8	13.1 ± 1.8	13.1 ± 1.8
Platelets (10^4/mm^3)	11.8 ± 6.7	11.8 ± 6.3	11.8 ± 6.6
AFP (ng/mL)	48.9 ± 224.7	24.0 ± 29.3	45.4 ± 193.9
DCP (mAU/mL)	66.8 ± 372.3	155.3 ± 620.4	92.4 ± 450.8
IL28B (TT : TG + GG)	133:57	51:20	184:77

All values are expressed as mean ± standard deviation.
AFP, α-fetoprotein; ALT, alanine transaminase; AST, aspartate aminotransferase; BMI, body mass index; DCP, des-γ-carboxy prothrombin; EV, esophageal varices; G1H, genotype 1 with high viral load; GGT, γ-glutamyltransferase; HCC, hepatocellular carcinoma; IFN, interferon; IL28B, interleukin 28B rs8099917 genotype; WBC, white blood cell.

significant association between the IL28B genotype and the incidence of adverse events.

Among patients in the G1H group, SVR was achieved in 27.1% (36/133) of those with the TT allele and 8.8% (5/57) of those with the TG or GG allele (Table 4). There was no statistically significant difference between IL28B genotype and viral response in patients with non-G1H.

Table 2 Summary of treatment and sustained virological response rates ($n = 261$)

	G1H, $n = 190$	Other than G1H, $n = 71$	All patients, $n = 261$
Dose reduction of IFN	$n = 98$ (51.6%)	$n = 36$ (50.7%)	$n = 134$ (51.3%)
Dose reduction of RBV	$n = 107$ (56.3%)	$n = 33$ (46.5%)	$n = 140$ (53.6%)
Treatment duration (weeks)			
Mean ± SD	45.3 ± 21.6	37.7 ± 19.6	43.2 ± 21.4
Median	48	28	48
SVR	$n = 41$ (21.6%)	$n = 43$ (60.6%)	$n = 84$ (32.2%)

G1H, genotype 1 with high viral load; IFN, interferon; RBV, ribavirin; SD, standard deviation; SVR, sustained virological response.

Table 3 Association between IL28B rs8099917 polymorphism and treatment response in 261 hepatitis C virus-related liver cirrhotic patients

IL28B	TT ($n = 184$)	TG + GG ($n = 77$)	*P*-value
SVR	68 (37.0%)	16 (20.8%)	0.013
Relapse and breakthrough	54 (29.3%)	11 (14.3%)	0.012
Non-responder	44 (23.9%)	43 (55.8%)	0.002
Discontinuation	18 (9.8%)	7 (9.1%)	1.000

IL28B, interleukin 28B rs8099917 genotype; SVR, sustained virological response.

Table 4 Sustained virological response associated between IL28B rs8099917 polymorphism and G1H in hepatitis C virus-related liver cirrhosis patients

IL28B	TT ($n = 184$)	TG + GG ($n = 77$)	*P*-value
G1H	36/133 (27.1%)	5/57 (8.8%)	0.004
Other than G1H	32/51 (62.7%)	11/20 (55.0%)	0.596

G1H, genotype 1 with high viral load; IL28B, interleukin 28B rs8099917 polymorphism.

Predictive factors associated with SVR

Differences in the characteristics of patients with SVR and those in whom SVR was not achieved are summarized in Table 5. Neither age, sex, alanine transaminase, aspartate aminotransferase, prothrombin activity, hemoglobin nor platelet counts appeared to significantly influence the chance of achieving SVR. The patients who achieved SVR had a lower body mass index, higher white blood cell count and higher serum albumin than those who did not, and were more likely to have non-G1H and the TT allele of IL28B rs8099917. Multivariate analysis identified that possession of the IL28B rs8099917 TT allele (OR = 2.85; 95% CI, 1.01–9.15; $P = 0.047$) and non-G1H (OR = 6.49; 95% CI, 1.77–26.43; $P = 0.005$) as significant determinants of SVR.

Treatment duration and efficacy in patients with G1H

Of the patients with G1H, 79 (41.6%) received less than 48 weeks of treatment. The number receiving 48–52 weeks, 53–72 weeks, over 72 weeks and unknown duration of treatment were 54 (28.4%), 41 (21.6%), 14 (7.4%) and two (1.1%), respectively. The median duration of treatment in patients who achieved RVR and cEVR was 48 weeks, but was significantly longer (66 weeks) in those with an LVR ($P < 0.001$). Table 6 shows the SVR rates of those with different IL28B genotypes and on-treatment viral response. The SVR rate in patients who achieved LVR was significantly lower than those who achieved RVR and cEVR ($P = 0.002$). Of the patients with G1H found to have the IL28B TG or GG genotype, none achieved RVR and only two achieved cEVR.

Predictors of SVR in patients with G1H and the TT allele

Patients with G1H and the TT allele who achieved SVR had higher platelet counts, higher serum albumin and had undergone over 48 weeks of treatment. Multivariate analysis identified platelet count (OR = 1.08; 95% CI, 1.01–1.18; $P = 0.047$), serum albumin (OR = 2.78; 95% CI, 1.14–7.42; $P = 0.031$) and over 48 weeks of treatment duration (OR = 2.53; 95% CI, 1.07–6.49; $P = 0.042$) as significant determinants of SVR (Table 7).

Predictors of SVR in patients with G1H and the TG or GG allele

Patients who had G1H and the TG or GG allele who achieved SVR had a higher total dose of ribavirin ($P = 0.011$) and more than 72 weeks of treatment duration ($P = 0.010$).

Treatment tolerability and adverse events

Table 8 illustrates details of the patients who experienced adverse events higher than grade 2. There were

Table 5 Factors associated with sustained virological response in hepatitis C virus-related liver cirrhosis patients

Factors	SVR (+), ($n = 84$)	SVR (−), ($n = 177$)	*P*-value	Multivariate analyses		
				Odds ratio	95% CI	*P*-value
BMI (kg/m^2)	22.9 ± 3.5	24.0 ± 3.3	0.019			
WBC (/μL)	4727 ± 2096	4168 ± 1376	0.013			
Albumin (g/dL)	3.83 ± 0.48	3.68 ± 0.46	0.018			
Other than G1H	$n = 43$ (51.2%)	$n = 28$ (15.8%)	<0.001	6.49	1.77–26.43	0.005
IL28B TT	$n = 68$ (81.0%)	$n = 116$ (65.5%)	0.012	2.85	1.01–9.15	0.047

P-values were obtained by logistic regression model.
BMI, body mass index; CI, confidence interval; G1H, genotype 1 with high viral load; IL28B, interleukin 28B rs8099917 polymorphism; SVR, sustained virological response; WBC, white blood cell.

Table 6 Sustained viral response rates between IL28B genotype and on-treatment viral response in the patients with G1H

	IL28B TT	IL28B TG/GG	All patients
RVR	7/7	0/0	7/7
	100%	0%	100%
cEVR	15/26	1/2	16/28
	57.7%	50%	57.1%
LVR	14/44	4/11	18/55
	31.8%	36.4%	32.7%

cEVR, complete early virological response (defined as serum HCV RNA negative at treatment week 12); G1H, genotype 1 with high viral load; HCV, hepatitis C virus; IL28B, interleukin 28B rs8099917; LVR, late virological response (defined as serum HCV RNA detectable at 12 weeks and undetectable at 36 weeks after the start of treatment); RVR, rapid virological response (defined as serum HCV RNA negative at treatment week 4).

Table 8 Adverse events higher than grade 2

	No. of patients (%)
Anemia	63 (24.1%)
Thrombocytopenia	31 (11.9%)
Leukopenia	19 (7.3%)
Rash and itching	17 (6.5%)
Fatigue and general malaise	15 (5.7%)
Gastrointestinal disorders	5 (1.9%)
Depression	5 (1.9%)
Development of hepatocellular carcinoma	3 (1.1%)
Respiratory disorders	3 (1.1%)
Liver decompensation	2 (0.8%)
Malignant neoplasm	2 (0.8%)
Interstitial pneumonia	2 (0.8%)
Cerebral hemorrhage	1 (0.4%)
Cerebral infarction	1 (0.4%)
Cholangitis	1 (0.4%)
Retinal hemorrhage	1 (0.4%)
Diabetes decompensation	1 (0.4%)
Palpitation	1 (0.4%)

two cases of liver decompensation, two cases of interstitial pneumonia, one case of cerebral hemorrhage and one case of cerebral infarction. The cause of death in two patients was decompensation of LC. In one patient, treatment was stopped after 4 weeks, and in another, treatment was stopped after 32 weeks because of hepatic failure. The IFN dose was reduced in 134 patients (51.3%), and the ribavirin dose was reduced in 140 patients (53.6%) and discontinued in 60 patients (23.0%). Among patients who had treatment discontinued, 27 patients (10.3%) had treatment withdrawn because of no virological response and 33 patients (12.6%) because of severe adverse events. In patients in whom treatment was discontinued, three patients had SVR and five had a relapse.

IL28B alleles predicting SVR in G1H group

The influence of IL28B rs8099917 genotype on SVR in G1H is shown in Figure 2. Overall, there were 84 patients (32.2%) who achieved SVR with IFN plus ribavirin in HCV-related LC. The SVR was 60.6% in those with non-G1H, and was not significantly influenced by IL28B rs8099917 genotype (the SVR in TT patients was 62.7% compared with 55.0% in TG or GG patients). In contrast, in patients with G1H, the SVR of patients with IL28B rs8099917 genotype TT was significantly higher than those with rs8099917 TG or GG (27.1% vs 8.8%, $P = 0.004$). In patients with G1H and IL28B TT, the SVR of those treated for over 48 weeks was significantly higher than those treated for less than 48 weeks (34.6% vs 16.4%, $P = 0.042$). In patients with G1H and IL28B TG/GG, the SVR of those treated for over 72 weeks was significantly higher than those treated for less than 72 weeks (37.5% vs 4.1%, $P = 0.010$).

DISCUSSION

WE FOUND THAT in Japanese patients with G1H HCV-related LC, the likelihood of achieving SVR with IFN plus ribavirin combination therapy was influ-

Table 7 Factors associated with sustained virological response in the patients with G1H and TT allele of IL28B rs8099917 ($n = 133$)

Factors	SVR (+) ($n = 36$)	SVR (−) ($n = 97$)	*P*-value	Multivariate analyses		
				Odds ratio	95% CI	*P*-value
Platelets (10^4/mm^3)	14.5 ± 11.5	10.6 ± 4.2	0.024	1.08	1.01–1.18	0.047
Albumin (g/dL)	3.92 ± 0.50	3.69 ± 0.46	0.018	2.78	1.14–7.42	0.031
Treatment duration, over 48 weeks	$n = 27$ (75%)	$n = 51$ (52.6%)	0.023	2.53	1.07–6.49	0.042

P-values were obtained by logistic regression model.
CI, confidence interval; G1H, genotype 1 with high viral load; IL28B, interleukin 28B; SVR, sustained virological response.

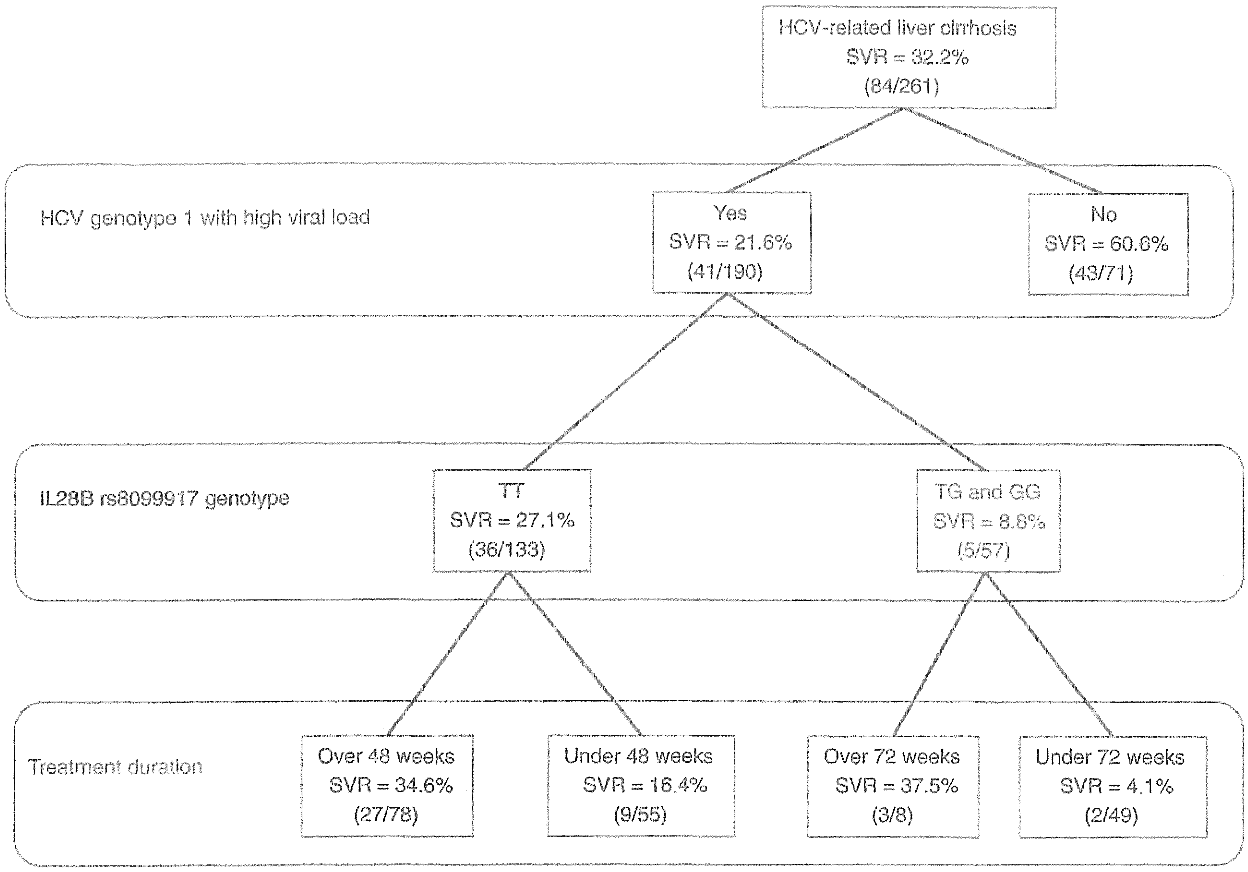

Figure 2 SVR in HCV-related liver cirrhosis patients treated with interferon plus ribavirin. In patients with G1H and the IL28B TT allele, the SVR rate of those who were treated for over 48 weeks was significantly higher than those treated for less than 48 weeks ($P = 0.042$). In patients with G1H and IL28B TG/GG, the SVR rate of patients treated for over 72 weeks was significantly higher than those treated for less than 72 weeks ($P = 0.010$). G1H, genotype 1 with high viral load; HCV, hepatitis C virus ; IL28B, interleukin 28B rs8099917; SVR, sustained virological response.

enced by a polymorphism at IL28B rs8099917. In contrast, SVR rates in non-G1H were higher than those in G1H, irrespective of IL28B genotype. This is the first report to demonstrate that an IL28B polymorphism can influence SVR rate in patients treated with IFN plus ribavirin combination therapy for G1H HCV-related LC. These results suggest that HCV genotypes, viral load and IL28B polymorphism should be taken into when determining antiviral therapy for HCV-related LC. In patients with HCV-related LC, IL28B genotyping may be a useful tool to determine the best antiviral therapy.

Recently, host genetic variation near the IL28B on chromosome 19, which encodes IFN-λ-3, have been shown to be associated with SVR to PEG IFN plus ribavirin in patients infected with HCV genotype 1.[11–13] Although some investigators have shown that IL28B polymorphisms are associated with a favorable response to treatment in patients with non-1 genotype infection, the association between the variants in IL28B and SVR in non-1 genotype-infected patients remains controversial.[19–25] IL28B polymorphisms are also a strong predictive factor for spontaneous HCV clearance.[26,27] However, the precise mechanism associated with the action of IL28B polymorphisms has not been fully elucidated.

Pegylated IFN plus ribavirin combination therapy has become the standard of care treatment for chronic HCV infection. The SVR rates range 42–46% in patients with HCV genotype 1 or 4 infection and 76–82% in patients with HCV genotype 2 or 3 infection, respectively.[9,28,29] However, in patients with HCV-related LC the SVR rate is even lower than in non-LC patients, reflecting reduced tolerance to the therapy.[8–10] Although patients with HCV-related LC are difficult to treat, patients who achieved SVR showed a lower rate of liver-related adverse outcomes and improved survival.[8–10] Moreover, a randomized controlled trial showed that patients with HCV-related LC who received long-term PEG IFN treatment had a lower risk of HCC than controls.[30] Thus, IFN treatment for HCV-related LC is an effective means of preventing HCC, irrespective of whether SVR is achieved. In this study, the SVR was very low in patients with G1H and the TG or GG allele. Therefore, for these patients, long-term administration of maintenance IFN should be considered to reduce the risk of developing of HCC even if SVR is unlikely to be achieved.

Patients with advanced liver disease have a higher rate of adverse events when taking IFN and ribavirin combination therapy than patients with mild disease. Adverse events, such as neutropenia, thrombocytopenia and anemia, often require dose reduction of IFN or ribavirin. Previous studies have demonstrated that in patients with HCV-related LC, the rate of dose reductions in IFN and ribavirin range 6.9–20.6% and 16.7–27.1%, respectively.[31–33] In our study, IFN and ribavirin dose reductions were needed in 51.3% and 53.6% of patients, respectively. These are higher than those reported in other studies, but the discontinuation rate was slightly lower (12.6%).[33] Many patients required reductions in the doses of IFN and/or ribavirin early in the treatment period because of adverse events, but ultimately were able to tolerate long-term administration. It might be safer to start low-dose antiviral therapy with IFN plus ribavirin in HCV-related LC and titrating the dose upward as tolerated with the aim of long-term treatment, rather than beginning with the full dose and risking adverse events that would curtail antiviral therapy.

In patients infected with HCV genotype 1, previous studies have demonstrated that SVR rates of late virological responders (HCV RNA detectable at 12 weeks and undetectable at 24 weeks after the start of treatment) could be improved when treatment was extended to 72 weeks, compared with the standard treatment duration of 48 weeks, largely as a result of reducing post-treatment relapse rates.[34–37] In this study, the SVR rate in patients who had an LVR was significantly lower than those who achieved RVR or cEVR. However, the duration of treatment in the patients with a LVR was significantly longer than those who achieved cEVR or RVR. Individual physicians determined the duration of treatment based on the time at which serum HCV RNA became undetectable, accounting for the improved SVR rates in those receiving extended courses. Nevertheless, the safety and effectiveness of more than 48 weeks of antiviral therapy in patients with HCV-related LC has not been examined. We found that patients with the IL28B rs8099917 genotype TT, treatment of more than 48 weeks achieved a higher SVR rate than treatment of less than 48 weeks, and in those with the TG or GG alleles SVR rates were greater in those who received more than 72 weeks of treatment. The response to treatment is a very important guide of treatment duration in HCV-related LC. Further prospective studies using larger numbers of patients matched for race, HCV genotype, viral load and treatment durations would be required to explore the relationships between IL28B polymorphism and the treatment response to combination therapy in patients with HCV-related LC.

Recently, new trials of IFN-free combination therapy with direct-acting antivirals (DAA) such as protease-inhibitor, non-structural (NS)5A inhibitor or NS5B polymerase inhibitor nucleotide analog have shown a strong antiviral activity against HCV.[38–40] A previous study reported that the IL28B genotype can affect the response to an IFN-free regimen, but this result has been unclear in other regimens.[38–40] In a study of Japanese patients with HCV genotype 1b infection, dual oral DAA therapy (NS5A inhibitor and NS3 protease inhibitor) without IFN achieved an SVR rate of 90.5% of 21 patients with no response to previous therapy and in 63.6% of 22 patients who had been ineligible for treatment with PEG IFN.[41] However, lack of a virological response to DAA was also seen in patients with no response or partial response to previous therapy. In these patients with viral resistance to DAA, the combination therapy with IFN and DAA may be a means of eliminating HCV, and IL28B genotyping may be a useful tool in determining the best antiviral therapy and duration of treatment.

This study had certain limitations. Selection bias cannot be excluded, considering the retrospective nature of the work. However, all patients had well-established cirrhosis and had received IFN plus ribavirin in hepatitis centers throughout Japan. Our patients received a variety of IFN treatments (IFN-α, IFN-β and PEG IFN), several different doses of IFN and ribavirin, and several treatment durations. In the intention-to-treat analysis, the overall SVR rate was 32.2%; in patients with G1H it was 21.6% but was 60.6% in those with non-G1H. Interestingly, the overall SVR rate in this study was similar to that found in previous studies of patients with advanced fibrosis or cirrhosis treated with IFN or PEG IFN plus ribavirin.[8–10] Thus, although there were some limitations, our findings contribute to providing valuable information to guide clinical decisions.

In conclusion, the combination therapy with IFN plus ribavirin in Japanese patients with non-G1H HCV-related LC was more effective than those with G1H and not influenced by IL28B polymorphism. However, in patients with G1H, IL28B polymorphism may be a strong predictive factor for SVR. Extending treatment may provide a better outcome in those with the IL28B TT allele treated for more than 48 weeks and in those with the TG/GG alleles treated for more than 72 weeks.

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