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Prevalence of type 2 diabetes mellitus in Japanese patients with hepatocellular carcinoma

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Abstract. The possibility has been raised in a number of cohort and case-control studies that diabetes mellitus (DM) may increase the risk of liver cancer, as well as that of cancer at other sites. To verify this possibility, we conducted a retrospective cohort study to determine the prevalence of type 2 DM in Japanese patients with hepatocellular carcinoma (HCC). A total of 1,251 patients with HCC, diagnosed at two major liver centers in the Nagasaki area, were consecutively recruited and categorized according to the etiology of HCC into four groups: HCC-B, HCC-C, HCC-BC and HCC-nonBC cases. Type 2 DM was diagnosed on the basis of standard criteria. The prevalence rate of HCC-nonBC and HCC-C was significantly higher than that of HCC-B, while the prevalence rate of HCC-nonBC was significantly higher than that of HCC-C. The prevalence of type 2 DM in HCC-B, HCC-C and HCC-nonBC patients under 66 years of age was 11, 31 and 32%, respectively, vs. 24, 22 and 40%, respectively, in patients over 66 years of age. In patients over 66 years of age, the prevalence of type 2 DM in HCC-B and HCC-nonBC cases was increased, whereas the prevalence of type 2 DM in HCC-C cases was significantly decreased. Our findings indicate that the effects of the interaction between type 2 DM and HCV increase the prevalence of HCC.

Introduction

Of the three leading causes of death in Japan – malignant neoplasms, cardiovascular diseases and cerebrovascular diseases – malignant neoplasms have been the leading cause of death in Japan since 1981. For the last 30 years, liver cancer has been the third leading cause of death by malignant neoplasms in men and, during the past decade, has ranked fifth in women (1-3). Hepatocellular carcinoma (HCC) accounts for 85-90% of cases of primary liver cancer, and chronic hepatitis B and C infections are the main cause of HCC. However, the prevalence of HCC in Japan in the liver of patients that are both hepatitis B surface antigen (HBsAg)- and hepatitis C virus (HCV)-RNA-negative has been increasing over the last 12 years (4).

Epidemiological findings have recently been reported proposing a link between type 2 diabetes mellitus (DM) and cancer in various organs (5,6). The possibility that DM may increase the risk of liver cancer, as well as cancer at other sites, has been raised in a number of cohorts and case-control studies (7-10). We carried out this retrospective study to determine the prevalence of type 2 DM in Japanese patients with HCC.

Patients and methods

Patients. A total of 1,251 patients with HCC diagnosed between January 1991 and December 2005 at the liver disease centers of the National Nagasaki Medical Center and Nagasaki University Hospital were consecutively recruited for this study. Informed consent was obtained from all patients. The diagnosis of HCC was based on the elevation of serum α-fetoprotein or des-γ-carboxy prothrombin levels, characteristic image findings obtained using ultrasonography, computerized tomography, magnetic resonance imaging and hepatic angiography, and/or histological diagnosis using tumor biopsy samples.

Etiology of HCC. The HCC cases were categorized according to etiology into four groups: HCC-B, hepatitis B virus surface antigen (HBsAg)-positive and hepatitis C virus (HCV)-RNA-negative; HCC-C, HCV-RNA-positive and HBsAg-negative; HCC-BC, both HBsAg- and HCV-RNA-positive; and HCC-nonBC, both HBsAg- and HCV-RNA-negative. A diagnosis of chronic HCV infection was based on the presence of both serum anti-HCV antibody and HCV-RNA detected by polymerase chain reaction (PCR), while a diagnosis of chronic hepatitis B virus (HBV) infection was based on the presence of HBsAg.

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Table I. Characteristics of the HCC patients.

	HCC-B	HCC-C	HCC-BC	HCC-nonBC	Total
No.	248	809	29	165	1,251
Gender					
Male	191	566	19	121	897
Female	57	243	10	44	354
Ratio (male/female)	3.4	2.3	1.9	2.8	2.5
Age (IQR), in years	57 (15)	67 (9)	65 (12)	67 (14)	66 (11)
<66	190	341	17	71	619
≥66	58	468	12	94	632
Child-Pugh grade					
A	95	70	80	67	412
B	111	213	240	292	1,134
C	8	8	9	11	46

Gender: HCC-B vs. HCC-C, p=0.031. Age: HCC-B vs. HCC-C, p<0.001; HCC-B vs. HCC-BC, p=0.022; HCC-B vs. HCC-nonBC, p<0.0001; HCC-C vs. HCC-BC, p=0.004; HCC-BC vs. HCC-nonBC, p=0.009. IQR, interquartile range.

Diagnosis of type 2 DM. Type 2 DM was diagnosed on the basis of the presence of hyperglycemia (≥200 mg/dl) in at least two postabsorptive samples, overt glycosuria, or both; or active treatment with insulin, oral hypoglycemic agents, or both. No consideration was given to minor alterations in glucose metabolism, such as impaired glucose tolerance based on an oral glucose tolerance test, in accordanc with World Health Organization criteria.

Statistical analysis. Data were analyzed by the Mann-Whitney U test for continuous ordinal data, and by the χ^2 test with Yates' correction and Fisher's exact test for associations between two qualitative variables. $p<0.05$ was considered statistically significant. Data analysis was performed with SPSS version 16.0 for Windows.

Results

Clinical features of the studied patients. As shown in Table I, of the 1,251 patients with HCC, 20% (248/1,251) were diagnosed with HCC-B, whereas 65% (809/1,251) had HCC-C and an additional 2% (29/1,251) had HCC associated with both viruses. In the remaining 165 patients (13%), no association was found between HCC and either of the viruses. Analyzing the patients with HCC by category revealed the male/female ratio in HCC-B, HCC-C, HCC-BC and HCC-nonBC to be 3.4, 2.3, 1.9 and 2.8, respectively. The male/female ratio in HCC-C was less than that in HCC-B. In addition, the median age of patients diagnosed with HCC-B, HCC-C, HCC-BC and HCC-nonBC was 57, 67, 65 and 67 years, respectively. The median age of patients diagnosed with HCC-B was significantly lower than that of the patients with other types of HCC. Among the patients with HCC, 25% (310/1,251) had type 2 DM, 3% (34/1,251) HCC-B, 16% (209/1,251) HCC-C, 1% (6/1,251) HCC-BC and 5% (61/1,251) HCC-nonBC.

Prevalence of type 2 DM by stratification according to etiology in patients with HCC. Cohorts of patients with HCC were divided according to etiology. Fig. 1 shows that the prevalence rate of type 2 DM in HCC-B, HCC-C, HCC-BC and HCC-nonBC was 14% (34/248), 26% (209/809), 37% (61/165) and 21% (6/29), respectively. The prevalence rate of HCC-nonBC and HCC-C was significantly higher than that of HCC-B (HCC-B vs. HCC-nonBC, p≤0.001; HCC-B vs. HCC-C, p≤0.001), while the prevalence rate of HCC-nonBC was significantly higher than that of HCC-C (HCC-C vs. HCC-nonBC, p=0.003).

The prevalence rate of type 2 DM was 25% in patients under 66 years of age (154/619) and 25% in patients over 66 years of age (156/632). Fig. 2 shows the age distribution of the prevalence rate for type 2 DM in HCC-B, HCC-C and HCC-nonBC cases. The prevalence rate of type 2 DM in HCC-B, HCC-C and HCC-nonBC was 11% (20/190), 31% (107/341) and 32% (23/71), respectively, in patients under 66 years of age, vs. 24% (14/58), 22% (102/468) and 40% (38/94), respectively, for those over 66 years of age. The prevalence rate of type 2 DM in HCC-B and HCC-nonBC patients over 66 years of age was increased, whereas that of HCC-C was significantly decreased.

Discussion

A nationwide health survey regarding the prevalence of DM in the general Japanese population conducted in 2006 indicated that the prevalence of DM in Japan was 12%. However, the prevalence rate of type 2 DM is higher in patients with HCC than in the general Japanese population. In this two major liver center-based cohort study designed to examine the prevalence of type 2 DM in HCC patients, 25% of patients with HCC had type 2 DM. Previous studies have suggested that DM is a potential risk factor for HCC (10-13). Inoue *et al* prospectively

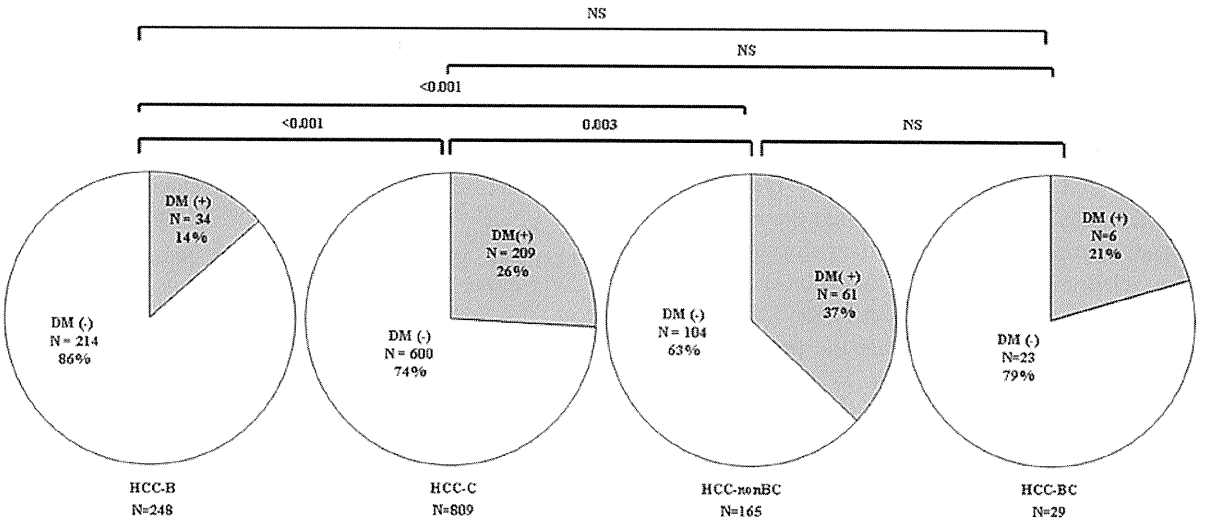

Figure 1. Prevalence rate of type 2 DM in HCC-B, HCC-C, HCC-BC and HCC-nonBC.

examined the association between a history of DM and the subsequent risk of cancer in a Japan Public Health Center-based prospective study, and found an increased risk of liver cancer in DM patients (12).

The present study found that the prevalence of type 2 DM was significantly higher in HCC-nonBC than in HCC-B and HCC-C patients. In particular, type 2 DM persisted in patients without chronic hepatitis virus infections; type 2 DM in these individuals may explain a relevant proportion of the observed cases of HCC. Previous studies have suggested that diabetes and/or non-alcoholic fatty liver disease account for at least a portion of these 'idiopathic' cases (14-16). Findings from the present study support the hypothesis that the presence of DM alone accounts for approximately 37% of cases of HCC-nonBC.

Investigations into the possible biological mechanisms of the association between type 2 DM and HCC-nonBC have been site-specific. However, these associations may be the result of metabolic and hormonal aberrations associated with type 2 DM, and common biological mechanisms may be at least partially associated with insulin and insulin-like growth factors (IGFs) (17).

The most obvious change in diabetic patients is reduced insulin sensitivity with compensatory hyperinsulinemia and elevated levels of IGF-1, which may in turn stimulate cell proliferation in the liver (18,19). At the same time, insulin activates the IGF-1 receptor, which is known to have a growth-promoting effect, including the modulation of cell cycle progression. Excess insulin may also indirectly affect the development of cancer by down-regulating the level of IGF-binding protein 1, which increases the level and bioavailability of total circulating IGF-1. Obesity and physical inactivity also cause hyperinsulinemia, and are thus also ultimately associated with cancer (17-20).

A survey of HCC-nonBC conducted between 1995 and 2003 in Japan by the Inuyama Hepatitis Research Group found that individuals with HCC-nonBC accounted for 9.3% of the general population (2). In the present study, we found the percentage of HCC-nonBC to be 14.1% in the Nagasaki area. Furthermore, the number and proportion of HCC-nonBC

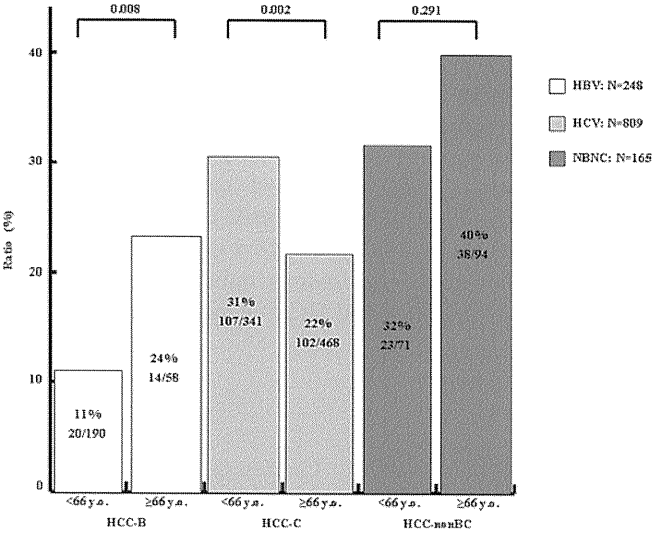

Figure 2. Age distribution for the prevalence rate of type 2 DM in HCC-B, HCC-C and HCC-nonBC.

cases gradually increased from 1981 to 2005 (4). According to an epidemiological study on DM by Nakano *et al*, the number of patients with DM has been gradually increasing since the development of an automotive society and the Westernization of the Japanese diet (21). Since the prevalence of DM increases with age, the proportion of individuals with DM aged 60 or above has exceeded two-thirds of the estimated total number of patients in Japan (7.40 million in 2002), which has a rapidly aging society (21). In other words, the number of individuals with type 2 DM is increasing in Japan, and these individuals are at high risk for HCC. Thus, the number of HCC-nonBC cases will increase in the next decade in Japan.

Approximately 60% of liver cancer cases in Japan are anti-HCV-positive (4). An experimental study revealed that HCV infection itself induces insulin resistance through the disturbance of the insulin intracellular signaling pathway by the hepatitis virus core protein (22). Liver fat deposition may contribute to insulin resistance, which in turn leads to a loss of the restraining effect of insulin on the production of glucose

by hepatocytes, thereby causing diabetes (23). Steatosis occurs more frequently in patients with chronic HCV infection than in those with chronic HBV infection; this may explain the increased risk of DM among HCV patients (24). Although we proposed possible explanations for the correlation between HCV infection and the prevalence rate of type 2 DM in patients in this study, it is also possible that the mechanism is multifactorial. A previous study identified chronic hepatitis B as having no relationship to DM, and on the basis of the results of this study, we arrive at the same conclusion (25,26).

Several studies have indicated that the progression from chronic hepatitis to cirrhosis and HCC is accelerated by dual HCV infection (11,27). The strong effect of DM on HCC in the absence of hepatitis infection suggests that, in addition to the hepatitis C causal pathway, HCC is mediated through the reduction of IGF-1 factors or IGF binding protein-3, caused by hyperinsulinemia. This in turn stimulates the proliferation of cancer cells, as demonstrated by Lagiou *et al* (28). In the present study, the prevalence rate of DM in patients with HCC-C was significantly higher in patients older than 66 years of age. Our findings demonstrate that the effects of the interaction between DM and HCV further the incidence of HCC.

In conclusion, the prevalence of HCC-nonBC and HCC-C was significantly higher than that of HCC-B, while the prevalence of HCC-nonBC was significantly higher than that of HCC-C. In patients over 66 years of age, the prevalence of type 2 DM in HCC-B and HCC-nonBC cases was increased, whereas the prevalence of type 2 DM in HCC-C cases was significantly decreased. Our findings indicate that the interaction between type 2 DM and HCV increases the prevalence of HCC.

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Original Article

Data mining reveals complex interactions of risk factors and clinical feature profiling associated with the staging of non-hepatitis B virus/non-hepatitis C virus-related hepatocellular carcinoma

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Aim: Non-hepatitis B virus/non-hepatitis C virus-related hepatocellular carcinoma (NBNC-HCC) is often detected at an advanced stage, and the pathology associated with the staging of NBNC-HCC remains unclear. Data mining is a set of statistical techniques which uncovers interactions and meaningful patterns of factors from a large data collection. The aims of this study were to reveal complex interactions of the risk factors and clinical feature profiling associated with the staging of NBNC-HCC using data mining techniques.

Methods: A database was created from 663 patients with NBNC-HCC at 20 institutions. The Milan criteria were used as staging of HCC. Complex associations of variables and clinical feature profiling with the Milan criteria were analyzed by graphical modeling and decision tree algorithm methods, respectively.

Results: Graphical modeling identified six factors independently associated with the Milan criteria: diagnostic year of HCC; diagnosis of liver cirrhosis; serum aspartate aminotransferase (AST); alanine aminotransferase (ALT); α-fetoprotein (AFP); and des-γ-carboxy prothrombin (DCP) levels. The decision trees were created with five variables to classify six groups of patients. Sixty-nine percent of the patients were

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within the Milan criteria, when patients showed an AFP level of 200 ng/mL or less, diagnosis of liver cirrhosis and an AST level of less than 93 IU/mL. On the other hand, 18% of the patients were within the Milan criteria, when patients showed an AFP level of more than 200 ng/mL and ALT level of 20 IU/mL or more.

Conclusion: Data mining disclosed complex interactions of the risk factors and clinical feature profiling associated with the staging of NBNC-HCC.

Key words: data mining, disease progression, hepatoma, non-viral hepatitis, tumor marker

INTRODUCTION

HEPATOCELLULAR CARCINOMA (HCC) is the fifth most common cancer and the third most common cause of cancer-related deaths worldwide.[1–3] Chronic infection with hepatitis B virus (HBV) or hepatitis C virus (HCV) is a risk factor for HCC. Recent developments in the management of patients with viral hepatitis have resulted in early detection of HCC and improvement of prognosis.[4–8]

The number of patients with non-HBV/non-HCV-related HCC (NBNC-HCC) has been increasing, and NBNC-HCC now accounts for 12–16% of all the HCC cases in Japan.[8,9] A variety of factors are involved in the development and progression of this cancer including age, sex, alcoholic liver disease and diabetes mellitus.[10–12] Therefore, neither early detection nor improved prognosis has been achieved in NBNC-HCC.[6] Radical treatment is applicable to patients with NBNC-HCC who meet the Milan criteria;[13] however, this cancer is often detected at an advanced stage. For earlier detection, it is important to understand the complex interactions of the risk factors and clinical feature profiling associated with the Milan criteria, a staging system for NBNC-HCC.

Data mining, a set of statistical techniques, uncovers meaningful patterns and interactions of variables from a large data collection even when there is no a priori hypothesis imposed.[13] Graphical modeling is an exploratory multivariate analysis of data mining that reveals complex associations between variables.[14] This analysis assumes that the response variable is influenced by multiple factors.[15] Therefore, different from results of univariate analysis, an association between a risk factor and an outcome variable may disappear or appear because of the effects of another set of variables known as "confounding factors".[16,17] Furthermore, its findings are visualized as a graph, which provides an idea of how variables interact and denotes the conditional independence structure between random variables.[15] Therefore, graphical modeling is now identified as a new approach to model clinical data.[18]

Decision tree making is another exploratory technique of data mining that represents a series of rules for classification by identifying priorities.[19–21] It is an explicit, quantitative and systematic approach to decision-making under conditions of uncertainty and allows clinicians to choose an option that maximizes the net benefit to the patient.[22] Recently, decision trees were used to reveal the clinical feature profiling for staging of pancreatic cancer[23] and ovarian cancer.[24] However, decision trees have never been applied to identify the clinical feature profiling associated with the staging of NBNC-HCC.

The aims of this study were to reveal complex interactions of the risk factors and clinical feature profiling associated with the staging of NBNC-HCC using data mining techniques.

METHODS

Patient database

BETWEEN 1995 AND 2006, a total of 10 133 patients were diagnosed with HCC at 23 institutions located in Kyushu, a high morbidity area of HCC in Japan. Among them, 1363 patients were diagnosed with NBNC-HCC according to the negative results of both serum hepatitis B surface antigen and serum anti-HCV antibody or HCV RNA.

In order to examine the clinical variables associated with the staging of NBNC-HCC, a database of 663 patients with NBNC-HCC at 20 institutions was created on the basis of the following variables: diagnostic year of HCC; age; sex; family history of liver disease; past history of blood transfusion; alcohol intake; diagnosis of liver cirrhosis; diagnosis of liver disease; diagnosis of diabetes mellitus; serum aspartate aminotransferase (AST) level; serum alanine aminotransferase (ALT) level; serum α-fetoprotein (AFP) level; serum des-γ-carboxy prothrombin (DCP) level; size of HCC; and number of HCC.

For practical use, alcohol intake, serum AFP level and serum DCP level were categorized as follows. Alcohol intake: none; 60 g/day or less; 60–100 g/day; or more than 100 g/day. AFP level: 20 ng/mL or less; 20–200 ng/mL; or more than 200 ng/mL. DCP level: 40 mAU/mL or less; 40–100 mAU/mL; or more than 100 mAU/mL.

The study protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki, as reflected by the approval of the Ethics Committee of the Kurume University School of Medicine.

Diagnosis and staging of HCC

The diagnosis of HCC was based on the clinical practice manual proposed by the Japan Society of Hepatology,[25] by using serum AFP and DCP levels and imaging techniques including ultrasonography, computerized tomography, magnetic resonance imaging, hepatic angiography and/or tumor biopsy. The Milan criteria (single nodule ≤5 cm or three nodules <3 cm) were used for the staging of HCC.[26]

Data mining

An association between the Milan criteria and each risk factor was examined by Student's *t*-test and χ^2-test. Because of the insufficient scientific evidence for testing specific clinical hypotheses, graphical modeling and decision trees were employed to explore complex associations between the Milan criteria and a set of risk factors.

MIM software (http://www.hypergraph.dk/) was used for graphical modeling. R package rpart (recursive partitioning and regression trees by Terry Therneau and Beth Atkinson; http://www.mayo.edu/biostatistics) was used to construct a decision tree algorithm. In order to evaluate the prediction error, the original data ($n = 663$) were randomly divided into a training dataset ($n = 442$) and a test dataset ($n = 221$). Ten-hold cross-validation was conducted to construct the initial tree on the basis of the training dataset; then, the optimal-size tree was constructed by examining a set of cost–complexity parameters. The overall prediction error rate as well as the sensitivity and specificity were calculated by applying the results of the decision tree algorithm to the test dataset.

RESULTS

Characteristics of patients with NBNC-HCC

THE PATIENTS' CHARACTERISTICS are summarized in Table 1. Family history of liver disease and history of blood transfusion were not noted in more than 80% of the patients. Approximately 40% of the patients did not have any etiology of chronic liver disease.

Univariate analysis of variables associated with the Milan criteria

Univariate analysis showed that diagnosis of liver cirrhosis, serum AST level, serum ALT level, serum AFP level and serum DCP level were significantly associated with the Milan criteria (Table 2).

Table 1 Characteristics of all patients

Variable	
n	663
Diagnostic year of HCC (years)	2002 ± 3
Age (years)	68.1 ± 9.9
Male/female	480/183
Family history of liver disease (yes/no/unclear)	79/547/37
History of blood transfusion (no/before 1989/after 1989/unclear)	584/29/22/28
Daily alcohol intake (none/<60 g/60–100 g/>100 g)	254/183/141/85
Etiology of chronic liver disease (none/alcohol/others)	296/188/179
Diagnosis of liver cirrhosis (yes/no)	260/403
Diagnosis of diabetes mellitus (no/yes without medication/yes with medication)	396/109/158
Serum AST level (U/L)	53.3 ± 51.3
Serum ALT level (U/L)	51.8 ± 49.9
Serum AFP level (ng/mL)	9397 ± 71066
Serum DCP level (mAU/mL)	8003 ± 37377
Size of HCC (cm)	5.0 ± 3.4
Number of HCC	2.8 ± 2.9

Data are expressed as the mean ± standard deviation or the number of patients.
AFP, α-fetoprotein; ALT, alanine aminotransferase; AST, aspartate aminotransferase; DCP, des-γ-carboxy prothrombin; HCC, hepatocellular carcinoma.

Graphical modeling

Complex interactions of the risk factors associated with the Milan criteria were visualized graphically (Fig. 1). Graphical modeling identified six independent factors directly associated with the Milan criteria: diagnostic year of HCC; diagnosis of liver cirrhosis; serum AST level; serum ALT level; serum AFP level; and serum DCP level (Fig. 1). Although alcohol intake, diagnosis of liver disease and diagnosis of diabetes mellitus were not directly associated with the Milan criteria, they were associated with the Milan criteria through diagnosis of liver cirrhosis (Fig. 1).

Decision tree algorithm

With the training dataset ($n = 442$), a decision tree algorithm was created by using five variables to classify six groups of patients (Fig. 2). A serum AFP level of 200 ng/mL or less was the cut-off value for the initial

Table 2 Univariate analysis of the variables associated with the Milan criteria

Variable	Statistical method	Test statistics	Degree of freedom (df)	*P*
Diagnostic year of HCC (years)	χ^2	13.4013	11	0.2679
Age (years)	Pooled	−1.07	661	0.2843
Sex	χ^2	0.2975	1	0.5854
Family history of liver disease	χ^2	1.7412	1	0.187
History of blood transfusion	χ^2	4.9527	2	0.084
Daily alcohol intake	χ^2	2.4158	3	0.4907
Liver cirrhosis	χ^2	28.9521	1	<0.0001
Diabetes mellitus	χ^2	0.926	2	0.6294
AST level (U/L)	Satterthwaite	3.06	387.51	0.0023
ALT level (U/L)	Satterthwaite	4.79	546.95	<0.0001
AFP level (ng/mL)	χ^2	63.1357	2	<0.0001
DCP level (mAU/mL)	χ^2	47.7161	2	<0.0001

Associations between the variables and the Milan criteria were analyzed by the indicated statistical methods. $P < 0.05$ was considered significant.
AFP, α-fetoprotein; ALT, alanine aminotransferase; AST, aspartate aminotransferase; DCP, des-γ-carboxy prothrombin; HCC, hepatocellular carcinoma.

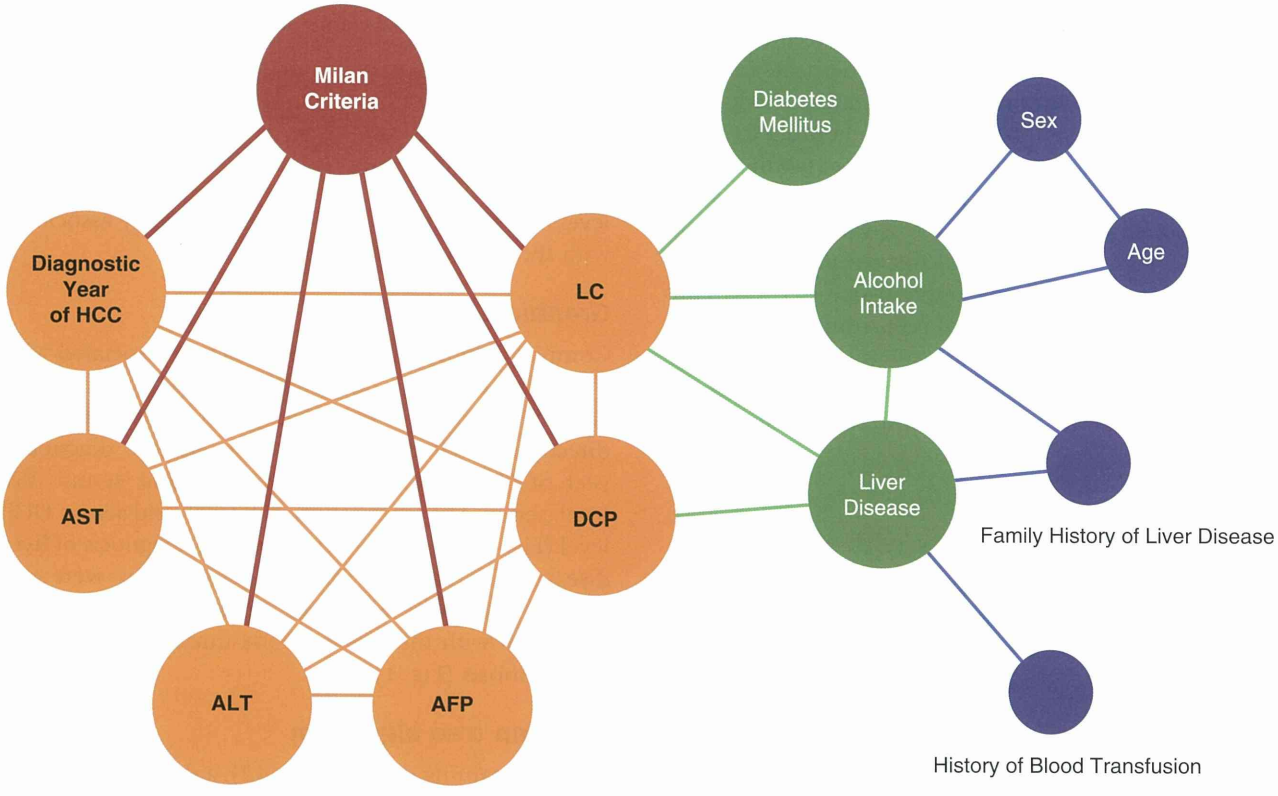

Figure 1 Graphical modeling of the interactions of the risk factors associated with the Milan criteria. AFP, α-fetoprotein; ALT, alanine aminotransferase; AST, aspartate aminotransferase; DCP, des-γ-carboxy prothrombin; HCC, hepatocellular carcinoma; LC, liver cirrhosis.

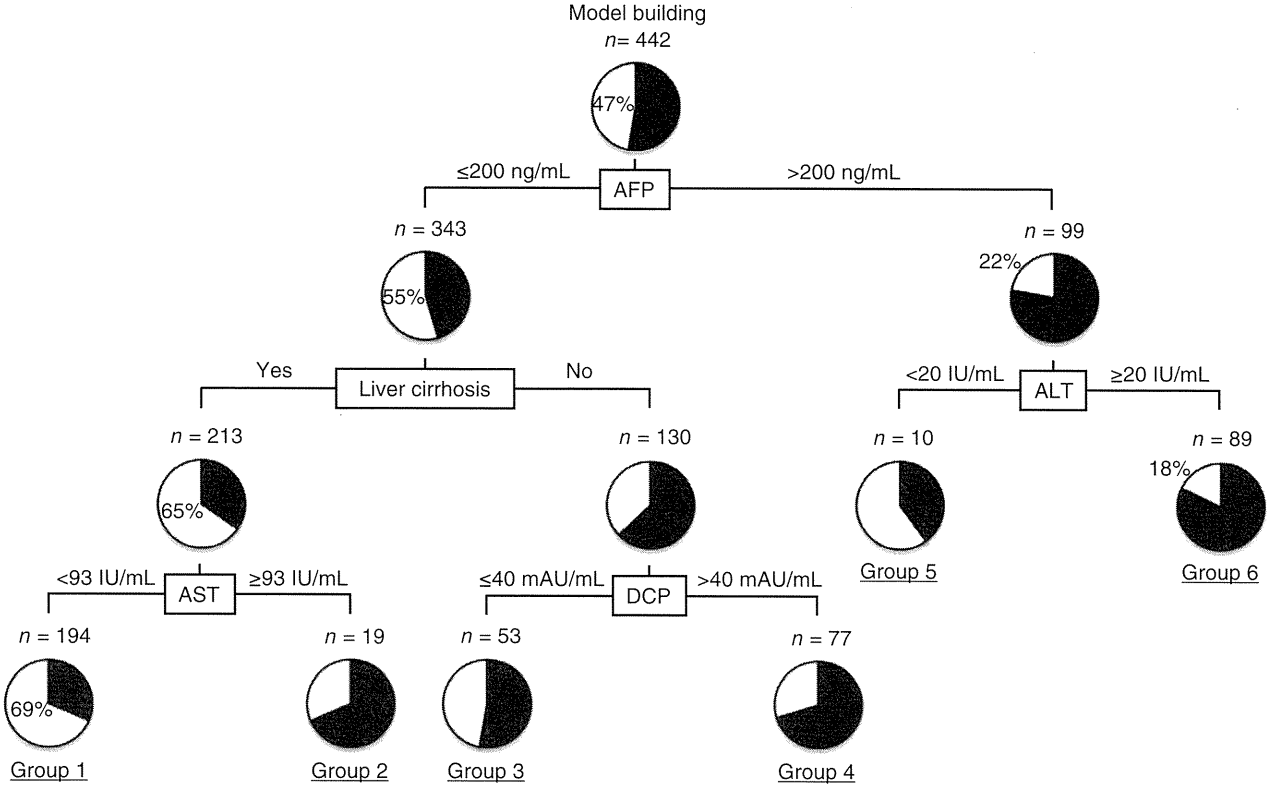

Figure 2 Decision tree algorithm of the variables associated with the Milan criteria. The patients were classified according to the indicated cut-off values of the variables. The pie graphs indicate the percentage of patients with HCC within (white)/beyond the Milan criteria in each group. AFP, α-fetoprotein; ALT, alanine aminotransferase; AST, aspartate aminotransferase; DCP, des-γ-carboxy prothrombin; HCC, hepatocellular carcinoma.

classification. Among the patients with an AFP level of 200 ng/mL or less, diagnosis of liver cirrhosis was used as the variable for the second division. Among the patients with liver cirrhosis, a serum AST level of less than 93 IU/mL was the cut-off value for the third division. Thus, 69% of the patients were within the Milan criteria, when the patients met all of the following conditions: AFP of 200 ng/mL or less; diagnosis of liver cirrhosis; and AST of less than 93 IU/mL (group 1; Fig. 2). On the other hand, only 18% of the patients were within the Milan criteria, when patients showed an AFP level of more than 200 ng/mL and an ALT level of 20 IU/mL or more (group 6; Fig. 2).

There were no significant differences in the patients' characteristics between the training dataset and the test dataset. Prediction error was obtained by applying the results of the decision tree algorithm to the test dataset. The sensitivity (proportion of patients with HCC correctly classified as beyond the Milan criteria) and specificity (proportion of patients with HCC correctly classified as within the Milan criteria) were 72.1% (75/104) and 68.4% (80/117), respectively; the overall prediction error rate was 29.8% (66/221).

DISCUSSION

IN THIS STUDY, we revealed the complex interactions of the risk factors associated with staging of NBNC-HCC using graphical modeling. In addition, we presented a decision tree algorithm to identify clinical feature profiling associated with the staging of NBNC-HCC.

Various factors seem to be intricately related to the progression of NBNC-HCC. In this study, by graphical modeling, we identified six variables directly associated with the Milan criteria: serum AST level; serum ALT level; serum AFP level; serum DCP level; diagnosis of liver cirrhosis; and diagnostic year of HCC. Chronic hepatic inflammation modulates many of the signaling cascades involved in cell proliferation, survival and invasion of

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

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

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

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

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

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

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

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Letter to the Editor

Successful pegylated interferon alpha2a monotherapy for hepatitis C virus infection in a transplanted patient who relapsed after the preceding course

T. Ichikawa, N. Taura, H. Miyaaki, M. Matsuzaki, S. Eguchi, M. Takatsuki, T. Kanematsu, K. Nakao. Successful pegylated interferon alpha2a monotherapy for hepatitis C virus infection in a transplanted patient who relapsed after the preceding course.
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Key words: HCV; IFN; liver transplantation

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To the Editor

The combination of pegylated interferon (peg-IFN) with ribavirin is the only treatment strategy at present for the treatment for hepatitis C virus (HCV) infection after liver transplantation (LT), but its effects are still incomplete. We successfully treated a patient with peg-IFN alpha2a (peg-IFN-α2a) monotherapy who showed a relapse after treatment with peg-IFN combined with ribavirin.

A 55-year-old female with liver cirrhosis and hepatocellular carcinoma within the Milan criteria was referred to Nagasaki University Hospital in December 2005. On admission, she had no ascites or hepatic encephalopathy and was determined to have Child–Pugh score 7 and modified end-stage liver disease score 12.5. She had repeatedly ruptured esophageal varices. LT surgery was successfully performed. After living-donor LT, tacrolimus and prednisone were administered at the standard doses used in the immunosuppressive protocol. Her laboratory data on post-operative day 15 were 58 U/L of aspartate aminotransferase (AST) and 110 U/L of alanine aminotransferase (ALT). A liver biopsy revealed fibrosis stage 1 and activity grade 2. The calcineurin inhibitor was switched to cyclosporine for IFN treatment with the combination of 80 μg of peg-IFN-α2b weekly with 600 mg of ribavirin daily. Her HCV was genotype 1a and 1,570,000 IU/mL at the start of IFN treatment. The IFN treatment was introduced at postoperative day 19. The first IFN treatment continued for 52 weeks, because the HCV-RNA titer had a 2-log decrease from the baseline at 12 weeks of IFN treatment and disappeared at 18 weeks, and HCV-RNA was positive at the end point of treatment.

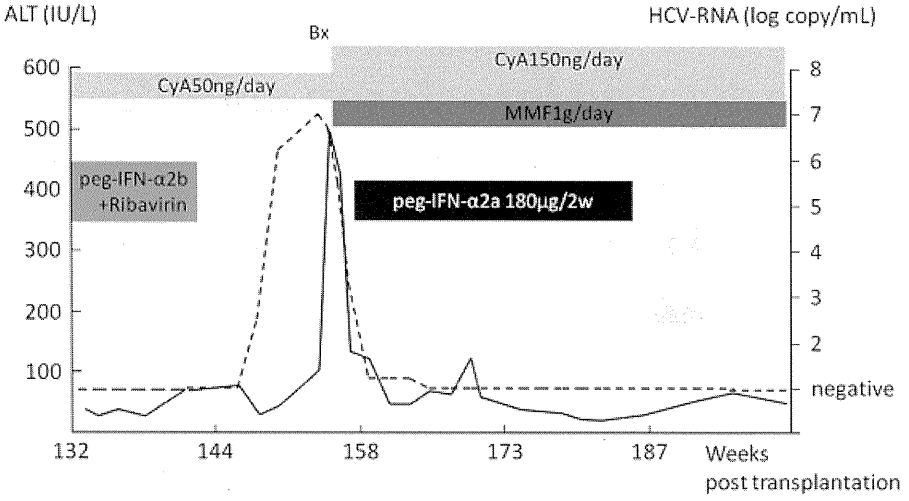

Fig. 1. Clinical course of rebound phenomenon after the second course of interferon (IFN) therapy. Solid lines are alanine aminotransferase (ALT) value (IU/L). Dotted lines are hepatitis C virus (HCV)-RNA value (log copy/mL). HCV-RNA is evaluated by the real-time polymerase chain reaction method. More than 1.2 log copy/mL of HCV-RNA is the sensitivity. Bx, liver biopsy; peg-IFN, pegylated interferon; w, weeks; CyA, cyclosporine (Neoral; Novartis Pharma, Tokyo, Japan); MMF, mycophenolate mofetil (Cellcept; Chugai, Tokyo, Japan).

After the first course, AST and ALT stabilized within the normal range and a liver biopsy revealed fibrosis stage 1 and activity score 1 at postoperative week 52. We waited for an improvement in the patient's side effects, and began the second treatment. The second course started at postoperative week 72, as her transplanted liver suffered from HCV-infected chronic hepatitis, according to the liver biopsy findings obtained at postoperative week 52. The second course was the combination of 90 μg of peg-IFN-α2a weekly with 200 mg of ribavirin daily for 9.5 g/dL of hemoglobin, because the early treatment of peg-IFN-α2b with ribavirin proved not to be effective (1).

An HCV-RNA qualitative assay, using the real-time polymerase chain reaction (PCR) method with a lower limit sensitivity of 15 IU/mL, was negative in serum at 20 weeks after IFN treatment. The HCV-RNA titer was positive in serum at 4 weeks after the end date of the second course, and reached a peak at 10 weeks after the second course. The ALT value indicated a relapse at 12 weeks. A liver biopsy revealed HCV-related chronic hepatitis. We introduced 180 μg of peg-IFN-α2a monotherapy once every 2 weeks, at 14 weeks after the second course.

HCV-RNA was negative by qualitative real-time PCR at 8 weeks after monotherapy. ALT was normalized at 4 weeks after monotherapy. The peg-IFN-α2a monotherapy was stopped at the treatment period of 6 months. HCV was negative at 6 months after the treatment end date, and she was determined to have a sustained viral response.

Some relapsing patients after IFN treatment show a flare up of HCV-RNA and ALT, the so-called 'rebound phenomenon' (2–6). ALT and HCV-RNA decrease again, after the rebound flare up. This is the best timing for induction of a second course of IFN (4, 5). The second course of IFN therapy has been reported to be IFN monotherapy for 6 months (4, 5). The second course of IFN monotherapy has a completion rate of 50% (4, 5). In our case, the third IFN monotherapy, but not the second IFN therapy, was induced at timing after rebound (Fig. 1).

The 'rebound phenomenon' is associated with immunological pressure against a rapid HCV increase (5, 7). A transplanted patient who shows relapse after the early treatment course must be treated quickly, because the HCV infection might be halted even by a single IFN treatment.

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Hepatology Research

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Original Article

Suppressor of cytokine signal 3 and IL28 genetic variation predict the viral response to peginterferon and ribavirin

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Aim: The aim of this study was to investigate the relationship among the expression of suppressor of cytokine signaling 3 (SOCS 3) in the liver, the SNPs in the IL28B locus, and the outcome of interferon therapy.

Methods: Prior to interferon treatment, we immunostained 67 liver specimens from chronic hepatitis C (CHC) patients who were receiving peginterferon alpha-2b/ribavirin therapy for suppressor of cytokine signaling 3 (SOCS3), and compared the expression of SOCS3, IL28 polymorphisms and other clinical factors between the patients and compared their eventual outcomes.

Results: Significant differences between the low SOCS3 group and high SOCS3 group were found in age, as well as in the platelet, transaminase, gamma-glutamyl transpeptidase levels. The incidence of high SOCS3 was not significantly different between subjects with the TT genotype and the TG genotype (TT : TG = 71%:29%, $P = 0.250$). In a multivariate analysis, age (≥65 years old) (odds ratio 0.221 [0.120–0.966], $P = 0.045$), IL28B gene (genotype TT) (odds ratio 5.422 [1.254–23.617], $P = 0.024$) and SOCS3 (high) (odds ratio 0.308 [0.104–0.948], $P = 0.040$) were significant predictors of the interferon response. In patients with the TT genotype, those with low SOCS3 immunostaining showed a high sustained virological response (69%), while the sustained virological rate was low (27%) in the patients with high SOCS3 immunostaining.

Conclusions: Using a combination of the SOCS3 immunostained area in the liver and the expression of IL28B single nucleotide polymorphisms might be a useful predictor of hepatitis C virus clearance by interferon therapy.

Key words: hepatitis C virus, IL28B, interferon, suppressor of cytokine signaling 3

INTRODUCTION

APPROXIMATELY 200 MISSION people worldwide are infected with hepatitis C virus (HCV). In Japan, about 2 million people are chronically infected, and HCV is the leading cause of hepatocellular carcinoma (HCC). The current standard care for chronic hepatitis C (CHC) is a combination of peginterferon-α (PEG-IFN) and ribavirin. This treatment is effective in approximately 40–50% of CHC patients with a high viral load of genotype 1.[1–5] This therapy is costly and frequently associated with side effects. Therefore, predicting the outcome of interferon therapy is important.

Several factors, such as gender, body mass index, the presence of steatosis and liver fibrosis, drug adherence and viral factors including the serum quantity of HCV RNA and HCV genotype have been reported to be significantly associated with the treatment outcome.[2,6–11] Among viral factors, Akuta *et al.* recently reported that the substitution of the HCV core amino acid was a predictor for the effect of interferon and ribavirin combination therapy.[2,12] Among the host factors, recent reports showed that genetic variations near the IL28 gene (rs8099917, rs1297860) on chromosome 19 were predictors of the virological response to 48-week PEG-IFN plus ribavirin combination therapy in individuals

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with HCV, and also affected the clinical outcome, including spontaneous clearance of HCV.[13–15]

We previously reported that the expression of suppressor of cytokine signaling 3 (SOCS3), which is related to insulin resistance, impairs the response to interferon treatment and might be a useful predictor of HCV clearance by interferon therapy.[16]

In this study, we examined the relationship among the expression of SOCS 3 in the liver, single nucleotide polymorphisms (SNPs) in the IL28B locus, and the outcome of interferon therapy.

METHODS

NEEDLE BIOPSIES OF the liver were obtained from 67 patients with positive HCV antibodies prior to interferon treatment at Nagasaki University Hospital and National Hospital Organization (NHO) Nagasaki Medical Center. Twenty of 67 cases were also examined in a previous study.[16] All patients with genotype 1b received weekly injections of PEG-IFN. The clinical data of the patients are summarized in Table 1. Liver biopsy was performed by needle puncture for diagnostic purposes. The diagnosis of each case was independently confirmed histologically by liver pathologists according to the Japanese chronic hepatitis classification criteria (New Inuyama classification). According to these criteria, mild activity was defined as A0 or A1, severe activity as A2 or A3, mild fibrosis as F0 or F1, and severe fibrosis as F2, F3, or F4. Fatty changes in >5% of all areas were defined as steatosis.

Table 1 Clinical backgrounds of the patients

Age	56.8 ± 9.3
Gender	Male : Female = 37:30
BMI (kg/m^2)	23.5 ± 2.9
Viral load (KIU/mL)	2320 ± 1519
White blood cell (/uL)	5074 ± 1713
Hemoglobin (mg/dL)	14.1 ± 1.3
Platelet (×10^3/uL)	167.3 ± 75.6
AST (IU/L)	77.1 ± 45.2
ALT (IU/L)	101.2 ± 56.3
γGTP (IU/L)	70.6 ± 65.5
HCV core 70 wild	40 cases
HCV core 91 wild	50 cases
Steatosis (>5%)	37 cases
A (0–1:2–3)	36:31
F (0~1:2~4)	22:45

ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; γGTP, gamma-glutamyl transpeptidase; HCV, hepatitis C virus.

All patients received PEG-IFN (Schering-Plough, Tokyo, Japan) + ribavirin (Schering-Plough, Tokyo, Japan) therapy for 48 weeks. The patients who were treated with a dose of PEG-IFN or ribavirin reduced by more than 20% were excluded from the study. PEG-IFN (1.5 μg/kg) was administered once per week, and the ribavirin dose was titrated according to body weight. A sustained virological response (SVR) was defined as undetectable HCV RNA at 6 months after the end of interferon treatment.

Of 38 patients who could not achieve an end-of-treatment response, 28 patients required a re-elevation of their viral loads regardless of the fact that the HCV-RNA levels were temporarily negative, and 10 patients did not achieve an HCV negative result during the entire treatment period.

SOCS3 immunohistochemistry

All tissue samples were fixed in 10% neutral buffered formalin and then embedded in paraffin, and 4 μm thick serial sections were cut from each paraffin block. In the immunohistochemical study, an anti-SOCS3 antibody (dilution 1:100, Affinity BioRegents, Golden, CO, USA) was used for SOCS3. Immunohistochemistry was performed with the labeled streptavidin biotinylate antibody (LSAB) method and a commercially available kit (Histofine, SAB-PO(R); Nichirei Corporation, Tokyo, Japan). The area immunostained for SOCS 3 was divided according to the number of immunoreactive cells per unit area. Immunoreactive cases were classified as those with less than 30% of the hepatocellular cells stained (low SOCS3 group) and those with 30% or more of the cells stained (high SOCS3 group), because our previous study showed that staining of more than 30% of the area was a significant predictor of viral clearance.[16]

Genetic variation near the IL28B gene

Genotyping for replication was performed by use of the Invader assay or direct sequencing. In this study, genetic variation near the IL28B gene (rs8099917), which was previously reported to be a predictor of the virological response was investigated.[13]

Statistical analysis

The SPSS 9.0 for Windows statistical software program was used to assess correlations among multiple variables. When appropriate, clinical and laboratory data

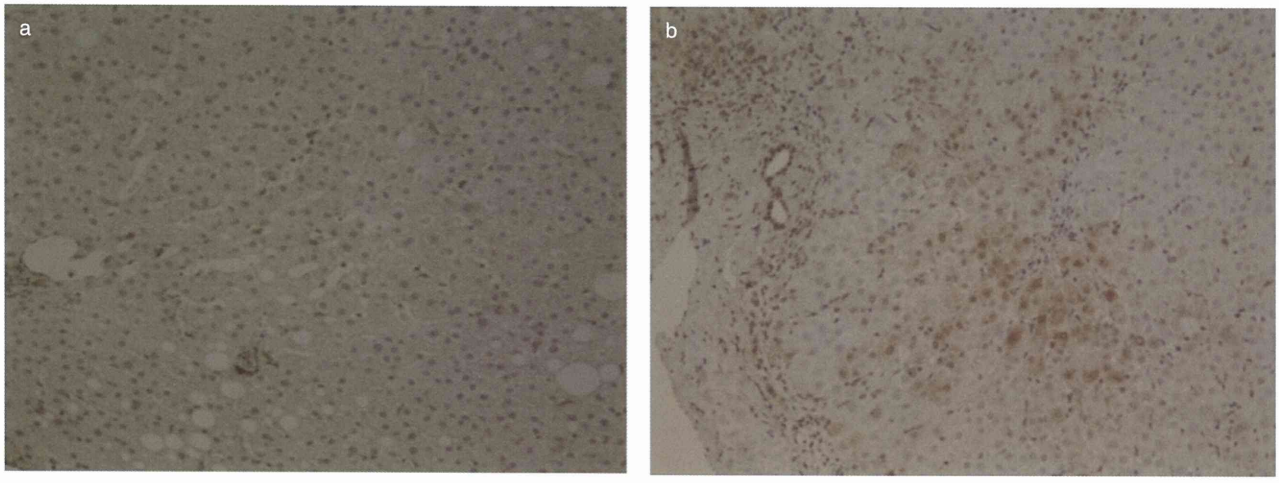

Figure 1 (a) This case showed less than 5% suppressor of cytokine signaling 3 (SOCS3) immunostained areas (low immunostaining). (b) This cases showed about 50% SOCS3 immunostaining areas (high immunostaining).

were compared with the Student's *t*-test or the Mann–Whitney test. A *P*-value of <0.05 was considered to be statistically significant.

RESULTS

Immunostaining of SOCS3 in the liver (Figs 1,2)

IMMUNOSTAINING FOR SOCS3 was mainly seen in the periportal area. Less than 30% SOCS3 immunostained areas were found in 36 cases (54%) and areas with 30% or more immunostaining for SOCS3 were found in 31 cases (46%).

The frequency and distribution of the SOCS3 expression are shown in (Fig. 2)

Correlation between SOCS3 immunostaining and clinicopathlogical factors

A significant difference between low and high SOCS3 groups was found in age (low : high = 54.5 ± 9.8:59.5 ± 8.1, $P = 0.028$), the levels of platelets (low : high = 189.5 ± 90.0:141.6 ± 41.3, $P = 0.009$), aspartate aminotransferase (AST) (low : high = 94.5. ± 56.0:62.1 ± 33.5, $P = 0.003$), alanine aminotransferase; (ALT) (low : high = 85.8 ± 52.4:119.0. ± 56.3, $P = 0.015$), gamma-glutamyl transpeptidase (γGTP) (low : high = 48.8 ± 53.5:94.7 ± 70.6, $P = 0.004$). The incidence of steatosis (low : high = 33%: 81%, $P = 0.001$), severe activity (low : high = 27%: 67%, $P = 0.001$) and sever fibrosis (low : high = 52%: 84%, $P = 0.006$) was significantly higher in the SOCS3 high

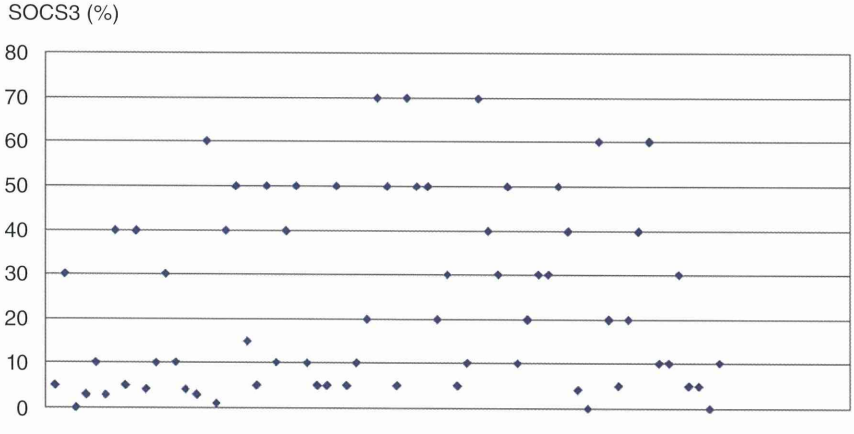

Figure 2 The distribution of the SOCS3 immunostaining area is shown.