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Anti-interferon-α neutralizing antibody is associated with nonresponse to pegylated interferon-α plus ribavirin in chronic hepatitis C

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SUMMARY. Pegylated interferon-α (PEG-IFN-α) plus ribavirin (RBV) treatment fails to achieve a sustained virological response (SVR) in approximately 20–50% of patients with chronic hepatitis C virus (HCV) infection. We assessed the contribution of an anti-IFN-α neutralizing antibody (NAb) on the nonresponse to treatment. NAbs were detected using an antiviral assay that assessed the neutralizing effects of serum samples against IFN. Serum samples were obtained at the end of the treatment and evaluated for the presence of NAbs using recombinant IFN-α as a standard. We studied 129 PEG-IFN-α/RBV-treated patients. In the 82 end-of-treatment responders, no NAbs were detected. Of the 47 patients who did not respond, seven (15%) were positive for NAbs. We also examined an additional 83 patients who had not responded to PEG-IFN-α treatment, and detected 12 with NAbs. Patients with good IFN-responsive characteristics, including HCV genotype 2/3 and major allele homozygotes for *interleukin-28B*, were included in the 19 patients with NAbs. No NAbs interfered with the antiviral activity of natural human IFN-β (nIFN-β) and re-treatement of patients with NAbs with nIFN-β/RBV achieved SVR. Our analyses revealed that the emergence of anti-IFN-α NAbs was a candidate causal factor of PEG-IFN-α-treatment failure. Therefore, these antibodies should be assayed in patients who do not respond to PEG-IFN-α therapy, and if detected, other effective treatments, i.e., medications that are not neutralized by anti-IFN-α NAbs, should be considered.

Keywords: anti-interferon-α neutralizing antibody, chronic hepatitis C, natural interferon-β, nonresponse, pegylated interferon-α.

INTRODUCTION

Currently, the standard therapy for chronic hepatitis C virus (HCV) infection is pegylated interferon-α (PEG-IFN-α) combined with ribavirin (RBV), whose sustained virological response (SVR) rate is approximately 50% in genotype 1 patients with a high viral load. However, 30–40% of patients remain HCV RNA-positive (nonresponders) at the end of therapy [1–3]. Many host characteristics (e.g. age, sex and *interleukin-28B* (*IL28B*) gene polymorphisms) and viral factors (e.g. genotype and mutations in the HCV RNA sequence) are reportedly associated with a nonresponse (NR) to PEG-IFN-α therapy; however, not all 'IFN-responsive' patients respond to PEG-IFN-α, indicating that additional IFN-unresponsive factor(s) may exist.

Anti-IFN neutralizing antibodies (NAbs), which bind to IFN and interfere with its biological activity by blocking its interaction with its receptor, were considered responsible for the failure of host-related recombinant IFN (rIFN) treatment [4]. The presence of anti-IFN NAbs is a common problem in patients who receive IFN treatment for conditions such as leukaemia, multiple sclerosis and chronic hepatitis C [5–8]. The prevalence and clinical effects of anti-IFN-β NAbs have been particularly well analysed in patients with multiple

Abbreviations: ALT, alanine aminotransferase; EVR, early virological response; HCV, hepatitis C virus; IL28B, interleukin-28B; ISDR, interferon sensitivity-determining region; NAb, neutralizing antibody; nIFN, natural interferon; NR, nonresponse; PCR, polymerase chain reaction; PEG-IFN-α, pegylated interferon-α; RBV, ribavirin; rIFN, recombinant interferon; RR, relapse response; SVR, sustained virological response; TRU, 10-fold reduction unit.

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sclerosis [8,9]. The European guidelines for multiple sclerosis patients recommend measuring NAbs during rIFN-β therapy and discontinuing rIFN-β therapy in NAb-positive patients [10].

Several studies have suggested that among chronic hepatitis C patients receiving rIFN-α (which was the first-choice treatment for these patients before the generation of PEG-IFN-α), the emergence of anti-IFN-α NAbs is higher in the NR group than in the responder group [5,11,12]. Meanwhile, the development of anti-IFN-α NAbs in patients receiving PEG-IFN-α has been poorly examined; the association between anti-IFN-α NAbs and NR to PEG-IFN-α therapy is unclear. Consequently, the guidelines from the American Association for the Study of Liver Diseases, the European Association for the Study of the Liver and the Japan Society of Hepatology for hepatitis C include no description of anti-IFN NAbs, and NAbs are not measured in PEG-IFN-α-treated patients. Thus, the clinical impact of anti-IFN-α NAbs has not been considered in PEG-IFN-α-treated patients.

In this report, to assess the association of NAbs with NR, we measured anti-IFN-α NAbs in the sera of chronic hepatitis C patients who received PEG-IFN-α/RBV treatment. We determined the emergence rate of NAbs and verified the clinical significance of NAb detection in PEG-IFN-α/RBV-treated patients. Additionally, we assessed the specific characteristics of anti-IFN-α NAb-positive patients. We also assessed the potential antiviral efficacy of other types of IFN in anti-IFN-α NAb-positive patients.

PATIENTS AND METHODS

Patients

The serum levels of anti-IFN NAbs were measured in 129 patients with chronic hepatitis C who received treatment with PEG-IFN-α2a/RBV or PEG-IFN-α2b/RBV from March 2005 to March 2009 at Hyogo College of Medicine Hospital (Study 1). To identify and assess the characteristics of additional anti-IFN-α NAb-positive patients, we also examined 83 NR patients who received PEG-IFN-α2a or PEG-IFN-α2b with or without RBV from April 2009 to December 2010 in Hyogo College of Medicine Hospital and 12 other hospitals in Japan (Study 2). All patients examined for anti-IFN NAb agreed to the research and some also agreed to genomic research. All patients provided written informed consent. This study was approved by the ethics committees of the appropriate institutional review boards in accordance with the Declaration of Helsinki (Hyogo College of Medicine Approved No. Hi-78, Hi-92 and Hi-112).

PEG-IFN-α2a (PEGASYS; Roche, Basel, Switzerland) was administered at 180 μg/week for 24–72 weeks together with 0.6–1.0 g/day RBV (COPEGUS; Roche). PEG-IFN-α2b (Pegintron; Merck, Whitehouse Station, NJ, USA) was administered at 60–150 μg/week for 24–72 weeks along with 0.6–1.0 g/day RBV (Rebetol; Merck). Natural human IFN-β (nIFN-β, FERON; Toray Industries, Tokyo, Japan) was administered at 6.0×10^6 IU/day for 4 weeks and 6.0×10^6 IU three times/week for the next 20–44 weeks (24–48 weeks in total) together with 0.6–1.0 g/day RBV (Rebetol; Merck). Serum samples were obtained at the end of the treatment and evaluated for the presence of anti-IFN-α NAbs.

Determination of patient characteristics

HCV RNA viral load (log copies/mL) was determined by real-time quantitative polymerase chain reaction (PCR, TaqMan PCR; Life Technologies Corporation, Carlsbad, CA, USA). Patients who tested positive for HCV RNA at the end of treatment were classified as the NR group. Patients who were negative for HCV RNA at the end of treatment were judged to be responders, and these patients were further classified into the relapse response (RR) and SVR groups. SVR patients were defined as those with undetectable serum HCV RNA at 24 weeks after the end of treatment, whereas RR patients were defined as those in which serum HCV RNA reappeared at 24 weeks after the end of treatment. Viral kinetics during PEG-IFN-α therapy were also examined by determining the number of patients with an early viral response (EVR, i.e. a $\geq$ 2 log reduction in HCV RNA compared with baseline HCV RNA at treatment week 12), null response (i.e. failure to have a $\geq$ 2 log reduction in HCV RNA compared with baseline HCV RNA during treatment), or breakthrough response (i.e. a $\geq$ 2 log elevation in HCV RNA at the end of treatment compared with the lowest HCV RNA level during treatment).

Prior to initiating PEG-IFN-α therapy, liver biopsies were taken and evaluated according to the Histology Activity Index score [13]. In HCV genotype 1b patients, AA70 and AA91 of the HCV core and NS5A [IFN sensitivity-determining region (ISDR)] sequences were determined according to previous reports [14]. Gene polymorphism analysis of *IL28B* was performed in patients who agreed to genomic analysis. A single nucleotide polymorphism (SNP) near the *IL28B* gene (rs8099917), which is significantly associated with IFN sensitivity, was examined as described previously [15]. *IL28B*-type of patients was classified into major-homo (T/T, major allele homozygotes), hetero (T/G, major/minor allele heterozygotes) or minor-homo (G/G, minor allele homozygotes).

Detection of anti-IFN-neutralizing antibodies

Anti-IFN NAbs were detected in the patients' sera by an antiviral assay using FL cells and Sindbis virus according to Kawade's method [16,17]. A positive result was defined as the ability to neutralize the antiviral activity of 10 laboratory units (LU)/mL of a standard IFN preparation. rIFN-α was used as the standard IFN to determine whether pa-

tients were positive or negative for anti-IFN-α NAbs. We used human rIFN-α2a (ProSpec, Rehovot, Israel) to detect anti-IFN-α NAbs in PEG-IFN-α2a-treated patients, and human rIFN-α2b (ProSpec) to detect anti-IFN-α NAbs in PEG-IFN-α2b-treated patients. The neutralizing activity of serum against PEG-IFN-α2a, PEG-IFN-α2b, natural human IFN-α (nIFN-α, Sumiferon; Dainippon Sumitomo Pharma, Osaka, Japan) and nIFN-β was also examined in each sample. Twofold serial dilutions (starting from 1:8) of serum samples in 60-μL volumes were incubated with 60 μL of 20 LU/mL IFN for 1 h at room temperature. Then, 100 μL of the solutions was added to FL cells in 96-well microtitre plates that were cultured in 100 μL medium/well from the previous day. The assay was performed in duplicate for each sample. After 18–24 h of cell culture, the cells were washed and challenged with the Sindbis virus at 37 °C for 22–24 h. The cells were fixed and stained using crystal violet with 10% ethanol and 6% formalin, and the absorbance was measured at 590 nm to determine cell viability. When the antiviral activity of IFN was disturbed in a serum concentration-dependent manner, the sample was adjudged as NAb-positive. For the NAb-positive samples, titres were calculated and expressed as 10-fold reduction units (TRU/mL) [16,18].

Statistical analysis

Univariate analyses were performed to elucidate significant factors for NR with PEG-IFN plus RBV therapy. Differences between groups were assessed using the chi-square test or Student's *t*-test. *P* values <0.05 were considered statistically significant.

RESULTS

Incidence of anti-IFN-α NAb in PEG-IFN-α/RBV-treated patients

Study 1 consisted of 54 SVR (41.8%), 28 RR (21.7%) and 47 NR (36.4%) patients. Fifty-one patients were treated with PEG-IFN-α2a, and 78 were treated with PEG-IFN-α2b; the former consisted of 21 SVR (41.2%), 14 RR (27.5%) and 16 NR (31.4%) patients, and the latter consisted of 33 SVR (42.3%), 14 RR (17.9%) and 31 NR (39.7%) patients. The baseline characteristics and viral kinetics of all 129 patients are shown in Table 1.

The presence of anti-IFN-α NAbs in Study 1 is shown in Table 2. The seven anti-IFN-α NAb-positive patients were all NR. One of the seven NAb-positive patients already had anti-

Table 1 Baseline characteristics and viral response in 129 patients with chronic hepatitis C examined for anti-IFN-α NAbs (Study 1)

Baseline characteristics	SVR	RR	NR
Patients (%)	54 (41.8%)	28 (21.7%)	47 (36.4%)
Mean age, years	51.7 ± 12.1	57.9 ± 11.3	60.4 ± 8.6
Sex, male/female (% male)	25/29 (46%)	13/15 (46%)	22/25 (47%)
Previous IFN-α therapy, +/− (% positive)	12/42 (22%)	11/17 (39%)	15/32 (32%)
Mean HCV RNA level, log copies/mL	6.0 ± 0.7	6.2 ± 0.6	6.3 ± 0.5
Mean ALT level, U/L	62.4 ± 44.4	53.0 ± 35.2	69.3 ± 46.2
Liver histology			
Grade (A), 0–1/2–3	26/25	11/15	24/19
Stage (F), 0–1/2/3–4	31/12/8	8/8/10	14/13/16
HCV genotype, 1/2	38/15	23/4	46/1
HCV core*			
AA70, wild-type/mutant	27/8	12/12	24/20
AA91, wild-type/mutant	24/11	14/10	34/12
HCV ISDR*, wild-type/mutant	23/10	18/6	38/8
IL28B allele (rs8099917), major-homo/hetero/minor-homo	23/9/0	15/5/1	18/21/1
Viral response			
EVR/non-EVR	54/0	28/0	18/29
Null response	–	–	23
Breakthrough	–	–	5

NAb, neutralizing antibody; SVR, sustained virological response; RR, relapse response; NR, nonresponse; ALT, alanine aminotransferase; ISDR, IFN sensitivity-determining region; IL28B, interleukin-28B; EVR, early virological response.
Mean ± SD or patient numbers are indicated in each category.
*HCV core and ISDR were examined in HCV genotype 1b patients.

Table 2 Incidence of anti-IFN-α NAbs in PEG-IFN-α/RBV-treated chronic hepatitis C patients

	Study 1 (n = 129)		
Anti-IFN-α NAbs	SVR	RR	NR
Negative	54	28	40
Positive	0	0	7
% for NR	–	–	14.9%
% for total	–	–	5.4%

NAbs, neutralizing antibodies; PEG-IFN-α, pegylated interferon-α; RBV, ribavirin; SVR, sustained virological response; RR, relapse response; NR, nonresponse.

IFN-α NAbs before the administration of PEG-IFN-α/RBV therapy. Of the NR patients, 14.9% were NAb-positive, and of all patients, 5.4% were NAb-positive. Of the seven NAb-positive patients, four were treated with PEG-IFN-α2a, and three were treated with PEG-IFN-α2b. All responders (SVR + RR) were NAb-negative.

In Study 1, univariate analyses revealed a significantly higher NR rate in patients with anti-IFN-α NAbs (P = 0.0001), high age (P = 0.0015), low red blood cell counts (P = 0.0073), high serum aspartate aminotransferase levels (P = 0.0129), low serum albumin levels (P = 0.0036), low serum total cholesterol levels (P = 0.0287), high HCV RNA levels (P = 0.0157), HCV genotype 1 (P = 0.0003) and *IL28B* hetero or minor-homo (P = 0.0091).

Among the 83 patients in Study 2, 32 received PEG-IFN-α2a with RBV, eight received PEG-IFN-α2a without RBV, and 43 received PEG-IFN-α2b with RBV, and all were NR. In Study 2, 12 patients were positive for anti-IFN-α NAbs.

Time course of HCV RNA, alanine aminotransferase (ALT) and NAb titres in anti-IFN-α NAb-positive patients

The temporal changes in the anti-IFN-α NAb titres during treatment were examined in 6 NAb-positive patients from Study 1. The time course of HCV RNA, ALT and anti-IFN-α NAb titres is shown for each patient in Fig. 1. In patients #1–3, HCV RNA steadily decreased by more than 2 log units until 20–30 weeks of treatment and thereafter gradually increased to pretreatment levels (Fig. 1a–c). These three patients were initially NAb-negative, but converted to NAb-positive after the HCV RNA levels reached their lowest point. In patients #4–6, the HCV RNA levels did not decrease by ≥2 log units during treatment (Fig. 1d–f). Patients #4 and #5 were initially NAb-negative, but converted to NAb-positive by the end of the treatment (Fig. 1d,e). Patient #6, who had received IFN-α therapy several times prior to PEG-IFN-α2b/RBV therapy, was NAb-positive before, during and after the treatment period (Fig. 1f).

Characteristics of the anti-IFN-α NAb-positive patients

The background details of the 7 (Study 1) and 12 (Study 2) NAb-positive patients are shown in Table 3. Patients with IFN-responsive backgrounds (i.e. low age, low HCV RNA levels, nonadvanced chronic liver disease, HCV genotype 2/3, wild type in core AA70 and AA91, mutation in ISDR or *IL28B* major-homo) were included in the NAb-positive patients. Of these 19 patients, nine were naïve patients who had not received IFN-α therapy prior to the initiation of PEG-IFN-α therapy.

In Study 1, five (17%) of the 29 patients who failed to obtain EVR were NAb-positive (Tables 1 & 3). Twenty-three patients exhibited a null response, of whom four (17%) were NAb-positive. Of the total five breakthrough patients, three (66%) were NAb-positive.

Neutralizing activity of anti-IFN-α NAb-positive sera against rIFN-α, PEG-IFN-α, nIFN-α and nIFN-β

The neutralizing effects of anti-IFN-α NAb-positive sera against IFN pharmaceuticals were examined and the titres were compared. The sera obtained from the 19 NAb-positive patients (Studies 1 and 2) at the end of treatment were examined, and the number of patients with high-titre sera (>1000 TRU/mL), moderate titre sera (100–1000 TRU/mL), low titre sera (<100 TRU/mL) or no neutralizing activity (negative) is shown in Fig. 2. All anti-IFN-α NAb-positive sera cross-reacted with rIFN-α2a and rIFN-α2b, and titres against rIFN-α2a and rIFN-α2b were nearly equal in each serum sample. All serum samples were also able to neutralize PEG-IFN-α2b; titres against PEG-IFN-α2b were at approximately the same level as titres against rIFN-α. The four serum samples that exhibited low titres against rIFN-α failed to neutralize PEG-IFN-α2a. In the other 15 sera, titres against PEG-IFN-α2a were much lower than titres against rIFN-α. Titres against nIFN-α were even lower than titres against PEG-IFN-α2a, and >50% of the sera could not neutralize nIFN-α. None of the sera exhibited neutralizing activity against nIFN-β.

Therapeutic effect of nIFN-β in anti-IFN-α NAb-positive patients

In 10 of the 19 anti-IFN-α NAb-positive patients, nIFN-β was administered in the presence of anti-IFN-α NAbs. The baseline characteristics and viral kinetics of these 10 patients are shown in Table 4.

Three patients received nIFN-β monotherapy, one of whom obtained complete EVR (cEVR, i.e. undetectable HCV RNA within 12 weeks of treatment), although HCV RNA had increased to baseline levels by the end of the treatment. This patient was HCV genotype 2b (*IL28B* was not determined). The other two patients, who were HCV genotype 1b and *IL28B* hetero, showed a null response to nIFN-β.

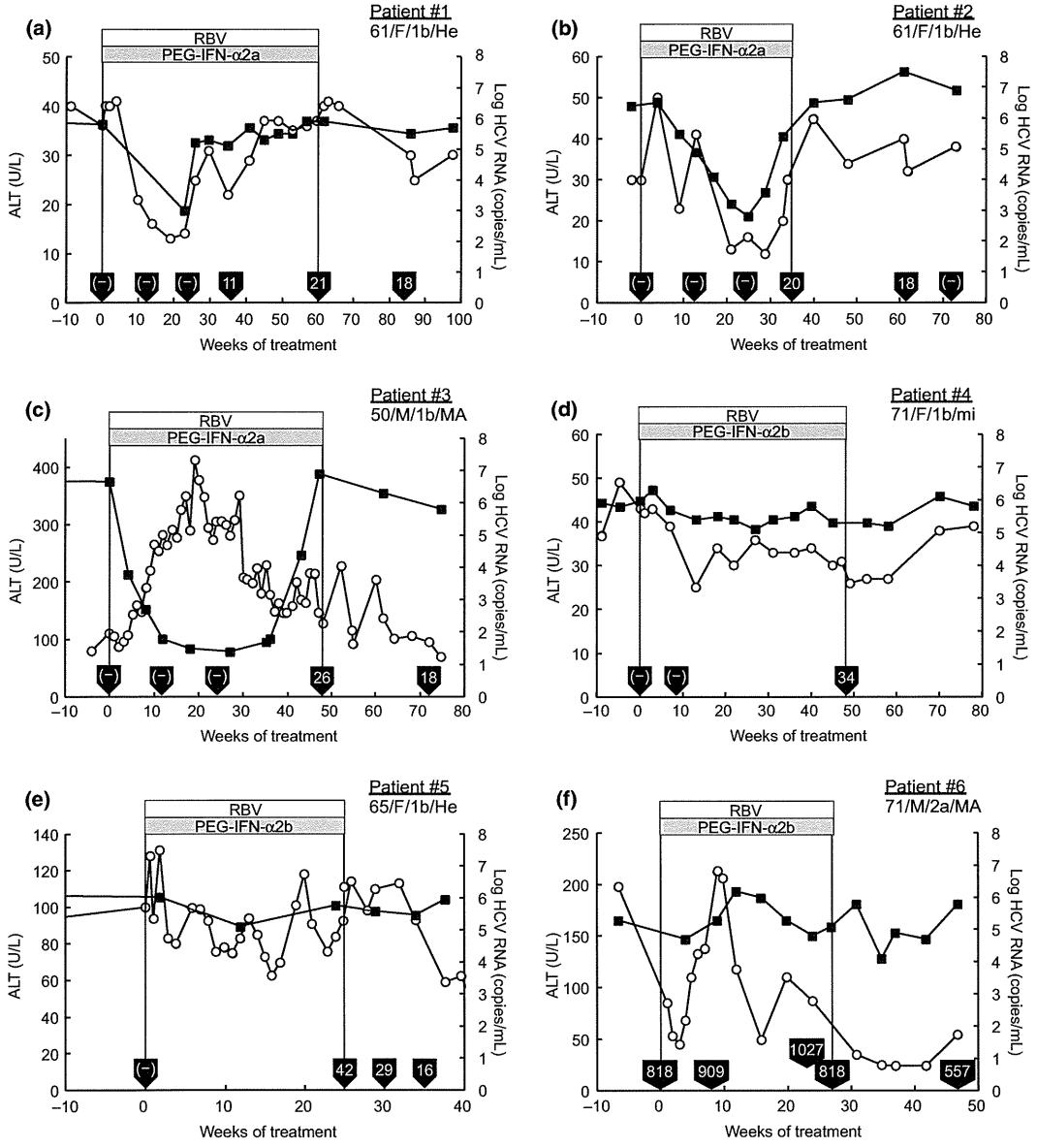

Fig. 1 Time course of hepatitis C virus (HCV) RNA, alanine aminotransferase (ALT) and anti-interferon-α (anti-IFN-α) neutralizing antibody (NAb) incidence in six NAb-positive patients (patients 1–6) from Study 1 who had been treated with pegylated interferon-α plus ribavirin (PEG-IFN-α/RBV) therapy. The filled squares and open circles indicate HCV RNA and ALT, respectively. The time course (weeks) after the initiation of PEG-IFN-α therapy is shown. The administered drugs and treatment periods are indicated on each graph. The results for anti-IFN-α NAbs are shown on the temporal axis, indicated as (−) if the patients were negative for NAbs or with the NAb titre value if NAbs were present. NAb titres are expressed as 10-fold reduction units (TRU/mL) against recombinant IFN-α2a or -α2b. The age (years), sex (M: male; F: female), HCV genotype and *IL28B*-type (MA: major-homo; He: hetero; mi: minor-homo) of each patient are indicated in the upper right of each graph.

Seven patients received nIFN-β/RBV combination therapy. Four of these patients achieved cEVR and maintained an undetectable HCV RNA status until the end of the treatment. Among these four patients, three achieved SVR and one was RR. The remaining three patients were NR, although one of these patients achieved EVR. Among the seven patients who received nIFN-β/RBV, the three SVR patients were all HCV genotype 2 and *IL28B* major-homo, whereas the remaining four patients (one was RR and three were NR) were HCV genotype 1b. The time course of HCV RNA, ALT and anti-IFN-α NAb titres during nIFN-β/RBV treatment and the previous PEG-IFN-α therapy are shown for three patients

Table 3 Baseline characteristics and viral response of the anti-IFN-α NAb-positive patients (n = 19)

Baseline characteristics	Study 1 (n = 7)	Study 2 (n = 12)
Age, mean ± SD/range	55.0 ± 7.8/50–71	58.8 ± 12.6/28–70
Sex, male/female	3/4	5/7
Previous IFN-α therapy, +/−	5/2	4/8
Mean HCV RNA level, log copies/mL	6.0 ± 0.4	6.0 ± 0.8
Mean ALT level, U/L	78.1 ± 40.2	99.0 ± 68.7
Liver histology, grade/stage		
Grade (A), 0–1/2–3	5/2	2/4
Stage (F), 0–1/2/3–4	4/1/2	2/2/2
HCV genotype, 1/2/3	6/1/0	6/5/1
HCV core*		
AA70, wild-type/mutant	3/3	4/1
AA91, wild-type/mutant	4/2	2/3
HCV ISDR*, wild-type/mutant	4/2	3/1
IL28B allele, major-homo/hetero/minor-homo	2/4/1	4/1/0
Viral response		
EVR/non-EVR	2/5	4/8
Null response	4	8
Breakthrough	3	4

NAb, neutralizing antibody; HCV, hepatitis C virus; ALT, alanine aminotransferase; ISDR, IFN sensitivity-determining region; IL28B, interleukin-28B; EVR, early virological response.
Mean ± SD or patient numbers are indicated in each category.
*HCV core and ISDR were examined in HCV genotype 1b patients.

(patients #6–8) in Fig. 3 and indicate the immediate disappearance of HCV RNA following nIFN-β/RBV therapy, unlike following PEG-IFN-α treatment.

DISCUSSION

This is the first study to examine the emergence of anti-IFN-α NAbs using a large number of PEG-IFN-α/RBV-treated patients and to indicate the association of anti-IFN-α NAbs with the response to PEG-IFN-α treatment. We report here that approximately 15% of NR patients with chronic hepatitis C treated by PEG-IFN-α/RBV were positive for anti-IFN-α NAbs. Notably, all NAb-positive patients encountered treatment failure at the end of the therapy. Furthermore, all IFN-α NAb-positive sera failed to neutralize nIFN-β *in vitro*, and nIFN-β/RBV therapy was effective in some IFN-α NAb-positive patients, unlike PEG-IFN-α-based therapy. These results are not direct proof of a role for NAbs in the failure of PEG-IFN-α treatment. However, they do indicate an association between these two factors. NAbs may be able to cause NR on their own, because a number of patients with IFN-responsive characteristics, for example, HCV genotype 2 and *IL28B* major-homo, were included in the NAb-positive group, and three of those patients (with genotype 2 and *IL28B* major-homo) achieved SVR by nIFN-β/RBV therapy. A gene polymorphism in *IL28B* was recently found to be highly associated with NR, but *IL28B* major-homo patients, the most responsive *IL28B*-type, do not always achieve SVR [15,19]. Therefore, it is possible that the presence of anti-IFN-α NAbs is the cause of NR in some *IL28B* major-homo patients. In the present study, 18 *IL28B* major-homo patients yielded NR, and two (13%) of these patients were NAb-positive. It was also shown that IFN treatment-naïve patients could develop anti-IFN-α NAbs by PEG-IFN-α/RBV therapy; five (16%) of the 32 naïve NR patients were NAb-positive. Thus, we should recognize that anti-IFN-α NAbs are a potential cause of NR during PEG-IFN-α/RBV therapy.

The rate of NAb-positive patients receiving rIFN-α treatment was reported to be 32% [11,20] to 52% [5] of NR. In the PEG-IFN-α2b/RBV-treated patients who had received IFN-α previously, anti-IFN-α NAbs were detected on the basis of the transcriptional activity of the IFN-inducible *Mx*-promoter; three of the 38 NR patients in that study were NAb-positive (7.9%) [21]. Only two case studies reported the actual detection of anti-IFN-α NAbs in PEG-IFN-α2a/RBV-treated patients [22,23]. Taking these reports together with our results, the NAb-emergence rate appears to be lower in PEG-IFN-α-treated patients than in rIFN-α-treated patients; this may be attributed to polyethylene glycol, which may hamper the host immune system from recognizing the IFN antigen. We did not find NAb-positive responders in our PEG-IFN-α/RBV-treated patients. However, in the previous reports, NAb-positive responders were identified in the rIFN-α-treated patients, although the number of NAb-positive

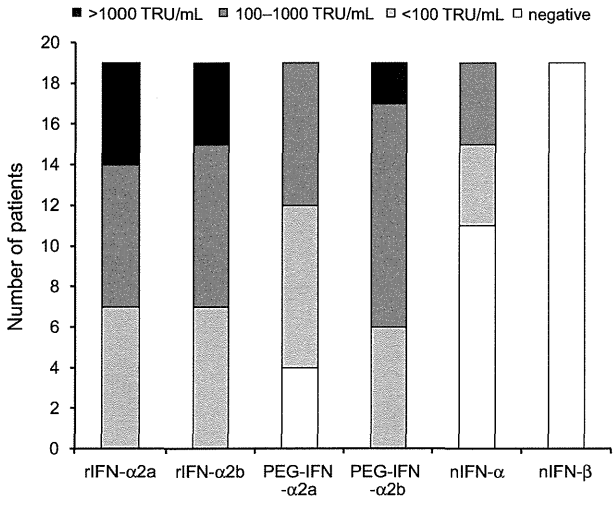

Fig. 2 Neutralizing effects of anti-IFN-α NAb-positive sera against IFN pharmaceuticals. Sera from the 19 NAb-positive patients (Studies 1 and 2) obtained at the end of treatment were examined for titres against rIFN-α2a, rIFN-α2b, PEG-IFN-α2a, PEG-IFN-α2b, nIFN-α and nIFN-β using an antiviral assay. The number of patients who exhibited high-titre sera (>1000 TRU/mL), moderate titre sera (100–1000 TRU/mL), low titre sera (<100 TRU/mL) or no neutralizing activity (negative) are shown.

responders was considerably less than the number of NAb-positive NR patients [5,11]. This difference may be attributed to the high sensitivity of the HCV RNA detection method used in the present study (TaqMan PCR), which enables the detection of NR patients at a higher efficiency than previously possible.

As mentioned, the reported NAb-positive rate has a wide range [5,11,20]; these differing findings may be due to the different sensitivities of the measurement methods used in each laboratory in addition to the different characteristics of the patients and the treatment procedures used in each institute. Here, we performed antiviral assays using 10 LU/mL rIFN-α (concentration defined by the measurement of actual IFN activity in our laboratory), which is more sensitive than PEG-IFN-α, as the standard IFN. We then observed NAbs in the NR group only. Our results show that the method used here is consistent with the clinical demand to screen patients who are unresponsive to IFN because of the presence of NAbs. We demonstrated a correlation between the emergence of NAbs and viral NR to PEG-IFN-α/RBV therapy, suggesting the need to measure anti-IFN-α NAbs in PEG-IFN-α-treated patients.

Breakthrough was representative viral kinetic of NAb-positive patients. The kinetics of HCV RNA breakthrough in NAb-positive patients indicated that the treatment was

Table 4 Baseline characteristics and viral response in 10 anti-IFN-α NAb-positive patients who received nIFN-β treatment with or without RBV

	nIFN-β (n = 3)		nIFN-β/RBV (n = 7)		
Baseline characteristics	SVR, RR	NR	SVR	RR	NR
Patients	0	3	3	1	3
Mean age, years	–	55.3	67.3	70	70.3
Sex, male/female	–	2/1	2/1	0/1	0/3
Mean HCV RNA level, log copies/mL	–	4.7	6.1	5.8	6.2
Mean ALT level, U/L	–	79.3	66.7	14	51.0
HCV genotype, 1/2	–	2/1	0/3	1/0	3/0
HCV core*					
AA70, wild-type/mutant	–	1/1	–	1/0	0/2
AA91, wild-type/mutant	–	2/1	–	1/0	1/1
HCV ISDR*, wild-type/mutant	–	1/1	–	0/1	0/1
IL28B allele (rs8099917), major-homo/hetero/minor-homo	–	0/2/0	3/0/0	1/0/0	0/0/1
Viral response					
EVR/non-EVR	–	1/2	3/0	1/0	1/2
Null response	–	2	–	–	2
Breakthrough	–	1	–	–	1

NAb, neutralizing antibody; nIFN-β, natural interferon-β; RBV, ribavirin; SVR, sustained virological response; RR, relapse response; NR, nonresponse; HCV, hepatitis C virus; ALT, alanine aminotransferase; ISDR, IFN sensitivity-determining region; IL28B, interleukin-28B; EVR, early virological response.
Mean or number of patients are indicated in each category.
*HCV core and ISDR were examined in HCV genotype 1b patients.

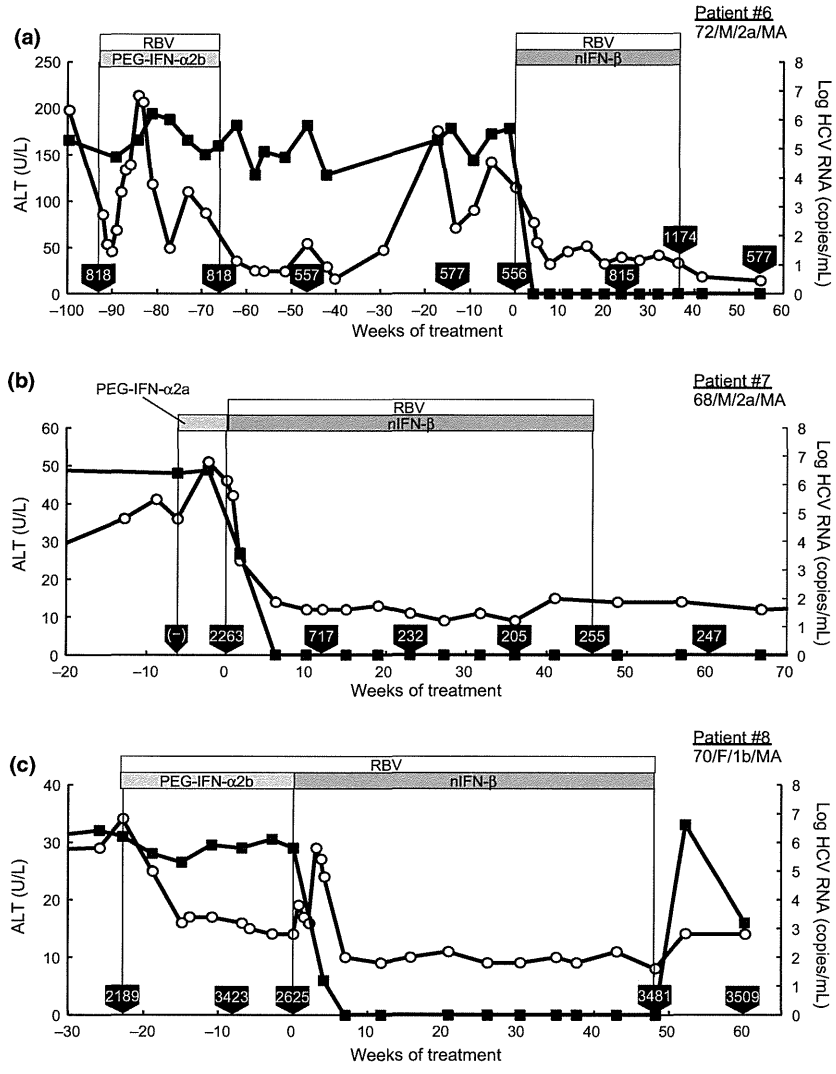

Fig. 3 Time course of HCV RNA, ALT and anti-IFN-α NAb incidence in three anti-IFN-α NAb-positive patients (patients 6–8) treated with natural human IFN-β (nIFN-β) plus RBV therapy. The filled squares and open circles indicate HCV RNA and ALT, respectively. The time course (weeks) before and after the initiation of nIFN-β therapy is shown. The administered drugs and treatment periods are indicated on each graph. The results for anti-IFN-α NAbs are shown on the temporal axis, indicated as (–) if the patients were negative for NAbs or with the NAb titre value if NAbs were present. NAb titres are expressed as 10-fold reduction units (TRU/mL) against recombinant IFN-α2a or -α2b. The age (years), sex (M: male; F: female), HCV genotype and *IL28B*-type (MA: major-homo; He: hetero; mi: minor-homo) of each patient are indicated in the upper right of each graph.

effective at its onset, but NAb generation began and subsequently inactivated the administered PEG-IFN-α at around the lowest level of HCV RNA; thereafter, the levels of HCV RNA increased again. Thus, patients who initially respond to PEG-IFN-α can potentially return to their original HCV RNA levels because of the emergence of NAbs. Meanwhile, patients who did not respond to PEG-IFN-α (non-EVR/null response) were also observed in the NAb-positive group. The potential causes of this unresponsiveness could be the presence of IFN-unresponsive characteristics (e.g. *IL28B* minor allele, HCV core mutation, etc.). NAbs could also be a major cause of viral unresponsiveness if the patients who are responsive to IFN (e.g. *IL28B* major allele, HCV genotype 2) had NAbs before treatment or developed NAbs immediately after the initiation of treatment because patients who experienced NAb-emergence during previous IFN-α therapy would develop NAbs earlier than treatment-naïve patients. Here, we suspect that the emergence of NAbs is a major cause of HCV breakthrough; therefore, the emergence of NAbs should be monitored from the point when the levels of HCV RNA begin to increase. We also propose that anti-IFN-α NAbs should be monitored in patients who fail to respond to PEG-IFN-α treatment. Post-treatment serum analysis would certainly confirm the presence of NAbs, even if they are undetectable during treatment.

All anti-IFN-α NAb-positive sera in this study effectively inactivated both rIFN-α2a and rIFN-α2b in the antiviral assays. All NAb-positive sera inhibited PEG-IFN-α2b and most of the sera inhibited PEG-IFN-α2a, suggesting that anti-IFN-α NAbs actually inactivate the injected medication *in vivo*. Meanwhile, NAb-positive sera with low titres could not inactivate PEG-IFN-α2a. This is because of the lower sensitivity of PEG-IFN-α2a compared with rIFN-α for NAb detection, which may be attributed to the large PEG molecule blocking NAb binding to PEG-IFN-α2a. Several reports demonstrated that nIFN-α, which consists of multiple IFN-α subtypes, is an effective therapy for anti-IFN-α NAb-positive chronic hepatitis C patients [11,23,24]. Indeed, the NAb-positive sera were less effective at neutralizing nIFN-α than PEG-IFN-α, as measured by the antiviral assay; however,

high-titre NAb sera effectively inactivated nIFN-α. Thus, anti-IFN-α NAbs probably reduce the bioactivity of any type of IFN-α, thereby resulting in therapeutic failure. Various protease inhibitors are in development as new medications for chronic hepatitis C [25] and have shown significant improvements in therapeutic efficacy when used in combination with PEG-IFN-α/RBV therapy [26]. However, approximately 40% of prior NR HCV genotype 1 patients still failed to achieve SVR in a clinical study of PEG-IFN-α/RBV with the protease inhibitor telaprevir [27]; this failure may be due to the emergence of anti-IFN-α NAbs in some patients. We performed PEG-IFN-α/RBV/protease inhibitor therapy in 1 anti-IFN-α NAb-positive patient, but it resulted in treatment failure. The viral load of this patient rapidly reduced after the initiation of the triple therapy (undetectable at week 4 of the therapy); however, HCV RNA was detected again at week 8 and subsequently increased to the pretreatment level (manuscript in preparation). This case suggests that anti-IFN-α NAbs could be a strong inhibitory factor against PEG-IFN-α/RBV/protease inhibitor therapy, which is used as a standard therapy in North America. Thus, any therapy based on PEG-IFN-α (even with new drugs) may be ineffective in anti-IFN-α NAb-positive patients; therefore, a medication that is not neutralized by anti-IFN-α NAb is essential to treat these patients.

Anti-IFN-α NAb-positive sera inhibited IFN-α, but did not inhibit nIFN-β, including sera with high titres. This fact indicates the higher efficacy of IFN-β, which has a different structure from IFN-α, in anti-IFN-α NAb-positive patients. nIFN-β is also a commonly used IFN to treat chronic HCV infection in Japan [28]; thus, we administered nIFN-β to anti-IFN-α NAb-positive patients. Although studies with larger numbers of patients are needed to confirm the efficacy of IFN-β therapy for anti-IFN-α NAb-positive patients, it was striking that among the 10 treated NAb-positive patients, none of whom had responded to previous PEG-IFN-α therapy, three patients achieved SVR following the co-administration of nIFN-β/RBV. These three patients were all HCV genotype 2 and *IL28B* major-homo, while the remaining seven patients, with characteristics such as genotype 1b or *IL28B* minor allele, failed to achieve SVR. These results suggest that the efficacy of nIFN-β therapy in NAb-positive patients is strongly affected by the other characteristics related to the IFN-response. One NAb-positive patient, who was HCV genotype 1b, wild type in core AA70 and AA91, ISDR mutation, and *IL28B* major-homo, achieved an end-of-treatment response, but then relapsed, suggesting that nIFN-β/RBV therapy is insufficient for treating HCV genotype 1b patients, even if they have other IFN-responsive characteristics. Combination therapy with RBV and protease inhibitors could ensure the efficacy of nIFN-β therapy or the development of PEG-IFN-β therapy might remarkably improve the efficacy.

Previous studies confirmed that host and viral factors are responsible for NR to PEG-IFN-α in chronic hepatitis C patients. Our study additionally revealed that the emergence of anti-IFN-α NAbs is a candidate causal factor for NR in a considerable number of these patients. Upon detection of anti-IFN-α NAbs during or after PEG-IFN-α treatment, other effective treatments should be considered, possibly IFN-β-based treatment in combination with RBV and other prospective new drugs.

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AUTHORS DECLARATION OF PERSONAL INTERESTS

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DECLARATION OF FUNDING INTERESTS

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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

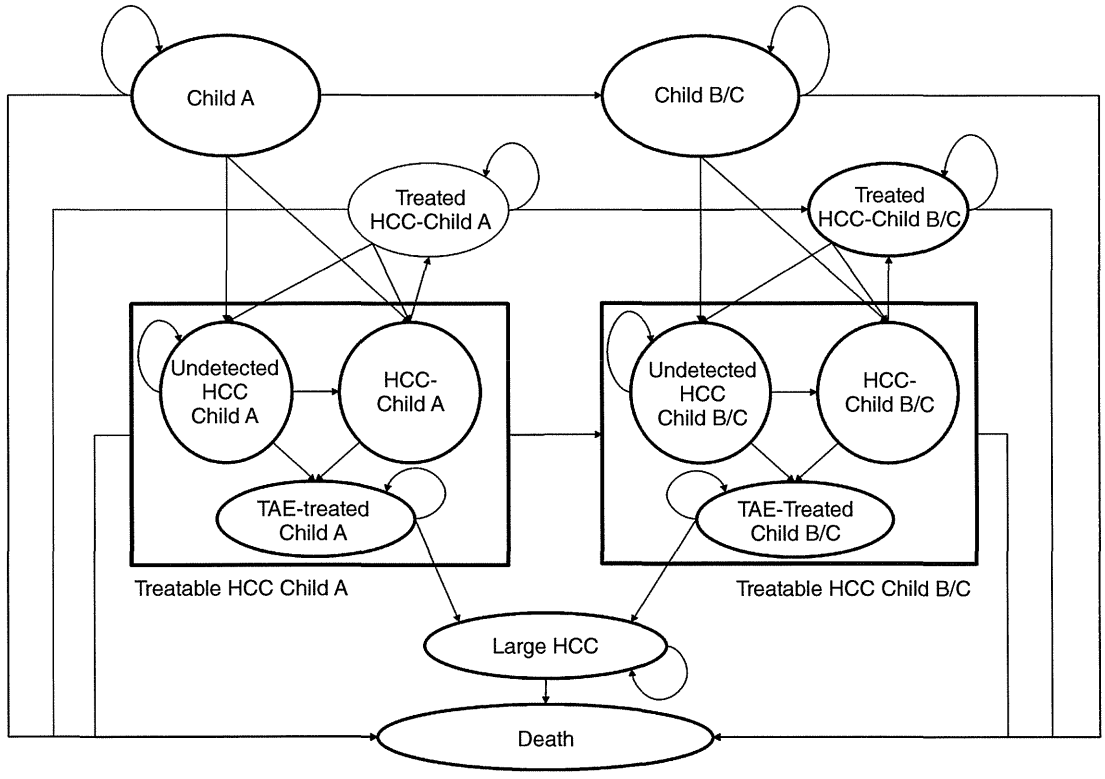

Figure 1 Natural history model. The arrows represent possible transitions during each 6 month cycle. Patients enter this model with Child A cirrhosis, and might develop Child B/C cirrhosis, hepatocellular carcinoma (HCC), both Child B/C and HCC, or death. If the health status does not change, then the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

more than three in number) are asymptomatic, and remain undetected until the surveillance is performed. Large tumors are symptomatic, and the patient can receive palliative treatment only;
2 there are no small HCCs that can be detected incidentally in the no surveillance group;
3 patients with positive surveillance tests undergo a confirmatory test. [CT and either MRI (70%) or liver biopsy (30%)];
4 the test performance is independent of previous test results;
5 compliance with the program is 100%; and
6 there is a small rate of false-positive diagnoses, which will be discovered before any treatment.

The tumor growth rate was calculated with the assumption of a doubling time of 120 days.[17,18,42]

Since one year's worth is different in the health status, health-states utility should be taken into account for cost effectiveness analysis. Thus, we obtained the health-state utility information from meta-analysis.[40] The survival and costs were also discounted at the commonly accepted annual rate of 3%, because time and cost of distant future are generally thought to be of less value than those of present time.

Cost

The cost data shown in Table 1 are from data published in Japan, because Sonazoid is currently available only in Japan.

The data were converted to US currency at the exchange rate of US$1.00 = JP¥90.00. The cost of transcatheter arterial embolization (TAE) was estimated by including health insurance reimbursement using the reimbursement data in our hospital, because there were no available national data.

Sensitivity analysis

The results obtained from this model depended on the values that were used in the study; therefore a one-way sensitivity analysis was performed on all variables.

RESULTS

Accuracy of our model

TO VALIDATE THE model's accuracy, we compared this model's survival rate with the cumulative survival rates of 417 compensated cirrhosis patients obtained from a large European cohort clinical study under surveillance.[43] When we set the annual incidence rate of HCC as 4% to fit the European model, these two survival curves were very similar, and the accuracy of our model was validated (Fig. 2).

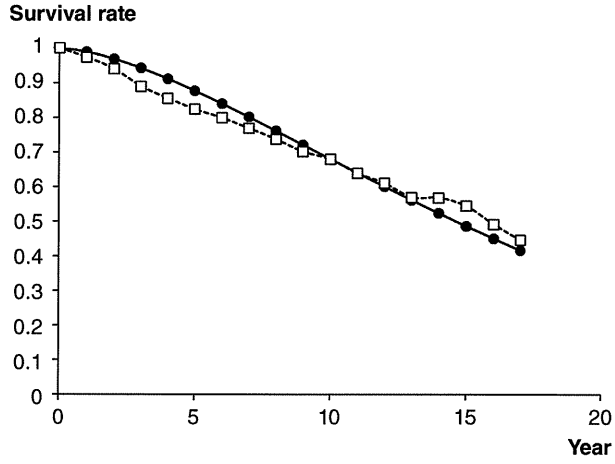

Figure 2 Comparison of the survival curves for compensated cirrhosis states between the one predicted by the current model and published data from a large cohort study.[43] Both data sources yielded similar curves. -●-, our model; -□-, Sangiovanni *et al.* 2004[43].

Baseline analysis

The expected life years of each group according to the starting age of the surveillance are shown in Figure 3.

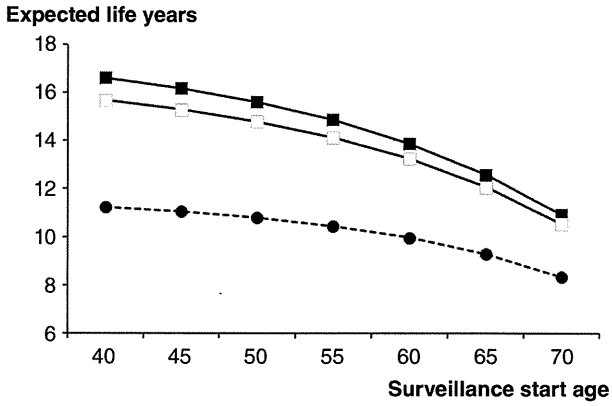

Figure 3 Expected life years according to surveillance at a starting age before it was discounted, and adjusted by health-state utilities. Although the expected life years decreased with age, both the ultrasonography (US) and contrast-enhanced ultrasonography (CEUS) surveillance groups increased the life expectancy even in 70-year-old patients. CEUS surveillance achieved the greatest gain in life expectancy in all analyzed age groups. -■-, CEUS Surveillance; -□-, US Surveillance; -●-, No Surveillance.

Table 2 Baseline analysis

Strategy	Total cost (US$)	Incremental cost (US$)	Expected life years (year)	QALY	Incremental QALY	ICER (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.

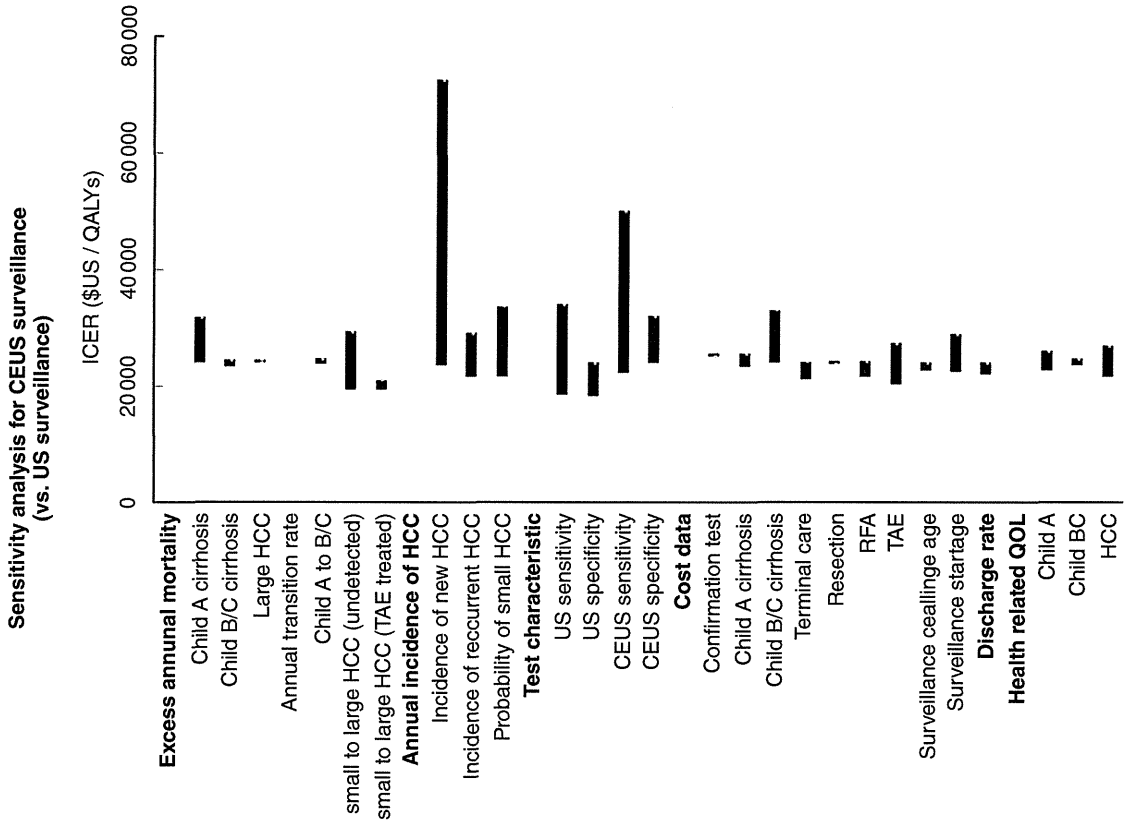

Figure 4 One way sensitivity analysis of the incremental cost-effectiveness ratio (ICER) for the contrast-enhanced ultrasonography (CEUS) surveillance group. When the ICER of the CEUS group was compared with the ultrasonography (US) group, the annual incidence rate of hepatocellular carcinoma (HCC) and 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

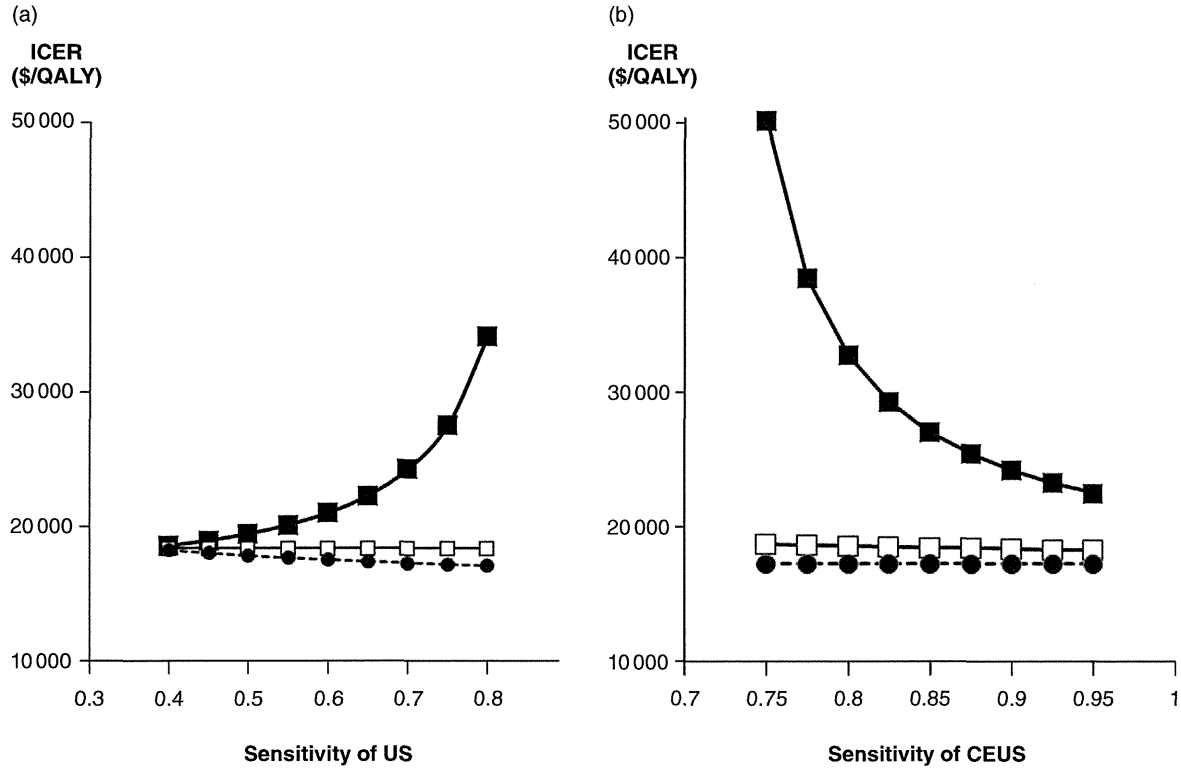

Figure 5 Effects of the sensitivity of ultrasonography (US) (A) and contrast-enhanced ultrasonography (CEUS) (B). When the incremental cost-effectiveness ratio (ICER) was compared with the no surveillance group, both US and CEUS surveillance groups were less than $US 20 000/quality adjusted life year (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

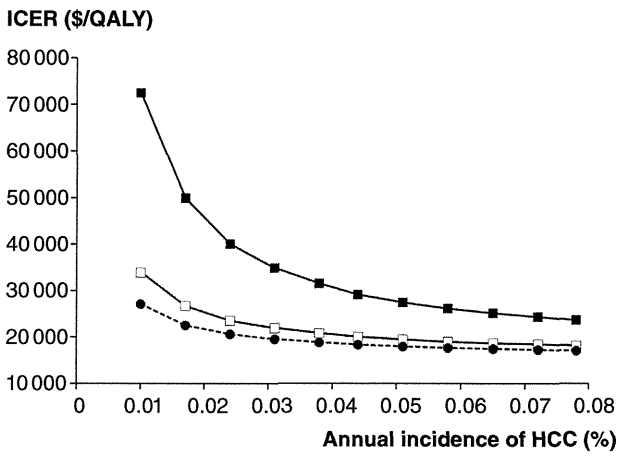

Figure 6 Effects of the annual incidence of hepatocellular carcinoma (HCC). The incremental cost-effectiveness ratio (ICER) values of the ultrasonography (US) and contrast-enhanced ultrasonography (CEUS) surveillance groups were less than $US 35 000/ quality adjusted life year (QALY) in all ranges as 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%,