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Sequences in the Interferon Sensitivity-Determining Region and Core Region of Hepatitis C Virus Impact Pretreatment Prediction of Response to PEG-Interferon Plus Ribavirin: Data Mining Analysis

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The aim of the present study was to clarify the significance of viral factors for pretreatment prediction of sustained virological response to pegylated-interferon (PEG-IFN) plus ribavirin (RBV) therapy for chronic hepatitis C using data mining analysis. Substitutions in the IFN sensitivity-determining region (ISDR) and at position 70 of the HCV core region (Core70) were determined in 505 patients with genotype 1b chronic hepatitis C treated with PEG-IFN plus RBV. Data mining analysis was used to build a predictive model of sustained virological response in patients selected randomly (n = 304). The reproducibility of the model was validated in the remaining 201 patients. Substitutions in ISDR (odds ratio = 9.92, $P < 0.0001$) and Core70 (odds ratio = 1.92, $P = 0.01$) predicted sustained virological response independent of other covariates. The decision-tree model revealed that the rate of sustained virological response was highest (83%) in patients with two or more substitutions in ISDR. The overall rate of sustained virological response was 44% in patients with a low number of substitutions in ISDR (0–1) but was 83% in selected subgroups of younger patients (<60 years), wild-type sequence at Core70, and higher level of low-density lipoprotein cholesterol (LDL-C) (≥120 mg/dl). Reproducibility of the model was validated ($r^2 = 0.94$, $P < 0.001$). In conclusion, substitutions in ISDR and Core70 of HCV are significant predictors of response to PEG-IFN plus RBV therapy. A decision-tree model that includes these viral factors as predictors could identify patients efficiently with a high probability of sustained virological response.
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KEY WORDS: data mining; decision-tree model; ISDR; core region; PEG-interferon

INTRODUCTION

The combination of pegylated-interferon (PEG-IFN) plus ribavirin (RBV) is currently the most effective therapy for chronic hepatitis C, but the rate of sustained virological response after 48 weeks of therapy is about 50% in patients with HCV genotype 1b and a high HCV

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RNA titer [Manns et al., 2001; Fried et al., 2002]. The most reliable means to predict sustained virological response is to monitor the viral response during the early weeks of treatment. The early virological response, defined as undetectable HCV RNA at week 12, is associated with a high rate of sustained virological response [Davis et al., 2003; Lee and Ferenci, 2008]. The rapid virological response, defined as undetectable HCV RNA at week 4 of therapy, is even more predictive of sustained virological response than the early virological response [Jensen et al., 2006; Yu et al., 2008; Izumi et al., 2010]. However, there is no established means that predicts the virological response before commencing treatment. Recent reports have revealed that single nucleotide polymorphisms located near the *IL28B* gene show a strong association with the response to PEG-IFN plus RBV therapy [Ge et al., 2009; Suppiah et al., 2009; Tanaka et al., 2009; Kurosaki et al., 2010c]. These findings indicate that the host factor is an important determinant of the treatment response. On the other hand, the present study's authors have reported that a stretch of 40 amino acids in the NS5A region of HCV, designated as the interferon sensitivity-determining region (ISDR), has a close association with the virological response to interferon mono-therapy [Enomoto et al., 1995, 1996; Kurosaki et al., 1997]. More recently, amino acid substitutions at positions 70 and 91 of the core region have been reported to be associated with response to PEG-IFN plus RBV combination therapy [Akuta et al., 2005, 2007a]. The impact of these HCV substitutions on treatment response is yet to be validated.

Decision-tree analysis is a core component of data mining analysis that can be used to build predictive models [Breiman et al., 1980]. This method has been used to define prognostic factors in various diseases such as prostate cancer [Garzotto et al., 2005], diabetes [Miyaki et al., 2002], melanoma [Averbook et al., 2002; Leiter et al., 2004], colorectal carcinoma [Zlobec et al., 2005; Valera et al., 2007], and liver failure [Baquerizo et al., 2003]. The major advantage of decision-tree analysis over logistic regression analysis is that the results of analysis are easy to understand. The simple allocation of patients into subgroups by following the flowchart form could define the predicted possibility of outcome [LeBlanc and Crowley, 1995].

Decision-tree analysis was used for the prediction of early virological response (undetectable HCV RNA within 12 weeks of therapy) to PEG-IFN and RBV combination therapy in chronic hepatitis C [Kurosaki et al., 2010a], and more recently for the pretreatment prediction of sustained virological response [Kurosaki et al., 2010b]. In the latter model, simple and noninvasive standard tests were used as parameters; specialized tests such as viral mutations and host genetics, or invasive tests such as liver histology, were not included because the aim of that model was for use in general medical practice, especially in some countries or areas where resources are limited. Thus, the impact of viral mutations or liver histology was not considered in that model.

The present study examined whether including viral substitutions in ISDR and the core region of HCV in the decision-tree model could improve its predictive accuracy over the previous model to identify chronic hepatitis C patients who are likely to respond to PEG-IFN plus RBV therapy.

MATERIALS AND METHODS

Patients

This multicenter retrospective cohort study included 505 chronic hepatitis C patients who were treated with PEG-IFN alpha-2b and RBV at Musashino Red Cross Hospital, Toranomon Hospital, Tokyo Medical and Dental University, Osaka University, Nagoya City University Graduate School of Medical Sciences, Yamanashi University, Osaka City University, and their related hospitals. The inclusion criteria were: (1) genotype 1b, (2) HCV RNA titer higher than 100 kIU[Q2]/ml by quantitative PCR (Cobas Amplicor HCV Monitor v 2.0, Roche Diagnostic Systems, CA[Q3]), (3) no co-infection with hepatitis B virus or human immunodeficiency virus, (4) no other causes of liver disease, (5) patients having undergone liver biopsy prior to IFN treatment, (6) number of substitutions in ISDR having been determined, (7) substitutions in the amino acid positions 70 and 91 of the core region having been determined, and (8) completion of at least 12 weeks of therapy. Patients were treated with PEG-IFN alpha-2b (1.5 μg/kg) weekly plus RBV. The daily dose of RBV was adjusted by weight: 600 mg for <60 kg, 800 mg for 60–80 kg, and 1,000 mg for >80 kg. For the analysis, patients were assigned randomly to either the model building (304 patients) or validation (201 patients) groups. There were no significant differences in the clinical backgrounds between these two groups (Table I). Informed consent was obtained from each patient. The study protocol conformed to the ethical guidelines of the Declaration of Helsinki and was approved by the institutional review committees of all concerned hospitals.

Laboratory Tests

Hematological tests, blood chemistry, and HCV RNA titer were analyzed before therapy and at least once every month during therapy. Sequences of ISDR and the core region of HCV were determined by direct sequencing after amplification by reverse transcription and polymerase chain reaction as reported previously. At position 70 of the core region (Core70), arginine was defined as the wild type, and glutamine or histidine was defined as the mutant type. At position 91 of the core region, leucine was defined as the wild type and methionine was defined as the mutant type, as described previously [Akuta et al., 2005]. Fibrosis and activity were scored according to the METAVIR scoring system [Bedossa and Poynard, 1996]. Fibrosis was staged on a scale of 0–4: F0 (no fibrosis), F1 (mild fibrosis), F2 (moderate fibrosis), F3 (severe fibrosis), and F4 (cirrhosis). Activity of necroinflammation was graded on a scale of

TABLE I. Comparison of Pretreatment Factors Between Model Building and Validation Patients

	Model (n = 304)	Validation (n = 201)	*P*-value
Age (years)	55.6 (9.4)	56.0 (12.2)	0.80
Male (%)	53 (%)	55 (%)	0.13
Body mass index (kg/m^2)	23.1 (3.1)	23.1 (4.0)	0.99
Albumin (g/dl)	4.0 (0.3)	4.0 (0.3)	0.47
Creatinine (mg/dl)	0.72 (0.15)	0.72 (0.14)	0.62
AST (IU/L)	63.3 (45.6)	58.9 (46.4)	0.91
ALT (IU/L)	78.7 (58.6)	74.5 (67.5)	0.68
GGT (IU/L)	53.2 (49.1)	57.4 (63.5)	0.43
Total cholesterol (mg/dl)	170.9 (32.6)	169.4 (34.1)	0.33
Triglyceride (mg/dl)	107.0 (44.7)	105.7 (48.0)	0.90
LDL-C (mg/dl)	95.5 (28.0)	96.4 (28.8)	0.34
White blood cell count (/μl)	4,902 (1,489)	4,906 (1,319)	0.86
Hemoglobin (g/dl)	14.1 (1.3)	14.3 (1.4)	0.09
Platelets (10^9/L)	164 (56)	172 (55)	0.68
HCVRNA (10^3 IU/ml)	1,859 (1,468)	2,021 (1,393)	0.09
ISDR mutations: ≥2 (%)	15 (%)	20 (%)	0.11
Core70: mutant (%)	36 (%)	29 (%)	0.22
Core91: mutant (%)	40 (%)	36 (%)	0.20
Fibrosis: F2–4 (%)	49 (%)	48 (%)	0.36
Activity: A2–3 (%)	42 (%)	34 (%)	0.10

AST, aspartate aminotransferase; ALT, alanine aminotransferase; GGT, gamma-glutamyltransferase; LDL-C, low-density-lipoprotein-cholesterol; ISDR, interferon sensitivity-determining region.
Data expressed as mean (SD).

0–3: A0 (no activity), A1 (mild activity), A2 (moderate activity), and A3 (severe activity). Sustained virological response was defined as undetectable HCV RNA by qualitative PCR with a lower detection limit of 50 IU/ml (Amplicor, Roche Diagnostic Systems) at week 24 after the completion of therapy.

Statistical Analysis

A database of pretreatment variables included hematological tests (hemoglobin level, white blood cell count, and platelet count), blood chemistry tests (serum levels of creatinine, albumin, aspartate aminotransferase, alanine aminotransferase (ALT), gamma-glutamyltransferase (GGT), total cholesterol, triglyceride, and low-density lipoprotein cholesterol (LDL-C)), viral factors (HCV RNA titer, number of substitutions in ISDR, substitutions in the amino acid positions 70 and 91 of the core region), histological findings (stage of fibrosis and grade of activity) and patient characteristics (age, sex, and body mass index). Based on this database, decision-tree analysis was used to define a predictive model for sustained virological response.

Student's *t*-test was used for the univariable comparison of quantitative variables and Fisher's exact test was used for the comparison of qualitative variables. For the multivariable analysis for factors associated with sustained virological response, logistic regression models with backward selection were used to identify independent predictors of sustained virological response. Variables that showed significant association with sustained virological response by univariable analysis were included in the multivariable analysis. IBM-SPSS software v.15.0 (SPSS, Inc., Chicago, IL) was used for these analyses. For the decision-tree analysis [Segal[Q4] and Bloch, 1989], the data mining software IBM SPSS Modeler 13 (IBM SPSS, Inc.) was used, as reported previously [Kurosaki et al., 2010a,b]. In brief, the software searched for the optimal split variables to build a decision-tree structure. The entire study population was first evaluated to determine the variables and cut-off points for the most significant division into two subgroups having different probabilities of sustained virological response. Thereafter, analysis was repeated on all subgroups in the same way until either no additional significant variable was detected or the sample size was below 20.

RESULTS

Generation of the Decision-Tree Model

The decision-tree analysis selected five predictive variables to produce six subgroups of patients (Fig. 1). The number of substitutions in ISDR was selected as the best predictor of sustained virological response. The possibility of achieving sustained virological response was 83% for patients with two or more substitutions in ISDR compared with 44% for patients with a single or no substitution. Among patients with a single or no substitution in ISDR, age, with an optimal cut-off of 60 years, was selected as the variable of second split. Patients younger than 60 had the higher probability of sustained virological response (55%) compared with those older than 60 years (31%). Among younger patients, amino acid substitution at Core70 was selected as the third variable of split—wild-type sequence being the predictor of favorable response compared with the mutant type (65% vs. 36%). Among patients with wild-type Core70, the level of serum LDL-C was selected as the fourth variable of split, with an optimal cutoff of

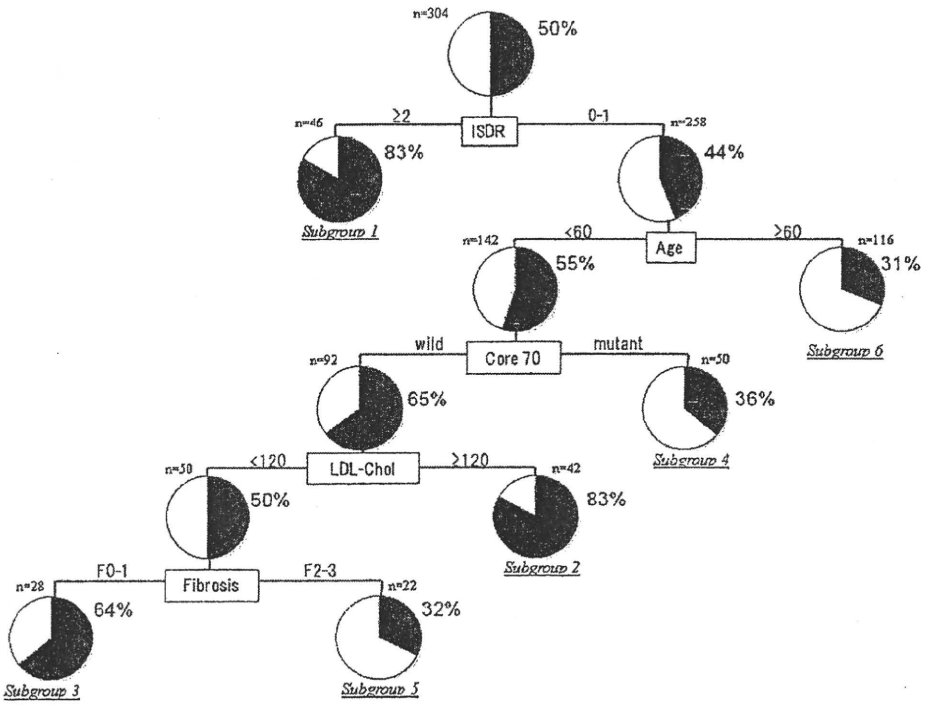

Fig. 1. Decision-tree model. Boxes indicate the factors used for splitting and the cutoff value for the split. Pie charts indicate the rate of sustained virological response for each group of patients after splitting. Terminal subgroups of patients discriminated by the analysis are numbered from 1 to 7. The rate of sustained virological response was >80% in subgroups 1 and 2, 64% in subgroup 3, and 31–36% in subgroups 4, 5, and 6. LDL-C represents low-density lipoprotein cholesterol and Core70 represents amino acid substitution at position 70 of the core region.

120 mg/dl. Patients with higher LDL-C level had the higher probability of sustained virological response (83% vs. 50%). The stage of fibrosis was selected as the final variable of split, with significant fibrosis (F2–4) being the predictor of lower sustained virological response probability (64% vs. 32%).

Among the six subgroups derived by this decision tree, the subgroup of patients with two or more substitutions in ISDR (subgroup 1) or with a single or no substitution in ISDR but younger than 60 years of age, having the wild-type Core70 and high serum level of LDL-C (≥120 mg/dl) (subgroup 2) showed the highest probability of sustained virological response (83%).

Validation of the Decision-Tree Model

The decision-tree model was validated using a validation dataset of 201 cases that were not included the model-building dataset. Each patient in the validation set was allocated to subgroups 1–6 using the flowchart form of the decision tree. The rates of sustained virological response were 75% for subgroup 1, 73% for subgroup 2, 65% for subgroup 3, 41% for subgroup 4, 46% for subgroup 5, and 33% for subgroup 6. The rates of sustained virological response for each subgroup of patients were correlated closely between the model building dataset and the validation dataset ($r^2 = 0.94$) (Fig. 2).

The six subgroups were reconstructed into three groups according to their rate of sustained virological response: the high-probability group consisted of subgroups 1 and 2, the intermediate-probability group consisted of subgroup 3, and the low-probability group consisted of subgroups 4, 5, and 6. The rate of sustained virological response in the high-probability group was high on a consistent basis: 83% for model-building patients and 74% for validation patients. The rate of sustained virological response in the intermediate-probability group was 64% for model building patients and 65% for internal validation patients. The rate of sustained virological response in the low-probability group was low on a consistent basis: 32% for model-building patients and 36% for internal validation patients (Fig. 3). Thirty percent of the patients were classified into the high-probability group and 10% of the patients were classified into intermediate-probability group, which means that about 40% of patients with higher than average probability of achieving sustained virological response were identified.

Effect of Dose Reductions of PEG-IFN and RBV

The possible effect of drug reductions was analyzed in the three groups of patients divided by decision tree (low-, intermediate-, and high-probability groups)

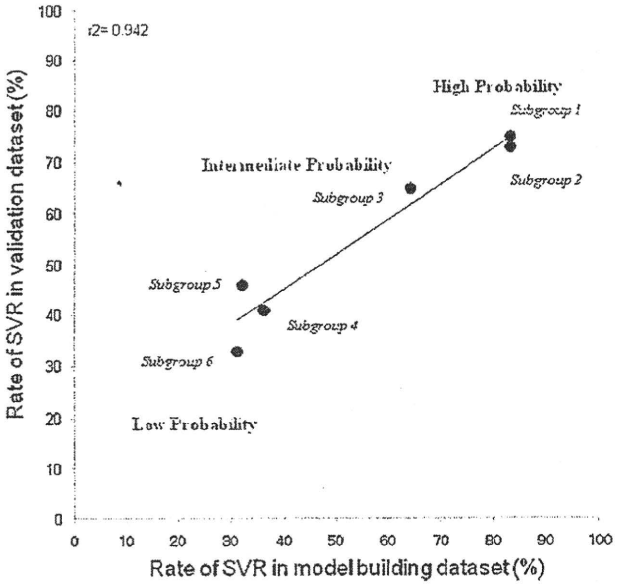

Fig. 2. Validation of the decision-tree analysis: Subgroup-stratified comparison of the rate of sustained virological response. Each patient in the validation set was allocated to subgroups 1–6 by following the flowchart form of the decision tree, and the rates of sustained virological response were then calculated and plotted for each subgroup. The x-axis represents the rate of sustained virological response in the model-building datasets and the y-axis represents the rate of sustained virological response in the validation datasets. The rates of achieving sustained virological response in each subgroup of patients correlated closely between the model-building dataset and the validation dataset (correlation coefficient: $r^2 = 0.94$).

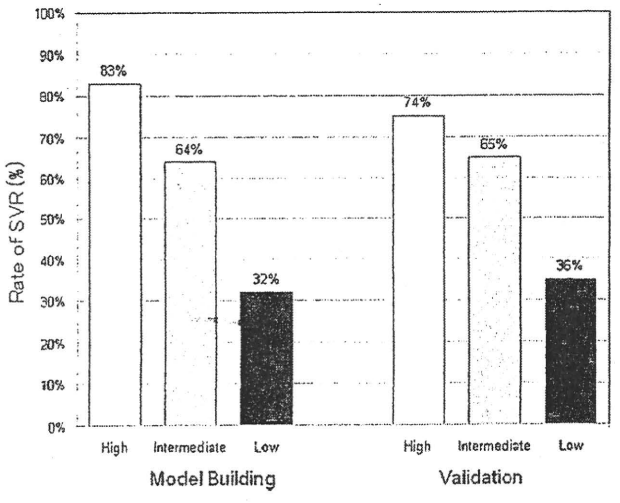

Fig. 3. Comparison of sustained virological response rates between groups divided by the decision tree. The rate of sustained virological response was compared between three groups of patients as divided by the decision-tree analysis. White, gray, and black boxes indicate the low-probability group (subgroup 4, 5, and 6), intermediate-probability group (subgroup 3), and high-probability group (subgroup 1 and 2), respectively. The rate of sustained virological response showed significant difference between the three groups.

(Fig. 4). Patients were stratified according to the cumulative drug exposure with PEG-IFN and RBV: the good adherence group consisted of patients who took ≥80% planned doses of both PEG-IFN and RBV; the poor adherence group consisted of patients who took <80% of planned doses of both PEG-IFN and RBV. Even after adjustment for drug adherence, the three groups of patients divided by decision-tree analysis still had low, intermediate, and high probability of achieving sustained virological response, respectively, indicating that this model predicts sustained virological response independent of drug exposure.

Multivariable Logistic Regression Analysis

Age, sex, serum levels of creatinine, ALT, GGT, LDL-C, hemoglobin, platelet count, HCV RNA titer, ISDR substitution, substitution at Core70, substitution at Core91, histological stage of fibrosis, and grade of activity were found to be associated with sustained virological response by standard univariable analysis. Multivariable analysis including these factors showed that age, sex, LDL-C levels, GGT levels, platelet count, ISDR substitution, and substitution at Core70 showed independent associations with sustained virological response (Table II). Substitution in ISDR had the highest odds ratio, at 9.92. Fibrosis, which was selected as a significant predictor of response in the decision-tree analysis, was not found to be an independent predictor of response in standard multivariable analysis, indicating that the decision-tree analysis could identify significant predictors that would apply specifically to selected patients.

DISCUSSION

The present study revealed that viral factors such as substitutions in ISDR and Core70 are significant and independent predictors of sustained virological response to PEG-IFN plus RBV in chronic hepatitis C. In a decision-tree model for the pretreatment prediction of sustained virological response, the number of substitutions in ISDR was the best predictor of sustained virological response, followed by younger age, wild-type sequence at Core70, higher level of LDL-C, and absent fibrosis. This decision-tree model could identify patients with high probability of sustained virological response (83%) among difficult-to-treat genotype 1b chronic hepatitis C patients. Using this model, rapid estimates of the response before treatment can be made by allocating patients to specific subgroups with a defined rate of response simply by following the flowchart form. Because more potent therapy, such as a combination of protease inhibitor, PEG-IFN, and RBV, is under clinical trial and may become available in the near future [Hezode et al., 2009; McHutchison et al., 2009], pretreatment prediction of the likelihood of sustained virological response may be useful for both patients and physicians to support clinical decisions whether to start current standard therapy or to wait for emerging new therapies.

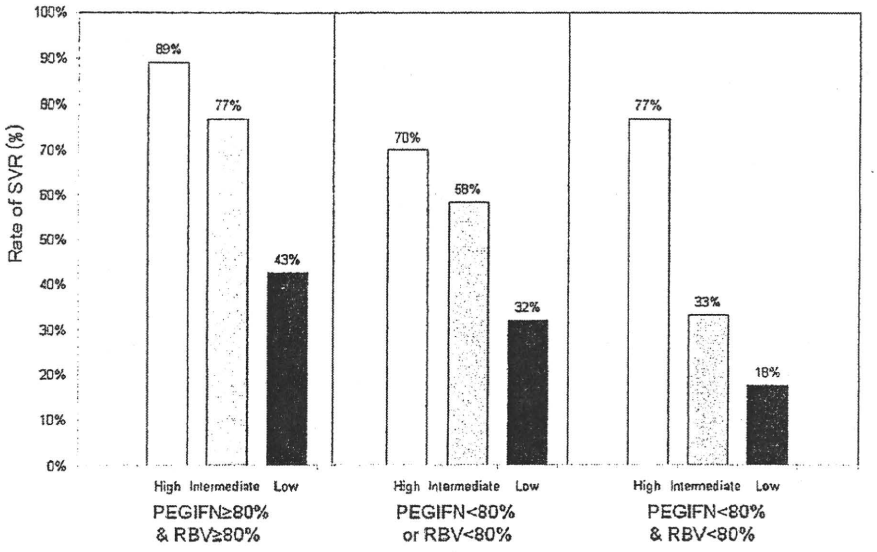

Fig. 4. Comparison of the rate of sustained virological response between the decision-tree groups stratified by drug adherence. The three groups of patients divided by the decision tree (black, gray, and white boxes indicating the low-, intermediate-, and high-probability groups, respectively) were further stratified according to cumulative drug exposure to PEG-IFN and RBV.

Two or more substitutions in ISDR had a strong impact on sustained virological response, because this factor was selected as a top variable in decision-tree analysis and had the highest odds ratio in multivariable analysis. Moreover, even among patients with unfavorable ISDR (0 or 1 mutation), younger patients (<60 years) with the wild-type sequence at Core70 and high level of LDL-C (≥120 mg/dl) had a high rate of sustained virological response. The sustained virological response rate of these two subgroups of patients was 83% in the model-building patients and 75% in the validation patients. Thus, patients with high possibility of sustained virological response could be extracted by the combined analysis of ISDR and Core70. These patients may be the best-suited candidates for treatment with the current combination therapy. Conversely, the following patients with 0–1 mutation in ISDR had a low probability of sustained virological response (32–35%): (1) older (>60 years); or (2) younger (<60 years) patients but having mutant-type sequence at Core70; or (3) younger (<60 years) patients having a wild-type sequence at Core70, but having a low level of LDL-C (<120 mg/dl) and advanced fibrosis. These patients may be advised to wait for a more effective therapy. Decision may be made on a case-by-case basis, taking into account the potential risk of disease progression while waiting.

In a previous decision-tree model using simple and noninvasive standard tests that are available readily worldwide [Kurosaki et al., 2010b], the rate of sustained virological response was at most 65–76% among those in the high-probability group. That model focused on use by general physicians in routine general practice, especially where specialized resources, such as liver biopsy or determination of viral sequences, are not available. In that model, younger age, male sex, higher platelet counts, lower alpha-fetoprotein (AFP) levels, and lower GGT levels were identified as favorable predictive parameters. Higher AFP levels and lower platelet counts that are hallmarks of advanced fibrosis [Shiratori and Omata, 2000; Akuta et al., 2007b] were associated with low probability of sustained virological response in that model. On the other hand, the present analysis aimed to clarify the significance of viral factors for pretreatment prediction of sustained virological response, and to build an advanced model that may be used by specialist physicians engaged in the

TABLE II. Multivariable Logistic Regression Analysis for Factors Associated With SVR

Parameter		Odds	95% CI	*P*-value
Age (years)	<60 vs. ≥60	2.28	1.31–3.94	0.003
Gender	Male vs. female	3.36	1.87–5.99	<0.0001
GGT (IU/L)	<40 vs. ≥40	2.65	1.45–4.85	0.002
LDL-C (mg/dl)	≥120 vs. <120	1.79	0.91–3.53	0.094
Platelets (109/L)	≥120 vs. <120	2.69	1.22–5.90	0.014
ISDR mutations	≥2 vs. 0–1	9.92	3.71–26.54	<0.0001
Core70	Wild vs. mutant	1.92	1.07–3.47	0.030

GGT, gamma-glutamyltransferase; LDL-C, low-density-lipoprotein-cholesterol; ISDR, interferon sensitivity-determining region.

treatment of hepatitis. In the present model, stage of fibrosis was selected as a predictive factor, but at lower level of significance than HCV mutations. The predicted rate of sustained virological response in the high-probability group of the present model is higher than that in the previous model (75–83% vs. 65–76%). These results indicate that substitutions in ISDR and Core70 were important pretreatment predictors of sustained virological response. Determination of these viral factors is not available readily in clinical practice, but is of value for improving the accuracy of pretreatment prediction of sustained virological response.

Substitutions in ISDR and Core70 have been reported previously to be associated with efficacy of IFN therapy. The association between the number of substitutions in ISDR and response to therapy was demonstrated originally in patients treated with IFN mono-therapy [Enomoto et al., 1995, 1996; Kurosaki et al., 1997], but recent studies have reported a positive correlation with PEG-IFN and RBV combination therapy as well [Munoz de Rueda et al., 2008; Shirakawa et al., 2008; Ikeda et al., 2009]. Another important viral factor relevant to treatment response is amino acid substitution in Core70. The sequence of this amino acid was reported originally to be associated with nonresponse to therapy [Akuta et al., 2005], but subsequent studies confirmed the positive correlation of a wild-type Core70 with sustained virological response [Akuta et al., 2009]. The multiple logistic regression analysis showed that ISDR and Core70 were independent factors associated with sustained virological response along with host factors. How these important viral factors and other host factors can be combined to predict response to PEG-IFN plus RBV is an important clinical question. Decision-tree modeling can make the response probability apparent by combining all these factors. Some factors that may be associated with treatment outcome, such as levels of ferritin or homocysteine, were not included. This may be a potential limitation of the present study.

It is of interest that a recent study by Li et al. [2010] has shown that a high serum level of LDL-C is linked to the *IL28B* major allele (CC in rs12979860). In that study, a high serum level of LDL-C was associated with sustained virological response, but it was no longer significant when analyzed together with the *IL28B* genotype in multivariate analysis. Thus, the association between treatment response and LDL cholesterol levels in the present study may reflect the underlining link of LDL cholesterol levels to the *IL28B* genotype. Recent reports indicate that the *IL28B* genotype and HCV substitutions are correlated closely [Akuta et al., 2010; Kurosaki et al., 2010c]. Still, Core70 [Akuta et al., 2010] or ISDR [Kurosaki et al., 2010c] were predictors of response to therapy independent of *IL28B* genotype. Future study is needed to elucidate the possible mechanisms underlying the association between HCV sequences and host genetic factors, and also the role of host and viral factors for the prediction of treatment response.

In conclusion, a data mining analysis emphasized the impact of substitutions in ISDR and Core70 on pretreatment prediction of sustained virological response to PEG-IFN plus RBV therapy. A decision-tree model that includes substitutions in ISDR and Core70 of HCV could identify patients with high probability of sustained virological response, and could thereby improve the predictive accuracy over predictions that are based on standard tests.

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Diagnostic and Treatment Algorithm of the Japanese Society of Hepatology: A Consensus-Based Practice Guideline

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Key Words

Hepatocellular carcinoma · Radiofrequency ablation · Surgical resection · Child-Pugh · JIS score

Abstract

In Japan, more than 70% of hepatocellular carcinomas (HCC) develop from hepatitis C virus infections and 15% are derived from hepatitis B infections. Since most have received close observation with e.g. ultrasound or enhanced computed tomography (CT) scan every 3–6 months before development of HCC, the HCC nodule was detected in the early stage in more than 60% of the patients. An algorithm for the HCC surveillance was shown as a Japanese clinical guideline of a scientific-based research group. At the joint symposium with JSH and the International Liver Cancer Association (ILCA), the algorithm of diagnosis and treatment for HCC was discussed using Answerpad. Several important discussions are described in this article.

Diagnosis of Early Hepatocellular Carcinoma

A consensus symposium of diagnosis and treatment for hepatocellular carcinoma (HCC) was held at the Annual Meeting of the Japanese Society of Hepatology (JSH) on June 4–5, 2009. This consensus-based practice guideline was a revision from that reported at the 2005 JSH Annual Meeting. More than 400 hepatologists including surgeons, radiologists and pathologists joined the symposium and consensus statements and recommendations were discussed using Answerpad. When more than 67% of the participants agreed with the statement, the statement was defined as established and described as a JSH consensus statement. More than 40 statements were discussed, which remain to be published.

In Japan, more than 70% of HCCs develop from hepatitis C virus infection and 15% are derived from hepatitis B infection. Since most had received close observation with e.g. ultrasound, enhanced computed tomography (CT) scan or enhanced magnetic resonance imaging (MRI) every 3–6 months before development of HCC, the HCC nodule was detected in the early stage in more than 60% of the patients. An algorithm for the HCC surveillance was shown as a Japanese clinical practice guideline of a scientific evidence-based research group supported

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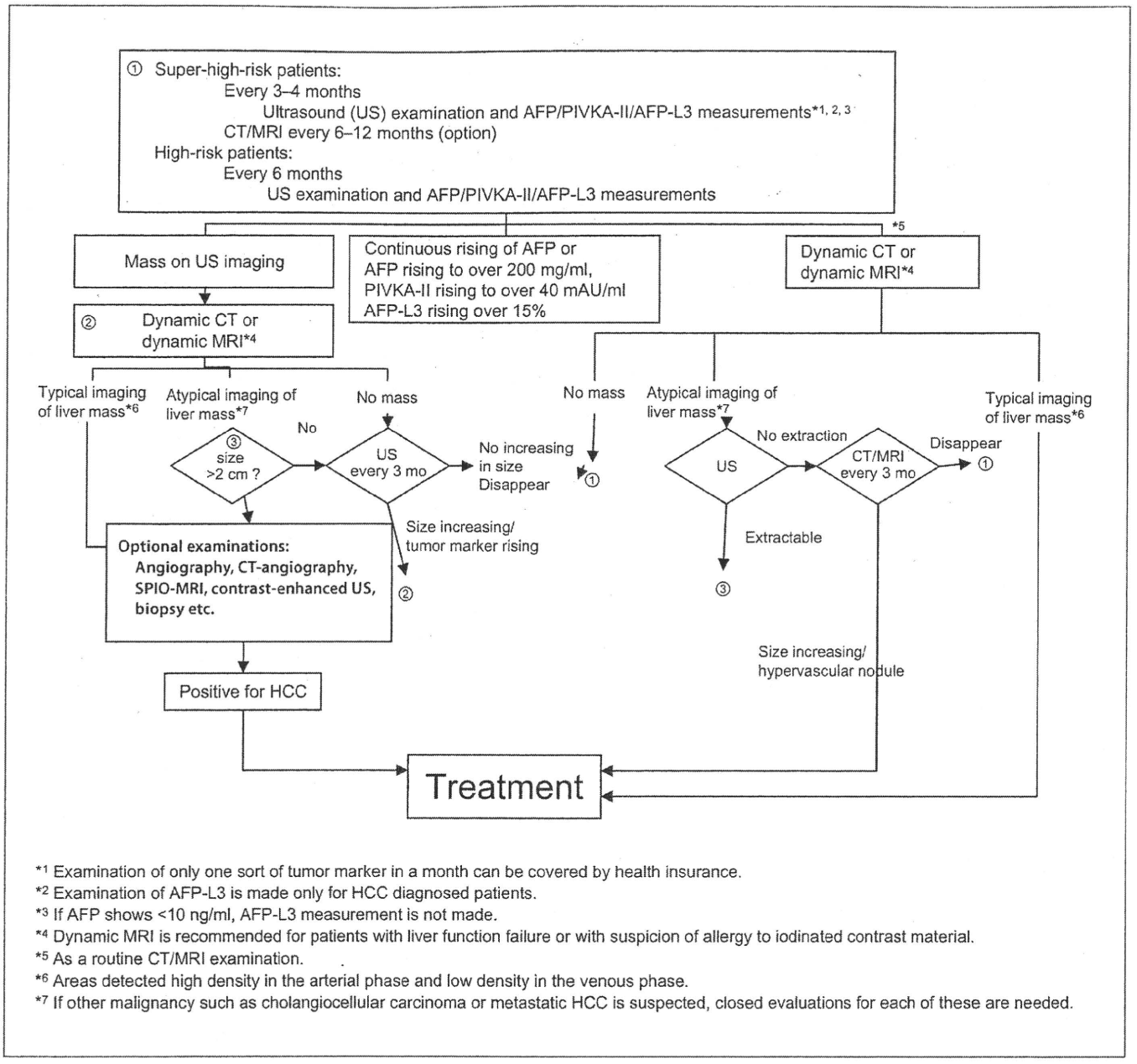

*1 Examination of only one sort of tumor marker in a month can be covered by health insurance.
*2 Examination of AFP-L3 is made only for HCC diagnosed patients.
*3 If AFP shows <10 ng/ml, AFP-L3 measurement is not made.
*4 Dynamic MRI is recommended for patients with liver function failure or with suspicion of allergy to iodinated contrast material.
*5 As a routine CT/MRI examination.
*6 Areas detected high density in the arterial phase and low density in the venous phase.
*7 If other malignancy such as cholangiocellular carcinoma or metastatic HCC is suspected, closed evaluations for each of these are needed.

Fig. 1. Algorithm for the HCC surveillance 2005 (Japanese clinical practice guideline of a scientific evidence-based research group supported by the Japanese Ministry of Health, Labor and Welfare [taken from 1].

by the Japanese Ministry of Health, Labor and Welfare (Head: M. Makuuchi) [1] in 2005 (fig. 1).

At the joint symposium with JSH and the International Liver Cancer Association (ILCA), the algorithm of diagnosis and treatment for HCC was discussed using Answerpad. Forty-five hepatologists, surgeons, radiologists and pathologists participated in this meeting and voted the statement. Eight important statements were discussed and voted by Answerpad. The results described compare them with those of the JSH consensus meetings.

Statement 1

A needle biopsy of the hypervascular HCC nodule with 1.5 cm should not be done.

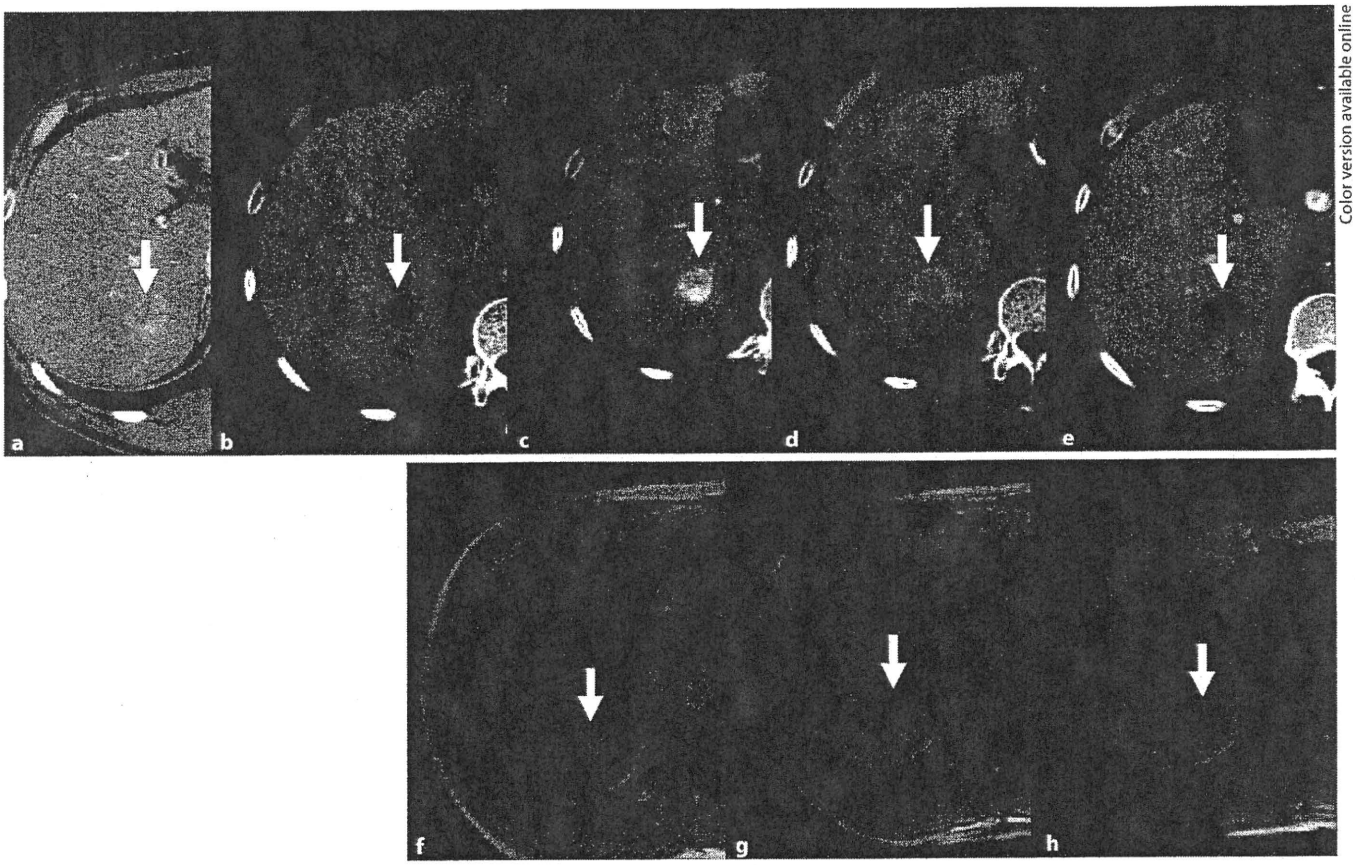

Fig. 2. Representative case of hypervascular early HCC in a 64-year-old male. There is a hypervascular nodule 1.8 cm in diameter in segment 7 during the arterial phase in the dynamic CT scan (**a**) which becomes a low-density area during the equilibrium phase (**b**). CTHA revealed a hypervascular region (**c**) which becomes a ringed enhancement, a so-called 'corona enhancement' in the late phase of CTHA (**d**). This nodule becomes a low-density area during CTAP (**e**). Gd-EPB-DTPA-enhanced MRI revealed a high-intensity area during the arterial phase (**f**) and a low-intensity area during the portal phase (**g**). Importantly, this nodule showed a low-intensity area in the T_1 hepatobiliary phase by Gd-EOB-DTPA-enhanced MRI (**h**).

A typical case is shown in figure 2. A hypervascular nodule was observed at the arterial phase in a contrast-enhanced CT scan with a diameter of 1.8 cm in segment 7, which becomes a hypovascular region in the equilibrium phase. This nodule was defined as a hypervascular region during CT during hepatic arteriography (CTHA) and low-density area during CT during arterioportography (CTAP). Gadolinium (Gd)-EOB-DTPA MRI was carried out and the nodule of segment 7 became a low-intensity area in the hepatobiliary phase. A needle biopsy gives important information concerning pathological differentiation grade and biomarker expression; however, implantation of neoplastic cells to the tract or seeding has been reported [2, 3].

This statement was agreed on by 78% of the participants, but 22% disagreed. At the JSH consensus meeting, 91% of the participants agreed with this statement, and only 9% disagreed. Thus, most of the hepatologists who participated in both consensus meetings did not agree to undergo needle biopsy of the nodule when the nodule is hypervascular.

Biopsy of the nodule was done under guided ultrasound, which revealed moderately differentiated HCC (fig. 3). This nodule was treated by radiofrequency ablation (RFA), and complete necrosis was obtained.

Statement 2

A needle biopsy of the nodule should be done to the arterial hypovascular nodule with 1.0 cm when the nodule becomes a low-intensity area in the hepatobiliary phase by Gd-EOB-DTPA-enhanced MRI.

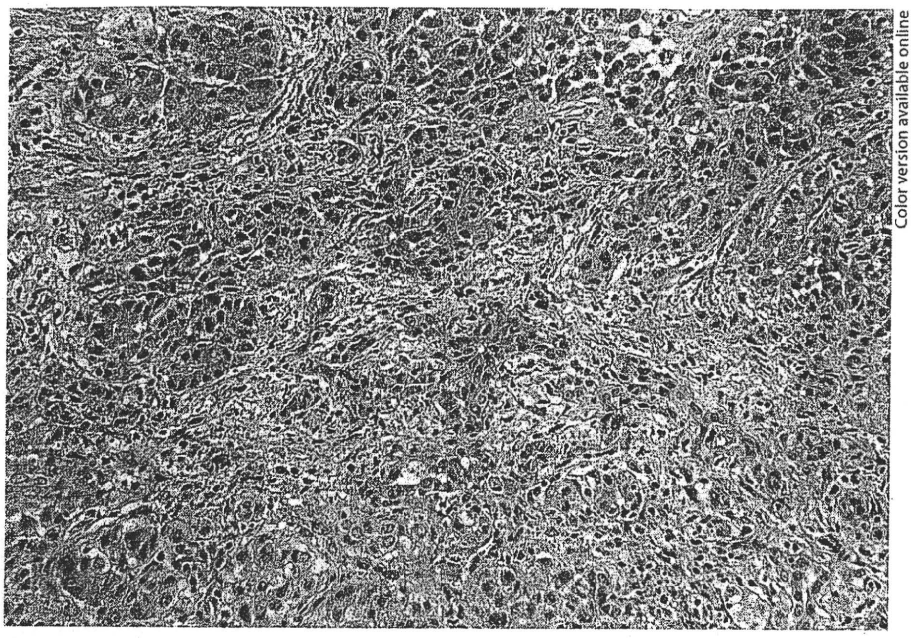

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Fig. 3. A needle biopsy of the nodule was done which revealed moderately differentiated HCC. HE. 200×.

Table 1. The JIS (Japan Integrated Score) was defined by adding the tumor TMN stage and Child-Pugh score

	Variable			
	0	1	2	3
Tumor stage (TMN)[1]	1	2	3	4
Child-Pugh score	A	B	C	

[1] Liver Cancer Study Group of Japan.

A typical case is shown in figure 4. The hypovascular nodule was detected at the arterial phase in a contrast-enhanced CT scan with a diameter of 1.5 cm in segment 8, which becomes also a hypovascular region in the equilibrium phase. This nodule was defined as a hypovascular region during CTHA and low-density area during CTAP. Gd-EOB-DTPA MRI was carried out, and the nodule of segment 8 became a low-intensity area in the hepatobiliary phase.

This statement was agreed on by 57% of the consensus meeting participants, but 43% disagreed with the statement. At the JSH consensus meeting, 47% of the participants agreed with this statement, and only 53% disagreed. Both of the voting results were similar.

A needle biopsy of the nodule was done which revealed well-differentiated HCC (fig. 5). When the hypovascular nodule was detected, it was difficult to obtain an accurate diagnosis without a needle biopsy and the strategy that was reported [4]. Since hypovascular nodules sometimes converted from malignant progression to overt HCC [5], it seems necessary to undertake a needle biopsy of the nodule.

Statement 3

For estimating the prognosis of patients with HCC, the most reliable staging system is the Japan Integrated Score (JIS).

The JIS scoring system was proposed by Kudo et al. [6] and was defined as adding the tumor TMN stage of the Japan Hepatocellular Cancer Study Group and Child-Pugh score as shown in table 1. In Japan, screening systems for the early detection of HCC have been established, e.g. periodic ultrasound, enhanced CT scan including measuring α-fetoprotein and prothombin induced by vitamin K deficiency. Thus, most HCC nodules were detected in the early stage. The JIS score has been validated in Japanese patients [7] and approved to be the best prognosis estimation of patients with HCC in Japan.

This statement was agreed on by 63% of the participants, but 37% disagreed at the ILCA consensus meeting. At the JSH consensus meeting, 71% of the participants agreed with this statement, and 29% disagreed.

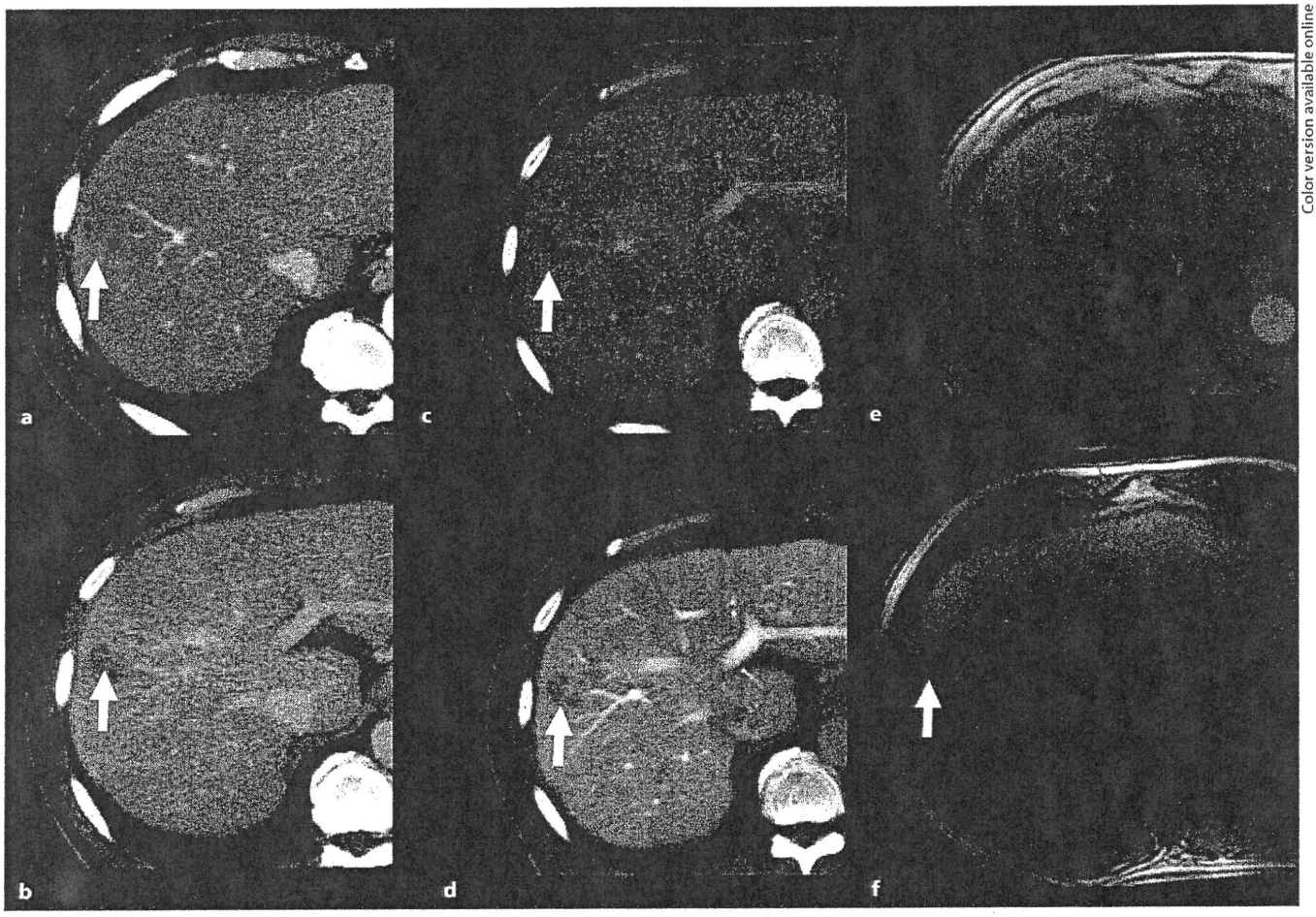

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Fig. 4. A representative case of hypovascular early HCC in a 75-year-old male. There is a hypovascular nodule with a diameter of 1.5 cm in segment 8 during the arterial phase in the dynamic CT scan (**a**) which becomes a low-density area during the equilibrium phase (**b**). CTHA also revealed a hypovascular region (**c**). This nodule becomes a low-density area during CTAP (**d**). Superparamagnetic iron oxide-enhanced MRI was carried out, but a nodular region was not detected in the T_2* MRI image (**e**). Gd-EPB-DTPA-enhanced MRI showed a low-intensity area in the T_1 hepatobiliary phase by Gd-EOB-DTPA-enhanced MRI (**f**).

Treatment Algorithm of HCC

The treatment algorithm was discussed at the JSH consensus meeting in 2005. At this meeting the treatment algorithm was established by initially dividing the patients according to extrahepatic spread, Child-Pugh score, and vascular invasion (fig. 6). Next, they were divided by the nodule number and the vascularity of the nodule. When the single nodule was identified as being hypovascular, intensive follow-up or ablation was recommended. When the patient had 1–3 hypervascular nodules <3 cm in diameter, they should be treated by surgical resection or RFA. When the nodules are >3 cm, they should be treated by surgical resection or transarterial chemoembolization (TACE). When the patients have 4 or more HCC nodules, they should be treated by TACE or transarterial embolization (TAE). If the patients have 3 or less nodules <3 cm or a single nodule <5 cm which are divided within the Milan criteria, liver transplantation should be considered if the patients are younger than 65 years of age. If invasion to the portal or hepatic vein was observed, they should be treated by surgical resection, TAI or TACE.

When the patients were classified as having poor liver function with Child-Pugh C and the HCC nodules are within the Milan criteria, liver transplantation should be considered. Otherwise, palliative care should be chosen.

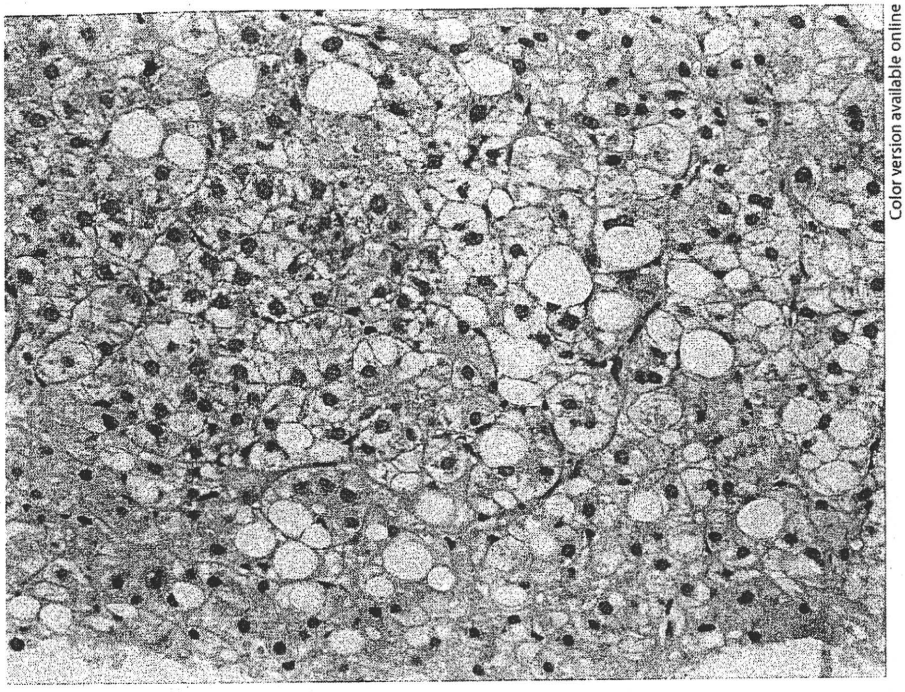

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Fig. 5. A needle biopsy of the nodule was done which revealed a well-differentiated HCC. HE. 200×.

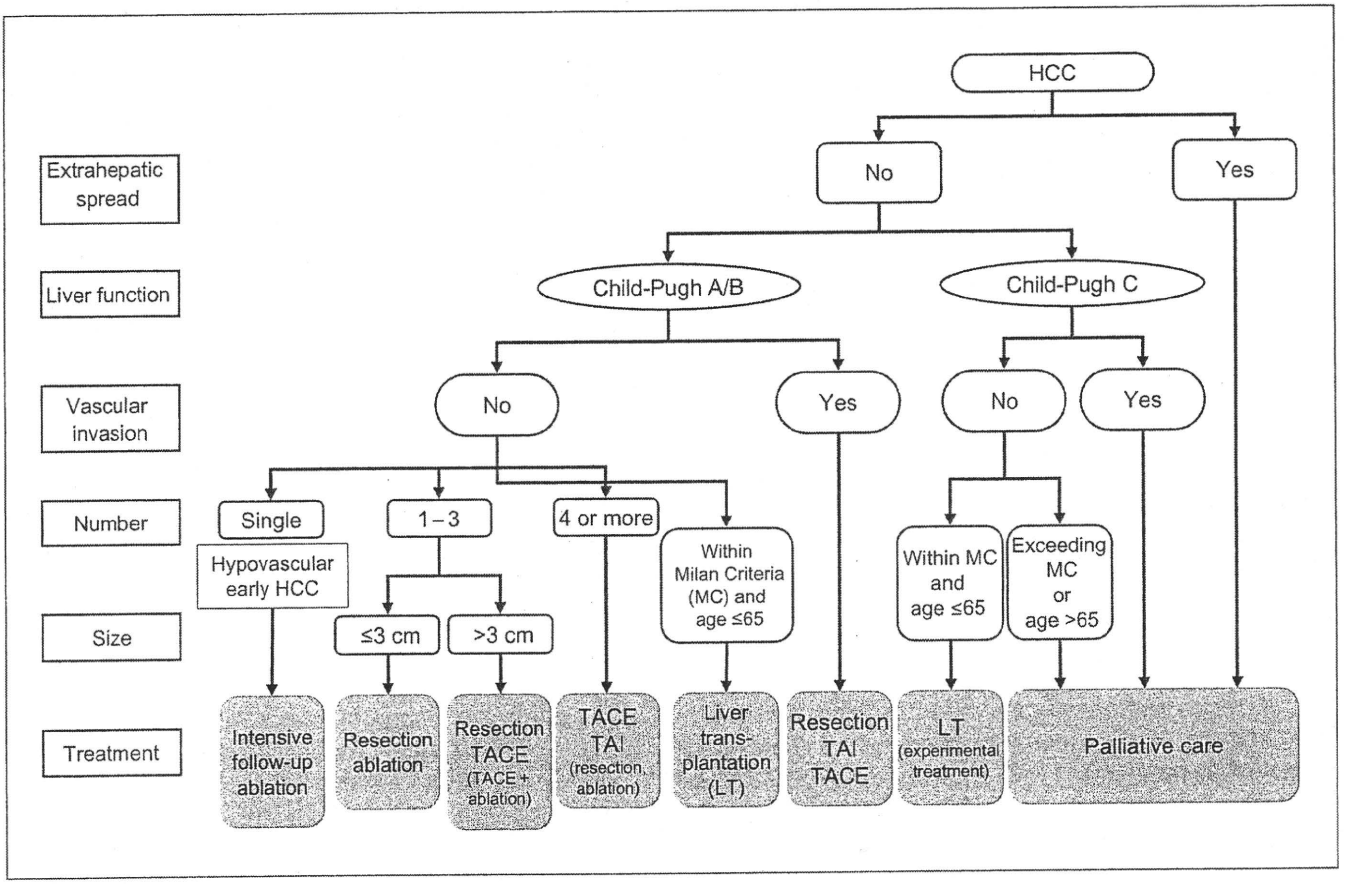

Fig. 6. Treatment algorithm for HCC (JSH consensus-based 2007).

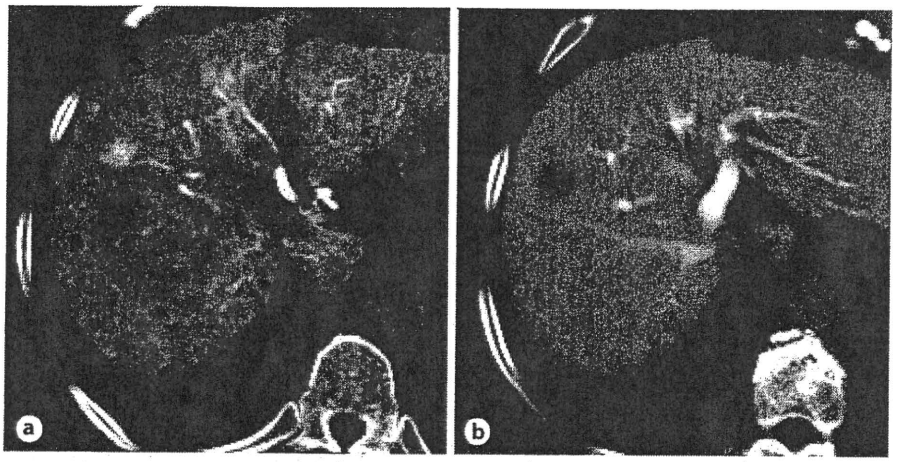

Fig. 7. A representative case with a hypervascular nodule by CTHA in segment 8 with a diameter of 1.8 cm (**a**). This nodule became a low-density area by CTAP (**b**). This patient has good liver function classified as Child-Pugh A.

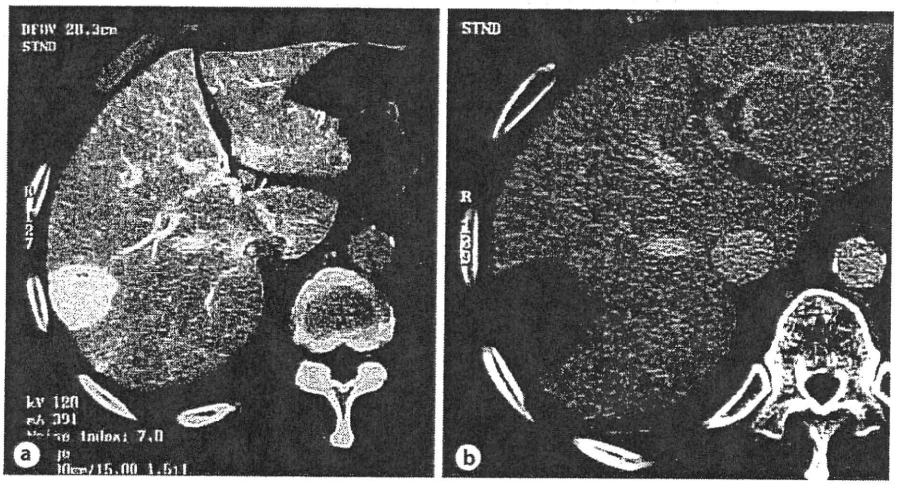

Fig. 8. A representative case with a hypervascular nodule by CTHA in segment 7 with a diameter of 3.0 cm (**a**). Locally complete curative necrosis was achieved by RFA (**b**).

Statement 4

Which treatment do you choose for the patient having a single HCC nodule <2 cm with Child-Pugh A?

A typical case of a 62-year-old male is shown in figure 7. A hypervascular small nodule was observed by CTHA at segment 8 with a diameter of 1.8 cm, which became a low-intensity area by CTAP. His liver function was classified as Child-Pugh A.

38% of the participants chose surgical resection, but 62% chose RFA. At the JSH consensus meeting, 44% of the participants chose surgical resection, but 56% chose RFA. When only surgeons were asked the same question, 80% of them chose surgical resection, but 20% chose RFA. This question was asked to only physicians at the JSH consensus meeting, and 32% chose surgical resection, and 68% chose RFA.

Overall survival was compared after surgical resection with RFA [8], in which no apparent difference was observed between the two groups. Thereafter, several reports including a large number of patients with HCC compared the survival or recurrence, but they are not randomized [9–11]. This is an important issue that needs to be clarified. Thus, randomized controlled trials are necessary including a large number of patients to clarify which treatment is superior between surgical resection and RFA.

Statement 5

Which treatment do you choose for a patient having a single HCC nodule <3 cm with Child-Pugh A?

A representative case of a 75-year-old male is shown in figure 8a. He had a single hypervascular nodule defined by CTHA at segment 7 with a diameter of 3 cm. His liver

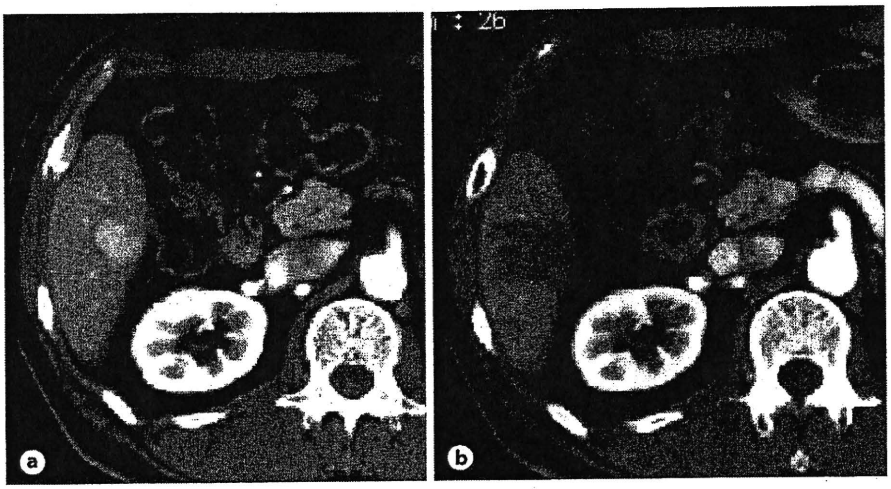

Fig. 9. A typical case with a single hypervascular HCC nodule in segment 6 with a diameter of 1.4 cm. He has a good liver function of Child-Pugh A (**a**). He was treated by RFA alone, and complete necrosis was obtained (**b**).

function was well preserved and he was classified as Child-Pugh A. The participants were asked this question. 74% of the participants chose surgical resection, but 26% chose RFA by the ILCA. At the JSH consensus meeting, 80% of the participants chose surgical resection, but 20% chose RFA as the first-line treatment.

Interestingly enough, most of the participants chose surgical resection when the nodule was as large as 3 cm. This hypervascular HCC nodule was treated by percutaneous RFA, and locally complete curative necrosis was obtained (fig. 8b).

Statement 6

RFA should be done after TACE to the hypervascular HCC nodule with a diameter of 2 cm.

A typical 62-year-old male with a 1.6-cm single hypervascular HCC nodule in segment 6 is shown in figure 9a. He has a good liver function with Child-Pugh A.

This statement was agreed on by 36% of the participants at the ILCA consensus meeting, but 64% disagreed. However, at the JSH consensus meeting, 51% of the participants agreed with this statement, but 49% disagreed.

This hypervascular HCC nodule was completely ablated by RFA alone (fig. 9b). It has been reported that TACE before RFA increased the ablated area, suggesting that overall survival will improve [12–14]; however, TACE may increase the adverse events by RFA. Whether TACE before RFA is beneficial for the patients should be examined by analyzing the overall survival of patients and comparing them to receiving TACE before RFA or without TACE before RFA.

Statement 7

Do you prescribe sorafenib as the first-line treatment option for the patients with advanced HCC in whom surgical resection, RFA or TACE is not indicated?

As sorafenib was approved in Japan in May 2009 [15], only a few hepatologists have experienced prescribing the medicine. Its usefulness after TACE in patients with advanced HCC is under investigation in the USA and Japan [16]. It will be included in the therapeutic algorithm for HCC, but it is still unclear to hepatologists to which patients the medicine should be prescribed.

At the ILCA consensus meeting, 61% of the participants agreed with this statement, but 30% disagreed. 9% of them did not have any opinion on the statement because they have no experience with sorafenib. At the JSH consensus meeting, 35% of the participants agreed with this statement, but 29% disagreed. 36% of the participants did not have any opinion because they have no experience with sorafenib.

The best indication for sorafenib should be investigated in the near future [17].

Statement 8

Overall survival should be the endpoint for the assessment of efficacy comparing ablation with surgical resection.

The recurrence rate after surgical resection or RFA was reported including a large number of patients, and the incidence of intrahepatic recurrence was higher after RFA than surgical resection [11]. However, overall survival was not different between the two groups. Thus, the problem is how to evaluate the outcome of surgical resection and RFA, and this question was proposed by hepatologists.

This statement was agreed on by 89% of the participants at the ILCA consensus meeting, but 11% disagreed with this statement. At the JSH consensus meeting, 84% of the participants agreed with this statement, and 16% disagreed. The outcome should be evaluated by both overall survival and incidence of recurrence.

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Disclosure Statement

The author declares that he has no financial conflict of interest.

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Original Article

Hepatic steatosis in chronic hepatitis C is a significant risk factor for developing hepatocellular carcinoma independent of age, sex, obesity, fibrosis stage and response to interferon therapy

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Aim: Hepatic steatosis is linked to development of hepatocellular carcinoma (HCC) in non-viral liver disease such as non-alcoholic steatohepatitis. The present study aimed to assess whether hepatic steatosis is associated with the development of HCC in chronic hepatitis C.

Methods: We studied a retrospective cohort of 1279 patients with chronic hepatitis C who received interferon (IFN) therapy between 1994 and 2005 at a single regional hospital in Japan. Of these patients, 393 had a sustained virological response (SVR) and 886 had non-SVR to IFN therapy. After IFN therapy, these patients were screened for development of HCC every 6 months. The average period of observation was 4.5 years.

Results: HCC developed in 68 patients. The annual incidence of HCC was 2.73% for patients with a steatosis grade of 10% or greater and 0.69% for patients with a steatosis grade of 0–9%. On multivariate analysis, higher grade of steatosis was a significant risk factor for HCC independent of older age, male sex, higher body mass index (BMI), advanced fibrosis stage and non-SVR to IFN therapy. The adjusted risk ratio of hepatic steatosis was 3.04 (confidence interval 1.82–5.06, $P < 0.0001$), which was higher than that of older age (1.09), male sex (2.12), non-SVR to IFN (2.43) and higher BMI (1.69).

Conclusion: Hepatic steatosis is a significant risk factor for development of HCC in chronic hepatitis C independent of other known risk factors, which suggest the possibility that amelioration of hepatic steatosis may prevent hepatocarcinogenesis.

Key words: hepatocellular carcinoma, interferon, steatosis, virological response.

INTRODUCTION

HEPATOCELLULAR CARCINOMA (HCC) is one of the most common cancers worldwide and its incidence has been increasing. This recent increase in HCC incidence may likely be attributed to the higher prevalence of non-alcoholic fatty liver disease (NAFLD) and hepatitis C virus (HCV) infection.[1]

Non-alcoholic fatty liver disease is characterized by hepatic steatosis with or without inflammation in the absence of excessive alcohol consumption. Several studies have indicated the etiological association between NAFLD and development of HCC.[2–4] Other studies have shown that obesity or diabetes, a common etiology of non-alcoholic hepatic steatosis, is associated with development of HCC.[5–7] Although the mechanism of carcinogenesis in NAFLD has not been determined, an animal model showed that obesity-related hepatic steatosis leads to the development of hepatic

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hyperplasia, suggesting the possibility that hepatic steatosis is a pre-malignant condition.[8]

Another important etiological agent for HCC is HCV infection. Because steatosis is a common pathological feature of HCV-infected patients,[9] the important question is whether steatosis influences the progression of liver disease in hepatitis C, by analogy with NAFLD. Several studies, including ours[10] indicated that hepatic steatosis promotes the progression of hepatic fibrosis.[11–15] The association between hepatic steatosis and the development of HCC in chronic hepatitis C has been proposed[16] and was confirmed in two studies[17,18] while another study failed to show such an association.[19] The present study was conducted to analyze the association between hepatic steatosis and development of HCC in a large cohort of chronic hepatitis C patients, which enabled to adjust for known risk factors for HCC.

METHODS

Patients

A TOTAL OF 1437 chronic hepatitis C patients were treated with interferon (IFN) at Musashino Red Cross Hospital between October 1994 and October 2005. Among them, 1279 patients who fulfilled the following inclusion criteria were enrolled in this study: (i) positive for HCV RNA by reverse-transcription polymerase chain reaction before IFN therapy; (ii) absence of other causes of liver disease, such as co-infection with hepatitis B virus, autoimmune hepatitis or primary biliary cirrhosis; (iii) had undergone liver biopsy within the 12 months prior to IFN treatment; (iv) were followed for more than 1 year after the completion of IFN therapy; and (v) absence of HCC during and within 1 year after the completion of therapy. A total of 158 patients were excluded: two patients who were positive for hepatitis B surface antigen, 97 patients lacking liver biopsy, 53 patients with less than 1 year's duration of follow up, and six patients who developed HCC within 1 year of the completion of IFN therapy. The study protocol conformed to the ethical guidelines of the Declaration of Helsinki and was approved by the institutional ethics review committee.

Patients were followed up by regular visits to our hospital every 1–3 months. Six patients died of liver-unrelated disease (two patients with gastric cancer and one patient each with lung cancer, colon cancer, pancreatic cancer and leukemia). There were 122 patients who were lost to follow up because of relocation. We included their data in the analysis, censored at the time of their last visit. The start of follow up was defined as the date of completion of first IFN therapy and the end of follow up was defined as the date of diagnosis of HCC or the date of the last visit. The average period of follow up was 4.5 years.

Clinical characteristics and laboratory data were collected at the most recent time point before liver biopsy. Diabetes mellitus was diagnosed based on a fasting plasma glucose concentration that exceeded 126 mg/dL, a casual plasma glucose concentration that exceeded 200 mg/dL, or the need for insulin or oral antihyperglycemic drugs. Information regarding alcohol consumption was obtained through an interview. Body mass index (BMI) was calculated using the following formula: weight in kilograms/height in meters squared. The baseline clinical features of patients at enrollment are summarized in Table 1.

Histological examination

Liver biopsy specimens were obtained from all patients before therapy. The median length of liver biopsy specimens was 13 mm (range 10–42 mm) and median number of portal tracts was 11 (range 4–30). Histological findings were re-evaluated recently by three independent pathologists who were blinded to the clinical details to ensure consistency over time. Fibrosis and activity were scored according to the METAVIR scoring system.[20] Fibrosis was staged on a scale of 0–4: F0 (no fibrosis); F1 (mild fibrosis: portal fibrosis without septa); F2 (moderate fibrosis: few septa); F3 (severe fibrosis: numerous septa without cirrhosis); and F4 (cirrhosis). Activity of necroinflammation was graded on a scale of 0–3: A0 (no activity); A1 (mild activity); A2 (moderate activity); and A3 (severe activity). Percentage of steatosis was quantified by determining the average proportion of hepatocytes affected by steatosis and graded on a scale of 0%, 1–9%, 10–29% and 30% or greater as reported previously.[10] All three pathologists assigned the same scale in 85% of cases for fibrosis staging, 87% for inflammation grading and 95% for steatosis grading. If there was discordance, the scores assigned by two pathologists were used for the analysis.

Screening for HCC

At enrollment, no patient had HCC or any suspicious lesion on abdominal ultrasonography or computed tomography. Patients were examined for HCC by abdominal ultrasonography or computed tomography at least every 6 months. Suspicious lesions were examined further by a triphasic contrast-enhanced computerized tomography or magnetic resonance imaging,