Table 3 Multivariate logistic regression analysis to identify independent predictive factors of RVR, cEVR, and SVR

	Odds ratio	95% CI	*p* value
RVR factors selected by stepwise method			
F stage			
F0–2/F3–4	2.924	0.988–8.696	0.053
HCV RNA load 0 week (KIU/mL)			
<1000/1000≤	2.151	1.130–4.082	0.020
ALT 0 week (IU/L)			
<60/60≤	2.165	1.127–4.149	0.020
Amino acid mutation of ISDR			
2≤/0–1	2.371	1.187–4.735	0.014
Amino acid substitution of core 91			
W/M	2.137	1.021–4.464	0.044
cEVR factors selected by stepwise method			
Gender			
Male/female	1.912	1.209–3.021	0.0055
F stage			
F0–2/F3–4	2.079	1.133–3.817	0.018
HCV RNA load 0 week (KIU/mL)			
<1000/1000≤	1.608	1.002–2.577	0.049
PLT count ($\times 10^4$/mm^3)			
15≤/<15	1.427	0.882–2.309	0.148
Amino acid mutation of ISDR			
2≤/0–1	2.512	1.407–4.485	0.0018
Amino acid substitution of core 70			
W/M	2.513	1.508–4.184	0.0004
Amino acid substitution of core 91			
W/M	1.965	1.241–3.115	0.004
SVR factors selected by stepwise method			
Gender			
Male/female	3.704	2.132–6.410	<0.0001
F stage			
F0–2/F3–4	1.812	0.888–3.690	0.103
HCV RNA load0 week (KIU/mL)			
<1000/1000≤	2.024	1.163–3.534	0.013
PLT count ($\times 10^4$/mm^3)			
15≤/<15	2.469	1.394–4.372	0.0019
Amino acid mutation of ISDR			
2≤/0–1	2.148	1.107–4.170	0.024
Amino acid substitution of core 70			
W/M	2.415	1.316–4.444	0.0045
Amino acid substitution of core 91			
W/M	1.433	0.828–2.481	0.199
PEG adherence (%)			
80≤/<80	1.562	0.834–2.926	0.164

W Wild, *M* Mutant

was a wild type but only 16% in patients with mutant at core 70. In female patients, no or one aa substitution in ISDR and $<15 \times 10^4$/mm^3 of PLT count, the SVR rates were as low as 10 or 8%, irrespective of aa substitution at core 70. SVR was only 24% in patients with substitution of core aa 70 even when the PLT count was $\geq 15 \times 10^4$/mm^3. In this study, the combination analysis of PLT count, ISDR, and core aa substitution was useful for predicting non-SVR.

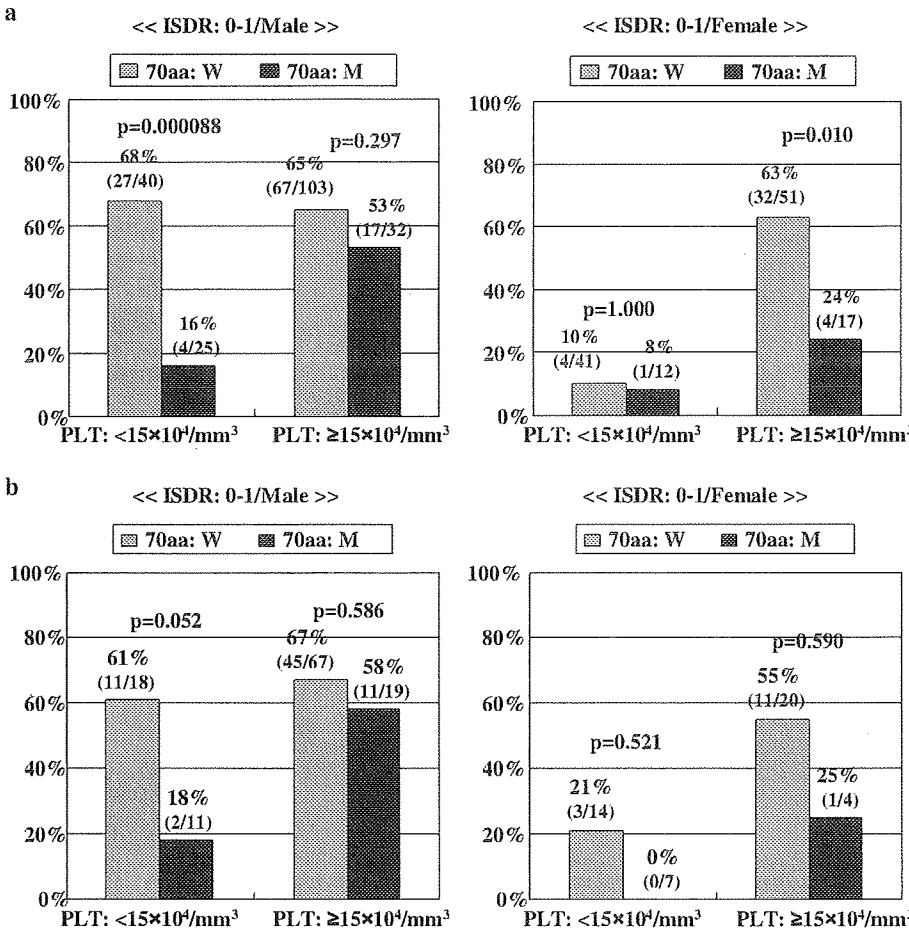

Fig. 2 Relationship between SVR rate and amino acid substitutions in the ISDR and core amino acids 70 and 91, PLT counts and gender difference. The two figures of **a** show the results of *Study 1* and the two figures of **b** show the results of *Study 2*. In male patients with no or only one amino acid (aa) substitution in the ISDR and PLT count of less than $15 \times 10^4/mm^3$, the SVR rate was 68% in those with wild type core aa 70, but only 16% in patients with mutant type of core aa 70, which is significantly different ($p = 0.000088$). There were no significant differences between wild type and mutant type of core aa 70 in the patients with no or one aa substitution in the ISDR and PLT count of over $15 \times 10^4/mm^3$. By contrast, in female patients with no or one aa substitution in the ISDR, there were no significant differences between wild type and mutant type of core aa 70 with PLT count of less than $15 \times 10^4/mm^3$, but there were significant differences between wild type and mutant type of core aa 70 with PLT counts of less than $15 \times 10^4/mm^3$ (**a**). For the patients maintaining over 80% adherences to both PEG-IFN and RBV, in males having no or one aa substitution in the ISDR and PLT counts of less than $15 \times 10^4/mm^3$, a wild type of core aa 70 could predict SVR with a positive predictive value (PPV) of 61% and negative predictive value (NPV) of 82% ($p = 0.052$). However, in male patients with PLT counts of over $15 \times 10^4/mm^3$, core aa 70 was not a useful marker for predicting SVR and non-SVR. The number of female patients with no or one aa substitution in ISDR was too small to reach a definite conclusion (**b**)

Study design 2

The basic features of 201 patients achieving 80% adherences to both PEG-IFN and RBV are as follows: the females were significantly ($p = 0.00006$) older than the males. Iron deposition in liver tissue, alcohol abuse, BMI, serum albumin level, serum ferritin level, and PLT count were significantly higher in males than females. Inflammatory activity was significantly ($p = 0.046$) higher in females than males (data not shown).

AA substitutions in the ISDR were as follows; in males 33 (22.3%) had two or more aa substitutions, in females 8 (15.1%) had two or more aa substitutions. The analysis of core aa position 70 and 91 sequences showed no significant differences in aa substitutions of either core aa 70 or 91 between males and females (data not shown).

In patients less than 60 years of age, SVR rate was significantly higher ($p = 0.0042$) in males than females, but no significant difference was noted between males and females over 60 years old. However, the number of patients over 60 years was small (Table 4).

Table 4 Univariate analysis to identify the significantly different factors between SVR and non-SVR (201 patients received over 80% adherences of both PEG-IFN and RBV)

Factors	Negative of HCV RNA after 24 weeks		*p* value
	(−)	(+)	
No. of patients	111 (55.2%)	90	
Gender			
Male	93 (62.8%)	55	0.00037
Female	18 (34.0%)	35	
Age			
Median (range)	51 (18–70)	56 (23–74)	0.00025
<60 years	91 (60.3%)	60	0.014
60 years≤	20 (40.0%)	30	
Age: <60 years			
Male	79 (66.4%)	40	0.0042
Female	12 (37.5%)	20	
Age: 60 years≤			
Male	14 (48.3%)	15	0.243
Female	6 (28.6%)	15	
F stage			
F0–2	103 (60.9%)	67	0.0012
F3–4	8 (25.8%)	23	
Grade (A factor)			
A0–1	80 (59.3%)	55	0.189
A2–3	31 (47.0%)	35	
HCV RNA load 0 week (KIU/mL)			
Median (range)	1300 (110–5000<)	1280 (130–5000<)	0.351
ALT 0 week (IU/L)			
Median (range)	74 (16–268)	67.5 (19–504)	0.752
BMI			
Median (range)	23.1 (17.3–31.0)	23.6 (16.1–33.9)	0.626
Alb (g/dL)			
Median (range)	3.95 (3.3–5.2)	3.9 (3.0–4.8)	0.079
LDL-C (mg/dL)			
Median (range)	96 (31–185)	97.5 (30–182)	0.865
T-Chol (mg/dL)			
Median (range)	170 (85–248)	170 (105–273)	0.624
PLT count ($\times 10^4$/mm^3)			
Median (range)	18.9 (8.7–30.9)	15.55 (7.2–28.4)	0.00003
<15	23 (35.9%)	41	0.00024
15≤	88 (64.2%)	49	
Amino acid mutation of ISDR			
0–1	84 (52.5%)	76	0.159
2≤	27 (65.9%)	14	
Amino acid substitution of core 70			
Wild	91 (61.5%)	57	0.0037
Mutant	20 (37.7%)	33	
Amino acid substitution of core 91			
Wild	73 (60.3%)	48	0.083
Mutant	38 (47.5%)	42	

Virological responses and aa substitution

The rates of RVR, cEVR, LVR, ETR and SVR in males and females were 12.5 versus 11.3% ($p = 1.000$), 59.6 versus 43.4% ($p = 0.053$), 74.3 versus 50.0% ($p = 0.0018$), 76.2 versus 66.7% ($p = 0.198$), and 62.8 versus 34.0% ($p = 0.00037$), respectively (data not shown). The backgrounds and characteristics of SVR and non SVR patients are shown in Table 4. There were significant differences in gender (male vs. female; $p = 0.00037$), age (<60 years vs. ≥60 years; $p = 0.014$), F stage (F0-2 vs. F3,4; $p = 0.0012$), PLT count ($<15 \times 10^4/mm^3$ vs. $15 \times 10^4/mm^3 \leq$; $p = 0.00024$), and substitution of core aa 70 (wild type vs. mutant, p = 0.0037) between SVR and non-SVR patients. The distribution of fatty change in liver tissue (≤10% vs. 11–33% vs. 34%≤; $p = 0.046$) and the grade of HOMA-IR (1.7 vs. 3.9, $p = 0.0018$) were significantly different between SVR and non-SVR (data not described in Table 4).

Factors affecting SVR by multivariate logistic regression analysis

Male gender ($p = 0.0006$), mild fibrosis stage ($p = 0.027$), and wild type of core aa 70 ($p = 0.043$) were independent predictors of SVR.

Valuable markers for predictions of sustained virological response to peginterferon and ribavirin therapy

Two or more aa mutations in the ISDR, wild type core aa 70, $\geq 15 \times 10^4/mm^3$ of PLT count, and male gender were selected statistically as independent predictors of SVR. We show here SVR rates of the patients having over 80% adherences to both PEG-IFN and RBV (Fig. 2b). In males having no or one aa substitution in the ISRD and PLT count of $<15 \times 10^4/mm^3$, wild type core aa 70 could predict SVR with a positive predictive value (PPV) of 61% and negative predictive value (NPV) of 82% ($p = 0.052$). In females, the SVR rate was very low in those who had substitution of core aa 70, but there was no significant difference between patients with wild type and substitution of core aa 70. The number of female patients was too small to provide a definite conclusion.

Discussion

The present multivariate logistic regression analysis revealed that male gender, low HCV RNA load, high PLT count, and two or more aa mutations in the ISDR and wild type core aa 70 were independent predictors for SVR. PLT count significantly decreased corresponding to the progression to the stage of liver fibrosis in CHC [9, 30, 31].

It has been considered that the low adherence level to PEG-IFN/RBV is a major cause of a significantly lower SVR rate in females and older patients [32]. The percentage of patients having over 80% adherences to both PEG-IFN and RBV was significantly lower in females than males, however, differences in the adherence to PEG-IFN/RBV between males and females were not independent predictive factors of non-SVR.

A recent report from Japan showed six or more mutations in the variable region 3 (V3) of nonstructural protein 5A (NS5A) plus upstream flanking region NS5A (aa 2334–2379), referred to as the IFN/RBV resistance determining region (IRRDR), was a useful marker for predicting SVR, but the ISDR sequence was not valuable for predicting SVR [33]. However, the number of subjects in that study was too small ($n = 45$) to reach an acceptable conclusion.

To elucidate the factors affecting low SVR rate in older female patients, we performed a multivariate logistic regression analysis using patients who achieved ≥80% adherence to both PEG-IFN and RBV. Male gender, stage of mild liver fibrosis, and wild type core aa 70 were independent predictors of SVR. In this study, blood concentration of RBV was determined in fewer than 50% of cases during treatment. Thus we cannot exclude the possibility of the effect of the blood concentration of RBV during treatment on the low SVR rate in females and older patients.

From the present analysis, it was clear that ALT, BMI, Alb, T. Chol, and adherence to RBV differed significantly between males and females, however, these factors were not independent predictors of SVR. There is a report that steatosis is an important cofactor that reduces the SVR rate in genotype 1 infected patients [34], however, such an effect was not seen in this study. Thus we could not identify the factors associated with a significantly lower SVR rate in females than males.

In the present multivariate logistic regression analyses, patients having wild type core aa 91 had significantly higher rates of RVR and cEVR, but not SVR, and patients with wild type core aa 70 had significantly higher rates of cEVR and SVR, but not RVR. Patients having two or more aa substitutions in the ISDR had significantly higher rates of RVR, cEVR, and SVR. Although several possibilities have been considered concerning the effects of aa substitutions of core protein on SVR in PEG-IFN/RBV therapy for CHC patients, the exact mechanisms have not yet been elucidated.

Recent reports have indicated that low serum IP-10 (interferon-γ inducible protein 10 kDa) [35], a higher HCV-specific CD8 cell proliferation potential [36], and a high ratio of Th1/Th2 [37] are good predictors of SVR to

PEG-IFN/RBV therapy. These results indicate the importance of immunological status and immunological response to treatment in patients difficult to treat with PEG-IFN/RBV therapy for CHC.

The present univariate analyses revealed that there were many factors relating to RVR, cEVR, and SVR including LDL-C, HOMA-IR, fatty change in liver tissue, and hyaluronic acid, however some of these factors had not been examined in some participating institutes. We consider that we must perform a prospective mass study using many factors including immunological aspects, viral factors, disease status, and therapeutic aspects to elucidate the reason that older female patients are resistant to a combination of PEG-IFN and RBV therapy in CHC with a high viral load genotype 1b.

In conclusion, our results demonstrated that wild type core aa 70, two or more aa mutations in the ISDR, low viral load, high PLT counts, and male gender are useful markers for predicting SVR.

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ORIGINAL ARTICLE

Fucosylated Fraction of Alpha-Fetoprotein as a Predictor of Prognosis in Patients with Hepatocellular Carcinoma After Curative Treatment

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Abstract
Aim The aim of this study was to evaluate the clinical usefulness of measuring the *Lens culinaris* agglutinin-reactive fraction of alpha-fetoprotein (AFP-L3) for prognostic predictor in patients with hepatocellular carcinoma (HCC).
Methods A total of 477 HCC patients who underwent percutaneous ablative therapy or hepatectomy were enrolled. Overall survival and recurrence-free survival were respectively evaluated retrospectively and prospectively. Multivariate analyses of clinical prognostic factors were performed by Cox's stepwise proportional hazard model.
Results AFP-L3 status was a statistically significant independent prognostic factor of long-term survival ($P = 0.013$) and recurrence-free survival ($P = 0.006$) in patients who underwent percutaneous ablative therapy. In contrast, AFP-L3 did not affect prognosis in patients who underwent hepatectomy.
Conclusions AFP-L3 had different impacts on prognosis in patients with HCC who underwent percutaneous ablative therapy and hepatectomy. Our results suggest that AFP-L3 positivity (≥15%) might be a promising indicator for choosing therapeutic modalities in HCC patients.

Keywords Alpha-fetoprotein · AFP-L3 · DCP (des-γ-carboxy prothrombin) · Hepatocellular carcinoma · Prognostic factor

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Introduction

Hepatectomy is a generally accepted method that improves the long-term outcome in patients with hepatocellular carcinoma (HCC) [1]. However, patients with HCC frequently have coexisting liver cirrhosis with impaired hepatic functional reserve, and this may prevent surgical intervention. On the other hand, percutaneous ablative therapies, including percutaneous ethanol injection (PEI), microwave coagulation therapy (MCT), and percutaneous radiofrequency ablation (RFA), have been developed and applied as alternative therapeutic options in cases of small HCC [2–8]. Recently, RFA has been performed as a first-line therapeutic option for early stage HCC; its survival outcomes are similar to those of hepatectomy [6–8]. However, a method for making the correct choice among therapeutic modalities to suit individual patients with early stage HCC remains to be determined.

The *Lens culinaris* agglutinin-reactive fraction of alpha-fetoprotein (AFP-L3) has been reported to be a specific marker for HCC [9–11]. Moreover, its level predicts the

malignant potential of HCC with subsequent unfavorable prognosis after treatment [12–16]. However, there have been few reports of the relationship between AFP-L3 status and prognosis in subgroups of HCC patients receiving different therapeutic modalities, such as hepatectomy and percutaneous ablative therapy.

The aim of this collaborative retrospective and prospective study was to evaluate the clinical usefulness of measuring AFP-L3 for prognostic predictor in patients with HCC after curative treatment.

Patients and Methods

Study Design

A total of 336 HCC patients underwent curative treatment at four participating hospitals (Niigata University Hospital, Ehime University Hospital, Shinsyu University Hospital, and Gunma University Hospital) from January 1998 to March 2005 and were investigated retrospectively. Of these patients, 232 underwent percutaneous ablative therapy and 104 underwent hepatectomy. Percutaneous ablative therapy comprised PEI in 90 patients, MCT in four patients, and RFA in 138 patients. Long-term survival data on these patients were confirmed as of the end of March 2005.

To evaluate the prognostic influence of AFP-L3 in two subgroups comparable for tumor extension, we prospectively investigated 189 patients diagnosed with early stage HCC initially at four hospitals from April 2005 to October 2007. We considered patients who had multiple (up to three) tumors measuring 3 cm or less in diameter as having early stage HCC. Forty-eight of 189 patients were excluded in this study, as they were received transcatheter treatment. As a result, 141 HCC patients, 99 who underwent percutaneous ablative therapy and 42 who underwent hepatectomy, were enrolled in the prospective study. Percutaneous ablative therapy comprised PEI in ten patients, MCT in two patients, and RFA in 87 patients. In these 141 patients, HCC recurrence was assessed by imaging modalities every 3 or 4 months after treatment and recurrence free survival was evaluated as of the end of December 2007. Informed consent was obtained from each patient, and the study protocol conformed with the ethical guidelines of the 1975 Declaration of Helsinki, as reflected in the a priori approval by our institution's human research committee.

Diagnosis of HCC and Laboratory Examination

In our study, the diagnosis was based essentially on imaging findings together with increments of tumor marker levels. We employed methods such as computed tomography (CT), magnetic resonance imaging, and CT during hepatic arteriography, considering hyperattenuation in the arterial phase with washout in the late phase to be a typical feature of HCC. In nine cases that showed atypical features on imaging, ultrasound-guided biopsies were performed.

Hepatic functional reserve was ranked by the criteria of the Child-Pugh scoring system. Serum alpha-fetoprotein (AFP) and des-gamma-carboxy prothrombin (DCP) were determined at each hospital by using commercially available kits. AFP-L3 percentage was measured at each hospital by liquid-binding assay (Wako Pure Chemical Industries Ltd, Osaka, Japan) [17]. AFP, AFP-L3, and DCP were measured in the same serum before treatment. Cut-off values for positivity for AFP, AFP-L3, and DCP were set at 20 ng/ml, 15%, and 40 mAU/ml, respectively, based on previous studies [18–20].

Treatment

Therapeutic modalities for individual patients were chosen according to hepatic functional reserve, tumor multiplicity, and tumor size. Percutaneous local ablative therapies were performed under a US-guided procedure, and its efficacy was evaluated with dynamic CT within a few days after treatment. Complete ablation of HCC was defined as non-enhancement of the lesion with surrounding liver parenchyma. Patients received additional sessions of an ablative therapy until the treatment was judged as complete. During the study, a Cool-tip RF System attached to a 200-W power generator (Radionics, Burlington, Massachusetts, USA) was the main device used for RFA treatment and Microtaze OT-110M (Alfresa-Pharma Co., Inc., Osaka, Japan) was used for MCT.

Statistical Analysis

Differences in the proportions of the independent binary variables were determined by Fisher's exact test. Continuous variables were compared by Student's *t*-test. Univariate survival and recurrence-free survival were determined by the Kaplan–Meier method. Log-rank test was used to test for equality of long-term survival and recurrence-free survival between the groups. Multivariate analyses of prognostic factors in the clinical features were performed by using Cox's stepwise proportional hazard model. The factors included for multivariate analyses were patient age, gender (female/male), HBsAg (negative/positive), Anti-HCV (negative/positive), Child-Pugh class (A/B, C), AFP (ng/ml) ($<20/\geq 20$), DCP (mAU/ml) ($<40/\geq 40$), AFP-L3 (%) ($<15/\geq 15$), tumor size (cm) ($<3/\geq 3$ or $\leq 2/>2$), and number of tumors (single/multiple). Statistical analyses were performed with SPSS 15.0 software (SPSS Japan Inc. Tokyo, Japan). A *P*-value of less than 0.05 was considered as statistically significant.

Results

Retrospective Study

Clinical Features of Patients Classified by Therapeutic Modality

A total of 336 HCC patients who underwent hepatectomy and percutaneous ablative therapy were investigated retrospectively. Patients who underwent percutaneous ablative therapy were characterized by older age ($P < 0.05$), positivity for antibody to hepatitis C virus (anti-HCV) ($P < 0.05$), and advanced Child-Pugh classification ($P < 0.05$). In contrast, patients who underwent hepatectomy were characterized by positivity for hepatitis B surface antigen (HBsAg) ($P < 0.05$), AFP-L3 ($P < 0.05$), and DCP ($P < 0.05$) elevation, as well as large tumor size ($P < 0.05$). No significant differences were observed between the two groups in terms of gender, AFP level, or number of tumors (Table 1A).

Univariate and Multivariate Analyses of the Factors Predicting Long-Term Patient Survival

The median observation time after treatment was 38.3 months (range, 1.0–146.2 months). Of the 232 patients who underwent percutaneous ablative therapy, 172 were alive and 60 had died from HCC, hepatic failure, and/or complications of cirrhosis. Of the 104 HCC patients who underwent hepatectomy, 68 were alive and 36 had died. The median survival time was 69.0 months in patients who had undergone percutaneous ablative therapy and 114.9 months in those who had undergone hepatectomy.

In the univariate analysis, anti-HCV status ($P = 0.034$), AFP status ($P = 0.007$), AFP-L3 status ($P = 0.001$), tumor size ($P = 0.001$), and number of tumors ($P = 0.045$) were significant prognostic factors of long-term survival in patients who underwent percutaneous ablative therapy. AFP status ($P = 0.011$), tumor size ($P = 0.006$), and number of tumors ($P < 0.001$) were significant prognostic factors in patients who underwent hepatectomy (Table 2).

Multivariate analysis by Cox's stepwise proportional hazard model revealed that tumor size ($P = 0.018$) and AFP-L3 status ($P = 0.013$) were significant independent prognostic factors for long-term survival in patients who underwent percutaneous ablative therapy. Tumor size ($P = 0.013$) and number of tumors ($P = 0.004$) were significant independent prognostic factors in patients who underwent hepatectomy (Table 3). We showed the long-term survival curves of two groups (with or without AFP-L3 elevation) in patients who underwent percutaneous ablative therapy and in those who underwent hepatectomy (Fig. 1). No significant difference in survival was observed

Table 1 Clinical features of patients with HCC classified by therapeutic modality in the retrospective and prospective studies

Variables	Percutaneous ablation ($n = 232$)	Hepatectomy ($n = 104$)
(A) Retrospective study		
Age (median, range)	68 (39–89)	65 (35–81)*
Gender		
Male	145 (62.5%)	66 (63.5%)
Female	87 (37.5%)	38 (36.5%)
HBsAg		
Negative	209 (90.1%)	73 (70.2%)
Positive	23 (9.9%)	31 (29.8%)*
Anti-HCV		
Negative	28 (12.1%)	45 (43.3%)
Positive	204 (87.9%)	59 (56.7%)*
Child-Pugh class		
A	177 (76.3%)	95 (91.3%)
B and C	55 (23.7%)	9 (8.7%)*
AFP (ng/ml)		
<20	65 (28.0%)	22 (21.2%)
≥20	167 (72.0%)	82 (78.8%)
DCP (mAU/ml)		
<40	149 (67.4%)	48 (51.1%)
≥40	72 (32.6%)	46 (48.9%)*
AFP-L3 (%)		
<15	181 (78.0%)	61 (58.7%)
≥15	51 (22.0%)	43 (41.3%)*
Tumor size (cm)		
<3	185 (79.7%)	33 (31.7%)
≥3	47 (20.3%)	71 (68.3%)*
Tumor number		
Single	148 (63.8%)	75 (72.1%)
Multiple	84 (36.2%)	29 (27.9%)
Variables	Percutaneous ablation ($n = 99$)	Hepatectomy ($n = 42$)
(B) Prospective study		
Age (median, range)	69 (36–85)	65 (40–80)
Gender		
Male	66 (66.7%)	24 (57.1%)
Female	33 (33.3%)	18 (42.9%)
HBsAg		
Negative	85 (85.9%)	29 (69.0%)
Positive	14 (14.1%)	13 (31.0%)*
Anti-HCV		
Negative	27 (27.3%)	15 (35.7%)
Positive	72 (72.7%)	27 (64.3%)
Child-Pugh class		
A	79 (79.8%)	39 (92.9%)
B and C	20 (20.2%)	3 (7.1%)

Table 1 continued

Variables	Percutaneous ablation ($n = 99$)	Hepatectomy ($n = 42$)
AFP (ng/ml)		
<20	64 (64.6%)	22 (52.40%)
≥20	35 (35.4%)	20 (47.6%)
DCP (mAU/ml)		
<40	63 (63.6%)	27 (64.3%)
≥40	35 (35.4%)	15 (35.7%)
AFP-L3 (%)		
<15	85 (85.9%)	33 (78.6%)
≥15	14 (14.1%)	9 (21.4%)
Tumor size (cm)		
≤2	63 (63.6%)	27 (64.3%)
>2	36 (36.4%)	15 (35.7%)
Tumor number		
Single	78 (78.8%)	34 (81.0%)
Multiple	21 (21.2%)	8 (19.0%)

HBsAg hepatitis B surface antigen, *HCV* hepatitis C virus, *AFP* alpha-fetoprotein, *DCP* des-gamma-carboxy prothrombin. Percentages are shown in parentheses

* $P < 0.05$ between groups by Fisher's exact test and Student's *t*-test

between the two AFP-L3 groups in patients who underwent hepatectomy ($P = 0.308$). In contrast, patients in the ablative therapy group whose AFP-L3 levels were below 15% lived significantly longer than those whose values were more than 15% ($P = 0.001$).

Prospective Study

Clinical Features of Patients with Early Stage HCC Classified by Therapeutic Modality

A total of 141 patients with early stage HCC were evaluated prospectively. Patients who underwent hepatectomy were characterized by positive for hepatitis B surface antigen (HBsAg) ($P < 0.05$). No significant differences were observed in age, gender, anti-HCV positivity, AFP status, AFP-L3 status, DCP status tumor size, and number of tumors between the two groups. Patients who underwent percutaneous ablative therapies tended to have an advanced Child-Pugh classification ($P = 0.055$) (Table 1B).

Univariate and Multivariate Analysis of the Factors Predicting Recurrence-Free Survival in Patients with Early Stage HCC

The median follow-up time after treatment was 12.0 months (range, 1.0–30.5 months). Among the 99 patients who underwent percutaneous ablation, recurrences were observed in 36 (36.4%). Among the 42 patients who underwent hepatectomy, recurrences were observed in six (14.3%).

In the univariate analysis, we found no significant difference in recurrence-free survival rates by pretreatment variables in patients who underwent percutaneous ablation, although AFP-L3 elevation ($P = 0.054$) tended to decrease recurrence-free survival. In contrast, tumor size ($P = 0.038$) and number of tumors ($P = 0.034$) were significant prognostic factors in patients who underwent hepatectomy (Table 2).

Although this prospective study was conducted over a short period of time, multivariate analysis of prognostic factors among the clinical features was performed and Cox's stepwise proportional hazard model revealed that HBsAg status ($P = 0.033$), DCP status ($P = 0.011$), and AFP-L3 status ($P = 0.006$) were significant independent prognostic factors of recurrence-free survival in patients who underwent percutaneous ablative therapies. On the other hand, we found no significant independent prognostic factors in patients who underwent hepatectomy (Table 3).

We showed recurrence-free survival rates between two groups—with or without AFP-L3 elevation—among

Table 2 Univariate analysis of the factors predicting long-term survival in the retrospective study and recurrence-free survival in the prospective study for patients who underwent percutaneous ablation and in those who underwent hepatectomy

Variables	Long-term survival		Recurrence-free survival	
	Percutaneous ablation *P*-value	Hepatectomy *P*-value	Percutaneous ablation *P*-value	Hepatectomy *P*-value
Gender (female/male)	0.907	0.525	0.225	0.194
HBsAg (negative/positive)	0.139	0.801	0.151	0.314
Anti-HCV (negative/positive)	0.034	0.963	0.194	0.171
Child-Pugh class (A/B,C)	0.083	0.235	0.293	0.487
AFP (ng/ml) (<20/≥20)	0.007	0.011	0.117	0.994
DCP (mAU/ml) (<40/≥40)	0.328	0.153	0.075	0.059
AFP-L3 (%) (<15/≥15)	0.001	0.308	0.054	0.530
Tumor size (cm) (<3/≥3)	0.001	0.006	0.063	0.038
Tumor number (single/multiple)	0.045	<0.001	0.667	0.034

HBsAg hepatitis B surface antigen, *HCV* hepatitis C virus, *AFP* alpha-fetoprotein, *DCP* des-gamma-carboxy prothrombin. *P*-value was calculated using Log-rank test

Table 3 Multivariate analysis of factors predicting long-term survival in the retrospective study and recurrence-free survival in the prospective study for patients who underwent percutaneous ablation and in those who underwent hepatectomy

Long-term survival			Recurrence-free survival		
Variables	Hazard ratio (95% CI)	*P*-value	Variables	Hazard ratio (95% CI)	*P*-value
Percutaneous ablation			Percutaneous ablation		
AFP-L3 (%)			HBsAg		
<15	1		Negative	1	
≥15	2.098 (1.169–3.765)	0.013	Positive	2.823 (1.090–7.310)	0.033
Tumor size (cm)			DCP		
<3	1		<40 (mAU/ml)	1	
≥3	1.998 (1.123–3.553)	0.018	≥40 (mAU/ml)	2.767 (1.267–6.046)	0.011
			AFP-L3		
			<15 (%)	1	
			≥15 (%)	3.463 (1.437–8.347)	0.006
Hepatectomy			Hepatectomy		
Tumor size (cm)			Tumor number		
<3	1		Single	1	
≥3	6.162 (1.457–26.064)	0.013	Multiple	4.654 (0.936–23.149)	0.060
Tumor number					
Single	1				
Multiple	3.170 (1.442–6.921)	0.004			

Hazard ratio and *P*-value were calculated using Cox's stepwise proportional hazard model

CI confidence interval, *AFP* alpha-fetoprotein, *HBsAg* hepatitis B surface antigen, *DCP* des-gamma-carboxy prothrombin

patients with early stage HCC who underwent percutaneous ablation and patients who underwent hepatectomy (Fig. 1). No significant difference was observed between groups with or without AFP-L3 elevation ($P = 0.53$) in patients who underwent hepatectomy. In contrast, a close-to-significant ($P = 0.054$) difference was observed between the groups of patients with and without AFP-L3 elevation who underwent percutaneous ablative therapy.

In summary, the results of the retrospective and prospective studies demonstrated that AFP-L3 status was a statistically significant prognostic factor of long-term survival and recurrence-free survival in patients who underwent percutaneous ablative therapy, but did not affect prognosis in patients who underwent hepatectomy.

Discussion

AFP-L3, a fucosylated species of AFP, is the product of alpha 1-6 fucosyltransferase (FUT8) in the presence of GDP-fucose. Our previous result revealed that FUT8 levels in HCC tissue were higher than those in the surrounding non-cancerous tissues and that FUT8 levels of HCC tissue increased in accordance with tumor dedifferentiation [21]. Several reports have shown the relationship between AFP-L3 status and histologic grade in HCC. Miyaaki et al. [16] showed that the frequency of poorly differentiated HCC was significantly higher in AFP-L3-positive patients than in AFP-L3-negative patients. Oka et al. [14] reported that AFP-L3-positive HCC was characterized by portal vein invasion and poorer differentiation, and that tumors in AFP-L3-positive HCC were advanced, even if they were small and the patient had a low serum AFP concentration. These results indicate the relationship between increased AFP-L3 level and increased degree of malignant behavior of HCC tissue.

Recurrence after treatment is an important factor affecting prognosis. Vascular invasion is an established adverse prognostic indicator of recurrence of HCC [22, 23]. Yamashita et al. [24] suggested that portal vein invasion is associated with AFP-L3 positivity, and that there is a strong possibility of intrahepatic invasion when there is positive conversion of this marker. Hayashi et al. [13] reported the relationship between AFP-L3 status and pattern of recurrence in patients with HCC. In their report, intrahepatic metastasis was significantly more common in AFP-L3-positive patients than in negative patients, although the recurrence rate of multicentric tumors did not differ significantly between the two groups with or without AFP-L3 elevation. From this point of view, hepatectomy—especially anatomical resection, which can remove venous tumor thrombi together with the primary lesion—is more suitable than local ablative therapies for the treatment of AFP-L3-positive patients.

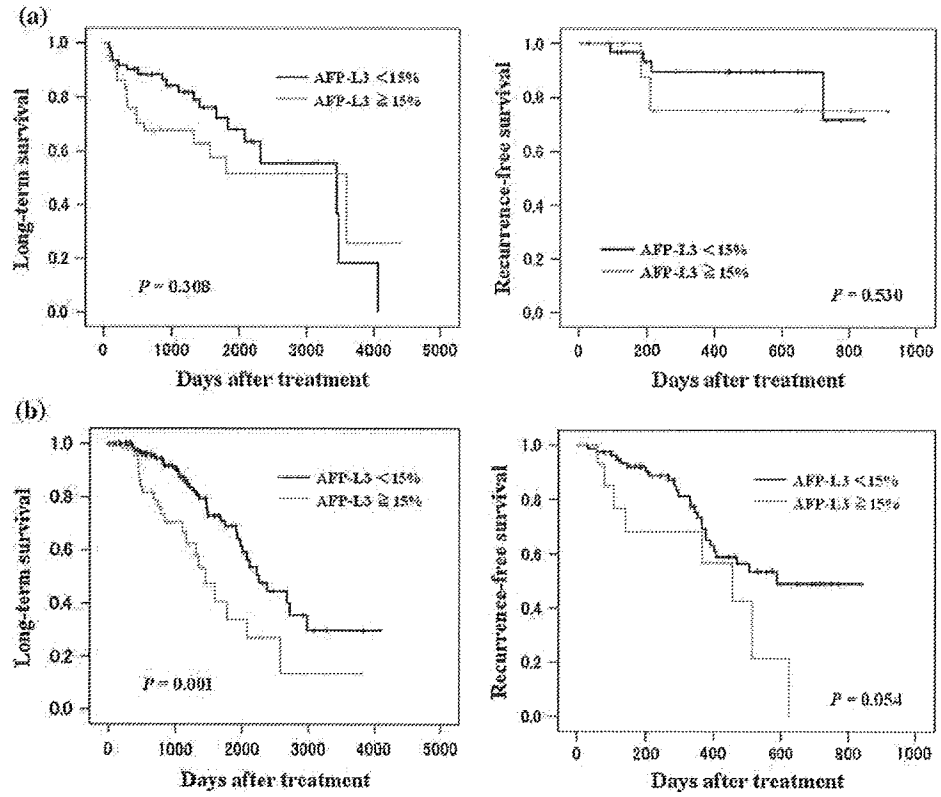

Fig. 1 Comparison of long-term survival rates and recurrence-free survival rates between patients with and without AFP-L3 elevation who underwent hepatectomy (**a**) and who underwent percutaneous ablation (**b**)

In our study, the pathological diagnosis was made by individual pathologists at each hospital. At Niigata University Hospital, 58 HCC patients underwent hepatectomy, of whom 23 had an elevated serum AFP-L3 level (≥15%) and the remaining 35 were negative for AFP-L3 (<15%). Among the 23 patients with AFP-L3 elevation, only two (8.7%) were diagnosed as having well-differentiated HCC on the basis of the resected specimens, 14 (60.9%) had moderately differentiated HCC, and seven (30.4%) had poorly differentiated HCC. In contrast, among the 35 patients who were negative for AFP-L3,. 7 (20.0%) were diagnosed as having well-differentiated HCC, 24 (68.6%) had moderately differentiated HCC, and only four (11.4%) had poorly differentiated HCC. Although no statistically significant differences were observed by Fisher's exact test, the group showing AFP-L3 elevation tended to have a poorer histopathological grading ($P = 0.141$). Only eight out of 331 patients who underwent percutaneous ablative therapy were diagnosed as having HCC on the basis of histological findings in four hospitals. Therefore, we were unable to investigate whether poorly differentiated tumors were more frequent in the groups who underwent percutaneous ablative therapy and hepatectomy. Portal vein invasion was investigated similarly in 58 patients, and was found to be present in six of 23 AFP-L3-positive patients and six of 35 AFP-L3-negative patients. No significant difference was observed between AFP-L3 and portal vein invasion in this limited investigation.

We demonstrated here in a multicenter retrospective study that AFP-L3 status was a significant prognostic factor affecting the long-term survival of patients who underwent percutaneous ablative therapy. In addition, to evaluate the prognostic influence of AFP-L3 in two subgroups comparable for tumor extension, we performed a multicenter prospective study to identify the prognostic factors for recurrence-free survival in patients with early stage HCC. Although this evaluation was conducted over a short period of time, we confirmed that AFP-L3 status was a significant prognostic predictor of recurrence-free survival in patients who underwent percutaneous ablative therapy, but it did not affect the prognosis of patients who underwent hepatectomy.

A number of studies have shown that AFP-L3 status is an independent prognostic factor in patients with HCC [12, 13, 15]. We previously reported that AFP-L3-positive (>15%) patients had a lower survival rate than negative (<15%) patients in subgroups with a low serum AFP concentration. Moreover, the statistically significant differences were more distinct in the subgroups with lower AFP concentrations [20]. However, the patients in these studies had received various treatments such as hepatectomy, RFA, and transcatheter arterial embolization, and

there have been few reports of the relationship between AFP-L3 status and prognosis in subgroups of HCC patients receiving different therapeutic modalities. Tateishi et al. [15] demonstrated that pre-treatment AFP-L3 positivity (>15%) was a significant predictor of HCC recurrence in patients who underwent curative ablation, and that AFP-L3 positivity after ablation was the strongest predictor of HCC recurrence by multivariate analysis. Although their study was performed in only one center and did not evaluate long-term survival, their results are compatible with ours.

Treatment of HCC patients with cirrhosis faces a dilemma in that minimization of damage to noncancerous liver tissue improves long-term survival, but incomplete treatment of subsequent HCC recurrences results in a poor prognosis. Accordingly, if a useful indicator of choice of therapeutic modality were to be available before the initial therapy, there would be several advantages in not only the treatment, but also the follow-up, of patients with HCC.

In conclusion, present results revealed that AFP-L3 had different impacts on prognosis in patients with HCC who underwent percutaneous ablative therapy and hepatectomy. Although this study was not a randomized control trial, AFP-L3 might be a promising scale to improve the prognostic estimate and appraisal of therapeutic outcome in patients with HCC.

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ORIGINAL ARTICLE—LIVER, PANCREAS, AND BILIARY TRACT

Regular surveillance by imaging for early detection and better prognosis of hepatocellular carcinoma in patients infected with hepatitis C virus

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Abstract
Purpose This study evaluated the usefulness of regular check-ups by ultrasonography and contrast-enhanced imaging for early detection of hepatocellular carcinoma (HCC) in a retrospective analysis.
Patients and methods From April 2001 to March 2007, 240 consecutive patients with HCC who were infected with hepatitis C virus (HCV) were divided into three groups. Patients diagnosed with HCC by repeated imaging constituted Group A (surveillance group). Group B comprised patients in whom HCC was detected during scheduled doctor visits for liver disease or other diseases such as diabetes. Group C comprised non-screened patients.
Results The prevalence of solitary tumors decreased from Group A through Group B to Group C (66, 48 and 24%, respectively, $P < 0.001$). The proportion of patients in stages I and II decreased from 83% (103/124) in Group A to 53% (42/79) in Group B and 24% (9/37) in Group C ($P < 0.001$). The proportion of patients who were treated with curative procedures, such as resection or ablation, was highest at 80% (99/124) in Group A, and lower at 53% (42/79) in Group B and 27% (10/37) in Group C ($P < 0.001$). The cumulative survival rate was better in Group A than B ($P < 0.05$), and in Group B than C ($P < 0.001$). Periodical medical check-ups without imaging did not necessarily detect early-stage disease, even when HCC-related markers including des-γ-carboxy prothrombin were tested.
Conclusions Regular surveillance with ultrasonography and contrast-enhanced imaging is useful for detecting early-stage HCC and increase chances for curative treatments in patients with HCV-related chronic liver disease.

Keywords Hepatocellular carcinoma · Early detection · Curative procedures · Survival rates · Surveillance

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Introduction

Hepatocellular carcinoma (HCC) is one of the most common malignancies worldwide [1], and patients with HCC continue to suffer an unsatisfactory prognosis. Surveillance for HCC should aim at decreasing mortality, and early detection is vital for therapeutic success. Serum levels of alpha-fetoprotein (AFP) and ultrasonography are widely accepted screening tests for early diagnosis of HCC [2–11].

However, serological markers including des-γ-carboxy prothrombin (DCP) and glycosylated AFP have shown limited success in detecting HCC in early stages [12–14]. Recent advances in imaging technologies allow the detection of early HCC, as reported in the guideline of the American Association for the Study of Liver Diseases [14]. Patients need to be surveyed for HCC, taking into consideration the incidence of HCC and cost-effectiveness. The discovery of hepatitis B virus (HBV) and hepatitis C virus (HCV), responsible for the majority of HCC cases [15], has enabled providers to identify the population at risk for HCC.

In Japan, HBV and HCV infections are associated with HCC in 15 and 80% of the cases, respectively [16, 17]. This retrospective study focused on HCV-associated HCC in Japan, and compared the efficacy of three methods for diagnosing HCC diagnosis. As the results show, regular repeated imaging was useful for early detection of HCC in patients infected with HCV.

Patients and methods

Patients

From April 2001 to March 2007, 338 consecutive patients were diagnosed with HCC in our institution. Among them, 240 patients infected with HCV were enrolled in this study. We retrospectively examined the procedure of diagnosis from clinical records and classified patients into one of three groups according to the method for diagnosing HCC. A total of 124 patients were diagnosed with HCC by regular imaging procedures such as ultrasonography, and they were categorized into the surveillance group (Group A). Hepatic damages such as rough surface pattern of the liver and dullness on the liver edge, as well as the detection of obvious varices on the first ultrasonography, led them to receive repeated imaging procedures. In 82% (102/124) of Group A patients, the interval between the latest imaging and diagnosis of HCC was within 6 months. The average interval between the latest imaging and diagnosis of HCC was 4.3 months [median, 3.6 months (range 2–11 months)]. They also received tests for HCC-related markers at least every 3 months. Group B comprised 79 patients who had been diagnosed with HCC during scheduled doctor visits for HCV-related liver disease or other diseases such as diabetes. These patients were not enrolled in a surveillance program at the time, and had not undergone any imaging procedures for at least 1 year before the diagnosis of HCC, while they received tests for HCC-related markers at least every 3 months. Among them, 26 patients received imaging due to elevated levels of HCC-related markers, such as AFP and DCP. In the remaining 53 patients in Group B, imaging was performed incidentally; they had not received imaging over the previous 1 year. The 37 patients who had not been screened for HCC were classified into Group C. They were diagnosed with HCC when symptoms developed (32 patients) or incidentally during a diagnostic workout for unrelated medical conditions such as traffic accident (5 patients). The study conformed to the ethical guidelines of the declaration of Helsinki, and was approved by the Institutional Review Board.

Surveillance strategy

Figure 1 outlines the surveillance program. Briefly, detection of any mass on ultrasonography instigated repeated imagings if the nodule diameter was up to 1 cm, or a dynamic study if the diameter exceeded 1 cm. HCC nodules are characterized by an intense contrast uptake during the arterial phase of dynamic computed tomography (CT) or magnetic resonance imaging (MRI), with the contrast washed away during the delayed or venous phase [12–14]. In the present study, the specific pattern of arterial uptake followed by venous washout was considered to represent HCC, since the value of "washout" in the venous phase has been recognized recently. If the vascular pattern on CT or MRI was not specific for HCC in a nodule with a diameter >1.5 cm, angiographically assisted CT or biopsy was undertaken to establish the diagnosis. Patients with nodules <1.5 cm in diameter who did not reveal HCC by angiographically assisted CT or biopsy underwent repeated surveillance procedures, and subsequent enlargement of the nodule during follow-ups indicated shifting to a dynamic study.

Diagnosis of cirrhosis

The diagnosis of chronic liver disease was made at the time of HCC detection by the following procedures.

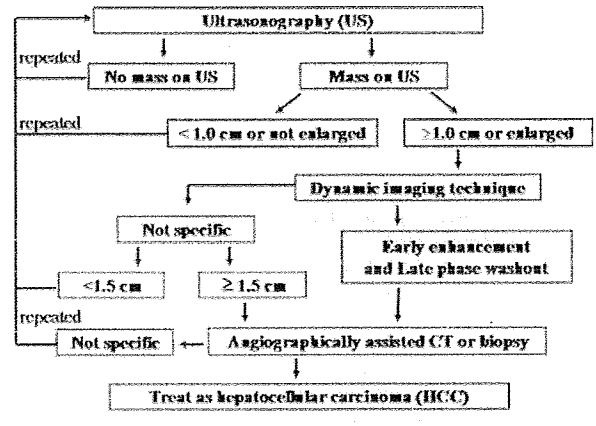

Fig. 1 Flow chart for the surveillance program including repeated imaging procedures

Histological findings were obtained in surgical specimens from 85 patients, and cirrhosis was diagnosed in 61 and chronic hepatitis or liver fibrosis in the remaining 24. Gastrointestinal varices in an additional 24 patients were considered diagnostic of cirrhosis. The remaining 131 patients were diagnosed to have cirrhosis according to the histologic scoring system [18].

Staging

Cancer stage was assessed by ultrasonography and dynamic CT or MRI. A total of 193 patients (80%) underwent angiography and/or angiographically assisted CT to obtain further details prior to resection, ablation or transarterial chemoembolization. In those patients, staging was also assessed by imaging on angiography and/or angiographically assisted CT. All patients underwent a chest X-ray, while additional investigations to detect metastases were performed only when extrahepatic involvement was suspected. Staging was not assessed by histologic findings on surgically resected specimens, even when they were available. Staging was determined according to the Liver Cancer Study Group of Japan classifications [19]. Staging was made also by the Milan criteria [20].

Treatment selection

Hepatic resection was indicated particularly to the patients with localized HCC who had maintained hepatic reserve capacity. When resection was contraindicated or refused by patients, the most appropriate treatment was selected according to the tumor status and liver function preserved [21]. Percutaneous ablation by ethanol injection [22] or radiofrequency ablation (RFA) [23] was considered in patients who had 1–3 nodules <3 cm in diameter, and were without vascular invasion or extrahepatic metastases. Transarterial chemoembolization [24] was offered to patients with either a paucifocal nodule not treatable by percutaneous ablation or multiple tumors not accompanied by thrombosis in main portal veins or extrahepatic metastasis. For the patients in Child-Pugh class C, transarterial chemoembolization was not recommended. In this study, resection and ablation were considered curative procedures based on their high efficacy.

Statistical analysis

The following 11 parameters were analyzed: age, sex, AFP, DCP, prothrombin activity, serum albumin level, total bilirubin level, liver state, tumor stage, HCC treatment and survival. Efficacy of the imaging program was evaluated by comparing clinical manifestation and prognosis among patients in the three groups. Differences in the distributions of tumor stage, tumor markers, and HCC treatment were evaluated by chi-squared test or Student's t test. Survival was calculated from the time of treatment start in patients who received it, and from the time of cancer diagnosis in patients without treatment. Data were censored at the time of death or the last follow-up visit. Survival was calculated according to the Kaplan–Meier method, and survival curves were compared by log-rank test. P values less than 0.05 were considered statistically significant.

Results

Background characteristics

There is no difference between Groups A and B in background of the patients except the programs with or without imaging. Table 1 details the background characteristics of all patients. Although the prevalence of cirrhosis was similar among the three groups, patients in Group C had poorer hepatic reserve with respect to albumin and total bilirubin levels ($P < 0.001$). The prevalence of non-cirrhotic liver in patients under 74 years was 26% (42/161), and 42% (33/79) in patients over 75 years. These differences were statistically significant ($P < 0.01$).

Features of HCC

The majority of HCC nodules were diagnosed by dynamic study including angiographically assisted CT, while HCC nodules in only 4 (1.7%) were confirmed by fine needle biopsy. Table 2 compares characteristics of HCC among the three groups. The frequency of solitary tumors was 66% (82/124) in Group A, 48% (38/79) in Group B, and 24% (9/37) in Group C, with a significant difference among three groups ($P < 0.001$). Nodules measuring less than 2 cm were detected in 64% (80/124) of patients in Group A, 25% (20/79) of those in Group B, and only 5% (2/37) of those in Group C ($P < 0.001$). The frequency of non-advanced tumor state decreased from 88% (109/124) in Group A, to 52% (41/79) in Group B, and to 27% (10/37) in Group C ($P < 0.001$). Cut-off values were set at 200 ng/ml and 40 mAU/ml, respectively, on AFP and DCP. In Group A, 47% (58/124) of the cases were negative for both, 46% (57/124) were positive for either, and 7% (9/124) were positive for both. In Group B, 11% (9/79) of the patients were negative for both, while 65% (51/79) were positive for ether, and 24% (19/79) were positive for both. In Group C, 11% (4/37) of the patients were negative for both, 57% (21/37) were positive for either, and 32% (12/37) were positive for both. These differences were statistically significant ($P < 0.001$). Thus, most patients in Groups B and C were positive for

Table 1 Background characteristics of patients

	Group A (surveillance) ($n = 124$)	Group B (scheduled doctor visits) ($n = 79$)	Group C (non-screened) ($n = 37$)	*P* value
Age at diagnosis of HCC (years)				
Median (range)	69.7 (49–89)	72.8 (49–87)	69.6 (50–87)	<0.05[b]
Gender				
Men	79 (64%)	52 (66%)	28 (76%)	NS
Women	45 (36%)	27 (34%)	9 (24%)	
History of blood transfusion	28 (22%)	19 (24%)	6 (16%)	NS
Excessive alcohol intake[a]	25 (20%)	20 (25%)	15 (49%)	NS
Liver state				
Not cirrhotic	34 (27%)	31 (39%)	10 (27%)	NS
Cirrhosis	90 (73%)	48 (61%)	27 (73%)	
Prothrombin activity (%)				
Median (range)	86 (48–125)	88 (57–135)	83 (39–124)	NS
Albumin (g/dl)				
Median (range)	3.6 (2.1–4.6)	3.8 (2.8–5.1)	3.4 (2.5–4.5)	<0.001[c]
Total bilirubin (mg/dl)				
Median (range)	0.9 (0.3–2.7)	0.8 (0.2–6.8)	1.4 (0.3–6.8)	<0.001[c]

NS not significant

[a] Excessive alcohol intake was defined as consumption of more 86 g alcohol/day

[b] Significant difference between Group B and Group A or Group C

[c] Significant difference between Group C and Group A or Group B

Table 2 Characteristics of the HCC nodule

	Group A (surveillance) ($n = 124$)	Group B (scheduled doctor visits) ($n = 79$)	Group C (non-screened) ($n = 37$)	*P* value
Solitary	82 (66%)	38 (48%)	9 (24%)	<0.001[b]
Size of main nodule				
–2 cm	80 (64%)	20 (25%)	2 (5%)	<0.001[b]
2.1–3 cm	31 (25%)	14 (18%)	6 (16%)	
3.1–5 cm	12 (10%)	33 (42%)	8 (22%)	
5.1 cm–	1 (1%)	12 (15%)	21 (57%)	
Vascular thrombus	4 (3%)	9 (11%)	10 (27%)	<0.001[b]
Distant metastases	1 (1%)	1 (1%)	5 (14%)	<0.001[c]
Tumor marker[a]				
Both negative	58 (47%)	9 (11%)	4 (11%)	<0.001[d]
Either positive	57 (46%)	51 (65%)	21 (57%)	
Both positive	9 (7%)	19 (24%)	12 (32%)	
Within the Milan criteria	109 (88%)	41 (52%)	10 (27%)	<0.001[b]

[a] HCC related tumor marker: AFP, DCP. Arbitrary cutoff values of 200 ng/ml and 40 mAU/ml were used for AFP and DCP, respectively

[b] Significant difference among all three groups

[c] Significant difference between Group C and Group A or Group B

[d] Significant difference between Group A and Group B or Group C

either or both AFP and DCP. Most patients in Group C who were in early tumor stages were diagnosed with HCC incidentally.

Figure 2a shows the distribution of tumor stages according to the Liver Cancer Study Group of Japan [19]. Proportions of patients in stages I and II were highest in the

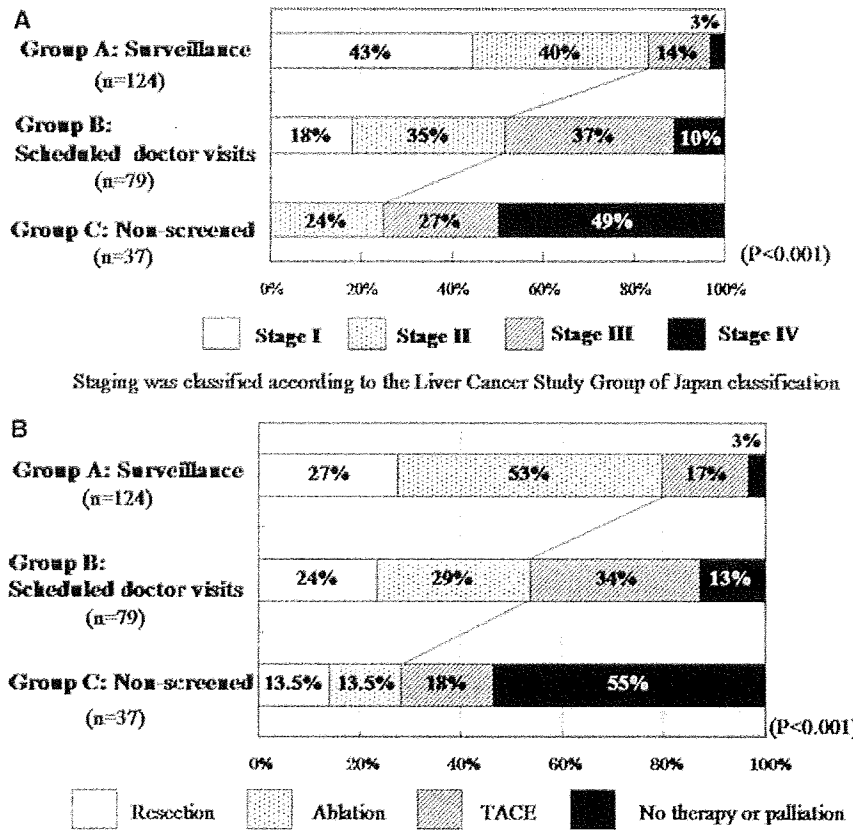

Fig. 2 **a** distribution of tumor stage according to the Liver Cancer Study Group of Japan [19]. **b** Distribution of treatment selected based on tumor stage and hepatic reserve

surveillance group (Group A); they decreased progressively through Group B to Group C ($P < 0.001$). The incidence of vascular thrombosis increased from 3% (4/124) in Group A to 11% (9/124) in Group B, and to 27% (10/37) in Group C ($P < 0.001$). Distant metastases were more frequent in Group C [14% (5/37)] than in Groups A and B [1% (1/124) and 1% (1/79), respectively] ($P < 0.001$). In Group A, the proportions of stages I and II was comparable between the patients with an interval between the latest imaging and diagnosis of HCC within 6 months and those with that of longer than 6 months [84% (86/102) vs. 77% (17/22)].

Treatment selection

Figure 2b shows the distribution of treatments selected based on the tumor stage and hepatic reserve. The proportion of patients treated with curative procedures, such as resection and ablation, was highest in Group A, and was lower in Groups B than C ($P < 0.001$). In Group C, the majority of patients received systemic chemotherapy or conservative care in hospice (palliation); most patients treated with curative procedures were diagnosed incidentally.

Survival

The median follow-up period was 35 months (range 3–94 months). During follow-ups, 148 patients died. Causes of death were cancer-related in 110 cases, liver failure in 6 (unrelated to treatment), gastrointestinal bleeding in 8, and others in the remaining 24. The distribution was similar between Groups A and B, while cancer-related causes were most prevalent (96%) in Group C. Figure 3a compares overall survival rates among the three groups. The cumulative survival rate was higher in group A than B ($P < 0.05$), and higher in group B than C ($P < 0.001$). Although survival rates of patients treated by curative procedures, such as resection and ablation, tended to be higher than the overall survival rate, there were no significant differences in the survival rates among patients in the three groups (Fig. 3b).

Discussion

For achieving better outcomes in patients with HCC, it is necessary to increase their eligibility for curative treatment. In the present study, 83% of patients under regular

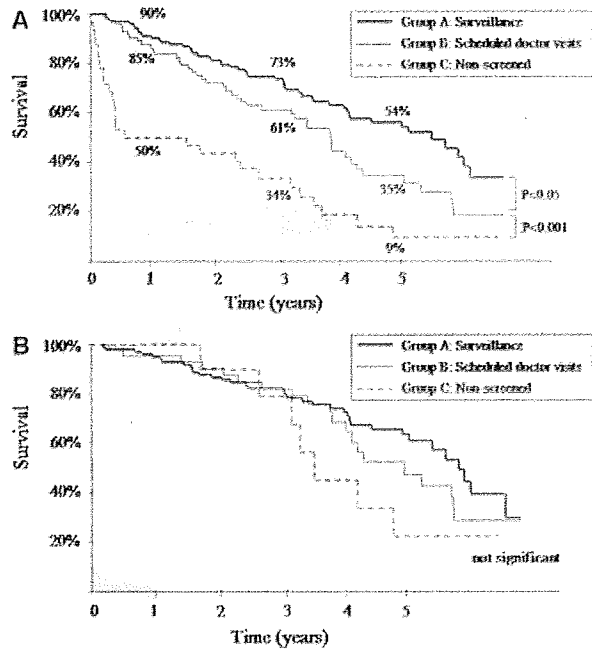

Fig. 3 **a** Survival rates in the three groups with different surveillance procedures. **b** Survival rates of the patients in three groups who had received curative treatments, such as resection and ablation

surveillance (Group A) were diagnosed with HCC at stage I or II, and the majority of them were indicated to curative treatments including surgical resection and RFA. As the results, patients in the surveillance group had a significantly better prognosis than those in the other groups without regular imaging screening (Group B) or none at all (Group C). Other reasons for the difference in prognosis among the three groups may include the following. Since the severity of underlying liver disease is a critical factor influencing the efficacy of surveillance programs, surveillance is reported to have few effects on improving the prognosis of patients with advanced cirrhosis [4, 10]. Although prevalence of cirrhosis was no different among the three groups, hepatic reserve was poorer in Group C than Group A or Group B. The dismal prognosis of patients in Group C, therefore, was attributed to either or both advanced tumor stage and poorer hepatic function. Indeed, analysis of only the patients who had received curative treatments, such as resection or ablation, revealed no significant differences in the survival among the three groups. However, the proportion of patients who had received curative treatment differed among the three groups with distinct diagnostic procedures.

Performance of surveillance would depend on the treatment selected and its efficacy. The 5-year survival of patients with a solitary HCC < 5 cm or up to 3 nodules <3 cm (Milan criteria [20]) exceeds 70% after transplantation, and that after resection surpasses 50% [12–14]. In general, transplantation offers the best long-term survival, and should be considered. In Japan, however, it is quite difficult for HCC patients to receive liver transplantation due to the shortage of donors [16], and liver resection is regarded as safe with less than 1% mortality [25]. Due to these background considerations, transplantation was not performed in the present study.

Should patients within the Milan criteria have undergone transplantation, differences in the outcome between Group A and Group B would have been reduced. In actuality, differences in the proportion of patients within the Milan criteria were lower that those in the distribution of stage I or II between them. The 5-year survival after resection was accomplished by 61% of patients with stage-II HCC and 73% of those with stage-I HCC; the staging was in accord with the definition of the Liver Cancer Study Group of Japan [16]. Thus, survival after resection in patients in Group A was comparable to that reported in transplanted patients within the Milan criteria. Indeed, the 5-year survival of patients in Group A who received curative treatments reached 63%. At present, the lack of sufficient liver donation is a worldwide problem in performing liver transplantation. Our results may indicate that surveillance by regular imaging can gain an excellent outcome where and when transplantation is hardly feasible, especially in patients with small HCC that can be treated by RFA or surgical resection.

With respect to HCC-related serological markers, most patients in Group A were negative for either AFP or DCP when they were diagnosed with HCC, in remarkable contrast to the majority of patients in Group B or C who were positive for either or both markers. In Group B, one-third of patients were tested for tumor markers during their scheduled doctor visits. However, the distribution of tumor stages was comparable between the patients with and without tumor-marker testing. Although yearly office visits would be helpful in early detection of HCC, periodical medical check-ups without screening by imaging may not necessarily detect early-stage disease, even if HCC-related markers such as AFP and DCP are tested for. This is the first report of poor performance of tumor markers including DCP in detecting early-stage HCC, and it suggests that various imaging procedures help detect HCC at a stage before levels of tumor markers elevate. Our results support the AASLD guideline that AFP alone should not be used for HCC screening when ultrasonography is not available [14]. On the other hand, it should be noted that 17% of patients in Group A in this study were diagnosed with HCC in stage III or IV, and 86% (18/21) of them were positive for either AFP or DCP. We therefore propose that HCC surveillance by regular imaging should be complemented with intermittent tests for tumor-markers, insofar as their elevated levels may reflect invisible nodules. As an

extension to this, repeated imaging with intermittent measurements of two different HCC-related tumor markers are included in the algorithm of the HCC surveillance program; it is described in Evidence-Based Clinical Practice Guidelines for HCC supported by the Japanese Ministry of Health, Labor and Welfare [26].

In a cirrhotic liver, small lesions detected by ultrasonography are likely to represent HCC. Even lesions not typical of cancer might transform into bona fide HCC during subsequent follow-ups. Generally, the incidence of HCC increases with the nodule size. In the present study, lesions >1 cm in diameter were examined by dynamic study, together with follow-ups by imaging at 3–6 month intervals, even when the appearance was atypical of HCC. Lesions >1.5 cm should be evaluated by dynamic study, preferably in combination with angiographically assisted CT or biopsy. Since the incidence of hypervascularity and moderately or poorly differentiated histology increases in HCC >1.5 cm [27–30], a 1.5-cm threshold in diameter may improve early diagnosis of HCC.

The AASLD guidelines recommend at-risk patients be screened by ultrasonography at 6–12-month intervals [14]. In our study, patients in Group B who had not undergone imaging for at least one year before the diagnosis often presented with advanced disease. A surveillance interval <12 months is therefore desirable. Although most patients in Group A were diagnosed with HCC within 6 months after the latest imaging, the proportion of stage I or II was similar between patients with the interval between the latest imaging and diagnosis of HCC below and above 6 months. However, optimal frequency of imaging was not determined in the present study. Further studies are required to determine the optimal screening interval.

Surveillance with imaging is feasible only in populations at risk for HCC, because radiological procedures are highly labor-intensive in comparison with serological testing. Major causes of cirrhosis in patients with HCC include HBV, HCV, alcoholic liver disease, exposure to aflatoxin, and possibly nonalcoholic steatohepatitis (NASH). Persistent infection with HBV or HCV is the most common cause of chronic liver disease including HCC, and increases the risk of HCC by approximately 20-fold. Heavy alcohol use and aflatoxin ingestion are environmental carcinogenic factors, and act synergistically with other risk factors [12–15]. In evaluating risks for HCC, geographic variations in incidence has to be taken into account. A recent study suggested an increased risk of HCC among patients with metabolic diseases such as diabetes or NASH [31–35]. However, the rate of HCC development in patients with NASH-related cirrhosis was significantly lower than that in those with HCV-related cirrhosis [33]. Thus, it remains uncertain how to assign surveillance programs to patients with metabolic disease.

In conclusion, surveillance programs including regular ultrasonography are useful for identifying HCC in early stages. HCC detected early is frequently indicated to curative treatments, such as resection and RFA, and is associated with better survival. Recently, several studies demonstrated that elderly patients infected with HCV developed HCC despite low-grade fibrosis stages [36, 37]. Elderly patients with HCV would be at high risk for the development of HCC, even though they do not show progression to cirrhosis. In the present study, most patients over 75 years were non-cirrhotic. Management of HCC should include early detection programs in all patients with HCV-related chronic liver disease including elderly patients in Japan.

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