TABLE 1. Reproducibility in the quantification of MA in human serum: analytical data

Sample	Individual Values			Mean ± SD
		pmol		
A	15.0	15.7	15.0	15.2 ± 0.38
B	14.4	15.6	15.5	15.2 ± 0.67
C	14.7	14.4	14.2	14.5 ± 0.29
D	15.6	15.6	14.7	15.3 ± 0.48
Mean ± SD				15.0 ± 0.58

MA was quantified in 50 μl of normal human serum.

and SRM measurement. The variances were not considered to be attributable to the sample preparation because the errors during sample preparation were not significantly larger than those between the measurements (**Table 2**). The inter-assay coefficients of variation for the between- and within-sample variations were 4.4% and 3.2%, respectively.

For the recovery experiments, known amounts of MA (a, 2a, 3a; a = 9.6 pmol) were spiked into 50 μl aliquots of the serum samples (n = 2). After the clean-up and derivatization procedures, SRM was carried out in triplicate for each sample. The recoveries of the known spiked amounts of MA ranged from 94.5% to 99.0%, with a mean of 96.0% (**Table 3**). In addition, the amount of endogenous MA found in unspiked 50 μl serum aliquots was within the 95% confidence limit for the estimated amount of MA calculated by orthogonal regression analysis, which also constituted an index for the precision and accuracy of the present method.

The circadian rhythm of MA levels in human sera

Figure 5 depicts the circadian rhythm of the serum concentrations of MA in a healthy male. Postprandial increases of MA concentrations (maximum 235% after dinner) were observed and the levels peaked between 2.5 and 6.5 h postmeal. The increase of MA concentration disappeared after skipping breakfast on the second day, which supports the idea that the diurnal pattern of serum MA concentrations is controlled mainly by food intake.

DISCUSSION

We describe a sensitive new LC-P-ESI-MS/MS method for the quantification of MA in serum. LC-N-ESI-MS/MS may be more suitable for the determination of negatively charged compounds, such as organic acids because the method does not require a derivatization step. However, as shown in Fig. 2, the sensitivity of N-ESI was not sufficient to quantify MA concentrations in a small volume of normal human serum.

Recently, we derivatized another organic acid, mevalonate, into mevalonyl-(2-pyrrolidin-1-yl-ethyl)-amide and measured it using LC-P-ESI-MS/MS (12). In this method, mevalonate was lactonized into mevalonolactone and then a tertiary amine moiety was introduced by a characteristic amidation reaction with a primary alkylamine. As a result, the tertiary amine moiety markedly promoted protonation and attomole levels of mevalonate were detected. In the present study, tertiary amine moieties were successfully introduced to MA by esterification with 3-hydroxy-1-methylpiperidine. Thus, the reaction for the synthesis of carboxylic esters by Shiina et al. (10) appears to be useful not only for the derivatization of alcohols (13) but also for that of carboxylic acids. This derivative, DMP-MA, exhibited $[M+H]^+$ as the base peak by P-ESI-MS and the detection limit by SRM was more than 100 times lower than that of underivatized MA by SRM with N-ESI mode.

The derivatization and purification steps in this method are very simple but it should be mentioned that there are two pitfalls to obtaining reliable and reproducible results. First, use of the anion exchange column cartridge gave unexpectedly high values of MA concentrations. Serum MA was extracted by this cartridge and interfering peaks on SRM chromatograms were markedly reduced by the addition of this purification step. However, the recoveries of known amounts of MA from this cartridge were always more than 100%, and additional experiments suggested that a significant amount of MA was produced from unknown substance(s) in organic solvents by this anion exchange column (data not shown). Plasma methylmalonic acid (MMA) and its isomer succinic acid (SA) are also known to be extracted by this column (14). We have derivatized MMA and SA into DMP-MMA and DMP-SA, respectively, and analyzed them by the same HPLC condition as that for DMP-MA. The SRM was conducted using m/z 313 → m/z 98 for both DMP-MMA and DMP-SA. The results showed that DMP-MMA and DMP-SA were much more hydrophobic than DMP-MA and both compounds were eluted during washout phase with 0.2% formic acid in acetonitrile (after 6 min).

Second, pH of the final sample solution should not be more than 7 because an alkaline condition easily hydrolyzes DMP-MA. After the derivatization step, most of the excess reagents and hydrophilic impurities were

TABLE 2. Reproducibility in the quantification of MA in human serum: ANOVA

Source	S	f	V	F_0	Relative SD
					%
Sample preparation	1.293	3	0.431	1.89	4.4
Error (SRM)	1.820	8	0.228		3.2
Total	3.113	11			
			$F(3,8,0.05)=4.07$		

S, residual sum of squares; f, number of degrees of freedom; f_1, $f_{\text{sample preparation}}$; f_2, f_{error}; V, unbiased variance; F_0, observed value following F distribution variance ratio ($V_{\text{sample preparation}}/V_{\text{error}}$); $F(f_1, f_2, \alpha)$, density function of F distribution with f_1 and f_2 degrees of freedom.

TABLE 3. Recovery of MA from human serum

Sample (X_0 + na) (n = 0,1,2,3)	Amount Added	Amount Found			Recovery[b] (Mean ± SD)	Estimated Amount ± 95% Confidence Limit[c]
	pmol				%	*pmol*
X_0	0	$\bar{X}_0 \pm SD = 15.0 \pm 0.6$[a]				14.1 ± 1.1
X_0 + a	9.6	23.6	25.2	25.0		
X_0 + a	9.6	24.2	23.6	23.3	94.5 ± 8.2	
X_0 + 2a	19.2	33.2	32.1	32.0		
X_0 + 2a	19.2	33.9	34.1	34.0	94.6 ± 5.1	
X_0 + 3a	28.8	43.8	43.9	43.0		
X_0 + 3a	28.8	44.1	43.6	43.2	99.0 ± 1.5	

Known amounts of MA were spiked into 50 μl of normal human serum before sample preparation.
[a] The value was obtained from Table 1.
[b] Recovery (%) = (amount found – $\bar{X}_0$) / amount added × 100.
[c] The estimated amount was calculated by orthogonal regression.

precipitated by the addition of n-hexane but significant amounts of 3-hydroxy-1-methylpiperidine and 4-dimethylaminopyridine were recovered with DMP-MA in the final residue of the extract. Therefore, it was necessary to dissolve the final residue in 1% formic acid in water to keep the pH of the solution less than 7. The mobile phase of the HPLC (0.2% formic acid in water) was not sufficient to neutralize the final extract.

The highly sensitive quantification of serum MA can be useful for monitoring of de novo FAS, also called de novo lipogenesis, in normal humans. The diurnal variation of serum MA levels in a healthy human (Fig. 5) was similar to the variation of de novo FAS determined in humans by continuous intravenous infusion of sodium [1-^{13}C]acetate and mass isotopomer distribution analysis (15, 16). According to Timlin et al. (16), de novo FAS peaked 4.2 h after ingestion of a meal whereas lipoprotein-triacylglycerol concentrations peaked at 2.0 h postmeal. Another study, by Hudgins et al. (15), showed that the maximum values of de novo FAS occurred in the evening, 3.0–9.0 h after the last meal, although the peak after every meal was not detected because a limited number of postprandial data points were obtained. In our data, postprandial increases of MA concentrations peaked between 2.5 h and 6.5 h after meals and the maximum value was observed in the night 6.0 h after dinner. In addition, the increase of MA concentration disappeared after skipping the meal. Thus, serum MA concentrations are regulated by food intake and appear to be a good marker that reflects de novo FAS in normal humans.

Because serum MA concentrations correlate well with de novo FAS, the most important enzyme that determines serum MA concentration is thought to be ACC, the rate-limiting enzyme in the fatty acid biosynthesis. In mammals, two ACC isoforms exist. Cytosolic ACC1 synthesizes malonyl-CoA, which participates in both de novo FAS and negative regulation of β-oxidation. In contrast, malonyl-CoA synthesized by mitochondrial ACC2 acts mainly as an inhibitor of β-oxidation (17). We cannot clarify at present which ACC contributes to serum MA concentration but both ACCs regulate de novo lipogenesis in a coordinated and complementary manner (18).

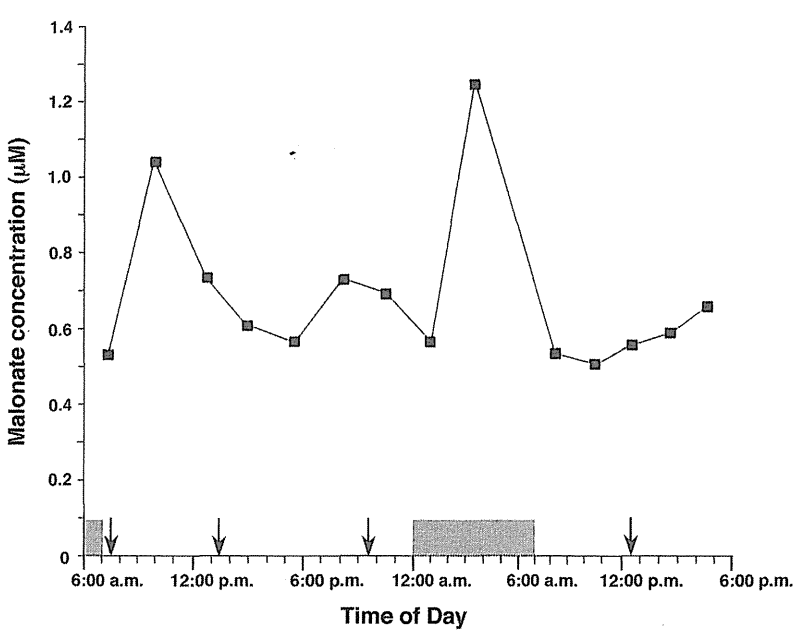

Fig. 5. The circadian rhythm of the serum levels of MA in a healthy volunteer. Blood samples were taken every 2–3 h. On the first day the volunteer consumed a normal hospital diet at 7:30 AM, 1:30 PM, and 9:30 PM (indicated by the arrows), and slept from 12:00 AM to 7:00 AM (indicated by the shaded box). On the second day the volunteer did not eat breakfast but consumed a normal hospital diet at 12:30 PM.

Under special conditions, however, other enzymes, MCD, and FAS can also be determinants of tissue malonyl-CoA levels and serum MA concentrations. For example, when MCD activity is reduced, such as with MCD deficiency, serum MA concentrations are elevated. Alternatively, when FAS is blocked by any drugs, such as C75 and cerulenin (2), MA concentrations increase in spite of reduced de novo FAS. Therefore, it is important to rule out the presence of such special conditions when we use serum MA as a biomarker for de novo FAS.

In summary, we developed a new method for the quantification of MA in human serum, which can be a good marker for de novo FAS. Derivatization of MA into DMP-MA allowed it to be quantified by LC-P-ESI-MS/MS with excellent sensitivity. Recovery and reproducibility experiments verified that this method provided highly reliable and reproducible analytical results.

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Original Article

The associated markers and their limitations for the primary screening of HCV carriers in public health examination

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Aim: Although the anti-hepatitis C virus (HCV) antibody test has been recommended to the whole Japanese population, most countries have not implemented it. The present study aims to re-evaluate the usefulness of markers examined in the general health examination for the initial screening of HCV carriers.

Methods: Of the overall population, 25 142 individuals (8876 males, 16 266 females) participated in health examinations with HCV tests in 2005, and the most commonly associated markers for HCV-positive subjects were explored by multivariate analysis, based on blood biochemical, physical, sphygmomanometric and hematological parameters. Thereafter, the efficiencies of the markers were estimated from a total population of 85 013 individuals (29 502 males, 55 511 females) in 2003–2005.

Results: The most significantly associated markers for HCV positivity were aspartate aminotransferase (AST) and alanine aminotransferase (ALT). Optimal limits of ALT and AST by receiver–operator characteristic (ROC) analysis were 24 and 27 IU (male, 33 and 28 IU; female, 22 and 26 IU), respectively. However, one-quarter of HCV carriers were not found to be positive using the optimal limits of aminotransferases.

Conclusion: The present study confirmed the limitation of serum aminotransferase levels as markers of HCV for primary screening. Therefore, at present, an anti-HCV antibody test is required for the efficient screening of HCV carriers in all health examinations.

Key words: aminotransferases, HCV, health examination

INTRODUCTION

INFECTION WITH HEPATITIS C virus (HCV) has been the leading cause of liver cirrhosis, and the consequent development of hepatocellular carcinoma, for the past few decades. The number of HCV carriers has increased worldwide. Indeed, the World Health Organization (WHO) estimates that about 180 million people, that is 3% of the world's population, are infected with HCV, and 3–4 million people are newly infected every year, 70% of whom develop chronic hepatitis.[1]

Based on early detection and treatment, it is very important to detect HCV carriers as early as possible, for example, in public health examinations. HCV carriers are diagnosed by the detection of HCV-RNA and/or anti-HCV antibody using the judgment system of HCV infection established since 2002 in Japan (Fig. 1). Generally, subjects who have abnormally high levels of serum alanine aminotransferase (ALT) as well as aspartate aminotransferase (AST) are recommend to take thorough examinations for liver diseases, including the HCV tests. However, there is an issue that most HCV carriers are considered to be asymptomatic and paucisymptomatic, and that approximately 30% of chronic HCV carriers persistently exhibit normal ALT levels (PNAL), while another 40% exhibit minimally elevated ALT levels.[2–6] Consequently, these asymptomatic and paucisymptomatic HCV carriers fail to be detected by the primary screening using serum ALT levels in public health examinations. Importantly, these asymptomatic HCV carriers with PNAL have significant histological

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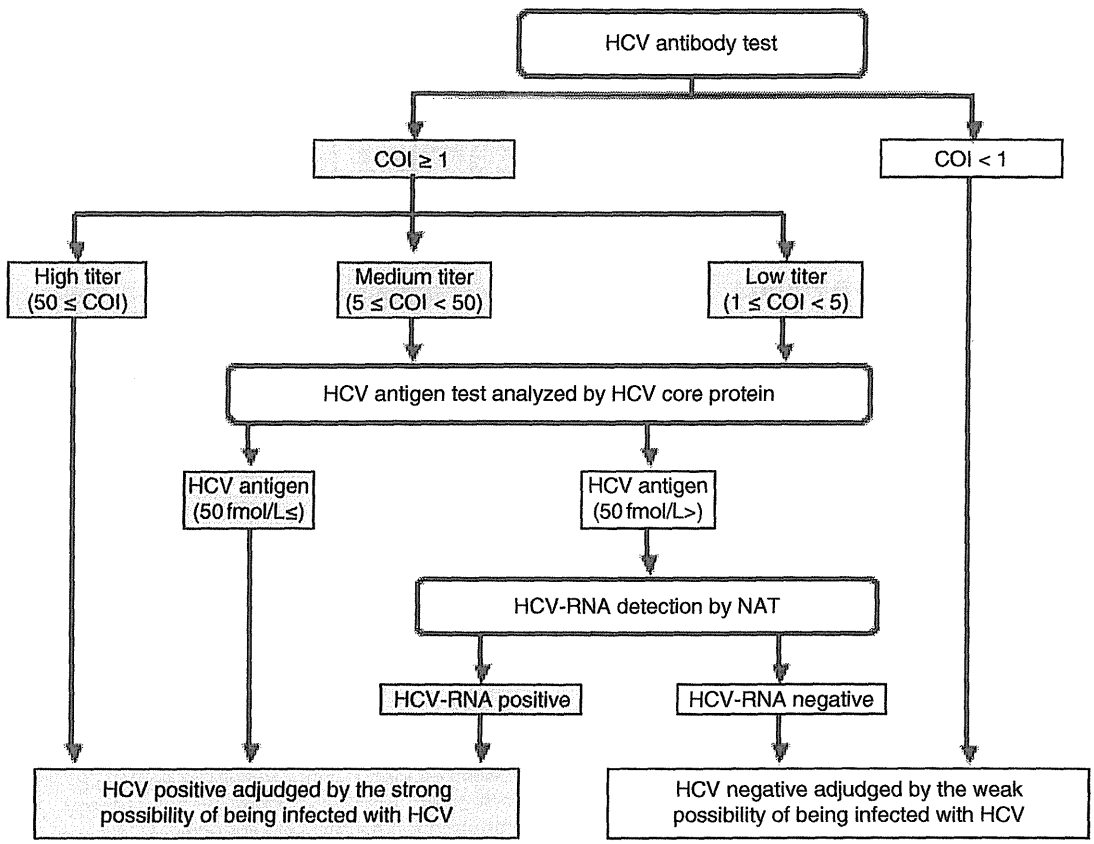

Figure 1 Flow chart showing the course of medical examination for hepatitis C virus (HCV). The diagnosis of HCV infection was conducted in accordance with the guidelines for the medical examination of HCV issued by the Japanese Ministry of Health, Labour and Welfare. COI, cut off index; HCV, hepatitis C virus; NAT, nucleic acid amplification test.

liver damage, similar to that in HCV carriers with raised ALT levels, and moderate to severe hepatitis has frequently been found in asymptomatic HCV carriers compared with HCV carriers with raised ALT levels.[7]

Accordingly, the optimal serum ALT limits for the screening of HCV carriers have been subject to debate.[8–10] However, what is considered a healthy range of ALT levels compared with liver disease differs between medical institutes, centers, hospitals, regions and countries. Almost all of the normal ALT ranges for liver disease are less than 40 IU,[7] however, Prati *et al.* reported that the upper limits of the healthy range differed between genders; 30 IU and 19 IU for males and females, respectively; calculated as the value of the 95th percentile in normal subjects from a population at the lowest risk for liver disease.[8] Furthermore, Okanoue *et al.* defined asymptomatic HCV carriers as those with PNAL less than 30 IU based on the histological fibrosis stage in a follow-up study.[11] In Japan, serum ALT levels under 35 IU had been considered to be within the healthy limit for diagnosis of liver diseases, but in 2008, the health limit was reduced to under 30 IU, for both ALT and AST, as suggesting liver disease in public health examinations, based on the guidance for antivirus therapy of HCV.[12] Based on these facts, it has not been actually clarified whether these markers are effective and whether the optimal limit points are useful or not for the detection of asymptomatic and paucisymptomatic HCV carriers. Therefore, in Japan, the anti-HCV antibody test has been recommended to the whole of the population during public health examinations.[13,14]

The purpose of the present study was to re-evaluate the effectiveness of serum aminotransferase levels as markers in the primary screening for HCV carrier detection in over 85 000 subjects in the annual public medical health examination for 3 years between 2003 and 2005.

METHODS

Population in the health examination

A TOTAL OF 85 013 individuals (29 502 males, 55 511 females), including non-employees, local residents, self-employed persons, farmers, housewives and retired persons participated in the biochemical examination of serum ALT and AST levels as part of the annual public health examination and HCV testing during the 3 years from 2003 to 2005 in Ibaraki Prefecture, Japan. HCV testing was carried out based in part on a project of urgent comprehensive countermeasures against hepatitis and HCC at the ages of 40, 45, 50, 55, 60, 65, or 70 for five years supported by the Japanese Ministry of Health, Labour and Welfare. In the health examination in 2005, in addition to the measurement of serum ALT and AST levels, 25 142 subjects (8876 males, 16 266 females) underwent examination of γ-GFP level, diastolic and systolic blood pressure, hemoglobin, hematocrit, red blood cell count (RBC), total cholesterol, triglyceride, glucose and glycohemoglobin (HbA_{1c}). Body mass index (BMI) was calculated by dividing the weight in kilograms by the square of the height in meters.[15] All of the health examinations with serum biochemical analyses, as well as the HCV tests, were carried out with the Ibaraki Health Service Association (Mito, Japan).

The determination of HCV carrier status

The determination of the presence or absence of HCV infection was performed in accordance with the guidelines for the medical examination of HCV issued by the Japanese Ministry of Health, Labour and Welfare, as summarized in Figure 1. Serum collected from the subjects during the medical examination was first measured for the HCV titer using a chemiluminescent enzyme immunoassay for HCV antibody (Lumipulse®, Fujirebio Inc, Tokyo, Japan). Subjects with serum HCV titer beneath a cut-off index (COI) of 1 were determined to be HCV negative. Those subjects with a COI > 1 were divided into three classes dependent on the levels of the HCV titer: low titer, COI under 5 and more than 1; medium titer, COI under 50 and more than 5; high titer, COI more than 50. The subjects in the high titer class were determined to be HCV positive. The subjects classified to the low and medium titers underwent the HCV antigen test analysis for the HCV core protein. Subjects with more than 50 fmol/L of HCV antigen were determined to be HCV positive. When the HCV antigen was under 50 fmol/L, a nucleic acid amplification test (NAT) was conducted for HCV-RNA detection. The subjects with positive and negative results by the NAT were finally determined to be HCV positive and negative, respectively.

Other investigated data in 2005

In the data from 2005, the most relevant factor for HCV positive status was determined statistically by multivariate analysis and the ROC curve. As a result of the ROC curve in 2005 (Table 1), the ROC curves for ALT and AST levels in serum were drawn from data for 3 years between 2003 and 2005 to evaluate the effective cut-off points to avoid false negative and positive findings for HCV.

Statistical analysis

Data are presented as the mean ± SE, the percentage and the percentiles. Significant differences were determined by unpaired Student's *t*-test or one-way ANOVA with Bonferonni's post-hoc test for comparisons between two groups or among multiple groups, respectively. The statistical analysis was performed using SPSS II software version 11.0 (SPSS Inc, Chicago, IL, USA). Multiple regression analyses were made using the stepwise method. The upper-left cut points for the HCV positive were chosen from likelihood value based on the ROC curve. ROC comparison was performed by calculation of the area under the curve and 95% confidence intervals using the technique described by Hanley and McNeil.[16]

RESULTS

Basic data, and the ROC and multivariate analyses of the health examinations in 2005

BASIC DATA OF all examined parameters in the health examinations in 2005 are shown in Table 2. The levels of serum ALT, AST and γ-GPT were significantly and markedly higher in the HCV positive than in the HCV negative subjects, for both genders. These serum levels were significantly higher in males than in females in all of the HCV negative and positive cases.

Table 1 presents the results of ROC and multivariate analyses in the respective parameters for HCV positive status from data in 2005. The most significant relevant parameter for HCV positive status was the serum AST level, followed by the serum ALT level. There were other significant parameters ($P < 0.05$), but the areas of the ROC curve for these parameters were less than 0.7. BMI and serum levels of triglyceride and total cholesterol

Table 1 Area under the receiver-operator characteristic (ROC) curve and multivariate analysis and the respective parameter for HCV positive subjects examined in 2005

Parameter	ROC curve area	SE	*P*-value	95% CI
AST	0.849	0.018	0.000	0.814–0.884
ALT	0.788	0.021	0.000	0.747–0.829
Hemoglobin	0.654	0.028	0.000	0.598–0.709
Age	0.642	0.026	0.000	0.591–0.692
Hematocrit	0.642	0.027	0.000	0.589–0.695
γ-GTP	0.622	0.028	0.000	0.508–0.677
Glucose	0.613	0.025	0.000	0.564–0.662
RBC	0.558	0.029	0.029	0.501–0.614
Systolic pressure	0.547	0.029	0.077	0.491–0.603
Diastolic pressure	0.528	0.028	0.289	0.473–0.583
Height	0.519	0.027	0.474	0.466–0.572
Weight	0.505	0.027	0.860	0.451–0.558
HbA1c	0.504	0.028	0.872	0.449–0.559
BMI	0.492	0.025	0.760	0.443–0.457
Triglyceride	0.409	0.025	0.001	0.361–0.330
Total cholesterol	0.278	0.027	0.000	0.226–0.330

ALT, alanine transaminase; AST, aspartate transaminase; BMI, body mass index; CI, confidence interval; HbA1c, glycohemoglobin; RBC, red blood cell count; γ-GTP, gamma-glutamyl transferase.

were related to HCV negative status but not HCV positive status. In particular, the total cholesterol level was the most relevant parameter for the HCV negative status. Based on the results of these analyses, the ROC curves for the serum AST and ALT levels of the HCV positive subjects among each gender were drawn from data for 3 years between 2003 and 2005.

As result of stepwise discrimination analysis, a combination of four parameters, AST, ALT, age and total cholesterol, gave the maximum likelihood. The established discrimination formula was as follows: $Z = 10.472 - 0.001 \times (\text{AST, IU}) + 0.027 \times (\text{ALT, IU}) + 0.057 \times (\text{age, year}) - 0.025 \times (\text{total cholesterol, mg/dL})$. However, the calculated predictive value for HCV positive ratio was only 6.61% using the formula.

HCV positive ratio and distribution of aminotransferases in HCV positive populations for 3 years

There were 787 HCV positive subjects (male, 406; female, 381) and the positive ratio was 0.93% (male, 1.38%; female, 0.69%) for 3 years between 2003 and 2005. The range of ages for the HCV positive subjects was 29–87 years old (male, 29–87 years; female, 40–84 years).

The distributions of serum ALT and AST levels were expressed as percentiles by age in the HCV positive populations for both genders (Fig. 2). In males, the levels of both aminotransferases, in particular ALT, were elevated in those aged less than 65 years, and there were large variations of these levels in all age ranges (Fig. 2a). However, there were no differences in the distribution of levels of both aminotransferases among the age ranges in females, and the variations of these levels were small compared to those in males (Fig. 2b).

The distribution of HCV positive subjects using the current limit points

Those who were detected as being HCV positive in 2003–2005 were divided into four cut-off ranges (A–D) by ALT and AST levels at 30 IU, that is the current limit point (Fig. 3). There was significant difference in the ratio balance of HCV positive between genders assessed by χ^2 analysis ($P < 0.0001$). In range A, which means the false-negative of HCV positive, the HCV positive rates of male and female were 25.9% and 47.0%, respectively. In range A, almost of half of female HCV positive subjects were classified as false-negative. In contrast, the ratios in range D were 56.3 and 39.8% in males and females, respectively, and over half of HCV positive subjects in males were included in range D. In males, the ratio in range B was 10.5% and higher than that (7.4%) in range C. In contrast, in females, the ratio in range B was only 4.3% and lower than that (9.0%) in range C.

Table 2 Basic data in all examined parameters in 2005

	Male				Female						
	Total	HCV (–)	HCV (+)		Total		HCV (–)		HCV (+)		
Population number	8876	8776	100	†	16266	‡	16178	‡	88	†	‡
ALT (IU/L)	27.2 ± 0.2	26.8 ± 0.2	68.0 ± 7.0	***	19.7 ± 0.1	***	19.6 ± 0.1	***	45.3 ± 0.1	***	**
AST (IU/L)	26.4 ± 0.1	26.0 ± 0.1	57.1 ± 4.3	***	22.5 ± 0.1	***	22.4 ± 0.1	***	46.9 ± 0.1	***	**
γ-GTP (IU/L)	53.6 ± 0.7	53.1 ± 0.7	101.1 ± 14.3	***	25.9 ± 0.2	***	25.9 ± 0.2	***	39.8 ± 0.2	*	***
Height (cm)	165.0 ± 0.1	165.0 ± 0.1	164.3 ± 0.6	ns	153.3 ± 0.0	***	153.3 ± 0.0	***	151.7 ± 0.0	*	***
Weight (kg)	65.3 ± 0.1	65.3 ± 0.1	63.7 ± 1.0	ns	54.3 ± 0.1	***	54.3 ± 0.1	***	53.4 ± 0.1	ns	***
BMI	24.0 ± 0.0	24.0 ± 0.0	23.6 ± 0.3	ns	23.1 ± 0.0	***	23.1 ± 0.0	***	23.2 ± 0.0	ns	ns
Systolic pressure (mmHg)	128.5 ± 0.2	128.6 ± 0.2	126.9 ± 2.0	ns	124.1 ± 0.1	***	124.0 ± 0.1	***	128.6 ± 0.1	*	ns
Diastolic pressure (mmHg)	81.1 ± 0.1	81.1 ± 0.1	78.7 ± 1.3	*	74.5 ± 0.1	***	74.5 ± 0.1	***	76.7 ± 0.1	ns	ns
Hemoglobin (g/dL)	32.0 ± 0.0	32.0 ± 0.0	32.3 ± 0.2	*	30.6 ± 0.0	***	30.6 ± 0.0	***	31.6 ± 0.0	***	*
Hematocrit (%)	95.5 ± 0.1	95.5 ± 0.1	96.2 ± 0.6	ns	91.7 ± 0.0	***	91.7 ± 0.0	***	94.3 ± 0.0	***	*
RBC ($\times 10^4$ cells/μL)	33.5 ± 0.0	33.5 ± 0.0	33.5 ± 0.1	ns	33.4 ± 0.0	***	33.4 ± 0.0	***	33.5 ± 0.0	ns	ns
Total cholesterol (mg/dL)	200.0 ± 0.4	200.4 ± 0.3	170.6 ± 3.1	***	210.6 ± 0.3	***	210.7 ± 0.3	***	191.1 ± 0.3	***	**
Triglyceride (mg/dL)	143.8 ± 1.2	144.0 ± 1.3	119.7 ± 12.3	***	110.5 ± 0.5	***	110.6 ± 0.5	***	101.2 ± 0.5	ns	ns
Glucose (mg/dL)	107.3 ± 0.3	107.2 ± 0.3	118.2 ± 4.2	*	99.3 ± 0.2	***	99.3 ± 0.2	***	108.9 ± 0.2	*	*
HbA_{1c} (%)	5.2 ± 0.0	5.2 ± 0.0	5.3 ± 0.1	ns	5.1 ± 0.0	***	5.1 ± 0.0	***	5.1 ± 0.0	ns	ns

Data are shown as mean ± SE. Significant differences were analyzed by the non-parametric Mann–Whitney *U*-test; $*P < 0.05$, $**P < 0.001$, $***P < 0.0001$. †: HCV (–) versus HCV (+), ‡: male versus female.

ALT, alanine transaminase; AST, aspartate transaminase; BMI, body mass index; HbA1c, glycohemoglobin; ns, no significance; RBC, red blood cell count; γ-GTP, gamma-glutamyl transferase.

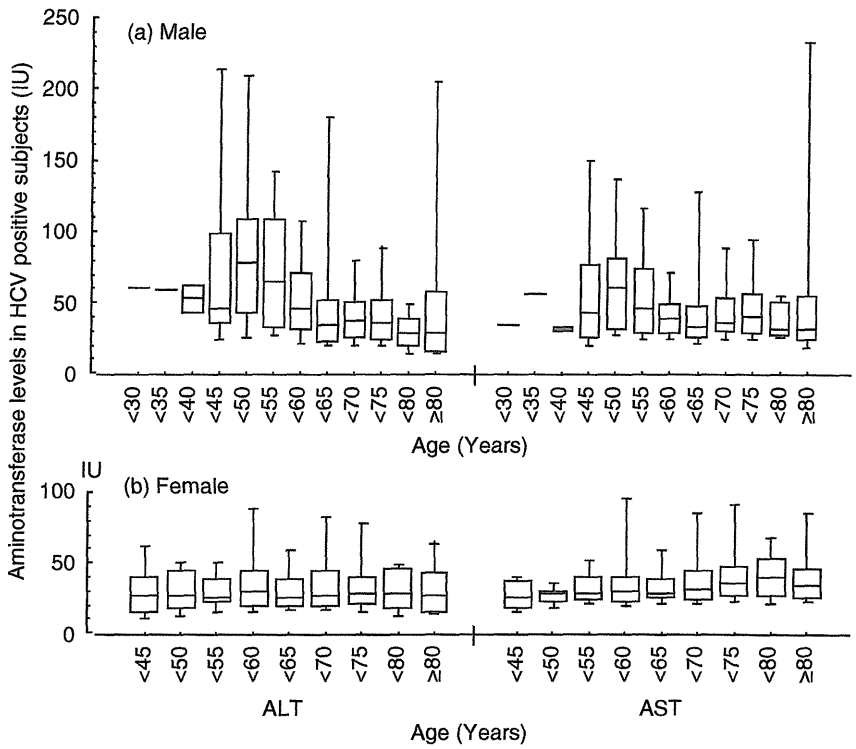

Figure 2 Distributions of serum levels of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) in the respective age ranges of hepatitis C virus (HCV) positive populations. The age ranges were divided into 5-year increments in males (a) and females (b). Data are shown as the 10th, 25th, 50th, 75th and 90th percentiles by box and whisker plots.

Optimal limits of aminotransferases by ROC curve

The ROC curves of serum ALT and AST levels for HCV positive subjects are shown by gender for 3 years in Figure 4. For both genders, the ROC curves for AST were positioned to the upper-left compared with those for ALT (Fig. 4a). The cut-off point (threshold) values in the uppermost left position on the curve were 24 IU (sensitivity 0.727: 1-specificity 0.280) and 27 IU (0.717: 0.222) for ALT and AST, respectively. Even in cases divided by gender, the ROC curves for AST for the respective gender was to the upper-left compared with those for ALT. In particular, the lower curve for ALT was remarkable compared to that for AST in males. The cut-off point values in the uppermost left (threshold) in males were 33 IU (sensitivity 0.577: 1-specificity 0.210) and 28 IU (0.761: 0.284) for ALT and AST, respectively, and in females were 22 IU (0.697: 0.256) and 26 IU (0.692: 0.202) for ALT and AST, respectively. These threshold values were defined as the proposed limit points in the present study.

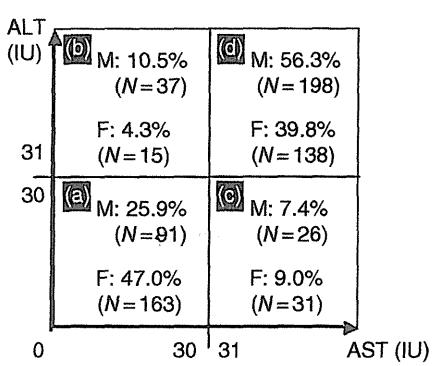

Figure 3 Hepatitis C virus (HCV) positive ratios by gender in the ranges cut off by the levels of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) at 30 IU. Data are shown as the percentage of HCV positive for the total HCV positive in each gender. The ranges less than 30 IU of both ALT and AST show the false-negative of HCV positive. χ^2 value = 40.836, $P < 0.0001$. The numbers in parenthesis show the HCV positive subjects. F, female; M, male; *N*, number.

Comparison of efficiency (sensitivity and specificity) among the three different limit points

The efficiency of the proposed limit points for serum ALT and AST levels were compared to those for the previous and current limit points in Japan based on the ratios of the true-positive and false-negative in the HCV positive

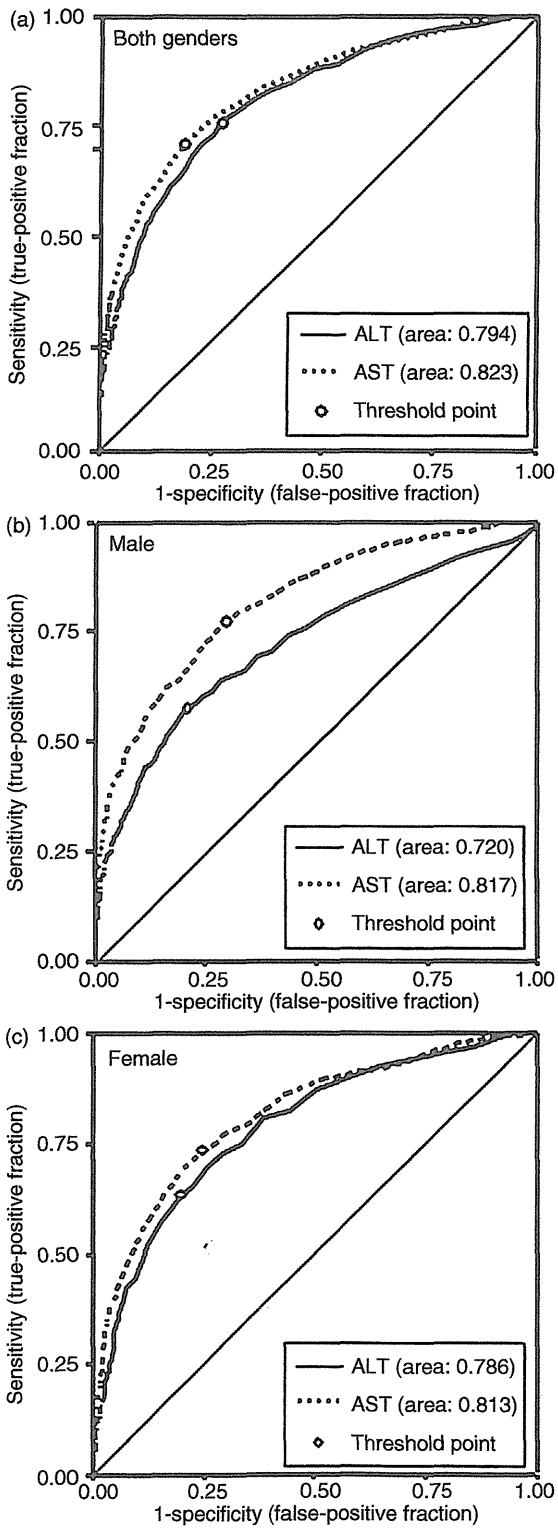

Figure 4 Receiver–operator characteristic (ROC) curves of sensitivity (true-positive fraction) plotted against 1-specificity (false-positive fraction) for serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels in the subjects that were diagnosed as hepatitis C virus (HCV) positive in examinations over 3 years for both genders (a) and by gender (male in (b), female in (c)). The cut-off point (threshold) values in the uppermost left of the curve (sensitivity: 1-specificity): both genders, ALT 24 IU (0.727: 0.280), AST 27 IU (0.717: 0.222); male, ALT 33 IU (0.577: 0.210), AST 28 IU (0.761: 0.284); female, ALT 22 IU (0.697: 0.256), AST 26 IU (0.692: 0.202). Area, area under the ROC curve.

group and of the true-negative and false-positive in the HCV negative group: the previous (ALT ≤ 35/AST ≤ 40), the current (ALT ≤ 30/AST ≤ 30) and the proposed limit points, by both genders (Fig. 5a) and each gender (males in Fig. 5b, females in Fig. 5c). For example, in case of the HCV positive group, the true-positive and false-negative ratios show the proportions of the HCV positive group outside and within the ranges, respectively, divided by the respective limit point.

As shown in Fig. 5, the true-positive and true-negative ratios were increased and decreased, respectively, depending on the limit points with the lower aminotransferase values. In both genders, shown in Fig. 5a, the true-positive ratio was improved to 76.7% with the proposed limit points. However, one-quarter of the HCV positive cases were judged as false-negative. In contrast, in the case of the HCV negative ratio, the true-negative ratio was decreased from 89.8% with the previous limit point to as low as 70.0% with the proposed limit point. Although the sum of true-positive and true-negative ratios with the proposed limit point was the highest among these cut-off points, the sum ratio was only 146.7%.

Likewise, in the case of males, shown in Fig. 5b, the true-positive ratio was improved with the proposed limit point, but only three-quarters. However, the true-negative ratios were decreased from 82.4% with the previous limit points to 69.9% with the proposed points. There were no marked differences in these ratios between the current and proposed limit points. In females, shown in Fig. 5c, the true-positive ratio was as low as 40% with the previous limit point. Although the true-positive ratio was remarkably improved with the proposed limit point, the ratio was only about 70%. In contrast, the true-negative ratio was as high as 94% with the previous limit point, but was down to three-quarters with the proposed limit point.

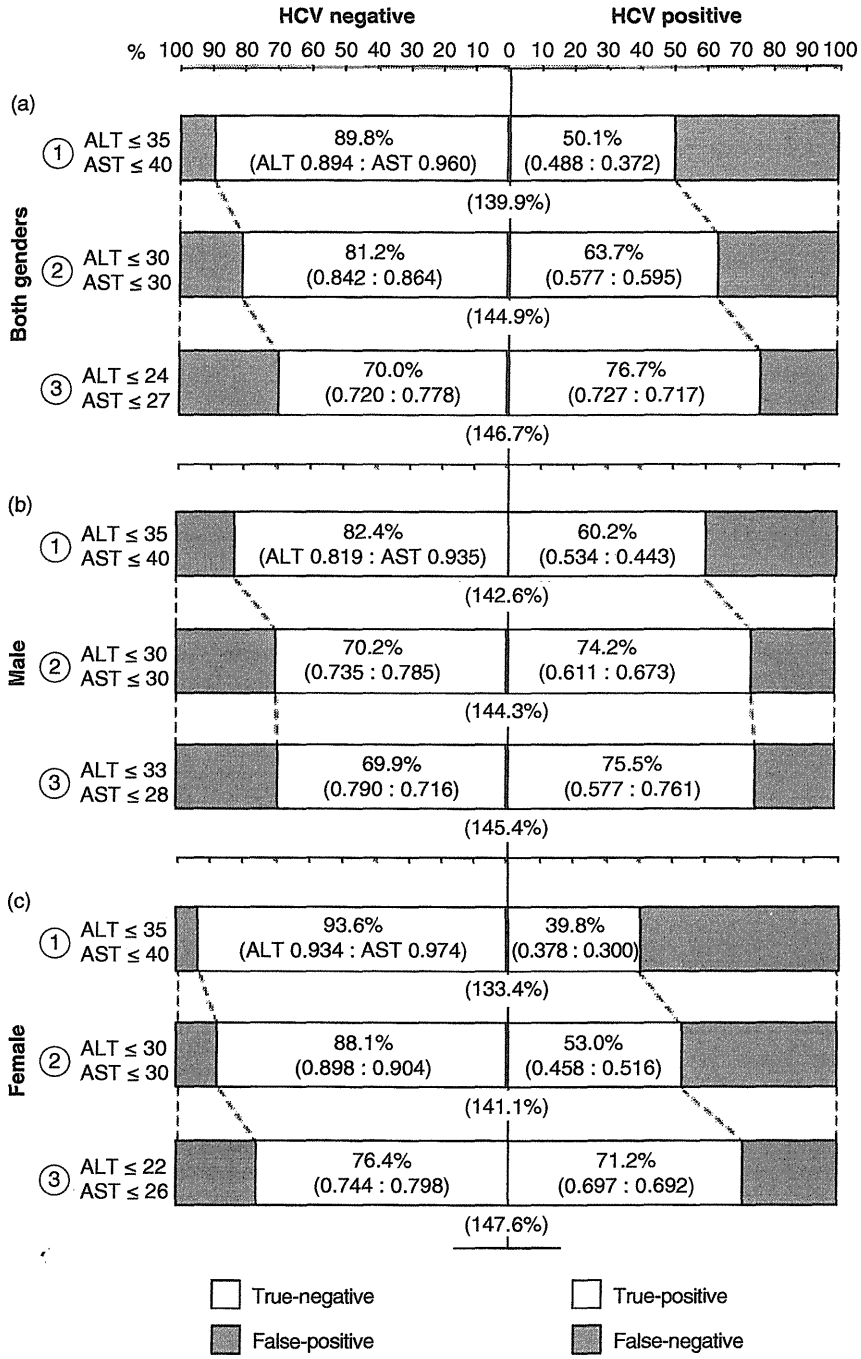

Figure 5 Percentages of true-positive among the HCV positive and true-negative among the HCV negative divided by the three set limit points of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels. (1) The previous limit points; (2) the current limit points; and (3) the proposed limit points. The true-negative and false-negative: the negative and positive ratios for HCV within the range set by the respective limit points. The false-positive and true-positive: the negative and positive ratios for HCV outside the range set by the respective limit points. The values in parentheses in the positive and negative columns indicate sensitivity (true-positive) and specificity (true-negative), respectively. In addition, the values in parentheses beneath the columns indicate the sum of the true-positive and true-negative.

DISCUSSION

THIS STUDY AIMED to re-evaluate the effectiveness of serum aminotransferases levels as a marker in the primary screening for HCV carrier detection in over 85 000 subjects in the annual public medical health examination. Before the evaluation, the optimal parameter obtained during public health examinations was also elucidated for HCV positive. Consequently, serum aminotransferases levels were confirmed as the optimal parameters related to an HCV positive diagnosis by the judgment system for HCV infection. Serum AST levels were more significantly associated with HCV positivity than ALT in both males and females. Furthermore, the optimal limit points (proposed limit points) for the healthy range derived from the ROC curve were different between genders (male, ALT33/AST28; female, ALT22/AST26). Based on a comparison of the proposed limit points to the previous (ALT ≤ 35/AST ≤ 40) and the current (ALT ≤ 30/AST ≤ 30) limit points in Japan, the efficiency of the proposed limit points was superior to the others. However, in males, 23.3% of HCV carriers were considered as negative (false-negative), although the true-positive ratio was increased by 15.3% from the previous limit point to the proposed limit point. In females, the true-positive ratio for the proposed limit point was markedly elevated by 31.4% compared to the previous limit point. Importantly, at least one-quarter of HCV carriers could not be captured for each of the genders, even when using these optimal conditions. Likewise, the incidence of false-positive among the HCV negative cases increased to 30 and 25% in males and females, respectively, with the optimal limit points. Therefore, one limitation of the use of aminotransferase levels as an indicator for HCV screening was found due to these low efficiencies. These results mean that almost one-quarter of male and half of female HCV carriers are already dropped from the thorough examinations and therapies when using the current limit points of serum aminotransferase levels.

Conry-Cantilena *et al.* have reported that 31 and 42% of HCV carriers exhibited PNAL (under ALT 41 IU) and minimally raised ALT levels (peak level of ALT less than 80 IU), respectively, in a follow-up study for 5 years.[2] The present study also demonstrated that the proportion of HCV carriers with PNAL (less than ALT 30 IU) was 33.3 and 56.0%, in males and females, respectively (Fig. 3). Although it is very important to determine how so many HCV carriers with PNAL can be screened in the health examinations, one of the markers associated with HCV positive status in the present study was ALT. Therefore, there are limitations to the use of ALT levels to screen for HCV with PNAL.

As the present study showed that AST rather than ALT levels were associated with HCV positive status, a significant relationship has been reported between AST, but not ALT, and liver damage, including portal inflammatory piecemeal necrosis and liver fibrosis.[17,18] In addition, Schiffman *et al.* observed that there was no correlation between baseline ALT activity, HCV-RNA level and liver histology in HCV patients with PNAL.[6] Puoti *et al.* described ALT levels as possibly being less important for the determination to undergo therapy than other factors, such as age, HCV genotype, liver histology, patient motivation, symptoms, extra-hepatic manifestations and co-morbid illnesses.[19] For hepatic fibrosis patients with not only HCV but also several other liver diseases, serum AST level has been suggested to be a better marker than ALT.[20–22] Assy and Minuk suggested possible reasons to explain why AST, but not ALT values, correlate with histological findings in patients with HCV.[18] One of the possible reasons is that co-existing non-viral-related fatty infiltration of the liver might contribute disproportionately to the elevation of ALT. Another is that HCV might destroy mitochondria, where AST exists as a specific enzyme, resulting in intracellular trophism. In addition, AST activity may be more stable than ALT activity.[23] However, it remains unclear why AST values correlate with hepatic histological features, in particular fibrosis, and further studies are therefore needed. Indeed, while there is no doubt that both ALT and AST are useful parameters for liver disease,[24] there are limitations of the use of ALT and AST as parameters for HCV screening in public health examinations.

In the present study, the serum aminotransferase levels were different between the genders, in agreement with previous studies.[7–9] Prati *et al.* indicated different normal ranges of ALT for liver disease between males and females (30 and 19 IU, respectively) based upon calculation of the 95th percentile of serum ALT levels in normal subjects with a population at lower risk for liver disease.[8] In general, both serum aminotransferase levels in females tend to be lower than those in males.[7,25–30] Puoti *et al.* speculated that different hormonal factors, such as estrogens, could act as modulators of some mechanisms and may explain the differences between genders,[25] based on the decreased ALT levels in pregnant and estrogen-treated females with chronic hepatitis C.[31,32] Indeed, in the present study, the aminotransferase levels in HCV positive females were independent of age; although these levels, especially ALT, were elevated in males aged less than 65 years. The current results may be

supported by these reports. Therefore, the cut-off points of ALT and AST might need to be lower than ALT in females, particularly in the younger age groups, for HCV screening.

The present study attempted to generate a useful equivalent for the detection of HCV carriers by logistic analysis. Ultimately, the equivalent, based on some significant parameters associated with HCV carriers led from the stepwise analysis shown in Table 2, could not efficiently detect HCV positive rates below 10%. Therefore, in the present study, the equivalent was not an effective tool for the screening of HCV carriers. In future, a more effective logistic equivalent should be considered using better parameters, including platelet counts,[9,12] in a larger population of both public and clinical examinations for the screening of HCV carriers, because the present judgment system for HCV infection needs some steps added with different specific equipment and techniques and the consequent costs. The effective logistic equivalent from the parameters used in public examination will be a good way from the viewpoint of cost effectiveness and will be available in developing countries. However, at present, there are no highly efficient methods that can be applied to the health examination for the screening of HCV carriers. Furthermore, as shown by the results of the present study, one-quarter of HCV carriers, in particular almost half of female carriers, have been diagnosed as healthy subjects and, therefore, have missed out on any treatments for HCV infection. This fact supports the recommendation of HCV tests, at least the anti-HCV antibody test, to all individuals during health examinations for the early detection of asymptomatic HCV carriers; this should be more important than cost considerations.

In conclusion, the limitation of serum aminotransferase levels as markers of HCV carriers for primary screening in public medical examinations was confirmed, because one-quarter of the HCV carriers were categorized as healthy subjects. Therefore, the current investigation supports the necessity to apply HCV testing to all individuals undergoing public medical examinations for early detection and early treatment and to prevent the spread of HCV.

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C型肝炎と肝癌

－肝炎から肝癌まで：
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松崎　靖司

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特集／感染症最前線

C型肝炎と肝癌
―肝炎から肝癌まで：とくに慢性C型肝炎治療の最近の知見―

東京医科大学 茨城医療センター 消化器内科 教授 松﨑 靖司

はじめに

我が国の肝疾患患者数は，C型ウイルス肝炎のキャリアーの方が200万～300万人とも言われ，その中で肝硬変の患者は全国で40万～50万人前後と推計されている。肝硬変単独の死亡数は年間17,000人とされ，肝癌による死亡数は３万人とされている。2000年を境に肝癌死亡数は横ばいになりつつある。インターフェロン治療の普及の効果が多少現れてきているのだろうか。肝癌の原因の80％はC型肝炎ウイルス（HCV）に起因する。肝癌撲滅を目指すには慢性肝炎から肝硬変への移行を阻止することが大命題である。HCV抗体陽性者の自然経過は，HCV暴露から高率に慢性化し，20～30年後に肝硬変，そして肝細胞癌（HCC）発癌へと移行することが明らかとなってきている。

以上のような現況から，B型肝炎ウイルス（HBV），HCV，アルコールあるいはその他の発癌因子のHCC発生への関与の解明は急務となってきているといっても過言ではない。C型慢性肝炎から不幸にも肝硬変になった患者さんの場合は肝癌の早期発見をし，早期治療することが重要課題である。

本稿では，肝癌撲滅を目指し，その目的達成のために特にC型慢性肝炎から肝硬変への移行を阻止するための治療法，インターフェロン（IFN）療法，ウルソデオキシコール酸（UDCA）療法，プロテアーゼ阻害剤について最新の知見を概説する。

HCV抗体陽性慢性肝炎患者における治療法

Ⅰ　インターフェロン療法

現在のC型慢性肝炎の治療はインターフェロン（IFN）が主流である。1991年にC型慢性肝炎に対してIFNが保険適応となり，2002年にリバビリンとの併用療法が保険適応となるまでは，全C型慢性肝炎患者のウイルス排除／陰性化（SVR）率は約30％であった。その後，2002～2004年まではリバビリン併用により効果も約45％に上がり，2006年現在は持続型IFN・リバビリン併用療法により，C型慢

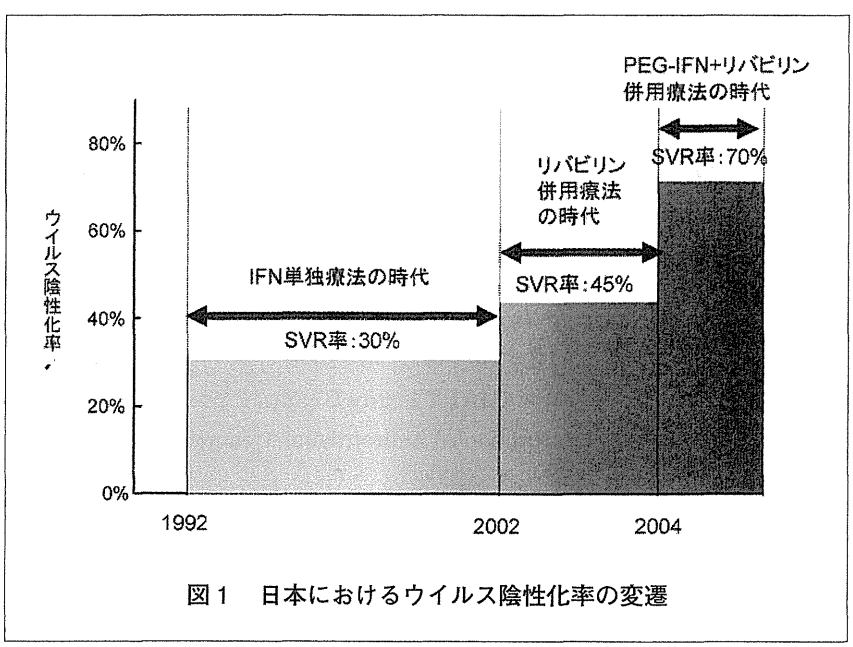

図１　日本におけるウイルス陰性化率の変遷

性肝炎の約70％でウイルス感染を断つことが可能である（**図1**）。しかし，すべてのC型慢性肝炎患者が治療の対象となるわけではないのが実状である。対象の選択には，年齢が60～65歳以下が望ましく，病期の進行度が軽いもの，線維化が強く起こらないような肝炎の活動性が弱いものが宿主側の良好な効果を得る要因となる。また，ウイルス側の要因として，ウイルス量が少ないこと（5.0logIU/mL未満），Genotype（G）の2a，2b（serotype 2群）は，genotype 1b（1群）に比べIFN効果が良い。これらより，1b高ウイルス群においてはSVR率が低く難治例とされてきた。その治療効果の推移は目を見張る。初期の難治例のSVR率が約2％であったのが，現在では約50％まで効果が上がったことは画期的なことである。最近は，G1のslow-responderの再燃率や，投与開始12週後にHCVRNAが陰性化せず，36週で陰性化した高ウイルス群，G1例での再燃率の低下を目指すために，これらの症例に対して72週間投与が適応されるに至った。これにより，48週間投与に比べSVR率が20％ほど上昇する[1)]。

1）持続型IFNの必要性と薬物動態の最適化

従来のIFNは急速に代謝され，半減期が非常に短いため，基本的には週3回注射されている。これは負担の大きな投与スケジュールである上に効果が弱い。単純にIFNの投与量を増やしても薬物動態プロファイルは劇的には変化せず，結局，週3回投与する必要がある（**図2**）。

そこで，IFNαのペグ化（PEG-IFN）の目的は，クリアランスを減らし，代謝を抑制し，*in vivo*における生物学的活性を維持あるいは改善することにより，IFNαの薬物動態を最適化することである。PEG・IFNは，従来のIFN治療の欠点を克服するために特別に設計された製剤である。2種類のPEG・IFNが現在使用されており，**表1**に示すよう違いがある。ペグイントロンの構造を示す。IFNα2bに平均分子量12KDのPEGつまりポリエチレングリコール1分子が共有結合した修飾タンパク質である。

ペグIFNα2bは，アミノ酸配列中のアミノ酸の1ヵ所に，1分子のペグが共有結合したものである。中でも，His^{34}へのペグの結合割合が50％を超えており，His^{34}に結合したペグIFNα2bはLys^{31}に結合したペグIFNα2bと比較して高い抗ウイルス活性が認められたことから，His^{34}への結合は，IFNα2bとIFNαレセプター（IFNAR2）との結合阻害を減少させる部位として好ましいと推測される。また，Lys^{31}に結合したペグIFNα2bは，他のペグ結合体と比較してウイルス活性が低いことが認められたことから，Lys^{31}への結合はIFNα

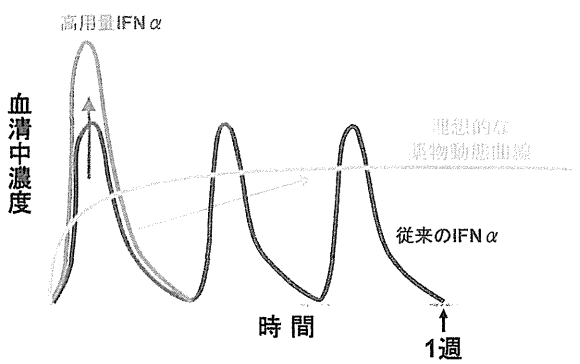

図2　IFNαの薬物動態の最適化

表1　PEG-IFNの種類

一般名	PEG-IFNα-2b	PEG-IFNα-2a
製品名（メーカー）	ペグイントロン（シェリング・プラウ）	ペガシス（中外製薬）
PEGの分子量	12KD	40KD
抗ウイルス作用	IFNα-2bの約28％	IFNα-2aの約7％
最高血中濃度（1回投与）	874pg/mL	10,700pg/mL
用量設定	体重別	固定
その他		毎投与時直前に血液検査が必要

各製品添付文書ほか

2bとIFNαレセプターとの結合を阻害している可能性が示唆されている[2]。

従来のIFNの薬物動態は，ペグ化IFN(PEG-IFN)のものとはかなり異なる。PEG-IFNの薬物動態は使用されるペグ化の種類，主に結合されるPEG分子の大きさと性質に応じて変化する。ペグIFNα2aの場合，40KDのPEGを結合させることで，小さな分布容積，低いクリアランス，長い半減期を持つ分子が得られる[3)~5)]。薬物動態曲線は，治療濃度への急速な上昇と，長時間続く最大濃度を示している。これは1週間を通して一定に保たれ，ピーク対トラフ比が低く，ピークに関係する副作用を排除し，7日間続く真のウイルス抑制を提供する（表2）。ペグIFNは週1回投与で安定した血中濃度を維持するので，忙しく通院が困難なため治療を受けられなかった患者さんにとって待ち望まれた薬剤と言える。また，血中濃度を維持することにより，従来のIFNでみられていたウイルスの再増殖の抑制が期待できる。

表2　PEG-IFN：薬物動態学的パラメータ

パラメータ	IFNα	PEG-IFNα-2b	PEG-IFNα-2a
分布容積（L）[1), 2)]	31～98	80*	6～14†
クリアランス（mL/h）	11,800～16,170	1,540	80
吸収半減期（h）[2)~5)]	2.3	4.6	50
ピーク対トラフ比[6)]	無限大	1/100	1/1.5～2.0
消失半減期（h）	2～5	≫40	77

*患者の体重によって調整
†患者の体重にかかわらず一定

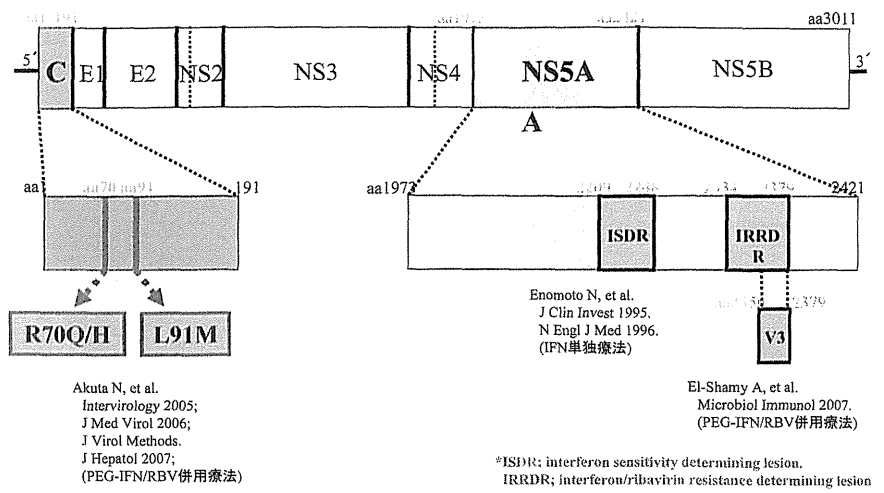

図3　IFN関連療法の治療感受性に影響する領域

2）ウイルス遺伝子からみた治療効果

HCVの構造中，非構造領域のNS5A領域の変異とIFN感受性との関連が指摘されている。図3は，従来報告されているIFN関連療法感受性に関与する領域である。まず，前述のごとくIFN単独療法に関してはNS5AのISDRが報告されている。またそのさらに3′側のV3 regionやそれを含むIRRDRという領域がRBV併用療法の治療効果予測に関与していることが最近報告されている。また最近，Coreの70・91番のアミノ酸置換がRBV併用療法で重要であることが報告されている[6)~9)]。

難治性肝炎に対し，これらの情報も加味しIFN投与法の改善や，多剤との併用など検討されているところである。

Ⅱ　肝庇護療法の位置付け

C型慢性肝炎の治療目標は肝癌の発現阻止にほかならない。この目標を達成するためにいくつかの治療法があるが，その中で一番はじめに考慮されるのは，抗ウイルス療法である。これはC型肝炎ウイルスを排除する治療法で，現在のところ国内外ともにペグインターフェロンとリバビリンの併用療法が標準療法とされている。しかしその有効率は完全なものとはいえないのが現状である。また，副作用の問題などで十分な抗ウイルス療法が行

えない場合も少なくない。このように抗ウイルス療法を行えない場合には，肝庇護療法にて肝炎を鎮静化し，肝発癌を抑制する必要がある。

肝庇護療法はHCVを排除しないものの，肝炎を鎮静化し肝細胞の再生を促すことにより，肝線維化進展を抑える治療法である。C型慢性肝炎で肝庇護療法の適応になるのは，肝炎の活動性のマーカーであるALTが異常値を示す患者さんで，抗ウイルス療法にてウイルス排除ができなかった患者さん，IFN療法の副作用により抗ウイルス療法を実施できない患者さん，実施できても規定の投与期間を完遂できない患者さん，また抗ウイルス療法を望まない患者さんなどが主な対象者となる。

肝庇護療法の歴史は古く，これまで多くの治療法が試みられている。その中でもウルソデオキシコール酸（UDCA）とグリチルリチン製剤の注射薬の先発品であるウルソと強力ミノファーゲンC（SNMC）は，有用性において科学的な根拠を有して使用されだした治療法である。

経口肝庇護療法の第一選択薬としては，UDCA（商品名：ウルソ）があげられる。UDCAは胆汁酸製剤であり，古来より動物性生薬として珍重された「熊胆」の成分である。本邦において1970年代後半より胆石溶解剤として使用されるようになった。ウルソはすでに，胆石溶解療法剤として1978年に600mg/日投与が保険適応認可となり，慢性肝疾患に対しては，原発性胆汁性肝硬変（PBC）に対して1999年に600～900mg/日が保険適応となっている。これらは本邦において，二重盲検試験により有効性が確認され認可された科学的な根拠に基づく治療法である[10), 11)]。UDCAのPBCに対する有効性は，1987年フランスのPouponらにより初めて示された[12)]。その後，著者らも同様にUDCAの原発性胆汁性肝硬変に対する有効性を報告した[13), 14)]。慢性肝炎をはじめこれら慢性肝疾患に対するUDCAの有効性の成績は，二重盲検試験により本邦を含め世界から報告された[15)～17)]。

作用機序については，我々は臨床例からの検討で原発性胆汁性肝硬変患者にUDCAを投与した時の血清胆汁酸分画の検討より，体内胆汁酸プールの変換の重要性を考えている[13), 14)]。UDCAの肝細胞保護作用に関しては，さまざまな角度より検討されている。しかし，いまだUDCA作用発現機序にはナゾの部分が多く存在しているのも実状である。以下に現在考えられている作用機序をまとめてみる。一つはUDCAの投与により細胞障害性の胆汁酸がUDCAに置き換わり肝細胞膜が保護される置換効果と考えられている。またUDCAには抗酸化作用，免疫調整作用，抗アポトーシス作用もあり，肝細胞の保護に働いているとも報告されている。これら複合的な機序により，PBCばかりでなく，C型慢性肝炎に対してもUDCAは肝機能の改善効果を発揮するものとされる。

2007年３月にウルソはC型慢性肝疾患に対する効能追加が承認された。以前からウルソは150mg/日の使用が可能であったが，今回二重盲検試験であるコントロール試験を国内63施設において実施した。その結果，ウルソ150mg/日投与群に比べ600mg/日および900mg/日投与群での投与開始４～24週後におけるAST，ALTおよびγ-GTP値の改善が有意の差をもって認められた。このような有効性が確認され，併せて安全性に問題ないことが確認され[17)]，承認に至った。現在，C型慢性肝疾患に対する効果的なウルソ投与量は600～900mg/日である。副作用については，胃不快感，下痢，便秘などの消化器症状が時にみられるが，その程度は軽微なものである。

以上のように，IFNやUDCAにより肝炎の進行を抑制し，肝硬変に移行することを少しでも抑制することが重要なポイントである。

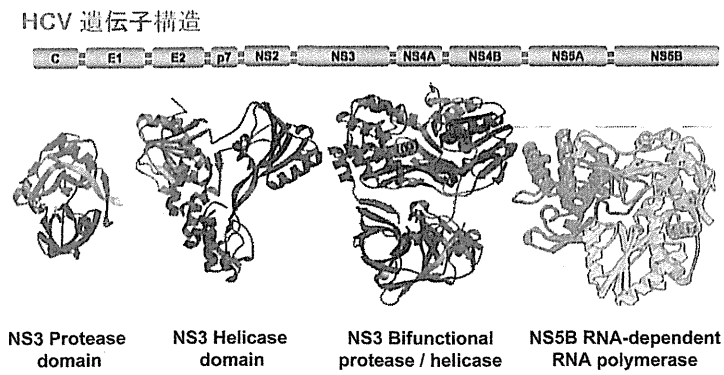

図４　C型肝炎のこれからの治療：特異的HCV酵素阻害剤

Ⅲ　新しい肝炎治療薬；プロテアーゼ阻害剤

HCVの増殖に際して，HCVが持つ３個の律速酵素が関与していることが明らかとなってきた。それはプロテアーゼ，ヘリカーゼ，ポリメラーゼである（図４）。この中でプロテアーゼに対する阻害剤の治験が現在一番先行している。Telaprevir（VX-950）はNS3.4A protease inhibitorである。TelaprevirとPEG・IFNの併用でSVRが極めて早いという成績がでている。近い将来，標準治療となりうるものであろう。

Ⅳ　肝癌早期発見のために

慢性C型肝炎の患者さんのフォローアップ方法であるが，基本的には早期発見をするためには，肝癌発癌高危険群をまず囲い込むことである。

HBs抗原陰性のHCV陽性肝細胞癌患者さん方の前向きな観察研究にて，抗HBs抗体，抗HBc抗体陽性，大量喫煙者において肝癌発癌が有意に高いことを我々は見出した[18]。さらにHBs抗原陰性のHCV陽性肝細胞癌患者さんの肝臓組織内のHBVDNAの存在を検討すると，50％近くにHBVDNAの一部が存在することを明らかにした[19]。HBs抗体やHBc抗体陽性の患者さんに特にこの現象が多いことが判明した。HBVの感染の既往者にも肝内にHBVが残っていることを示唆するものである。

C型慢性肝疾患において，肝癌の早期発見には，高齢の男性の肝硬変患者で，特に抗-HBV抗体陽性者（抗-HBsまたは抗-HBc），大量喫煙者においては，３～６ヵ月ごとの超音波検査により特に綿密に観察することが重要である。

Ⅴ　地域医療連携と肝炎対策事業

自治体の肝炎対策を基本に，啓発活動や医療連携などを構築していかねばならない時代である。我々は茨城県における平成15～17年に住民基本健診とHCV検診を受診した85,013人（男性29,5002人，女性55,511人）のデータから，血清ALT・AST値のHCV陽性率に対する判定値の有用性を検討した。その結果，血清トランスアシナーゼ（ALT・AST）値を用いた基本健診検査項目に頼ったHCVスクリーニングでは，陽性者の約1/4から1/2がすでに漏れていることが浮き彫りとなり，改めて基本健診でのHCV抗体検査が不可欠であることが確認された[20]。患者の掘り起こしをせねばならぬところである。

我々は平成14年度より行政と連携して取り組んできた。慢性C型肝炎・肝硬変・肝癌征圧モデル自治体において，節目検診終了２年目の今年度の継続受診状況を調査した結果，節目検診受診６年後で75％，５年後で96％と高い確率でフォローアップが確立されていることが確認された。このような患者動向調査も重要な今後の課題である。

今後の課題として，１）潜在性C型肝炎患者の発掘，２）肝炎患者のフォローアップの充実，３）高い継続受診率の維持による病態の進展抑制，４）IFN療法の普及が挙げられる。そのために，医師のための研修会の開催，患者・市民への情報の普及と啓発，肝疾患診療連絡協議会の稼働を通して，医療連携