start of therapy, probably before the therapeutic effect of ribavirin manifests. Among 38 patients with RVR, 16 (group C) were found to have a viral load of less than 3.7 log IU/mL in week 1. It was believed that these patients constitute super-high responders to peginterferon; and that a high SVR rate may be reached when the duration of the peginterferon plus ribavirin therapy is curtailed to less than 12 weeks. The viral load of the two patients who had discontinued treatment in weeks 17 and 19 was less than 3.7 log IU/mL in week 1, and both converted to SVR after discontinuation of treatment. Further studies on a larger scale are needed.

CONCLUSION

Rapid virologic responder is a predictive factor for SVR in peginterferon plus ribavirin therapy. However, it was proven that the RI that was computed from the early viral kinetics in this study is the first predictive factor for SVR as a substitute for RVR by multiple logistic regression analysis. Patients with a RI less than 1.0 and a viral load of less than 3.7 log IU/mL (below the detectable level) in week 1 are also considered to be super-high responders to peginterferon plus ribavirin, thus constituting a group for whom the treatment period may be shortened. Further studies on a larger scale are necessary.

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ORIGINAL ARTICLE—LIVER, PANCREAS, AND BILIARY TRACT

Effective prediction of outcome of combination therapy with pegylated interferon alpha 2b plus ribavirin in Japanese patients with genotype-1 chronic hepatitis C using early viral kinetics and new indices

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Abstract
Background The rates of sustained virologic response (SVR) and relapse with pegylated interferon alpha 2b (peginterferon) plus ribavirin in patients with genotype-1 chronic hepatitis C (CHC) are approximately 50 and 30%, respectively. We investigated whether SVR and transient response (TR) can be differentiated during treatment using new indices calculated from early viral kinetics and the timing of when hepatitis C virus (HCV)-RNA becomes undetectable.
Methods Peginterferon alpha 2b (1.5 μg/kg per week) plus weight-based ribavirin (600–1,000 mg/day) were administered to 141 patients with genotype-1 CHC for 48 weeks. The HCV-RNA loads were measured at baseline, 24 h, week 1, and week 2. The rebound index (RI, viral load at week 1 divided by viral load at 24 h) and the second rebound index (RI-2nd, viral load at week 2 divided by viral load at 24 h) were calculated.
Results With SVR, the viral load was reduced at 24 h, did not rise during week 1 (RI $\leq$ 1.0), and was significantly reduced at week 2 ($P < 0.05$). Viral loads with TR and non-response increased at week 1. The SVR rate was 90% with RI $\leq$ 1.0, 96% with rapid viral responders, and 93% with RI-2nd <0.7 and week 8 early viral responders. The SVR rate with these 3 groups was 90% and administration for 48 weeks was recommended. With other groups, the SVR rate was 23% and the TR rate was 77%. Administration for 72 weeks was therefore recommended.
Conclusions We distinguished SVR from TR during treatment using two indices (RI and RI-2nd) and the timing of HCV-RNA negativity.

Keywords Chronic hepatitis C · Pegylated interferon plus ribavirin · Early viral kinetics · Rebound index · Genotype 1

Abbreviations

SVR	Sustained virologic response
TR	Transient response
NR	Non-response
RI	Rebound index
RI-2nd	Second rebound index
RVR	Rapid viral responder
W8EVR	Week 8 early viral response
W12EVR	Week 12 early viral response
LVR	Late viral responder
NVR	Non-viral responder

Introduction

The first choice of treatment of genotype 1 chronic hepatitis C (CHC) is combination therapy with pegylated interferon alpha 2b (peginterferon) and ribavirin. The duration of treatment for genotype 1 is 48 weeks [1, 2]. Factors predictive of sustained virologic response (SVR) to

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peginterferon plus ribavirin include genotype, viral load, age, histology, and amino acid substitutions in the hepatitis C virus (HCV) [3–6]. None of these predictive factors is adequate in predicting SVR in patients with genotype 1 and a high viral load prior to treatment.

With the current standard of care of peginterferon plus ribavirin administered for 48 weeks, the SVR rate in patients with genotype 1 and a high viral load is about 50% [1, 2]. It has been recognized, however, that the SVR rate increases if the duration of treatment is extended to 72 weeks [7–9]. The result of treatment with peginterferon plus ribavirin is SVR, transient response (TR), or non-response (NR). At the end of treatment, about 80% of patients are HCV RNA-negative, but about 30% of these patients relapse (TR) after the end of treatment, resulting in an actual SVR rate of about 50%. To increase the SVR rate, this incidence of relapse must be reduced; to achieve this, the duration of treatment needs to be extended to 72 weeks for patients who may potentially relapse after the end of treatment [7]. Differentiation between SVR and TR is therefore essential during treatment.

HCV RNA negativity status at weeks 4 and 12 during treatment is important in predicting SVR [6, 10–12], with reduction in the SVR rate observed if HCV RNA is not undetectable by week 12. In other words, for the early determination of the therapeutic efficacy of peginterferon plus ribavirin treatment, HCV RNA negativity by week 4 (rapid viral responder: RVR), HCV RNA negativity by week 12 (early viral responder: EVR), and HCV RNA negativity by week 24 (late viral responder: LVR) are considered important. EVR is a better predictor of SVR than the predictive factors that can be determined prior to treatment. EVR is therefore considered to be an index of therapeutic effect in the early stage of peginterferon plus ribavirin treatment. In a recent trend, a duration of treatment of 72 weeks is being selected when HCV RNA is detected at week 12 but is undetectable at week 24. However, distinguishing SVR from TR during treatment is difficult by these two time points when HCV RNA is not detected.

For a more accurate determination of SVR and TR during treatment, HCV RNA was examined at week 8 in addition to weeks 4, 12, and 24, and the SVR rate was examined based on HCV RNA negativity at these time points. Early viral kinetics up to week 2, considered to be the index of the therapeutic effect of peginterferon alone, were also evaluated and two new indices were defined. Distinguishing SVR from TR during peginterferon treatment was possible by combining these new indices and the timing of HCV RNA negativity and allowed the assignment of patients to 48- or 72-week treatment as a result.

Patients and methods

A total of 149 patients with genotype 1 CHC were treated with peginterferon plus ribavirin at the Shin-Kokura Hospital between December 2004 and May 2006. Of these patients, treatment was interrupted in 8 patients, so this study was conducted on the remaining 141 patients who completed 48 weeks of treatment. Eighty were male and 61 were female, with ages ranging from 27 to 70 years (mean: 53.2 ± 10.8), and 109 individuals were naïve to interferon therapy. The viral load at enrollment exceeded 100,000 IU/ml. The results of liver biopsy conducted within 6 months of enrollment confirmed chronic hepatitis (F1-F3), and diagnosis was based on the scoring system of Desmet et al. [13]. All patients received 1.5 μg/kg of peginterferon alpha-2b (PegIntron, Schering-Plough, Osaka, Japan) administered subcutaneously once a week in combination with ribavirin (Rebetol, Schering-Plough, Osaka, Japan) administered orally at a daily dose of 600–1,000 mg based on body weight (600 mg for patients weighing less than 60 kg, 800 mg for those weighing 60–80 kg, and 1,000 mg for those weighing more than 80 kg).

Peginterferon was administered at 9:00 in the morning for the initial, second, and third doses. The HCV loads were measured immediately before the start of treatment, at 24 h post-dose, and at weeks 1 and 2. The coefficient derived by dividing the viral load at week 1 by that at 24 h was defined as the rebound index (RI), while the coefficient derived by dividing the viral load at week 2 by that at 24 h was called the second rebound index (RI-2nd). The patients were divided into the following 3 groups based on RI and RI-2nd: RI-A group (RI $\leq$ 1.0), RI-B group (RI > 1.0 and RI-2nd < 0.7), and RI-C group (RI > 1.0 and RI-2nd $\geq$ 0.7).

The qualitative test for HCV RNA was conducted 6 times (at weeks 4, 8, 12, and 24, at the end of treatment, and at week 24 after the end of treatment). Patients who were HCV RNA-negative by week 4 were considered rapid viral responders (RVR), patients who were HCV RNA-negative between weeks 5 and 12 were considered early viral responders (EVR), and patients HCV RNA-negative between weeks 13 and 24 were considered late viral responders (LVR). EVR was further divided into week 8 EVR (HCV RNA-negative between weeks 5 and 8, W8EVR) and week 12 EVR (HCV RNA-negative between weeks 9 and 12, W12EVR). Patients HCV RNA-positive at week 24 were considered non-viral responders (NVR). Patients who remained HCV RNA-negative up to 24 weeks after the end of treatment were considered to have achieved SVR. Patients HCV RNA-negative by week 24 of treatment but who became positive again after the end of treatment were considered TR. Patients who failed to achieve HCV RNA negativity by the end of treatment were

considered NR. None of these patients were HCV RNA-negative between weeks 25 and 48.

Sera were collected from the patients before and during treatment and frozen for determination of viral loads by a quantitative HCV RNA PCR assay (COBAS Amplicor HCV Monitor Test v2.0 using a 10-fold dilution method, Roche Diagnostics, Tokyo, Japan), which has a low threshold of quantitation of 5,000 IU/ml and an outer limit of quantitation of 5,100,000 IU/ml. A qualitative test for serum HCV RNA was performed using Amplicor-HCV kit version 2.0 (Roche Diagnostics, Tokyo, Japan) and the results were labeled positive or negative. The lower limit of detection was 50 IU/ml. All testing was performed at a single reference laboratory. The HCV genotype was determined by a type-specific primer from the core region of the HCV genome. Genotyping was carried out as described previously [14].

Criteria for exclusion were [1] clinical or biochemical evidence of hepatic decompensation and advanced cirrhosis identified by ascites, encephalopathy, or hepatocellular carcinoma [2], white blood cell count of less than 3,000/mm^3 and platelet count of less than 50,000/mm^3 [3], concurrent liver disease other than hepatitis C (hepatitis B surface antigen- or human immunodeficiency virus-positive), [4] excessive active alcohol consumption over 60 g/day or drug abuse, [5] severe psychiatric disease, or [6] antiviral or corticosteroid therapy within the 12 months prior to enrollment. Both peginterferon alpha-2b and ribavirin were discontinued if the hemoglobin level, white blood cell count, or platelet count fell below 8.5 g/dl, 1,000/mm^3 and 25,000/mm^3, respectively. Treatment was discontinued if severe general fatigue, hyperthyroidism, interstitial pneumonia, or severe hemolytic problems developed, if continuation of treatment was judged not to be possible by the attending physician, or if the patient no longer desired to continue treatment.

Informed consent

The study protocol was approved by the Institutional Ethics Committee of Shin-Kokura Hospital, and all patients gave informed consent to participate in this study. The study was conducted in accordance with the ethical guidelines of the Declaration of Helsinki and International Conference on Harmonization Guidelines for Good Clinical Practice.

Statistical analysis

Differences between viral loads between two groups were analyzed using the Student's *t* test and Mann–Whitney rank-sum test. We conducted analysis using the Kruskal Wallis test for three-group (SVR, TR, and NR) and five-group (RVR, W8EVR, W12EVR, LVR and NVR) comparisons. All statistical analyses were conducted on a Macintosh computer using StatView 5.0 (Abacus Concepts, Berkeley, CA, USA). *P* values of <0.05 were considered to be statistically significant.

Results

Baseline characteristics of patients grouped by SVR, TR and NR

SVR was observed in 72 patients (51.1%), TR in 40 patients (28.4%), and NR in 29 patients (20.6%). The characteristics at enrollment of patients showing SVR, TR, or NR are presented in Table 1. The mean age of SVR, TR, and NR patients was 50.8, 56.6, and 56.0 years, respectively. There were no significant inter-group differences in mean age, gender, or pre-treatment test results (alanine aminotransferase, hemoglobin level, platelet count, and viral load).

Table 1 Baseline characteristics of patients by response (SVR, TR, and NR)

	SVR $n = 72$	TR $n = 40$	NR $n = 29$	Total $n = 141$	*P* value[a]
Age (years)	50.8 (11.3)	56.6 (8.6)	56 (10.4)	53.2 (10.8)	0.084
Male (%)	41 (56)	24 (52)	19 (65)	84 (57)	
Laboratory					
ALT (IU/l)	88 (82)	86 (53)	94 (78)	90 (74)	0.808
Hemoglobin level (g/dl)	14.5 (1.4)	14.6 (1.3)	14.6 (1.0)	14.5 (1.3)	0.917
Platelet count ($\times 10^4$/mm^3)	19 (6)	17 (6)	20 (10)	19 (7)	0.707
HCV RNA loads ($\times 10^3$ IU/ml)	2299 (1634)	2228 (1344)	2390 (1501)	2298 (1524)	0.953
Body mass index (kg/m^2)	23.6 (5.7)	24.8 (2.9)	23.9 (3.6)	24 (4.9)	0.536

Values are represented as means with standard deviation in parentheses or as absolute values with percentages in parentheses

SVR sustained virologic response, *TR* transient response, *NR* non response, *ALT* alanine aminotransferase

[a] Kruskal Wallis Test

Early viral kinetics, RI and RI-2nd relative to SVR, TR and NR

Viral kinetics up to the first two weeks after the start of treatment are shown for the SVR, TR, and NR groups (Fig. 1 and Table 2). The viral load at 24 h for the SVR and TR groups (226,000 and 229,000 IU/ml) was reduced significantly compared to the NR group (523,000 IU/ml) ($P \leq 0.05$). The differences of the viral loads for three groups at weeks 1 and 2 were significant ($P \leq 0.0001$). The viral load of the SVR group at weeks 1 and 2 was significantly lower than that of the TR group ($P < 0.01$ and $P < 0.05$). The former was reduced at week 1 with no increases thereafter, and the viral load at week 2 (76,000 IU/ml) was significantly lower than at 24 h (226,000 IU/ml, $P < 0.01$). The viral load of the TR group rose to 397,000 IU/ml at week 1 and was reduced to 169,000 IU/ml at week 2. This reduction was not significant when compared against that at 24 h (229,000 IU/ml). The viral load of the NR group rose again to 1,206,000 IU/ml at week 1 and was reduced to 615,000 IU/ml at week 2, which was still higher than that at 24 h (523,000 IU/ml).

RI and RI-2nd for the SVR, TR and NR groups are shown in Table 2. RI for the SVR group (0.8) was below 1.0. The differences of the RI for the three groups were significant ($P \leq 0.0001$). RI-2nd for the SVR, TR and NR groups was 0.3, 0.8, and 1.3, respectively, with the highest value observed with the NR group. The differences of the RI-2nd for three groups were significant ($P \leq 0.0001$). RI-2nd for the TR group was significantly higher than for the SVR group ($P < 0.05$).

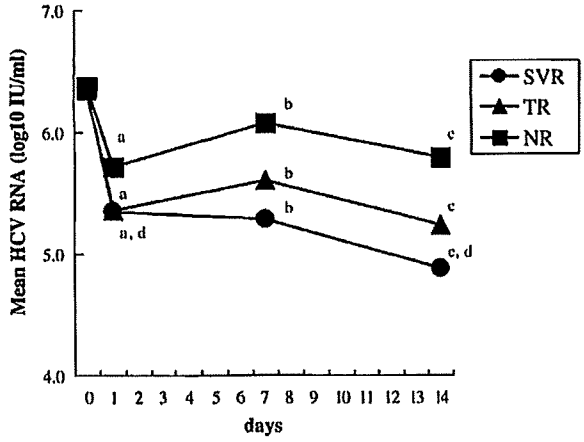

Fig. 1 HCV-RNA kinetics during the first 2 weeks of treatment by SVR (*black circle*), TR (*black triangle*), and NR (*black square*). a $P < 0.05$, b $P < 0.0001$, c $P < 0.0001$ (Kruskal Wallis test), d $P < 0.01$ (hour 24 vs. week 2 in SVR). *SVR* sustained virologic response, *TR* transient response, *NR* non-response

SVR rates, early viral kinetics, RI and RI-2nd relative to the timing of HCV RNA negativity

RVR, W8EVR, W12EVR, LVR, and NVR were observed in 26 (18.4%), 31 (22.0%), 31 (22.0%), 24 (17.0%), and 29 (20.6%), respectively. The SVR rate with the RVR, W8EVR, W12EVR, and LVR groups was 96.2% (25/26), 83.9% (26/31), 54.8% (17/31), and 16.7% (4/24), respectively. None in the NVR group exhibited the absence of HCV-RNA at the end of treatment. The HCV RNA kinetics for the RVR, W8EVR, W12EVR, LVR, and NVR groups up to week 2 of treatment are shown in Fig. 2 and Table 3. The viral load of the RVR group was rapidly reduced to 143,000 IU/ml by 24 h, with a further drop to 55,000 IU/ml at week 1. At week 2, the viral load was reduced to 8,000 IU/ml, which was significantly less than that at 24 h ($P < 0.001$). The viral loads for the W8EVR and W12EVR groups were reduced to 186,000 IU/ml and 134,000 IU/ml, respectively, by 24 h but rose to 231,000 IU/ml and

Table 2 Kinetics of HCV RNA during the first 2 weeks of treatment by response (SVR, TR, and NR)

	SVR ($n = 72$)		TR ($n = 40$)		NR ($n = 29$)		P value[a]
	Mean	SD	Mean	SD	Mean	SD	
HCV loads (×1000 IU/ml)							
Before treatment	2299	(1634)[b]	2228	(1344)	2390	(1501)	0.9538
Hour 24	226	(328)	229	(249)	523	(518)	0.0102
Week 1	190	(302)	397	(399)	1206	(811)	<0.0001
Week 2	76	(193)[b]	169	(249)	615	(617)	<0.0001
Rebound index	0.8	(0.9)	2.9	(3.1)	3.1	(1.8)	<0.0001
Rebound index 2	0.3	(0.6)	0.8	(0.7)	1.3	(1.1)	<0.0001

Values represent means with ranges in parentheses

[a] Kruskal Wallis Test

[b] $P < 0.01$ (hour 24 vs. week 2 in SVR)

Abbreviations *SVR* sustained virologic response, *TR* transient response, *NR* non response, *SD* standard deviation

277,000 IU/ml, respectively, at week 1. The viral load of the W8EVR group at week 2 was reduced to 49,000 IU/ml, which was significantly less than that at 24 h ($P < 0.001$). The viral load of the W12EVR group, on the other hand, was 119,000 IU/ml, which was not significantly less than that at 24 h. The viral load of the LVR group at 24 h (563,000 IU/ml) was higher than that of RVR, W8EVR, or W12EVR. The viral load rose further to 674,000 IU/ml at week 1, and although a reduction to 361,000 IU/ml was observed at week 2, it was still significantly greater than with RVR, W8EVR, and W12EVR ($P < 0.001$, $P < 0.001$, and $P < 0.01$, respectively). The viral load (523,000 IU/ml) of the NVR group at 24 h was similar to that of the LVR group. It rose after one week and was not low in the second week (615,000 IU/ml) compared to that at 24 h. The differences of the viral loads for five groups were significant at hour 24, week 1, or week 2 ($P \leq 0.0001$).

RI and RI-2nd of the RVR, W8EVR, W12EVR, LVR, and NVR groups are shown in Table 3. RI of RVR (0.4) was the lowest and was lower than that of W8EVR (2.3), W12EVR (2.4), LVR (1.5), and NVR (3.1). RI-2nd of NVR (1.3) was the highest, being higher than RVR (0.1), W8EVR (0.5), W12EVR (0.7), and LVR (0.7) ($P < 0.001$, $P < 0.01$, $P < 0.05$, and $P < 0.01$, respectively). The mean RI-2nd for other than NVR was below 0.7. The differences of the RI or RI-2nd for five groups were significant ($P \leq 0.0001$).

SVR, TR, and NR rates relative to RI and RI-2nd

The 3 groups (RI-A, RI-B, and RI-C) and 4 groups (RVR, W8EVR, W12EVR, and LVR) were combined and then divided into 12 groups, with SVR, TR, and NR grouped by

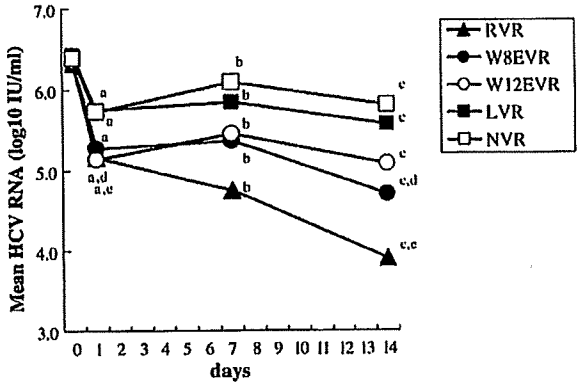

Fig. 2 HCV RNA kinetics by RVR (*black triangle*), W8EVR (*black circle*), W12EVR (*white circle*), LVR (*black square*), and NVR (*white square*) during the first 2 weeks of treatment a $P < 0.001$, b $P < 0.0001$, c $P < 0.0001$ (Kruskal Wallis test), d $P < 0.001$ (hour 24 vs. week 2 in W8EVR), e $P < 0.001$ (hour 24 vs. week 2 in RVR). *RVR* rapid viral response, *W8EVR*, week 8 early viral response, *W12EVR* week 12 early viral response, *LVR* late viral response, *NVR* non-viral response

RI and RI-2 and by RVR, W8EVR, W12EVR, and LVR (Fig. 3). The SVR, TR, and NR rates were 90.2% (46/51), 9.8% (5/51), and 0% (0/51), respectively, with RI-A (RI $\leq$ 1.0), 55.6% (25/45), 40.0% (18/45), and 4.4% (2/45), respectively, with RI-B (RI > 1.0, RI-2nd < 0.7), and 2.2% (1/45), 37.8% (17/45), and 60.0% (27/45), respectively, with RI-C (RI > 1.0, RI-2nd $\geq$ 0.7). The SVR rate for RI-A and RVR was 90.2% (46/51) and 96.2% (25/26), respectively. The SVR rate for RI-B and W8EVR was 93.3% (14/15). The SVR rate for the patients in these 3 areas was 89.7% (61/68), suggesting that they represent the population for which 48-week treatment is appropriate. Among the 112 patients who became HCV RNA-negative at week 24, 60.7% (68/112) were in the above 48-week regimen area. In particular, the SVR rate (2.2%) from RI-C was very low and the TR and NR rates were 37.8% and 60.0%, respectively. Among W8EVR, W12EVR, and LVR in the range outside that for a 48-week regimen, the SVR rate (25.0%, 11/44) was low but the TR rate (75.0%, 33/44) was high. Thus extension of the treatment period was considered necessary, and a 72-week regimen was recommended.

Discussion

This is the first study in which SVR, TR, and NR, in response to 48 weeks of peginterferon plus ribavirin treatment, were successfully distinguished in the early stage of treatment. This was possible by using new indices (rebound index: RI, and second rebound index: RI-2nd) calculated from early viral kinetics and the timing of when HCV RNA becomes undetectable. This allows for the TR group to be treated for 72 weeks, potentially raising the SVR rate.

In the treatment of genotype 1 CHC with peginterferon plus ribavirin, relapse occurs in about 30% of patients after the end of treatment [1, 2]. In LVR (late viral responders), in particular, the percentage of relapse is high (59%) after 48 weeks of treatment [7]. It is vital to reduce the relapse rate (TR rate) and raise the SVR rate. This requires (1) dose increase and (2) prolongation of the period of treatment. In Japanese patients, the dose of peginterferon must often be reduced because of the onset of such adverse events as neutropenia, thrombocytopenia, and malaise, and thus dose increase is not a feasible option. The dose of ribavirin must also be reduced in some patients due to ribavirin-induced anemia, and likewise, any increase in dose is not feasible [15]. Thus extending the duration of treatment to 72 weeks is considered necessary, and it becomes essential to distinguish the population for which 48-week treatment is adequate from the population for which 72-week treatment is necessary. In a previous study, HCV RNA-negativity was determined at weeks 12 and 24 [7]. SVR was noted in

Table 3 Kinetics of HCV RNA during the first 2 weeks of treatment by response (RVR, W8EVR, W12EVR, LVR, or NVR)

	RVR ($n = 26$)		W8 EVR ($n = 31$)		W12 EVR ($n = 31$)		LVR ($n = 24$)		NVR ($n = 29$)		P value[a]
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	
HCV loads (×1000 IU/ml)											
Before	2102	(1636)[b]	2271	(1536)[c]	2259	(1744)	2625	(1278)	2390	(1501)	0.9172
24 h	143	(182)	186	(191)	134	(95)	563	(558)	523	(518)	0.0007
1 week	55	(66)	231	(213)	277	(325)	674	(503)	1206	(811)	<0.0001
2 week	8	(5)[b]	49	(86)[c]	119	(264)	361	(299)	615	(617)	<0.0001
Rebound index	0.4	(0.3)	2.3	(3.6)	2.4	(2.8)	1.5	(0.6)	3.1	(1.8)	<0.0001
Rebound index 2	0.1	(0.1)	0.5	(0.6)	0.7	(1.4)	0.7	(0.4)	1.3	(1.1)	<0.0001

RVR rapid viral response, *W8EVR* week 8 early viral response, *W12EVR* week 12 early viral response, *LVR* late viral response, *NVR* non viral response, *SD* standard deviation

[a] Kruskal Wallis Test

[b] $P < 0.001$ (hour 24 vs. week 2 in RVR)

[c] $P < 0.001$ (hour 24 vs. week 2 in W8 EVR)

18% even when treatment was continued to week 48 in patients who were HCV RNA-positive at week 12 but HCV RNA-negative at week 24 (LVR) [7]. If treatment is continued for 72 weeks, these patients will receive drugs unnecessarily for an extra 24 weeks. On the other hand, in this study, the SVR rate with W8EVR and W12EVR was 84% and 55%, respectively. The SVR rate for the overall EVR (W8EVR + W12EVR) was 69%, with a relapse rate of 31%. By treating these relapsed patients for 72 weeks, a higher SVR can be expected.

For more effective treatment with peginterferon plus ribavirin, indices besides the currently used index (the time to HCV RNA negativity) should be introduced and evaluated. In this study, we succeeded in differentiating the populations to be treated for 48 and 72 weeks more accurately by measuring the viral loads up to week 2 after the start of treatment and calculating two new indices (rebound index and second rebound index). Unrelated to ribavirin, lowering the dose of peginterferon at the early stage of treatment reduces the SVR rate [16]. In other words, the therapeutic effect of peginterferon, independent of that of ribavirin at the early stage of treatment, is expected to be responsible for SVR and EVR, which is believed to occur before ribavirin takes effect. Early viral kinetics were determined up to week 2, which are believed to express the therapeutic effect of peginterferon. The serum concentration of peginterferon alpha 2b peaks after 24 h, followed by a gradual decline [17, 18]. The viral load is therefore reduced by 24 h but increases in week 1 [19, 20]. A large dose of peginterferon at each administration results in a marked reduction in the viral load at 24 h but the viral load increases in week 1 regardless of the dose. In the responder group, the viral load continues to decline each week thereafter [20]. This trend is also seen with peginterferon monotherapy [17]. On the other hand, in the SVR group, in particular the RVR group, it was noted that a number of patients did not experience an increase in the viral load at week 1. As shown in Fig. 1, in SVR, the viral load does not increase at week 1, while a return of viral loads is seen in TR and NR. The viral loads of SVR and TR were lower than that of NR at week 2. The viral load in week 1 divided by the viral load at 24 h was therefore defined as the rebound index (RI). The RI of SVR is 0.8 (less than 1.0), which is less than that for TR or NR. Among the RI of RVR, W8EVR, W12EVR, LVR, and NVR, only that of RVR was below 1.0. Among the 26 RVR patients, 24 (92%) exhibited RI-A (RI: $\leq$ 1.0) without a rise in week 1 (Fig. 3). The SVR rate with RI-A was 90%. It was believed that this group (RI-A, RI: $\leq$ 1.0) was composed of high responders to peginterferon. Because no decline in the viral load is noted in non-responders after week 2 [20], the viral load at week 2 divided by the viral load at 24 h was defined as the second rebound index (RI-2nd). Compared with SVR and TR, RI-2nd with NR was high. The patients with high RI-2nd were suspected to be poor responders or non-responders to peginterferon. RI-2nd of those other than NVR was below 0.7; and therefore 0.7 was adopted as the reference value for RI-2nd.

Based on the results of our study (Fig. 3), the SVR rate was very high (about 90%) in 3 areas of SVR: RI-A (RI: $\leq$ 1.0), RVR, and RI-B (RI-2: $<$ 0.7) and W8EVR. These are believed to represent the areas for which 48-week treatment is recommended. About 60% of the 112 patients who became HCV RNA-negative within 24 weeks were in this area, and the SVR rate of the remaining 40% was low (25%) while the TR rate was high (75%). It was therefore thought that 72-week treatment is needed for these patients.

In peginterferon and ribavirin treatment, the status of EVR is important. When HCV RNA-negativity is not achieved by week 12, the SVR rate becomes very low [10].

	RVR N=26	W8EVR N=31	W12EVR N=36	LVR N=26	NVR N=29	Total N=148
RI>1.0, RI-2nd≥0.7 RI-C, N=52		△△△	△△△△ ○△△△△△	△△△△△△ ○△△△△△	□□□ □□□□□□ □□□□□□ □□□□□□ □□□□□□	SVR:3.9% TR:44.2% NR:51.9%
RI>1.0, RI-2nd<0.7 RI-B, N=45	○△	○○△ ○○○○○○ ○○○○○○	△△ ○△△△△△ ○○○○○○	△△△△△△ ○○○△△△	□□	SVR:55.6% TR:30.0% NR:4.4%
RI≤1.0 RI-A, N=51	○○○○○○ ○○○○○○ ○○○○○○ ○○○○○○	△ ○○○○○○ ○○○○○○	○○○△△△ ○○○○○○	○△		SVR:90.2% TR:9.8% NR:0.0%
Total N=148	SVR:96.2% TR:3.8% NR:0.0%	SVR:83.9% TR:16.1% NR:0.0%	SVR:47.2% TR:52.8% NR:0.0%	SVR:19.2% TR:80.8% NR:0.0%	SVR:0.0% TR:0.0% NR:100%	SVR:49.3% TR:31.1% NR:19.6%

Fig. 3 SVR, TR, and NR by RI and RI-2nd as well as RVR, W8EVR, W12EVR, LVR, and NVR. *SVR* white circle, *TR* white triangle, *NR* white square, *SVR* sustained virologic response, *TR* transient response, *NR* non-response, *RI* rebound index, *RI-2nd* rebound index second, *RVR* rapid viral response, *W8EVR* week 8 early viral response, *W12EVR* week 12 early viral response, *LVR* late viral response, *NVR* non-viral response

In our study, SVR was low (below 20%) among the LVR who became negative for HCV RNA between weeks 12 and 24. Mangia et al. reported that to raise the SVR rate, treatment for 48 weeks is needed if HCV RNA becomes negative at week 8, while treatment for 72 weeks is needed if HCV RNA negativity is observed at week 12 [21]. In our study, the SVR rate with RVR was 96% and was also very high (84%) with W8EVR achieving HCV RNA negativity between 5 and 8 weeks. On the other hand, the SVR rate was low (55%) in patients who became HCV RNA-negative between weeks 9 and 12. These findings suggested that in treating Japanese patients with CHC with peginterferon plus ribavirin for 48 weeks, EVR should be qualified at week 8 rather than at week 12. Therefore, for evaluation, EVR patients who became HCV RNA-negative by week 8 were classified as W8EVR and those who became HCV RNA-negative by week 12 were classified as W12EVR. Early viral kinetics of both W8EVR and W12EVR indicated a rebound at week 1 but the viral load of W8EVR at week 2 was significantly lower than that at 24 h. On the other hand, the reduction in the viral load of W12EVR at week 2 was not significant when compared against that at 24 h. A significant reduction in the viral load was observed with RVR and W8EVR at week 2 compared to that at 24 h, and the SVR rates were correspondingly very high. It was believed that the reduction in the viral load at week 2 is important.

Real-time PCR assay is now commonly used and is more sensitive for detecting serum HCV than the COBAS Amplicor HCV Monitor assay. Its use may have allowed viral detection for a longer period of time, possibly resulting in the number of RVR and EVR patients being reduced while the SVR rate in RVR and EVR patients was increased. Examination of the SVR rate by the timing of HCV RNA negativity using real time PCR assay will be necessary in the future.

Reduction in the duration of treatment is being investigated for the good responders to peginterferon plus ribavirin treatment. In RVR patients who achieve HCV RNA-negativity at week 4, the SVR rate is reported to be 89% when treatment is continued for 24 weeks [11]. In this study, all patients in the RI-A (RI $\leq$ 1.0) and RVR area became SVR; thus they were believed to be extremely good responders. A more detailed investigation with a larger number of subjects is necessary to elucidate the question of a reduction in the duration of therapy.

The explanation of early viral kinetics by SVR, TR, and NR is highly complex and is impractical in clinical use. In this study, RI and RI-2nd calculated from early viral kinetics were used. It is believed that the simplified RI and RI-2nd are effective indices to determine the therapeutic efficacy of peginterferon therapy alone. By combining these two new indices and the indices for therapeutic efficacy of peginterferon plus ribavirin (RVR, W8EVR, W12EVR and LVR), SVR was distinguished from TR during treatment. With the aid of these indices, it is believed that a more effective peginterferon plus ribavirin treatment will be possible. We used these new indices in this study, and the measurement of HCV RNA levels was conducted using the COBAS Amplicor HCV Monitor assay. Since the range of detection of HCV is narrow with this assay, there were many patients with pretreatment HCV levels above the limit of detection. The timing of HCV RNA negativity and examination based on the HCV levels at week 1 and week 2 needs to be conducted using real time PCR assay in future studies. A larger scale study should be conducted to examine the duration of treatment for patients who are on reduced doses of peginterferon and ribavirin.

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EDITOR'S CHOICE

Visceral fat accumulation is an independent risk factor for hepatocellular carcinoma recurrence after curative treatment in patients with suspected NASH

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ABSTRACT

Background and Aim: Visceral fat accumulation reportedly increases the risk of hepatocellular carcinoma (HCC) development in patients with chronic liver disease. However, it has not beeen fully elucidated whether visceral fat accumulation increases the risk of HCC recurrence after curative treatment in patients with suspected non-alcoholic steatohepatitis (NASH). Therefore this was investigated in the current study.

Methods: 62 patients with naïve HCC with suspected NASH were enrolled. All were curatively treated with percutaneous radiofrequency ablation between 1999 and 2006. The visceral fat area (VFA) was determined in each patient from CT images, taken at the time of HCC diagnosis. Patients were divided into two groups based on VFA: the high VFA group (>130 cm^2 in males, >90 cm^2 in females, n = 27) and the others (n = 35). The effects of VFA on HCC recurrence were analysed together with other factors including patients' background, tumour-related factors and liver function-related factors.

Results: The cumulative recurrence rates differed significantly between the two groups; 15.9, 56.5 and 75.1% at 1, 2 and 3 years, respectively, in the high VFA group, and 9.7, 31.1 and 43.1%, respectively, in the controls (p = 0.018). Multivariate analysis indicated visceral fat accumulation (risk ratio 1.08, per 10 cm^2, p = 0.046) and older age (risk ratio 1.06 per 1 year, p = 0.04) as independent risk factors of HCC recurrence.

Conclusions: Visceral fat accumulation is an independent risk factor of HCC recurrence after curative treatment in patients with suspected NASH.

Hepatocellular carcinoma (HCC) is the fifth leading cause of cancer death worldwide, accounting for >500 000 deaths annually, showing an increasing incidence throughout the world.[1 2] HCC usually develops in patients with advanced liver fibrosis due to chronic liver diseases such as chronic hepatitis B, chronic hepatitis C, alcoholic liver disease[2 3] and haemochromatosis.[4] In addition, non-alcoholic steatohepatitis (NASH) is considered to be another important liver disease preceding cirrhosis and HCC. Non-alcoholic fatty liver disease (NAFLD), increasing in prevalence in Western countries as well as in Japan because of the increasing prevalence of obesity,[5] is shown to be a clinical condition that may progress to NASH and, subsequently, HCC.[6]

Recently, obesity has also been reported to be a risk factor of HCC development in patients with chronic liver diseases other than NASH.[7–9] Emerging data have indicated that neuroendocrine systems that regulate weight and energy metabolism play a pivotal role in the development of hepatic steatosis.[10] Several mechanisms have been proposed by which accumulation and anatomic distribution of fat are related to the development of hepatic steatosis and fibrosis.[11] Eguchi *et al* reported that the severity of fatty liver was positively related to visceral fat accumulation and insulin resistance in both obese and non-obese NAFLD patients without viral hepatitis.[11] This suggests that hepatic fat infiltration in NAFLD may be influenced by visceral fat accumulation independently of body mass index (BMI).

Based on these findings, we hypothesised that the previously reported relationship between BMI and the risk of HCC was mediated by visceral fat accumulation, and the latter is directly associated with HCC development. Considering the low incidence of HCC among patients with NAFLD in general, the study will require a very large cohort, where it would be difficult to measure visceral fat accurately in each subject. On the other hand, abdominal CT, with which visceral fat accumulation can be calculated, is performed routinely on HCC patients. Since recurrence of HCC is very frequent, the effect of visceral fat can be evaluated in a relatively small cohort. Thus, we conducted this study enrolling consecutive, non-viral non-alcoholic patients with naïve HCC who received curative radiofrequency ablation (RFA) treatment to evaluate the impact of visceral fat accumulation on HCC recurrence.

PATIENTS AND METHODS

Patients

A total of 777 patients received RFA as the treatment for naïve HCC at the authors' institution between January 1999 and December 2006. There were 596 patients positive for hepatitis C virus HCV antibody (HCVAb), 74 patients positive for hepatitis B surface antigen (HBsAg) and 6 patients positive for both HCVAb and HBsAg. A past history of alcohol consumption of >20 g ethanol per day was found in another 27 patients, and intractable ascites, which may interfere with visceral fat measurement, was found in 12 on CT scan at diagnosis. Excluding these patients, we enrolled the remaining 62 patients as a cohort and analysed the relationship between visceral fat accumulation and intrahepatic recurrence of HCC. All of these patients were negative for antimitochondrial antibody and antinuclear antibody. Haemochromatosis was not diagnosed in

any of them. Diagnosis of diabetes mellitus was based on medical history or a 75 g oral glucose tolerance test. Dyslipidaemia was defined as blood total cholesterol concentration >220 mg/dl or triglyceride >150 mg/dl, or a history of taking oral drugs for dyslipidaemia. BMI was calculated as body weight in kilograms (kg) divided twice by body height in metres (m), which was also routinely measured at the first visit of each patient.

Diagnosis of HCC

Contrast-enhanced dynamic CT was performed on patients with suspected HCC detected by ultrasonography. Multidetector row CT with 4, 8 or 16 detector rows (Aquilion 4/16; Toshiba, Tokyo, Japan; LightSpeed Qx/I, LightSpeed Ultra; GE Healthcare, Milwaukee, Wisconsin, USA) was used. Images were obtained in early arterial, late arterial and equilibrium phases at 28, 40 and 120 s after starting bolus injection of iodinated contrast material. Images were reconstructed with a section thickness of 5 mm and a reconstruction interval of 5 mm (a section thickness of 2–2.5 mm, an interval of 1.5–2 mm and a field of view (FOV) of 24–35 cm for arterial phases). The diagnosis of HCC was based on typical findings on CT—that is, hyperattenuation in the arterial phase and hypoattenuation in the equilibrium phase.[12 13] Ultrasound-guided biopsy of the non-tumorous liver was performed in most cases, except for patients with a risk of haemorrhage such as severe thrombocytopenia. Background tissue was pathologically graded based on NAFLD activity score[14] and Ishak fibrosis staging.[15] Ultrasound-guided tumour biopsy was also applied when CT findings were inconclusive regarding HCC. HCC tissue was pathologically graded based on Edmondson–Steiner criteria.[16]

Measurement of visceral fat, subcutaneous fat and waist circumference

Fat tissue area and waist circumference were measured in each patient by analysing a CT image at the level of the umbilicus with the software Slim Vision (KGT, Tokyo, Japan).[17] The subcutaneous fat area (SFA) was defined as the sum of extraperitoneal fat area between the skin and muscle on the CT image, which showed attenuation ranging from −150 to −50 Housefield units. The visceral fat area (VFA) was defined as the sum of the intraperitoneal fat area showing the same attenuation. The patients were divided into two groups according to the cut-off levels of VFA recommended by Oka *et al* for metabolic syndrome in the Japanese population[18]: the high VFA group (defined as VFA >130 cm^2 in males and VFA >90 cm^2 in females, n = 27) and the controls (n = 35).

Treatment and follow-up

The indication criteria for RFA consisted of total bilirubin concentration <3.0 mg/dl, platelet count >$50\times10^5/mm^3$ and prothrombin activity >50%. Patients with portal vein tumour thrombosis, massive refractory ascites or extrahepatic metastasis were excluded. In general, RFA was performed on patients with three or fewer lesions, each <3.0 cm in diameter. However, we also performed ablation on patients who did not meet these criteria when complete ablation could be anticipated in all tumours without deteriorating liver function. The detailed procedure was meticulously described elsewhere.[19] In brief, percutaneous RFA was performed using internally cooled tip RF electrodes with a 2 or 3 cm long exposed metallic tip (Radionics, Burlington, Massachusetts, USA) on patients after intravenous administration of pentazocine (30 mg), hydroxyzine (25 mg) and atropine (0.5 mg). After local anaesthesia, the electrode was inserted under ultrasound guidance. During ablation, the tip temperature was monitored and kept below 20 °C with cold saline. In the case of 3 cm tip electrodes, the output started at 60 W, was increased at 20 W/min until tissue impedance overshot and then was decreased by 20 W and maintained for 12 min. With 2 cm tip electrodes, the output started at 40 W, was increased at 20 W/min until impedance overshot and then was maintained for 6 min.

After RFA, dynamic CT was performed with a section thickness of 0.5 cm to evaluate treatment efficacy. Complete ablation was defined as hypoattenuation of the whole lesion together with the surrounding liver parenchyma as a safety margin. Patients received additional RFA until complete ablation was confirmed for each HCC nodule.

Major complications were defined as those which, if left untreated, might threaten the patient's life, lead to substantial morbidity and disability, or result in hospital admission or substantially lengthen hospital stay according to the previously described guideline.[20–22]

The follow-up consisted of monthly blood tests and monitoring of tumour markers (alpha-fetoprotein (AFP), lens culinaris agglutinin-reactive fraction of AFP (AFP-L3), and des-gamma-carboxy prothrombin (DCP)) at the outpatient clinic, with ultrasonography and dynamic CT scan performed every 4 months. Tumour recurrence was diagnosed based on the same criteria applied to the naïve HCC. Intrahepatic HCC recurrence was classified as recurrence either at a site distant from the primary tumour or adjacent to the treated site (local tumour progression).

Analysis of recurrence

The end points consisted of the interval between the first ablation and the detection of HCC recurrence, death without recurrence or the last examination until 31 December 2007, whichever came first. Death without recurrence was treated as censored data. Cumulative recurrence of HCC was estimated using the Kaplan–Meier method and compared with log-rank test. The effect of VFA on recurrence was assessed with a multivariate Cox proportional hazard regression model controlling for factors shown to be significant in univariate analysis.[23] We compared the number and size of tumours at the time of recurrence between the high VFA group and the controls. Changes in VFA between the time of first diagnosis of HCC and at recurrence were also compared in patients with HCC recurrence.

Analysis of survival

Survival time was defined as the interval between the first treatment and death or the last visit to the outpatient clinic up to 31 December 2007. A cumulative survival curve was plotted by the Kaplan–Meier method, and the difference between the groups was assessed by log-rank test. The effect of VFA on survival was assessed by multivariate Cox proportional hazard regression adjusted for factors shown to be significant in univariate analysis.

Statistical analysis

We used the following variables obtained at the initial RFA in the analysis of recurrence and survival: age, gender, tumour factors including size and number of nodules, BMI, VFA, SFA, waist circumference, aspartate aminotransferase (AST) level,

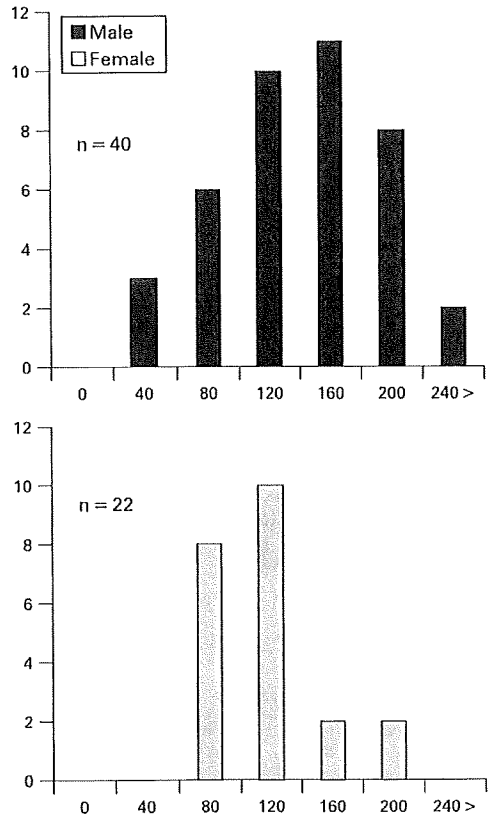

Figure 1 The mean value of the visceral fat area among male and female patients was 120.3 and 93.5cm², respectively.

platelet counts, HCC-specific biomarkers—that is, AFP, DCP and AFP-L3—and liver function as classified by Child–Pugh scores (5–6 points, class A; 7–9 points, class B; and 10–12 points, class C) based on albumin, total bilirubin, prothrombin activity, and the presence of ascites or hepatic encephalopathy. Nominal categorical data were represented by corresponding binary dummy variables. Categorical variables were compared by χ^2 test (not ordered) or Cochran–Armitage test (ordered). Continuous variables were compared with unpaired Student t test (parametric) or Wilcoxon test (non-parametric). Data processing and analysis were performed using the S-PLUS 2000 (MathSoft, Seattle, Washington, USA).

RESULTS

Patient profile

The patients were divided into two groups according to the cut-off levels of VFA recommended by Oka *et al* for metabolic syndrome in the Japanese population.[18] The distribution of VFA in the present study, when shown separately in men and women, was similar to the previous data (fig 1). The mean VSA (SD) among male patients was 120.3 (56.6) cm² and that among female patient was 93.5 (37.8) cm² ($p = 0.036$). The baseline characteristics of each group were shown in table 1. The mean follow-up period was 3.4 (1.9) years in the high VFA group and 3.5 (1.9) years in controls ($p = 0.95$). The mean age was 67.5 (8.9) years in the high VFA group and 69.6 (9.7) years in controls ($p = 0.37$). The proportion of obese patients was significantly larger in the high VFA group ($p = 0.001$). However, there were no significant differences in the prevalence of comorbidities, diabetes mellitus and dyslipidaemia ($p = 0.52$ and $p = 0.78$, respectively). Hepatitis B core antibody (HBcAb) was positive in 8 (29.6%) patients in the high VFA group and 10 patients (28.6%) in the controls ($p = 0.93$). Other parameters were not significantly different between the two groups.

Histology of the non-tumorous liver

Histological diagnosis of NASH is often difficult at the time of HCC development due to decreased fat deposition in hepatocytes, especially when cirrhosis has been established. Histology of the non-tumorous liver was available in 60 of 62 patients (26 of the high VFA group and 34 of the controls), and we compared NAFLD activity score and fibrosis stages between the two groups (table 2). There were 6 (23.1%) patients who were diagnosed as having NASH (NAFLD activity score ≥5) among the high VFA group and 7 (20.6%) patients among the controls ($p = 0.92$). Cirrhosis was identified in 18 (69.2%) patients among the high VFA group and 20 (58.8%) patients in the controls ($p = 0.43$).

Percutaneous ablation

Complete ablation was achieved in all patients, and major complications were identified in 3/27 (11.1%) in the high VFA group and 1/35 (2.9%) in the controls ($p = 0.19$): neoplastic seeding (n = 1), liver infarction (n = 2) and liver abscess (n = 1). There was no treatment-related death. Although statistical consideration may not be appropriate because of the small size of the studied population, the safety and efficacy of RFA were considered to be acceptable in spite of obesity frequently found in the studied patients.

Analysis of recurrence

By the end of the follow-up, tumour recurrence was identified in 15/27 (55.6%) patients in the high VFA group and in 11/35 (31.4%) in the controls. The cumulative recurrence rates at 1, 2 and 3 years were 15.9, 56.5 and 75.1%, respectively, in the high VFA group and 9.7, 31.1 and 43.1%, respectively, in the controls (fig 2). The difference between the two groups was statistically significant ($p = 0.018$). Local tumour progression was found only in two patients in the high VFA group, who were treated as censored in the analysis of recurrence. The diameter of

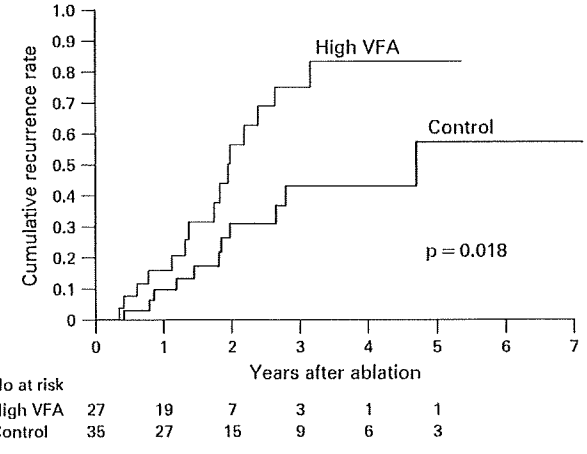

Figure 2 The cumulative recurrence rates at 1, 2 and 3 years were 15.9, 56.5 and 75.1%, respectively, in the high visceral fat area (VFA) group and 9.7, 31.1 and 43.1%, respectively, in the controls ($p = 0.018$).

Table 1 Baseline characteristics of patients

Variables (No)	High VFA group (n = 27)	Control group (n = 35)	p Value
Age*	67.5 (8.9)	69.6 (9.7)	0.37§
Male	16 (59.3%)	24 (68.6%)	0.59‡
Diabetes mellitus	13 (48.1%)	14 (40.0%)	0.52‡
Dyslipidaemia	7 (25.9%)	8 (22.9%)	0.78‡
BMI (kg/m²)*	26.9 (3.8)	23.8 (3.2)	0.001§
VFA (cm²)*	152.1 (45.2)	79.0 (29.9)	0.001§
SFA (cm²)*	180.2 (96.0)	113.9 (56.2)	0.001§
Waist circumference (cm)*	92.5 (10.0)	84.2 (10.6)	0.001§
Serum albumin (g/dl)†	3.9 (3.5–4.2)	3.8 (3.4–4.1)	0.40§
AST (IU/l)†	39.0 (27.5–50.0)	38.0 (24.5–53.5)	0.94§
Total bilirubin (mg/dl)†	0.8 (0.6–1.0)	0.8 (0.6–1.5)	0.31§
Platelet count (×10³/μl)	135 (102–187)	137 (122–189)	0.96§
Prothrombin activity (%)†	75.0 (67.5–83.8)	78.0 (63.3–88.8)	0.68§
Child–Pugh classification			0.30¶
Class A	22 (81.5%)	26 (74.3%)	
Class B	5 (8.5%)	6 (17.1%)	
Class C	0 (0%)	3 (8.6%)	
HBcAb positive	8 (29.6%)	10 (28.6%)	0.93‡
Tumour size (mm)*	32.1 (15.1)	27.6 (15.8)	0.26§
Number of nodules			0.45‡
UninodularUninodular	16 (59.3%)	24 (68.6%)	
MultinodularMultinodular	11 (40.7%)	11 (31.4%)	
AFP >100 ng/ml	4 (14.8%)	6 (17.1%)	0.80‡
DCP >100 mAU/ml	5 (18.5%)	7 (20.0%)	0.88‡
AFP-L3 >15%	4 (14.8%)	6 (17.1%)	0.80‡

*Expressed as mean (SD). †Expressed as median (25th–75th percentiles). ‡χ^2 tests. §Unpaired Student t test. ¶ Cochran–Armitage test.
AFP, alpha-fetoprotein; AFP-L3, lens culinaris agglutinin-reactive fraction of AFP; AST, aspartate transaminase; BMI, body mass index; DCP, des-gamma-carboxy prothrombin; HBcAb, hepatitis B core antibody; SFA, subcutaneous fat area; VFA, visceral fat area.

recurrent tumour nodules was 18.9 (7.1) mm in the high VFA group and 23.7 (10.8) mm in the controls (p = 0.30), and the number of nodules was 1.4 (0.8) in the high VFA group and 2.2 (1.8) in the controls ($P = 0.42$). By the end of follow-up, a total of 26 patients had HCC recurrence. The mean VFA at the first diagnosis of HCC and that at recurrence were 121.5 (52.0) and 129.5 (79.8) cm², respectively (p = 0.27 by paired t test).

Univariate analysis identified older age (p = 0.006) and VFA (p = 0.022) as significant predictors for recurrence after curative ablation (table 3). BMI, SFA, waist circumference, comorbidity with diabetes mellitus, presence of dyslipidaemia and other variables did not show statistical significance at the $p<0.05$ level. Gender, although not significant in univariate analysis, was included in the multivariate analysis because the distribution of VFA was significantly different between men and women (fig 1). We also included comorbidity with diabetes mellitus and dyslipidaemia as variables to elucidate whether VFA was an independent risk factor for HCC recurrence. As a result, only age and VFA retained significance in the multivariate analysis (table 4).

Table 2 Histology of the non-tumorous liver

Variables (No)	High VFA group (n = 26)	Control group (n = 34)	p Value
NAFLD activity score			0.92*
0–2	17 (65.4%)	22 (64.7%)	
3–4	3 (11.5%)	5 (14.7%)	
5–8 (NASH)	6 (23.1%)	7 (20.6%)	
Cirrhosis	18 (69.2%)	20 (58.8%)	0.43*

*χ^2 tests.
NAFLD, non-alcoholic fatty liver disease; NASH, non-alcoholic steatohepatitis; VFA, visceral fat area.

Analysis of survival

There were 14 deaths during the observation period: 6 deaths in the high VFA group and 8 deaths in the control group. The causes of death were HCC progression in 9, hepatic failure in 2, upper gastrointestinal bleeding in 1 and liver-unrelated causes in 2 patients. There was no significant difference in cumulative survival rate between the two groups (p = 0.99).

DISCUSSION

Currently in Japan, HCV infection is the most common background liver disease of HCC, followed by HBV infection and alcoholic liver disease. Primary biliary cirrhosis and other chronic liver diseases are an uncommon cause of HCC. The underlying liver disease in the remaining HCC cases, constituting about 5% of the total, is usually not determined, with histological examination revealing only cryptogenic cirrhosis. NASH is thought to be responsible for a substantial portion of such HCC cases, but characteristic pathology is rarely present at the stage of HCC development.

HCC is characterised by extremely frequent intrahepatic recurrence even after successful curative treatments.[24] Both surgical hepatectomy and medical ablation are locoregional treatment in that the background liver diseases are left untreated. Liver transplantation forms an exception, after which recurrence is rare if the indication is appropriate. Two modes of intrahepatic recurrence have been distinguished: de novo carcinogenesis and intrahepatic metastasis.[25] The factors responsible for the development of primary HCC, such as age,

Table 3 Univariate analysis of recurrence after curative ablation

Variables	OR (95% CI)	p Value
Age (per 1 year)	1.06 (1.02 to 1.10)	0.006
Male vs female	0.81 (0.37 to 1.76)	0.60
Diabetes mellitus	1.49 (0.69 to 3.22)	0.30
Dyslipidaemia	2.01 (0.69 to 5.86)	0.20
BMI (per 1.0 kg/m^2)	1.04 (0.92 to 1.16)	0.53
VFA (per 10 cm^2)	1.07 (1.01 to 1.14)	0.022
SFA (per 10 cm^2)	1.02 (0.97 to 1.07)	0.40
Waist circumference (per 1.0 cm)	1.01 (0.98 to 1.05)	0.56
Serum albumin (per 1.0 g/dL)	0.64 (0.30 to 1.38)	0.25
AST (per 10 IU/l)	0.95 (0.80 to 1.13)	0.54
Total bilirubin (per 1.0 mg/dl)	0.44 (0.14 to 1.37)	0.16
Platelet counts (per 10×10^3/μl)	0.96 (0.90 to 1.03)	0.25
Prothrombin activity (per 1.0%)	0.99 (0.97 to 1.02)	0.67
HBcAb positive	1.68 (0.67 to 4.18)	0.27
Tumour size (per 1.0 mm)	1.00 (0.98 to 1.03)	0.84
Multinodular vs. Uninodular	1.57 (0.70 to 3.50)	0.27
AFP >100 ng/ml	1.58 (0.63 to 4.02)	0.33
DCP >40 mAU/ml	2.12 (0.98 to 4.62)	0.056
AFP-L3 >15%	1.47 (0.59 to 3.68)	0.41

AFP, alpha-fetoprotein; AFP-L3, lens culinaris agglutinin-reactive fraction of AFP; AST, aspartate aminotransferase; BMI, body mass index; DCP, des-gamma-carboxy prothrombin; HBcAb, hepatitis B core antibody; SFA, subcutaneous fat area; VFA, visceral fat area.

sex, fibrosis stage and the presence of viral hepatitis, will also affect de novo carcinogenesis,[9] whereas the factors related to the primary HCC, such as the size and number of tumours, pathological grade and the presence of vascular invasion, will affect the possibility of intrahepatic metastasis.[26 27]

In the present study, multivariate analysis showed older age and accumulation of visceral fat as independent risk factors for recurrence of non-B non-C non-alcoholic HCC after curative ablation. The former factor was compatible with previous reports, whereas the relationship between visceral fat accumulation and the risk of HCC recurrence was a new finding in the present study. It may be suspected that visceral fat accumulation affected the efficacy of RFA. However, there were only two cases of local tumour progression, the most common sequel of insufficient ablation, which was not considered as an event but was censored in the present study. Thus, patients with high VFA showed a higher incidence of intrahepatic recurrence distant from the primary lesion, suggesting that visceral fat accumulation was associated with metachronous de novo carcinogenesis.

In the current study the risk of recurrence of non-B non-C HCC was associated with VFA but not with BMI, suggesting that non-B non-C hepatocarcinogenesis is more strongly associated with visceral fat accumulation than with BMI. In contrast to BMI, visceral fat accumulation is considered to be directly causative, through disturbing the adipocytokine balance of insulin resistance, which is a major cause of hepatic fat accumulation.[28] Excessive fat in the liver will lead to hepatocellular injury and possibly result in hepatocarcinogenesis through the direct cellular toxicity of excessive free fatty acids, oxidant stress and lipid peroxidation, or another mechanism.[10] Furthermore, visceral fat accumulation-induced insulin resistance causes hyperinsulinaemia. Insulin has growth-promoting properties and increases free insulin-like growth factor levels, which plays an important role in tumour growth and differentiation.[29 30] Visceral fat accumulation may be involved in both tumour initiation and promotion or progression steps through these mechanisms.

Table 4 Multivariate analysis of recurrence after curative ablation

Variables	OR (95% CI)	p Value
Age (per 1 year)	1.07 (1.03 to 1.13)	0.003
Diabetes mellitus	2.04 (0.89 to 4.63)	0.093
Dyslipidaemia	1.99 (0.66 to 6.06)	0.225
VFA (per 10 cm^2)	1.08 (1.01 to 1.17)	0.036
Male vs female	1.90 (0.84 to 4.33)	0.126

VFA, visceral fat area.

Since the present study examined recurrence of HCC, the results may not be applicable to primary hepatocarcinogenesis. In particular, there is the possibility that BMI and comorbidity with diabetes mellitus are significantly associated with the risk of HCC among patients with non-B non-C non-alcoholic liver diseases in general. Since the presence of chronic hepatic inflammation is a prerequisite for hepatocarcinogenesis, BMI and comorbidity with diabetes mellitus, which are associated with the risk of NASH, could still be used as an indicator of the risk of HCC development among patients with NAFLD.[10] On the other hand, the subjects of the present study, who had developed HCC, had a definite risk of de novo carcinogenesis. BMI and comorbidity with diabetes mellitus are not a significant predictor among such patients.

The distribution of visceral fat is different between males and females,[31] and the present study showed similar data: average VFA was different between men (120.3 (56.6) cm^2) and women (93.5 (37.8) cm^2, p = 0.036). According to this result, we defined the high VFA group as VFA >130 cm^2 in males and VFA >90 cm^2 in females. These values are equal to the cut-offs recommended by Oka *et al* for metabolic syndrome in the Japanese population.[32] Thus, it can be speculated that mechanisms involved in metabolic syndrome played a greater role in the hepatocarcinogenesis among patients with high VFA than in that among the others.

In conclusion, visceral fat accumulation was shown to be an independent risk factor of recurrence in patients with non-B non-C non-alcoholic HCC treated with curative ablation. Visceral fat accumulation plays a significant role in de novo hepatocarcinogenesis among such patients, and it remains to be seen whether reduction of visceral fat decreases HCC recurrence.

Competing interests: None.

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Influence of anti-HBc seropositivity on the risk of hepatocellular carcinoma in HCV-infected patients after adjusting for confounding factors

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SUMMARY. It is controversial whether past hepatitis B virus infection constitutes an additional risk of hepatocellular carcinoma (HCC) among patients with hepatitis C virus (HCV). The incidence of HCC between 1994 and 2004 was analysed among 1262 patients who were only positive for HCV. The cumulative incidence of HCC was assessed by Kaplan–Meier analysis and the difference between two groups was assessed by the log-rank test. The effect of anti-HBc positivity on the risk of HCC was assessed with multivariate Cox proportional analysis. Anti-HBc was positive in 522 (41.4%) patients. The proportion of male patients (56.7 *vs* 46.8%, $P < 0.001$) and mean age (60.8 *vs* 56.9 years, $P < 0.001$) were significantly higher in the anti-HBc positive group. HCC developed in 339 patients (mean follow-up 7.0 years), with cumulative incidence rates at 3, 5 and 10 years of 12.7, 24.5 and 41.9% in the anti-HBc positive group and 10.6, 17.7 and 33.4% in the negative group, respectively ($P = 0.005$). However, anti-HBc seropositivity did not reach statistical significance in multivariate analysis including age and gender (hazard ratio, 1.06; 95% CI, 0.85–1.31; $P = 0.63$). Anti-HBc positivity and HCC incidence were confounded by male gender and older age.

Keywords: anti-HBC, chronic hepatitis C, HCV, occult HBV infection.

INTRODUCTION

Hepatocellular carcinoma (HCC) is the fifth leading cause of cancer worldwide, accounting for more than 500 000 deaths annually. HCC usually develops in patients with advanced chronic liver diseases, the aetiology of which varies in each geographical area. Chronic hepatitis C virus (HCV) infection is a major cause of HCC in Japan and southern European countries and is an increasing cause in the USA [1,2], whereas hepatitis B virus (HBV) infection is the dominant cause of HCC in southeast and east Asian countries.

In Japan, the prevalence of HCV and that of HBV infection is 1.5%, accounting for about 75 and 15% cases of HCC, respectively [3,4]. While the prevalence of HBV infection is thought to have remained fairly constant, except for generations after the advent of neonatal vaccination, HCV infection seems to have spread through blood transfusion and other practices mainly in 1950s and 1960s [5], when stringent infection control was yet to be introduced. At that period, HBV could be transmitted, sometimes concomitantly with HCV. In contrast to HCV however, chronic HBV infection was rarely established in this fashion because in adults hepatitis due to HBV is usually acute and transient [6,7].

Although there are few reliable statistics, a large proportion of HCV-infected patients in Japan have a past history of HBV infection, as indicated by serum hepatitis B core antibody (anti-HBc) positivity and hepatitis B surface antigen (HBsAg) negativity. It has been demonstrated that HBV DNA may be present in a latent form even after serum HBsAg becomes negative, which is refer to as occult HBV infection [8,9]. Anti-HBc is considered as a surrogate marker of such latent carriers [10]. Since co-infection with HCV and HBV has been reported to be associated with an accelerated risk of HCC [11,12], the relationship between occult HBV infection and HCV related HCC has been extensively reviewed but remains controversial [13–15].

Abbreviations: ALT, alanine aminotransferase; anti-HBc, hepatitis B core antibody; anti-HBs, hepatitis B surface antibody; BMI, body mass index; CI, confidential interval; CT, computed tomography; HBsAg, hepatitis B virus surface antigen; HCC, hepatocellular carcinoma; HBV, hepatitis B virus; HCV, hepatitis C virus; HIV, human immunodeficiency virus; IFN, interferon; OD, odds ration; SVR, sustained virological response.

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Previous large-scale epidemiological studies in Japan showed that serum anti-HBc was positive in as many as 50% of patients positive for HCV and negative for HBsAg [16,17]. Some previous studies have defined the relation between anti-HBc seropositivity and increased risk of HCC among HCV-positive patients [16,17]. However, anti-HBc seropositivity in Japan is known to be higher among old, male patients, who are at a higher risk of HCC development regardless of anti-HBc status [18]. Thus, the evaluation of the effects of anti-HBc positivity on the risk of HCC development requires meticulously careful adjustment for other possible risk factors. For this purpose longitudinal studies are preferable to cross-sectional ones because certain characteristics may change after HCC development. In the present study, we sought to determine the risk of HCC development attributable to anti-HBc seropositivity among a cohort of patients with HCV-related chronic liver diseases who were followed up at the authors' institution [19].

PATIENTS AND METHODS

Patients

Between January 1994 and December 2004, a total of 1954 HCV RNA positive patients, excluding those with HCC or a past history of it, visited our liver clinic. We analysed 1262 of them, excluding 87 patients with concomitant HBsAg positivity, 169 patients whose status of anti-HBc was not available, and 423 patients whose visit was only for consultation purposes. Thirteen patients with intractable ascites were also excluded since they were considered to have advanced liver disease. The remaining 1262 patients were divided into two groups based on anti-HBc status: 522 patients were positive for anti-HBc and 740 patients, negative. Blood tests and physical examination were performed and a questionnaire was filled in at their first visit. Heavy alcohol consumption was defined as taking alcohol of over 80 g/day [20,21]. Body mass index (BMI) was calculated as body weight in kilograms (kg) divided twice by body height in meters (m). Diagnosis of diabetes mellitus was based on medical history or 75 g oral glucose tolerance test [22]. Human immunodeficiency virus (HIV) antibody was not routinely tested for because the prevalence of HIV co-infection among HCV positive patients is very low in Japan [23].

Laboratory tests

At study entry, serum samples from each patient were tested for serologic markers of HCV. HCV antibody was assessed by using second generation anti-HBc, and anti-HBs were determined by using commercial enzyme immunoassay kits. Results of the anti-HBc assays were expressed as the percentage of inhibition, and the specimen was considered to be anti-HBc positive when the percentage of inhibition exceeded 50% [16]. Serum samples, randomly selected from frozen sera stored at −40°C of both anti-HBc positive and negative patients (each $n = 50$), were tested for HBV-DNA by real-time polymerase chain reaction [24], which has a detection limit of 0.7 log copy/mL of HBV-DNA.

Patients follow-up and diagnosis of HCC

Each patient was screened for HCC with ultrasonography at or immediately after the first visit. Those in whom HCC suspected nodules were detected, contrast enhanced computed tomography (CT) was performed. We used multidetector row CT with 4, 8 or 16 detector rows (Aquilion 4/16; Toshiba, Tokyo, Japan; LightSpeed Qx/I, LightSpeed Ultra; GE Healthcare, Milwaukee, WI, USA). Images were obtained in early arterial, late arterial and equilibrium phases at 28, 40 and 120 s after starting bolus injection of iodinated contrast material. Images were reconstructed with a section thickness of 5 mm and a reconstruction interval of 5 mm (a section thickness of 2–2.5 mm, an interval of 1.5–2 mm, and a field of view (FOV) of 24–35 cm for arterial phases). HCC was diagnosed by dynamic CT, considering hyperattenuation in the arterial phase with washout in the late phase [25]. When HCC diagnosis was not clear, ultrasound-guided tumour biopsy was performed. The grade of malignancy was evaluated based on the Edmondson–Steiner criteria [26]. Patients finally diagnosed as having HCC were excluded from this study. Afterwards, patients were followed up at the outpatient clinic with blood tests including tumour markers and ultrasonography at every 3–6 months. CT was performed when it was considered necessary. The observation was terminated on 30 June 2006.

Characteristics of anti-HBc positive patients

Clinical and laboratory factors observed at the first visit to outpatient clinic were compared between anti-HBc positive and negative patients. The relation between anti-HBc positivity and other clinical and laboratory factors were also analysed by using an unconditional multivariate logistic regression model with anti-HBc seropositivity as the outcome variable.

Risk factors for HCC development

Factors recorded at the first visit, including anti-HBc status, were analysed for the association with HCC development by using univariate and multivariate Cox's proportional hazard regression models. Observation time was defined as the interval between the first visit and detection of HCC. Death without HCC development was treated as a censor. To evaluate possible confounding factors, patients were stratified based on the factors significantly associated with anti-HBc positivity and subset analyses were performed in each stratum for the risk of HCC development.

Statistical analysis

Data were expressed as the median and range (25–75th percentiles) unless otherwise indicated. Continuous variables were compared by unpaired *t*-test (parametric) or Mann–Whitney *U*-test (non-parametric), and categorical variables were compared by chi-square test. A *P*-value <0.05 on two-tailed tests was considered significant. Cumulative incidence of HCC was estimated using the Kaplan–Meier method. The variables analysed with Cox's proportional hazard regression models were: age, gender, BMI, heavy alcohol drinking, comorbidity with diabetes mellitus, serum concentration of albumin and total bilirubin, alanine aminotransferase (ALT) levels, prothrombin time, platelet counts, and positivity of anti-HBc, all of which were obtained at entry. Logistic regression was performed using the same variables, except that anti-HBc positivity was the outcome variable. Multichotomous categorical variables were represented by corresponding binary dummy variables. Data processing and analysis were performed by using the S-PLUS 2000 software (MathSoft Inc., Seattle, WA, USA).

RESULTS

Patient profile

The study population consisted of a total of 1262 patients, 642 male and 620 female with a median age of 59.8 (52.5–66.8) years. Serum anti-HBc was positive in 522 patients (41.4%). Heavy overweight alcohol consumption was found in 63 (5.0%), diabetes mellitus was noted in 118 (9.4%), and there were 269 (21.3%) overweight patients (BMI ≥ 25 kg/m^2). HBV-DNA was detected in none of the tested sera.

During the follow-up period, a total of 252 patients received interferon (IFN) and 36 of them achieved a sustained virological response (SVR) (Table 1). The proportion of patients who received interferon therapy was not significantly different between the patients positive for anti-HBc and those negative ($P = 0.72$ by exact test). There was no significant difference in the SVR rates between the two groups ($P = 0.27$ by exact test).

Characteristics of anti-HBc positive patients

As shown in Table 1, anti-HBc positive patients were significantly older ($P < 0.001$), male ($P < 0.001$), and had more advanced liver damage, as indicated by marginally but still significantly lower serum albumin concentration ($P < 0.001$) and platelet counts ($P < 0.001$), than anti-HBc negative ones. The proportion of heavy alcohol drinkers was also significantly larger in the former group ($P = 0.037$). All of these factors are known to be associated with of risk of HCC development among chronic hepatitis C patients.

Incidence of HCC

At the time of enrolment, no patients remained undiagnosed of HCC because patients with ambiguous diagnosis were closely followed up with imaging and underwent tumour biopsy if necessary. HCC developed in 339 patients during a mean follow up period of 7.0 years. The cumulative incidence rates at 3, 5 and 10 years were 11.4, 20.5 and 36.9%, respectively. Among anti-HBc positive patients, 160 of 522 patients presented with HCC, with an incidence rate of 5.3% per person-year and cumulative incidence rates at 3, 5 and 10 years of 12.7, 24.5 and 41.9% respectively. In the meantime HCC developed in 179 of 740 anti-HBc negative patients with an incidence rate of 4.0% per person-year and cumulative incidence rates of 10.6, 17.7 and 33.4%, respectively at the times stated above (Fig. 1). The crude incidence rates differed significantly between the two groups ($P = 0.005$ by the log-rank test).

Table 1 Patient baseline characteristics

Variables	Anti-HBc positive $n = 522$	Anti-HBc negative $n = 740$	P
Age* (years old)	60.8 ± 9.6	56.9 ± 12.4	<0.001‡
Male, *n* (%)	296 (56.7%)	346 (46.8%)	<0.001§
Drinking >80 g/day, *n* (%)	34 (6.5%)	29 (3.9%)	0.037§
Diabetes mellitus, *n* (%)	51 (9.8%)	67 (9.1%)	0.67§
BMI ≥ 25 kg/m^2, *n* (%)	97 (18.6%)	172 (23.2%)	0.05§
Serum albumin† (g/dL)	4.0 (3.7–4.2)	4.1 (3.8–4.3)	<0.001‖
Total bilirubin† (mg/dL)	0.7 (0.5–0.9)	0.7 (0.6–0.9)	0.32‖
ALT† (IU/mL)	65.0 (39.3–102)	61.0 (35.0–99.0)	0.10‖
Prothrombin time activity† (%)	83.3 (71.8–96.4)	85.3 (74.4–100)	0.011‖
Platelet count† × 10^3 (/μL)	142 (101–180)	155 (110–204)	<0.001‖
Patients who received IFN, *n* (%)	101 (19.3%)	150 (20.3%)	0.72§
Patients who achieved SVR, *n* (%)	11 (2.1%)	25 (3.4%)	0.27§

*Expressed as mean ± standard deviation (SD). †Expressed as median (25–75th percentiles). ‡Unpaired Student's *t*-test. §Chi-square tests. ‖Mann–Whitney *U*-test. BMI, body mass index; ALT, alanine aminotransferase.

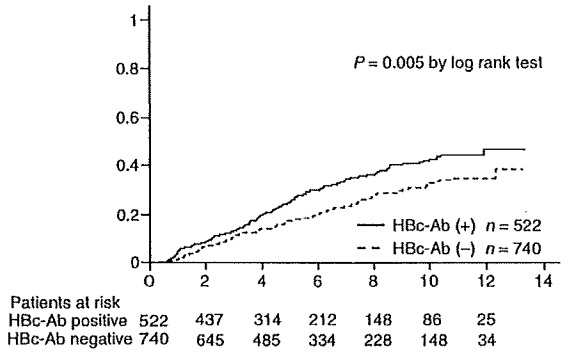

Fig. 1 Cumulative incidence of HCC among total 1262 patients, divided by HBcAb status. Dashed HBcAb negative patients; solid: HBcAb positive patients. The incidence rates differed significantly between the two groups ($P = 0.005$ by the log-rank test).

Risk factors for HCC development

Univariate analyses showed that anti-HBc positivity was associated with the risk of HCC, together with older age, male gender, heavy alcohol intake, comorbidity with diabetes mellitus, higher BMI, lower serum albumin level, lower total bilirubin level, higher ALT level, lower prothrombin time activity, and lower platelet counts (Table 2). We performed multivariate proportional hazard regression using these variables found significant in univariate analysis. All variables retained statistical significance except for total bilirubin level, comorbidity with diabetes mellitus, and anti-HBc positivity (Table 3). Anti-HBcb seropositivity showed a non-significant hazard ratio of 1.06 ($P = 0.63$) in the multivariate analysis. These three factors were each strongly associated with other significant factors. Total bilirubin level was strongly correlated with lower albumin level ($r = 0.24$, $P < 0.001$), higher ALT level ($r = 0.14$, $P < 0.001$), lower prothrombin time activity ($r = 0.35$, $P < 0.001$), and lower platelet counts ($r = 0.28$, $P < 0.001$). Multivariate logistic regression indicated that the comorbidity with diabetes mellitus was strongly associated with older age [odds ratio (OR), 1.03 per 1 year; 95% confidence interval (CI), 1.02–1.04; $P < 0.014$], male gender (OR, 2.34; 95% CI, 2.12–2.56; $P < 0.001$), and heavy alcohol consumption (OR, 2.05; 95% CI, 1.71–2.38; $P = 0.032$). Anti-HBc seropositivity was strongly associated with older age (OR, 1.03 per 1 year; 95% CI, 1.02–1.04; $P < 0.001$) and male gender (OR, 1.51; 95% CI, 1.36–1.59; $P = 0.007$).

Analysis of confounding factors

The association between anti-HBc positivity and the risk of HCC found in the univariate analysis was suspected to be spurious from the results of multivariate analysis. However, it was also possible that the effect of anti-HBc positivity on the risk of HCC development was masked by the effects of other factors, especially age and gender, which were strong risk factors of HCC development and were also strongly

Table 2 Risk factors for HCC development – univariate analysis

Variables	HR (95% CI)	*P*
Age (years old) (per 1 year)	1.07 (1.06–1.08)	<0.001
Male	1.90 (2.26–2.37)	<0.001
Drinking >80 g /day	3.22 (1.55–4.57)	<0.001
Diabetes mellitus	1.58 (1.17–2.51)	0.003
BMI (per 1 kg/m^2)	1.05 (1.02–1.08)	0.003
Serum albumin (per 1 g/dL)	0.20 (0.16–0.25)	<0.001
Total bilirubin (per 1 mg/dL)	1.87 (1.55–2.25)	<0.001
ALT (per 1.0 IU/mL)	1.003 (1.002–1.005)	<0.001
Prothrombin time activity (%)	0.97 (0.96–0.98)	<0.001
Platelet count (per 1 × 10^3/μL)	0.85 (0.83–0.87)	<0.001
Anti-HBc positivity	1.36 (1.10–1.68)	0.005

BMI, body mass index; ALT, alanine aminotransferase; HBcAb, hepatitis B core antibody

Table 3 Risk factors for HCC development – multivariate analysis

Variables	HR (95% CI)	*P*
Age (years old) (per 1 year)	1.07 (1.05–1.08)	<0.001
Male	1.98 (1.57–2.50)	<0.001
Drinking >80 g/day	1.85 (1.29–2.65)	<0.001
Diabetes mellitus	1.15 (0.84–1.56)	0.38
BMI (per 1 kg/m^2)	1.06 (1.03–1.10)	<0.001
Serum albumin (per 1 g/dL)	0.37 (0.27–0.50)	<0.001
Total bilirubin (per 1 mg/dL)	0.81 (0.60–1.10)	0.17
ALT (per 1.0 IU/mL)	1.003 (1.001–1.004)	<0.001
Prothrombin time activity (%)	0.98 (0.98–0.99)	<0.001
Platelet count (per 1 × 10^3/μL)	0.89 (0.87–0.92)	<0.001
HBcAb positivity	1.06 (0.85–1.31)	0.63

BMI, body mass index; ALT, alanine aminotransferase; HBcAb, hepatitis B core antibody.

associated with anti-HBc positivity. Thus we performed stratified subset analyses as follows.

First, we divided the 1262 patients according to gender and analysed cumulative incidence of HCC among 642 male patients (296 anti-HBc positive) and 620 female patients (226 anti-HBc positive) separately. Among the male patients, the cumulative incidence rates of HCC at 3, 5 and 10 years were 17.1, 34.0 and 53.2% in the anti-HBc positive group, and 11.9, 21.6 and 40.2% in the anti-HBc negative group, respectively ($P = 0.003$ by the log-rank test). However, anti-HBc positivity did not retain statistical significance (OR, 1.02; 95% CI, 0.72–1.43; $P = 0.93$) in multivariate analysis. Among the female patients, the cumulative incidence rates at 3, 5 and 10 years were 7.0, 11.9 and 29.0% in the anti-HBc positive group and 8.9, 14.7 and 27.2% in the anti-HBc negative group, respectively ($P = 0.92$, Fig. 2). Anti-HBc positivity was not significant in multivariate analysis (OR, 1.24; 95% CI, 0.85–1.81; $P = 0.27$).

Second, we divided the 642 male patients into two age groups, the over 60 year olds (302 patients, 172anti-HBc positive) and those below (340 patients, 124 anti-HBc positive). Among the older male patients, the cumulative incidence rates at 3, 5 and 10 years were 21.9, 42.3 and 65.0% in the anti-HBc positive group, and 19.5, 36.9 and 60.0% in the anti-HBc negative group, respectively ($P = 0.51$, Fig. 3). Anti-HBc positivity was not found to be significant by multivariate analysis (OR, 1.02; 95% CI, 0.71–1.43; $P = 0.92$). Among the younger male patients, the cumulative incidence rates at 3, 5 and 10 years were 13.0, 23.2 and 36.5% in the anti-HBc positive group, and 7.3, 12.6 and 29.6% in the anti-HBc negative group, respectively ($P = 0.092$, Fig. 4). Anti-HBc positivity was not found significant by multivariate analysis (OR, 1.23; 95% CI, 0.77–1.53; $P = 0.27$).

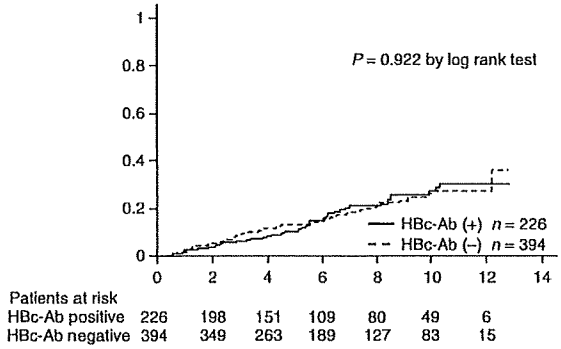

Fig. 2 Cumulative incidence of HCC among 620 female patients, divided by HBcAb status. Dashed HBcAb negative; solid: HBcAb positive. The incidence rates did not differ significantly between the two groups ($P = 0.922$ by the log-rank test).

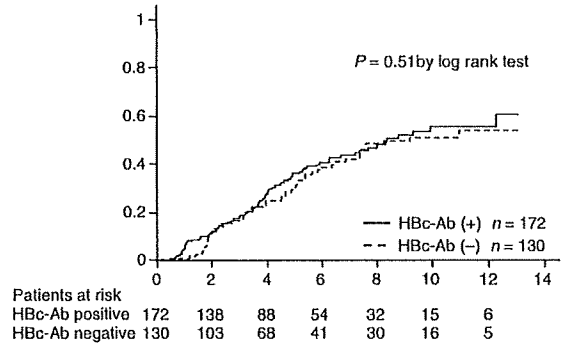

Fig. 3 Cumulative incidence of HCC among 302 male patients older than 60 years. Dashed HBcAb negative; solid: HBcAb positive. The incidence rates did not differ significantly between the two groups ($P = 0.51$ by the log-rank test).

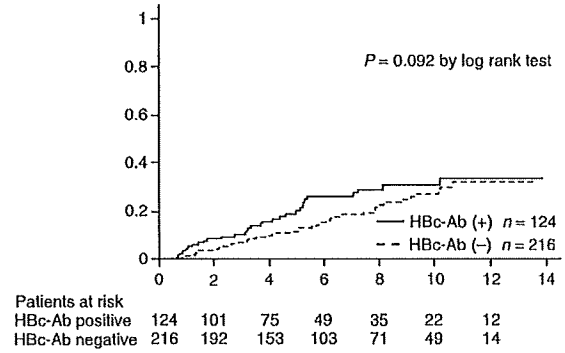

Fig. 4 Cumulative incidence of HCC among 340 male patients younger than 60 years. *Dashed* HBcAb negative; *solid* HBcAb positive. The incidence rates did not differ significantly between the two groups ($P = 0.092$ by the log-rank test).

DISCUSSION

In previous studies from Japan, anti-HBc seropositivity among patients with HCV-related chronic liver disease was reported to be about 50% [16,17], which was compatible with the seropositivity found in the present study. As reported in other studies [27,28], the cumulative incidence of HCC in the present study, when crudely compared, was significantly higher among anti-HBc positive patients than among negative ones. However, in the present study, the seropositivity for anti-HBc was significant as a risk factor of HCC only in univariate analysis and did not remain statistically significant in multivariate analysis. With logistic regression analysis we showed that anti-HBc positivity was strongly associated with older age and male gender, both of which are strong risk factors of HCC development among patients with HCV-related liver disease, as shown in the