Clinical cases. The multifidus muscle of a 45-year-old healthy man is shown in Figure 1a. This individual showed no muscle steatosis (IMAC = –0.44). Figure 1b shows the multifidus muscle of a 45-year-old man with simple steatosis (Matteoni's classification = 2). The muscle in this individual shows increased muscle fat storage (IMAC = –0.26). Figure 1c shows the multifidus muscle of a 45-year-old man with NASH (Matteoni's classification = 3, Brunt's staging = 3), with extensive muscle fat storage (IMAC = –0.08).

Statistical analysis. Descriptive statistics (means and standard deviations) were calculated for all continuous variables, and frequencies were calculated for categorical variables. Differences between two groups were compared by the Mann–Whitney *U*-test. Pearson's correlation coefficient analysis and Spearman's correlation by rank analysis were used to compare IMAC and FIB4 index or NAFIC score. Differences and correlations among five groups were performed by Kruskal–Wallis analysis of variance followed by the Scheffe and Tukey–Kramer post-hoc test. Comparisons between before and after NASH therapy were tested using the Wilcoxon signed-rank test.

Effects of improvements in IMAC were evaluated by changes in the variable (Δ = variable after intervention – variable before intervention). Spearman's rank correlation was used to compare the Δ IMAC and each Δ parameters.

Multivariate logistic regression analysis was used to identify independent factors associated with the severity of NAFLD and NASH stage. Differences were considered significant at $P < 0.05$. All analyses were carried out using IBM SPSS (Version 19.0; SPSS, Inc., Tokyo, Japan).

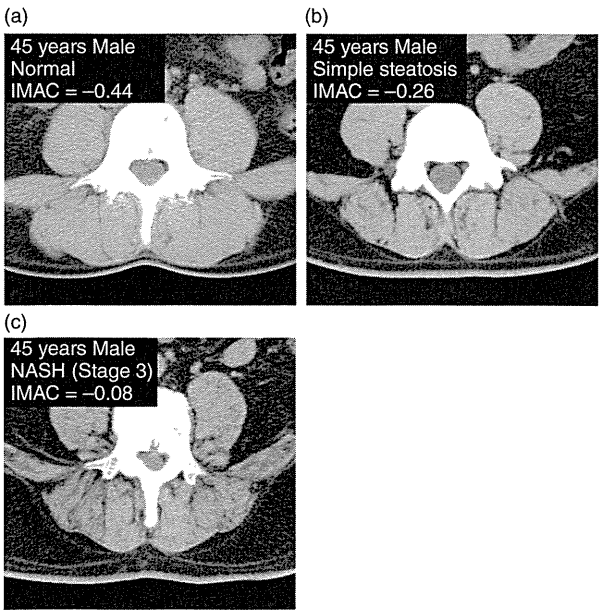

Figure 1 Cross-sectional computed tomographic images of the subfascial muscular tissue in the multifidus muscle taken at the umbilical level in a normal individual (a), a patients with simple steatosis (b), and a patient with non-alcoholic steatohepatitis (c).

Results

Characteristics of the patients with NAFLD. The clinical and biochemical characteristics of the patients enrolled in this study are summarized in Table 1. There were 208 patients (95 women and 113 men) with a mean age of 51 years; the women were significantly older than men. Bodyweight, VFA, ALT, γ-GTP, and serum albumin were significantly higher in men than in women, whereas ALP, L/S ratio, and IMAC were significantly higher in women than in men.

Characteristics of patients with NAFLD defined according to Matteoni's classification. BMI, AST, ALT, FPG, insulin, HOMA-IR, and IMAC were significantly higher in patients with types 3–4 NAFLD than in patients with types 1–2 NAFLD. By contrast, γ-GTP, QUICKI, and platelet count were significantly higher in patients with types 1–2 NAFLD than in those with types 3–4 NAFLD. There were no significant differences in the other characteristics between the two groups of patients (Table 2). As indicated in Table 3, multivariate logistic regression analysis indicated that risk factors associated with the severity of NAFLD were IMAC, aging, ALT, and HOMA-IR

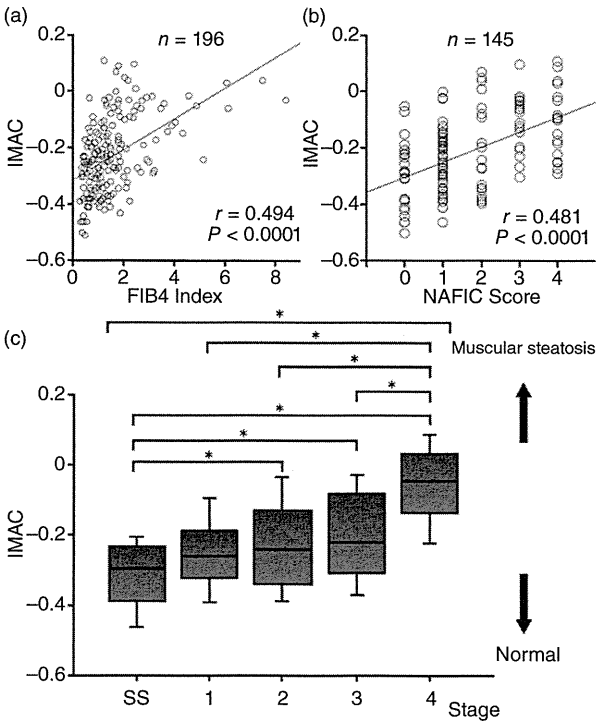

Figure 2 Intramuscular adipose tissue content (IMAC) was correlated with FIB4 index (a: $r = 0.494$, $P < 0.01$) and NAFIC score (b: $r = 0.481$, $P < 0.01$). (c) Comparison between IMAC and histopathological stage determined using Matteoni's and Brunt's classification. Histopathological stage determined by both classifications was significantly correlated with IMAC (*$P < 0.01$).

Table 1 Baseline characteristics of the patients with NAFLD subjects

Variables	All patients ($n = 208$)	Males ($n = 113$)	Females ($n = 95$)	*P* value
Age (years)	51.0 ± 15.2	44.7 ± 14.2	58.6 ± 12.7	< 0.0001
Weight (kg)	74.1 ± 16.9	81.8 ± 16.7	64.9 ± 11.7	< 0.0001
BMI (kg/m^2)	28.3 ± 4.6	28.9 ± 4.9	27.7 ± 4.2	0.1432
VFA (cm^2)	149.0 ± 55.3	159.6 ± 58.4	136.5 ± 48.6	< 0.01
AST (IU/L)	49.4 ± 34.5	48.3 ± 36.3	50.7 ± 32.3	0.2762
ALT (IU/L)	77.0 ± 60.0	84.3 ± 64.3	68.4 ± 53.5	< 0.05
ALP (IU/L)	263.0 ± 137.1	256.0 ± 169.8	271.3 ± 82.8	< 0.01
γ-GTP (IU/L)	89.2 ± 96.0	105.0 ± 115.2	70.4 ± 60.6	< 0.01
TC (mg/dL)	209.4 ± 43.3	207.5 ± 45.8	211.5 ± 40.3	0.2093
TG (mg/dL)	180.8 ± 135.9	191.3 ± 156.4	168.1 ± 105.4	0.0704
FPG (mg/dL)	113.8 ± 35.1	109.5 ± 31.2	118.9 ± 38.9	0.0913
Insulin (μg/mL)	15.6 ± 10.3	17.0 ± 12.5	13.8 ± 6.0	0.4907
Insulin resistance				
HOMA-IR	4.4 ± 3.3	4.6 ± 3.7	4.1 ± 2.5	0.9278
QUICKI	0.32 ± 0.04	0.32 ± 0.04	0.32 ± 0.03	0.9187
Platelet count (× 10^4/μL) ($n = 196$)	22.9 ± 6.9	22.5 ± 6.3	23.4 ± 7.5	0.5768
Serum albumin (g/dL)	4.6 ± 0.4	4.7 ± 0.4	4.4 ± 0.4	< 0.01
L/S ratio	0.83 ± 0.31	0.76 ± 0.28	0.92 ± 0.31	< 0.0001
IMAC	−0.23 ± 0.13	−0.31 ± 0.10	−0.14 ± 0.11	< 0.0001

Data are means ± standard deviation.

Statistical analysis was performed using the Mann–Whitney *U*-test. Differences were considered significant at $P < 0.05$.

ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; FPG, fasting plasma glucose; HOMA-IR, homeostasis model assessment-insulin resistance; IMAC, intramuscular adipose tissue content; L/S ratio, liver-spleen attenuation ratio; NAFLD, non-alcoholic fatty liver disease; QUICKI, quantitative insulin sensitivity check index; TC, total cholesterol; TG, triglyceride; VFA, visceral fat area; γ-GTP, γ-glutamyl transpeptidase.

(IMAC: OR = 3.671, $P < 0.05$; Age: OR = 2.895, $P < 0.05$; ALT = 6.222, $P < 0.01$; HOMA-IR = 3.035, $P < 0.05$).

Characteristics of patients with NASH defined according to Brunt's classification. Age and AST were significantly higher in patients with stages 3–4 NASH than in those with stages 1–2 NASH. By contrast, IMAC was significantly greater in patients with stages 3–4 than in those with stages 1–2 NASH. There were no significant differences in the other factors between the two groups of patients (Table 2). As shown in Table 4, multivariate logistic regression analysis indicated that risk factors associated with the severity of NASH were IMAC and aging (IMAC: OR = 2.444, $P < 0.05$; Age: OR = 2.355, $P < 0.05$).

Comparing the IMAC to severity model of NAFLD/NASH and histopathological stage. IMAC and severity model of NAFLD/NASH in each groups of patients are compared in Figure 2a,b. IMAC was correlated with FIB4 index ($r = 0.494$, $P < 0.01$) and NAFIC score ($r = 0.481$, $P < 0.01$). IMAC and histopathological stage in both groups of patients are compared in Figure 2c. Histopathological activity determined by simple steatosis and Brunt's classification was significantly correlated with IMAC ($P < 0.01$).

Effects of lifestyle interventions on IMAC in patients with NASH. The effects of the prescribed treatment on the clinical characteristics of 21 patients with NASH (13 women and 8 men) are shown in Table 5. The mean intervention period at 24.0 months, in some cases, was administering pharmacotherapy with a sulfonylurea, α-glucosidase inhibitor, or hypocholesterolemic drug. Overall, 11 patients showed improvements in IMAC during the treatment period. These patients also showed significant decreases in bodyweight, BMI, SFA, ALT, γ-GTP, total cholesterol, triglyceride, FPG, 180-PG, Insulin, and HOMA-IR following treatment, while the QUICKI and L/S ratio increased significantly. The histopathological changes determined by Matteoni's and Brunt's classifications and NAS have been improved in comparison with pretreatment biopsy. By contrast, among patients with no improvement in IMAC, improvement of total cholesterol was observed, but platelet count decreased significantly, and no significant changes were found in the other factors. The histopathological changes and NAS were not improved in comparison with pretreatment biopsy.

Effects improvements in IMAC on other parameters. As shown in Table 6, the ΔIMAC was significantly correlated with Δ weight ($\rho = 0.507$, $P < 0.05$), Δ BMI ($\rho = 0.512$, $P < 0.05$), Δ SFA ($\rho = 0.653$, $P < 0.01$), Δ triglyceride ($\rho = 0.458$, $P < 0.05$), Δ FPG ($\rho = 0.678$, $P < 0.01$), Δ Insulin ($\rho = 0.466$, $P < 0.05$), Δ HOMA-IR ($\rho = 0.493$, $P < 0.05$), Δ QUICKI ($\rho = -0.571$, $P < 0.01$), Δ L/S ratio ($\rho = -0.714$, $P < 0.01$), histopathological assessments (Δ steatosis, $\rho = 0.800$; Δ lobular inflammation, $\rho = 0.686$; Δ ballooning, $\rho = 0.769$; $P < 0.01$ for each), and Δ NAS ($\rho = 0.800$, $P < 0.01$). The Δ IMAC evaluation did not indicate a correlation between Δ VFA, Δ ALT, Δ total cholesterol, Δ platelet count, and Δ serum albumin.

Table 2 Comparison of biochemical parameters according to stage of NAFLD/NASH determined by Matteoni's and Brunt's classifications

Variables	Simple steatosis ($n = 38$)	NASH ($n = 170$)	*P* value*	Stage 1–2 ($n = 104$)	Stage 3–4 ($n = 66$)	*P* value**
Sex (male/female)	24/14	89/81	0.2278	58/46	31/35	0.2656
Age (years)	47.2 ± 15.6	51.9 ± 15.0	0.0829	49.2 ± 14.5	56.2 ± 14.8	< 0.01
Weight (kg)	72.8 ± 15.6	74.3 ± 17.2	0.7317	75.4 ± 17.4	72.7 ± 16.9	0.4204
BMI (kg/m^2)	26.9 ± 4.1	28.7 ± 4.7	< 0.05	28.8 ± 4.9	28.4 ± 4.4	0.7993
VFA (cm^2)	134.2 ± 40.4	149.4 ± 54.0	0.6732	149.4 ± 54.0	157.0 ± 63.2	0.6732
AST (IU/L)	39.3 ± 26.0	51.7 ± 39.8	< 0.01	49.7 ± 39.8	54.9 ± 28.3	< 0.01
ALT (IU/L)	61.6 ± 50.8	80.4 ± 69.8	< 0.01	83.2 ± 69.8	76.1 ± 45.7	0.7363
ALP (IU/L)	236.6 ± 62.2	268.8 ± 148.0	0.1754	251.1 ± 77.1	296.4 ± 214.6	0.0964
γ-GTP (IU/L)	93.5 ± 136.2	88.3 ± 85.2	< 0.05	92.5 ± 99.5	81.8 ± 56.2	0.9025
TC (mg/dL)	221.2 ± 40.1	206.7 ± 43.6	0.0548	210.8 ± 42.9	200.6 ± 44.3	0.0892
TG (mg/dL)	156.8 ± 72.6	186.3 ± 146.1	0.5567	189.7 ± 166.8	180.8 ± 107.0	0.8761
FPG (mg/dL)	103.0 ± 35.1	116.2 ± 34.8	< 0.01	114.3 ± 34.4	119.3 ± 35.4	0.2988
Insulin (μg/mL)	10.8 ± 7.8	16.7 ± 10.5	< 0.0001	17.0 ± 11.8	16.2 ± 7.7	0.6029
Insulin resistance						
HOMA-IR	2.8 ± 2.5	4.8 ± 3.3	< 0.0001	4.8 ± 3.5	4.7 ± 2.9	0.7002
QUICKI	0.35 ± 0.05	0.32 ± 0.03	< 0.0001	0.32 ± 0.03	0.32 ± 0.03	0.7260
Platelet count (× 10^4/μL) ($n = 196$)	24.9 ± 6.5	22.4 ± 6.9	< 0.05	23.3 ± 6.9	21.0 ± 6.8	0.0894
Serum albumin (g/dL)	4.5 ± 0.4	4.6 ± 0.4	0.2087	4.6 ± 0.4	4.5 ± 0.5	0.1806
L/S ratio	0.89 ± 0.34	0.82 ± 0.30	0.2056	0.79 ± 0.30	0.86 ± 0.29	0.1392
IMAC	−0.31 ± 0.11	−0.22 ± 0.13	< 0.01	−0.24 ± 0.12	−0.18 ± 0.14	< 0.05
Histopathological assessment						
Fibrosis (0/1/2/3/4)	(38/0/0/0/0)	(0/36/68/58/8)		(0/36/68/0/0)	(0/0/0/58/8)	

**P* values are shown for comparisons between simple steatosis and NASH; ***P* values are shown for comparisons between stages 1–2 and 3–4. Data are means ± standard deviation.

Statistical analysis was performed using the Mann–Whitney *U*-test. Differences were considered significant at $P < 0.05$.

ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; FPG, fasting plasma glucose; HOMA-IR, homeostasis model assessment-insulin resistance; IMAC, intramuscular adipose tissue content; L/S ratio, liver-spleen attenuation ratio; NAFLD, non-alcoholic fatty liver disease; NASH, non-alcoholic steatohepatitis; QUICKI, quantitative insulin sensitivity check index; TC, total cholesterol; TG, triglyceride; VFA, visceral fat area; γ-GTP, γ-glutamyl transpeptidase.

Table 3 Multivariate analyses of clinical factors associated with the severity of NAFLD (SS *vs* NASH) in Matteoni's and Brunt's classifications

Variables	OR	95% CI	*P* value
IMAC (SS *vs* NASH)	3.671	1.084–11.722	< 0.05
Age (males > 45 years, females > 50 years)	2.895	1.045–8.021	< 0.05
Sex (male *vs* female)	1.683	0.604–4.687	0.3192
ALT (≥ 50)	6.224	2.009–19.277	< 0.01
HOMA-IR (≥ 2.2)	3.035	1.084–8.493	< 0.05
L/S ratio (< 0.9)	0.715	0.239–2.144	0.5498
VFA (≥ 100 mm^2)	0.671	0.201–2.234	0.5154

ALT, alanine aminotransferase;CI, confidence interval; HOMA-IR, homeostasis model assessment-insulin resistance; IMAC, intramuscular adipose tissue content; L/S ratio, liver-spleen attenuation ratio; NAFLD, non-alcoholic fatty liver disease; NASH, non-alcoholic steatohepatitis; OR, odds ratio; SS, simple steatosis; VFA, visceral fat area.

Table 4 Multivariate analyses of clinical factors associated with the severity of NASH (stage1–2 *vs* 3–4) in Matteoni's and Brunt's classifications

Variables	OR	95% CI	*P* value
IMAC (stage 1–2 *vs* 3–4)	2.444	1.118–5.341	< 0.05
Age (males > 45 years, females > 55 years)	2.355	1.042–5.321	< 0.05
Sex (male *vs* female)	1.577	0.719–3.457	0.2554
ALT (≥ 50)	1.864	0.766–4.536	0.1699
HOMA-IR (≥ 2.2)	0.847	0.314–2.287	0.7438
L/S ratio (< 0.9)	1.187	0.501–2.810	0.6964
VFA (≥ 100 mm^2)	1.160	0.575–4.457	0.3675

ALT, alanine aminotransferase; CI, confidence interval; HOMA-IR, homeostasis model assessment-insulin resistance; IMAC, intramuscular adipose tissue content; L/S ratio, liver-spleen attenuation ratio; NASH, non-alcoholic steatohepatitis; OR, odds ratio; VFA, visceral fat area.

Discussion

In this study, we objectively evaluated skeletal muscle steatosis by measuring IMAC and found that skeletal muscle steatosis increased significantly with increasing stage of NASH. Multivariate analysis also suggested that there was a relationship between the stage of NASH and IMAC. These results suggest the presence of a physiological link between the liver and skeletal muscle in patients with NASH.

IMAC is significantly different between female and male. We hypothesized that gender differences in the IMAC may be affected by type of muscle fibers. Muscle fibers are classified into type I, type IIa, and type IIb fibers. Previous studies have reported that the

Table 5 Characteristics of all patients with NASH at baseline, and of patients with/without improvements in IMAC before and after the intervention

Variables	All patients ($n = 21$)	Patients with improvements in IMAC ($n = 11$)			Patients with no improvement in IMAC ($n = 10$)		
	Baseline	Before	After	*P* value	Before	After	*P* value
Sex (male/female)	8/13	4/7	4/7		4/6	4/6	
Age (years)	52.4 ± 16.9	48.9 ± 13.5	50.2 ± 13.7	< 0.01	56.2 ± 19.9	59.3 ± 20.6	< 0.01
Weight (kg)	73.5 ± 16.8	77.5 ± 14.8	72.3 ± 15.6	< 0.05	69.0 ± 18.6	68.0 ± 17.7	0.4838
BMI (kg/m^2)	28.7 ± 4.1	30.1 ± 4.1	28.1 ± 4.7	< 0.05	27.3 ± 3.7	26.9 ± 3.4	0.4833
VFA (cm^2)	144.6 ± 47.7	136.3 ± 33.2	113.8 ± 54.2	0.0754	153.6 ± 60.5	144.8 ± 51.5	0.9594
SFA (cm^2)	254.3 ± 98.3	298.7 ± 100.0	261.7 ± 116.9	< 0.05	205.4 ± 72.8	205.7 ± 84.3	0.9594
ALT (IU/L)	104.1 ± 97.0	90.5 ± 63.5	31.4 ± 14.0	< 0.01	119.0 ± 126.4	70.8 ± 50.0	0.1392
γ-GTP (IU/L)	91.6 ± 78.0	81.1 ± 40.5	34.5 ± 18.5	< 0.01	103.1 ± 106.9	88.9 ± 112.2	0.2839
TC (mg/dL)	233.8 ± 43.9	249.7 ± 37.9	198.9 ± 43.5	< 0.01	216.2 ± 45.1	176.7 ± 28.2	< 0.05
TG (mg/dL)	187.2 ± 123.3	215.6 ± 159.0	102.9 ± 35.9	< 0.01	155.9 ± 60.1	155.9 ± 90.5	0.8785
FPG (mg/dL)	117.6 ± 37.1	129.0 ± 44.3	113.6 ± 43.1	< 0.05	105.0 ± 23.4	110.2 ± 17.8	0.0926
60-PG (mg/dL)	230.1 ± 65.5	234.9 ± 68.6	163.6 ± 61.0	0.0910	225.2 ± 66.0	209.0 ± 48.7	0.4618
180-PG (mg/dL)	155.7 ± 55.4	160.5 ± 47.3	106.0 ± 31.0	< 0.05	150.1 ± 67.1	156.8 ± 58.9	0.7150
Insulin (μg/mL)	16.8 ± 6.9	18.2 ± 7.0	11.1 ± 6.1	< 0.05	15.3 ± 6.3	18.3 ± 11.7	0.3329
Insulin resistance							
HOMA-IR	5.1 ± 3.0	6.0 ± 3.3	3.3 ± 2.3	< 0.05	4.1 ± 2.4	4.9 ± 2.9	0.2411
QUICKI	0.31 ± 0.03	0.30 ± 0.03	0.33 ± 0.04	< 0.05	0.32 ± 0.04	0.31 ± 0.03	0.0926
Platelet count (× 10^4/μL)	22.7 ± 5.7	24.8 ± 5.3	24.8 ± 7.3	0.7211	20.4 ± 5.5	18.9 ± 5.7	< 0.05
Serum albumin (g/dL)	4.7 ± 0.4	4.6 ± 0.5	4.4 ± 0.2	0.5606	4.8 ± 0.4	4.7 ± 0.4	0.6771
L/S ratio	0.78 ± 0.32	0.61 ± 0.27	1.07 ± 0.12	< 0.01	0.97 ± 0.25	0.93 ± 0.25	0.1846
IMAC	−0.21 ± 0.15	−0.20 ± 0.16	−0.25 ± 0.18	< 0.01	−0.21 ± 0.14	−0.17 ± 0.15	< 0.01
Histopathological assessment							
Fibrosis (0/1/2/3/4)	(0/5/6/10/0)	(0/2/4/5/0)	(1/3/6/1/0)	0.0702	(0/3/2/5/0)	(0/2/4/3/1)	0.5637
Steatosis (0/1/2/3)	(0/9/5/7)	(0/1/5/5)	(1/7/2/1)	< 0.05	(0/8/0/2)	(0/6/2/2)	0.1573
Lobular inflammation (0/1/2/3)	(0/10/8/3)	(0/2/7/2)	(2/8/0/1)	< 0.01	(0/8/1/1)	(0/6/3/1)	0.3173
Ballooning (0/1/2)	(0/9/12)	(0/1/10)	(1/8/2)	< 0.01	(0/8/2)	(0/3/7)	< 0.05
NAS	5.1 ± 1.7	6.7 ± 0.5	3.0 ± 1.0	< 0.01	3.3 ± 0.7	4.3 ± 0.9	< 0.05

Data are means ± standard deviation. *P* values are for comparisons between types 1–2 and 3–4. Statistical analysis was performed by Wilcoxon signed-rank test, and differences were considered significant at $P < 0.05$. 60-PG, 60 min post-challenge plasma glucose; 180-PG, 180 min post-challenge plasma glucose; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; FPG, fasting plasma glucose; HOMA-IR, homeostasis model assessment-insulin resistance; IMAC, intramuscular adipose tissue content; L/S ratio, liver-spleen attenuation ratio; NAFLD, *non-alcoholic fatty liver disease*; NAS, NAFLD *activity score*; NASH, *non-alcoholic steatohepatitis*; QUICKI, *quantitative insulin sensitivity* check index; SFA, subcutaneous fat area; TC, total cholesterol; TG, triglyceride; VFA, visceral fat area; γ-GTP, γ-glutamyl transpeptidase.

a type IIb fibers in obese women had increased than in a type I fiber, and exercise therapy transformed muscle fibers from type IIb to type IIa.[30–32] However, the distribution of fiber types is unclear in a gender; in addition, our study did not perform histopathological evaluation. Further studies are needed to the distribution of fiber types related to the IMAC.

A previous study demonstrated that the intramuscular lipid content in the femoral biceps determined by ^{1}H-magnetic resonance spectroscopy (^{1}H-MRS) was associated with aging and obesity.[33]

Previous other studies have demonstrated a relationship between liver and skeletal muscle steatosis determined by ^{1}H-MRS in patients with NAFLD or type 2 diabetes and discusses the significance of diet and exercise therapy.[34–36] Our results using IMAC determined by CT are agreement with these earlier reports phenomenologically, although there was no comparative study between ^{1}H-MRS and CT for evaluation of skeletal muscle steatosis.

Several other reports have demonstrated a relationship between NAFLD and the risk of cardiac diseases, as well as physiological links between visceral fat accumulation, the liver, and muscle steatosis in metabolic syndrome, suggesting that these factors are associated with the risk of cardiac diseases.[15,37] However, a relationship between the severity of fibrosis in NASH and skeletal muscle has not yet been demonstrated.

Previous studies have reported that the composition of muscle tissue and the physiological activity of cells are influenced by exercise and weight control in patients with type 2 diabetes mellitus and NAFLD.[14,34,35,38] It was also reported that muscle atrophy is caused by an age-associated decrease in exercise, coupled with a subsequent increase in interstitial adipose tissue and the deposition of lipids, primarily neutral fat, in skeletal muscle cells.[10,39] Studies using mice have reported that skeletal muscle glucose absorption is associated with glucose tolerance and insulin resistance.[40,41] Our study showed that IMAC has improved in patients with a significant amelioration in glucose metabolism in the post-challenge 180-min plasma glucose with the amelioration of insulin resistance and histopathological changes. In patients with liver cirrhosis, abnormal glucose tolerance and insulin resistance were reported to be related to abnormal glucose transporter type 4 (GLUT4) expression in skeletal muscle.[42] GLUT4 is an insulin-regulated glucose transporter expressed in adipose tissue and skeletal muscle, and is primarily responsible for insulin-stimulated glucose uptake into cells.

Table 6 Relationships between Δ IMAC and Δ parameters in the intervention patients

Variables	All patients ($n = 21$)	
	ρ	P
Δ Weight (kg)	0.507	< 0.05
Δ BMI (kg/m^2)	0.512	< 0.05
Δ VFA (cm^2)	0.307	0.1739
Δ SFA (cm^2)	0.653	< 0.01
Δ ALT (IU/L)	0.227	0.3194
Δ γ-GTP (IU/L)	0.420	0.0625
Δ TC (mg/dL)	−0.013	0.9325
Δ TG (mg/dL)	0.458	< 0.05
Δ FPG (mg/dL)	0.678	< 0.01
Δ Insulin (μg/mL)	0.466	< 0.05
Insulin resistance		
Δ HOMA-IR	0.493	< 0.05
Δ QUICKI	−0.571	< 0.01
Δ Platelet count (× 10^4/μL)	−0.162	0.4514
Δ Serum albumin (g/dL)	−0.070	0.7077
Δ L/S ratio	−0.714	< 0.001
Histopathological assessment		
Δ Fibrosis (0/1/2/3/4)	0.419	0.0870
Δ Steatosis (0/1/2/3)	0.800	< 0.001
Δ Lobular inflammation (0/1/2/3)	0.686	< 0.01
Δ Ballooning (0/1/2)	0.769	< 0.001
Δ NAS	0.800	< 0.001

Data are means ± standard deviation. Δ = variable after intervention − variable before intervention. Spearman's rank correlation was used to correlate continuous or discrete variables $P < 0.05$.
ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; FPG, fasting plasma glucose; HOMA-IR, homeostasis model assessment-insulin resistance; IMAC, intramuscular adipose tissue content; L/S ratio, liver-spleen attenuation ratio; NAFLD, nonalcoholic fatty liver disease; NAS, NAFLD activity score; NASH, nonalcoholic steatohepatitis; QUICKI, quantitative insulin sensitivity check index; SFA, subcutaneous fat area; TC, total cholesterol; TG, triglyceride; VFA, visceral fat area; γ-GTP, γ-glutamyl transpeptidase.

Recently, it was proposed that physiologically active materials derived from skeletal muscle, such as interleukin-6 (IL-6), should be referred to as myokines. IL-6 released from skeletal muscle in response to exercise was reported to enhance glucose absorption via GLUT4 by increasing phosphatidylinositol 3-kinese expression in the muscle. Additionally, IL-6 was also reported to enhance 5′ adenosine monophosphate-activated protein kinase-regulated lipid oxidation by increasing the expression of signal transducer and activator of transcription 3 (STAT3), and increase hepatic glucose absorption.[43,44] Several studies demonstrated that exercise therapy improved not only liver function and insulin resistance but also IL-6 in NAFLD/NASH patients.[32,45] However, IL-6 concentrations are chronically increased in obese and insulin-resistant patients and may induce insulin resistance.[46] Recently, IL-6 was reported to increase the expression of insulin receptor substrate 2 and enhance insulin sensitivity by activating STAT3.[47] According to that study, if IL-6 is continuously increased, STAT3 is not activated because of negative feedback exerted by suppressor of cytokine signaling 3, for example, a signaling pathway that can even induce insulin resistance.[47]

Our present results are generally in agreement with these earlier reports. Dietary and exercise therapy increases fatty acid oxidation and glucose absorption in skeletal muscle, and alleviates skeletal muscle steatosis and insulin resistance. We previously reported that visceral fat accumulation and skeletal muscle steatosis are independent factors for the severity of fatty liver.[10] We believe that controlling visceral fat accumulation and muscle steatosis by diet and exercise therapy enhances hepatic insulin sensitivity, attenuates fatty liver, and reverses liver fibrosis.

However, our study has several limitations that should be discussed. First, the severity of NASH and skeletal muscle steatosis must be evaluated serially. We must also determine the differences in skeletal muscle steatosis between patients with or without the severity of NASH must be clarified. The relationship between myokines that affect skeletal muscle steatosis, such as IL-6, and the severity of liver fibrosis must also be evaluated. In conclusion, our study suggested that substitution of adipose tissue in skeletal muscle was associated with the severity of NAFLD/NASH. From these results, we think that interventions aimed at improved hepatic status, as well as systemic metabolic disorders, should be considered when developing new therapeutic strategies for NASH/NAFLD.

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Original Article

Evaluation narcotic analgesic use and survival time in terminal stage liver diseases compared with lung cancer: a retrospective chart review

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Hepatocellular carcinoma (HCC) and liver cirrhosis are fatal diseases. This study aimed to investigate survival time and palliative care in terminal HCC and/or liver cirrhosis compared with lung cancer. Between January 2004 and December 2010, we enrolled 116 patients with terminal cirrhosis and/or HCC or lung cancer admitted to a municipal hospital in Japan; 48 had liver cirrhosis, 35 HCC and 33 lung cancer. By retrospective chart review, we evaluated: (i) rate of usage of narcotic analgesics and (ii) survival time from onset of coma (Glasgow Coma Scale less than 8). Time between coma and death was significantly shorter in the liver disease patients (cirrhosis and/or HCC: 7.0 h) than in lung cancer (44.0 h, $p = 0.045$). Total bilirubin was higher in HCC compared with cirrhosis ($p<0.01$). Rate of usage of narcotic analgesics was higher in lung cancer (20/33: 60.6%) than in liver disease (17/83: 20.5%, $p<0.01$); analgesics were used more frequently in HCC than in liver cirrhosis ($p<0.01$). These results suggest that liver cirrhosis and HCC patients do not always require palliative care and that survival time from onset of coma due to liver disease was not prolonged compared with lung cancer.

Key Words: **hepatocellular carcinoma, liver cirrhosis, palliative care, coma, narcotic analgesics**

Hepatocellular carcinoma (HCC) is the sixth most common malignant disease and has the third highest mortality worldwide.[1] In Japan, more than 50% of cases of HCC are due to hepatitis C virus, approximately 45,000 patients are diagnosed with HCC each year and approximately 34,000 patients per year die with HCC.[2] Supportive care of HCC and/or liver cirrhosis, including nutritional support, has been improved recently, but it has not been clearly demonstrated whether the quality of life and prognosis of these patients have improved. Terminal care of HCC and/or liver cirrhosis is important; in Japan, terminal care is mainly provided in municipal hospitals rather than the hospital to which the patient was initially referred.

The questions most commonly asked by the patient and family during terminal care are "how long do I have?" and "how long does he/she have?", indicating that estimated survival time is an important issue in terminal care. The survival time between onset of coma and death might be shorter in HCC than in other malignant diseases and the usage rate of narcotic analgesics might be less, but these factors have not been investigated in previous studies.

This retrospective study in a municipal hospital evaluated: (i) the rate of usage of narcotic analgesics and (ii) the survival time from onset of coma due to HCC and/or liver cirrhosis. These factors were compared with lung cancer, which has a high mortality of approximately 70,000 per year in Japan.[3]

Materials and Methods

We enrolled patients with terminal cirrhosis, terminal HCC or terminal lung cancer with performance status (PS)[4] greater than 3 who were admitted to Eguchi Hospital between January, 2004 and December, 2010 because the family could no longer care for them. PS was determined as follows: 0 – fully active, able to perform all pre-disease activities without restriction; 1 – restricted in physically strenuous activities but ambulatory and able to perform light or sedentary tasks (e.g. light housework, office work); 2 – ambulatory and capable of all self-care but unable to work, active for more than 50% of waking hours; 3 – capable of only limited self-care, confined to bed or chair for more than 50% of waking hours; 4 – completely disabled and unable to perform self-care, confined to bed or chair; or 5 – dead. All patients gave informed consent that their treatment would be limited to terminal care and that resuscitation would not be attempted in an emergency. Narcotic analgesics were applied as required. We retrospectively evaluated the patients' main symptoms on admission, their consciousness level and the duration between onset of coma (Glasgow Coma Scale less than 8) and death.[5]

The data in Tables 1and 2 were evaluated by the chi-square test for independence, the Mann-Whitney U test or the Kruskal-Wallis test. The data in Fig. 1 and 2 were evaluated by the chi-square test. Statistical analysis was performed using IBM SPSS Statistics ver. 19. Differences were considered significant if the probability of the difference occurring by chance was less than 5 in 100 ($p<0.05$).

Results

The background characteristics of the patients are shown in Table 1. There were 48 cases of terminal liver cirrhosis, 35 cases of terminal HCC and 33 cases of terminal lung cancer. Patients in the lung cancer group were older (82.0 ± 11.0 years) than those in the two liver disease groups (69.5 ± 11.9 years and 72.0 ± 10.2 years, respectively, both $p<0.001$). Men predominated in both the lung cancer and the liver disease groups, but there was no significant difference in sex ratio among the groups. Duration of hospitalization did not differ among the three groups. Cause of

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Table 1. Background characteristics of the liver disease and lung cancer patients

	Cirrhosis (n = 48)	HCC (n = 35)	Lung cancer (n = 33)	p value
Age (years)	69.5 ± 11.9	72.0 ± 10.2	82.0 ± 11.0	<0.001
Sex (Men/Women)	28/20	26/9	21/12	ns
Period of hospitalization (days)	42.5 ± 89.6	28.0 ± 99.3	33.0 ± 62.9	ns
Cause of cirrhosis (HBV/HCV/alcohol/others)	6/28/9/5	4/27/1/3	—	ns
Child-Pugh (A/B/C)	1/12/31	4/7/23	—	ns

HBV, hepatitis B virus; HBC, hepatitis C virus; ns, not significant.

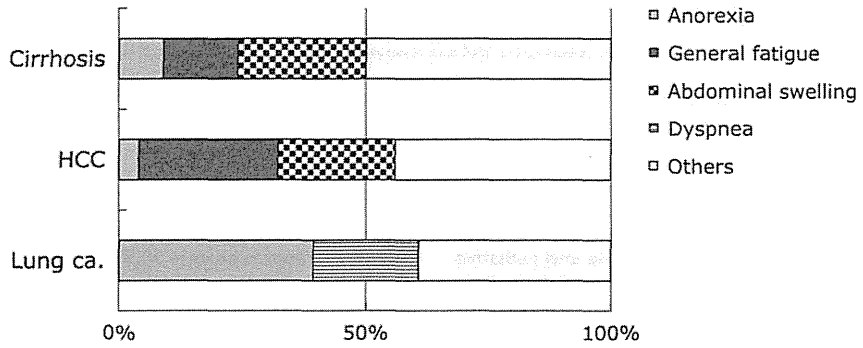

Fig. 1. Major complaint motivating hospitalization in liver cirrhosis, hepatocellular carcinoma (HCC) and lung cancer (Lung ca.).

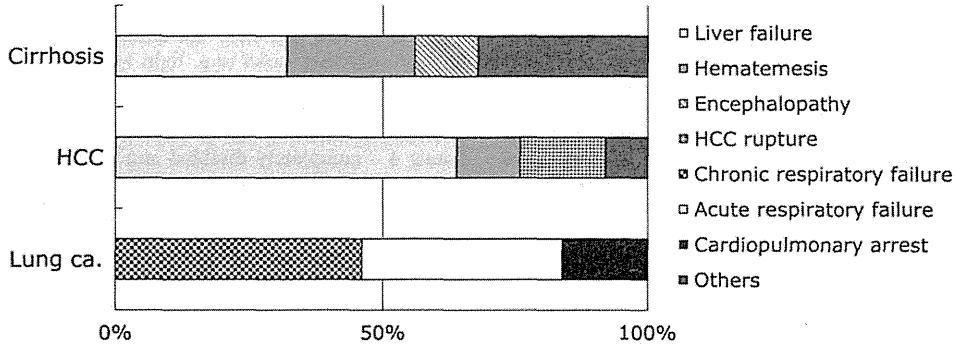

Fig. 2. Main cause of coma in liver cirrhosis, hepatocellular carcinoma (HCC) and lung cancer (Lung ca.).

liver cirrhosis and Child–Pugh class did not differ between the liver cirrhosis and HCC groups.

Fig. 1 shows the main complaints that led directly to hospitalization. In liver cirrhosis and HCC patients, these were general fatigue (15% and 28%, respectively) and abdominal swelling, mainly due to ascites (26% and 24%); the main complaints in the lung cancer patients were anorexia (38%) and dyspnea (21%).

Fig. 2 shows the direct cause of coma. In liver cirrhosis and HCC patients, the most common cause was liver failure (cirrhosis: 32%, HCC: 64%). Hematemesis and encephalopathy induced coma in the cirrhosis patients (24% and 12%, respectively) and hematemesis and rupture of HCC were risk factors in the HCC patients. Respiratory failure (chronic: 46%, acute: 38%) and cardiopulmonary arrest were the main causes of coma in the lung cancer patients.

Survival time from onset of coma and use of narcotic analgesics are shown in Table 2. Time between coma and death was significantly shorter in the liver disease patients (cirrhosis and/or HCC: 7.0 h) compared with the lung cancer patients (44.0 h, $p = 0.045$), with no significant difference between cirrhosis and HCC. Total bilirubin was higher in HCC compared with cirrhosis, but there was no difference in the rate of ascites. Rate of usage of narcotic analgesics was significantly higher in the lung cancer patients (20/33: 60.6%) than in the liver disease patients (17/83: 20.5%, $p<0.01$). Analgesics were used more frequently in HCC than in liver cirrhosis ($p<0.01$).

Discussion

The results of the present study indicate that: (i) the rate of usage of narcotic analgesics in the terminal stage was significantly lower in patients with liver cirrhosis and/or HCC (17/83: 20.5%) compared with lung cancer patients (20/33: 60.6%, $p<0.01$); and (ii) the time between onset of coma and death was significantly

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Table 2. Time from onset of coma to death and rate of usage of narcotic analgesics in each group

	Cirrhosis ($n = 48$)	HCC ($n = 35$)	Lung cancer ($n = 33$)	$p^{\dagger}$	$p^{\dagger\dagger}$	$p^{\S}$	$p^{\P}$
Duration from coma to death (hours)	10.5 (0.5–192)	5.0 (0.5–168)	44.0 (1.0–528)	0.045	0.06	0.09	0.31
Using rate of narcotic drugs (yes/no)	4/44	13/22	20/13	<0.01	0.053	<0.01	<0.01
Serum total bilirubin just before death (mg/dL)	4.15 (0.6–31.7)	11.1 (0.4–32.1)	—	—	—	—	<0.01
Ascites (yes/no)	41/6	29/6	—	—	—	—	ns

Data are the median (range). †Lung cancer compared with liver cirrhosis and HCC, ††lung cancer compared with HCC, §lung cancer compared with liver cirrhosis, ¶liver cirrhosis compared with HCC. HCC, hepatocellular carcinoma; ns, not significant.

shorter in liver disease patients (cirrhosis and/or HCC: 7.0 h) compared with lung cancer patients (44.0 h).

Metastasis from HCC might not be common compared with cancers originating in other organs.[6] Recently, survival with HCC has been prolonged by advances in therapeutic approaches that have delayed metastasis to the late phase of the disease.[7,8] A recent report from Japan observed metastasis from HCC to lung, bone, lymph nodes, adrenal gland, brain and peritoneum; the most common site was the lung.[9] Lung metastases did not commonly lead to serious clinical symptoms; by contrast, metastasis to bone was not common (2–15%) but caused severe pain, walking difficulties and paralysis of the lower half of the body.[10] The low incidence of serious complications from metastasis of HCC might be a major reason for the low frequency of use of narcotic analgesics compared with lung cancer, in which severe clinical symptoms in the early stage lead to early introduction of palliative care.[11]

Highly impaired liver function might explain why the time between onset of coma and death was significantly shorter in the liver disease patients compared with the lung cancer patients. Surgical resection of up to two-thirds of the liver is possible even in cirrhosis patients if their total bilirubin is within the normal range,[12] so coma does not occur in liver cirrhosis and/or HCC until the terminal stage of the disease. In contrast, lung cancer is complicated by dysfunctions of ventilation, pulmonary function and gas exchange,[13] which lead to coma in the early stage of the disease.

Few studies have focused on the terminal stage of liver cirrhosis and/or HCC. The present study suggests that these patients do not always require palliative care and that the survival time from onset of coma is not prolonged. The limitations of this study, such as the small number of patients, the retrospective chart review and the comparison with lung cancer only, warrant further investigations.

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Original Paper

Survival Advantage of Radiofrequency Ablation for Hepatocellular Carcinoma: Comparison with Ethanol Injection

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ABSTRACT

Background/Aims: The aims of this study were to compare long-term prognosis of patients with hepatocellular carcinoma (HCC) treated with radiofrequency ablation (RFA) and percutaneous ethanol injection (PEI). **Methodology:** Two hundred and thirteen patients with HCC were initially treated with PEI or RFA at Saga University Hospital between 1990 and 2004. The present study included 190 patients: 98 treated with PEI from 1990 to 1999, and 92 with RFA from 2000 to 2004. The association of treatment method with survival prognosis was evaluated by multivariate analysis. **Results:** There were no significant differences in gender, etiology, and tumor stage between the two groups. Five-year survival rate in the PEI group was 40% and 51% in the RFA group. According to tumor stage, there were no differences in 5-year survival rate between the two groups for tumor stage I and III. For stage II patients, RFA had better survival than PEI (48% *vs.* 28%, $p = 0.03$). Multivariate analysis indicated that RFA was more effective for long-term survival than PEI in patients with tumor stage II ($p = 0.04$). **Conclusions:** Compared to PEI, RFA improved survival in patients with stage II HCC, indicating a therapeutic advantage of RFA.

Key Words: Hepatocellular carcinoma; Radiofrequency ablation; Percutaneous ethanol injection; Cause of death; Survival prognosis.

Abbreviations: Hepatocellular carcinoma (HCC); Percutaneous Ethanol Injection (PEI); Radiofrequency Ablation (RFA); Randomized Controlled Trials (RCTs); Ultrasonography (US); Computed Tomography (CT); Magnetic Resonance Imaging (MRI); Hepatitis B Surface Antigen (HBsAg); Hepatitis C Virus (HCV); Aspartate Aminotransferase (AST); Alanine Aminotransferase (ALT); γ-glutamyl Transpeptidase (GGT); α–Fetoprotein (AFP).

INTRODUCTION

In Japan, hepatocellular carcinoma (HCC) has been treated according to 2009 clinical practice guidelines (1). According to these guidelines, resection or local therapy is adopted for patients with Child-Pugh class A or B, ≤3 tumors, and ≤30 mm tumor diameter. Currently, percutaneous ethanol injection (PEI) or radiofrequency ablation (RFA) is generally selected for local therapy (2-5). Several randomized controlled trials (RCTs) or quasi-RCTs that have investigated the differences in safety and efficacy between PEI and RFA have been reported (6-11). Although RFA sometimes has severe complications such as obstructive jaundice caused by bile duct injury or hepatic infarction due to vascular ablation, most studies have indicated that there are no significant differences in complications between the two therapeutic approaches (8,10,11). Ablation-site recurrence in patients treated with RFA is less frequent than in patients treated with PEI (7-11), which might lead to superior overall survival for RFA compared to PEI (8-10). However, some studies have indicated that there are no significant differences in survival prognosis between the two treatment methods (7,11).

One problem with these previous RCTs is that the observation period was short-term (22.4–37.2 mo), and there have been few studies that have evaluated the long-term prognosis of RFA and PEI. The aim of this historical comparison was to compare PEI with RFA from the point of view of long-term survival rate.

METHODOLOGY

Patients

Two hundred and thirteen patients with HCC were initially treated with PEI or RFA at Saga University Hospital between 1990 and 2004. The present study enrolled 190 patients who were followed up for >3 years: 98 patients treated with PEI from 1990 to 1999, and 92 patients treated with RFA from 2000 to 2004. Median observation period in the PEI group was 54.4 (range: 2.5–157.4) mo, and 54.9 (range: 5.2–119.2) mo in the RFA group. Local therapy for HCC was indicated for patients with ≤3 nodules, each with a maximum diameter of 30 mm.

Diagnosis and staging of HCC

HCC was diagnosed by using at least two imaging tests including ultrasonography (US), dynamic computed tomography (CT), dynamic magnetic resonance imaging (MRI) and/or angiographic CT. Tumor stages were classified according to the article published by the Liver Cancer Study Group of Japan (12). This method includes the following three conditions: i) tumor diameter <20 mm; ii) single tumor; and iii) no vascular invasion. When all the three conditions were met, the tumor was classified as stage I. When two conditions were met, the tumor was classified as stage II. When one condition was met, the tumor was classified as stage III. When none of the conditions were met, the tumor was classified as stage IV.

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TABLE 1. Patient characteristics of PEI and RFA groups.

Factors	PEI (n = 98)	RFA (n = 92)	*p* value
Gender			
Female / Male	41 / 57	34 / 58	0.492
HCC occurrence age	64.4 ± 7.6 (43-82)	67.0 ± 7.9 (47-80)	0.022
Etiology			
HCV / HBV / B+C / NBNC	83/ 8 / 2 / 5	80 / 5 / 3 / 4	0.833
Child-Pugh class			
A / B / C	55 / 39 / 4	66 / 26 / 0	0.025
Tumor stage			
I / II / III	41 / 39 / 18	37 / 46 / 9	0.166
Tumor diameter (mm)	21 ± 7 (8-30)	18 ± 6 (8-30)	0.003
Platelet count ($\times 10^4/\mu L$)	8.4 ± 3.9 (3-22.3)	9.1 ± 4.6 (2.5-28.3)	0.293
Prothrombin activity (%)	70.3 ± 21.1 (26-149)	74.6 ± 11.9 (36.5-101.1)	0.107
Albumin (g/dL)	3.5 ± 0.5 (2.4-4.5)	3.7 ± 0.5 (2.5-4.6)	0.060
Total bilirubin (mg/dL)	1.3 ± 0.7 (0.2-4.2)	1.1 ± 0.6 (0.4-3.1)	0.020
AST (IU/L)	79 ± 42 (18-246)	69 ± 41 (23-253)	0.129
ALT (IU/L)	69 ± 47 (12-237)	64 ± 42 (14-241)	0.414
GGT (IU/L)	78 ± 65 (15-397)	92 ± 92 (15-564)	0.255
AFP (ng/mL)	296 ± 1432 (2-12454)	212 ± 765 (3-5740)	0.638

Data are expressed as means ± SD or as the number of patients. Figures in parentheses indicate range.

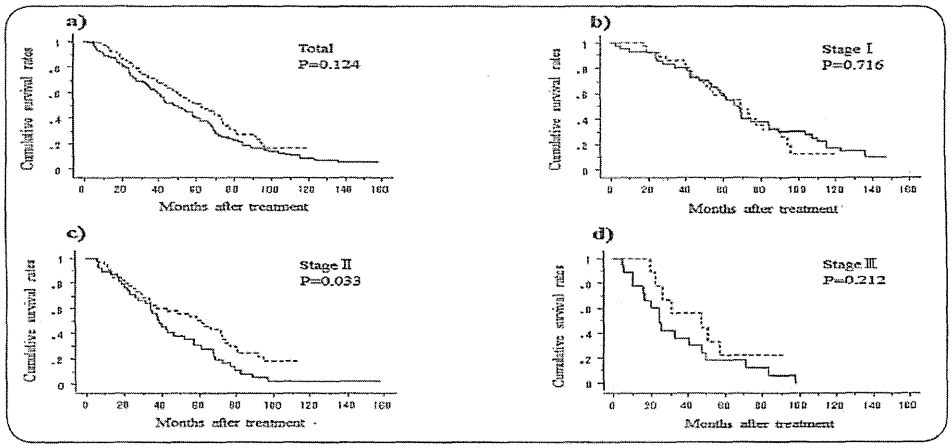

FIGURE 1. Cumulative survival curves in RFA group (---) and PEI group (—). (a) Total; (b) stage I; (c) stage II; (d) stage III.

Procedure of PEI and RFA

PEI was performed using real-time US under intramuscular administration of pentazocine and regional anesthesia of the puncture site. A 21-gauge needle was used for purpose of liver puncture and ethanol injection. According to the distribution of ethanol, tumor size, and the patient's condition, ethanol was injected at a dose of 1–4 mL per session once or twice weekly. The number of sessions was decided on the basis of the required dose of ethanol calculated by tumor volume on CT or MRI.

RFA was performed using the following three RF systems under general, sometimes local, anesthesia. From January to December 2000, 12 patients underwent RFA using an RF 2000 generator system (Radio Therapeutics, Mountain View, CA, USA) with 10 expandable hook-shaped electrode tines, and 17 patients underwent RFA using a model 500PA generator system (Rita Medical Systems, Mountain View, CA, USA) with four expandable hook-shaped electrode tines (Model 30). From January 2001 to December 2004, 63 patients underwent RFA with a cool-tip RF system (Radionics, Burlington, MA, USA) with a 17-gauge cooled-tip electrode with a 2- or 3-cm metallic tip. Ablation procedures were performed according to a globally standardized regimen (13).

TABLE 2. Patient characteristics of PEI and RFA groups (stage I HCC).

Factors	PEI (n = 41)	RFA (n = 37)	*p* value
Gender			
Female / Male	14 / 27	15 / 22	0.560
HCC occurrence age	63.2 ± 8.5 (43-82)	66.4 ± 8.2 (48-79)	0.099
Etiology			
HCV / HBV / B+C / NBNC	34 / 4 / 1 / 2	31 / 2 / 1 / 3	0.849
Child-Pugh class			
A / B / C	27 / 12 / 2	32 / 5 / 0	0.078
Tumor diameter (mm)	16 ± 4 (8-20)	14 ± 4 (8-20)	0.076
Platelet count ($\times 10^4/\mu L$)	8.7 ± 3.9 (3-22.3)	9.3 ± 4.9 (2.2-3.6)	0.602
Prothrombin activity (%)	75.3 ± 20.4(37-149)	77.7 ± 12.4 (36.5-101)	0.548
Albumin (g/dL)	3.7 ± 0.4 (2.5-4.5)	3.8 ± 0.5 (2.9-4.6)	0.109
Total bilirubin (mg/dL)	1.2 ± 0.5 (0.5-2.6)	1.0 ± 0.4 (0.4-1.9)	0.033
AST (IU/L)	77 ± 47 (18-246)	70 ± 43 (23-219)	0.482
ALT (IU/L)	70 ± 43 (12-201)	63 ± 40 (14-147)	0.527
GGT (IU/L)	65 ± 41 (16-189)	86 ± 76 (15-408)	0.148
AFP (ng/mL)	66 ± 140 (2-730)	125 ± 261 (4-1400)	0.244

Data are expressed as means ± SD or as the number of patients. Figures in parentheses indicate range.

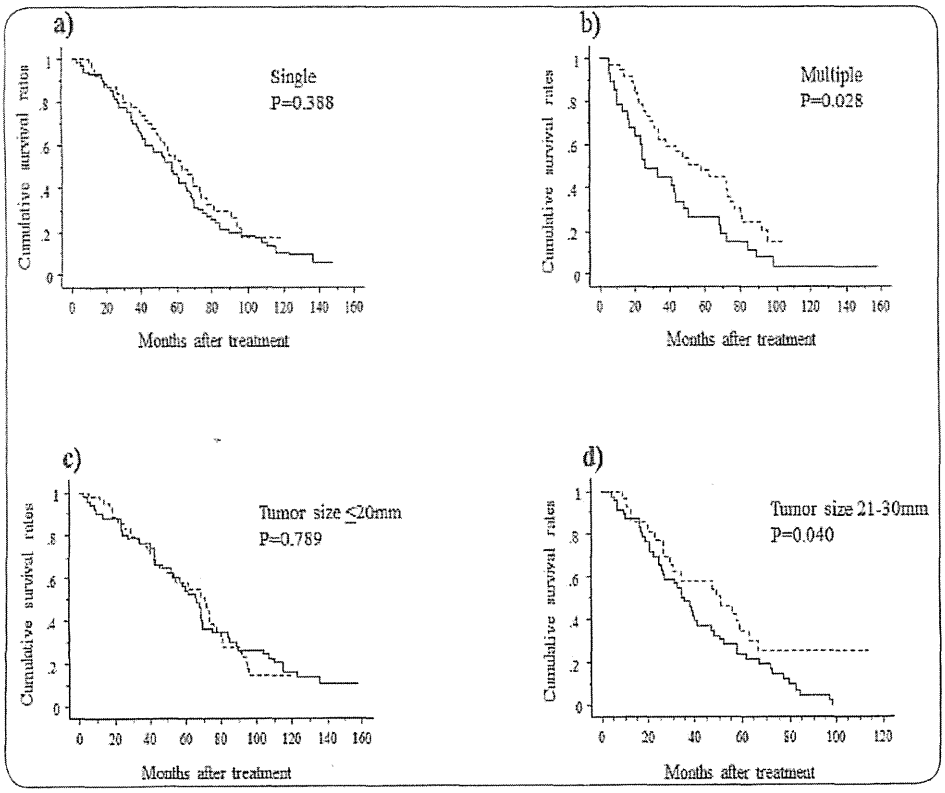

FIGURE 2. Cumulative survival curves in RFA group (---) and PEI group (—). (a) Single tumor; (b) two or three tumors; (c) tumor diameter ≤20 mm; (d) tumor diameter 21–30 mm.

TABLE 3. Patient characteristics of PEI and RFA groups (stage II HCC).

Factors	PEI (n = 39)	RFA (n = 46)	*p* value
Gender			
Female / Male	20 / 19	17 / 29	0.184
HCC occurrence age	64.7 ± 7.2 (53-82)	67.6 ± 7.8 (48-80)	0.081
Etiology			
HCV / HBV / B+C / NBNC	32 / 3 / 1 / 3	40 / 3 / 2 / 1	0.647
Child-Pugh class			
A / B / C	17 / 21 / 1	27 / 19 / 0	0.245
Tumor diameter (mm)	24 ± 6 (10-30)	21 ± 5 (11-30)	0.009
Platelet count ($\times 10^4/\mu L$)	7.8 ± 4.0 (3.3-18.9)	9.1 ± 4.7 (3.2-28.3)	0.185
Prothrombin activity (%)	66 ± 20.6 (26-103)	71.7 ± 11.3 (41.1-98)	0.127
Albumin (g/dL)	3.3 ± 0.5 (2.7-4.2)	3.5 ± 0.5 (2.5-4.3)	0.092
Total bilirubin (mg/dL)	1.4 ± 0.7 (0.2-3.1)	1.2 ± 0.6 (0.4-3.1)	0.350
AST (IU/L)	72 ± 40 (18-175)	70 ± 43 (26-253)	0.866
ALT (IU/L)	58 ± 38 (15-188)	65 ± 47 (17-241)	0.486
GGT (IU/L)	73 ± 62 (15-304)	101 ± 107 (18-564)	0.165
AFP (ng/mL)	77 ± 164 (2-792)	291 ± 1045 (3-5740)	0.241

Data are expressed as means ± SD or as the number of patients. Figures in parentheses indicate range.

TABLE 4. Patient characteristics of PEI and RFA groups (stage III HCC).

Factors	PEI (n = 18)	RFA (n = 9)	*p* value
Gender			
Female / Male	7 / 11	2 / 7	0.387
HCC occurrence age	66.4 ± 5.9 (58-82)	66.3 ± 7.3 (55-77)	0.976
Etiology			
HCV / HBV / B+C / NBNC	17 / 1 / 0 / 0	9 / 0 /0 / 0	0.471
Child-Pugh class			
A / B / C	11 / 6 / 1	7 / 2 / 0	0.607
Tumor diameter (mm)	28 ± 3 (22-30)	24 ± 4 (21-30)	0.013
Platelet count ($\times 10^4/\mu L$)	9.1 ± 3.7 (3.4-17.2)	8.2 ± 2.6 (5.4-13.3)	0.541
Prothrombin activity (%)	68.7 ± 22.6 (38-111)	76.9 ± 9.7 (56.4-87)	0.335
Albumin (g/dL)	3.6 ± 0.5 (2.4-4.3)	3.6 ± 0.6 (2.7-4.4)	0.877
Total bilirubin (mg/dL)	1.5 ± 0.9 (0.5-4.2)	1.0 ± 0.5 (0.5-1.8)	0.188
AST (IU/L)	102 ± 56 (20-198)	63 ± 21 (39-101)	0.073
ALT (IU/L)	93 ± 63 (15-237)	58 ± 26 (33-104)	0.146
GGT (IU/L)	120 ± 96 (29-397)	65 ± 62 (25-206)	0.162
AFP (ng/mL)	1278 ± 3202 (4-12454)	160 ± 263 (4-682)	0.340

Data are expressed as means ± SD or as the number of patients. Figures in parentheses indicate range.

Laboratory data

We collected laboratory data just before initial treatment including hepatitis B surface antigen (HBsAg), hepatitis C virus (HCV) antibody, platelet count, prothrombin activity, albumin, aspartate aminotransferase (AST), alanine aminotransferase (ALT), total bilirubin and γ-glutamyl transpeptidase (GGT) and α-fetoprotein (AFP). Etiology of liver disease was decided as follows: patients with positive HBsAg were HBV; patients with positive HCV antibody were HCV; patients with both HBsAg and HCV antibody were HBV+HCV (B+C); and patients with negative for HBsAg and HCV antibody were non-HBV non-HCV (NBNC).

Statistical analysis

Comparisons of clinical characteristics between the two treatment groups were made using the unpaired Student's *t*-test for continuous variables and the χ^2 test for categorical data. The survival periods were estimated using the Kaplan-Meier method, and the survival curves

were compared using the log-rank test. Factors related to survival prognosis were analyzed by using multivariate Cox proportional hazards model. Two-tailed p values <0.05 were considered significant. Statistical analyses were performed with SPSS Statistics ver. 19 (SPSS Japan, Tokyo, Japan).

RESULTS

Baseline characteristics

Clinical characteristics of the patients are shown in **Table 1**. Although there were no significant differences in gender, etiology of liver disease, platelet count, prothrombin activity, albumin, AST, ALT, GGT and AFP between the PEI and RFA groups, the RFA group was older, and Child-Pugh score and total bilirubin level of the RFA group were lower than those of the PEI group. Although tumor diameter in the PEI group was longer than that in the RFA group, there was no difference in stage classification of HCC.

Cumulative survival rates overall and according to tumor stage

Regarding overall cumulative survival rates, 5-year survival rates were 40% and 51% in the PEI and RFA group, respectively (**Figure 1a**), and there was no difference between the two groups. **Tables 2-4** show the clinical characteristics of the patients categorized by tumor stage. There were no significant differences in gender, age, etiology, Child-Pugh class, platelet count, prothrombin activity, albumin, AST, ALT, GGT and AFP between the RFA and PEI group for each tumor stage. Total bilirubin level in tumor stage I was higher in the PEI than RFA group. Regarding the cumulative survival rate at each stage, the RFA group had significantly better survival compared to the PEI group for tumor stage II (**Figure 1c**, $P = 0.033$). This better survival in the RFA group was not detected for tumor stage I and stage III (**Figure 1b** and **1d**). Five-year survival rates in the two groups were as follows: PEI, 60% and RFA, 59% for stage I; PEI, 28% and RFA, 48% for stage II; and PEI, 18% and RFA, 22% for stage III.

Comparison of causes of death

During the observation period, 156 of 190 patients died (PEI: 93, RFA: 63). Causes of death were identified in 112 patients. Fifty-nine patients died from growth of HCC (PEI: 38, RFA: 21); 29 from hepatic failure (PEI: 16, RFA: 13); seven from extrahepatic rupture of HCC (PEI: 5, RFA: 2); and six from rupture of esophageal or gastric varices (PEI: 3, RFA: 3). Eleven patients died from causes other than liver-related diseases: six from infectious disease; two from other carcinomas; two from perforation of the gastrointestinal tract; and one from rupture of aortic aneurysm. Distribution in causes of death did not differ between the PEI and PFA groups, and it was similar according to stage of tumor (data not shown).

Cumulative survival rates according to tumor size and number

Figure 2 is the Kaplan-Meier curve with regard to the number of tumors and tumor size. There was no significant difference in cumulative survival rate between the two groups in patients with a single tumor or tumor diameter <20 mm (**Figure 2a** and **2c**). The RFA group had better survival than the PEI group in patients with multiple tumors or tumor diameter 21-30 mm (**Figure 2b** and **2d**).

TABLE 5. Univariate analysis of factors for survival periods in stage II HCC patients.

Variables	Odds ratio	95%CI		p value
Gender: Female	1.19	0.742	1.893	0.476
HCC occurrence age: ≥66	1.24	0.777	1.984	0.365
Child-Pugh class: B, C	1.62	1.016	2.591	0.043
Etiology: non HBV	2.30	0.718	2.296	0.161
Treatment: PEI	1.66	1.037	2.658	0.035
Tumor diameter: ≥20 mm	1.00	0.973	1.034	0.851
Platelet: <7.7×10⁴/μL	1.46	0.893	2.371	0.132
AST: ≥70 IU/L	1.28	0.784	2.091	0.323
ALT: ≥80 IU/L	0.81	0.463	1.432	0.476
GGT: ≥100 IU/L	0.91	0.516	1.597	0.738
AFP: ≥100 ng/ml.	1.32	0.734	2.355	0.358

AST: aspartate aminotransferase; ALT: alanine aminotransferase; GGT: gamma-glutamyl transpeptidase; AFP: α-fetoprotein.

TABLE 6. Multivariate analysis of factors for survival periods in stage II HCC patients.

Variables	Odds ratio	95%CI		p value
Gender: Female	1.24	0.742	- 2.064	0.414
HCC occurrence age: ≥66	1.32	0.774	- 2.235	0.311
Child-Pugh class: B, C	1.55	0.907	- 2.642	0.109
Etiology: non HBV	3.88	0.887	- 16.988	0.072
Treatment: PEI	1.84	1.023	- 3.295	0.042
Tumor diameter: ≥20 mm	0.98	0.947	- 1.013	0.231

Analysis for factors associated with survival in tumor stage II patients

There was a significant difference in survival prognosis of tumor stage II patients between the two groups; therefore, we analyzed factors associated with survival in stage II patients. As a result of the univariate analysis for factors including gender, age, Child-Pugh class, tumor diameter, etiology of liver disease, initial treatment method (PEI or RFA), platelet count, AST, ALT, GGT and AFP, the initial treatment method and Child-Pugh class were significant factors associated with survival prognosis (**Table 5**). Multivariate analysis adjusted by gender, age, Child-Pugh class, tumor diameter, and etiology of liver disease showed that RFA for initial treatment was a significant factor for good survival prognosis in patients with stage II HCC ($p = 0.042$, **Table 6**).

DISCUSSION

Our comparative study demonstrated that long-term survival in patients treated with RFA was superior to that with PEI in patients with stage II HCC, although this advantage was not observed when the outcome was evaluated in the overall or other stage patients.

An important feature of RFA is that a larger ablated area is obtained from one treatment session compared to PEI (6,8). It is speculated that the difference in sur-

vival of stage II HCC between the two treatment methods might have originated from the extent of the ablated margin. Previous pathological studies have shown that one fifth to one third of small HCCs, ≤3.0 cm in diameter, had satellite lesions that were not detected during pretreatment evaluation (14,15). Although several studies have indicated that ablation-site recurrence in patients treated with RFA was less than that in those treated with PEI (7-11), there are some reports that RFA was not significantly better than PEI for tumors <20 mm in diameter (8,9,16). Regarding survival prognosis, although some studies have indicated no significant difference between these two treatment methods (7,11), many more studies have indicated that RFA treatment results in less local recurrence than does PEI, which results in a longer survival period (8-10). These studies suggest that RFA might be more effective for HCC >20 mm in diameter compared with PEI with regard to local regulation and subsequent improved survival. This agrees with our result that RFA has a better prognosis than PEI for HCC of 21–30 mm diameter.

HCC stage II contains multiple tumors ≤20 mm in size, as well as single tumors >20 mm in size. Our result indicated that RFA was more effective against multiple (2 or 3 tumors) HCC than PEI was. We cannot explain the reason for this result from our data, although it is speculated that it resulted from the completeness of local ablation of each tumor.

The superiority of RFA was not observed in tumor stage I and stage III. We speculate that stage I HCC could be cured by PEI, similar to RFA, because the tumor size was small (<20 mm), and stage III HCC could not be regulated by not only PEI but also RFA, because these tumors were multiple and >20 mm in diameter. From the point of view of cost benefit, PEI might be a better selection for local treatment of stage I HCC, because the cost of PEI is much lower than that of RFA. For stage III HCC, we should consider combination therapy with other treatment methods including transarterial chemoembolization.

It is noteworthy that the observation period (median: 54.6 mo) of the present study was longer than that of previous studies indicating the advantage of RFA over PEI, and causes of death were examined. It was revealed that there was no difference in cause of death between the two treatment methods.

The limitation of the present study was that it was not prospective and not an RCT. The data were selected from a retrospective chart review for historical comparison, and the local regulatory rate could not be investigated. Moreover, because the follow-up period was different, the survival of the patients might have been influenced by several other factors, including progress in supportive care and innovation of another treatment for HCC. The PEI group included some cases treated with RFA for second and subsequent treatment after initial treatment with PEI. However, many previous studies have indicated that the initial therapy strongly affects the survival prognosis of HCC. Moreover, because PEI has rarely been selected for initial therapy for HCC in our country, it is almost impossible to compare long-term survival between PEI and RFA at present.

In conclusion, this historical comparative study suggests that RFA is appropriate compared to PEI for stage II HCC patients evaluated for long-term survival.

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An automated rapid detection system using the quenching probe method for detecting interleukin 28B and inosine triphosphatase single nucleotide polymorphisms in chronic hepatitis C

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SUMMARY. Single nucleotide polymorphisms (SNPs) in the interleukin 28B gene (*IL28B*) are good pretreatment predictors of anti-hepatitis C virus (HCV) therapy with interferon. SNPs of the inosine triphosphatase (*ITPA*) gene are associated with reduced haemoglobin levels during treatment with ribavirin. The i-densy (Arkray, Inc.), which is based on the quenching probe (QP) method, automatically detects target genes in blood samples by fluorescence quenching within 100 min. Using a QP and primer set, a gene amplification response is generated that can quickly and easily detect a specific gene's arrangement by fluorometry. The present study was conducted to compare the utility of i-densy (QP method) with that of conventional direct sequencing (DS) for detecting SNPs in the *IL28B* and *ITPA* genes in chronic hepatitis C patients. Between June 2011 and January 2012, 73 consecutive patients underwent genotyping of IL28B, and 54 patients underwent genotyping of ITPA. All of the patients were seropositive for HCV-RNA. The IL28B and ITPA genotypes were tested for bi-allelic polymorphisms in rs8099917 (T/T, T/G and G/G; minor allele, G) and rs1127354 (C/C, C/A and A/A; minor allele, A), respectively. The results obtained with the QP method were identical to those obtained with the conventional DS method. The frequency of the *IL28B* genotypes TT, GT and GG were 74%, 24.7% and 1.4%, respectively, and those of the *ITPA* genotypes CC, AC and AA were 68.5%, 29.6% and 1.9%, respectively. These results indicate that the i-densy using the QP method can automatically, quickly and easily identify genotypes of *IL28B* and *ITPA*.

Keywords: fluorometry, IL28B, inosine triphosphatase, quenching probe.

BACKGROUND AND AIM

Recent reports have shown that single nucleotide polymorphisms (SNPs) in the interleukin 28B (*IL28B*) gene, which encodes IFN-lambda 3, are good pretreatment predictors for peginterferon α (Peg-IFNα) plus ribavirin (RBV) therapy and triple therapy [1–3]. Additionally, a recent genome-wide association study showed a strong association between reductions in haemoglobin levels during RBV treatment and the SNP rs6051702 in the inosine triphosphatase (*ITPA*) gene [4]. Therefore, easy and rapid detection of SNPs in *IL28B* or *ITPA* is important for routine clinical practice and to guide treatment decisions. As previously reported, the quenching probe (QP) method is extremely effective in detecting the *KRAS* mutations in lung adenocarcinoma [5]. Following the addition of a QP and a primer set, a gene amplification response is generated, and the gene's arrangement is quickly and easily detected by fluorometry. In the present study, we compared the utility of the QP method with that of the conventional DS method for detecting *IL28B* and *ITPA* SNPs in blood samples from chronic hepatitis C patients.

Abbreviations: DS, direct sequencing; HCV, hepatitis C virus; ITPA, inosine triphosphatase; QP, quenching probe; RBV, ribavirin.

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MATERIALS AND METHODS

Patients and blood samples

Between June 2011 and January 2012, 73 consecutive patients underwent *IL28B* genotyping, and 54 patients underwent ITPA genotyping. All patients were seropositive

for HCV-RNA. Venous blood samples were collected and stored at 4 °C.

Detection of IL28B and *ITPA* by direct sequencing

Human genomic DNA was extracted from peripheral blood using a blood DNA extraction kit (QIAGEN, Tokyo, Japan). Allelic typing was performed by real-time PCR with the Applied Biosystems 104 prIsM dye terminator cycle sequencing method on an ABI 105 PRISM 3100 Genetic Analyzer (Applied Biosystems, Foster City, CA, USA) using a fluorescein amidite-labelled SNP primer for the locus rs8099917 (Applied Biosystems). The *IL28B* polymorphism (rs8099917) and the *ITPA* polymorphism (rs1127354) were detected by PCR amplification with specific primers according to the manufacturer's instructions. The possible genotypes for each bi-allelic polymorphism in rs8099917 are T/T, T/G and G/G (minor allele, G). *ITPA* was genotyped at the polymorphic site for rs1127354 on chromosome 20 using the ABI TaqMan allelic discrimination kit and the same system as for *IL28B* genotyping. The possible genotypes in rs1127354 are C/C, C/A and A/A (minor allele, A).

Detection of IL28B and *ITPA* by the quenching probe method

The i-densy fully automated genotyping system (ARKRAY, Inc., Kyoto, Japan) was used. In this system, genomic DNA is purified from a blood sample using an FTA® matrix (Whatman, Middlesex, UK) followed by PCR amplification of the target SNPs. The SNP genotypes are determined by monitoring the fluorescence intensity of a QP. Each QP contains cytosine at its 5′ or 3′ end, which is labelled with a guanine quench fluorophore. When the QP hybridizes to its target DNA, its fluorescence is quenched by the guanine residue in the target that is complementary to the modified cytosine. The system performs these processes automatically and can genotype up to three SNP sites using four blood samples. For this study, the forward and reverse PCR primers and guanine QP (J-Bio21, Tokyo, Japan) were 5′-caacatggagagttaaagtaagtcttgtatttcacc-3′, 5′-cagctaccaaaactgtatacagcatggttc-3′ and 5′-ctgtgagcaatgtcaccc-3′, respectively, for *IL28B*, and 5′-aagtgttctcttttctcttggaacag-3′, 5′-ag aa(or g)acatacggtcaattttctgtg-3′ and 5′-gcatgtaaacttatctcc-3′, respectively, for *ITPA*. For *IL28B*, PCR consisted of

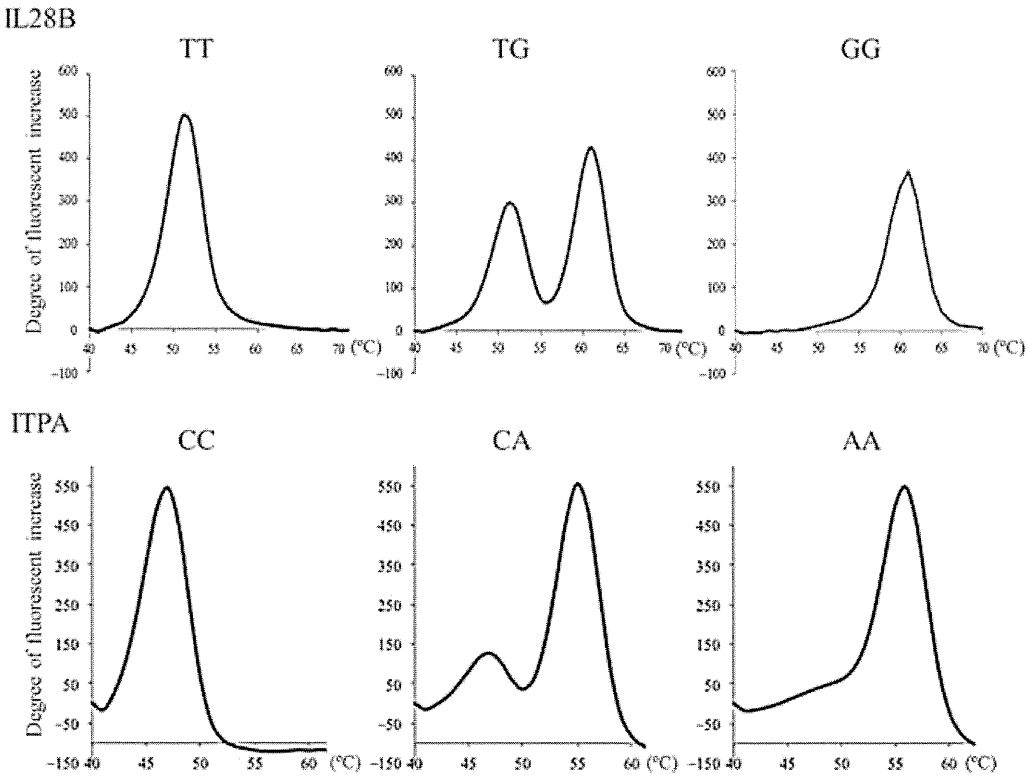

Fig. 1 Genotyping of the *IL28B* and *ITPA* SNPs by the quenching probe (QP) method. The temperature was gradually increased from 40 to 95 °C, and the increased fluorescence of the probe was measured. The excitation and emission wavelengths were 445–480 and 520–555 nm, respectively, for *IL28B*, and 587–700 and 520–555 nm, respectively, for *ITPA*. The TT and GG genotype of *IL28B* were detected as a single fluorescent peak at 53 and 62 °C. The heterozygous genotype TG was detected as a double peak at 53 and 62 °C. The CC and AA genotypes of *ITPA* were detected as single peak at 48 and 56 °C, respectively. The CA genotype was detected as a double fluorescent peak at 48 and 56 °C.

initial denaturation for 1 min at 95 °C, and 50 cycles of denaturation at 95 °C for 1 s and annealing at 60 °C for 30 s. For *ITPA*, PCR consisted of initial denaturation at 95 °C for 1 min, and 50 cycles of denaturation at 95 °C for 1 s and annealing at 64 °C for 30 s. After the PCR was complete, melting temperature (T_m) analyses were performed. The SNPs were identified based on differences in temperature and fluorescence.

RESULTS AND CONCLUSION

By differentiating the fluorescence intensities by the temperature, the T_m was obtained within 100 min (Fig. 1). All 73 patients were successfully genotyped for *IL28B*. The results obtained with the QP, and conventional DS methods were identical. Fifty-four patients were successfully genotyped for *ITPA*, and the results were identical with both methods. The frequencies of TT, GT and GG for *IL28B* were 74%, 24.7% and 1.4%, respectively. The frequencies of CC, AC and AA for *ITPA* were 68.5%, 29.6% and 1.9%, respectively. In conclusion, the QP method, with its high sensitivity, effectiveness and speed, allows for the determination of *IL28B* and *ITPA* genotypes in clinical settings.

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＜原　著＞

1型高ウイルス量C型慢性肝炎に対するPEG-IFNα-2a＋Ribavirin療法の治療成績—九州多施設共同研究—

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要旨：九州内肝臓専門医療機関の多施設研究により，1型，高ウイルスC型慢性肝炎（CHC）に対するペグインターフェロン（PEG-IFN）α-2a＋リバビリン（RBV）療法の有効性，安全性について検討を行なった．総数は320例，抗ウイルス効果判定症例は288例，安全性評価症例は310例．持続ウイルス陰性化（SVR）率はIntention-to-treatで53.1%，Per Protocol Set（44週間以上投与）で59.6%であった．13〜36週目のRNA陰性化例では，延長投与が有効であった．SVRに寄与する因子は，治療前では年齢，BMI，総コレステロール，ウイルス量であったが，治療開始後では12週目までのウイルス陰性化のみであった．有害事象による治療中止例は14例（4.5%）であり，難治性CHCに対するPEG-IFNα-2a＋RBV療法は安全性が高く，50%以上のSVR率が期待できる．

索引用語：　難治性C型慢性肝炎　ペグインターフェロンα-2a＋リバビリン療法
抗ウイルス効果　多施設共同研究　九州

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はじめに

C型慢性肝炎に対するインターフェロン（IFN）療法は，2004年12月にPEG-IFNα-2bとリバビリン（RBV）の併用療法，2007年3月にPEG-IFNα-2aとRBVの併用療法が認可され，本邦においても世界の標準治療を行うことが可能となった[1)2)]．

PEG-IFNα-2aとRBVの併用療法の国内第III相臨床試験において，genotype 1b，高ウイルス量（100 KIU/mL以上）のC型慢性肝炎に対して，48週間投与にて初回治療例は59.4%，前治療無効・再燃例に対する再治療においては54.0%のウイルス学的著効（sustained virological response：SVR）が得られている[3)]．

近年，ウイルス陰性化時期別に投与期間を調整するresponse-guided therapyの有効性が示され，genotype 1の初回症例に対するPEG-IFNα-2a＋RBV療法において，投与開始4週時HCV-RNA陰性例（rapid virological response：RVR）は24週間投与で48週間投与と同等のSVR率であり，12週時陽性かつ24週時陰性例（late virological response）では48週間より72週間投与の方がより高いSVRが得られた，と報告されている[4)5)]．

しかし，これらの成績のほとんどは治験や海外での研究によるもので，またHCV-RNAの測定は以前のア

ンプリコア法で行われたものである．2007年12月により感度の高いHCV-RNA測定法であるTaqMan PCR法が本邦で認可されたが[6]，PEG-IFNα-2a＋RBV療法における治療中のTaqMan PCR法によるウイルス動態や，市販後のいわゆる「real world」におけるウイルス陰性化時期別の治療効果，および延長投与の有効性に関しては，未だ多数例で解析した報告が少ない．

さらに，PEG-IFN＋RBV療法においては，高齢者，女性などの背景因子の違いによる有効性の低下が問題となっており[7]，これらについても多数例での検討で明らかにする必要がある．

以上の背景により，九州内の肝疾患専門医療機関で構成される九州肝疾患治療戦略研究会では，セログループ1，高ウイルス量のC型慢性肝炎に対するPEG-IFNα-2a＋RBV療法の有効性・安全性の検証，およびTaqMan PCR法によるウイルス動態と有効性の関係を明らかにするため多施設共同研究を行った．

C型肝炎の治療においてはDirect Acting Antiviral（DAA）製剤を使用したさらに強力な抗ウイルス治療の時代に入りつつあるが，これまで長い間標準治療法としての位置を占めてきたPEG-IFN＋RBV療法の我が国における治療成績をまとめておくことは，今後の治療法を考える上で重要であると考え，報告する．

対象と方法

1）対象患者

セログループ1（またはgenotype 1a/1b）かつ高ウイルス量（血中HCV-RNA≧5.0 Log IU/mL）のC型慢性肝炎患者で，以下の選択基準を満たし，かつ除外基準に抵触しない患者を対象とした．過去のIFN治療歴は問わないこととした．

2）選択基準

上記対象のうち，①年齢が20歳以上の患者，②投与開始前の臨床検査値で白血球数3,000/μL以上，好中球数1,500/μL以上，血小板数90,000/μL以上，ヘモグロビン量12 g/dL以上の基準を満たした患者，③試験の参加にあたり十分な説明を受けた後，十分な理解の上，文書による患者本人の自由意思による同意が得られた患者，を選択した．

3）除外基準

選択基準を満たす患者のうち，以下の除外基準のひとつでも当てはまる患者は除外した．①妊婦，妊娠している可能性のある婦人又は授乳中の婦人，②PEG-IFNα-2aまたは他のIFN製剤に対し過敏症の既往歴のある患者，③リバビリン又は他のヌクレオシドアナログに対し，過敏症の既往歴のある患者，④コントロール困難な心疾患（心筋梗塞，心不全，不整脈等）のある患者，⑤異常ヘモグロビン症（サラセミア，鎌状赤血球性貧血等）の患者，⑥慢性腎不全又はクレアチニンクリアランスが50 mL/分以下の腎機能障害のある患者，⑦重度のうつ病，自殺念慮又は自殺企図等の重度の精神病状態にある患者又はその既往歴のある患者，⑧重度の肝機能障害のある患者，⑨自己免疫性肝炎の患者，⑩ワクチン等生物学的製剤に対し過敏症の既往歴のある患者，⑪小柴胡湯を投与中の患者，⑫担当医師が本研究の対象者として不適当であると認めた患者．

4）PEG-IFNα-2a（Pegasys®）およびRBV（Copegus®）の投与方法

PEG-IFNα-2aは1回180μgを週1回皮下投与し，RBVは体重60 kg未満であれば600 mg/日，60 kg以上80 kg未満であれば800 mg/日，80 kg以上であれば1,000 mg/日内服を原則とした．なお，PEG-IFNα-2a，RBVとも主治医の判断により，減量しての投与開始も可能とした．

投与期間は原則48週間とし，主治医の判断により投与開始12週後のHCV-RNAが陽性の場合は，72週までの延長投与も可能とした．以下，44—55週間投与を標準投与，56週間以上の投与を延長投与と呼ぶこととする．

5）減量・中止基準

好中球数，血小板数，ヘモグロビン量の減少が発現した場合には，添付文書の基準を参考に用量調整することとした．その他副作用など，患者の状態により医師の判断で減量してもよいものとした．以下に該当する場合，その後の試験を中止し適切な処置を行うこととした．①添付文書の中止基準に該当する臨床検査値異常が発現した場合，②病勢の明らかな進行が認められた場合，③有害事象のため投与の継続が困難な場合，④被験者が投与の中止を希望した場合，⑤何らかの理由により来院しなくなった場合，⑥その他，試験責任（分担）医師の医学的判断により中止の必要性を認めた場合．

6）検査項目

開始前，治療中は4週間毎および治療終了時，治療終了後24週時にHCV-RNA（ロシュ COBAS TaqMan HCV），白血球数，好中球数，赤血球数，ヘモグロビン，ヘマトクリット，血小板数，総蛋白，アルブミン，AST，ALT，LDH，アルカリフォスファターゼ，γGTPを測定した．