Figure 1. Cumulative overall survival (OS) in the aged group (n=179) and the control group (n=279). The median OS intervals were 9.7 months (95% confidence interval [CI], 7.5-12.0 months) in the aged group and 8.2 months (95% CI, 6.9-9.6 months) in the control group (*P*=0.641).
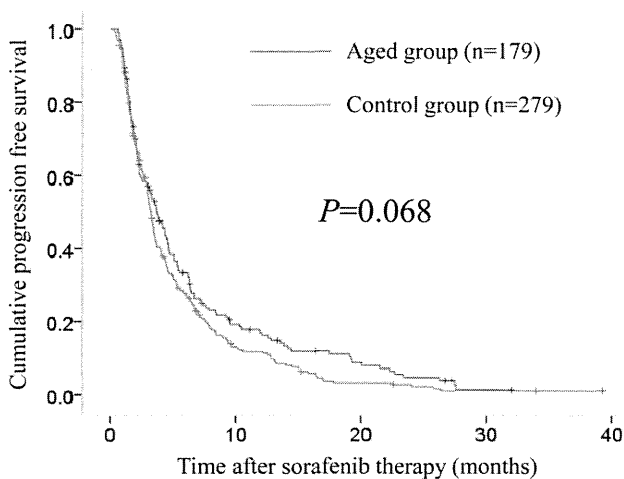

Figure 2. Cumulative progression free survival (PFS) in the aged group (n=179) and the control group (n=279). The median PFS intervals were 3.8 months (95% CI, 2.9-4.6 months) in the aged group and 3.3 months (95% CI, 3.0-3.6 months) in the control group (*P*=0.068).

Treatment duration, treatment discontinuation rate and dose reduction rate in the two groups

In patients with initial dose of sorafenib of 800 mg/day (n=51 in the aged group and n=132 in the control group), the median treatment durations were 3.1 months (range, 0.1-30.0 months) in the aged group and 3.2 months (range, 0.2-40.4 months) in the control group (*P*=0.629). Treatment discontinuation rates were 90.2% (46/51) in the aged group and 92.4% (122/132) in the control group (*P*=0.764). Dose reduction rates were 62.7% (32/51) in the aged group and 57.6% (76/132) in the control group (*P*=0.616).

In patients with reduced initial dose of sorafenib (n=128 in the aged group and n=147 in the control group), the median treatment durations were 3.3 months (range, 0.1-32.1 months) in the aged group and 3.8 months (range, 0.1-29.0 months) in the control

group (*P*=0.381). Treatment discontinuation rates were 89.8% (115/128) in the aged group and 89.1% (131/147) in the control group (*P*>0.999). Dose reduction rates were 42.2% (54/128) in the aged group and 29.9% (44/147) in the control group (*P*=0.043), suggesting that aged group patients with reduced initial dose of sorafenib had significantly higher dose reduction rate than control group patients.

Treatment tumor response rate

The best treatment tumor response rates during follow-up period were: CR in 4 patients, PR in 23, SD in 61, PD in 50 and not evaluated (NE) in 41, respectively, in the aged group; CR in 2 patients, PR in 38, SD in 97, PD in 98 and NE in 44, respectively, in the control group. The objective response rates (ORRs) were 15.1% (27 out of 179 patients) in the aged group and 14.3% (40 out of 279 patients) in the control group (*P*=0.892). The disease control rates (DCRs) were 49.2% (88 out of 179 patients) in the aged group and 49.1% (137 out of 279 patients) in the control group (*P*>0.999). (Table 2)

Table 2. Best treatment response rate in the aged group and the control group.

	Aged group	Control group	*P* value[a]
Complete response	4 (2.2%)	2 (0.7%)	
Partial response	23 (12.8%)	38 (13.6%)	
Stable disease	61 (34.1%)	97 (34.8%)	
Progressive disease	50 (27.9%)	98 (35.1%)	
Unavailable response	41 (22.9%)	44 (15.8%)	
Disease control rate	88/179 (49.2%)	137/279 (49.1%)	>0.999
Objective response rate	27/179 (15.1%)	40/279 (14.3%)	0.892

[a]; Fisher's exact test

Treatment response according to Edmondson grade

In HCC patients with Edmondson grade I (n=29; n=16 in the aged group and n=13 in the control group), the ORRs were 18.8% (3/16) in the aged group and 30.8% (4/13) in the control group (*P*=0.667), while the DCRs were 62.5% (10/16) in the aged group and 76.9% (10/13) in the control group (*P*=0.454). In HCC patients with Edmondson grade II (n=37; n=13 in the aged group and n=24 in the control group), the ORRs were 23.1% (3/13) in the aged group and 4.2% (1/24) in the control group (*P*=0.115), while the DCRs were 46.2% (6/13) in the aged group and 29.2% (7/24) in the control group (*P*=0.472). In HCC patients with Edmondson grade III (n=23; n=4 in the aged group and n=19 in the control group), the ORRs were 25.0% (1/4) in the aged group and 5.3% (1/19) in the control group (*P*=0.324), while the DCRs were 25.0% (1/4) in the aged group and 36.8% (7/19) in the control group (*P*>0.999).

Univariate and multivariate analyses of factors contributing to OS

In the univariate analysis, Child-Pugh classification ($P<0.001$), BCLC stage ($P<0.001$), portal vein invasion ($P<0.001$), extrahepatic spread ($P<0.001$), EOCG PS ($P=0.001$), AST $\geq$50 IU/L ($P<0.001$), ALP $\geq$400 IU/L ($P<0.001$), GGT $\geq$90 IU/L ($P<0.001$), lactose dehydrogenase (LDH) $\geq$240 IU/L ($P<0.001$), alpha-fetoprotein (AFP) $\geq$200 ng/mL ($P<0.001$) and des-γ-carboxy prothrombin (DCP) $\geq$700 mAU/mL ($P<0.001$) were significant factors contributing to OS. (Table 3) In the multivariate analysis involving 12 factors with $P<0.1$ in the univariate analyses, Child-Pugh classification ($P=0.005$), causes of liver disease (viral) ($P=0.001$), portal vein invasion ($P=0.007$), extrahepatic spread ($P=0.002$), GGT $\geq$90 IU/L ($P<0.001$), LDH $\geq$240 IU/L ($P<0.001$), AFP $\geq$200 ng/mL ($P<0.001$) and DCP $\geq$700 mAU/mL ($P=0.002$) were significant factors contributing to OS. The hazard ratios (HRs) and 95% CIs for these factors are detailed in table 4.

Univariate and multivariate analyses of factors contributing to PFS

In the univariate analysis, Child-Pugh classification ($P=0.002$), BCLC stage ($P=0.023$), portal vein invasion ($P=0.005$), AST $\geq$50 IU/L ($P=0.002$), ALP $\geq$400 IU/L ($P=0.001$), GGT $\geq$90 IU/L ($P<0.001$), LDH $\geq$240 IU/L ($P<0.001$), AFP $\geq$200 ng/mL ($P<0.001$) and DCP $\geq$700 mAU/mL ($P<0.001$) were significant factors associated with PFS. (Table 3) In the multivariate analysis involving 10 factors with $P<0.1$ the univariate analysis, Child-Pugh classification ($P=0.031$), GGT $\geq$90 IU/L ($P=0.008$), LDH $\geq$240 IU/L ($P=0.043$), AFP $\geq$200 ng/mL ($P=0.009$) and DCP $\geq$700 mAU/mL ($P=0.009$) were significant factors linked to PFS. The HRs and 95% CIs for these factors are detailed in table 4.

Causes of death in the two groups

One hundred and twenty seven patients (70.9%) in the aged group and 215 (77.1%) patients in the control group died during the follow-up period. The causes of death in the aged group were as follows: HCC progression (90 patients); liver failure (19 patients); miscellaneous (15 patients); and unknown causes (3 patients). In the control group the causes of death were: HCC progression (178 patients); liver failure (13 patients); miscellaneous (17 patients); and unknown causes (7 patients).

Table 3. Univariate analyses of factors contributing to overall survival (OS) and progression free survival (PFS).

Variables	n	OS P value[a]	PFS P value[a]
Age ($\geq$75 years), yes/no	179/279	0.641	0.068
Gender (male), yes/no	369/89	0.353	0.828
Child-Pugh classification, A/B	374/84	<0.001	0.002
BCLC stage, B/C	163/295	<0.001	0.023
Causes of liver disease (viral), yes/no	337/121	0.054	0.134
Portal vein invasion, yes/no	107/351	<0.001	0.005
Extrahepatic spread, yes/no	195/263	<0.001	0.394
EOCG PS 0, yes/no	346/112	0.001	0.291
AST ($\geq$50 IU/L), yes/no	251/207	<0.001	0.002
ALT ($\geq$50 IU/L), yes/no	206/252	0.270	0.346
ALP ($\geq$400 IU/L), yes/no[b]	220/229	<0.001	0.001
GGT ($\geq$90 IU/L), yes/no[c]	209/241	<0.001	<0.001
LDH ($\geq$240 IU/L), yes/no[d]	202/237	<0.001	<0.001
Platelets ($\geq$12×10^4/mm^3), yes/no[e]	224/233	0.259	0.658
AFP ($\geq$200 ng/mL), yes/no[f]	211/238	<0.001	<0.001
DCP ($\geq$700 mAU/mL), yes/no[g]	217/224	<0.001	<0.001
Initial dose of sorafenib (800 mg/day), yes/no	183/275	0.950	0.788
Initial dose of sorafenib based on BW $\geq$8.4 mg/kg/day, yes/no	222/236	0.470	0.187

BCLC; Barcelona Clinic Liver Cancer, ECOG PS; Eastern Cooperative Oncology Group Performance Status, AST; aspartate aminotransferase, ALT; alanine aminotransferase, ALP; alkaline phosphatase, GGT; gamma glutamyl transpeptidase, LDH; lactose dehyrogenase, AFP; alpha-fetoprotein, DCP; des-γ-carboxy prothrombin, BW; body weight, [a] log-rank test, [b] missing values, n=9, [c] missing values, n=8, [d] missing values, n=19, [e] missing values, n=1, [f] missing values, n=9, [g] missing values, n=17

Table 4. Multivariate analyses of factors contributing to overall survival (OS) and progression free survival (PFS).

Variables	OS HR	OS 95% CI	OS P value[a]	PFS HR	PFS 95% CI	PFS P value[a]
Age ($\geq$75 years)				0.926	0.746-1.151	0.490
Child-Pugh classification, A/B	0.658	0.491-0.882	0.005	0.741	0.564-0.972	0.031
BCLC stage, B/C	0.952	0.632-1.434	0.815	0.840	0.660-1.070	0.158
Causes of liver disease (viral)	0.628	0.472-0.836	0.001			
Portal vein invasion	0.657	0.485-0.891	0.007	0.947	0.719-1.248	0.699
Extrahepatic spread	0.599	0.433-0.828	0.002			
EOCG PS, 0/1,2	0.785	0.581-1.060	0.115			
AST ($\geq$50 IU/L)	1.140	0.858-1.514	0.368	1.025	0.809-1.298	0.840
ALP ($\geq$400 IU/L)	0.960	0.740-1.246	0.760	1.008	0.799-1.271	0.946
GGT ($\geq$90 IU/L)	0.609	0.472-0.786	<0.001	0.729	0.578-0.921	0.008
LDH ($\geq$240 IU/L)	0.558	0.434-0.719	<0.001	0.794	0.635-0.992	0.043
AFP ($\geq$200 ng/mL)	0.601	0.474-0.763	<0.001	0.749	0.604-0.930	0.009
DCP ($\geq$700 mAU/mL)	0.676	0.529-0.863	0.002	0.766	0.616-0.952	0.016

HR; hazard ratio, CI; confidence interval, BCLC; Barcelona Clinic Liver Cancer, ECOG PS; Eastern Cooperative Oncology Group Performance Status, AST; aspartate aminotransferase, ALP; alkaline phosphatase, GGT; gamma glutamyl transpeptidase, LDH; lactose dehyrogenase, AFP; alpha-fetoprotein, DCP; des-γ-carboxy prothrombin, [a] Cox proportional hazard model.

Serious adverse events (SAEs)

Grade 3 or more SAEs as defined by CTCAE were observed in 51 patients (28.5%) in the elderly group and 69 patients (24.7%) in the control group (*P*=0.385): rash (5.7% [10/175] *vs.* 2.2% [6/274], *P*=0.066), hand-foot syndrome (6.9% [12/175] *vs.* 4.4% [12/275], *P*=0.285), diarrhea (2.3% [4/174] *vs.* 2.2% [6/277], *P*>0.999), fever (1.1% [2/177] *vs.* 1.4% [4/278], *P*>0.999), fatigue (4.0% [7/175] *vs.* 2.5% [7/276], *P*=0.412), hypertension (0.6% [1/175] *vs.* 1.8% [5/276], *P*=0.412), gastrointestinal bleeding (1.7% [3/175] *vs.* 1.4% [4/276], *P*>0.999), liver damage (9.7% [17/175] *vs.* 13.0% [36/276], *P*=0.299) and lung injury (4.0% [7/174] *vs.* 0% [0/276], *P*=0.001). (Table 5)

Table 5. Treatment related serious adverse events of grade 3 or more in the aged group and the control group.

Adverse events	Aged group	Control group	
	Grade 3 or more SAEs	Grade 3 or more SAEs	*P* value[a]
Overall	51/179 (28.5%)	69/279 (24.7%)	0.385
Rash[b]	10/175 (5.7%)	6/274 (2.2%)	0.066
Hand foot syndrome[c]	12/175 (6.9%)	12/275 (4.4%)	0.285
Diarrhea[d]	4/174 (2.3%)	6/277 (2.2%)	>0.999
Fever[e]	2/177 (1.1%)	4/278 (1.4%)	>0.999
Fatigue[f]	7/175 (4.0%)	7/276 (2.5%)	0.412
Hypertension[g]	1/175 (0.6%)	5/276 (1.8%)	0.412
Gastrointestinal bleeding[h]	3/175 (1.7%)	4/276 (1.4%)	>0.999
Liver damage[i]	17/175 (9.7%)	36/276 (13.0%)	0.299
Lung injury[j]	7/174 (4.0%)	0/276 (0%)	0.001

SAEs; serious adverse events, [a] Fisher's exact test, [b] missing values, n=9, [c] missing values, n=8, [d] missing values, n=7, [e] missing values, n=3, [f] missing values, n=7, [g] missing values, n=7, [h] missing values, n=7, [i] missing values, n=7, [j] missing values, n=8

Subgroup analyses according to Child-Pugh classification

In patients with Child-Pugh A (n=152 in the aged group and n=222 in the control group), the median OS intervals were 11.3 months (95% CI, 9.0-13.6 months) in the aged group and 9.3 months (95% CI, 7.0-11.7 months) in the control group (*P*=0.690). The median PFS intervals were 4.2 months (95% CI, 3.5-5.0 months) in the aged group and 3.3 months (95% CI, 3.0-3.6 months) in the control group (*P*=0.047), suggesting that the aged group patients with Child-Pugh A had significantly higher PFS rate compared with the control group. In patients with Child-Pugh B (n=27 in the aged group and n=57 in the control group), the median OS intervals were 4.9 months (95% CI, 2.8-7.0 months) in the aged group and 4.4 months (95% CI, 3.3-5.4 months) in the control group (*P*=0.704). The median PFS intervals were 1.6 months (95% CI, 0.2-3.0 months) in the aged group and 2.6 months (95% CI, 1.3-3.8 months) in the control group (*P*=0.554).

Subgroup analyses according to BCLC stage

In patients with BCLC stage B (n=63 in the aged group and n=100 in the control group), the median OS intervals were 14.6 months (95% CI, 9.6-19.7 months) in the aged group and 15.0 months (95% CI, 11.9-18.0 months) in the control group (*P*=0.530). The median PFS intervals were 3.8 months (95% CI, 2.6-5.1 months) in the aged group and 4.2 months (95% CI, 3.2-5.3 months) in the control group (*P*=0.768). In patients with BCLC stage C (n=116 in the aged group and n=179 in the control group), the median OS intervals were 7.9 months (95% CI, 5.4-10.3 months) in the aged group and 6.1 months (95% CI, 5.0-7.2 months) in the control group (*P*=0.269). The median PFS intervals were 3.6 months (95% CI, 2.4-4.7 months) in the aged group and 2.9 months (95% CI, 2.4-3.3 months) in the control group (*P*=0.046), indicating that the aged group patients with BCLC stage C had significantly higher PFS rate than the control group patients.

Subgroup analyses according to initial dose of sorafenib

We further analysed clinical outcomes according to initial dose of sorafenib since the proportion of patients with initial dose of sorafenib of 800 mg/day in the aged group was significantly lower than that in the control group. In patients with initial dose of sorafenib of 800 mg/day (n=51 in the aged group and n=132 in the control group), the median OS intervals were 12.0 months (95% CI, 7.8-16.3 months) in the aged group and 7.1 months (95% CI, 5.3-8.9 months) in the control group (*P*=0.332). The median PFS intervals were 4.2 months (95% CI, 3.3-5.1 months) in the aged group and 3.2 months (95% CI, 2.9-3.5 months) in the control group (*P*=0.079). In patients with reduced initial dose of sorafenib (n=128 in the aged group and n=147 in the control group), the median OS intervals were 9.3 months (95% CI, 7.1-11.6 months) in the aged group and 9.2 months (95% CI, 6.9-11.6 months) in the control group (*P*=0.850). The median PFS intervals were 3.6 months (95% CI, 2.7-4.5 months) in the aged group and 3.4 months (95% CI, 2.9-3.9 months) in the control group (*P*=0.253).

Baseline characteristics and clinical outcomes in the aged and control groups after propensity score matching

Baseline characteristics in the two groups (aged group: n=132, control group: n=132) after propensity score matching are demonstrated in Table 6. In all analysed variables, no significant differences were observed. The median OS intervals were 10.7 months (95% CI, 8.0-13.4 months) in the aged group and 9.5 months (95% CI, 6.6-12.4 months) in the control group
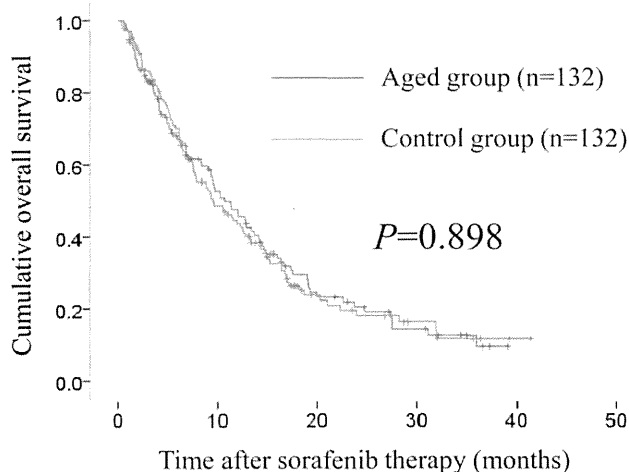
(*P*=0.898). (Fig. 3) The median PFS intervals were 3.8 months (95% CI, 2.6-5.1 months) in the aged group and 3.8 months (95% CI, 2.9-4.8 months) in the control group (*P*=0.407). (Fig. 4)

Clinical outcome in the two groups according to different cut-off age

When using cut-off age of 80 years (n=81 in patients aged ≥80 years and n=377 in patients aged <80 years), the median OS intervals were 9.3 months (95% CI, 7.4-11.3 months) in the aged group and 8.8 months (95% CI, 7.5-10.1 months) in the control group (*P*=0.827), while the median PFS intervals were 3.8

months (95% CI, 2.2-5.4 months) in the aged group and 3.4 months (95% CI, 3.1-3.7 months) in the control group (*P*=0.668). When using cut-off age of 70 years (n=249 in patients aged ≥70 years and n=209 in patients aged <70 years), the median OS intervals were 10.1 months (95% CI, 8.5-11.8 months) in the aged group and 7.7 months (95% CI, 6.2-9.2 months) in the control group (*P*=0.950), whereas the median PFS intervals were 3.7 months (95% CI, 3.1-4.4 months) in the aged group and 3.1 months (95% CI, 2.8-3.4 months) in the control group (*P*=0.046).
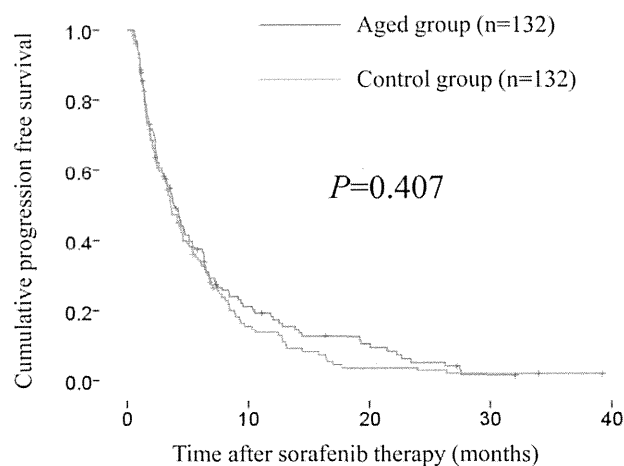

Figure 3. Cumulative overall survival (OS) in the aged group (n=132) and the control group (n=132) after propensity score matching. The median OS intervals were 10.7 months (95% CI, 8.0-13.4 months) in the aged group and 9.5 months (95% CI, 6.6-12.4 months) in the control group (*P*=0.898).

Figure 4. Cumulative progression free survival (PFS) in the aged group (n=132) and the control group (n=132) after propensity score matching. The median PFS intervals were 3.8 months (95% CI, 2.9-4.8 months) in the aged group and 3.6 months (95% CI, 2.9-4.3 months) in the control group (*P*=0.407).

Table 6. Baseline characteristics between the aged group and the control group after propensity score matching.

Variables	Aged group (n=132)	Control group (n=132)	*P* value
Age (years)	79.4 ± 3.3	64.1 ± 6.2	<0.001[a]
Gender, male/female	101 / 31	108 / 24	0.363[b]
Child-Pugh A / B	110 / 22	115 / 17	0.488[b]
Causes of liver disease			
B/C/non B and non C/B and C	6 / 85 / 41 / 0	7 / 85 / 40 / 0	>0.999[b]
BCLC stage B/C	48/84	42/90	0.516[b]
ECOG PS, 0/1/2	94/35/3	95/34/3	>0.999[b]
AST (IU/L)	65.7 ± 64.6	60.8 ± 35.6	0.296[a]
ALT (IU/L)	45.8 ± 38.1	48.0 ± 35.2	0.622[a]
Total bilirubin (mg/dL)	0.93 ± 0.45	0.89 ± 0.47	0.422[a]
Albumin (g/dL)	3.50 ± 0.46	3.54 ± 0.50	0.458[a]
ALP (IU/L)[c]	443.9 ± 238.0	469.3 ± 360.6	0.505[a]
GGT (IU/L)[d]	112.6 ± 129.0	143.0 ± 164.6	0.101[a]
LDH[e]	248.0 ± 76.1	252.4 ± 101.5	0.696[a]
Prothrombin time (%)	85.7 ± 18.2	88.1 ± 16.7	0.261[a]
Platelets ($\times 10^4$/mm³)[f]	13.1 ± 6.0	13.9 ± 6.3	0.285[a]
AFP (ng/mL)[g]	6779 ± 26576	15102 ± 67697	0.191[a]
DCP (mAU/mL)[h]	13873 ± 75599	21164 ± 80945	0.457[a]
Initial dose of sorafenib (mg/day)			
800mg/600mg/400mg/200mg	44 / 0 / 82 / 6	48 / 2 / 76 / 6	0.593[b]

Data are expressed as number or mean ± standard deviation. BCLC; Barcelona Clinic Liver Cancer, ECOG PS; Eastern Cooperative Oncology Group Performance Status, AST; aspartate aminotransferase, ALT; alanine aminotransferase, ALP; alkaline phosphatase, GGT; gamma glutamyl transpeptidase, LDH; lactose dehyrogenase, AFP; alpha-fetoprotein, DCP; des-γ-carboxy prothrombin, [a] unpaired t test, [b] Fisher's exact test, [c] missing values, n=7, [d] missing values, n=6, [e] missing values, n=11, [f] missing values, n=1, [g] missing values, n=2, [h] missing values, n=8

Discussion

To the best of our knowledge, this is the largest study comparing clinical outcomes and safety between aged and non-aged HCC patients treated with sorafenib. [29-32] Current guidelines for the management of HCC do not satisfy strategies according to age. [2, 3] Few studies assessed the clinical outcomes in HCC patients treated with sorafenib based on age. [29, 30, 32] With the aging population, HCC in the elderly represents a significant health burden. In Japan, the proportion of elderly patients with HCC and their average age is increasing. These trends have led to a rising demand in our country for investigations related to the outcome of sorafenib therapy in elderly HCC patients: hence the reasons for the current comparative study.

In our results, the aged group patients had comparable OS rate, PFS rate, DCR and ORR as compared with the control group patients. The difference in the two groups in terms of sorafenib related SAEs of grade 3 or more did not reach significance except for the development of lung injury. In subgroup analyses, in patients with Child-Pugh A and in those with BCLC-C, the median PFS intervals in the aged group were significantly longer than those in the control group and in all other subgroup analyses, no significant difference in the two groups was observed in terms of OS and PFS. Furthermore, in the propensity score matched cohorts, no significant difference in the two groups was found in terms of OS and PFS and when using different cut-off age (80 years or 70 years), and similar results were obtained. Systemic anticancer therapy in aged patients with malignancies tends to be viewed with skepticism owing to the greater frequency of treatment related SAEs in aged than in younger patients. However, our results suggest that aged HCC patients treated with sorafenib had comparable prognosis and well tolerable drug related toxicity compared with younger HCC patients treated with sorafenib, which are in line with results reported by Jo, et al. [40] Since our study regarding effect of sorafenib on clinical outcome stratified by age is the largest that has been published so far and includes unselected cases by fourteen centers scattered throughout in Japan, our study results faithfully reflect what actually occurs in clinical practice.

The prevalence of aged subjects in our population was higher than in other previous reports. [11-26] This was possibly due to a lower proportion of patients with HBV infection who often develop HCC in younger age as compared with those with HCV infection and the shift towards older ages of HCC occurrence in Japan. The proportion of male patients in the aged group was almost significantly lower than

that in the control group (P=0.053). This may have been associated with a larger female elderly population because of their longer life expectancy. Furthermore, the observations of significantly lower hemoglobin level, lower BW and higher serum creatinine levels in the aged group of this study may well reflect the actual situations in aged HCC patients in clinical practice.

In aged group, the difference in patients with initial dose of sorafenib of 800 mg/day and those with reduced dose of sorafenib did not reach significance in terms of OS (P=0.445) and PFS (P=0.691). Iavarone M, et al reported that the effectiveness of half-dosed sorafenib may have implications for tailored therapy in HCC patients. [41] Since in aged HCC patients, high frequency of sorafenib related SAEs were expected when given an initial dose of sorafenib of 800 mg/day, leading to treatment discontinuation or interruptions, reduced initial dose of sorafenib can be considered in elderly patients for avoiding SAEs although further examination is needed to confirm these results.

As described earlier, in patients with Child-Pugh A and in those with BCLC-C, PFS intervals in the aged group were significantly longer than that in the control group. These findings might be associated with the slower cancer growth in aged patients or to a higher susceptibility of vasculature to antiangiogenic agents in aged patients. [42] On the other hand, it is of interest that GGT level was the significant predictor linked to both OS and PFS in our multivariate analysis. Several studies reported that a high level of GGT was related to a higher incidence of HCC progression, which are in line with our results. [43, 44] As for other significant predictors observed in our multivariate analyses, our study results were consistent with previous reports. [7, 9, 29, 30, 32, 38]

It is noteworthy that sorafenib related lung injury of grade 3 or more occurred in 7 aged patients, whereas no such lung injury was observed in the control group and 2 out of 7 died due to respiratory failure. The reasons for these results are unclear, however, during sorafenib therapy, caution should be exercised for lung injury especially in aged patients.

Although several studies have examined the predictive factors linked to the response to sorafenib in advanced HCC patients, the factors predicting a favorable response remained unclear. [45] However, recent studies demonstrated that polymorphisms of VEGF and its receptor genes may regulate angiogenesis and tumor growth and they may influence OS and PFS in HCC patients undergoing sorafeinb therapy. [46, 47] In addition, Lee, et al. reported that differences in the incidence of sorafenib-related hand foot skin reaction in HCC patients treated with sorafenib

may have been caused by ethnic differences in genetic polymorphisms of the tumor necrosis factor-alpha, VEGF, and uridine diphosphate glucuronosyltransferase 1 family-polypeptide A9 genes. [48] Although such polymorphisms were not tested in the current analyses, these may be associated with clinical outcome in elderly HCC patients treated with sorafenib and in this regard, further investigations will be required.

This study included several limitations. First, our study is a retrospective observational study, although the major strength of our study is a large sample size. Second, the initial sorafenib dose varied among individual patients, leading to bias. Third, various therapies were performed after discontinuation of sorafenib in some patients, also potentially leading to bias in concerning their OS. Lastly, our study cohort is limited to Japanese patients with relatively low BW in contrast to patients in Western countries. Hence, our results cannot be extended to patients with other racial cohorts and caution should be exercised when interpreting these results. Thus, further prospective studies will be necessary. However, our results indicated that in HCC patients treated with sorafenib, life expectancy, disease progression, treatment efficacy and SAEs are unaffected by age over 75 years. In conclusion, aged HCC patients treated with sorafenib had comparable clinical outcomes compared with younger HCC patients treated with sorafenib. Sorafenib therapy for HCC should not be determined solely based on age.

Acknowledgement

Conflicts of interest

The authors declare that they have no conflicts of interest.

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Original Article

Clinical significance of pretreatment serum interferon-gamma-inducible protein 10 concentrations in chronic hepatitis C patients treated with telaprevir-based triple therapy

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Aim: We aimed to determine whether pretreatment serum interferon-γ-inducible protein (IP)-10 concentration can predict response to telaprevir (TVR)-based triple therapy in patients with genotype 1 chronic hepatitis C (CHC), and to examine the effects of IP-10 concentration on liver histology.

Methods: Baseline IP-10 concentrations were measured in 97 patients with genotype 1 CHC treated with TVR-based triple therapy, and the associations between baseline IP-10 and treatment outcome were assessed by univariate and multivariate analyses. Associations between baseline serum IP-10 concentration and laboratory data and liver histological findings were also investigated.

Results: Median IP-10 concentration in these patients was 461.83 pg/mL (range, 151.35–4297.62). Multivariate analysis showed that IL28B genotype ($P = 0.025$) and IP-10 level ($P = 0.004$) were factors significantly predictive of rapid virological response (RVR), whereas in pretreatment factors only,

IL28B genotype ($P = 0.001$) and liver fibrosis ($P = 0.035$) were independent predictors of sustained virological response. Using a cut-off IP-10 concentration of 460 pg/mL, patients with IL28B risk allele and low IP-10 had a significantly higher RVR rate than those with high IP-10 ($P = 0.005$). IP-10 concentration was significantly correlated with liver fibrosis ($P = 0.001$) and inflammation activity ($P = 0.006$) and had the highest areas under the curve for liver histological findings.

Conclusion: Baseline serum IP-10 level is a useful predictor of virological response in patients with genotype 1 CHC treated with TVR-based triple therapy, especially in patients with IL28B risk allele. IP-10 was well correlated with liver fibrosis and inflammation.

Key words: chronic hepatitis C, histology, IL28B, interferon-gamma-inducible protein-10, telaprevir, treatment response

INTRODUCTION

CHRONIC HEPATITIS C virus (HCV) infection affects approximately 170 million people worldwide and is the most common cause of chronic liver disease.[1] Of these HCV-infected individuals, 20–30% eventually develop cirrhosis or hepatocellular carcinoma (HCC). In Japan, approximately 30 000 persons per year die from HCC, with 70–80% of these deaths ascribed to HCV. Thus, reducing HCV infection can prevent HCC.[2–4]

Telaprevir (TVR) is a direct acting antiviral (DAA) that inhibits the non-structural 3/4A serine protease of HCV

and was recently approved to treat patients with chronic hepatitis C (CHC).[5–10] Phase 2 and 3 studies in both treatment-naïve and treatment-experienced patients with genotype 1 CHC have shown significantly higher sustained virological response (SVR) rates following treatment with TVR-containing triple therapy than with pegylated interferon (PEG IFN) and ribavirin (RBV) combination therapy.[5–10] TVR in combination with PEG IFN and RBV is now considered the standard of care for patients infected with HCV genotype 1.[11] Single nucleotide polymorphisms (SNP) on chromosome 19 (rs8099917) near the IL28B region have been reported to be highly associated with SVR in patients with genotype 1 CHC treated with either TVR-based triple therapy or PEG IFN and RBV.[12–14]

The host immune response plays a significant role in HCV clearance. Activation of the immune system involves the release of pro- and anti-inflammatory molecules measurable in serum samples.[15] However, HCV-specific immunity often fails to eradicate HCV. This inability to control HCV infection leads to the recruitment of inflammatory cells into the liver parenchyma.[15,16] Cytokines and chemokines, which regulate inflammation and immunity in HCV-infected patients, are potential markers of treatment efficacy[15,16] and may play significant roles in viral clearance.[16] Chemokines are also involved in lymphocyte differentiation, leukocyte activation, regulation of the T-helper (Th)1/Th2 balance, angiogenesis and fibrogenesis.[15] Interferon-γ-inducible protein (IP)-10, a T-cell-specific CXC chemokine of 77 amino acids in its mature form, targets the CXCR3 receptor, attracts natural killer (NK) cells, T lymphocytes and monocytes, and may be a prognostic marker in patients infected with HCV genotype 1.[16–18] Intrahepatic and serum IP-10 levels have been reproducibly linked to the extent of HCV-related liver fibrosis.[19–21] Additionally, IP-10 is a valid surrogate marker of IFN-stimulated gene activation, which predicts a more pronounced early phase decline in HCV RNA and an increased SVR rate in patients treated with PEG IFN and RBV combination therapy.[18,22–24]

Previous studies have shown that pretreatment IP-10 concentrations were closely associated with SVR rate in response to PEG IFN and RBV in patients with HCV genotype 1, with high systemic IP-10 concentrations at the onset of treatment predictive of poorer outcomes.[17,18,25] IL28B genotype in combination with IP-10 concentration is useful for predicting SVR in patients with HCV genotype 1 with PEG IFN and RBV.[26] It has not been determined, however, whether IL28B genotype in combination with baseline IP-10 is useful in predicting outcomes in HCV-infected patients treated with TVR-based triple therapy.[27] This study was therefore designed to determine whether baseline serum IP-10 concentration is predictive of response to TVR-based triple therapy in patients with HCV genotype 1, and to examine the association between pretreatment serum IP-10 concentration and other baseline patient characteristics.

METHODS

Patients

BETWEEN JANUARY 2012 and April 2013, 105 DAA-naïve patients with CHC were treated with TVR-based triple therapy at the Department of Gastroenterology and Hepatology, Osaka Red Cross Hospital, Japan; the Division of Hepatobiliary and Pancreatic Disease, Department of Internal Medicine, Hyogo College of Medicine, Hyogo, Japan; and the Department of Hepatology, Osaka City University Hospital, Osaka, Japan. Pretreatment serum samples had been obtained from 100 of these patients and stored at −80°C. Three patients co-infected with HCV and hepatitis B virus were excluded; thus, 97 patients were analyzed. All patients analyzed had compensated liver disease, were infected with HCV genotype 1, were naïve to DAA treatment, had no evidence of HIV infection, and had a serum HCV RNA concentration of more than 5.0 log IU/mL. Liver biopsy samples obtained from 85 patients (87.6%) before treatment were coded and scored using the METAVIR scoring system by a single pathologist in each hospital.[28] Advanced fibrosis was defined as the presence of F3 or F4 fibrosis. The associations between baseline serum IP-10 concentration and the clinical characteristics and virological responses of patients were analyzed retrospectively.

This study was conducted according to the ethical guidelines of the 1975 Declaration of Helsinki and was approved by the ethics committee of each participating facility. Written informed consent was obtained from all patients prior to treatment.

Treatment schedule

All patients analyzed were scheduled to receive TVR (Telavic; Mitsubishi Tanabe Pharma, Osaka, Japan) in combination with PEG IFN-α-2b (Peg-Intron; MSD, Tokyo, Japan; 1.5 μg/kg per week) and weight-based RBV (Rebetol; MSD; total doses of 600 mg/day, 800 mg/day and 1000 mg/day for patients weighing less than <60 kg, 60–80 kg and >80 kg, respectively, according to

Japanese guidelines) for 12 weeks, followed by PEG IFN-α-2b and RBV for 12 weeks. TVR was initiated at a dose of 750 mg every 8 h (2250 mg/day) or 500 mg every 8 h (1500 mg/day), with the dose determined by each attending physician based on each patient's baseline characteristics such as age and bodyweight.[29]

Dose reductions for hematological side-effects were based mainly on the information supplied by each drug manufacturer. Grade 2 or higher adverse events, such as malaise, fever, anorexia and light-headedness, resulted in TVR reductions of 750 mg/day, PEG IFN reductions of 10–20 μg/week, and RBV reductions of 200 mg/day as soon as possible, until symptom severity decreased to grade 1 or below. None of the patients received erythropoietin or granulocyte-macrophage colony-stimulating factor during treatment. Patients with grade 1 (several sites or localized to one site) or 2 (diffuse skin eruption involving up to 50% of the body surface) dermatological adverse events were managed at the discretion of the physicians at each hospital. TVR was discontinued in patients who experienced a progressive grade 3 dermatological adverse event (rash with the appearance of substantial systemic signs or symptoms or involving >50% of the body surface), but these patients continued to receive PEG IFN-α-2b and RBV, if possible.

Virological evaluations

Hepatitis C virus RNA concentrations were measured using the TaqMan HCV assay (COBAS TaqMan HCV assay; Roche Molecular Diagnostics, Tokyo, Japan) with lower and upper limits of quantification of 15 IU/mL and 6.9×10^7 IU/mL (range, 1.2–7.8 log IU/mL), respectively. HCV genotype was determined using a HCV Genotype Primer Kit (Institute of Immunology, Tokyo, Japan). Amino acid substitutions in core 70/91 were assayed as described.[30]

Previous virological responses to IFN-based therapy included prior relapse, undetectable HCV RNA at the end of treatment but detectable HCV RNA 24 weeks or less later and the reappearance of HCV RNA at any time during treatment after a virological response (breakthrough). Patients whose HCV RNA never became undetectable during treatment were defined as non-responders.

Assessment of treatment efficacy

Rapid virological response (RVR) was defined as undetectable serum HCV RNA at week 4 of treatment. End of treatment response (ETR) was defined as undetectable

HCV RNA at the end of therapy. SVR12 was defined as undetectable HCV RNA 12 weeks after the completion of treatment.[31] All methods of assessing treatment efficacy were defined according to guidelines.[32,33] Even if treatment was discontinued before the assigned schedule because of side-effects or non-compliance with therapy, patients were considered SVR12 if serum HCV RNA was undetectable at 12 weeks of follow up. During follow up, clinical, biochemical and qualitative serum HCV RNA parameters were determined every 1–3 months.

Genotyping for SNP near IL28B (rs8099917) and quantification of serum IP-10

Genetic polymorphisms in tagged SNP located near IL28B (rs8099917) were determined by direct sequencing of polymerase chain reaction-amplified DNA. IP-10 was measured in serum samples collected at baseline, prior to initiation of TVR-based triple therapy, using commercially available Quantikine human CXCL10/IP-10 immunoassay kits (R&D Systems, Minneapolis, MN, USA). Samples with IP-10 concentration of more than 779.22 pg/mL were diluted 1:10 and reanalyzed.

Statistical analysis

Variables were compared between groups by Spearman's rank correlation coefficient r_s test, Fisher's exact test and the Mann–Whitney U-test, as applicable. The influence of various factors on response to TVR-based triple therapy was evaluated by univariate analysis. Virological response was analyzed on an intention to treat basis. Factors associated with RVR, defined as $P < 0.1$ in univariate analyses, were entered into multivariate logistic regression analysis. Additionally, only pretreatment factors associated with SVR12, with $P < 0.1$ in univariate analyses, were entered into multivariate analysis, because the aim of this study was to evaluate the impact of pretreatment IP-10 on the ability of pretreatment factors to predict response to treatment. Data were analyzed using SPSS for Windows. All statistical analyses were based on two-sided hypothesis tests with a significance level of $P < 0.05$. Furthermore, receiver–operator curves (ROC) were constructed to investigate the superiority of IP-10 level over measurements of platelet counts and aspartate aminotransferase (AST) and alanine aminotransferase (ALT) concentrations to predict histological liver fibrosis and activity. Areas under the ROC (AUC) were used to estimate the probability.

RESULTS

Baseline characteristics and association between liver histology findings and baseline serum IP10

THE BASELINE CHARACTERISTICS of the 97 patients enrolled in the present study (56 male, 41 female) are shown in Table 1. Median baseline serum IP-10 concentration was 461.83 pg/mL (range, 151.35–4297.62). The IP-10 concentration was significantly higher in the 22 patients with (median, 570.06 pg/mL; range, 209.66–4297.62) than in 63 without (median, 394.64 pg/mL; range, 151.35–1146.43) ($P = 0.001$) advanced fibrosis (F3/F4) (Fig. 1a). Similarly, the IP-10 concentration was significantly higher in the 40 patients with (median, 532.59 pg/mL; range, 151.35–1768.81) than in the 45 without (median, 355.06 pg/mL; range, 155.53–4297.62) ($P = 0.006$) moderate/severe activity (METAVIR score A2/A3) (Fig. 1b).

Association between baseline laboratory data and IP-10 concentration

We also examined the correlations between baseline laboratory data and IP-10 concentrations using Spearman's rank correlation coefficient r_s test. Platelet count ($r_s = -0.289$, $P = 0.004$), AST concentration ($r_s = 0.510$, $P < 0.001$) and ALT concentration ($r_s = 0.345$, $P = 0.001$) were all significantly correlated with IP-10 concentration (Fig. 2). None of the other laboratory parameters, including white blood cell count, hemoglobin level, body mass index and HCV RNA concentration, was significantly correlated with IP-10, whereas age tended to correlate with IP-10 concentration ($r_s = 0.200$, $P = 0.050$).

Table 1 Baseline characteristics ($n = 97$)

Variables	$n = 97$
Age (years)†	57.3 ± 9.8
Sex (male/female)	56/41
HCV RNA (log IU/mL)	6.7 ± 0.6
Body mass index (kg/m^2)	23.9 ± 3.4
Bodyweight (kg)‡	62.8 ± 11.8
White blood cell (/mm^3)	5067 ± 1565
Hemoglobin (g/dL)	14.2 ± 1.4
Platelets (×10^4/mm^3)	16.5 ± 5.3
AST (IU/L)	56.8 ± 42.1
ALT (IU/L)	62.8 ± 45.5
Serum creatinine (mg/dL)	0.73 ± 0.16
Previous IFN therapy, naïve/relapse/ non-responder	38/39/20
Fibrosis (F0–2/F3–4/unknown)	63/22/12
Activity (A0–1/A2–3/unknown)	45/40/12
Core 70 (wild/mutant/compete/ equivocal/NT)	45/21/2/5/24
Core 91 (wild/mutant/compete/ equivocal/NT)	47/20/1/5/24
IL-28B, rs8099917 (TT/non-TT/ unknown)	67/27/3
Initial dose of telaprevir (mg/day) (2250 mg/1500 mg)	65/32

Values are expressed as mean ± standard deviation.
†Twenty-two patients (22.7%) were ≥65 years old.
‡Seventeen patients (17.5%) had bodyweights ≤50 kg.
ALT, alanine aminotransferase; AST, aspartate aminotransferase; HCV, hepatitis C virus; NT, not tested.

AUC of platelet count and IP-10 level for advanced fibrosis (F3 or F4)

The AUC of platelet count and IP-10 concentration for advanced fibrosis were 0.577 ($P = 0.283$; 95% confi-
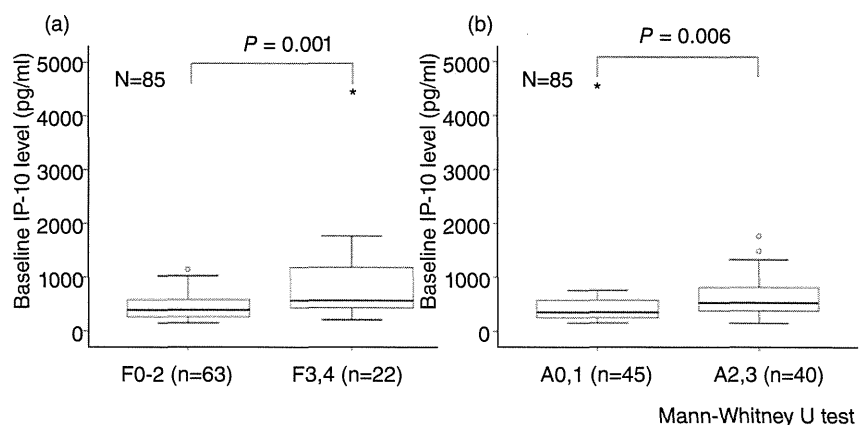

Figure 1 Association between baseline interferon-γ-inducible protein (IP)-10 concentration and liver fibrosis (F) and inflammation (A) in the 85 assessable patients.
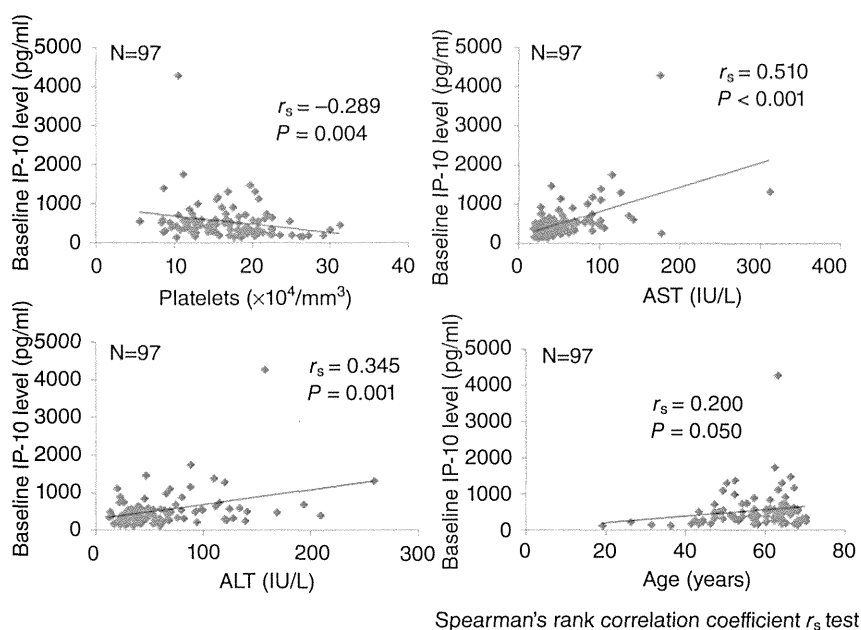
Figure 2 Association between baseline interferon-γ-inducible protein (IP)-10 concentration and baseline platelet count, aspartate aminotransferase (AST) and alanine aminotransferase (ALT) concentrations, and age in the 97 treated patients.

Spearman's rank correlation coefficient r_s test

dence interval [CI], 0.443–0.712) and 0.731 ($P = 0.001$; 95% CI, 0.611–0.851), respectively, indicating that IP-10 concentration was a better pretreatment predictor of advanced liver fibrosis than platelet count.

AUC of AST, ALT and IP-10 concentrations for severe activity (A2 or A3)

The AUC of AST, ALT and IP-10 concentrations for severe activity were 0.627 ($P = 0.045$; 95% CI, 0.503–0.750), 0.540 ($P = 0.523$; 95% CI, 0.414–0.666) and 0.673 ($P = 0.006$; 95% CI, 0.557–0.790), respectively, indicating that IP-10 concentration was a better pretreatment predictor of severe liver inflammation than AST and ALT levels.

Previous IFN therapy and pretreatment serum IP10 level

The IP-10 concentration was significantly lower in the 38 IFN-treatment-naïve patients (median, 331.86 pg/mL; range, 151.35–1333.57) than in the 39 patients who relapsed (median, 529.29 pg/mL; range, 169.58–4297.62; $P = 0.005$) and the 20 non-responders (median, 583.42 pg/mL; range, 278.38–1768.81; $P = 0.001$). IP-10 concentrations, however, did not differ significantly in relapsers and non-responders ($P = 0.154$) (Fig. 3a).

Association between IL28B genotype and pretreatment serum IP10

IL28B genotype (rs8099917) was tested in 94 patients, including 67 with IL28B TT and 27 with IL28B non-

TT. In terms of IP-10 level, there was no significant difference between patients with IL28B TT (median, 414.67 pg/mL; range, 169.58–4297.62) and those with IL28B non-TT patients (median, 534.97 pg/mL; range, 151.35–1768.81) ($P = 0.294$) (Fig. 3b).

Association between core amino acid 70/91 and pretreatment serum IP10

Core amino acid 70/91 was tested in 73 patients. In terms of core 70, they included wild type in 45 patients, mutant type in 21, competent type in two and equivocal in five. In terms of core 91, they included wild type in 47 patients, mutant type in 20, competent type in one and equivocal in five. In terms of IP-10 level, there was no significant difference between patients with core 70 wild type (median, 455.05 pg/mL; range, 151.35–1490.87) and those with core 70 mutant type (median, 533.44 pg/mL; range, 190.76–1768.81) ($P = 0.286$). Similarly, patients with core 91 wild type did not have significantly higher IP-10 level (median, 531.74 pg/mL; range, 190.76–1768.81) than those with core 91 mutant type (median, 374.97 pg/mL; range, 151.35–765.16) ($P = 0.058$).

Assessment of treatment response and treatment discontinuation and association between treatment response and pretreatment serum IP-10

In three patients (3.1%), RVR was not evaluated because of missing data. Thus, RVR was evaluated in 94 patients,
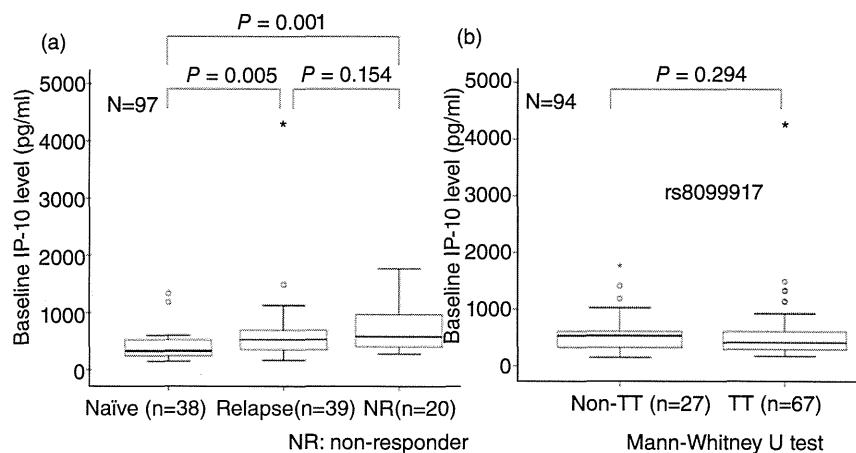
Figure 3 Associations between base-line interferon-γ-inducible protein (IP)-10 concentration and (a) prior interferon (IFN) response (n = 97) and (b) IL28B genotype (rs8099917) (n = 94) (b). IL28B genotype was not assessed in three patients.

71 (75.5%) of whom achieved RVR. Eighty-one (83.5%) of 97 patients achieved ETR. In two patients, SVR12 was not evaluated: one patient discontinued treatment because of a PEG IFN-related psychiatric disorder, and one selected to discontinue treatment, with both lost to follow up. Of the 95 evaluable patients, 71 (74.7%) achieved SVR12.

Nineteen patients (19.6%) discontinued all study drugs: three for renal dysfunction; two each for severe general fatigue and loss of appetite, grade 3 or higher rash and patient discretion; and one each for thyrotoxicosis, severe anemia, deterioration of liver function, gastrointestinal bleeding, pneumonia, acute heart failure, HCC development, PEG IFN-related psychiatric disease and an unexpected accident.

Baseline serum IP-10 concentration was significantly lower in the 71 patients who achieved RVR (median, 394.64 pg/mL; range, 151.35–4297.62) than in the 23 who did not (median, 583.55 pg/mL; range, 209.66–1768.81) ($P = 0.001$). Additionally, IP-10 concentration tended to be lower in the 71 patients who achieved SVR12 (median, 434.48 pg/mL; range, 151.35–4297.62) than in the 24 who did not (median, 549.71 pg/mL; range, 209.66–1768.81) ($P = 0.097$).

Association between IL28B genotype and treatment response

Of the 67 patients with IL28B TT, 53 achieved RVR, 11 did not and three were undetermined. Of the 27 patients with IL28B non-TT, 15 achieved RVR and 12 did not. RVR rate was significantly higher in patients with IL28B TT than non-TT genotypes (82.8% [53/64] vs 55.6% [15/27], $P = 0.009$). ETR (92.5% [62/67] vs 59.3% [16/27], $P < 0.001$) and SVR12 (84.6% [55/65]

vs 48.1% [13/27], $P = 0.001$) rates were also significantly higher in patients with IL28B TT than non-TT genotypes. All three patients not evaluated for IL28B SNP achieved RVR, ETR and SVR12.

Treatment response in treatment-naïve patients, relapsers and non-responders

Of 38 treatment-naïve patients, 31 (81.6%) each achieved RVR and SVR12. Of the 39 relapsers, three were not evaluated for RVR and two for SVR12. RVR was achieved by 29 of 36 evaluable patients (80.6%) and SVR12 by 31 of 37 (83.8%). Of the 20 non-responders, 11 (55%) achieved RVR and nine (45.0%) achieved SVR12.

Treatment response according to IL28B genotype and pretreatment serum IP-10 level

Patients were dichotomized relative to the median IP-10 concentration (461.83 pg/mL), with those having 460 pg/mL or more, and those with less than 460 pg/mL IP-10, defined as the high and low IP-10 groups, respectively. Of the 35 IL28B TT patients with low IP-10, 31 (88.6%) achieved RVR (31/35), and of the 29 IL28B TT patients with high IP-10, 22 (75.9%) achieved RVR ($P = 0.203$). Of the 11 IL28B non-TT patients with low IP-10, 10 (90.9%) achieved RVR (10/11), whereas, of the 16 IL28B non-TT patients with high IP-10, five (31.3%) achieved RVR ($P = 0.005$), indicating that IP-10 concentration was predictive of RVR in patients with IL28B non-TT genotypes. SVR12 rates were similar in IL28B TT patients with low (85.3% [29/34]) and high (83.9% [26/31]) baseline IP-10 ($P > 0.999$), as

well as in IL28B non-TT patients with low (63.6% [7/11]) and high (37.5% [6/16]) IP-10 ($P = 0.252$).

Factors contributing to RVR

Univariate analysis showed that HCV RNA of 6.8 log IU/mL or more ($P = 0.041$), IL28B genotype ($P = 0.009$) and IP-10 concentration ($P = 0.001$) were significant baseline predictors of RVR (Table 2). Multivariate analysis involving four factors with $P < 0.1$ in univariate analysis showed that IL28B genotype ($P = 0.025$) and IP-10 concentration ($P = 0.004$) were independent predictors of RVR. The hazard ratios (HR) and 95% CI for these factors are detailed in Table 2.

Factors contributing to SVR12

Univariate analysis showed that liver histology (F0–2 vs F3/4; $P = 0.034$), RVR ($P < 0.001$), IL28B genotype ($P = 0.001$) and discontinuation of all study drugs ($P < 0.001$) were significant predictors of SVR12 (Table 3). Multivariate analysis involving four factors (only pretreatment factors) with $P < 0.1$ in univariate analysis showed that IL28B genotype ($P = 0.001$) and platelet count ($P = 0.035$) were significant predictors of SVR12. The HR and 95% CI for these factors are detailed in Table 3.

RVR and SVR12 rates according to initial dose of TVR

Rapid virological response rates were similar in patients with initial TVR doses of 2250 mg/day (74.6% [47/63]) and 1500 mg/day (77.4% [24/31]). SVR12 rates in these two groups were also similar (74.6% [47/63] vs 75.0% [24/32]).

Treatment response according to IL28B genotype and pretreatment serum IP-10 level in patients with initial TVR dose of 2250 mg/day

Of the 65 patients who initially received 2250 mg/day TVR, 41 were IL28B TT, 21 were IL28B non-TT and three were undetermined. RVR (83.3% [20/24] vs 80.0% [12/15], $P > 0.999$) and SVR12 (81.8% [18/22] vs 94.1% [16/16], $P = 0.363$) rates were similar in IL28B TT patients with low and high IP-10. In contrast, the RVR rate was significantly higher in IL28B non-TT patients with low than high IP-10 (88.9% [8/9] vs 33.3% [4/12], $P = 0.024$), whereas SVR12 rate in patients with IL28B non-TT and low IP-10 was not significantly higher than that in patients with IL28B non-TT and high IP-10 (66.7% [6/9] vs 33.3% [4/12], $P = 0.198$).

Table 2 Univariate and multivariate analysis of factors contributing to RVR ($n = 94$)

Variables	Univariate analysis		Multivariate analysis		
	n	P-value	HR	95% CI	P-value§
Age	94	0.120†			
Sex (male/female)	55/39	>0.999‡			
Body mass index	94	0.604†			
Previous interferon therapy (yes/no)	56/38	0.331‡			
Liver histology					
F0–2/F3, 4	22/60	0.331‡			
A0, 1/A2, 3	44/38	0.193‡			
IL28B genotype, rs8099917 (TT/non-TT)	64/27	0.009‡	0.277	0.090–0.851	0.025
AST	94	0.372†			
ALT	94	0.447†			
Platelet count	94	0.075†	0.953	0.313–2.904	0.932
HCV RNA	94	0.041†	2.221	0.753–6.549	0.148
Pretreatment serum IP-10 level	94	0.001†	5.431	1.693–17.427	0.004

RVR was not evaluated in three patients.
†Mann–Whitney U-test.
‡Fisher's exact test.
§Logistic regression analysis.
95% CI, 95% confidence interval; ALT, alanine aminotransferase; AST, aspartate aminotransferase; HCV, hepatitis C virus; HR, hazard ratio; IP-10, interferon-γ-inducible protein-10; RVR, rapid virological response.

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Table 3 Univariate and multivariate analyses of factors contributing to SVR12 ($n = 95$)

Variables	Univariate analysis		Multivariate analysis		
	n	P-value	HR	95% CI	P-value§
Age	95	0.267†			
Sex (male/female)	54/41	>0.999‡			
Body mass index	95	0.246†			
Previous interferon therapy (yes/no)	57/38	0.238‡			
Liver histology					
F0–2/F3, 4	22/62	0.034‡	3.730	1.096–12.698	0.035
A0, 1/A2, 3	44/40	0.599‡			
Rapid virological response (yes/no)	69/23	<0.001‡			
IL28B genotype, rs8099917 (TT/non-TT)	65/27	0.001‡	0.130	0.038–0.438	0.001
Treatment discontinuation of all study drugs (yes/no)	17/78	<0.001‡			
AST	95	0.659†			
ALT	95	0.260†			
Platelet count	95	0.094†	1.006	0.305–3.322	0.992
HCV RNA	95	0.810†			
Pretreatment serum IP-10 level	95	0.097†	1.714	0.541–5.177	0.181

SVR12 was not evaluated in two patients.
†Mann–Whitney U-test.
‡Fisher's exact test.
§Logistic regression analysis.
Multivariate analyses included only pretreatment factors with $P < 0.1$ in univariate analyses.
95% CI, 95% confidence interval; ALT, alanine aminotransferase; AST, aspartate aminotransferase; HCV, hepatitis C virus; HR, hazard ratio; IP-10, interferon-γ-inducible protein-10; SVR12, undetectable HCV RNA 12 weeks after the completion of treatment.

Treatment response according to IL28B genotype and pretreatment serum IP-10 level in patients with initial TVR dose of 1500 mg/day

Of the 32 patients who initially received 1500 mg/day TVR, 26 were IL28B TT and six were IL28B non-TT. In terms of RVR and SVR12 rates, the difference between patients with IL28B TT and low IP-10 and those with IL28B TT and high IP-10 was not significant (RVR, 100% [11/11] vs 71.4% [10/14], $P = 0.105$; SVR12, 91.7% [11/12] vs 71.4% [10/14], $P = 0.330$). Because of the small sample size ($n = 6$), we did not perform subgroup analyses of patients with IL28B non-TT.

DISCUSSION

TO OUR KNOWLEDGE, few studies have examined the effects of pretreatment serum IP-10 concentration on virological responses in genotype 1 CHC patients treated with TVR-based triple therapy.[27] Baseline IP-10 has been found predictive of treatment outcomes in HCV genotype 1-infected patients treated with PEG IFN and RBV.[17,18,25] IL28B SNP has also been associated with virological responses to antiviral treatment

in HCV-infected patients.[12,13] However, currently, data on combining these predictors in patients with genotype 1 HCV infection treated with TVR-based triple therapy are limited; hence, the reason for the current study.

Our multivariate analyses showed that pretreatment serum IP-10 concentration was a significant predictor of RVR, but not of SVR12. In patients with the IL28B risk allele, the RVR rate was significantly higher in those with low than high IP-10 concentrations. The SVR12 rate also tended to be higher in the former subgroup, although the difference did not reach statistical significance, probably due to the small sample size. Similar results were observed in patients receiving initial TVR doses of 1500 and 2250 mg/day per protocol. These results suggest that, in patients with HCV genotype 1 treated with TVR-based triple therapy, baseline IP-10 level is useful for predicting virological response, especially in those with the IL28B risk allele who are considered difficult to treat.

We found that pretreatment serum IP-10 differed significantly ($P = 0.001$) in patients who did and did not achieve RVR. Low systemic IP-10 was found to predict a favorable first-phase decline in HCV RNA and RVR during treatment with PEG IFN and RBV.[22,34] Furthermore, among 45 HCV-infected patients treated with

TVR-based triple therapy, low pretreatment IP-10 level was associated with a very rapid virological response (undetectable HCV RNA at 2 weeks).[27] Although treatment regimen or timing of virological evaluation differed in this and previous studies, the results were generally similar.

Assessments of the associations between baseline IP-10 and other baseline clinical characteristics showed that IP-10 concentration correlated significantly with liver fibrosis and inflammation. IP-10 was significantly correlated with platelet count, reflecting fibrosis, and AST and ALT concentrations, reflecting inflammation. Furthermore, our AUC results showed that IP-10 levels were closely related to liver histological findings, confirming that IP-10 level is useful for predicting the extent of liver disease.[19,20] Circulating IP-10 concentrations were found to correlate with intrahepatic levels of IP-10 mRNA.[22] Higher intrahepatic IP-10 mRNA may attract inflammatory cells into the liver, leading to the progression of liver fibrosis and inflammation. Higher circulating IP-10 levels may result in the accumulation of effector T cells in the liver, with the selective pressure imposed by this accumulation fostering the outgrowth of immune escape HCV mutants that are more difficult to eradicate with PEG IFN and RBV combination therapy.[17] It is of interest that age was almost significantly correlated with baseline IP-10 level in our study ($r_s = 0.200$, $P = 0.050$). Asahina *et al.* demonstrated that advanced age was related to advanced liver histological findings.[35]

Although a previous study reported significant differences in serum IP-10 concentrations between patients with different IL28B genotypes,[36] we did not observe similar findings. The reasons for these discrepancies are unclear, although they may have been due to racial differences. IP-10 concentrations were significantly lower in treatment-naïve than in relapsing patients and non-responders. This may have been due to the lower rates of F3/F4 liver fibrosis among treatment-naïve (21.1% [8/38]) than relapsing patients (24.2% [8/33]) and non-responders (42.9% [6/14]); and to the lower rates of A2/A3 liver inflammation in treatment-naïve patients (42.1% [16/38]) than in relapsers (48.5% [16/ 33]) and non-responders (57.1% [8/14]).

We found that RVR and SVR12 rates were comparable in patients with reduced initial TVR dose of 1500 mg/ day and those with initial TVR dose of 2250 mg/day. Although investigating the impact of initial TVR dose on treatment outcomes was beyond the scope of this analysis, reduced initial dose of TVR may be as effective as the standard dose in some patients with HCV genotype 1.

This study had several limitations, including its retrospective design and relatively small sample size. Moreover, treatment outcomes were missing for some patients, which may have introduced bias. Additionally, adherence to each study drug was not assessed, which may have also led to bias. Lastly, IFN-free regimens based on several DAA are expected to be approved in the near future. Pretreatment factors that may predict virological responses in patients receiving TVR-based triple therapy may thus be inapplicable in patients treated with these new regimens. Hence, our study results should be interpreted with caution and further, larger prospective studies will be required. However, our results demonstrated that pretreatment serum IP-10 level was associated with virological response in patients with genotype 1 CHC undergoing TVR-based triple therapy, and combined evaluation of IP-10 and IL28B genotype may improve prognostication of virological response. In addition, IP-10 correlated well with liver histological findings.

In conclusion, we found that pretreatment serum IP-10 concentration correlated with liver fibrosis and inflammation in patients with HCV genotype 1 treated with TVR-based triple therapy and was predictive of virological responses, especially in patients with the IL28B risk allele.

ACKNOWLEDGMENTS

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Review Article

Treatment for hepatocellular carcinoma in Japan over the last three decades: Our experience and published work review

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Hepatocellular carcinoma (HCC) is one of the most common causes of cancer-related mortality worldwide. In the last few decades, there has been a marked increase in therapeutic options for HCC and epidemiological characteristics at HCC diagnosis have also significantly changed. With these changes and advances in medical technology and surveillance program for detecting earlier stage HCC, survival in patients with HCC has significantly improved. Especially, patients with liver cirrhosis are at high risk of HCC development, and regular surveillance could enable early detection of HCC and curative therapy, with potentially improved clinical outcome. However, unfortunately, only 20% of HCC patients are amenable to curative therapy (liver transplantation, surgical resection or ablative therapies). Locoregional therapies such as radiofrequency ablation, percutaneous ethanol injection, microwave coagulation therapy and transcatheter arterial chemoembolization play a key role in the management of unresectable HCC. Currently, molecular-targeted agents such as sorafenib have emerged as a promising therapy for advanced HCC. The choice of the treatment modality depends on the size of the tumor, tumor location, anatomical considerations, number of tumors present and liver function. Furthermore, new promising therapies such as gene therapy and immunotherapy for HCC have emerged. Approaches to the HCC diagnosis and adequate management for patients with HCC are improving survival. Herein, we review changes of epidemiological characteristics, prognosis and therapies for HCC and refer to current knowledge for this malignancy based on our experience of approximately 4000 HCC cases over the last three decades.

Key words: hepatocellular carcinoma, progress, surveillance, three decades, treatment

INTRODUCTION

HEPATOCELLULAR CARCINOMA (HCC) is one of the most common causes of cancer-related mortality worldwide in terms of incidence with 626 000 new cases per year, accounting for 5.7% of all new cancer cases.[1–6] HCC represents more than 90% of primary liver cancer.[1–6] Annual incidence rates of HCC are highest in sub-Saharan Africa and East Asia, where approximately 85% of all cases occur.[1,6] This malignancy tends to occur in livers damaged through chronic infection with hepatitis B and C or alcohol abuse on a background of cirrhosis.

The therapies of HCC have markedly changed in the last few decades.[1–6] Furthermore, in our country, epidemiological characteristics such as age, disease stage at HCC diagnosis and causes of background liver disease for HCC have also significantly changed in the last few decades. With these changes and advances in medical technology such as diagnostic imaging and surveillance programs for detecting earlier stage HCC, survival in patients with HCC has significantly improved. Especially, patients with liver cirrhosis (LC) are at high risk of HCC development. The initiation of surveillance for HCC involves identifying high-risk populations for

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HCC development that would benefit from cancer screening.[7] Regular surveillance for these high-risk populations could enable early detection of HCC and curative therapy, with potentially improved clinical outcome.[7] However, unfortunately, only 20% of HCC patients are amenable to curative therapy (liver transplantation [LT], surgical resection [SR] or ablative therapies). HCC often recurs even after curative therapy and survival in HCC patients with advanced stage remains poor.[1,2,4,6] Locoregional therapies such as radiofrequency ablation (RFA), percutaneous ethanol injection (PEI), microwave coagulation therapy and transcatheter arterial chemoembolization (TACE) play a key role in the management of unresectable HCC. These non-surgical treatments for HCC have also significantly improved in the last few decades and have shown survival benefits for selected patients with HCC.[2,4,8-11] Currently, molecular-targeted agents such as sorafenib have emerged as promising therapies for advanced HCC.[5] The choice of the treatment modality depends on the size of the tumor, tumor location, anatomical considerations, number of tumors present and liver function.[2,4-11] Furthermore, new promising therapies such as gene therapy and immunotherapy for HCC have emerged.[12,13] Approaches to the HCC diagnosis and adequate management for patients with HCC are improving survival.

In this article, we review changes of epidemiological characteristics, prognosis and therapies for HCC and refer to current knowledge for this cancer based on our experience of approximately 4000 HCC cases over the last three decades. Because our experience included vast number of HCC cases, our data will well reflect actual situations of HCC therapy in Japan.

CURRENT TRENDS IN HCC PATIENTS

BASELINE CHARACTERISTICS IN 4165 patients diagnosed with HCC between 1981 and 2013 in our hospital are shown in Table 1. Current annual trends of age and sex in HCC patients are shown in Figure 1. For over the last three decades, the average age in patients diagnosed with HCC has risen from approximately 60 years to 70 years and the proportion of female HCC patients has been slightly increasing. An aging society means that the number of elderly patients with cancer is predicted to rise in the future.[14,15] HCC patients are not an exception. In Japan, 75-year-old men and women have an average expected lifespan of approximately 5 and 10 years, respectively, and Japan has the greatest longevity in the world.[16] The increased longevity of the population means that more aged HCC patients

Table 1 Baseline characteristics of 4165 patients diagnosed with hepatocellular carcinoma (HCC) between 1981 and 2013 in our hospital

	n (%) or mean ± SD
Male/female	2954 (70.9%)/1211 (29.1%)
Age, mean ± SD (range) (years)	66.2 ± 9.5 (17–95)
Child–Pugh classification	
Child–Pugh A/B/C	2571 (65.4%)/1095 (27.9%)/ 265 (6.7%)
Cause of liver disease	
B/C/B and C/non-B, non-C	460 (12.0%)/2734 (71.4%)/83 (2.2%)/551 (14.4%)
Background liver	
LC/CH/fatty liver/normal liver	3073 (75.5%)/860 (21.1%)/11 (0.3%)/125 (3.1%)
HCC stage	
Stage I/II/III/IVA/IVB	722 (18.1%)/1467 (36.8%)/ 1175 (29.5%)/501 (12.6%)/ 121 (3.0%)

CH, chronic hepatitis; HCC, hepatocellular carcinoma; LC, liver cirrhosis; SD, standard deviation.

are to be expected in the coming years. The proportion of elderly patients with HCC and their average age are increasing in Japan.[15,17,18] The age at HCC diagnosis has increased in parallel with the increased proportion of elderly patients infected with HCV.[19,20] These trends have thus led to a rising demand in our country for investigations related to clinical characteristics and outcomes of therapy in elderly patients with HCC.
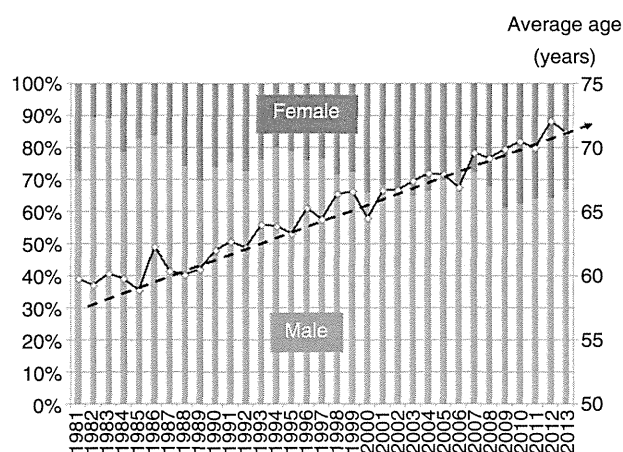

Figure 1 Annual trends in patients with hepatocellular carcinoma (1981–2013, age and sex, Osaka Red Cross Hospital, Japan).