Vascular and Interventional Radiology • Original Research

Impact of a Unified CT Angiography System on Outcome of Patients with Hepatocellular Carcinoma

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OBJECTIVE. The purpose of our study was to evaluate the impact of a unified CT angiography (CTA) system for the management of patients with hepatocellular carcinoma (HCC).

SUBJECTS AND METHODS. A total of 1,312 patients with HCC who had been diagnosed and treated between 1990 and 2003 were studied. The clinical characteristics and survival rate were compared between patients who underwent pretreatment evaluation of tumor progression by a unified CTA system and those who underwent evaluation by a conventional angiography system. In addition, the survival rates for 438 patients who underwent transcatheter arterial chemoembolization (TACE) as initial treatment were compared between patients who were treated using a unified CTA system and those who were treated without the system.

RESULTS. Overall, the survival rate was higher in patients who underwent pretreatment examination using a unified CTA system than in those who underwent examination without it ($p < 0.0001$). The survival rate was higher when focusing on patients with HCC of stage I ($p = 0.0093$). In patients who underwent TACE as an initial treatment, the survival rate was higher in patients treated by TACE using a unified CTA system than in those without it ($p = 0.0023$).

CONCLUSION. The more accurate and detailed pretreatment evaluation of HCC progression using a unified CTA system contributed to the improvement of survival of patients with HCC. In addition, it contributed to the improved efficacy of TACE with an increased survival rate.

Hepatocellular carcinoma (HCC) is one of the most common malignancies, especially in southern and eastern Asia. The incidence of HCC is also increasing in the United States [1, 2]. The prognosis of patients with HCC has improved because of improvements in the management of such patients, including increased early detection of HCC, development of novel treatment options and enhanced treatment techniques, and the accurate evaluation of tumor progression [3]. Detailed and accurate evaluation of the progression of HCC, including the determination of the size, number, and location of tumors at diagnosis and the accurate evaluation of treatment response, is an important factor in the management of patients with HCC and contributes to the overall improved outcome.

Examination of liver tumor with CT during angiography, that is, CT during arterial portography (CTAP) [4] and CT during hepatic arteriography (CTHA) [5], reportedly provides precise and detailed information, including the size, number, and location of HCC in the entire liver and vascular invasion by HCC [6–8]. In addition, in patients who underwent transcatheter arterial chemoembolization (TACE) as a treatment for HCC, the confirmation of the feeding arteries toward the tumor using CT in addition to digital subtraction arteriography (DSA) will improve the accuracy of the selection of arteries that should be embolized. Also, combination of TACE with simultaneous evaluation of its results using CT will improve the treatment efficacy.

A unified CT angiography (CTA) system was developed in 1996 [9, 10] and has been used in several liver centers in Japan. This system integrates CT and angiography and allows immediate and repeated CT examination during angiography examinations or the TACE procedure. We have applied this system for all angiography examinations or the TACE procedure for HCC in our institution since July 1997. In the present study, we evaluated the usefulness of a unified CTA system for the management of patients with HCC.

Keywords: hepatocellular carcinoma, survival, transcatheter arterial chemoembolization, tumor progression, unified CT angiography system

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Subjects and Methods

Patients and Angiography Examination

A total of 1,312 patients were diagnosed with an initial HCC (not a recurrence) and underwent treatment for HCC at our institution between 1990 and 2003. The background characteristics of the study patients are shown in Table 1 (348 women and 964 men; mean age, 65.5 ± 9.3 years). Patients were diagnosed with HCC on the basis of histologic examination of tumor tissue taken from resected or biopsy specimens in 401 cases (30.6%). In the remaining 911 patients, diagnosis was made on the basis of clinical criteria [11, 12]: a pertinent clinical background (association with liver cirrhosis or viral hepatitis) and typical imaging findings. Typical imaging features of HCC include a mosaic pattern with a halo on B-mode sonographic images, hypervascularity on angiographic images, and a high-density mass on arterial phase dynamic CT images with a low-density mass on portal phase dynamic CT images obtained with a helical or MDCT scanner. CTAP and CTHA were performed when typical findings for HCC were not obtained by means of dynamic CT or angiography before July 1997, at which time a unified CTA system was introduced in our institution. CTAP and CTHA were performed in all patients after July 1997, and T1- and T2-weighted imaging associated with superparamagnetic iron oxide–enhanced MRI was performed when typical findings for HCC were not obtained by means of CTAP and CTHA during this period. In cases without typical features on imaging studies, a biopsy was performed to confirm the diagnosis of HCC. Of the 1,312 patients, 294 (22.4%) underwent hepatectomy, 271 (20.7%) were treated by locoregional ablative therapy including ethanol injection, microwave thermocoagulation, or radiofrequency ablation; and 438 (33.4%) were treated using TACE.

All patients underwent angiography for the diagnosis of HCC before treatment, regardless of the treatment options applied and including patients who did not undergo treatment as a consequence. The size, number, and location of tumors and portal vein invasion by the tumor were evaluated at angiography through hepatic arteriography and arterial portography. Angiography was performed without a unified CTA system in 603 patients before July 1997. CTAP was performed only when necessary for the diagnosis of HCC, with transportation of the patient from angiography to CT during this period. After July 1997, angiography was performed using the unified CTA system in all 709 patients. CTAP and CTHA were performed for all patients during this period. Patients were followed up from 0.4 months to 100.8 months (median, 16.9 months) at our institution.

The study protocol was approved by the hospital ethics committee and was in compliance with the Helsinki Declaration. Written informed consent was obtained from all patients before the study for use of the clinical, laboratory, and outcome data.

A Unified CTA System

A unified CTA system was introduced in our institution in July 1997. The unified system of helical CT and angiography (Interventional-CT, Toshiba Medical Systems) [9, 10] consists of helical CT (X-Vision Real, Toshiba Medical Systems) and angiography units in which DSA equipment with a C-arm (Angiorex, BLA-800A, Toshiba Medical Systems) is arranged in a linear configuration to form a common couch, facilitating quick transportation of the patient from one unit to the other for CT, including CTAP and CTHA, without risking dislodgment of the catheter.

TACE

Individual decisions regarding treatment of HCC were determined by the treating physicians and according to each patient's wishes. Patients were assessed initially for eligibility for hepatectomy. Principally, patients with three or fewer HCC tumors were recommended to undergo hepatectomy when they had Child-Pugh class A liver function. Those who declined or were deemed not eligible for hepatectomy were considered for locoregional ablative therapy with percutaneous ethanol injection, percutaneous microwave thermocoagulation, or radiofrequency ablation. Locoregional ablative therapy was selected as a treatment in patients with three or fewer HCC tumors that were < 3 cm in maximum size. Patients who were not eligible for either hepatectomy or locoregional ablative therapy and had no extrahepatic spread or portal vein thrombosis were offered TACE. This treatment policy was constant throughout the study period. No patient underwent liver transplantation as a treatment for HCC.

As a result, a total of 438 patients underwent TACE as an initial primary treatment for HCC. TACE was performed immediately after angiography for the diagnosis of HCC or at the latest within 2 weeks after diagnosis. TACE was performed solely with an angiography apparatus in 219 patients before July 1997 and with a unified CTA system in the other 219 patients after July 1997.

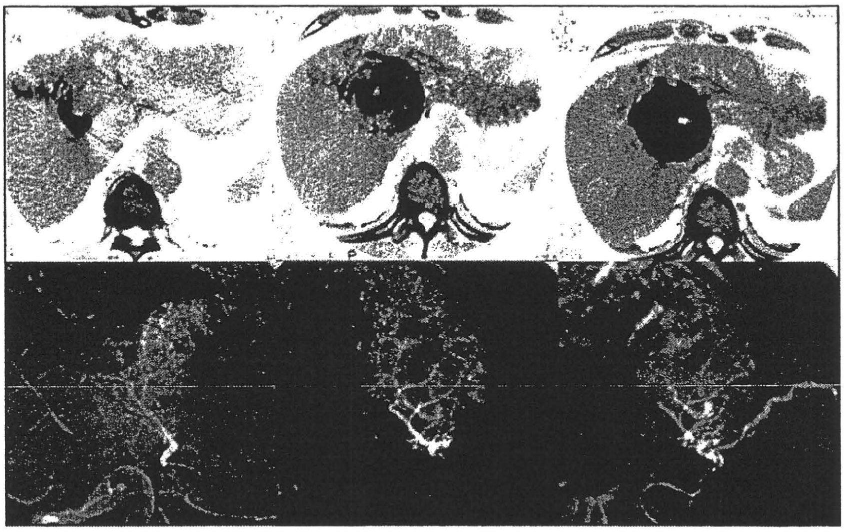
Before TACE, a baseline angiography with arterial portography and hepatic arteriography was performed in all patients. Hepatic arteriography in the right anterior oblique position was performed if deemed necessary. On the basis of the baseline study findings, the feeding artery was determined. Subsequently, a 2.5- or 2.8-French, 135-cm-long microcatheter (Tracker-18 or Renegade, Boston Scientific) with a double angle–shaped 0.016-inch, 180-cm-long guidewire covered by hydrophilic polymer (Radifocus, Terumo) was advanced through a 5-French catheter into the peripheral portion of the feeding artery as close to the lesion as possible. If the predicted noncancerous hepatic portion to be embolized was large enough

TABLE 1: Background Characteristics of Study Patients (n = 1,312)

Parameter	Value
Age (y)	65.5 ± 9.3
Sex (%)	
M	964 (73.5)
F	348 (26.5)
Total bilirubin (mg/dl)	1.3 ± 1.9
Albumin (g/dl)	3.3 ± 0.6
Prothrombin time (%)	81.9 ± 18.6
Child-Pugh classification	
A	794 (60.5)
B	402 (30.6)
C	116 (8.9)
Maximum tumor size (cm)	
< 2	442 (33.7)
2–5	469 (35.7)
> 5	401 (30.6)
Number of tumors	
Single	643 (49.0)
Multiple	669 (51.0)
Portal vein invasion	
Absent	1,025 (78.1)
Present	287 (21.9)
Tumor stage[a]	
I	288 (21.9)
II	421 (32.2)
III	315 (24.0)
IV	288 (21.9)
Initial treatment	
Surgery	294 (22.4)
Locoregional ablative therapies[b]	271 (20.6)
Transcatheter arterial chemoembolization	438 (33.4)
Other	97 (7.4)
None	212 (16.2)

Note—Data in parentheses are percentages.
[a]TNM tumor stage according to the Liver Cancer Study Group of Japan.
[b]Locoregional ablative therapies include percutaneous ethanol injection, percutaneous microwave thermocoagulation, and radiofrequency ablation.

A



B

Fig. 1—72-year-old man who underwent transcatheter arterial chemoembolization (TACE) for hepatocellular carcinoma (HCC).
A, Angiography image shows three arteries (*arrows* 1, 2, and 3) feeding to HCC.
B, TACE images show each artery was embolized with mixture of anticancer agent and lipiodol followed by gelatin sponge particles (lower row, numbers correspond to numbered arteries in A) that was confirmed by retention of lipiodol on CT images (upper row) immediately after TACE. TACE procedure was completed after confirmation of retention of lipiodol in entire HCC tumor.

to deteriorate the hepatic functional reserve, the catheter tip was advanced to a more peripheral part of the feeding artery. TACE was performed with an injection into the feeding artery of an emulsion of 50 mg of farmorubicin hydrochloride (Epirubicin, Adria) dissolved in 5 mL of iopamidol (Iopamiron, 370 mg I/mL, Schering Tokyo) mixed with 5 mL of iodized oil (Lipiodol Ultra-Fluid, Guerbet), followed by an injection of gelatin sponge particles (Gelfoam, Upjohn). The end point of TACE was cessation of arterial blood flow. If deposition of iodized oil in the lesion was inadequate, another feeding artery was sought and TACE was performed again.

In 219 patients who underwent TACE with a unified CTA system after July 1997, both CTAP and CTHA examinations were performed for all patients as the baseline study. When the catheter tip was advanced into the peripheral portion of the feeding artery, follow-up CT arteriography was performed before TACE to confirm the possible feeding artery supplying the targeted lesion and the expected extent of embolization of the noncancerous portion. The deposition of the iodized oil in the lesion was examined using unenhanced CT immediately after TACE. If deposition of iodized oil in the lesion was inadequate on CT, another feeding artery was sought and CT arteriography was performed again to confirm the feeder supplying the lesion with an undeposited portion before performing TACE again (Fig. 1). When the feeding artery was not found by arteriography through the hepatic artery, other arteries that were not the branch of the hepatic artery, including the infraphrenic artery, renal artery, or adrenal artery, were examined as the possible feeding artery (Fig. 2). The complete deposition of iodized oil in the lesion was confirmed using unenhanced CT to complete treatment.

Statistical Analyses

Numeric data are expressed as mean ± SD values unless otherwise specified. Differences in proportions of the number of patients between groups were analyzed by a chi-square test. Differences in quantitative values were analyzed by the Student's t test if the data were normally distributed; otherwise, differences were analyzed by the Mann-Whitney U test.

In the analysis of overall study patients, the date of HCC diagnosis was defined as time zero for calculations of patient survival. In the analysis of patients who underwent TACE, the date of TACE was defined as time zero for calculations of patient survival. Surviving patients and patients who died from causes other than liver disease were censored. Patients who died from HCC-related causes or liver failure were not censored. The Kaplan-Meier method was used to calculate survival rates, and the log-rank test was used to analyze differences in survival. The Cox proportional hazards model was used for multivariate analysis for factors that influenced patient survival. The variables analyzed were age, sex, Child-Pugh class, TNM tumor stage according to the Liver Cancer Study Group of Japan (see Appendix 1) [13], and the use of the unified CTA system for TACE. The JMP statistical software package, version 4.0 (SAS Institute), was used for all statistical analyses. All p values were derived from two-tailed tests, and $p < 0.05$ was accepted as statistically significant.

Results

Characteristics and Survival Rates of Patients Examined by Angiography With or Without a Unified CTA System

The background characteristics of patients who underwent angiographic evaluation for HCC with a unified CTA system and those who underwent angiographic evaluation for HCC without it are compared in Table 2. With regard to liver function at the diagnosis of HCC, patients examined with a unified CTA system had lower serum albumin levels ($p = 0.0079$) than those examined without it. As for the progression of HCC, the prevalence of patients with multiple tumors at diagnosis tended to be higher in patients examined with a unified CTA system than those examined without it ($p = 0.0864$). However, the prevalence of findings at the earliest stage of HCC (HCC stage I according to the Liver Cancer Study Group of Japan [13]) was higher in patients examined with a unified CTA system than those without it ($p = 0.0337$). In contrast, the prevalence of findings at the most advanced HCC (HCC stage IV) was higher in patients examined without a uni-

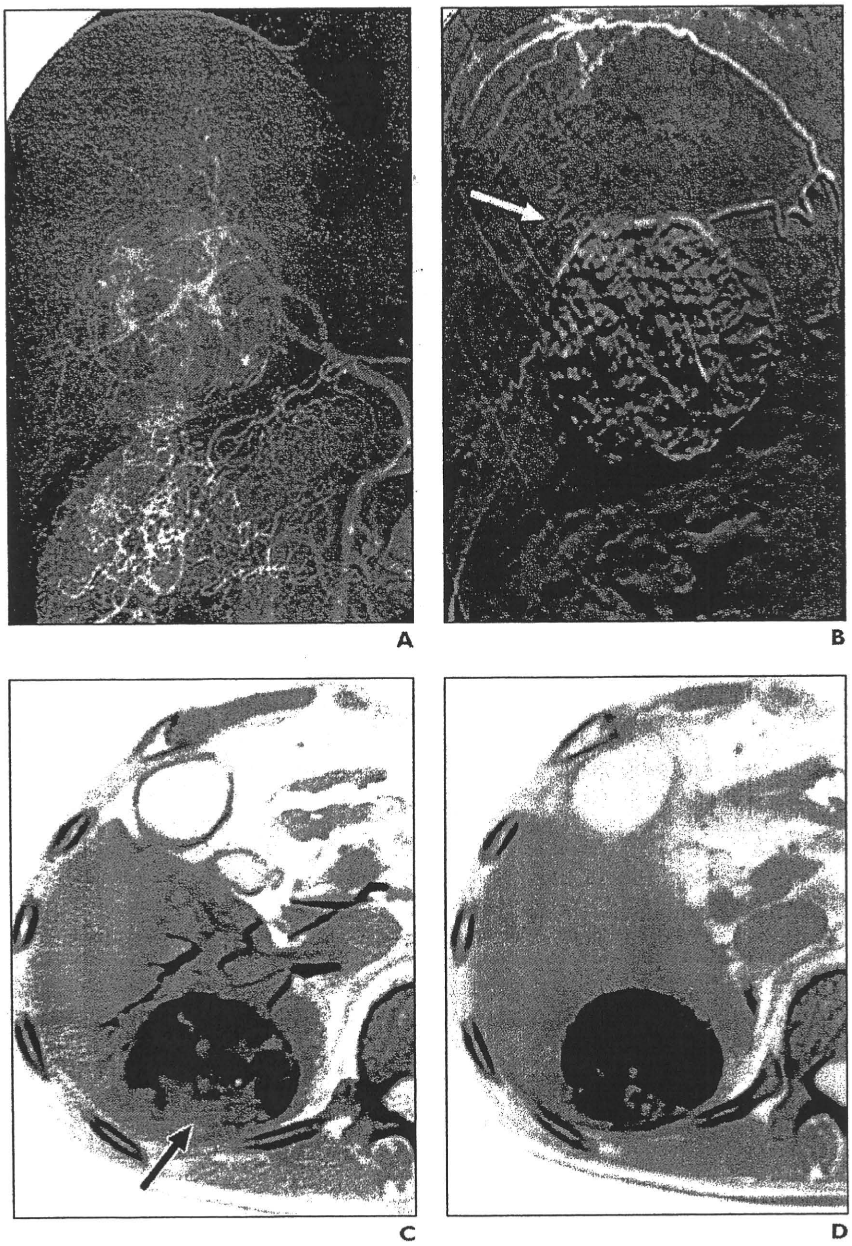


Fig. 2—64-year-old woman who underwent transcatheter arterial chemoembolization (TACE) for hepatocellular carcinoma (HCC) with feeding from the extrahepatic artery.
A and B, Angiography images show HCC was fed from branches of hepatic artery and from branch of right infraphrenic artery (*arrow*, **B**).
C, CT image shows that there was part of HCC that lacked retention of lipiodol after embolization of branches of hepatic artery (*arrow*).
D, CT image shows retention of lipiodol in entire HCC tumor after embolization of branch of right infraphrenic artery, and TACE procedure was completed.

fied CTA system than those examined with it ($p = 0.0012$).

The overall survival rate of patients who underwent angiography with a unified CTA system was significantly higher than that of patients who underwent angiography without it ($p < 0.0001$) (Fig. 3). When focusing on patients with stage I HCC tumor progression (single HCC with a maximum diameter of < 2 cm and without portal vein invasion), the survival rate of 172 patients who underwent pretreatment examination with a unified CTA system was significantly higher than that of 116 patients who underwent the examination without it ($p = 0.0093$) (Fig. 4). In contrast, no significant difference was found in survival rates between patients examined with a unified CTA system and those without it when focusing on patients with HCC at stages II, III, or IV, respectively (data not shown).

Characteristics and Survival Rates of Patients Who Underwent TACE With or Without a Unified CTA System

TACE was performed as a treatment for initial HCC (not a recurrence) solely with an angiography apparatus in 219 patients before July 1997 and with a unified CTA system in the other 219 patients after July 1997. The background characteristics of patients who underwent TACE with and without a unified CTA system are shown in Table 3. Patients who underwent TACE with a unified CTA system were older than those who underwent TACE without it ($p < 0.0001$). With regard to liver function, patients treated by TACE with a unified CTA system had higher serum albumin levels ($p = 0.0203$) but higher 15-minute retention of indocyanine green ($p = 0.0461$) than those treated by TACE without a unified CTA system. As for the progression of HCC, the prevalence of patients with HCC greater than 5 cm in diameter was higher in patients treated by TACE without a unified CTA system than those treated by TACE with it ($p = 0.0085$). Consequently, the prevalence of patients with stage IV HCC was higher in patients treated by TACE without a unified CTA system than those treated with it ($p = 0.0078$). Locoregional ablative therapy (one or two sessions) was performed as an additional treatment with TACE within 2 weeks after the TACE procedure in seven patients treated by TACE without a unified CTA system and in 10 patients treated by TACE with the unified CTA system. Fourteen patients who underwent TACE without a unified CTA system for treatment of initial HCC received TACE with a unified CTA system as a treatment of recurrent HCC.

The rate of local control without local recurrences was compared between patients who underwent TACE with and without a unified CTA system, excluding patients with stage IV HCC, which is far advanced and usually cannot be controlled by TACE. Local control was achieved by TACE in 84 of

TABLE 2: Study Patients Examined With and Without a Unified CT Angiography (CTA) System

Patients Characteristics (n = 1,312)	Without Unified CTA System (n = 603)	With Unified CTA System (n = 709)	p
Age (y)	65.6 ± 9.2	65.3 ± 9.4	0.6270
Sex			0.9582
M	443 (73.5)	521 (73.5)	
F	160 (26.5)	188 (26.5)	
Total bilirubin (mg/dL)	1.3 ± 1.9	1.4 ± 2.1	0.6348
Albumin (g/dL)	3.4 ± 0.6	3.3 ± 0.6	0.0079
Prothrombin time (%)	81.7 ± 19.1	82.1 ± 18.1	0.9876
Child-Pugh classification			0.9137
A	369 (61.2)	425 (59.9)	
B	181 (30.0)	221 (31.2)	
C	53 (8.8)	63 (8.9)	
Maximum tumor size (cm)			0.5104
< 2	213 (35.3)	229 (32.3)	
2–5	211 (35.0)	258 (36.4)	
> 5	179 (29.7)	222 (31.3)	
Number of tumors			0.0864
Single	311 (51.6)	332 (46.8)	
Multiple	292 (48.4)	377 (53.2)	
Portal vein invasion			0.2327
Absent	480 (79.6)	545 (76.9)	
Present	123 (20.4)	164 (23.1)	
Tumor stage[a]			0.0055
I	116 (19.2)	172 (24.2)	
II	188 (31.2)	233 (32.9)	
III	142 (23.6)	173 (24.4)	
IV	157 (26.0)	131 (18.5)	

Note—Data in parentheses are percentages.
[a]TNM tumor stage according to the Liver Cancer Study Group of Japan.

173 (48.6%) patients treated by TACE without a unified CTA system and in 126 of 198 (63.6%) patients treated by TACE with a unified CTA system. The local control rate of patients who underwent TACE with a unified CTA system was significantly higher than that of patients who underwent TACE without it (p = 0.0048). The survival rate was also compared between patients who underwent TACE with and without a unified CTA system, excluding patients with stage IV HCC (Fig. 5). The survival rate of patients who underwent TACE with a unified CTA system was significantly higher than that of patients who underwent TACE without it (p = 0.0023). This difference in survival rate was maintained when excluding 17 patients who underwent locoregional ablative therapy as an additional treatment after TACE (p = 0.0094) (Fig. 6). We conducted multivariate analysis for the factors that influenced patient survival rate (Table 4). The use of a unified CTA system on TACE had an independent impact on increasing survival rates (p = 0.0387) as did Child-Pugh class and TNM tumor stage.

Discussion

The efficacy of CT during angiography—that is, CTAP and CTHA—has been shown to be beneficial for accurate evaluation of HCC [6–8]. Although one study reported a high rate of false-positive findings of HCC tumors with this method [14], this high false-positive rate could be attributed to the lack of analysis for the coronal enhancement that is observed in the late phase of CTHA in HCC [15]. The unified CTA system made it easy to perform both CTAP and CTHA without transporting a patient from the angiography

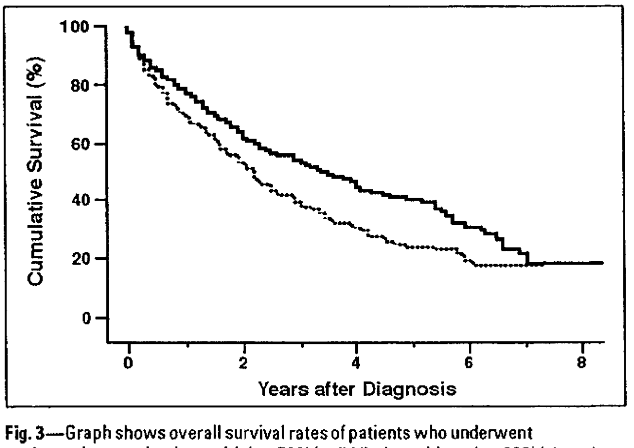


Fig. 3—Graph shows overall survival rates of patients who underwent angiography examinations with (n = 709) (*solid line*) or without (n = 603) (*dotted line*) unified CT angiography (CTA) system. Survival rate of patients who underwent angiography with unified CTA system was significantly higher than that of patients who underwent angiography without it (p < 0.0001).

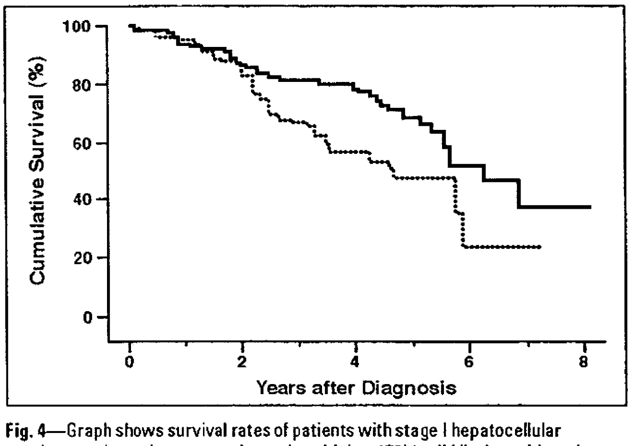


Fig. 4—Graph shows survival rates of patients with stage I hepatocellular carcinoma who underwent angiography with (n = 172) (*solid line*) or without (n = 116) (*dotted line*) unified CT angiography (CTA) system. Survival rate of patients who underwent angiography with unified CTA system was significantly higher than that of patients evaluated by angiography without it (p = 0.0093).

TABLE 3: Study Patients Who Underwent Transcatheter Arterial Chemoembolization (TACE) With and Without Unified CT Angiography (CTA) System

Patient Characteristics (n = 438)	Without Unified CTA System (n = 219)	With Unified CTA System (n = 219)	p
Age (y)	63.3 ± 8.5	68.3 ± 8.9	< 0.0001
Sex			0.0963
M	173 (79.0)	157 (71.7)	
F	46 (21.0)	62 (28.3)	
Total bilirubin (mg/dl)	1.2 ± 1.1	1.1 ± 0.7	0.3214
Albumin (g/dl)	3.2 ± 0.6	3.3 ± 0.5	0.0203
Prothrombin time (%)	81.4 ± 19.0	80.0 ± 16.3	0.4643
15-minute retention of ICG	24.2 ± 14.4	26.5 ± 13.1	0.0461
Child-Pugh classification			0.3458
A	119 (54.3)	134 (61.2)	
B	90 (41.1)	77 (35.2)	
C	10 (4.6)	8 (3.6)	
Maximum tumor size (cm)			0.0085
< 2	48 (21.9)	53 (24.2)	
2–5	92 (42.0)	116 (53.0)	
> 5	79 (36.1)	50 (22.8)	
Number of tumors			0.9203
Single	76 (34.7)	78 (35.6)	
Multiple	143 (65.3)	141 (64.4)	
Portal vein invasion			0.2843
Absent	191 (87.2)	199 (90.9)	
Present	28 (12.8)	20 (9.1)	
Tumor stage[a]			0.0078
I	16 (7.3)	24 (11.0)	
II	73 (33.3)	78 (35.6)	
III	84 (38.4)	96 (43.8)	
IV	46 (21.0)	21 (9.6)	

Note—Data in parentheses are percentages. ICG = indocyanine green.
[a]TNM tumor stage according to the Liver Cancer Study Group of Japan.

apparatus to the CT scanner and vice versa and allowed these examinations to be performed easily on all patients. Use of the unified CTA system allowed CTAP and CTHA for all patients during angiography, resulting in an increase in the accuracy of the evaluation of HCC progression.

The prevalence of patients with stage I HCC (earliest stage HCC) was higher in patients examined with a unified CTA system than in patients examined without it. The surveillance system for the early detection of HCC has greatly improved in Japan during the study period [3, 16]. This strongly contributed to the increase in the percentage of findings of stage I HCC at the time of diagnosis in patients who underwent examination with a unified CTA system because HCC was diagnosed in these patients after July 1997 when the surveillance system was improved compared with the period before July 1997.

Despite the increase in the percentage of patients with HCC of an earlier stage, the prevalence of patients with multiple tumors at diagnosis tended to be higher in patients who underwent examination with a unified CTA system than in those who underwent examination without it. This indicates that the detailed examination with CTAP and CTHA enabled the detection of minute HCC that had not been detected with conventional CT, sonography, or DSA, resulting in an increase in the number of HCC findings at diagnosis. The accurate evaluation of the progression of HCC at the time of diagnosis could contribute to the appropriate choice of treatment technique and to a decrease in the amount of HCC sites that are missed for treatment. Consequently, the overall survival rate of patients with HCC examined with a unified CTA system was significantly higher than that of patients with HCC examined without the unified CTA system. The significantly higher survival rate in patients examined with a unified CTA system was especially evident when focusing on patients with stage I HCC. This indicates that the higher survival rate in patients examined with a unified CTA system is not simply due to the increase in detection of early HCC that arose with improved surveillance during the period of July 1997 through 2003 compared with the period of 1990 through July 1997. This indicates that patients with stage I HCC examined with the unified CTA system were more strictly evaluated and, therefore, were more accurately classified as stage I HCC in comparison with the patients with stage I HCC examined without a unified CTA system. However, there were several developments in the diagnostic and treatment technology during the study period, and these factors might also have contributed to the higher survival rate of patients with HCC examined using a unified CTA system.

TACE was initially used to treat HCC by Doyon et al. [17] in 1974 and in Japan was applied to inoperable HCC using gelatin sponge particles and anticancer agents [18]. In the mid 1980s, lipiodol was newly introduced, primarily to enhance the therapeutic effect [19–23]. TACE with the injection of a mixture of an anticancer agent and lipiodol followed by embolization with gelatin sponge particles is now the mainstay treatment of choice for noncurative HCC [24–29]. Although the survival benefits of TACE have been controversial [30–33], recent randomized controlled trials showed the survival benefits of TACE compared with a control population [34, 35].

The ideal TACE for HCC should be a superselective catheterization into the feeding artery followed by an injection of the emulsion of iodized oil and anticancer agent deposited only in the targeted lesion with no washout of iodized oil. This should be achieved in only one session, and the noncancerous liver tissue should remain completely free from unnecessary embolization.

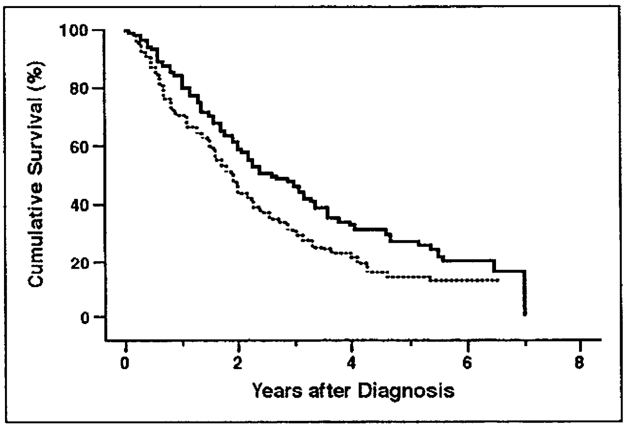


Fig. 5—Graph shows survival rates of patients, excluding those with stage IV hepatocellular carcinoma, who underwent transcatheter arterial chemoembolization (TACE) with (n = 219) (*solid line*) or without (n = 219) (*dotted line*) unified CT angiography (CTA) system. Survival rate of patients who underwent TACE with unified CTA system was significantly higher than that of patients who underwent TACE without unified CTA system (p = 0.0023).

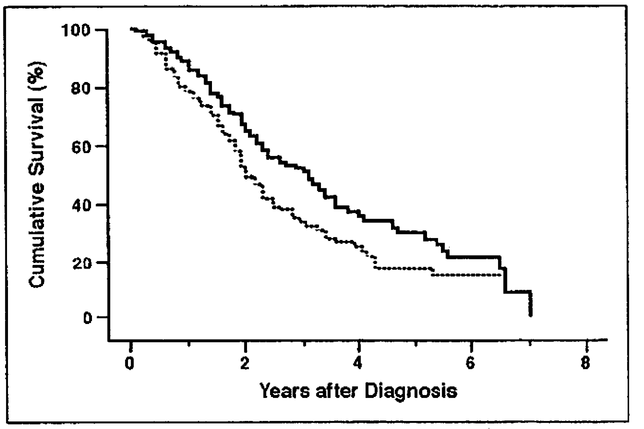


Fig. 6—Graph shows survival rates of patients who underwent only transcatheter arterial chemoembolization (TACE) with (n = 188) (*solid line*) or without (n = 167) (*dotted line*) unified CT angiography (CTA) system, excluding patients who had stage IV hepatocellular carcinoma and patients who underwent additional treatment (percutaneous ethanol injection, percutaneous microwave thermocoagulation, or radiofrequency ablation) after TACE. Survival rate of patients who underwent TACE with unified CTA system was significantly higher than that of patients who underwent TACE without unified CTA system (p = 0.0094).

TABLE 4: Multivariate Analysis for the Factors That Influenced Survival Rate of Patients Who Underwent TACE

Factor	Parameter Estimate	Standard Error	Chi-Square	Risk Ratio (95% CI)	p
Age	0.0141	0.0095	2.24	1.0142 (0.9956–1.0332)	0.1348
Sex					
M				1	
F	0.0253	0.0889	0.08	1.0256 (0.8581–1.2165)	0.7765
Child-Pugh class					
A				1	
B	0.3379	0.0767	19.09	1.4020 (1.2063–1.6294)	< 0.0001
C	0.2761	0.1633	2.50	1.3180 (0.9308–1.7762)	0.1135
Tumor stage[a]					
Stage I				1	
Stage II	0.1232	0.1385	0.83	1.1311 (0.8727–1.5071)	0.3631
Stage III	0.3392	0.1370	7.00	1.4038 (1.0875–1.8664)	0.0082
Use of unified CT angiography system					
No				1	
Yes	–0.1571	0.0759	4.28	0.8546 (0.7365–0.9917)	0.0387

[a]TNM tumor stage according to the Liver Cancer Study Group of Japan.

TACE was originally developed with the introduction of a unique carrier of anticancer agents, iodized oil, gelatin sponge particles, and a microcatheter that makes segmental [27] or subsegmental [28] TACE possible. However, without the assistance of sectional imaging such as CT at TACE, superselective catheterization into the correct feeding artery to obtain complete necrosis of the entire HCC lesion has not always been successful. As a result, inadvertent mistaken embolization has sometimes occurred. In such a mistaken embolization, the targeted lesion was not embolized. Instead, the noncancerous hepatic portion that should have been preserved was embolized, resulting in unnecessary liver damage. This kind of mistaken embolization usually is not found immediately after the TACE procedure but rather after completion of the TACE procedure at the follow-up CT. With the advent of a unified CTA system, this difficulty of performing TACE with a conventional angiography apparatus has been overcome, and several advantages have been added. Using selective arteriography of the feeding artery followed by confirmation using CT arteriography and repeating these studies after advancing the microcatheter to a more distal artery, it has become possible to achieve targeted TACE, strengthening the effect of TACE on the targeted lesion and reducing damage to the surrounding noncancerous portion. Indeed, the local control rate by TACE was significantly higher in patients who underwent TACE with a unified CTA system than in patients who underwent TACE without it.

The survival rate of patients treated by TACE with a unified CTA system was higher than that of patients treated by TACE without a unified CTA system. The technique for subsegmental and superselective TACE of hepatic arteries was established in Japan in the beginning of the 1990s [27, 28], which overlaps the entire period of the present study. Microcatheters were constantly used for TACE procedures throughout the study period. The use of farmorubicin hydrochloride as an anticancer agent, iodized oil, and gelatin sponge particles was also constant throughout the study period. These factors, therefore, could not contribute to the difference in the survival rate between patients treated by TACE with a unified CTA system and those treated without it. The result of the multivariate analysis showed that the use of a unified CTA system independently influenced the increase in the patient survival rate as did the TNM tumor stage and Child-Pugh class. Therefore, the improvement of targeted TACE with the use of a unified CTA system contributed to the improvement in survival of patients treated with TACE.

In conclusion, the use of a unified CTA system contributed to the accurate evaluation of tumor progression in patients with HCC, resulting in the appropriate choice of treatment options and improved management of patients. In patients who underwent TACE for the treatment of HCC, a unified CTA system contributed to the increase in the patient survival rate by improving the targeted TACE by enhancing treatment efficacy and reducing mistaken embolization.

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APPENDIX 1: TNM Stage Classification of the Liver Cancer Study Group of Japan [13]

T factor	I, single; II, < 2 cm; III, no vascular involvement
T1	Fulfilling three factors
T2	Fulfilling two factors
T3	Fulfilling one factor
T4	Fulfilling no factors
Stage	
I	T1 N0 M0
II	T2 N0 M0
III	T3 N0 M0
IV-A	T4 N0 M0, or any T N1 M0
IV-B	Any T N0 or N1 M1

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Incidence of hepatocellular carcinoma in hepatitis C carriers with normal alanine aminotransferase levels☆

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Background/Aims: This study sought to identify the independent risk factors involved in the development of hepatocellular carcinoma (HCC) in patients with chronic hepatitis C virus (HCV) infection who have normal alanine aminotransferase (ALT) levels.

Methods: A total of 519 patients with average ALT integration values less than or equal to 40 IU/L over 10 years were included. Baseline ultrasound was done in all patients and 68 patients underwent liver biopsy at the start of this study. Factors associated with the cumulative incidence of HCC were determined.

Results: HCC occurred in 48 of 519 patients (9.2%). The following factors were significantly associated with the incidence of HCC: age > 65 years (adjusted hazard ratio: 2.006 [95% confidence interval: 1.078–3.733]), ALT > 20 IU/L (6.242 [1.499–25.987]), platelet count < 15.0 × 10^4/m^3 (2.675 [1.407–5.085]), total bilirubin > 1.2 mg/dL (2.798 [1.257–6.228]), ALP > 338 IU/L (2.486 [1.327–4.657]), and total albumin < 3.5 g/dl (2.707 [1.177–6.223]). The 5- and 10-year cumulative incidences of HCC were 4.4% and 26.5% in patients with ALT > 20 IU/L and platelet count < 15.0 × 10^4/m^3, respectively.

Conclusions: High ALT level and low platelet count are closely associated with the development of hepatocarcinogenesis. Therefore, individuals within this group are candidates for antiviral therapy.

Keywords: Hepatocellular carcinoma; Hepatocarcinogenesis; Hepatitis C virus; Normal ALT; Average integration value

1. Introduction

Hepatitis C virus (HCV) infection is widespread and often leads to chronic hepatitis, cirrhosis, or hepatocellular carcinoma (HCC). In Japan, deaths from HCC have increased annually and in the 1990's exceeded 30,000 [1], with 75–80% of HCC cases related to HCV infection [2]. HCC develops in 6–8% of patients with HCV-related cirrhosis every year in Japan [3–5]. A persistent necroinflammatory process and subsequent proliferation of hepatocytes (as observed by increased DNA synthesis) are important for the development of HCC in patients with HCV [6–10]. Thus, appropriate treatment of chronic HCV infection is needed to prevent the development of HCC.

Tarao et al. reported that maintenance of low alanine aminotransferase (ALT) levels may prevent hepatic carcinogenesis [9,10]. They reported that 27 of 33 patients (81.8%) with persistently high ALT levels (annual average ALT ⩾ 80 IU/L) developed HCC, whereas only 12 of 41 (29.3%) patients with persistently low ALT levels

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(annual average ALT < 80 IU/L) developed HCC. There was a statistically significant difference between the two groups [10].

ALT activity is the most widely used laboratory parameter in the evaluation of necroinflammatory activity in liver disease [11–13]. However, measurement of the annual mean value or simple arithmetic mean is problematic for the following reason: if the ALT level is high, the measurement interval shortens, whereas if the ALT level is low, the interval lengthens. As a result, the arithmetic mean value becomes greater and greater in patients with increased ALT levels due to the increased number of measurements being taken. For this reason, in a previous report we suggested that it would be more useful to measure the time integral of the ALT level ("integration value") [14]. The average ALT integration value was well correlated to the cumulative incidence of hepatic carcinogenesis. However, it is well known that HCC occurs in some HCV carriers with normal ALT values. There is little information about how many patients with normal ALT develop HCC. This study, therefore, sought to identify the independent risk factors associated with the development of HCC in chronically infected HCV patients with average ALT integration values less than or equal to 40 IU/L.

2. Materials and methods

2.1. Patient selection

A total of 4620 patients who tested positive for HCV visited the Department of Gastroenterology at Ogaki Municipal Hospital, Japan, between September 1995 and August 2004. After analyzing each patient's long-term prognosis, we selected 519 patients for further study who (1) had tested positive for HCV RNA for at least 6 months; (2) displayed no evidence of hepatitis B virus (HBV) infection; (3) had no other possible causes of chronic liver disease (i.e., alcohol consumption lower than 80 g/day, no history of hepatotoxic drug use, and negative tests for autoimmune hepatitis, primary biliary cirrhosis, hemochromatosis, and Wilson's disease); (4) had a follow-up period of greater than 3 years; (5) had no evidence of HCC for at least 3 years from the start of the follow-up period; (6) had no history of therapy involving interferon and/or ribavirin; (7) had ALT measurements taken more than twice in 1-year; and (8) had average ALT integration values less than or equal to 40 IU/L.

All patients had follow-up examinations at least every 6 months. During each examination, the following parameters were measured at least every 6 months: prothrombin time (PT), ALT, aspartate aminotransferase (AST), platelet count, γ-glutamyl transpeptidase (γ-GTP), total bilirubin, alkaline phosphatase (ALP), cholinesterase, total protein, albumin, and total cholesterol. ALT, AST, γ-GTP, and ALP were expressed as average integration values [14]. When we explained ALT as an example, the integration value of ALT was calculated as follows: $(y_0+y_1) \times x_1/2+(y_1+y_2) \times x_2/2+(y_2+y_3) \times x_3/2+(y_3+y_4)$ x $x_4/2+(y_4+y_5) \times x_5/2+(y_5+y_6) \times x_6/2+(y_6+y_7) \times x_7/2+(y_7+y_8) \times x_8/2$ (Fig. 1). We calculated the area of a trapezoid with ALT value and the measurement interval and added the values. We divided the integration value of ALT by the observation period to obtain the average integration value (Fig. 2). In addition, patients were classified into two groups according to the change pattern of ALT: persistently normal ALT group and intermittently normal ALT group. The persistently normal ALT group includes patients with persistently normal ALT values less than or equal to 40 IU/L during follow-up period. The intermittently normal ALT group includes patients with temporary ALT fluctuations but the average integration value was less than or equal to 40 IU/L. We also recorded PT, platelet count, total bilirubin, cholinesterase, total protein, albumin, and total cholesterol values at the time of entry into the study. HCV genotype was determined by PCR using genotype-specific primers [15] and HCV RNA was quantified (Amplicor 2; Diagnostics K.K., Tokyo, Japan) [16].

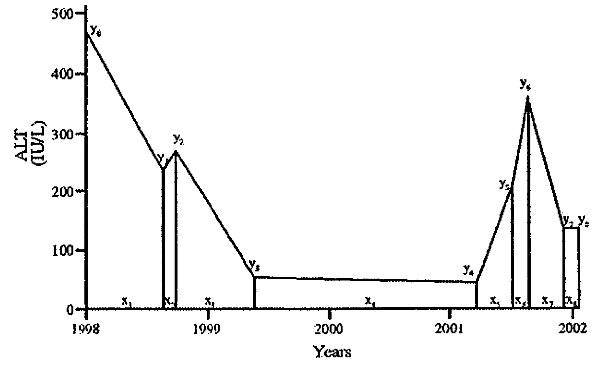

Fig. 1. Integration value of alanine aminotransferase (ALT). The integration value of ALT was calculated as follows: $(y_0+y_1) \times x_1/2+(y_1+y_2) \times x_2/2+(y_2+y_3) \times x_3/2$ + $(y_3+y_4) \times x_4/2+(y_4+y_5) \times x_5/2+(y_5+y_6) \times x_6/2+(y_6+y_7) \times x_7/2+(y_7+y_8) \times x_8/2$. We divided the integration value of ALT by the observation period and expressed it as an average integration value.

Histological confirmation was obtained in 68 out of 519 patients. The degree of fibrosis was staged according to Desmet et al. as follows; F0, no fibrosis; F1, mild fibrosis; F2, moderate fibrosis; F3, severe fibrosis; F4, cirrhosis [17].

Ultrasound (US) was performed in all patients at the start of the follow-up period for the evaluation of liver fibrosis. The diagnosis of cirrhosis was performed according to typical ultrasound findings, e.g. liver surface nodularity, increased echogenicity and echotexture of the liver parenchyma, and signs of portal hypertension (splenomegaly > 120 mm, dilated portal vein diameter >12 mm, patent collateral veins, or ascites) [18,19].

To detect early-stage HCC, ultrasonography, computed tomography (CT), magnetic resonance imaging (MRI), digital subtraction angiography (DSA), and/or measurement of tumor markers (i.e. AFP, *Lens culinaris* agglutinin-reactive AFP, and des-γ-carboxyprothrombin) were

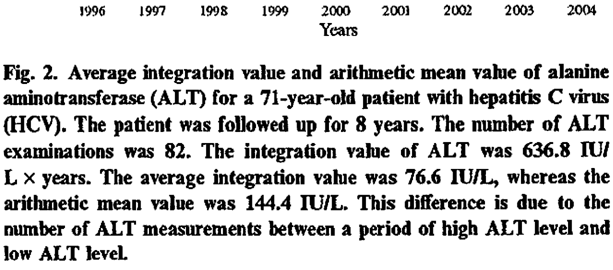

Fig. 2. Average integration value and arithmetic mean value of alanine aminotransferase (ALT) for a 71-year-old patient with hepatitis C virus (HCV). The patient was followed up for 8 years. The number of ALT examinations was 82. The integration value of ALT was 636.8 IU/L × years. The average integration value was 76.6 IU/L, whereas the arithmetic mean value was 144.4 IU/L. This difference is due to the number of ALT measurements between a period of high ALT level and low ALT level.

performed for all patients, at least every 6 months. Blood biochemistry data used in this study were obtained over a 1-year prior to HCC development. The median follow-up period was 8.8 years (range, 3.0–13.3 years). A total of 14,347 blood examinations were performed, with the median number of examinations per patient being 22 (range, 6–158). Study of each patient ended in December 2007 or on the date of HCC identification, whichever came earlier. Diagnosis of HCC was confirmed via histologic examination (resected specimens $n = 13$ or liver biopsy $n = 7$) or via typical characteristics radiological findings such as hypervascularity at DSA or hyperattenuation at CT during hepatic arteriogrophy [20] in addition to US, CT, and MRI ($n = 28$).

2.2. Statistical analysis

Statistical analyses were performed using the Statistical Program for Social Science (SPSS version 11.5 for Windows; SPSS Japan Inc., Tokyo, Japan). Continuous variables are expressed as median (range). The Kruskal–Wallis test was used to assess continuous variables with a skewed distribution, and the χ^2-test was used to assess categorical variables. An actuarial analysis of the cumulative incidence of hepatocarcinogenesis was performed using the Kaplan–Meier method, and differences were tested by a log-rank test. The Cox proportional hazard model and forward selection method were used to estimate the hazard ratio of HCC development associated with the following parameters: age (⩽65 years or >65 years), sex (woman or man), body mass index (<25.0 kg/m^2 or ⩾25.0 kg/m^2), HCV genotype (type 1 or type 2), viral concentration (⩽100 KIU/mL or >100 KIU/mL), PT (⩽70% or >70%), average ALT integration value (⩽20 IU/L or >20 IU/L), average AST integration value (⩽40 IU/L or >40 IU/L), platelet count ($<15.0 \times 10^4$/mm^3 or $\geqslant 15.0 \times 10^4$/mm^3), average γ-GTP integration value (⩽56 IU/L or >56 IU/L), total bilirubin (⩽1.2 mg/dL or >1.2 mg/dL), average ALP integration value (⩽338 IU/mL or >338 IU/mL), cholinesterase (<431 IU/mL or ⩾431 IU/mL), total protein (<6.5 g/dL or ⩾6.5 g/dL), albumin (<3.5 g/dL or ⩾3.5 g/dL), and total cholesterol (<130 mg/dL or ⩾130 mg/dL). We used the lower or upper limit of the reference values at our institute as cut-off values for PT, ALT, AST, platelet count, γ-GTP, total bilirubin, ALP, cholinesterase, total protein, albumin, and total cholesterol. Statistical significance was defined as $p < 0.05$.

The study protocol was approved by the Ethics Committee at Ogaki Municipal Hospital and performed in compliance with the Helsinki Declaration.

3. Results

3.1. Patient characteristics

HCC developed in 48 of 519 patients (9.2%) in this follow-up study. The 5- and 10-year cumulative incidences of hepatocellular carcinoma were 2.0% and 11.2%, respectively. Profiles and data from the 519 patients with normal ALT values are summarized in Table 1.

3.2. Factors associated with the incidence of hepatic carcinogenesis

Factors significantly associated with the incidence of HCC on univariate analysis are listed in Table 2. The following associations were statistically significant: age > 65 years, ALT > 20 IU/L, intermittently normal ALT, platelet count $< 15.0 \times 10^4$/m^3, γ-GTP > 56 IU/L, total bilirubin > 1.2 mg/dL, ALP > 338 IU/L, total protein < 6.5 g/dL, albumin < 3. 5 g/dl, high fibrous stage, and presence of cirrhosis. Hepatic carcinogenesis occurred at significantly higher rates in patients with average ALT integration value greater than 20 IU/L ($n = 402$) than in patients with average ALT integration value less than or equal to 20 IU/L ($n = 117, p = 0.011$, Fig. 3). Hepatic carcinogenesis occurred at significantly higher rates in patients with platelet counts less than 15.0×10^4/mm^3 ($n = 179$) than in patients with platelet counts greater than or equal to 15.0×10^4/mm^3 ($n = 340, p < 0.001$, Fig. 4).

Factors associated with the incidence of HCC as determined by the Cox proportional hazard model and the forward selection method are listed in Table 3, and are as follows: age > 65 years, ALT > 20 IU/L, platelet count $<15.0 \times 10^4$/m^3, total bilirubin > 1.2 mg/dL, ALP > 338 IU/L, and total albumin <3.5 g/dl were significantly associated with the incidence of HCC.

Table 1
Patient characteristics.

Age (years)	66 (18–88)
Sex (W/M)	290/229
BMI (kg/m^2)	22.4 (14.1–34.6)
HCV Genotype (1/2/unknown)	239/129/159
Viral concentration (KIU/mL)	285 (1–30,000)
Prothrombin time (%)	96.0 (21–145)
ALT (IU/L)[a]	27.4 (7.3–40.0)
Persistently normal ALT(+/−)[b]	148/371
AST (IU/L)[a]	31 (9–127)
Platelet ($\times 10^4$/mm^3)	17.2 (2.4–58.8)
γ-GTP (IU/L)[a,c]	23 (6–192)
Total bilirubin (mg/dL)	0.6 (0.3–4.7)
ALP (IU/L)[a]	247 (84–907)
Cholinesterase (IU/L)	264 (55–600)
Total protein (g/dL)	7.3 (4.5–9.2)
Albumin (g/dL)	4.1 (2.1–5.2)
Total cholesterol (mg/dL)	165 (72–290)
Fibrosis (F0/F1/F2/F3/F4)[c]	7/38/10/6/7
Cirrhosis (−/+)[a,d]	432/87
Follow up period (years)	8.8 (3.0–13.3)
Hepatocaricinogenesis (+/−)	48/471

Values are expressed as median (range). W, women; M, men; BMI, body mass index; HCV, hepatitis C virus; ALT, alanine aminotransferase; AST, asparate aminotransferase; GTP, glutamyl transpeptidase; ALP, alkaline phosphatase.
[a] Average integration value.
[b] Persistently normal ALT values less than or equal to 40 IU/L.
[c] Staging of chronic hepatitis according to Desmet et al. [17].
[d] Cirrhosis diagnosed by ultrasound findings.

3.3. Group classification according to average ALT integration value and platelet counts

HCV carriers with normal ALT levels were divided into four groups (A: ALT ⩽ 20 IU/L and platelet count $\geqslant 15.0 \times 10^4$/mm^3 [$n = 82$]; B: ALT > 20 IU/L and platelet count $\geqslant 15.0 \times 10^4$/mm^3 [$n = 258$]; C: ALT ⩽ 20 IU/L and platelet count $< 15.0 \times 10^4$/mm^3 [$n = 35$]; D: ALT > 20 IU/L and platelet count $< 15.0 \times 10^4$/mm^3 [$n = 144$], Table 4). Age, total bilirubin, ALP, and fibrous staging in Group D were higher than in the other groups ($p < 0.001$). Group D

Table 2
Factors associated with hepatocarcinogenesis (univariate analysis).

		Hazard ratio (95% CI)	P
Age (years)	⩽ 65	1	
	> 65	2.420 (1.326–4.414)	0.004
ALT (IU/L)[a]	⩽ 20	1	
	> 20	6.263 (1.520–25.808)	0.011
AST (IU/L)[a]	⩽ 40	1	
	> 40	3.194 (1.799–7.111)	<0.001
Persistently normal ALT[b]	Presence	1	
	Absence	2.426 (1.031–5.709)	0.042
Platelets ($\times 10^4/m^3$)	⩾15.0	1	
	< 15.0	4.297 (2.357–7.834)	<0.001
γ-GTP (IU/L)[a]	⩽ 56	1	
	> 56	2.521 (1.368–4.645)	0.003
Total bilirubin (mg/dL)	⩽ 1.2	1	
	> 1.2	5.563 (2.832–10.927)	<0.001
ALP (IU/L)[a]	⩽ 338	1	
	> 338	3.180 (1.740–5.811)	<0.001
Total protein (g/dL)	⩾6.5	1	
	< 6.5	2.550 (1.005–6.466)	0.049
Albumin (g/dL)	⩾3.5	1	
	< 3.5	3.543 (1.756–7.150)	<0.001
Staging[c]	F0, F1	1	
	F2, F3, F4	20.339 (2.575–160.656)	0.004
Cirrhosis[d]	Absence	1	
	Presence	10.003 (5.597–17.878)	<0.001

P-values and hazard ratios were calculated by Cox proportional hazard model. ALT, alanine aminotransferase; AST, asparate aminotransferase; GTP, glutamyl transpeptidase; ALP, alkaline phosphatase.
[a] Average integration value.
[b] Persistently normal ALT values less than or equal to 40 IU/L.
[c] Staging of chronic hepatitis according to Desmet et al. [17].
[d] Cirrhosis diagnosed by ultrasound findings.

showed the highest rate of hepatic carcinogenesis, followed by Groups B and C, as compared with Group A (Fig. 5). The 5- and 10-year cumulative incidences of HCC were 4.4% and 26.5% in Group D, respectively.

3.4. Change of platelet count in patients with HCC development in Groups A and B

Table 5 shows the profile of patients in Groups A and B who developed HCC. In 12 out of 16 patients (75.0%), platelet counts decreased less than $15 \times 10^4/mm^3$ during

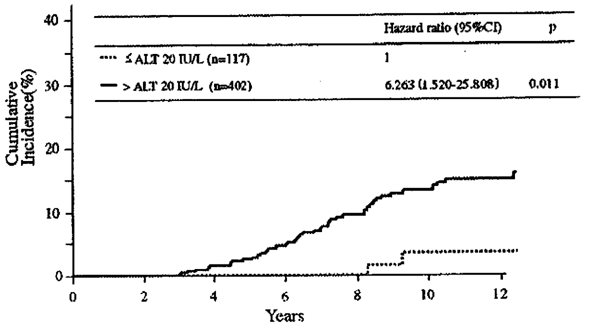

Fig. 3. Incidence of hepatocarcinogenesis as a function of average integration serum alanine aminotransferase (ALT) levels. The 5- and 10-year cumulative incidences of HCC were 0.0% and 3.6% in patients with average ALT integration value less than or equal to 20 IU/L ($n = 117$) and 2.6% and 13.3% in patients with average ALT integration value greater than 20 IU/L ($n = 402$), respectively. Hepatic carcinogenesis occurred at significantly higher rates in the latter group than in the former group ($p = 0.011$).

the follow-up period (2.3 years [0.9–9.5 years]) prior to HCC development.

4. Discussion

We previously showed that increased liver inflammation, as assessed by increased ALT levels, is associated with increased risk for development of HCC in patients with HCV infection [14]. This suggests that suppression of inflammation, as assessed by maintenance of a low ALT level, could inhibit HCC development in HCV carriers. However, some patients develop HCC, even if their ALT levels are within the normal range. It is, therefore, important to identify candidates for antiviral therapy in patients with normal ALT levels.

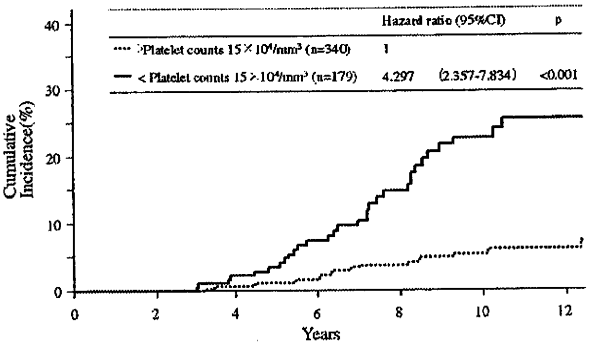

Fig. 4. Incidence of hepatocarcinogenesis as a function of serum platelet levels. The 5- and 10-year cumulative incidences of HCC were 1.2% and 5.4% in patients with platelet counts equal to or greater than $15.0 \times 10^4/mm^3$ ($n = 340$) and 2.5% and 22.9% in patients with platelet counts less than $15.0 \times 10^4/mm^3$ ($n = 179$), respectively. Hepatic carcinogenesis occurred at significantly higher rates in the latter group than in the former group ($p < 0.001$).

Table 3
Factors associated with hepatocarcinogenesis (multivariate analysis).

		Hazard ratio (95% CI)	P
Age (years)	⩽ 65	1	
	> 65	2.006 (1.078–3.733)	0.028
ALT (IU/L)[a]	⩽ 20	1	
	> 20	6.242 (1.499–25.987)	0.012
Platelets ($\times 10^4/m^3$)	⩾15.0	1	
	< 15.0	2.675 (1.407–5.085)	0.003
Total bilirubin (mg/dL)	⩽ 1.2	1	
	> 1.2	2.798 (1.257–6.228)	0.012
ALP (IU/L)[a]	⩽ 338	1	
	> 338	2.486 (1.327–4.657)	0.004
Albumin (g/dL)	⩾3.5	1	
	< 3.5	2.707 (1.177–6.223)	0.019

P-values and hazard ratios were calculated by Cox proportional hazard model. ALT, alanine aminotransferase; ALP, alkaline phosphatase.
[a] Average integration value.

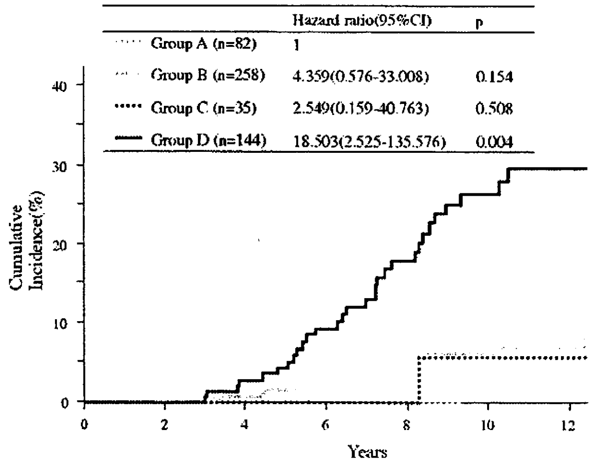

Fig. 5. Cumulative hepatocarcinogenesis as a function of platelet count and average integration serum alanine aminotransferase (ALT) levels. Patients were classified into four groups according to average ALT integration value and platelet count (A: ALT ⩽20 IU/L and platelet counts ⩾ 15.0 × 10^4/mm^3 [n = 82]; B: ALT > 20 IU/L and platelet counts ⩾ 15.0 × 10^4/mm^3 [n = 258]; C: ALT ⩽ 20 IU/L and platelet counts <15.0 × 10^4 /mm^3 [n = 35]; D: ALT > 20 IU/L and platelet counts < 15.0 × 10^4/mm^3 [n = 144]). The 5- and 10-year cumulative incidences of HCC were 0.0% and 2.9% in Group A, 1.6% and 6.2% in Group B, 0.0% and 5.9% in Group C, and 4.4% and 26.5% in Group D, respectively. Group D showed the highest rate of hepatocarcinogenesis compared to Groups A–C.

ALT concentration is the most commonly used variable in the assessment of liver disease [21–23]. ALT level fluctuates within individual patients. Therefore, repeated measurement of this parameter is important for accurate interpretation of the data. The arithmetic mean value of a series of measurements is often the value used for analysis; however, this value can be greatly affected by the period of time between measure-

Table 4
Baseline characteristics between 4 groups.

	Group A	Group B	Group C	Group D
ALT	⩽20 IU/L	>20IU/L	20 IU/L	>20 IU/L
Platelet	⩾15 × 10^4/m^3 (n = 82)	⩾15 × 10^4/m^3 (n = 258)	<15 × 10^4/m^3 (n = 35)	<15 × 10^4/m^3 (n = 144)
Age (years)[a]	62 (21–87)	65 (18–87)	69 (48–88)	67 (41–87)
Sex (W/M)[b]	58/24	138/120	18/17	76/68
BMI (kg/m^2)	20.8 (15.8–26.8)	22.0 (14.1–34.6)	21.4 (17.9–33.3)	20.5 (14.3–31.1)
HCV Genotype (1/2)	28/25	140/67	6/7	65/30
ALT (IU/L)[c]	17.1 (9.3–20.0)	29.0 (20.1–40.0)	16.8 (7.3–20.0)	32.4 (20.1–40.0)
Persistently normal ALT (+/−)[a,d]	56/26	52/206	20/15	20/124
Viral concentration (KIU/mL)	82.5 (0.51–4900)	360 (0.54–30,000)	290 (1.6–1000)	270 (0.55–5000)
Platelet ($\times 10^4/m^3$)	20.7 (15.0–58.8)	19.6 (15.0–56.8)	12.5 (3.7–14.9)	10.55 (2.4–14.9)
Total bilirubin (mg/dL)[a]	0.4 (0.4–4.4)	0.5 (0.2–3.7)	0.6 (0.2–4.6)	0.7 (0.2–4.7)
ALP (IU/L)[a]	229 (114–417)	238 (84–623)	249 (147–437)	274 (158–907)
Albumin (g/mL)	4.1 (2.7–5.1)	4.2 (2.3–4.8)	4.0 (2.6–4.7)	3.9 (2.1–5.2)
Staging (F0/F1/F2/F3/F4)[b,e]	1/9/0/1/0	3/17/4/1/0	3/2/2/1/1	0/10/3/4/6
Cirrhosis(−/+)[a,f]	80/2	256/12	24/11	82/62
Hepatocaricinogenesis (+/−)[a]	1/81	15/243	1/34	31/113

P-values were calculated by Kruskal-Wallis test or χ^2-test. BMI, body mass index; HCV, hepatitis C virus; ALT, alanine aminotransferase; ALP, alkaline phosphatase.
[a] $P < 0.001$.
[b] $P < 0.05$.
[c] Average integration value.
[d] Persistently normal ALT values less than or equal to 40 IU/L.
[e] Staging of chronic hepatitis according to Desmet et al. [17].
[f] Cirrhosis diagnosed by ultrasound findings.

Table 5
Characteristics of patients that developed HCC in Groups A and B.

No.	Sex	Age (years)	Average ALT integration value (IU/L)	Platelet count at entry ($10^4/mm^3$)	Platelet counts decreased[a]	Duration (years)[b]
1	Male	79	23.2	16.9	Yes	0.6
2	Male	82	37.9	18.7	Yes	0.6
3	Male	69	17.2	15.1	Yes	0.6
4	Female	77	24.0	15.4	Yes	0.8
5	Female	63	39.2	15.5	Yes	2.3
6	Female	68	31.1	19.2	Yes	2.3
7	Male	67	35.6	15.7	Yes	2.3
8	Male	70	37.5	20.4	Yes	3.8
9	Male	85	25.0	16.3	Yes	3.9
10	Male	67	28.6	56.8	Yes	4.9
11	Male	70	23.8	16.7	Yes	6.7
12	Male	55	39.1	17.5	Yes	9.5
13	Male	82	25.5	23.8	No	
14	Female	73	28.9	16.1	No	
14	Female	73	28.9	16.1	No	
16	Male	73	27.5	22.9	No	

ALT; alanine aminotransferase.
[a] Platelet counts decreased under $15 \times 10^4/mm^3$.
[b] Duration from the time at entry to the date platelet counts decreased under $15 \times 10^4/mm^3$.

ments. Therefore, we used the time integral of the ALT level to determine the value for analysis. Because this determination is strongly affected by the follow-up period, we divided the average integration value by the time of follow-up. We have previously argued that the average integration value is more meaningful than the arithmetic mean value [14]. In the present study, the average integrated value of ALT increased along with the incidence of HCC. The cumulative incidence of hepatocarcinogenesis was 6.242-fold higher (1.499–25.987) in patients with average ALT integration values greater than 20 IU/L than in patients with average ALT integration value less than or equal to 20 IU/L. Kim et al. reported that the adjusted hazard ratio of mortality from liver disease for patients with ALT concentrations of 20–29 IU/L and 30–39 IU/L were 2.9 (95% confidence interval 2.4–3.5) and 9.5 (7.9–11.5) in men and 3.8 (1.9–7.7) and 6.6 (1.5–25.6) in women compared to that for patients with ALT concentrations <20 IU/L [23]. Furthermore, we evaluated the change pattern of ALT: persistently normal ALT group and intermittently normal ALT group. Although the intermittently normal ALT group is the factor significantly associated with the incidence of HCC on univariate analysis, this factor was not selected on multivariate analysis.

The present study also reveals that a low platelet count is a predictive factor for the development of HCC. Cirrhosis is an established risk factor for HCC in patients with HCV [2–10]. US produce detailed cross-sectional images of the liver and its surrounding structures. We attempted to distinguish cirrhosis patients from non-cirrhosis patients according to typical ultrasound findings [19,20]. The presence of cirrhosis diagnosed by US was strongly associated to the increased incidence of HCC on univariate analysis. Anatomical constraints and interobserver variability, however, remain limiting factors. Because of this, we excluded the factor of cirrhosis diagnosed by US from the multivariate analysis. In this study, histological confirmation was obtained in only 68 patients (13.1%). F2, F3, and F4 showed the higher incidence rate of HCC than F0 and F1 on univariate analysis. It is thought that this study had limitations because the liver histologies were not obtained in many cases. Over the past 50 years, percutaneous liver biopsy has become the primary tool for diagnosing and staging liver disease, and its techniques, indications, and contraindications have been well characterized. However, it is not practical to perform biopsies on all patients who do not receive active treatment because of the potential complications that might arise from this procedure. Furthermore, results often differ depending on the pathologist, and liver fibrosis results from liver biopsy specimens do not always reflect the fibrosis grade of the entire liver. It is likely that low platelet counts account for a large proportion of cirrhosis cases, suggesting that HCC may develop in patients with progressive or advanced liver disease. Platelet count is a useful marker for the diagnosis of cirrhosis. Lu et al. reported that the best cut-off platelet count for a diagnosis of cirrhosis is $15.0 \times 10^4/mm^3$ [24]. We adopted this cut-off level in this study.

Older age, high total bilirubin, high ALP, and low albumin were also significantly associated with incidence of HCC. Increases in conjugated bilirubin are highly specific for disease of the liver or bile ducts [25]. However, only total bilirubin was measured in this series and total bilirubin > 1.2 mg/dL was found in only 35 cases (6.7%). ALP is found in many organs (i.e. kidney, liver, bone, ileal mucosa, and placenta) and has many isoenzymes. Measurement of other associated enzymes (such as γ-GTP) is necessary for correct evaluation of liver function [25]. Therefore, we did not use these parameters in further analyses. Albumin is the most abundant plasma protein produced by hepatocytes. The rate of albumin production is dependent on several factors, including the number of functioning hepatocytes. Plasma albumin gradually falls with progression to cirrhosis [25]. Ten of the 53 patients (18.9%) with albumin less than 3.5 g/dL developed HCC during the follow-up period. We concluded that low platelet count and hypoalbuminemia were confounding factors for identifying cirrhosis. For this study, we selected platelet count as a parameter for further analyses.

We divided patients into four groups according to the average ALT integration value and platelet count in the present study. Patients in Group D (ALT > 20 IU/L and platelet counts $<15.0 \times 10^4/\text{mm}^3$) showed the highest rate of hepatocarcinogenesis (21.5%) compared with Groups A–C. In addition, platelet counts decreased in 12 of 16 patients in Groups A and B who developed HCC. Therefore, it is important to evaluate not only ALT levels but also platelet counts in order to predict hepatic carcinogenesis precisely.

In conclusion, relatively high ALT levels and low platelet counts are closely associated with the development of hepatocarcinogenesis in patients infected with HCV. Therefore, this group is a candidate for antiviral therapy, even if their ALT values are within the current normal range.

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CLINICAL STUDIES

Efficacy of peginterferon-α-2b plus ribavirin in patients aged 65 years and older with chronic hepatitis C

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Keywords
elderly patient – hepatitis C virus – peginterferon – ribavirin – sustained virological response

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Abstract

Objectives: The aim of this study was to evaluate the efficacy and indication of combination therapy with ribavirin plus peginterferon-α-2b in chronic hepatitis C virus (HCV) patients aged 65 years and older. *Methods:* Five hundred and ninety-one consecutive HCV patients were treated with combination therapy. These patients were divided into elder patients (≥ 65 years) ($n=115$) and younger patients (< 65 years) ($n=476$). The clinical characteristics, sustained virological response (SVR) rates and discontinuation rates were compared between the two groups. *Results:* Compared with younger patients, baseline haemoglobin levels and baseline platelet counts were significantly lower ($P < 0.0001$, $P=0.013$ respectively) and fibrosis was more advanced in elderly patients ($P=0.0310$). Moreover, the SVR rate was significantly lower (37.4 vs. 51.5%; $P=0.0067$) while the combination therapy discontinuation rate was significantly higher (32.2 vs. 17.0%; $P=0.0003$) in elderly patients. A multivariate analysis revealed that HCV load and genotype were significantly associated with an SVR in elderly patients. An SVR was achieved in over 50% of elderly male patients with genotype 1 and HCV RNA concentrations under 2 000 000 IU/ml. In contrast, the SVR rate was under 30% in elderly male patients with genotype 1 and with HCV RNA concentrations over 2 000 000 IU/ml and in all elderly female patients with genotype 1. *Conclusions:* The SVR rate was lower in elderly patients than in younger patients. However, in elderly patients combination therapy was most beneficial for genotype 1 patients, male patients with HCV RNA concentrations < 2 000 000 IU/ml and patients with genotype 2.

Hepatitis C virus (HCV) infection is a widespread viral infection that often leads to chronic hepatitis, cirrhosis and hepatocellular carcinoma (HCC). The need for chronic HCV therapies for elderly patients is increasing in Japan and is expected to rise in the US and other Western countries (1). Moreover, HCC has become a recent and growing problem in elderly patients with chronic hepatitis C.

Sustained virological responders who are negative for serum HCV RNA 6 months after interferon (IFN) treatment are reported to be likely to remain in virological and biochemical remission with histological improvement (2, 3). Moreover, IFN therapy reduces the risk of HCC among virological or biochemical responders (4–6). Ribavirin is now generally used in combination with IFN to treat chronic hepatitis C, and this combination therapy is reportedly more effective than IFN monotherapy, with a higher rate of HCV eradication (7–10).

It is important to determine whether elderly hepatitis C patients should be treated with IFN. Arase *et al.* (11) reported that HCV clearance after IFN therapy significantly reduced the risk of HCC and death in older hepatitis C patients. In addition, Veldt *et al.* (12) reported that a sustained virological response (SVR) to treatment is associated with improved clinical outcomes in the general population with chronic hepatitis C and advanced fibrosis.

Several studies have shown that IFN monotherapy has comparable efficacy in elderly and younger patients with chronic hepatitis C (13, 14). IFN and ribavirin combination therapy has greater efficacy than IFN monotherapy

(7, 9). However, elderly patients with genotype 1 and high HCV loads have a lower SVR rate than younger patients because of higher dose reduction rates and discontinuation rates because of ribavirin-related anaemia (15, 16). In a previous study, we examined patients with a similar background, except for age, and found that treating chronic hepatitis C with combination therapy was comparably effective between patients ≥ 60 years old and those < 60 years old, although the ribavirin discontinuation rate was higher among older patients (17). Similar results were obtained from chronic hepatitis patients treated with peginterferon and ribavirin; although the probability of a positive response to peginterferon-α plus ribavirin combination therapy was decreased for genotype 1- or 4-infected patients older than 40 years, patients older than 65 years had a response rate similar to those aged 40–64 years (18). There are few reports on the efficacy of ribavirin and peginterferon in the elderly patients with chronic hepatitis C. Moreover, no study has determined which patients will benefit from combination therapy among elderly patients with chronic hepatitis C. This study was designed to examine the background and treatment efficacy of peginterferon and ribavirin combination therapy according to gender in older patients with chronic hepatitis C and to identify which patients will achieve an SVR in this patient population.

Methods

Patients

This nonrandomized, prospective study was originally discussed in December 2004 by a committee composed of members from Nagoya University Hospital and 63 affiliated hospitals in Japan. Diagnostic criteria for chronic hepatitis C patients, peginterferon and ribavirin regimens and follow-up protocols were determined. Patients were divided by age into two groups: those aged ≥ 65 years and those aged < 65 years. Patients were compared with respect to background and treatment efficacy according to gender and tolerability of combination therapy with peginterferon and ribavirin. The study protocol was approved by the ethics committee of each hospital, and written informed consent was obtained from each patient before therapy.

Five hundred and ninety-one consecutive patients with chronic hepatitis C were treated with peginterferon and ribavirin combination therapy between December 2004 and February 2007 at 64 institutions: Nagoya University Hospital and affiliated hospitals. The indications for treatment were under 75 years old, positive for antibody to HCV and a serum HCV RNA level $> 100\,000$ IU/ml by a quantitative PCR assay (Amplicor GT-HCV Monitor version 2.0; Roche Molecular Systems, Pleasanton, CA, USA) within 12 weeks preceding the treatment. In Japan, combination with peginterferon and ribavirin therapy for patients with an HCV RNA level $> 100\,000$ IU/ml (high viral load in Japan) was approved for medical insurance coverage. Exclusion criteria included pretreatment haemoglobin (Hb) levels < 10 g/dl, positive for serum hepatitis B surface antigen, drug addiction, alcohol abuse, autoimmune hepatitis, primary biliary cirrhosis, human immunodeficiency virus, coexisting serious psychiatric or medical illness and pregnancy. To exclude any patient bias, only complete cohorts from each hospital were enrolled. HCV genotypes were determined by PCR with genotype-specific primers that were described previously by Ohno *et al.* (19). All genotyping was performed at one institution.

All patients were treated with 1.5 μg peginterferon-α-2b (Pegintron®; Schering-Plough K. K., Osaka, Japan) per kilogram of body weight subcutaneously once weekly for 24 weeks for genotype 2 patients and for 48 weeks for genotype 1 patients. When the virus was eradicated between 16 and 24 weeks from the beginning of treatment, the treatment duration was prolonged up to 72 weeks for genotype 1 patients. Treatment was discontinued when a patient's Hb concentration declined below 8.5 g/dl because of drug-induced haemolytic anaemia or when a patient's white blood cell count declined below $1000/mm^3$, the neutrophil count declined below $500/mm^3$ or the platelet count declined below $50\,000/mm^3$. Some patients discontinued treatment because the virus could not be eradicated after 24 weeks, as determined by the physician. We considered these cases to be discontinued. Oral ribavirin (Rebetol; Schering-Plough K. K.) was administered for the same duration as peginterferon at 600 mg/day for patients who weighed < 60 kg, 800 mg/day for those who weighed > 60 kg but < 80 kg and 1000 mg/day for those who weighed > 80 kg during the treatment period. The dose of ribavirin was reduced by 200 mg/day when the patient's Hb concentration declined below 10 g/dl because of drug-induced haemolytic anaemia. Ribavirin was discontinued when peginterferon therapy was discontinued. In Japan, peginterferon and ribavirin combination therapy was not approved for medical insurance coverage until November 2004.

Liver histology

Pretreatment liver biopsy specimens were analysed for fibrosis on a scale of F0–F4 (F0, no fibrosis; F1, portal fibrosis without septa; F2, few septa; F3, numerous septa without cirrhosis; and F4, cirrhosis) and for necroinflammatory activity on a scale of A0–A3 (A0, no histological activity; A1, mild activity; A2, moderate activity; and A3, severe activity) (20).

Assessment of efficacy

A virological response was assessed using a qualitative HCV RNA assay with a lower detection limit of 100 IU/ml (Amplicor HCV version 2.0; Roche Molecular Systems). According to the qualitative HCV RNA results, the responses were defined as follows: SVR (no HCV RNA detected at the end of the 24-week follow-up period after completion of treatment), relapse (no HCV RNA at the end of treatment and reappearance of serum HCV RNA during the 24-week follow-up period) or nonresponse (persistent positive serum HCV RNA throughout treatment).

Comparison of characteristics and efficacy of treatment according to age

Patients were divided by age into two age groups: (1) ≥ 65 years old ($n=115$) and (2) < 65 years old ($n=476$). The following baseline parameters were compared between the two groups: gender ratio, age, body weight, body mass index (BMI), alanine aminotransferase (ALT) levels, γ-glutamyl transpeptidase (GGT), Hb levels, platelet counts, HCV genotype and viral load, histological activity and fibrosis. The SVR rate, rapid virological response (RVR) (HCV RNA negative by a qualitative assay at week 4) rate, early virological response (HCV RNA negative by a qualitative assay at week 12) rate and end of treatment virological response (ETR) rate were obtained by an intention-to-treat (ITT) analysis and per-protocol (PP) analysis, and the ribavirin or peginterferon reduction rate and combination therapy discontinuation rate were compared between the two age groups.

Comparison of treatment efficacy between patients who did and did not achieve a sustained virological response

To identify factors that predict an SVR among patients treated with combination therapy, we first determined the factors associated with an SVR in combination therapy with respect to the same factors above baseline parameters by a univariate analysis. Next, we identified the factors associated with an SVR in combination therapy, including gender, age, BMI, baseline serum ALT, GGT, Hb, platelet counts, genotype and HCV RNA, using a multivariate stepwise analysis with forward inclusion methods.

Comparison of treatment efficacy between older patients who did and did not achieved a sustained virological response

To identify elderly patients who may particularly benefit from combination therapy, we determined factors associated with an SVR using a univariate analysis of the same background factors as above. Then we determined factors associated with an SVR in elderly patients treated with combination therapy by a multivariate stepwise analysis with forward inclusion methods. In addition, we analysed the virological responses to combination therapy according to the age and gender of patients infected with each genotype because the age distribution of the treated patients differed according to gender.

Statistical analysis

Values are expressed as means ± standard deviation (SD). Between-group differences in the mean quantitative values were analysed using Student's t-test, and differences in nonparametrical data were analysed using the Mann–Whitney U-test. Differences in proportions were tested by a χ^2-test. The SVR rate between age generations in females was assessed using Fisher's exact test. Multiple logistic regression analysis was used to identify factors related to SVR. Statistical analyses were performed using SPSS software version 16.0 (SPSS Japan Inc., Tokyo, Japan) for multiple logistic regression analysis and SAS software (SAS Institute Inc., Cary, NC, USA) for another analysis. All P values were two-tailed, and $P < 0.05$ was considered statistically significant.

Results

Patient characteristics

A total of 658 patients were screened, and 591 patients were enrolled in this study (Fig. 1). The patients included 327 men and 264 women aged 20–74 years (mean ± SD, 54.7 ± 11.6). Patients ≥ 65 years old comprised 19.5% of the patient population (115/591). The clinical characteristics of the two study groups are shown in Table 1. Body weight was significantly lower in patients aged ≥ 65 years than that in patients aged < 65 years ($P=0.0006$). Hb levels and platelet counts were significantly lower in patients aged ≥ 65 years than those in patients aged < 65 years ($P < 0.0001$ and $P=0.0013$ respectively). The fibrosis stage was more advanced in patients aged ≥ 65 years than that in patients aged < 65 years ($P=0.0310$).

Response to therapy

The ribavirin dose reduction rate was significantly higher in patients aged ≥ 65 years than that in patients aged < 65 years ($P=0.00013$) (Table 2), while the peginterferon dose reduction rate did not differ significantly between the two groups. The treatment discontinuation rate in patients aged ≥ 65 years was significantly higher than that in patients < 65 years ($P=0.0003$). As a result, the SVR rate by ITT analysis in patients aged ≥ 65 years was significantly lower than that in patients aged < 65 years ($P=0.0067$). However, the SVR and ETR rate by PP analysis were not significantly different between the two groups.

The factors associated with an SVR were determined by univariate analysis. The SVR rate was significantly higher in male patients than that in female patients ($P=0.0153$) (Table 3). Age was significantly lower in patients who achieved an SVR than in patients who did not achieve an SVR ($P < 0.0001$). Hb levels and platelet counts were significantly higher in patients who achieved an SVR than those in patients who did not achieved an SVR ($P=0.0202$ and $P=0.0002$ respectively). The HCV load in patients who achieved an SVR was significantly lower than that in patients who did not achieved an SVR

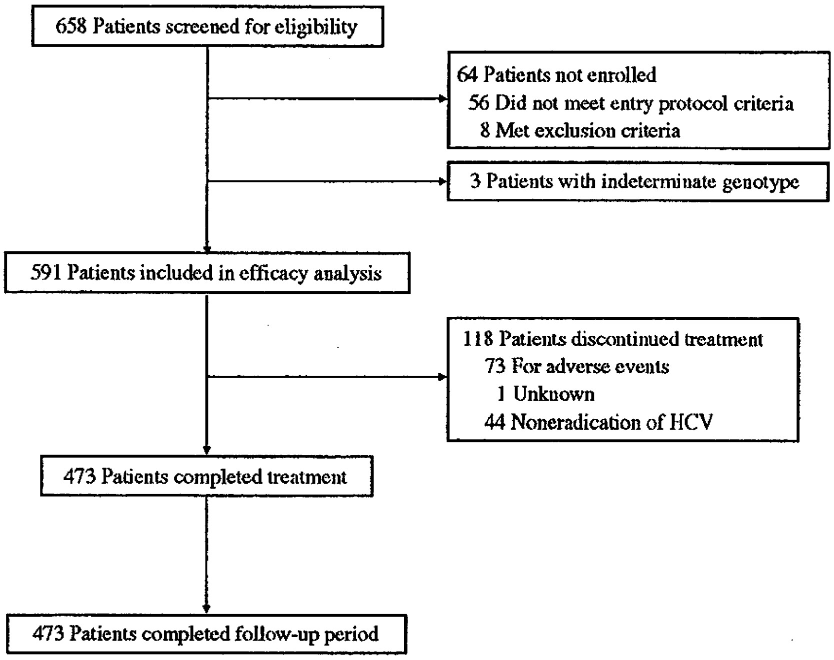

Fig. 1. Flow chart for patient selection.

Table 1. Baseline clinical characteristics of patients treated with combination therapy

	Total patients ($n=591$)	Patients aged <65 years ($n=476$)	Patients aged ≥ 65 years ($n=115$)	*P* value
Sex ratio (male/female)	327/264	270/206	57/58	0.1659
Age (years)	54.7 ± 11.6	51.5 ± 10.6	67.9 ± 2.2	< 0.0001
Body weight (kg)	60.1 ± 11.3	60.9 ± 11.4	56.7 ± 10.1	0.0006
Body mass index	22.9 ± 3.2	22.9 ± 3.2	22.9 ± 3.2	0.9221
Baseline serum ALT (IU/L)	64.8 ± 57.3	66.5 ± 60.6	57.7 ± 40.4	0.1425
GGT (IU/L)	57.8 ± 76.7	58.9 ± 78.9	53.3 ± 67.3	0.4880
Haemoglobin (g/dl)	14.1 ± 1.3	14.2 ± 1.4	13.7 ± 1.2	< 0.0001
Platelets ($\times 10^4/\mu l$)	17.7 ± 5.7	18.0 ± 5.9	16.1 ± 4.3	0.0013
Genotype (1/2)	467/124	374/102	93/22	0.5870
HCV RNA (kIU/ml)	1863.3 ± 1456.3	1896.4 ± 1454.9	1726.2 ± 1460.5	0.2611
Activity (A0/A1/A2/A3)	16/255/141/19	13/202/115/13	3/53/26/6	0.6053
Fibrosis (F0/F1/F2/F3/F4)	37/228/107/56/5	31/191/83/37/3	6/37/24/19/2	0.0310

ALT, alanine aminotransferase; GGT, γ-glutamyl transpeptidase; HCV RNA, hepatitis C virus RNA; kIU, kilo international units.

($P=0.0132$). The rate of genotype 2 patients who achieved an SVR was significantly higher than that in patients who did not achieve an SVR ($P<0.0001$). The fibrosis stage was more advanced in patients who did not achieve an SVR than that in those who did achieve an SVR ($P=0.0186$).

The factors associated with an SVR in combination therapy were determined by multivariate analysis (Table 4). Age [$P<0.0001$, odds ratio 0.959 (0.942–0.975)] and genotype [$P<0.0001$, odds ratio 0.415 (0.255–0.676)] were significantly associated with an SVR. Including the RVR (ITT) factor after starting treatment, the factors associated with an SVR in combination therapy were determined using a multivariate analysis (Table 5). Age [$P<0.0001$, odds ratio 0.961 (0.944–0.978)] and RVR [$P<0.0001$, odds ratio 8.168 (4.511–14.789)] were significantly associated with an SVR.

Then, we separately analysed male and female patients in different age groups. The virological responses to combination therapy according to the gender of genotype 1 patients are shown by age groups in Figure 2. In both males and females, the SVR rate decreased with age, and the SVR rates of patients <40 years old were over 50%. In patients aged ≥ 65 years, the SVR rate of female patients was lower than that in patients aged <65 years and was lower than that of male patients aged ≥ 65 years [20.8% (10/48) vs. 40.5% (64/158) and 20.8% (10/48) vs. 42.2% (19/45); $P=0.0261$ respectively].