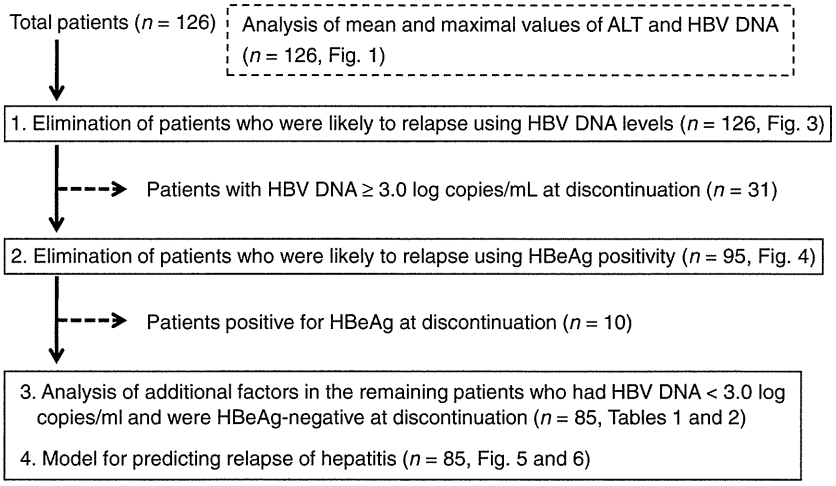

Figure 2 The progression of analyses in the present study and population structure of each analysis.

both negative for HBeAg and whose serum HBV DNA was lower than 3.0 log copies/mL at NA cessation. Table 1 shows the comparison of clinical and virological backgrounds between the 53 relapse and 32 non-relapse patients using univariate analysis. Age and gender distributions were similar between the groups. Approximately 75% of the 85 patients had HBV genotype C, but the distribution of genotypes did not differ between the groups. Approximately 90% of patients were being treated with LVD alone at the time of discontinuation, compared with 6% of patients being given ETV. The median duration of NA treatment was about two times longer in patients without relapse. Levels of both HBsAg and HBcrAg were significantly lower in non-relapse patients than in relapse patients at the time of NA discontinuation. The difference between serum HBsAg was also significant at the initiation of NAs, but not that of HBcrAg. As only patients with HBV DNA lower than 3.0 log copies/mL were analyzed, the majority of these cases showed levels below the 2.6 log copies/mL lower detection limit of the Amplicor assay at NA discontinuation. We therefore also tested HBV DNA with a TaqMan assay, which had a higher sensitivity than the Amplicor assay, in 43 patients whose serum samples were available. The prevalence of patients having a negative detection signal did not differ between the two groups. The number of

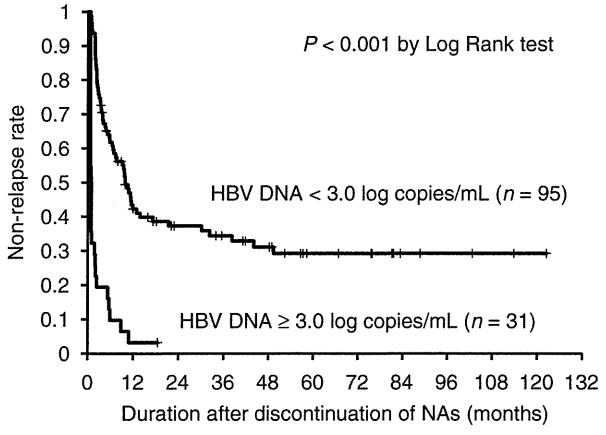

Figure 3 Comparison of non-relapse rates using the Kaplan–Meier method between 31 patients with serum hepatitis B virus (HBV) DNA equal to or higher than 3.0 log copies/mL and 95 patients with serum HBV DNA lower than 3.0 log copies/mL at the time of nucleos(t)ide analog (NA) discontinuation.

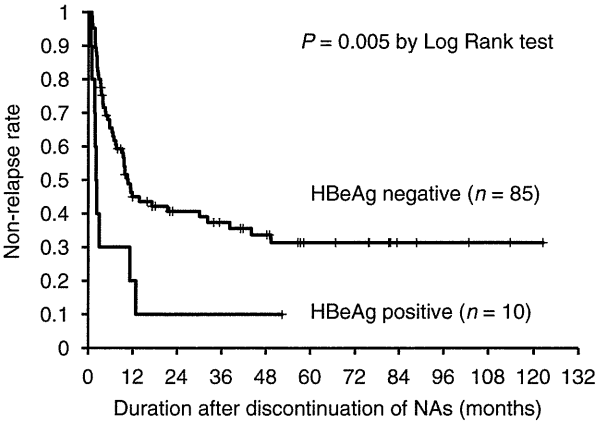

Figure 4 Comparison of non-relapse rates using the Kaplan–Meier method between 10 patients with detectable hepatitis B e antigen (HBeAg) and 85 patients without detectable HBeAg at the time of nucleos(t)ide analog (NA) discontinuation.

Table 1 Comparison of clinical and virological backgrounds between patients with and without relapse of hepatitis at initiation and discontinuation of nucleos(t)ide analogs (NAs)

Background	Non-relapse patients ($n = 32$)	Relapse patients ($n = 53$)	*P*-value
At initiation of NAs			
Age (years)†	47 (17–75)	48 (26–74)	>0.2
Gender (M : F)	23:9	32:21	>0.2
ALT (IU/L)†	183 (9–1182)	187 (20–2052)	>0.2
Genotype (A : B : C : UD)	1:2:21:8	0:3:44:6	0.193
HBeAg (positive)‡	11 (34%)	16 (30%)	>0.2
HBV DNA			
Amplicor assay (log copies/mL)†	6.2 (<2.6–>7.6)	6.5 (<2.6–>7.6)	0.099
HBsAg (log IU/mL)†	2.7 (0.1–4.3)	3.3 (1.6–3.9)	0.018
HBcrAg (log U/mL)†	5.2 (<3.0–>6.8)	5.6 (<3.0–>6.8)	>0.2
At discontinuation of NAs			
Age (years)†	50 (21–78)	49 (26–79)	>0.2
NAs (LVD : LVD+ADV : ETV : ADV)	28:1:3:0	50:0:2:1	>0.2
Duration of NA treatment (months)†	36 (4–129)	17 (4–84)	0.007
Follow-up period after discontinuation of NAs (months)†	45 (6–123)	12 (1–111)	0.002
ALT (IU/L)†	16 (7–38)	20 (9–65)	0.002
HBV DNA			
Amplicor assay (log copies/mL)†	<2.6 (<2.6–2.9)	<2.6 (<2.6–2.9)	>0.2
TaqMan assay (negative signal)‡	5 (23%) ($n = 22$)	3 (14%) ($n = 21$)	>0.2
TaqMan assay (negative or positive signal)‡	13 (59%) ($n = 22$)	13 (62%) ($n = 21$)	>0.2
HBsAg (log IU/ml)†	2.0 (<–1.5–4.3)	3.1 (0.6–4.0)	0.001
HBcrAg (log IU/mL)†	3.4 (<3.0–4.9)	4.3 (<3.0–>6.8)	0.003

†Data are expressed as the median (range)
‡Data are expressed as a positive number (%)
ADV; adefovir dipivoxil; ALT, alanine aminotransferase; ETV, entecavir; HBcrAg, hepatitis B core-related antigen; HBeAg, hepatitis B e antigen; HBsAg, hepatitis B surface antigen; LVD, lamivudine; UD, undetermined.

patients with a negative detection signal or a positive signal also did not vary significantly. The follow-up period after discontinuation of NAs was significantly shorter in patients with relapse than in those without because formal follow-up ended once patients relapsed. The median period of follow-up was 45 months in patients without relapse.

Multivariate analyses revealed that a shorter duration of NA treatment and higher levels of HBsAg and HBcrAg at discontinuation were significantly associated with the occurrence of hepatitis relapse (Table 2). The cut-off values that showed the highest significance by ROC analysis were 1.9 log IU/mL for HBsAg (AUC = 0.707, $P = 0.001$), 4.0 log U/mL for HBcrAg (AUC = 0.692, $P = 0.003$), and 16 months (AUC = 0.674, $P = 0.007$) for treatment duration.

Model for predicting relapse of hepatitis using levels of HBsAg and HBcrAg

The existence of a second cut-off value was suggested by ROC analysis for both of HBsAg (2.9 log IU/mL) and HBcrAg (3.0 log IU/mL) to discriminate between

Table 2 Multivariate analysis of factors associated with relapse of hepatitis after discontinuation of nucleos(t)ide analogs (NAs)

Factor	Hazard ratio	95%CI	*P*-value
HBsAg at discontinuation ≥ 1.9 log IU/mL	5.21	1.87–14.55	0.002
HBcrAg at discontinuation ≥ 4.0 log U/mL	2.20	1.25–3.87	0.006
Duration of NA treatment ≥ 16 months	0.54	0.31–0.93	0.027

CI, confidence interval; HBcrAg, hepatitis B core-related antigen; HBsAg, hepatitis B surface antigen.

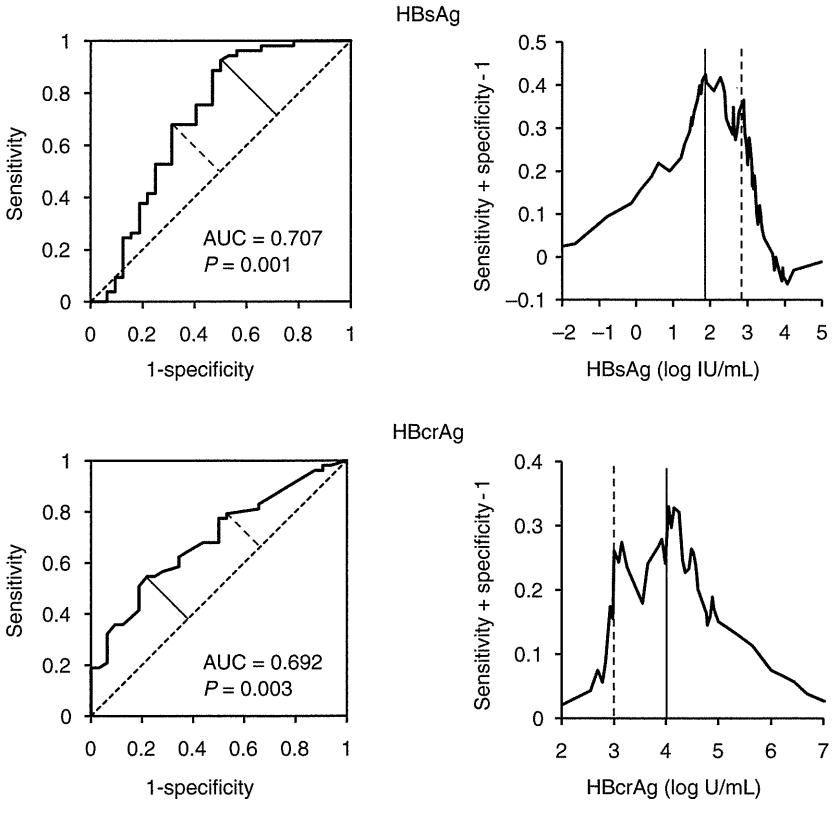

Figure 5 Receiver operating characteristic curve (ROC) analysis of hepatitis B surface antigen (HBsAg) and hepatitis B core-related antigen (HBcrAg) to discriminate between patients with and without hepatitis relapse. The existence of two inflection points is suggested for both HBsAg and HBcrAg. Short diagonal lines indicate main inflection points and short broken diagonal lines indicate second inflection points. Vertical lines indicate actual values of antigens that correspond to the main inflection points and vertical broken lines indicate actual values of antigens that correspond to the second inflection points.

patients with and without relapse (Fig. 5). Thus, we set cut-off values as 1.9 and 2.9 log IU/mL for HBsAg and 3.0 and 4.0 log U/mL for HBcrAg in our model for predicting hepatitis relapse.

We tentatively defined three groups using the sum of the scores for HBsAg and HBcrAg levels at the time of NA discontinuation for our model. Conversions were made by assigning a score of 0 for an HBsAg level lower than 1.9 log IU/mL, 1 for a level from 1.9 to 2.8 log IU/mL, and 2 for a level equal to or higher than 2.9 log IU/mL. HBcrAg was scored as 0 for a level lower than 3.0 log U/mL, 1 for a level from 3.0 to 3.9 log U/mL, and 2 for a level equal to or higher than 4.0 log U/mL. Overall, group 1 consisted of patients with a total score of 0, group 2 of patients with a total score of 1 or 2, and group 3 of patients with a total score of 3 or 4.

Patients whose HBV DNA was lower than 3.0 log copies/mL and in whom HBeAg was negative at the time of NA discontinuation were assigned to one of the three groups. Figure 6 shows the comparison of non-relapse rates among the three groups using Kaplan–Meier analysis, which differed significantly. The non-relapse rate was approximately 90% in group 1, as low as 10% in group 3, and intermediate in group 2. When factors associated with relapse were analyzed in group 3 patients, an age of over 40 years at the time of discontinuation was calculated as a significant factor (hazard

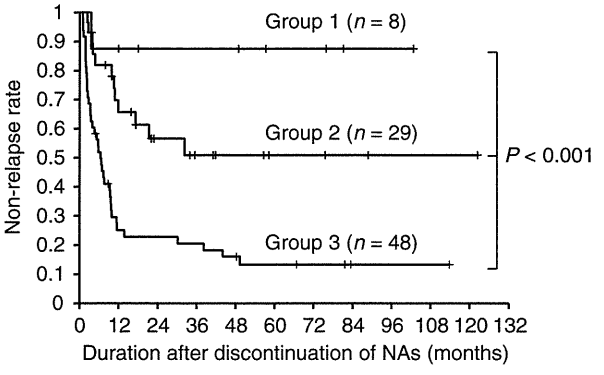

Figure 6 Comparison of non-relapse rates using the Kaplan–Meier method among three groups classified by the sum of the scores of hepatitis B surface antigen (HBsAg) and hepatitis B core-related antigen (HBcrAg) levels at the time of nucleo-s(t)ide analog (NA) discontinuation.

ratio = 5.25, range 2.37–11.65, $P < 0.001$). No significant factors were associated with relapse in group 2 patients.

DISCUSSION

THE EUROPEAN ASSOCIATION for the Study of the Liver recommends continuation of NA treatment until HBsAg is cleared.[25] Liu *et al.* came to a similar conclusion in their study of chronic hepatitis B patients treated with LVD.[14] Indeed, the clearance of HBsAg is a reliable marker for the safe discontinuation of NAs, but the rate of patients who can clear HBsAg is relatively low (1–3%/year).[26–28] Thus, additional factors associated with relapse of hepatitis B after discontinuation of NAs were analyzed in the present study to better identify candidates who could achieve drug-free status. Such studies are relatively few, possibly because patients who discontinue NAs prematurely often experience severe complicating relapse and hepatic failure.[9] Although prospective studies are desirable to obtain accurate results, retrospective studies, such as ours, are also necessary to minimize the risk of adverse complications.

Since HBV cannot be completely eradicated in hosts, the primary goal in treating chronic hepatitis B is to convert symptomatic patients into inactive carriers in whom HBeAg is negative (usually anti-HBe-positive), serum HBV DNA is low, and serum ALT is normal.[1,2,18,29] Thus, we set the clinical conditions of a successful discontinuation of NAs as serum HBV DNA level below 4.0 log copies/mL and ALT below 30 IU/L following NA cessation. Patients who satisfy these conditions are not recommended for treatment by the Japanese guidelines for hepatitis B,[18] and it is also widely accepted that the risk of developing cirrhosis or complicating hepatocellular carcinoma is very low in such patients.[30,31] We used our cohort's mean and maximal values of HBV DNA and ALT for relapse analyses. Mean values were useful for evaluating relapse of hepatitis as a whole since parameter levels often fluctuated after discontinuation, and maximal values were used to evaluate relapse in a real-time fashion during the follow-up period. It is noteworthy that the mean and maximal values correlated very closely for both HBV DNA and ALT. The mean HBV DNA value of 4.0 log copies/mL corresponded to the maximal HBV DNA value of 5.7 by ROC analysis, and similarly the mean ALT value of 30 IU/L corresponded to the maximal ALT value of 79 IU/L. Thus, relapse of hepatitis B was judged to occur when serum ALT became higher than 79 IU/L or when serum HBV DNA surpassed 5.7 log copies/mL after the time of NA discontinuation. Such criteria may also be useful for physicians to detect relapse at an early phase and avoid the occurrence of severe reactivation or unnecessary discontinuation of NAs.

It is generally understood that patients with a higher level of HBV DNA at the time of NA discontinuation are likely to relapse, but this cut-off value has not been analyzed sufficiently. Our findings using ROC analysis showed that patients with levels lower than 3.0 log copies/mL have a good possibility to achieve successful discontinuation. The presence of HBeAg is also generally accepted as a reliable factor to predict relapse of hepatitis. Our study showed that patients with detectable HBeAg at the time of NA discontinuation were likely to relapse, even if their HBV DNA levels were lower than 3.0 log copies/mL. Therefore, we next analyzed additional factors associated with a relapse of hepatitis after discontinuation of NAs by selecting patients who met both of these criteria.

Nucleos(t)ide analog treatment produces a rapid decrease in serum HBV DNA by suppressing reverse transcription of pregenomic HBV RNA. However, the key intrahepatic HBV replicative intermediate, covalently closed circular DNA (cccDNA), tends to remain and is capable of reinitiating replication once NAs are ceased.[32] Measurement of HBV cccDNA has been reported to be useful for monitoring and predicting responses to antiviral treatments.[33] However, its measurement is difficult in the clinical setting as it requires a liver biopsy. Due to the mechanism of action of NAs mentioned above, serum HBV DNA does not reflect intrahepatic HBV cccDNA in patients undergoing NA treatment.[34] To address this, quantitative measurement of HBV antigens has been reported to be useful for predicting the effect of antiviral treatment in patients with chronic hepatitis B. Although HBsAg is usually used as a serum marker for the diagnosis of HBV infection, several groups have shown that HBsAg levels can also be reflective of the response to peg-interferon in chronic hepatitis B.[28,35,36] The HBcrAg assay measures serum levels of HB core and e antigens simultaneously using monoclonal antibodies that recognize the common epitopes of these two denatured antigens. Since the assay measures all antigens transcribed from the pre-core/core gene, it is regarded as core-related.[37] Serum HBcrAg has been reported to accurately reflect intracellular levels of HBV cccDNA even during NA treatment,[24,34,38] and was found to be useful for identifying patients who were likely to show relapse of hepatitis after the discontinuation of NAs.[39,40] It is possible that levels of HBsAg and HBcrAg have different roles in

monitoring antiviral effects because the transcription of these two antigens are regulated by alternative enhancer-promoter systems in the HBV genome.[3] Therefore, we analyzed both of these antigens to elucidate their ability to predict relapse of hepatitis after discontinuation of NAs.

Multivariate analysis demonstrated that levels of HBsAg and HBcrAg at the time of NA discontinuation were independent factors significantly associated with relapse of hepatitis. Thus, we believe these factors can also be applied for predicting relapse in patients whose HBV DNA is lower than 3.0 log copies/mL and whose HBeAg is negative at NA discontinuation. HBV DNA levels were further analyzed using a highly sensitive assay based on real-time polymerase chain reaction (PCR). However, even the level of a negative signal did not ensure successful discontinuation of NAs. The results obtained here indicate that the combined use of HBV-related antigens are useful makers for monitoring the effect of anti-viral treatment in ways different from HBV DNA. Finally, since prolonged NA administration was also a significant factor associated with safe discontinuation, physicians are advised to continue patient treatment for at least 16 months for the best possible outcome.

From our data, a tentative model for predicting relapse of hepatitis after discontinuation of NAs was constructed using levels of HBsAg and HBcrAg at discontinuation. A negative result for HBeAg and HBV DNA lower than 3.0 log copies/mL at the time of NA discontinuation are the essential conditions in this system. Levels of HBsAg and HBcrAg were each converted into scores from 0 to 2 partly because two cut-off values were needed for each antigen and partly because a scoring system may be more convenient for clinical use. The sum of the two scores, which ranged from 0 to 4, was used to prospect relapse. We found that group 1 patients who had a low score (0) could be recommended to discontinue NAs because nearly 90% of this group achieved successful discontinuation. Further analysis of factors associated with relapse are needed for group 2 patients who had middle range scores (1 or 2), since the odds of achieving successful discontinuation were approximately 50%. Continuation of NA treatment is recommended for group 3 patients having high scores (3 or 4) because nearly 90% of this group relapsed. However, this recommendation may be reconsidered in patients younger than 40 years; such cases tended to have a lower relapse rate in group 3. It is also noteworthy that relapse occurred mainly during the first and second years following NA discontinuation in all groups, similarly to a report by Liu *et al.*[14] Thus, clinicians should be vigilant in the early phase after discontinuation.

This study has several limitations. The patients who discontinued NAs were recruited retrospectively, and thus the decision to halt NA treatment was made by individual physicians without uniformly established criteria. Based on this, prospective studies are required to confirm our results. Furthermore, as over 90% of the patients we enrolled had genotype C and over 90% of cases were treated with LVD until discontinuation, the results obtained here can not be applied directly to other HBV genotypes or other types of NAs.

In conclusion, the present study showed that maximal levels of serum ALT and HBV DNA were useful for defining relapse patients after discontinuation of NAs. Along with serum HBV DNA of less than 3.0 log copies/mL and negative serum HBeAg, serum levels of HBsAg and HBcrAg at the time of NA discontinuation were able to predict relapse of hepatitis B and should therefore be considered when establishing uniform guidelines regarding the safe withdrawal of NA treatment. To this end, NA administration of more than 16 months is advisable to achieve successful discontinuation.

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Original Article

Cost-effectiveness analysis on the surveillance for hepatocellular carcinoma in liver cirrhosis patients using contrast-enhanced ultrasonography

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Aim: Sonazoid is a new contrast agent for ultrasonography (US). Contrast-enhanced ultrasonography (CEUS) using Sonazoid enables Kupffer imaging, which improves the sensitivity of hepatocellular carcinoma (HCC) detection. However, there are no studies on the cost-effectiveness of HCC surveillance using Sonazoid.

Methods: We constructed a Markov model simulating the natural history of HCV-related liver cirrhosis (LC) patients, and compared three strategies (no surveillance, US surveillance and CEUS surveillance). The transition probability and cost data were obtained from published data. The simulation and analysis were performed using TreeAge pro 2009 software.

Results: When compared to the no surveillance group, the US and CEUS surveillance groups increased the life expectancy by 1.67 and 1.99 quality-adjusted life-years (QALY), respectively, and the incremental cost effectiveness ratio (ICER) were 17 296 $US/QALY and 18 384 $US/QALY, respectively. These results were both less than the commonly-accepted threshold of $US 50 000/QALY. Even if the CEUS surveillance group was compared with the US surveillance group, the ICER was $US 24 250 and thus cost-effective. Sensitivity analysis showed that the annual incidence of HCC and CEUS sensitivity were two critical parameters. However, when the annual incidence of HCC is more than 2% and/or the CEUS sensitivity is more than 80%, the ICER was also cost-effective.

Conclusions: Contrast-enhanced ultrasonography surveillance for HCC is a cost-effective strategy for LC patients and gains their longest additional life years, with similar degree of ICER in the US surveillance group. CEUS surveillance using Sonazoid is expected to be used not only in Japan, but also world-wide.

Key words: contrast-enhanced ultrasonography, cost-effective analysis, hepatocellular carcinoma, Sonazoid, surveillance

INTRODUCTION

HEPATOCELLULAR CARCINOMA (HCC) is the fifth most common neoplasm in the world.[1] Although many environmental factors, including aflatoxins and alcohol,[2,3] have been implicated in the development of HCC, hepatitis B virus and hepatitis C virus (HCV) are the most important factors associated with the progression from chronic hepatitis to cirrhosis, and eventually to HCC.[4] Surveillance for HCC is recommended in patients with chronic liver injury to detect small-sized HCCs, which can be efficiently treated.[5] Ultrasonography (US) is a major surveillance method, because it provides low cost, real-time and non-invasive detection. However, there are some problems associated with this surveillance approach. It is known that the annual incidence of HCC increases with the degree of fibrosis.[6] Unfortunately, an increase in fibrosis makes US surveillance substantially more difficult, because the

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intrahepatic echo patterns in US become rough with advanced fibrosis.

Recently, a novel intravenous contrast medium for US, "Sonazoid", has become available in Japan. This strategy of using US with Sonazoid dramatically improves the sensitivity in the diagnosis of hepatic malignancy.[7] Thus, contrast-enhanced ultrasonography (CEUS) using Sonazoid can effectively detect HCCs that are usually overlooked by B-mode, which is currently used for observation. Therefore, this new contrast medium would be desirable for use in HCC surveillance. However, it is almost five times more expensive than the conventional observational approach in Japan. Until now, the surveillance for HCC using this novel agent has not been evaluated with regard to its cost-effectiveness, and this is the focus of the current study.

METHODS

WE USED TREE Age Pro 2009 (Tree Age Software Inc., William-stown, MA, USA) software to construct a Markov model, and estimated the cost-effectiveness of a surveillance program for HCC. The transition probabilities used in the analysis are listed in Table 1. The age specific mortality rate was obtained

Table 1 Values used in the analyses

Variable	Base value	Range	References
Excess annual mortality			
Child A Cirrhosis	0.02	0.00–0.08	8–11
Child B/C Cirrhosis	0.13	0.07–0.40	
Large HCC	0.90	0.50–1.00	12–14
Annual transition rate			
Child A to Child B/C	0.04	0.02–0.08	8,10,15,16
Small HCC to Large HCC (Undetected)*	0.30	0.10–0.60	17–19
Small HCC to large HCC (TAE treated)*	0.10	0.02–0.20	20–22
Annual incidence of HCC			
Incidence of new HCC	0.07	0.01–0.08	6,8,23–27
Incidence of HCC after curative treatment	0.20	0.10–0.37	13,25,28
Probability of small HCC at diagnosis	0.90	0.66–1.00	23,29
Test characteristics			
US			
Sensitivity	0.70	0.40–0.80	30–32
Specificity	0.90	0.70–0.90	
CEUS			
Sensitivity	0.90	0.80–0.95	7
Specificity	0.95	0.80–0.95	
Cost data			20,23,31,33–37
US	61		
CEUS	248		
Confirmation test	862	170–1 100	
LC	587	300–1 200	38
Decompensated LC	6 422	6 422–23 000	38
Terminal care	5 556	5 000–42 000	38
Resection	19 390	12 000–40 000	39
RFA	10 333	35 000–11 000	39
TAE	7 778	35 000–12 000	
Health-related QOL			40
Child A	0.75	0.66–0.83	
Child B/C	0.66	0.46–0.86	
HCC	0.64	0.44–0.86	

*Per 6 months. The costs were $US/6 months, and the baseline cost has been adjusted to US dollars (Currency rate: $1.00 = \90.00). CEUS, contrast-enhanced ultrasonography; HCC, hepatocellular carcinoma; LC, liver cirrhosis; QOL, quality of life; RFA, radio-frequency ablation; TAE, transcatheter arterial embolization; US, ultrasonography.

from the homepage of the Japanese Ministry of Health, Labour, and Welfare.

Decision model

We estimated the long-term outcomes of different treatments by modifying a previously published computer simulation model[41] using current data on the natural history of chronic hepatitis C in Japan (Fig. 1). Each cycle consisted of 6 months. During each cycle, patients died according to the population-based mortality.

The decision tree for our analysis was composed of three arms: (i) the no surveillance group or "no surveillance" (ii) the B-mode US surveillance group or "US group", and (iii) the CEUS surveillance group or "CEUS group".

Assumptions 1 (program)

Based on the limited information available in the literature, the following assumptions were made:

1 the transition data from liver cirrhosis (LC) to decompensated LC are constant regardless of the patient's age and prior history of HCC;
2 the progression from compensated to decompensated cirrhosis is irreversible;
3 the incidence of HCC is the same in compensated versus decompensated cirrhosis.
4 the probabilities of HCC recurrence and growth remain constant over time;
5 surgery is not performed in patients with a background of decompensated cirrhosis or HCC recurrence; and
6 liver transplantation is not the first-choice for HCC therapy because it is still very rare in Japan.

Assumptions 2 (surveillance)

With regard to surveillance, the following assumptions were made:

1 HCC can be divided into two categories: "small" and "large". Small tumors (1–5 cm in diameter, and no

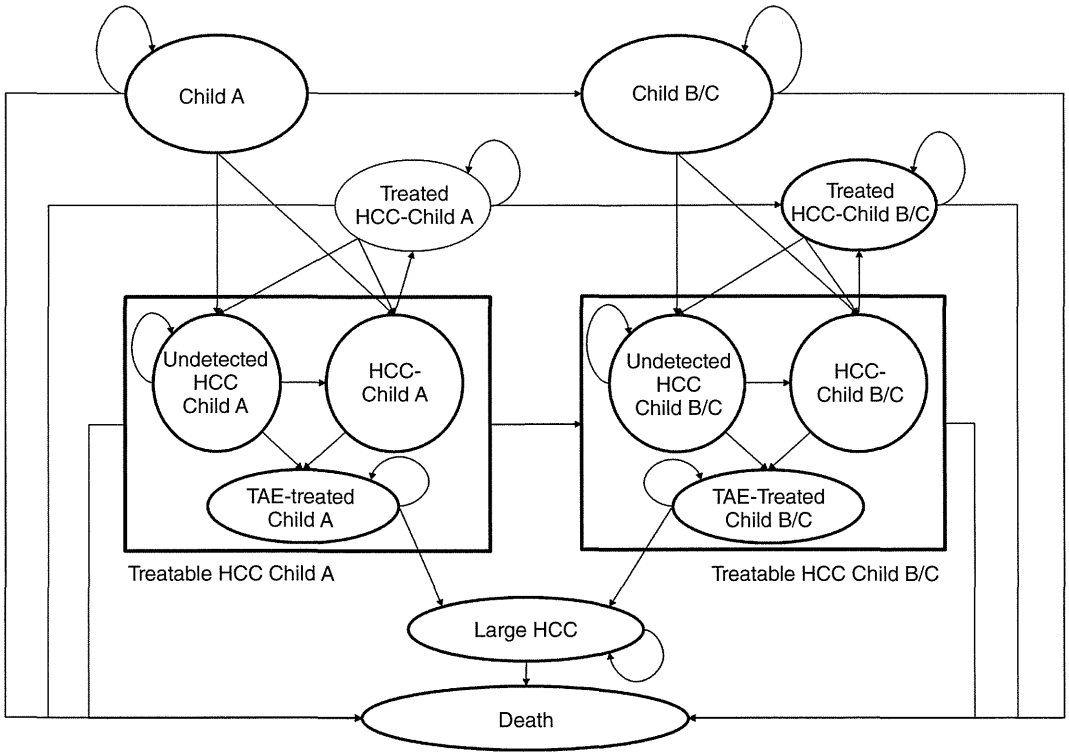


Figure 1 Natural history model. The arrows represent possible transitions during each 6 month cycle. Patients enter this model with Child A cirrhosis, and might develop Child B/C cirrhosis, hepatocellular carcinoma (HCC), both Child B/C and HCC, or death. If the health status does not change, then the patients remain in the same state of health. Surveillance and treatment strategies were superimposed on this model. TAE, transcatheter arterial embolization.

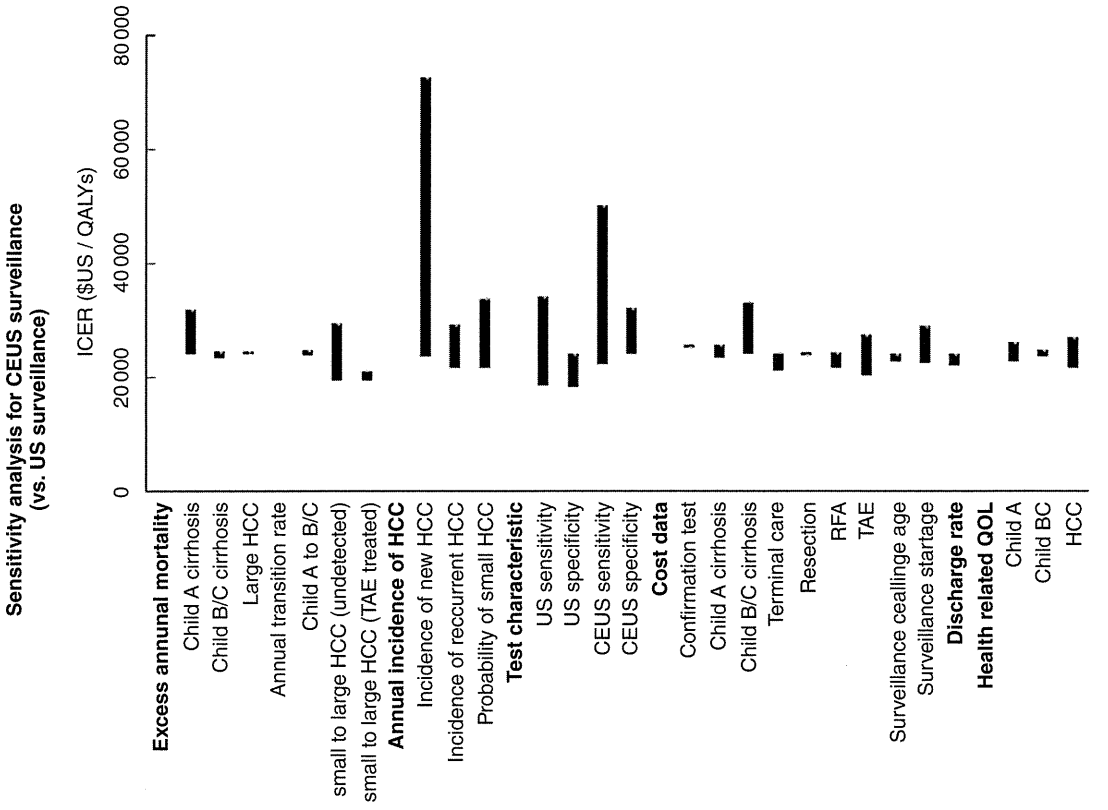


Figure 4 One way sensitivity analysis of the incremental cost-effectiveness ratio (ICER) for the contrast-enhanced ultrasonography (CEUS) surveillance group. When the ICER of the CEUS group was compared with the ultrasonography (US) group, the annual incidence rate of hepatocellular carcinoma (HCC) and CEUS sensitivity were critical parameters in this model. QALY, quality adjusted life year; RFA, radio-frequency ablation; TAE, transcatheter arterial embolization.

Sonazoid is composed of a hard shell containing bubbles, and produces stable, non-linear oscillations in the low-power acoustic field. Because of this feature, Sonazoid provides detailed perfusion features during vascular imaging in the vascular phase, and Kupffer imaging in the post-vascular phase at least 10 min after injection, without collapsing the bubbles. Specifically, Sonazoid CEUS is stable for at least 3 h after injection and allows for multiple and real time scans, because the Sonazoid microbubbles are phagocytosed by Kupffer cells.[44] In contrast, malignant hepatic tumors including HCC contain few or no Kupffer cells, which lead to clear negative contrast as a perfusion defect in Kupffer imaging.[45,46] Thus, surveillance for HCC using Sonazoid is especially useful for LC patients whose liver parenchyma have become roughened by fibrosis. For these reasons, the trend towards the use of US contrast agents in Japan has changed dramatically from Levovist to Sonazoid after it became commercially-available in 2007.

A recent study on the cost-effectiveness of surveillance for HCC reported the sensitivity of US at only 28.6% for detecting middle-sized HCC (between 2 and 5 cm in diameter).[47] The sensitivity of US depends on the skill of the operator, especially in LC patients, in which the intrahepatic echo patterns become roughened with advanced fibrosis. In sensitivity analysis, the US sensitivity was an important factor. When the US sensitivity is expected to be low due to patient physiologic factors such as obesity, CEUS surveillance is recommended. US technicians whose skill may not achieve the average level are also advised to perform additional CEUS using Sonazoid.

Contrast-enhanced ultrasonography sensitivity was a critical factor for cost-effectiveness. When the CEUS group was compared with the US group, and CEUS surveillance was not cost-effective if CEUS sensitivity was lower than 75% (Fig. 5b). As noted earlier, CEUS using Sonazoid is effective for Kupffer imaging, and it

Table 2 Baseline analysis

Strategy	Total cost (US$)	Incremental cost (US$)	Expected life years (year)	QALY	Incremental QALY	IECR (US$/QALY)
No surveillance	29 142	–	10.45	6.18	–	–
US surveillance	58 064	28 922	14.13	7.85	1.67	17 296†
CEUS surveillance	65 726	36 584	14.86	8.17	1.99	18 384† (24 250‡)

†Compared with the no surveillance group.
‡Compared with the US surveillance group.
CEUS, contrast-enhanced ultrasonography; ICER, incremental cost effective rate; QALY, quality-adjusted life-year.

Both the US group and the CEUS group could extend their additional life years as compared with the no US group, regardless of age. The CEUS group could also extend their additional life years as compared with the US group. The biggest difference in expected life years between the US and CEUS groups was 0.93 at an age of 40 years. The superiority of surveillance with CEUS over US was also seen in the 70 year-old patients group. If the sensitivity of US was lower than 50%, then CEUS could extend their additional life years by 2 years and more as compared with the US group.

In the no surveillance group, 55 year-old patients (base value) with compensated HCV-related cirrhosis are expected to live 10.45 life years. When surveillance for HCC with conventional US or CEUS was used in these patients, their expected life years increased by 3.68 years and 4.41 years, respectively. Since the discount rate and health-related utility should be considered in cost-effective analysis, we showed the results of the baseline cost-effectiveness analysis in Table 2. Even though the additional expected life years became small when the program was analyzed while considering the discount rate and health-related utility, in comparison to having no surveillance, the US and CEUS groups still showed an increase in QALYs, 1.67 and 1.99 QALYs, respectively.

Next, the incremental cost-effectiveness ratio (ICER) was estimated, which is a measure of the extra cost incurred to save one year of life. The ICER of the US and CEUS groups, as compared to the no surveillance group, were $US 17 296/QALY and $US 18 384/QALY, respectively. These values were well below $US 50 000/QALY, which is commonly considered to be the cost-effective threshold. Even when the CEUS group was compared with the US group, the ICER of the CEUS group was $US 24 250/QALY, and was also cost-effective.

Sensitivity analysis

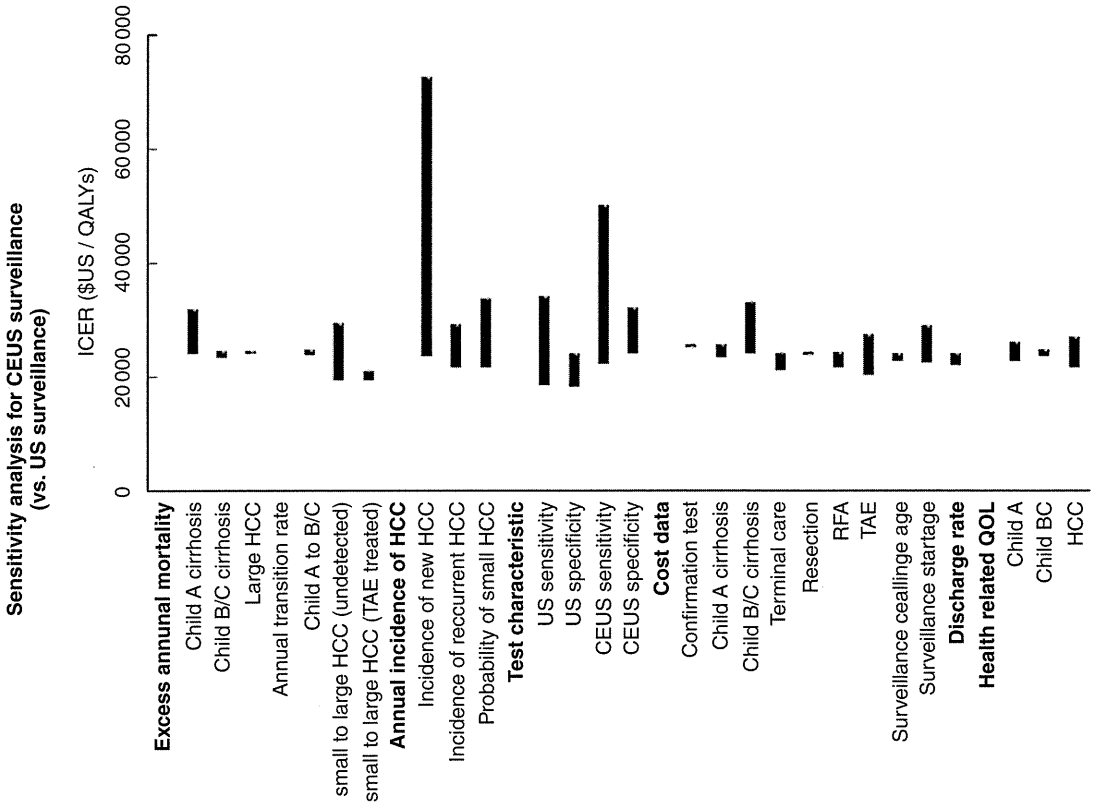
The above results depended largely on the baseline values used in this model, but the estimates of these parameters vary in the published literature. We therefore examined the effects of changing the value of each parameter through sensitivity analysis (Fig. 4). After performing the sensitivity analysis on all parameters in this model, three important parameters emerged in CEUS surveillance compared with US surveillance: the annual HCC incidence rates, and the CEUS sensitivity, and the US sensitivity.

Figure 5a shows the differences of ICERs in varying the US sensitivity. The ICERs of the US and CEUS groups were also less than US$ 20 000, and cost-effective when compared with the no surveillance group. On the other hand, when the CEUS group was compared with the US group, the ICER of the CEUS group increased as the US sensitivity increased up to almost the CEUS sensitivity. However, if the US sensitivity was 80%, then the ICER was $US 34 143, and still less than the threshold of $US 50 000/QALY. If the US sensitivity was lower than 60%, then the ICER of the CEUS group was almost $US 20 000, and thus was more cost-effective. On the other hand, CEUS sensitivity was especially affected when the CEUS group was compared with the US group, and the ICER rose sharply when the CEUS sensitivity was lower than 80% (Fig. 5b).

DISCUSSION

IN THE PRESENT study, we analyzed the cost-effectiveness of CEUS for HCC surveillance using Sonazoid in liver cirrhosis patients, and demonstrated that CEUS surveillance could cost effectively extend the expected life years, even compared with the US surveillance.

Currently, there are only two US contrast agents, Sonazoid and Levovist, which can be used for Kupffer imaging in the post-vascular phase. However, Levovist bubbles are very fragile, and are collapsed by US emissions easily. Therefore, Kupffer imaging in the post-vascular phase using Levovist needs to be performed by a single sweep scan of the liver, which is insufficient for surveillance.



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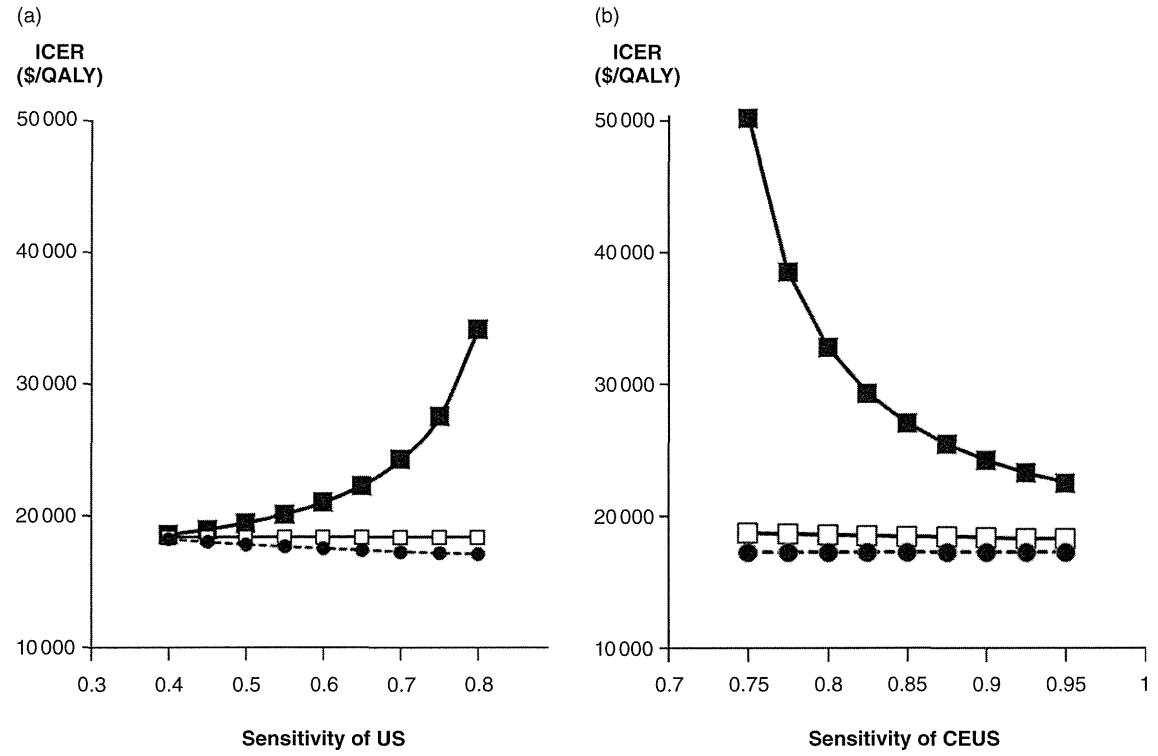

Figure 5 Effects of the sensitivity of ultrasonography (US) (A) and contrast-enhanced ultrasonography (CEUS) (B). When the incremental cost-effectiveness ratio (ICER) was compared with the no surveillance group, both US and CEUS surveillance groups were less than $US 20 000/quality adjusted life year (QALY) in all ranges. The ICER of the CEUS surveillance group achieved $US 50 000/QALY when the sensitivity of CEUS was lower than 0.75. -■-, CEUS (vs US surveillance); -□-, CEUS (vs no surveillance); -●-, US (vs no surveillance).

has been reported to have high sensitivity.[7] This helps technicians to detect the HCC more easily. Thus, a greater than 75% sensitivity represents a reasonable value for Sonazoid CEUS.

The incidence of HCC is the most critical parameter in decision-making for the surveillance of patients with cirrhosis. In our baseline analysis, we selected 7% as a baseline value because most studies in Japan reported 5–8% as the incidence of HCC.[6,26,48,49] This rate is slightly higher than the one in the United States and Europe, where incidence rates are reported from 1.5 to 4%.[8,27] Figure 6 shows how the incidence rate affects the ICER. When the ICER of the CEUS group was compared with the US group, it increased as the rate decreased. However, when the rate was 2%, the ICER of the CEUS group was still less than $50 000/QALY.

Although our results enable us to evaluate the effectiveness of CEUS surveillance, the study has some limitations. First, Sonazoid is available only in Japan. Thus, there are only Japanese published reports for analysis. On the other hand, our baseline data of US sensitivity 70% could be affected by the regional difference, and might be estimated lower than in the Japanese one. However, even if the US sensitivity was as high as 80%, ICER was still lower than $US 40 000 when CEUS surveillance was compared with US surveillance (Fig. 5a).

Similarly, our results were analyzed based on some hypothesis. Thus, the validation is desirable but is difficult because there are also ethical issues. For the solution of the problems, we performed the sensitivity analysis with the widest possible range using many representative reports. As the results of our analysis, we could indicate that the parameters except the HCC incidence rate, US sensitivity and CEUS sensitivity have little impact on cost-effectiveness.

In summary, our analysis suggests that surveillance for HCC in patients with compensated HCV-related cirrhosis by CEUS using Sonazoid was a cost-effective strategy. Since this cost-effectiveness decreased when the HCC incidence rate was low, this strategy should be selected

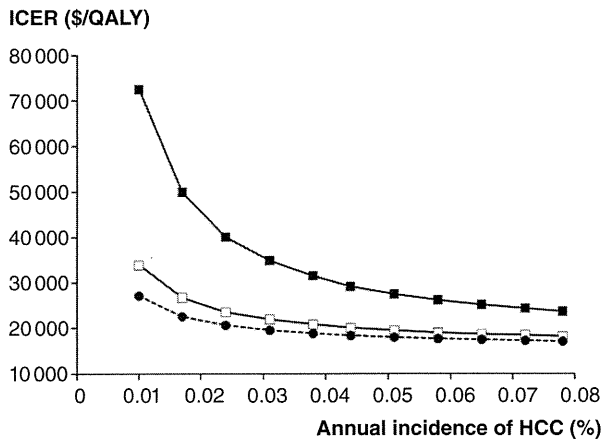

Figure 6 Effects of the annual incidence of hepatocellular carcinoma (HCC). The incremental cost-effectiveness ratio (ICER) values of the ultrasonography (US) and contrast-enhanced ultrasonography (CEUS) surveillance groups were less than $US 35 000/ quality adjusted life year (QALY) in all ranges as compared with the no surveillance group. However, the ICER of the CEUS surveillance group achieved $US 50 000/QALY as compared with the US surveillance group when the incidence was lower than 0.016. -■-, CEUS (vs no surveillance); -□-, CEUS (vs US surveillance); -●-, US (vs no surveillance).

considering of the influence of patient factors such as age, gender and fibrosis grade.

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BRIEF ARTICLE

Elevation of the glycated albumin to glycated hemoglobin ratio during the progression of hepatitis C virus related liver fibrosis

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Author contributions: Aizawa N and Enomoto H contributed equally to this work; Enomoto H and Nakamura H designed and proposed the research; all authors approved the analysis and participated in drafting the article; Aizawa N, Enomoto H, Saito M, Iwata Y, Tanaka H, Ikeda N, Sakai Y, Takashima T, Iwai T, Moriwaki E, Shimomura S and Iijima H treated the patients, performed the liver biopsies and collected the clinical data; all authors were involved in the histological evaluation and the final histopathological results were confirmed by Enomoto H and Imanishi H; Aizawa N, Enomoto H and Nishiguchi S performed the statistical analysis; Enomoto H, Imanishi H and Nakamura H wrote the manuscript; all authors were involved in the manuscript revision and approved the final version of the manuscript.

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Abstract

AIM: To analyze the relationship between the glycated albumin (GA) to glycated hemoglobin (HbA1c) ratio and the histological grading of liver fibrosis.

METHODS: The study retrospectively included consecutive hepatitis C virus positive chronic liver disease patients (n = 142) who had undergone percutaneous liver biopsy between January 2008 and March 2010 at our institution. The ratios of GA/HbA1c were calculated in all patients to investigate the relationship with the degree of the liver fibrosis. The values of the aspartate aminotransferase-to-platelet ratio index (APRI), an excellent marker for the evaluation of liver fibrosis, were also calculated. In addition, we combined the ratio of GA/HbA1c and the APRI in order to improve our ability to detect the presence of significant liver fibrosis.

RESULTS: Sixty-one (43%) patients had either no fibrosis or minimal fibrosis (METAVIR score: F0-F1), while 25 (17%) had intermediate fibrosis (F2). Fifty-six (39%) patients had severe fibrosis (F3-F4) and 27 of them had cirrhosis (F4). The mean values of the GA/HbA1c increased with the progression of the fibrosis (F0-1: 2.83 ± 0.24, F2: 2.85 ± 0.24, F3: 2.92 ± 0.35, F4: 3.14 ± 0.54). There was a significant difference between the F0-F1 *vs* F4, F2 *vs* F4, and F3 *vs* F4 groups ($P < 0.01$, $P < 0.01$, $P < 0.01$ and $P < 0.05$, respectively). The GA/HbA1c ratio was significantly higher in the patients with cirrhosis (F4) than in those without cirrhosis (F0-F3) (3.14 ± 0.54 *vs* 2.85 ± 0.28, $P < 0.0001$). The GA/HbA1c ratio was also significantly higher in the patients with severe fibrosis (F3-F4) than in those without severe liver fibrosis (F0-F2) (3.03 ± 0.41 *vs* 2.84 ± 0.24, $P < 0.001$). Furthermore, the GA/HbA1c ratio was also significantly higher in the patients with significant fibrosis (F2-F4) than in those without significant liver fibrosis (F0-F1) (2.98 ± 0.41 *vs* 2.83 ± 0.24, $P < 0.001$). The diagnostic performance of the increased GA/HbA1c ratio (> 3.0) was as follows: its sensitivity and specificity for the detection of liver cirrhosis (F4) were 59.3% and 70.4%, respectively and its sensitivity and specificity for the detection of severe liver fibrosis (F3-F4) were 50.0% and 74.4%,

respectively. With regard to the detection of significant fibrosis (F2-F4), its sensitivity was 44.4% and its specificity was 77.0%. Although even the excellent marker APRI shows low sensitivity (25.9%) for distinguishing patients with or without significant fibrosis, the combination of the APRI and GA/HbA1c ratio increased the sensitivity up to 42.0%, with only a modest decrease in the specificity (from 90.2% to 83.6%).

CONCLUSION: The GA/HbA1c ratio increased in line with the histological severity of liver fibrosis, thus suggesting that this ratio is useful as a supportive index of liver fibrosis.

Key words: Glycated albumin; Glycated hemoglobin; Liver fibrosis; Liver biopsy; Hepatitis C virus

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INTRODUCTION

Glycated proteins are known to reflect the plasma glucose level and glycated hemoglobin (HbA1c) is used as a standard index of glycemic control in patients with diabetes mellitus[1,2]. Since the lifespan of erythrocytes is about 120 d, HbA1c reflects the glycemia for the recent few months[3]. Glycated albumin (GA) is another index of glycemic control which correlates with the plasma glucose levels during the past few weeks because the turnover of albumin is about 20 d[4,5]. Although the ratio of GA/HbA1c is usually close to 3, the value changes based on the patient's condition[6]. In patients with chronic liver disease (CLD), hypersplenism causes a shortened lifespan of erythrocytes, leading to lower HbA1c levels relative to the plasma glucose level. In contrast, the turnover periods of serum albumin in CLD patients is prolonged in order to compensate for the reduced production of albumin. Therefore, the GA levels in CLD patients are higher relative to the degree of glycemia[6].

Since HbA1c shows lower and GA shows higher values in CLD patients, the GA/HbA1c ratio is thought to be high in patients with liver cirrhosis. Indeed, the GA/HbA1c ratio in patients with CLD has been reported to show an inverse correlation with some indicators of hepatic function (including the hepaplastin test, cholinesterase and bilirubin) independent of the mean plasma glucose levels, thus suggesting that the GA/HbA1c ratio increases as the liver cirrhosis progresses[7]. However, it has not been examined whether the GA/HbA1c ratio correlates with the histological fibrotic stage in CLD patients.

Hepatitis C virus (HCV) is one of the main causes of liver cirrhosis and hepatocellular carcinoma and knowledge about the progression of liver fibrosis is important. In the present study, we analyzed the relationship between the histological grading of liver fibrosis and the GA/HbA1c ratio in 142 patients with HCV-related CLD. Our findings suggest that the GA/HbA1c ratio is associated with the progression of liver fibrosis and cirrhosis in HCV-positive patients.

MATERIALS AND METHODS

Patients

We retrospectively studied HCV-positive CLD patients (n = 142) who had undergone percutaneous liver biopsy between January 2008 and March 2010 at our institution who met the following conditions: (1) HCV infection diagnosed by detectable HCV antibodies and HCV RNA in serum; and (2) blood samples were obtained on the same day of the liver biopsies. Patients with the following conditions were excluded from the study: the presence of other liver diseases, hepatocellular carcinoma, immunosuppressive therapy, hepatitis B virus co-infection and those with insufficient liver tissue for staging of fibrosis. The present study did not include patients whose GA/HbA1c ratios could have been influenced by poorly controlled diabetes.

The routine studies, including platelet counts, prothrombin time international normalized ratio (PT-INR), liver functional tests [alanine transaminase (ALT), aspartate aminotransferase (AST), alkaline phosphatase and total bilirubin] were performed. Since the index calculated by the combination of GA and HbA1c (CLD-HbA1c: defined as the average of the measured HbA1c and GA/3) was reported to be a good indicator for the evaluation of the mean plasma glucose level in patients with CLD[8], HbA1c and GA were also routinely measured in all patients. The values of GA and HbA1c were determined in the same sample and on the same day as the liver biopsies were performed. The AST-to-platelet ratio index (APRI), an excellent marker for the evaluation of liver fibrosis, was also calculated based on the formula proposed by Wai *et al*[9]: APRI = [(AST level/upper limit of normal)/platelet counts (10^9/L)] × 100. Written informed consent regarding the liver biopsy and retrospective use of clinical data was obtained from all patients on admission. This study was approved by the ethics committees of the institutional review board.

Liver biopsy

Liver biopsy examinations were performed using the

Table 1 Characteristics of the patients

Age (yr)	60 (19-78)
Gender (male/female)	60/82
AST (IU/L)	37.5 (14-328)
ALT (IU/L)	36 (10-388)
γ-GTP (IU/L)	29 (7-259)
ALP (IU/L)	217 (97-556)
Total bilirubin (mg/dL)	0.7 (0.1-2.1)
Albumin (g/dL)	3.96 ± 0.36
Hemoglobin (g/dL)	13.4 ± 1.8
Platelet ($\times 10^4$/mm^3)	15.9 ± 5.5
PT-INR	1.04 ± 0.07

AST: Aspartate aminotransferase; ALT: Alanine transaminase; ALP: Alkaline phosphatase; PT-INR: Prothrombin time international normalized ratio.

standard procedures and all liver specimens were evaluated by well-trained pathologists at our institute, with evaluation of the fibrosis stage and activity grade according to the METAVIR scoring system[10]. Fibrosis was staged on a scale of 0-4 (F0: no fibrosis, F1: portal fibrosis without septa, F2: portal fibrosis with rare septa, F3: numerous septa without cirrhosis, F4: liver cirrhosis). The histological evaluation of the biopsy samples was also routinely performed in our department. All authors participated in the conference about the histological evaluation and the final results were confirmed by two authors (Enomoto H and Imanishi H) who received training for histological studies.

Statistical analysis

In the present study, we attempted to clarify whether the GA/HbA1c ratio was associated with liver fibrosis and cirrhosis. The data for the comparisons among the groups "F0-1 *vs* F2 *vs* F3 *vs* F4" was analyzed by non-repeated measurements ANOVA and statistical significance was further examined by the Student-Newman-Keuls test. We compared the "F0-F3 (no cirrhosis) *vs* F4 (cirrhosis)", "F0-F2 (no - intermediate fibrosis) *vs* F3-F4 (severe fibrosis)" and "F0-F1 (no approximately minimal fibrosis) *vs* F2-F4 (significant fibrosis)" groups. The differences in the baseline characteristics and GA/HbA1c ratios of the groups were evaluated. Quantitative variables were expressed as the mean ± SD and those with an abnormal distribution were expressed as the median values (range). Statistical analysis was performed using Student's *t* test or the Mann-Whitney *U* test, as appropriate.

RESULTS

Characteristics of patients and clinical data

From January 2008 to March 2010, a total of 142 patients with HCV were consecutively included in the present study, based on the inclusion and exclusion criteria as described in the "Patients and Methods" section. The characteristics of the study population are summarized in Table 1. The population consisted of 60 (42%) males and 82 (58%) females, and the age of patients ranged from 19 to 78 years old (median 60). According to the METAVIR liver fibrosis staging[10], 56 (39%) patients had significant fibrosis (F3-F4) and 27 (19%) had cirrhosis (F4).

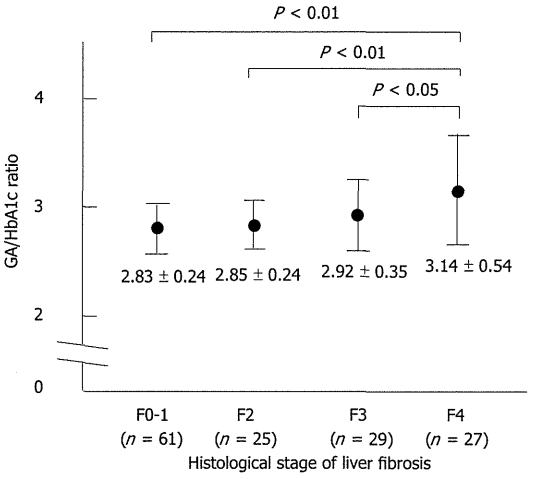

Figure 1 The glycated albumin/glycated hemoglobin ratio in relation to the METAVIR fibrosis score in patients with hepatitis C virus-related chronic liver disease. The glycated albumin (GA)/glycated hemoglobin (HbA1c) ratio increased as the fibrosis progressed. There was a significant difference between the F0-F1 *vs* F4, F2 *vs* F4, and F3 *vs* F4 groups.

The GA/HbA1c ratio in patients with HCV

The GA/HbA1c ratio in patients with CLD has been reported to show an inverse correlation with certain indicators of hepatic function. As shown in Figure 1, the mean values of the GA/HbA1c increased with the progression of the fibrosis stage, suggesting that the GA/HbA1c ratio was associated with the histological severity of liver fibrosis.

Comparing the F0-F3 (no cirrhosis) and F4 (cirrhosis) groups, we found that there was a significant difference in several parameters which correlated with hepatic function; that is, higher AST, ALT, γ-GTP alkaline phosphatase (ALP) and PT-INR levels and also a lower platelet count, and albumin values in the presence of cirrhosis (Table 2; left). However, no significant difference was observed in other parameters such as age and gender, which were not related to the hepatic function. Between the two groups, the GA/HbA1c ratio was significantly higher in patients with cirrhosis (Figure 2A), thus suggesting that the GA/HbA1c ratio is associated with the cirrhotic changes in the liver.

Next, we examined whether the GA/HbA1c ratio differed in patients with or without severe liver fibrosis. Comparing the F0-F2 (without severe fibrosis) and F3-F4 (with severe fibrosis) groups, we found significant differences, with higher AST, ALT, γ-GTP, ALP and PT-INR values and a lower platelet count, and albumin values in the presence of severe fibrosis (Table 2; middle). In patients with severe liver fibrosis, the GA/HbA1c ratio was significantly higher (Figure 2B) than that in patients without severe fibrosis, suggesting that the GA/HbA1c ratio

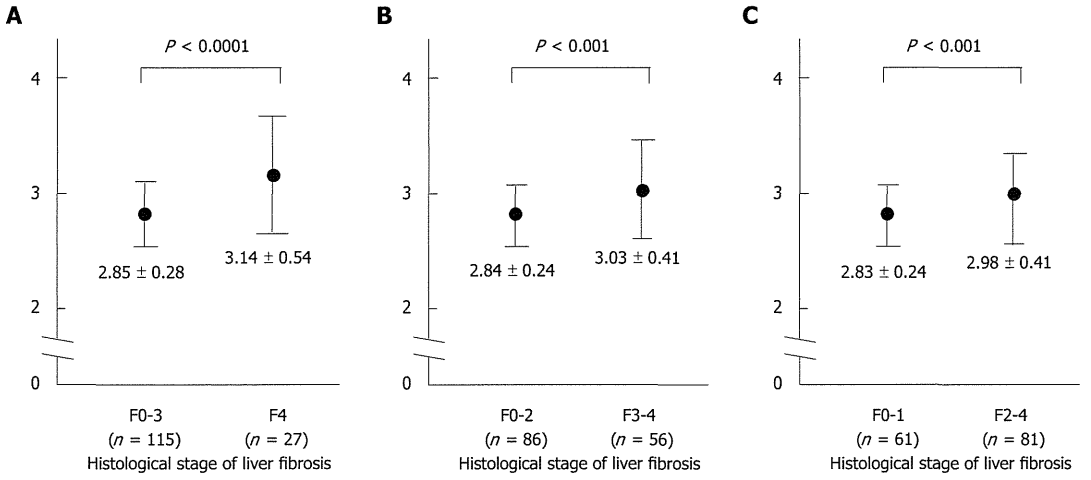

Figure 2 The glycated albumin/glycated hemoglobin ratio in patients with hepatitis C virus-related chronic liver disease. A: A comparison between the F0-F3 (no cirrhosis) group and F4 (cirrhosis) group. The glycated albumin (GA)/glycated hemoglobin (HbA1c) ratio was higher in patients with cirrhosis than that in non-cirrhotic patients; B: The comparison between the F0-F2 (no or intermediate fibrosis: without severe fibrosis) group and the F3-F4 (severe fibrosis) group. The GA/HbA1c ratio was higher in the patients with significant fibrosis than that in the patients with no or minimal fibrosis; C: A comparison between the F0-F1 (no or minimal fibrosis: without significant fibrosis) group and the F2-F4 (significant fibrosis) group. The GA/HbA1c ratio was higher in the patients with significant fibrosis than in those with either minimal fibrosis or none at all.

Table 2 Characteristics of the patients (F0-F3 *vs* F4), (F0-F2 *vs* F3-F4) and (F0-F1 *vs* F2-F4)

	F0-F3 (*n* = 115)	F4 (*n* = 27)	*P* value	F0-F2 (*n* = 86)	F3-F4 (*n* = 56)	*P* value	F0-F1 (*n* = 61)	F2-F4 (*n* = 81)	*P* value
Age (yr)	60 (19-78)	62 (23-78)	NS	60 (19-78)	62 (23-78)	NS	60 (19-78)	62 (23-78)	NS
Gender (male/female)	48/67	12/15	NS	31/55	29/37	NS	25/36	35/46	NS
AST (IU/L)	35 (14-195)	50 (20-328)	< 0.001	32 (14-175)	46 (20-328)	< 0.001	32 (14-104)	42 (18-328)	< 0.001
ALT (IU/L)	38 (10-388)	47 (10-310)	< 0.05	31.5 (10-388)	48 (10-310)	< 0.01	31 (11-388)	46 (10-310)	< 0.01
γ-GTP (IU/L)	25 (7-183)	50 (12-259)	< 0.001	22 (7-183)	42.5 (12-259)	< 0.0001	22 (8-183)	36 (7-259)	< 0.01
ALP (IU/L)	207 (97-490)	267 (133-556)	< 0.001	186 (97-465)	275 (133-556)	< 0.0001	207 (97-465)	258 (101-556)	< 0.001
Total bilirubin (mg/dL)	0.7 (0.1-1.6)	0.7 (0.3-2.1)	NS	0.7 (0.1-1.6)	0.8 (0.3-2.1)	NS	0.7 (0.1-1.6)	0.7 (0.3-2.1)	NS
Albumin (g/dL)	4.02 ± 0.31	3.70 ± 0.43	< 0.001	4.03 ± 0.32	3.84 ± 0.37	< 0.01	4.05 ± 0.31	3.89 ± 0.38	< 0.01
Hemoglobin (g/dL)	13.5 ± 1.7	12.8 ± 2.0	NS	13.5 ± 1.8	13.3 ± 1.7	NS	13.7 ± 1.7	13.2 ± 1.8	NS
Platelet ($\times 10^4/mm^3$)	16.5 ± 5.3	13.2 ± 5.9	< 0.001	17.2 ± 5.2	13.8 ± 5.5	< 0.001	17.2 ± 4.8	14.9 ± 5.9	< 0.05
PT-INR	1.03 ± 0.05	1.08 ± 0.06	< 0.001	1.02 ± 0.05	1.07 ± 0.06	< 0.001	1.02 ± 0.05	1.05 ± 0.08	< 0.05

AST: Aspartate aminotransferase; ALT: Alanine transaminase; ALP: alkaline phosphatase; PT-INR: Prothrombin time international normalized ratio.

also correlates with the progression of liver fibrosis.

We also examined whether the GA/HbA1c ratio differed in patients with or without significant liver fibrosis. When we compared the F0-F1 (no or minimal fibrosis: without significant fibrosis) and F2-F4 (with significant fibrosis) groups, we also found significant differences, with higher AST, ALT, γ-GTP ALP and PT-INR values and a lower platelet count and albumin values in the presence of significant fibrosis (Table 2; right). In patients with significant liver fibrosis, the GA/HbA1c ratio was significantly higher than that in patients without significant fibrosis (Figure 2C).

Although the GA/HbA1c ratio is usually about 3, we found that the ratio increased in line with the progression of liver fibrosis (Figure 2). We therefore evaluated the diagnostic performance of the increased GA/HbA1c ratio (> 3.0) for the detection of patients with cirrhosis (F4), severe fibrosis (F3-F4) and significant fibrosis (F2-F4) (Table 3). Its sensitivity for the detection of liver cirrhosis was 16/27 (59.3%) and the specificity was 81/115 (70.4%). With regard to the detection of severe fibrosis, the sensitivity of the increased GA/HbA1c ratio (> 3.0) was 28/56 (50.0%) and its specificity was 64/86 (74.4%). With regard to the detection of significant fibrosis, the sensitivity of the increased GA/HbA1c ratio (> 3.0) was 36/81 (44.4%) and its specificity was 47/61 (77.0%).

Combination of the GA/HbA1c ratio and APRI for the detection of significant liver fibrosis

As described above, the GA/HbA1c ratio in patients with significant liver fibrosis was higher than that in patients without significant fibrosis. However, the differences were small and the GA/HbA1c ratio had difficulty in distinguishing between F1 and F2.

Several biomarkers for the evaluation of fibrosis have