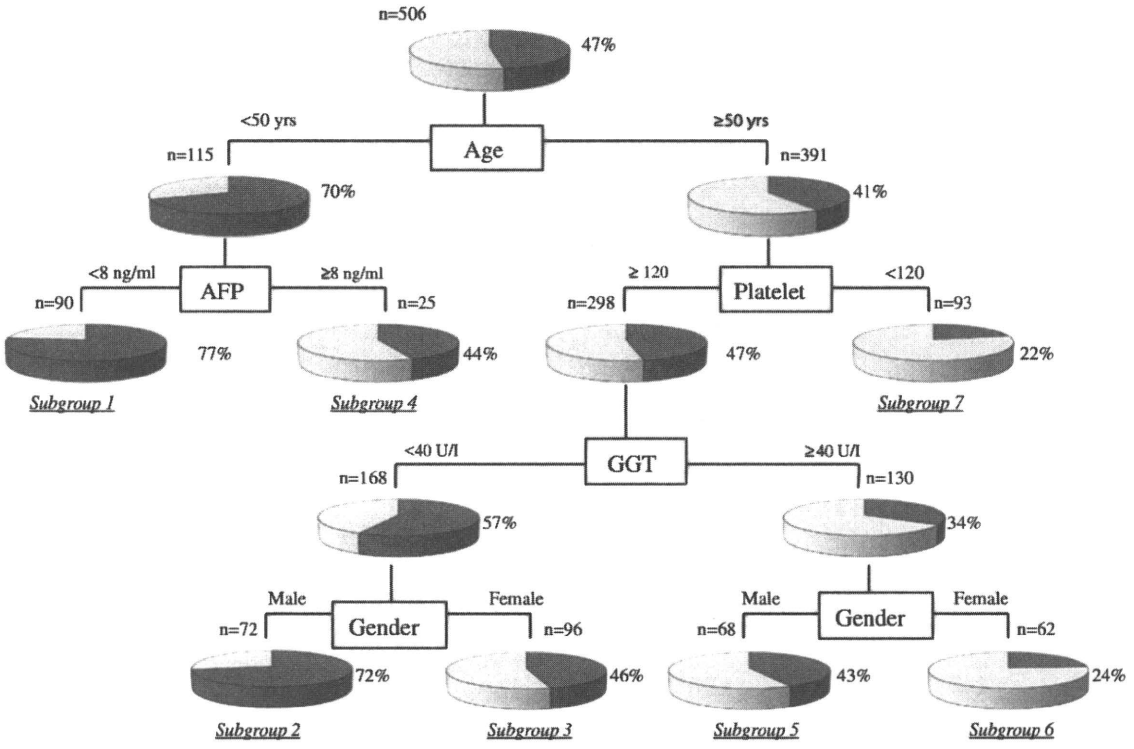

Fig. 1 Decision tree analysis. *Boxes* indicate the factors for splitting and the cutoff value for the split. *Pie charts* indicate the rate of SVR for each group. Terminal subgroups of patients discriminated by the analysis are numbered from 1 to 7. *AFP* alpha-fetoprotein, *GGT* gamma-glutamyltransferase

younger than 50. Among patients younger than 50, the level of serum AFP, with an optimal cutoff of 8 ng/ml, was selected as the variable of second split. Patients with lower AFP levels had a higher probability of SVR (77 vs. 44%). Among older patients, platelet count was selected as the second variable of split, with an optimal cutoff of 120×10^9/l. Patients with higher platelet counts had a higher probability of SVR (47 vs. 22%). Among patients with platelet counts higher than 120×10^9/l, GGT was selected as the third variable of split with an optimal cutoff of 40 IU/l. Patients with a lower GGT level had a higher probability of SVR (57 vs. 34%). Gender was selected as the fourth variable of split, with male gender being a predictor of a higher SVR probability (72 vs. 46% in patients with GGT levels <40 IU/l and 43 vs. 24% in those with GGT ≥40 IU/l). HCVRNA load was included in the analysis but was not selected as a significant variable.

The probabilities of SVR for the 7 subgroups derived by this process were highly variable. The subgroup of young patients (<50 years) with low serum AFP (<8 ng/ml) (subgroup 1) or the subgroup of older (≥50 years) male patients with high platelet counts (≥120×10^9/l) and low serum GGT (<40 IU/l) (subgroup 2) showed the highest probability of SVR (72 and 77%), while the subgroup of older (≥50 years) patients with low platelet counts (<120×10^9/l) (subgroup 7) and older (≥50 years) female patients with high serum GGT (subgroup 6) showed the lowest probability of SVR (22 and 24%).

Validation of the decision tree

The results of the decision tree analysis were validated with an internal validation dataset of 295 cases, which was independent of the model building dataset. Each patient in the validation set was allocated to subgroups 1–7 using the flow-chart form of the decision tree. The rates of SVR were 77% for subgroup 1, 71% for subgroup 2, 55% for subgroup 3, 44% for subgroup 4, 41% for subgroup 5, 17% for subgroup 6, and 30% for subgroup 7. The rates of SVR for each subgroup of patients were closely correlated between the model building dataset and the internal validation dataset ($r^2 = 0.925$) (Fig. 2a).

To further confirm the universality of the results, data collected from 524 patients by a collaborating study group were used for external validation. Thus, the dataset used for external validation was completely independent of the

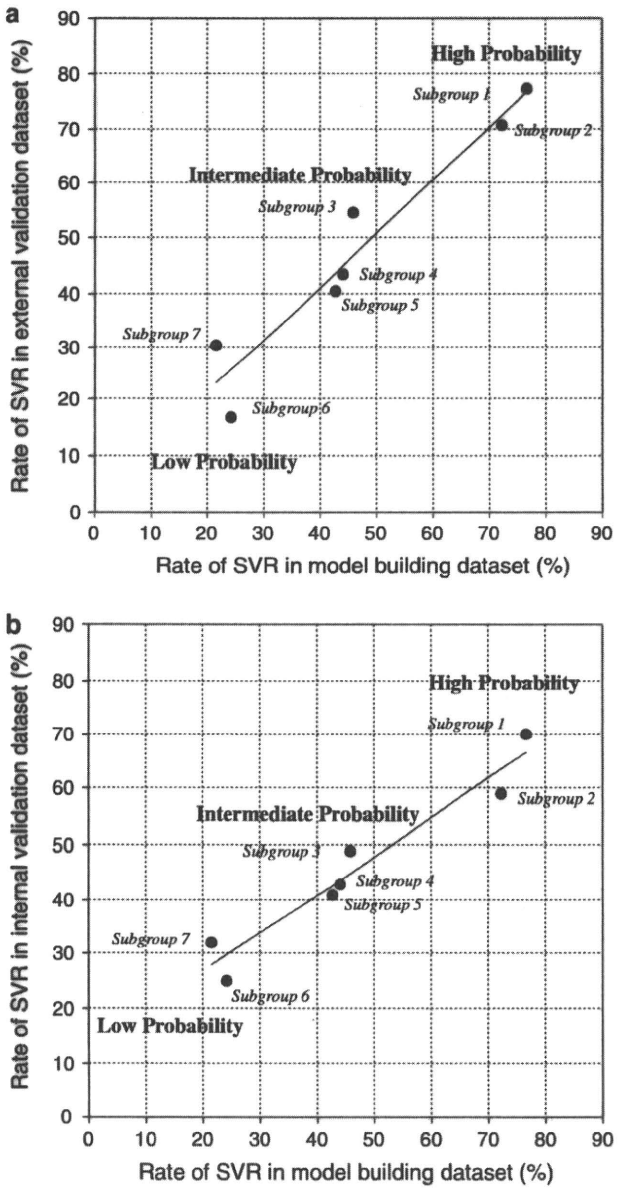

Fig. 2 Validation of the decision tree analysis by an internal and external validation dataset: subgroup-stratified comparison of the SVR rate. The rate of SVR in each subgroup was plotted. The *X* axis represents the model building, and the *Y* axis represents the validation datasets. **a** Internal validation and **b** external validation. There was a close correlation between the model building and the internal validation dataset (correlation coefficient $r^2 = 0.925$) and between the model building and the external validation dataset (correlation coefficient $r^2 = 0.936$)

original dataset used for model building. Each patient in the external validation set was allocated to subgroups 1–7 using the flow-chart form of the tree. The rates of SVR were 70% for subgroup 1, 59% for subgroup 2, 49% for subgroup 3, 43% for subgroup 4, 41% for subgroup 5, 25% for subgroup 6, and 32% for subgroup 7. The rates of SVR for each subgroup of patients were closely correlated

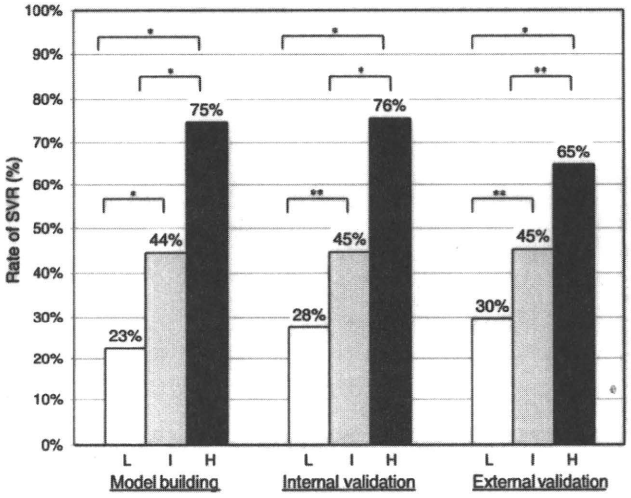

Fig. 3 Comparison of SVR rates between groups divided by the decision tree. The rate of SVR was compared among the 3 groups of patients divided by the decision tree analysis (*white*, *gray* and *black boxes*, indicating a low (*L*), intermediate (*I*) and high (*H*) probability group, respectively). The rate of SVR was significantly different among the 3 groups. $*p < 0.0001$, $**p < 0.001$

between the model-building dataset and the validation dataset ($r^2 = 0.936$) (Fig. 2b).

Construction of 3 groups according to the probability of SVR

Seven subgroups were reconstructed into 3 groups according to their predicted rates of SVR: the high probability group consisted of subgroups 1 and 2, the intermediate probability group consisted of subgroups 3, 4 and 5, and the low probability group consisted of subgroups 6 and 7. The rate of SVR was significantly different among the 3 groups (Fig. 3). The rate of SVR in the high probability group was consistently high: 75% for model building patients, 76% for internal validation patients and 65% for external validation patients. Conversely, the rate of SVR in the low probability group was consistently low: 23% for model building patients, 28% for internal validation patients and 30% for external validation patients. The rate of SVR in the intermediate probability group was 44% for model building patients, 45% for internal validation patients and 45% for external validation patients. Since 28–32% of patients were classified as high probability and 30–32% were classified as low probability, roughly 60% of patients were classified as having either a high or low probability of achieving SVR.

Effect of dose reductions of PEG-IFN and RBV on SVR

The cumulative dose of PEG-IFN and RBV was not included as a variable of analysis since the present study

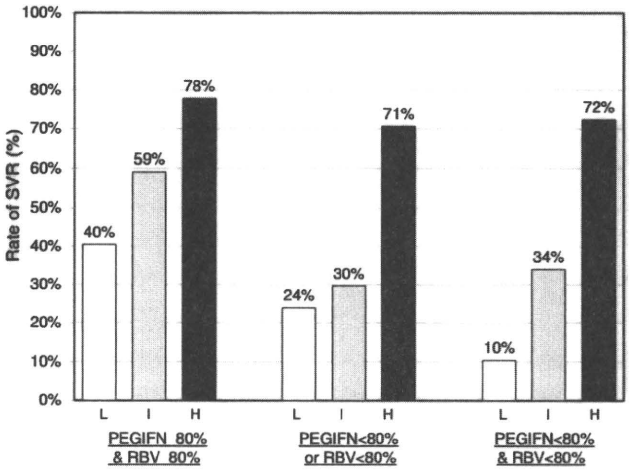

Fig. 4 Comparison of SVR rates among groups stratified by drug adherence. The 3 groups of patients divided by the decision tree analysis (*white*, *gray* and *black boxes* indicating a low (*L*), intermediate (*I*) and high (*H*) probability group, respectively) were further stratified according to the cumulative drug exposure of PEG-IFN and RBV. The good adherence group (≥80% planned dose of both PEG-IFN and RBV) had a higher rate of SVR compared with the poor adherence group (<80% planned dose of both PEG-IFN and RBV) in the low ($p = 0.0003$) and intermediate ($p = 0.007$) but not in the high probability group ($p = 0.53$)

aimed to develop a pre-treatment model for the prediction of response. To analyze the possible effect of drug reductions on the result of the decision tree analysis, 3 groups of patients divided by the decision tree analysis (low, intermediate and high probability group) were further stratified according to the cumulative drug exposure of PEG-IFN and RBV (Fig. 4). Even after adjustment for adherence, 3 groups of patients still had low, intermediate and high probability of achieving SVR, respectively. Of note, the good adherence group (≥80% planned dose of both PEG-IFN and RBV) had higher rates of SVR compared with the poor adherence group (<80% planned dose of both PEG-IFN and RBV) in the low ($p = 0.0003$) and intermediate ($p = 0.007$) probability group, but not in the high probability group ($p = 0.53$).

Factors associated with SVR by multivariate logistic regression analysis

We also explored the factors associated with SVR using a standard statistical analysis. By univariate analysis, age, gender, serum albumin, creatinine, alanine aminotransferase, GGT, red blood cell count, hemoglobin, hematocrit, platelet count and AFP were found to be associated with SVR (Table 2). HCVRNA load was not associated with SVR. By multivariate analysis, age, gender, GGT and platelet count were found to be independently associated with SVR (Table 3). Of note, AFP, which was selected as a significant predictor of response in the decision tree analysis, was not found to be an independent response predictor in the standard multivariate analysis. This indicates a unique feature of the decision tree analysis; i.e., it could identify significant predictors that specifically apply to selected patients, in this case patients younger than 50 years old.

Relationships between decision tree model and stage of fibrosis or HCV RNA load

Liver biopsy was performed in 664 patients. The distribution of fibrosis in three probability groups differed significantly. Advanced fibrosis (F3 or F4) was higher in the low probability group (39%) compared to the intermediate probability group (13%) ($p < 0.0001$) and to the high probability group (6%) ($p < 0.0001$). Advanced fibrosis was also higher in the intermediate group compared to the high probability group ($p = 0.01$). AFP was significantly associated with liver fibrosis stage: medians of AFP levels were 4.9, 5.9, 13.0 and 18.6 for F1, F2, F3 and F4, respectively ($p < 0.0001$, Spearman's rank correlations). Lower platelet counts correlated with advanced fibrosis stages (data not shown). The SVR rate was higher in the high probability group compared to the intermediate or low probability group after stratification by HCV RNA load. Among patients with low HCVRNA load (<400,000 IU/ml), the rate of SVR was 93, 59 and 50% for the high, intermediate and low probability group, respectively ($p = 0.002$ for high vs. intermediate and $p < 0.001$ for high vs. low probability groups). Among patients with a high HCVRNA load (≥400,000 IU/ml), the rate of SVR was 73, 42 and 21% for the high, intermediate and low probability group, respectively ($p < 0.001$ for high vs. low, high vs. intermediate and intermediate vs. low probability groups).

Discussion

Currently, the combination of PEG-IFN and RBV is the recommended therapy for chronic HCV infection. The rate of SVR with 48 weeks of therapy is around 50% in patients with HCV genotype 1b and a high HCV RNA titer [2, 3]. To date, the virological response during therapy is the most reliable means for predicting the likelihood of SVR [2, 24, 25]. More potent therapy, such as a triple combination of protease inhibitor, PEG-IFN and RBV, is being evaluated in clinical trials but is not readily available [26, 27]. Under the circumstances, pre-treatment prediction of the likelihood of SVR may be useful for both patients and physicians to support clinical decisions as to whether to start PEG-IFN/RBV therapy or delay treatment until a new more effective therapy becomes available.

Table 2 Comparison of pre-treatment factors between patients with and without sustained virological response (SVR) among the model building dataset ($n = 506$)

	SVR ($n = 240$)	Non-SVR ($n = 266$)	p
Age (years)	54 (25–75)	60 (36–73)	<0.0001
Male gender[a]	151/240 (63%)	171/266 (41%)	<0.0001
Body mass index (kg/m^2)	22.5 (16.8–32.0)	22.6 (15.5–33.3)	0.244
Albumin (g/dl)	4.1 (3.2–5.0)	4 (2.7–4.9)	0.004
Creatinine (mg/dl)	0.7 (0.44–1.14)	0.69 (0.39–1.47)	<0.0001
AST (IU/l)	59 (11–370)	61 (17–261)	0.457
ALT (IU/l)	58 (11–413)	53 (11–316)	0.031
GGT (IU/l)	31 (10–322)	43 (12–328)	0.005
Total cholesterol (mg/dl)	175 (87–297)	171 (73–274)	0.184
Triglyceride (mg/dl)	105 (36–474)	105 (33–294)	0.992
White blood cell count (/μl)	4,600 (2,200–10,900)	4,425 (1,800–10,810)	0.479
Neutrophils (/μl)	2,507 (667–7,870)	2,423 (900–7,281)	0.321
Red blood cell count (/μl)	455 (336–577)	441 (313–564)	0.001
Hemoglobin (g/dl)	14.3 (10.2–17.6)	13.9 (9.4–17.9)	0.004
Hematocrit (%)	42.1 (13.3–53.7)	41.2 (30.7–52.0)	0.031
Platelets (10^9/l)	178 (81–380)	142 (60–320)	<0.0001
AFP (ng/ml)	4.3 (0.9–680)	6.4 (1.9–468)	0.041
HCVRNA (10^3 IU/ml)	1,400 (100–5,100)	1,700 (100–5,100)	0.659
Fibrosis stage: F3–4[a]	21/198 (11%)	52/219 (24%)	<0.0001

Data expressed as median (range) unless otherwise indicated

AST aspartate aminotransferase, *ALT* alanine aminotransferase, *GGT* gamma-glutamyltransferase, *AFP* alpha-fetoprotein

[a] Data expressed as number/available data (percentage)

Table 3 Multivariate logistic regression analysis for factors associated with sustained virological response (SVR)

	Odds	95% CI	p value
Age (years)	0.96	0.94–0.98	0.001
Platelets (10^9/l)	1.09	1.04–1.14	<0.0001
ALT (IU/l)	1.01	1.00–1.01	0.001
GGT (IU/l)	0.99	0.98–0.99	<0.0001
Male gender	2.92	1.87–4.55	<0.0001

GGT gamma-glutamyltransferase

Using the data mining analysis, we constructed a simple decision tree model for the pre-treatment prediction of response to PEG-IFN/RBV. The analysis highlighted 5 variables relevant to response: age, gender, platelet count, AFP and GGT. Classification based on these variables identified subgroups of patients with high probabilities of achieving SVR among difficult to treat genotype 1b chronic hepatitis C patients. The reproducibility of the model was confirmed by the independent internal and external validation datasets. An advantage of the decision tree analysis over traditional regression models is that the decision tree model is user-intuitive and can be readily interpreted by medical professionals without any specific knowledge of statistics. Patients can be allocated to specific subgroups with a defined rate of response simply by following the flow-chart form. Using this model, an estimate of the response before treatment can be rapidly obtained, which may facilitate clinical decision making. Thus, this model could be readily applicable to clinical practice.

According to the results of the decision tree analysis, patients were categorized into 3 groups: the rate of SVR was 23–30% for the low probability group, 44–45% for the intermediate probability group and 65–76% for the high probability group. About 30% of patients were each categorized in the high and low probability group and the remaining 40% of patients in the intermediate probability group. These results support the evidence-based approach for selecting an optimum treatment strategy for individual patients. For example, patients in the high probability group may be the most suitable candidates for PEG-IFN/RBV therapy, while patients in the low probability group may be advised to wait for a future therapy, such as the combination of protease inhibitor, PEG-IFN and RBV. However, the estimation of low probability should not be used to preclude patients from therapy, and the final decision should be made on a case-by-case basis, taking into consideration the acceptance by the patient of a low likelihood of response and the potential risk of disease progression while waiting for a future therapy.

Another important finding was that poor adherence to drugs lowered the rate of SVR in the low and intermediate probability groups, which implies that effort should be made to maintain ≥80% of the planned dose of PEG-IFN and RBV in those patients. On the other hand, the rate of SVR was high irrespective of drug adherence in the high probability group. Whether shorter duration of therapy is sufficient in this group of patients should be confirmed in future study.

The variables used in the decision tree have been previously reported to associate with the efficacy of IFN therapy. Younger age and male gender are associated with a favorable response [28]. Lower platelet count is a hallmark of advanced fibrosis in chronic hepatitis C and is reported to be associated with poor response to IFN [29]. AFP is usually used for the screening or the diagnosis of hepatocellular carcinoma, but recent studies suggest an association between higher AFP levels and poor response to IFN therapy [30–33]. Previous report speculated that higher expression of AFP by hepatic progenitor cells may be associated with non-response to therapy [30]. Another report speculated that AFP levels predict poor response to therapy through the underlining link to advanced liver fibrosis [31]. Our data support the latter speculation since advanced fibrosis was associated with elevation of AFP levels. Fibrosis of the liver is an important predictor of response, but we did not include this factor in the decision tree analysis since liver biopsy may not always be available in general practice. As a result, two predictive factors that correlate with fibrosis stage (platelet counts and AFP) were selected in the model, and three probability groups reflected the different distribution of fibrosis stage. GGT is reported to be associated with insulin resistance and hepatic steatosis [34–37], a factor that confers resistance to IFN therapy [38–44]. What is unique to the present study is the visualization of response probability by combining these factors and its high reproducibility revealed by a high-quality validation of the model by internal and external validation datasets that were completely independent of the model building dataset. Since factors used in the model were clinical parameters that are readily available by the usual workup of patients, this model could be immediately applicable to clinical practice without imposing costs for additional examinations.

A potential limitation of this study is that data mining analysis has an intrinsic risk of showing relationships that fit to the original dataset but are not reproducible in different populations. Although internal and external validations showed that our model had high reproducibility, we recognize that further validation on a larger external validation cohort, especially in populations other than Japanese, may be necessary to further verify the reliability of our model.

In conclusion, we built a pre-treatment model for the prediction of virological response to PEG-IFN/RBV. Because this decision tree model was made up of simple variables, it can be easily applied to clinical practice. This model may have the potential to support decisions about patient selection for PEG-IFN/RBV based on a possibility of response weighed against the potential risk of adverse events or costs.

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Authors' Contribution:
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B Data Collection
C Statistical Analysis
D Data Interpretation
E Manuscript Preparation
F Literature Search
G Funds Collection

The incidence of hepatocellular carcinoma associated with hepatitis C infection decreased in Kyushu area

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Summary

Background: The incidence of hepatocellular carcinoma (HCC) in Japan has still been increasing. The aim of the present study was to analyze the epidemiological trend of HCC in the western area of Japan, Kyushu.

Material/Methods: A total of 10,010 patients with HCC diagnosed between 1996 and 2008 in the Liver Cancer study group of Kyushu (LCSK), were recruited for this study. Cohorts of patients with HCC were categorized into five year intervals. The etiology of HCC was categorized to four groups as follows; B: HBsAg positive, HCV-RNA negative, C: HCV-RNA positive, HBsAg negative, B+C: both of HBsAg and HCV-RNA positive, non-BC: both of HBsAg and HCV-RNA negative.

Results: B was 14.8% (1,485 of 10,010), whereas 68.1% (6,819 of 10,010) had C, and 1.4% (140 of 10,010) had HCC associated with both viruses. The remaining 1,566 patients (15.6%) did not associate with both viruses. Cohorts of patients with HCC were divided into six-year intervals (1996–2001 and 2002–2007). The ratio of C cases decreased from 73.1% in 1996–2001 to 64.9% in 2002-2007. On the other hand, B and -nonBC cases increased significantly from 13.9% and 11.3% in 1996-2001 to 16.2% and 17.6% in 2002-2007, respectively.

Conclusions: The incidence of hepatocellular carcinoma associated with hepatitis C infection decreased after 2001 in Kyushu area. This change was due to the increase in the number and proportion of the HCC not only nonBC patients but also B patients.

key words: hepatitis virus • hepatocellular carcinoma • Japan

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Background

The three leading causes of death in Japan are malignancy neoplasms, cardiovascular diseases, and cerebrovascular diseases. Since 1981, malignant neoplasms have been the leading cause of death in Japan. For the last 30 years, liver cancer has been the third leading cause of death from malignant neoplasms in men. In women, liver cancer has ranked fifth during the past decade [1]. Hepatocellular carcinoma (HCC) accounts for 85% to 90% of primary liver cancers[2] and the age-adjusted HCC mortality rate has increased in recent decades in Japan [3]. Similarly, a trend of increasing rates of HCC has been reported from several developed countries in North America, Europe and Asia [4,5]. HCC often develops in patients with liver cirrhosis caused by hepatitis B virus (HBV), hepatitis C virus (HCV), excessive alcohol consumption, or nonalcoholic fatty liver disease. Of the hepatitis viruses which cause HCC, HCV is predominant in Japan [6–9].

Although the age-adjusted incidence of HCC has increased in Japan, sequential changes in etiology of HCC patients between 2001 and 2008 are not fully understood [10]. To clarify factors affecting epidemiological changes in Japanese HCC patients, especially the recent trend of HCC, we analyzed the epidemiological trend of HCC in the western area of Japan, Kyushu area.

Material and Methods

Patients

A total of 10,010 patients with HCC diagnosed between 1996 and 2008 in the Liver Cancer study group of Kyushu (LCSK), were recruited for this study. The diagnosis of HCC was based on AFP levels and imaging techniques including ultrasonography (USG), computerized tomography (CT), magnetic resonance imaging (MRI), hepatic angiography (HAG), and/or tumor biopsy. The diagnostic criteria for HCC were either a confirmative tumor biopsy or elevated AFP (>20 ng/mL) and neovascularization in HAG and/or CT.

Etiology of HCC

A diagnosis of chronic HCV infection was based on the presence of HCV-RNA detected by polymerase chain reaction (PCR), whereas diagnosis of chronic HBV infection was based on the presence of hepatitis B surface antigen (HBsAg). The etiology of HCC was categorized to four groups as follows; **B:** HBsAg positive, HCV-RNA negative, **C:** HCV-RNA positive, HBsAg negative, **B+C:** both of HBsAg and HCV-RNA positive, **nonBC:** both of HBsAg and HCV-RNA negative.

Statistical analysis

The data were analyzed by the Mann-Whitney test for the continuous ordinal data, the x2 test with Yates' correction and the Fisher exact test for the association between two qualitative variables. The standard deviation was calculated based on the binomial model for the response proportion. $P<0.05$ was considered statistically significant.

Results

Clinical features of the studied patients

A total of 10,010 patients with HCC were diagnosed at our study group from 1996 to 2008. Table 1 show that the proportion of patients diagnosed with **B** was 14.8% (1,485 of 10,010), whereas 68.1% (6,819 of 10,010) had **C**, and an additional 1.4% (140 of 10,010) had HCC associated with both viruses. The remaining 1,566 patients (15.6%) did not associate with both viruses. In analysis of patients in HCC by category, the median age of patients at diagnosis of **B** was 57 years old significant younger than other types HCC(**C: 69, nonBC: 70,.B+C 65 years old).**

As shown in Figures 1 and 2, the number and ratio of **B** cases remained unchanged from1996 to 2001 and thereafter increased and plateaued, whereas **C** rapidly increased from 1996 to 2000 and thereafter decreased and plateaued. In addition, the number and ratio of the **nonBC** cases has increased continued gradually and continued in this study period.

Change of etiology in patients with HCC during the period 1996–2007 with 6-years intervals

Cohorts of patients with HCC were divided into six-year intervals (1996–2001 and 2002–2007). Table 2 show that the incident rate of **C** decreased significantly from 73.1% in 1996-2001 to 64.9% in 2002-2007 (1996–2001 vs. 2002–2007, $p=<0.001$). On the other hand, the incident rate of **B** and **-nonBC** increased significantly from 13.9% and 11.3% in 1996-2001 to 16.2% and 17.6% in 2002-2007, respectively. Not only the incident rate but also number of **B** and **-nonBC** became larger in same 6 years periods.

Table 3 shows that male/female ratio of **C** and **-nonBC** decreased significantly from 2.2 and 4.0 in 1996-2001 to 1.8 and 2.7 in 2002-2007, respectively ($p<0.001$). The ratio became clearly smaller, indicates an increase in female patients with **C** and **nonBC.** On the other hand, the male/female ratio of **B** patients did not significantly change during the period. The median age at diagnosis of **B, C,** and **nonBC** in six-year intervals were significant increase from 56 to 58, from 67 to 71 and from 68 to 71 years of age during the period.

Discussion

Our study was the twenty-three major liver center-based study designed to examine the sequential change in the background of HCC patients during the past 13 years, 1996–2008. More than 80% of our patients had chronic HBV or HCV infections. During this observation period, the number and proportion of HCC-C reached a peak in 2000 and thereafter decreased and became stabilized. Previous studies from Japan reported that the proportion of the HCC patients with HCV infection had been increased and reached a plateau in the period of 1981–2001 [1,3,10–12]. However, in our study, the number and proportion of the HCC patients with HCV infection cases decreased in 2001–2008. The reason may be explained as follows; interferon therapy for chronic hepatitis C may have been associated with a decreased incidence of HCC [13–17]. Oral supplementation with a oral branched-chain amino acids has been useful in the prevention HCC [18]. Finally, the chronically HCV-infected

Table 1. The characteristic of HCC patients during the period of 1996–2008.

Age (y.o.)	B		C		nonB		B+C		Total
	Male	Female	Male	Female	Male	Female	Male	Female	
0–	1	0	0	1	0	0	0	0	2
10–	4	1	0	0	0	2	0	0	7
20–	6	2	1	0	1	1	0	0	11
30–	31	5	4	0	11	3	2	0	56
40–	204	22	130	12	32	15	12	0	427
50–	507	66	728	145	167	32	31	6	1,682
60–	287	118	1836	741	411	102	35	13	3,543
70–	140	64	1775	947	483	133	22	14	3,578
80–	9	18	271	214	97	65	1	4	679
90–	0	0	9	5	9	2	0	0	58
Total	**1,189**	**296**	**4,754**	**2,065**	**1,211**	**355**	**103**	**37**	10,010
	1,485 (4.8%)		6,819 (68.1%)		1,566 (15.6%)		140 (1.4%)		
Median	57	63	67	70	68	70	61	68	67
	57		69		70		65		
Mean	56	64	68	71	69	71	62	68	67
	58		68		68		63		
Range	1–87	14–89	27–94	0–93	28–96	17–90	36–82	55–82	0–96
	1–89		0–94		17–96		36–82		

Age: B vs. C $p \leq 0.001$; B vs. B+C $p \leq 0.001$; B vs. nonBC $p \leq 0.001$; C vs. BC $p \leq 0.001$; C vs. nonBC $p = 0.043$; BC vs. nonB+C $p \leq 0.001$. IQR – interquartile range; SD – standard deviation.

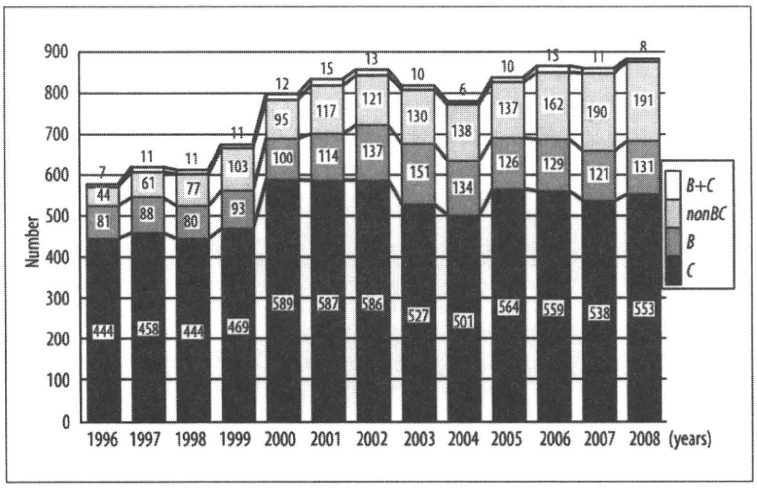

Figure 1. Sequential changes in the number of HCC patients categorized by etiology during the period 1996–2008.

population is aging in Japan. Yoshizawa et al. reported that age-specific prevalence for the presence of HCVAb among ~300,000 voluntary blood donors from Hiroshima in 1999 clearly increased with the age, reaching the highest proportion of 7% in individuals who were more than 70 years old [10,19]. In this study, the median age of the HCC patients with HCV infection steadily increased from 67 to 71 years of age during the studied period. In a word, HCV infected people become older with years in Japan and they were regarded as a high risk for HCC.

The prevalence rate of HBV in Kyushu area has been reported to be higher than other area in Japan [1]. In Kyushu area, 95% of patients with chronic HBV infection had HBV genotype C except for Okinawa [20]. HBV genotype C is thought to be associated with higher incidence of HCC

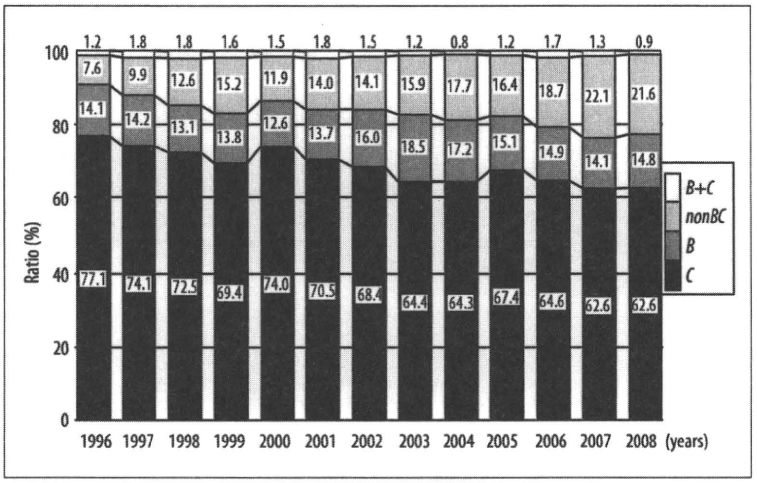

Figure 2. Sequential changes in the ratio of HCC patients categorized by etiology during the period 1996–2008.

Table 2. Change of etiology in patients with HCC during the period 1996–2007 with 6-years intervals.

Period		1996–2001	2002–2007	P value
Number		3,023	4,173	
Sex	Male	2,162	2,849	
	Female	861	1,324	
Ratio (male/female)		2.5	2.2	0.003
Age (y.o.) (IQR)		66 (14)	69 (12)	<0.001
Hepatitis virus (%)	B	13.9	16.2	
	C	73.1	64.9	
	B+C	1.7	1.3	
	nonBC	11.3	17.6	0.001

QR – interquartile range.

Table 3. The median age and male/female ratio of HCC patients during the period of 1996–2007.

Period		1996–2001	2002–2007	P value
B				
Age (y.o.) (IQR)		56 (14)	58 (15)	0.001
Sex	Male	331	519	
	Female	88	157	
Ratio (male/female)		3.8	3.3	0.391
C				
Age (y.o.) (IQR)		67 (9)	71 (11)	<0.001
Sex	Male	1,524	1,753	
	Female	687	955	
Ratio (male/female)		2.2	1.8	0.002
nonBC				
Age (y.o.) (IQR)		68 (12)	71 (13)	<0.001
Sex	Male	273	534	
	Female	69	201	
Ratio (male/female)		4.0	2.7	0.012

QR – interquartile range.

compared with other HBV genotypes [21]. In the present study, the incident rate of HCC patients with HBV infection became larger in this study period. To explain this change, we must consider from two viewpoints. The one is that the number of patients with HCC caused by HCV infection decreased, the other is that the proportion of chronic HBV infected patients who have reached the age of developing HCC is relatively high as described below.

Nationwide health survey for HBsAg in the over 40 years of age population had been done between 2002 and 2006 in Japan. This survey reports indicated that the average HBsAg prevalence was 1.2% in the total Japanese population patients with chronic HBV infection [10] and the age-specific prevalence of HBsAg was higher in the group aged between 50 (1.4%) and 55 years (1.5%). In the HCC patients with HBV genotype C, the mean age was 55 years in Japan [20]. This overlap between age-specific prevalence and hepatocellular carcinogenic age would be associated with the increase of HCC patients with HBV infection. Nucleoside analogue reverse transcriptase inhibitor (NARTI) therapy effectively reduces the incidence of HCC in chronic hepatitis B patients [22,23]. However, Interferon therapy for chronic hepatitis C started from 1992, whereas NARTI therapy for HBV started from 2000 in Japan [24,25]. Hence, HBV associated HCC will probably decrease in Japan during the next 10 to 20 years.

The survey of HCC patients associated with nonBC infection in Japan was conducted by Inuyama Hepatitis Research Group from 1995 to 2003. The ratio of HCC patients with nonBC accounted 9.3% [1]. In the present study, the ratio of HCC patients with nonBC was 14.1%. Furthermore, the number and the proportion of HCC patients with nonBC have been gradually increasing in the periods. The current two studies account for the increase in number and proportion of HCC patients with nonBC. First, Lai et al. reported

that type 2 diabetes increases the risk of developing HCC in those who are HCV negative or have a high level of total cholesterol [26]. Second, Nakano et al. reported that epidemiological studies on diabetes mellitus revealed that the number of patients with diabetes mellitus is gradually increasing in Japan along with development of car society and westernization of food intake. Since prevalence of diabetes mellitus increases with aging, proportion of individuals with diabetes mellitus aged over 60 has exceeded two-third of estimated total number of patients (7.40 million in 2002) in Japan where aging of society is rapidly progressing [27]. In a word, the number of type 2 diabetes people is increasing in Japan and they were regarded as a high risk for HCC. Then, the number and the proportion of HCC patients with nonBC have been increased recent twelve years in Japan.

It is known that 2 to 4 decades of chronic HCV infection are required to develop cirrhosis and subsequent HCC [28–31]. The number of HCC cases has increased in Japan, because individuals infected with HCV during the past have grown old and have reached the cancer-bearing age. The prevalence of HCV infection in young Japanese individuals is low and the incidence of HCVAb is very low because of preventative actions against HCV infection such as the screening of blood products for HCV and the use of sterile medical equipment [32]. Additionally, we showed that the number and proportion of patients with HCC-C cases decreased, whereas the number and ratio of HCC-nonBC steadily increased during the studied period. These findings may be expected that the incidence of HCC patients with nonBC in Japan may continue to increase even after the consequence of the HCV epidemic level off, a country that is far advanced with regard to HCC patients with HCV infection, in the near future.

Conclusions

In summary, HCC patients had increased from 1996 to 2000 and this increase was originated from HCC patients with HCV infection. The number and proportion of HCC patients with HCV infection reached a peak in 2000 and thereafter decreased and became stabilized. The incidence of hepatocellular carcinoma associated with hepatitis C infection decreased after 2001 in Kyushu area. This change was due to the increase in the number and proportion of the HCC not only nonBC patients but also B patients.

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ORIGINAL ARTICLE

Integrated fibrosis scoring by ultrasonography predicts the occurrence of hepatocellular carcinoma in patients with chronic hepatitis C virus infection

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Abstract
Purpose This study was performed to elucidate whether evaluating the liver surface, edge, and texture by high-resolution ultrasonography is useful for predicting the occurrence of hepatocellular carcinoma (HCC) in patients with hepatitis C virus (HCV)-associated chronic liver diseases (CLDs)
Methods The integrated fibrosis stage (a comprehensive value of scores for liver edge, surface, and texture) of 337 patients with HCV-associated CLDs was evaluated, at entry, by ultrasonography (US), as a US score. The patients were followed up prospectively (mean observation period was 16.4 months; range 2.8–36.2 months) for the occurrence of HCC by US or helical CT at 3-month intervals. A total of 140 patients received interferon therapy, and the occurrence of HCC was compared between those with and without interferon therapy
Results The annual incidence of HCC was 1.1, 5.5, and 10.2% in low, middle, and high US score groups, respectively. Univariate analysis showed that age, serum levels of total bilirubin, alpha-fetoprotein (AFP), platelet count, albumin, total cholesterol, and the US score were associated with HCC occurrence in the patients. A multivariate proportional hazard model revealed that only the middle and high US scores ($p = 0.0922$, hazard ratio 4.006, 95% CI 0.796–20.153 and $p = 0.008$, hazard ratio 7.991, 95% CI 1.721–37.10, respectively) and elevated AFP ($p = 0.031$, hazard ratio 2.774, CI 1.097–7.014) were independently associated with HCC occurrence. Our US scoring based on evaluation of the liver surface, edge, and texture was clearly and strongly associated with the occurrence of HCC in patients with HCV-associated CLDs, and with the higher occurrence rate of HCC in patients with higher US scores
Conclusion Thus, US is a good tool for evaluating the fibrosis stage of the liver, and may therefore be useful in designing an optimum follow-up interval for each patient with HCV-associated CLD.

Keywords Hepatocellular carcinoma · Hepatitis C virus · Fibrosis · High and low-frequency probe · US score

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Introduction

More than 95% of hepatocellular carcinoma (HCC) cases in Japan are associated with chronic liver diseases (CLDs).

Moreover, most cases of CLD in Japan are related to hepatitis viruses, and, importantly, 70–80% of these are associated with HCV. CLD is classified into five fibrosis stages, i.e., F0, F1, F2, F3, and F4. It has been reported that the incidence of HCC correlates with such stages, i.e., the higher the fibrosis stage, the higher the incidence of HCC [1–5]. Accordingly, a precise assessment of the fibrosis stage of CLDs would provide a means of evaluation of the risk of HCC occurrence. The pathological examination of specimens obtained by liver biopsy has been the gold standard of staging in CLD. However, liver biopsy is an invasive technique that may lead to a number of adverse events, for example peritoneal bleeding. Therefore, liver biopsy is not usually performed on an OPD basis. Furthermore, a biopsy cannot be performed in cirrhotic cases, because of the high risk of massive bleeding.

The recently introduced transient elastography, which is used for measurement of liver stiffness, is a promising tool in clinical practice. Transient elastography, however, is not suitable for evaluating intermediate stages of fibrosis. On the other hand, ultrasonography (US) is an indispensable imaging modality for liver diseases in clinical practice. Currently, a number of methods for evaluation of chronic hepatitis or cirrhosis have been reported [6–10]. However, no methods are objective or effective enough to make a precise staging diagnosis of CLDs. In a previous study, we analyzed the liver stage by evaluating the liver edge, liver surface, and liver parenchymal texture using a high-quality US device [11]. The combined values of the three factors were referred to as the US score, which showed a high correlation with hepatic fibrosis evaluated by liver biopsy. In this study, we prospectively assessed the risk of HCC occurrence in HCV-associated CLDs utilizing the US score.

Patients and methods

Three hundred and thirty-seven HCV-related CLD patients who underwent ultrasonographic examination at the National NHO Nagasaki Medical Center between 2002 and 2006 were included in this study. Patients consisted of 142 males and 195 females with an average age of 63.2 years. The mean observation period was 16.4 months (range 2–36.2 months). Patient characteristics are shown in Table 1.

A total of 140 patients received interferon (IFN) treatment. A sustained virological response (SVR) was defined as negative for HCV RNA at 6 months after the end of treatment. Forty-one patients (29.3%) showed an SVR and 99 (70.7%) did not (non-SVR).

The patients were followed-up every 3 months, and detection of a newly developed nodule(s) in the liver which was subsequently confirmed as HCC was the end point of the study.

Table 1 Patients' characteristics

	Mean ± SD
Age (years)	63.2 ± 9.63
Male/female	142/195
Total bilirubin (mg/dl)	0.9 ± 0.46
AST (IU/L)	57.8 ± 37.2
ALT (IU/L)	60.8 ± 47.6
AFP (ng/ml)	16.2 ± 31.0
PLT (10^4/mm^3)	14.4 ± 0.61
Albumin (g/dl)	4.1 ± 0.60
Total cholesterol (mg/dl)	173.5 ± 36.2
Genotype (1, 2, unknown)	(118, 31, 188)
US score low/middle/high	131/90/116

US system

The patients were studied ultrasonically using a real-time apparatus (HDI 5000 Sono CT; Philips, USA) with a 2–5 MHz convex array transducer C5-2 (low frequency probe) and a 5–12 MHz convex array transducer L12-5 (high frequency probe).

US findings and the scoring system

US scores were determined using the method proposed by Nishiura et al. [11]. Briefly, liver edge, liver surface, and liver parenchymal texture, were graded as follows:

1. liver edge: score 0 for sharp (Fig. 1a), score 1 for mildly blunted (Fig. 1b), score 2 for blunted (Fig. 1c);
2. liver surface: score 0 for smooth (Fig. 2a), score 1 for mildly irregular (Fig. 2b), score 2 for irregular (Fig. 2c), score 3 for highly irregular (Fig. 2d); and
3. liver parenchymal texture: score 0 for fine (Fig. 3a); score 1 for mildly coarse (Fig. 3b), score 2 for coarse (Fig. 3c), score 3 for highly coarse (Fig. 3d).

The total score of each was determined separately for the right and left lobes, and the mean value was regarded as the US score. The US examiners (TN, HW) were both certified by the Japan Society of Ultrasonics in Medicine and were unaware of the clinical details of the patients.

Diagnosis of hepatocellular carcinoma

Diagnosis of HCC was made using the typical enhancement pattern by contrast-enhanced CT and/or by histological analysis of specimens obtained from hepatic tumor biopsy.

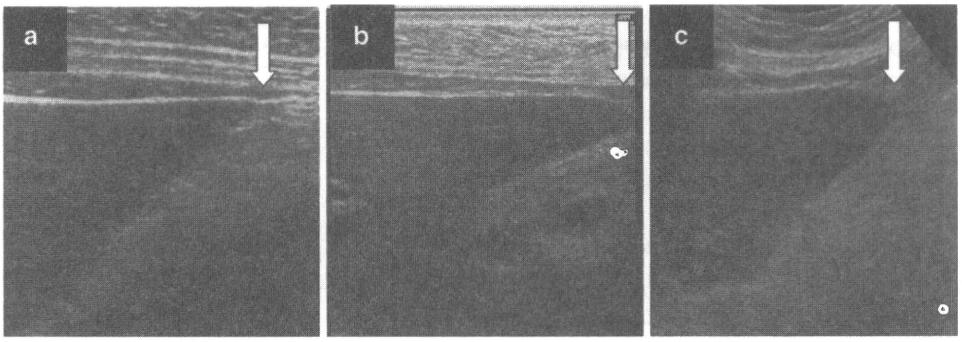

Fig. 1 Results for the ultrasonographic features of the liver edge; **a** sharp edge with a high-frequency probe, **b** mildly blunted edge with a high-frequency probe, and **c** blunted edge with a low-frequency probe

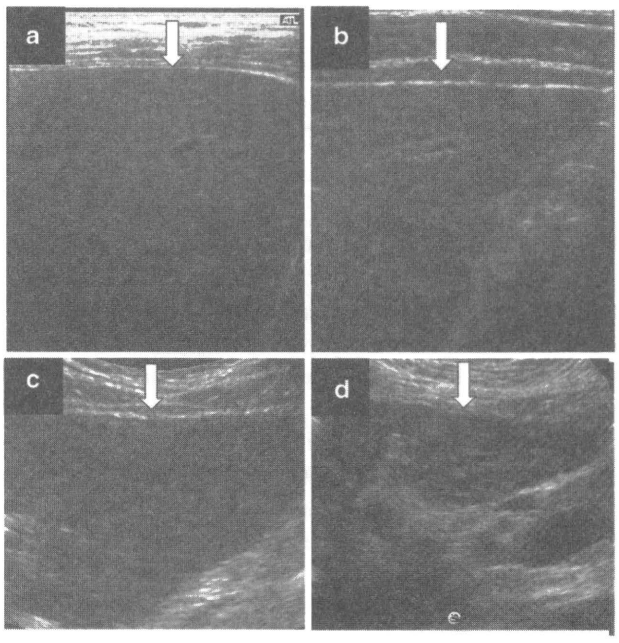

Fig. 2 Results for the ultrasonographic features of the liver surface; **a** a smooth surface with a high-frequency probe, **b** a mildly irregular surface with a high-frequency probe, **c** an irregular surface with a low-frequency probe, and **d** a highly irregular surface with a low-frequency probe

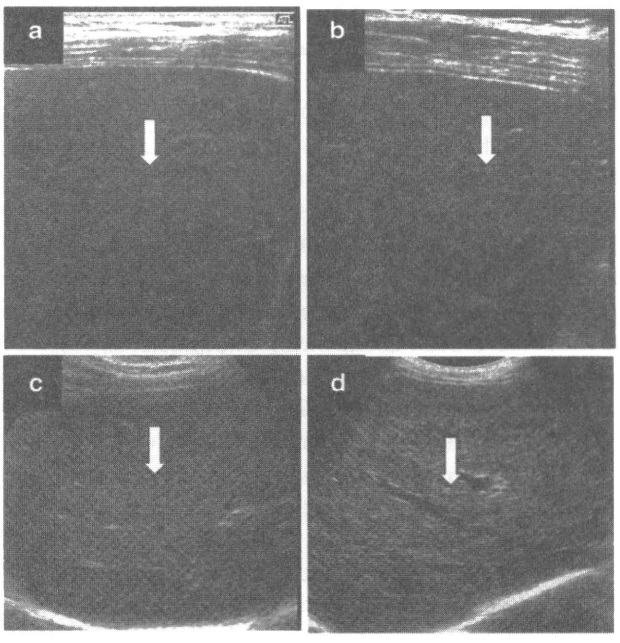

Fig. 3 Scores for the ultrasonographic features of the liver parenchymal texture; **a** fine parenchymal texture with a high-frequency probe, **b** a mildly coarse parenchymal texture with a high-frequency probe, **c** a coarse parenchymal texture with a low-frequency probe, and **d** a highly coarse parenchymal texture with a low-frequency probe

Statistical analysis

The patients were categorized by US score. The cumulative occurrence of HCC was described using the Kaplan–Meier method. Univariate statistical difference between the categories was tested by log rank test, Student's *t* test, and chi-squared analysis, where appropriate. Cox's multivariate promotional hazard model was used to determine independent factors for HCC occurrence.

Results

Occurrence of hepatocellular carcinoma

During follow-up, HCC developed in 32 patients (9.5%). The mean age of patients in whom HCC was detected was 66 years (range 50–80 years), and that in patients in whom HCC was not detected was 63 years (range 30–88 years).

US score and HCC occurrence

Observed patient US scores varied from 0.0 to 8.0. The number of patients with a score of 3.5 or less, 4.0, 4.5, 5.0, 5.5, 6.0, 6.5, 7.0, 7.5, and 8.0 at baseline was 92, 39, 27, 41, 22, 50, 13, 30, 5, and 18, respectively. No HCC was observed in patients with a score of 3.5 or less during the period. The number of patients in whom HCC was found was 2 (5.1%), 0 (0%), 3 (7.3%), 3 (13.6%), 9 (18.0%), 3 (23.1%), 6 (20.0%), 1 (20.0%), and 5 (27.8%) for patients with US scores of 4.0, 4.5, 5.0, 5.5, 6.0, 6.5, 7.0, 7.5, and 8.0, respectively.

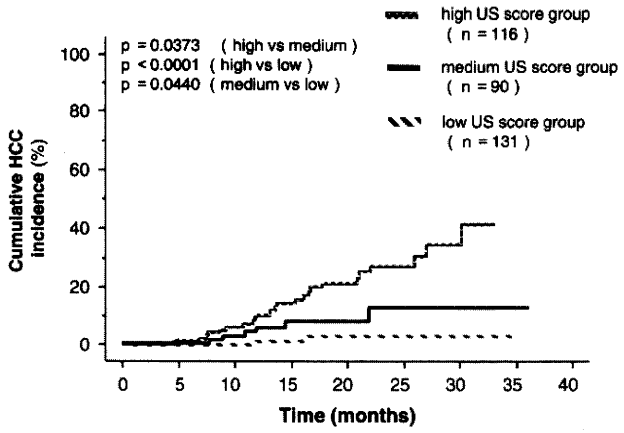

Fig. 4 Cumulative HCC occurrence by integrated US score

US score as a predictive indicator of HCC development

To examine whether the US score has predictive power for HCC development, we subclassified the US score into three groups: low (4.0 or below), middle (>4.0 and <6.0), and high (6.0 or above). Groups with low, middle, and high US scores consisted of 131, 90, and 116 patients, respectively, and HCC was detected in 2 (1.5%), 6 (6.7%), and 24 (20.7%) patients in the respective groups.

The diameter of HCC was 24 mm on average with a range of 16–32 mm in the low score group, 17 mm (8–36 mm) in the middle score group, and 15 mm (8–24 mm) in the high score group. A solitary HCC was found in one case in the low score group, five cases in the middle score group, and 20 cases in the high score group. Multiple HCCs were found in one case in the low score group, one case in the middle score group, and four cases in the high score group.

Figure 4 shows the cumulative occurrence of HCC by US score group. The annual HCC occurrence rates were 1.1, 5.5, and 10.2% in the low, middle, and high US score groups, respectively. The annual rate of HCC occurrence was significantly higher in the high US score group than in the middle US score group ($p < 0.0001$). The difference between the middle US score group and the low US score group was also significant ($p = 0.0373$).

Table 2 shows the patient demographic data according to the occurrence of HCC. Univariate analyses revealed that age, serum levels of total bilirubin, alpha-feto protein (AFP), platelet count, albumin, total cholesterol, and the US score were associated with HCC occurrence. A multivariate proportional hazard model revealed that only the middle and high US scores ($p = 0.0922$, hazard ratio 4.006, 95% CI 0.796–20.153 and $p = 0.008$, hazard ratio 7.991, 95% CI 1.721–37.10, respectively) and elevated AFP ($p = 0.031$, hazard ratio 2.774, CI 1.097–7.014) were independently associated with HCC occurrence.

Table 2 Factors associated with HCC occurrence on the basis of monovariate analysis

	HCC (−)	HCC (+)	*P* value
Age (years)	62.9 ± 9.8	66.8 ± 7.5	0.029[a]
Male/female	131/174	11/21	0.874[b]
Total bilirubin (mg/dl)	0.9 ± 0.4	1.2 ± 0.6	0.001[a]
ALT (IU/L)	59.6 ± 47.6	72.0 ± 46.8	0.163[a]
AFP (ng/ml)	14.4 ± 29.0	33.2 ± 43.3	0.001[a]
PLT (10^4/mm^3)	14.9 ± 0.61	10.6 ± 0.38	0.001[a]
Albumin (g/dl)	4.2 ± 0.6	3.7 ± 0.6	<0.001[a]
Total cholesterol (mg/dl)	174.9 ± 36.6	159.7 ± 29.3	0.023[a]
HCV genotype (1, 2, unknown)	(109, 29, 167)	(9, 2, 21)	0.81[b]
US score low/middle/high	129/84/92	2/6/24	<0.001[b]

[a] Student's *t* test

[b] Chi-squared test

HCC incidence in IFN-untreated patients

One hundred and ninety-seven patients did not receive IFN treatment during the study period. Among such patients, the number of low, middle, and high US scores was 80, 48, and 69, respectively. HCC was found in 2 (2.5%), 5 (10.4%), and 15 (21.7%) patients in the respective groups. The annual incidence of HCC was significantly higher in the high US score group than in the low US score group ($p = 0.0023$). The differences between low and middle US score groups, and between middle and high US score groups, were not significant ($p = 0.0628$ and $p = 0.3281$, respectively) (Table 3).

HCC incidence in IFN-treated patients

One-hundred and forty patients received IFN treatment during the study period. The number of patients with low, middle, and high US scores was 51, 42, and 47, respectively. HCC was found in 0 (0%), 1 (2.3%), and 9 (19.1%) patients in the respective groups. In the SVR group ($n = 41$), the number of patients with low, middle, and high US scores was 22 (43.2%), 12 (28.6%), and 7 (14.9%), respectively. In the non-SVR group ($n = 99$), the number of patients with low, middle, and high US scores was 29 (56.8%), 30 (71.4%), and 40 (85.1%), respectively. HCC was found in none of the SVR patients whereas in the non-SVR group HCC was found in 0 (0%), 1 (3.3%), and 9 (22.5%) patients in the respective groups during the observation period.

Discussion

A variety of risk factors for the development of HCC in HCV-associated CLDs have been reported. Fibrosis is

Table 3 Multivariate analysis for factors independently associated with HCC occurrence

	Hazard ratio	95% CI	*P* value
Age (years)			
<65	1	0.627–2.724	0.475
≥65	1.307		
Sex			
Female	1	0.396–1.900	0.721
Male	0.867		
Total bilirubin (mg/dl)			
<1.0	1	0.505–2.286	0.853
≥1.0	1.074		
ALT (IU/L)			
<48	1	0.347–1.915	0.639
≥48	0.815		
AFP (ng/ml)			
<30	1	1.097–7.014	0.031*
≥30	2.774		
PLT ($\times 10^4$/mm^3)			
≥13	1	0.438–2.443	0.938
<13	1.035		
Albumin (g/dl)			
≥3.6	1	0.678–3.703	0.289
<3.6	1.584		
Total cholesterol (mg/dl)			
≥170	1	0.552–2.714	0.619
<170	1.224		
US score			
<4.0	1		
≥4.0, <6.0	4.006	0.796–20.153	0.0922*
≥6.0	7.991	1.721–37.10	0.008**

considered to be an important risk factor [12, 13], and many lines of evidence indicate that the incidence of HCC is different depending on the fibrosis stage defined by histological examination.

A histological examination has traditionally been the gold standard for defining the histological stage. A liver biopsy, however, is an invasive modality that is normally performed on an inpatient basis. Moreover, there is also a risk of sampling error. A liver biopsy specimen represents a very limited area of the liver, and two distinct samples from a single patient may sometimes show different results. US, on the other hand, yields a general picture of the liver and may have an advantage in the assessment of diffuse hepatic change, which is often seen in chronic HCV infection.

This study shows that the proposed US score is a useful tool for predicting the occurrence of HCC in patients with HCV. The annual incidence of HCC in the patients in this study was 1.1, 5.5, and 10.2% in the low, middle, and high US score groups, respectively. We further assessed which of liver edge, liver surface, and liver parenchymal texture correlated with the occurrence of HCC. None of these either strongly or independently correlated with HCC occurrence in comparison with the others, thus indicating that a comprehensive assessment of these is important for predicting the occurrence of HCC (data not shown). The low US score group consisted of patients with a larger HCC diameter and fewer solitary HCC compared with the medium and high US score groups. It seems to be difficult, however, to evaluate the significance of this observation because the sample size of the low US score group is too small.

In a previous study, we reported that the US score strongly correlated with histological findings [11]. The sensitivity, specificity, and positive likelihood ratio of the early stage of fibrosis (F0–F2) were 91, 94%, and 15.95, respectively. We also showed that the US score was powerful in distinguishing advanced chronic hepatitis (F3) from cirrhosis (F4). A US score of 6.0 or above suggested the presence of liver cirrhosis (F4) with a sensitivity, specificity, and positive likelihood ratio of 98, 91%, and 11.05, respectively [14]. Based on these results, it seems reasonable to classify the US score in the low, middle, and high groups as we did in the study. Several studies from Japan have revealed that the annual incidence of HCC was 0.5, 2, 5, and 8% in F1, F2, F3, and F4, respectively [5]. In the current study, the highest US score (6.0 or above) group showed a higher risk of HCC than that of the histological stage F4, indicating that the group consisted of a selected group of patients with an exceedingly high risk of HCC. In addition, the incidence of HCC was 18.0% (9/50) and 22.7% (11/66) in patients with a score of 6.0 and 6.5 or above, respectively, and it was dramatically high, i.e., 27.8% (5/18), in patients with a US score of 8.0.

These results suggest that even in the same F4 stage, there are several subcategories with different risks of HCC occurrence. Such a difference cannot be evaluated by liver biopsy because liver cirrhosis (F4) is a single and final category of CLDs and, more importantly, because such an invasive examination is contraindicated in advanced cirrhosis. To the best of our knowledge, there been no report of the potential risk of HCC occurrence in liver cirrhosis evaluated by liver biopsy.

Taken together, the US score may have an advantage over liver biopsy in predicting HCC, especially for advanced CLDs including liver cirrhosis. Many studies have reported independent risk factors of HCC in HCV-associated CLDs. Such factors include fibrosis, alcohol intake, age, sex, history of blood transfusion, the platelet count, and biochemical data [1, 3, 15, 16]. The fibrosis stage is an important risk factor, undoubtedly; however, in the current study the US score seemed to be associated

more strongly with the occurrence of HCC. The advantage of the US system we utilized is highlighted by its high resolution, which enables us to make a detailed observation of the parenchymal texture and to obtain a gross overview including both the right and left lobes. Although the US score correlates well with the fibrosis stage [11], this score may also be independently associated with HCC occurrence, which needs to be clarified in an extended study with a larger number of patients.

It has been reported that IFN treatment suppresses the occurrence of HCC in chronic hepatitis C [17]. In accordance with previous studies, patients treated with IFN showed a lower incidence of HCC, which was significant in the low and middle US score groups but not in the high US score group, suggesting initiation of carcinogenesis before INF treatment in the high US score group. In the IFN-untreated group, there was no statistical difference among low, middle, and high US score groups, most likely because of the limited number of patients.

The US score may vary depending on the quality of the US device and examiners. We have not assessed whether a different device produces different US scores; however, the utilization of both low and high-frequency probes enables more objective evaluation and minimizes inter-examiner variations [11]. We believe that a highly reliable and reproducible US score can be obtained when the examination is completed by a single examiner with a single device. US examination is noninvasive and does not require as much time as a liver biopsy and the subsequent histological examination. We propose standardization of such a scoring system, so that studies in a large number of patients with CLDs can be completed. This may lead to a more precise prediction of HCC occurrence and more appropriate management of patients with HCV-associated liver diseases. Furthermore, it would be valuable to follow up the US score over time to elucidate whether the progression speed assessed by US is associated with the incidence of HCC.

Recent advances in US technology are remarkable. Owing to improvements in the spatial resolution and digitization of the US signal, more detailed and vivid visualization of parenchymal texture or liver surface is now possible. High-frequency probes have enabled objective assessment of liver surface and texture. US examination using the US score would have advantages not only for patients for whom liver biopsy is contraindicated but also for patients who require longitudinal follow-up. Furthermore, it may be possible to identify an optimum follow-up interval in each patient with CLD according to the risk of HCC occurrence on an OPD basis. As technology advances and this type of study continues to grow in popularity, more precise predictions may thus become available in the future.

The recently introduced transient elastography, which can be used for liver stiffness measurement, is a promising tool for dynamic evaluation of disease progression in clinical practice. Transient elastography, however, should not be viewed as a surrogate of liver biopsy or other invasive procedures [18] because it is not suitable for intermediate stages of fibrosis [19]. Nevertheless, the usefulness of the US score should not be underestimated. First, the installation of elastography is costly, so it is usually only available in tertiary institutes. Second, our preliminary study indicated that the correlation between the US score and histological findings in chronic hepatitis C was higher than that of transient elastography (data not shown). Further study is needed to compare the usefulness and cost effectiveness of such modalities.

In conclusion, the incidence of HCC in HCV-associated CLDs was 1.1, 5.5, and 10.2% in the low, middle, and high US score groups, respectively. Moreover, in the high US score group, which represents liver cirrhosis, a higher score was associated with a higher incidence of HCC. US score, the integrated evaluation of the fibrosis stage of the liver described herein, is useful for the prediction of HCC in HCV-associated CLDs.

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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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Key words: hepatitis virus, hepatocellular carcinoma, diabetes mellitus

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