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What Is the Most Effective Drug Delivery System for Cisplatin during the Treatment of Hepatic Tumors with Single-Session Transcatheter Chemotherapy? A Pilot Study

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Background/Aims: The aim of this study was to determine the pharmacodynamics of cisplatin following three different treatment procedures for intrahepatic arterial infusion therapy for hepatocellular carcinoma (HCC). **Methods:** We divided 13 HCC patients into the following three groups: group A, lone injection of cisplatin (n=3); group B, combined injection of cisplatin and lipiodol, with embolization using small gelatin cubes (GCs) (n=5); and group C, injection of suspended lipiodol with cisplatin powder, with embolization using small GCs (n=5). In each group, the free cisplatin concentration in the hepatic vein was measured at 0, 5, 10, and 30 minutes. **Results:** The mean free cisplatin concentrations were as follows. For group A, the mean was 48.58 μg/mL at 0 minute, 7.31 μg/mL at 5 minutes, 5.70 μg/mL at 10 minutes, and 7.15 μg/mL at 30 minutes. For the same time points, for group B, the concentrations were 8.66, 4.23, 3.22, and 1.65 μg/mL, respectively, and for group C, the concentrations were 4.81, 2.61, 2.52, and 1.75 μg/mL, respectively. The mean area under the curve $(AUC)_{0\text{-infinity}}$ for the free cisplatin concentration was 7.80 in group A, 2.48 in group B, and 2.27 in group C. The $AUC_{0\text{-infinity}}$ for the free cisplatin concentration gradually decreased, from group A to group C. **Conclusions:** These results indicate that the combination of lipiodol and small GCs may be useful for delaying cisplatin drainage from the liver. **(Gut Liver 2013;7:576-584)**

Key Words: Cisplatin; Attention; Carcinoma, hepatocellular; Drug delivery

INTRODUCTION

Hepatocellular carcinoma (HCC) is one of the most common neoplasms in Africa and in Asia, including Japan. It was established recently that more than 80% of cases with HCC have liver cirrhosis, and therefore a routine check-up for cirrhotic patients using ultrasound (US) usually detects small HCCs. However, due to the association between cirrhosis and tumor multiplicity, surgical resection is performed in only 20% of cases or less.[1,2] Transcatheter arterial chemoembolization (TACE) has been reported to be an effective palliative treatment for patients with unresectable HCC.[3-10] Although repeated TACE is one of the most potent therapies for unresectable HCC, resistance to this therapy often results after repeated therapy, with the long-term survival rates achieved after 3 years not being sufficiently high.

Platinum analogues are effective against many malignant tumors, and in recent years have been used in the treatment of HCC. For example, there are numerous reports that cisplatin is effective for advanced HCC and that combination therapy of cisplatin and lipiodol may be especially effective.[11-18]

Our group has reported previously that the rate of complete or partial response in cases of epirubicin TACE-resistant patients was significantly higher in patients treated with a platinum-analogue used TACE compared with a single hepatic arterial injection (HAI) without embolization.[19]

It is thought that the measurement of cisplatin concentration in samples collected from the hepatic veins after intrahepatic infusion is a useful method for determining differences in the curative effect of different treatment methods for cisplatin.

However, to our knowledge, there is no information on cisplatin concentration in the hepatic vein following different treatment methods. The aims of this study were therefore to measure total (protein-bound and unbound) and free (protein unbound)-cisplatin concentration in the hepatic vein and to carry out a pharmacokinetic analysis on the three kinds of drug delivery

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methods.

MATERIALS AND METHODS

1. Study population and ethical considerations

From 2007 to 2008, we carried out a prospective study on total and free cisplatin concentration in samples collected from the hepatic and peripheral veins during transcatheter arterial cisplatin chemotherapy in 13 patients with HCC. All the patients were considered to have an unresectable HCC at the time of diagnosis. Before treatment with the platinum analogue, all the patients underwent an evaluation consisting of a medical history, physical examination, measurement of tumor size, performance status, chest radiograph, liver imaging (computed tomography [CT], US, and digital subtraction angiography [DSA]), complete blood count, and blood chemistry. The diagnosis of HCC was established on the basis of the findings of the US, CT, and DSA.

A total of 13 patients were enrolled in the study using the following inclusion criteria: 1) typical hypervascular HCC observed in all imaging modalities; 2) Child-Pugh A or B classification; 3) performance status of 0 to 1; 4) adequate liver function with a bilirubin level ≤5 mg/dL; 5) sufficient hematopoietic function with a platelet count of >25,000 mm^3 and leukocyte count >2,000 mm^3; 6) an expected survival time of at least 3 months.

At first, if the patients had advanced portal vein invasion (tumor thrombus reaching the main trunks of the portal vein) or a severe arterioportal shunt, they were treated using only transcatheter arterial infusion of cisplatin (group A). The remaining patients were informed of the two other methods for administering cisplastin and the appropriate method was then chosen. One group received a combined injection of cisplatin and lipiodol, with embolization in small gelatin cubes (GCs) (group B), while the other group received an injection of suspended lipiodol with cisplatin powder, with embolization in small-GCs (group C). As a result, three patients were assigned to group A, five to group B, and five to group C (Fig. 1). The clinical background, laboratory data, and tumor characteristics of the patients are summarized in Tables 1 and 2.

The physicians in charge explained the purpose and method of this clinical trial to each patient, who provided their informed consent prior to participation.

The study was approved by Institutional Review Board of our hospital.

2. Details of treatment procedures

Hydration of the patients was performed through a peripheral line. The femoral artery was catheterized under local anesthesia, and a catheter then inserted superselectively into the hepatic artery that supplied the target tumor, followed by injection of cisplatin (IA-call; Nippon Kayaku, Tokyo, Japan) with or without lipiodol (Lipiodol Ultrafluide; Laboratoire Guerbet, Aulnay-sous-Bois, France) and 1-mm GCs (Gelpart; Nippon Kayaku). The dose of cisplatin was 100 mg/body administered over 20 minutes under careful fluoroscopic guidance.

In group A, only cisplatin was administered using transcatheter arterial infusion; in group B, cisplatin and lipiodol were first divided into six to eight parts and injected mutually, followed

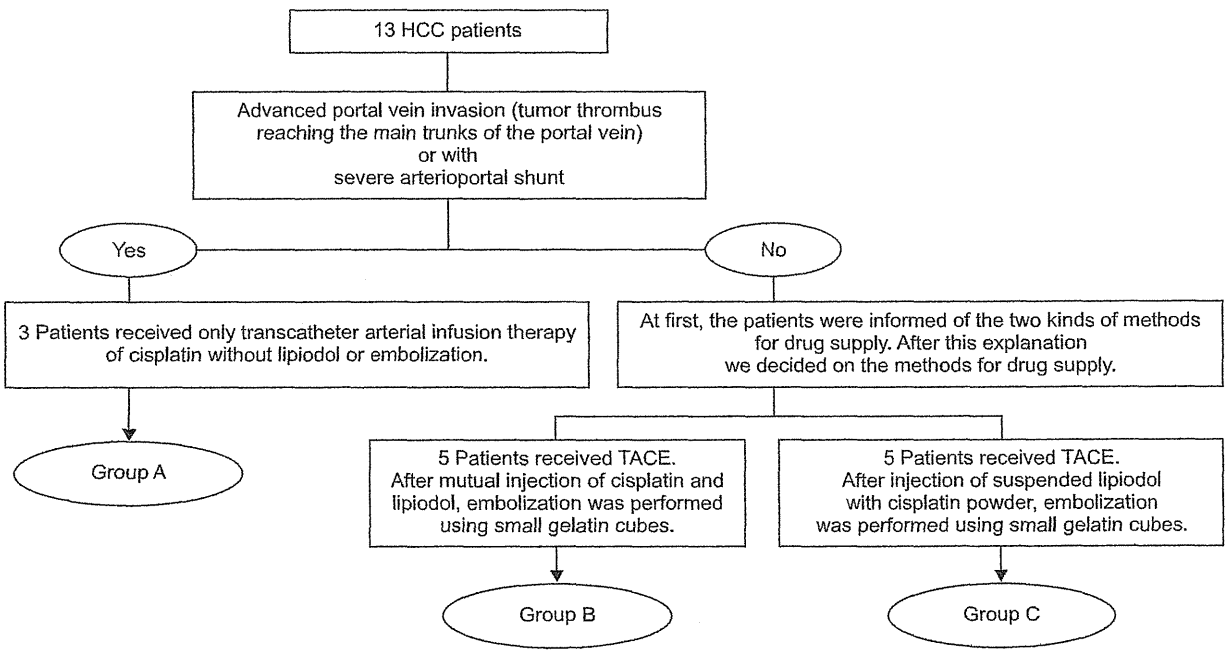

Fig. 1. Distribution of patients receiving cisplatin by three different administration procedures.
HCC, hepatocellular carcinoma; TACE, transcatheter arterial chemoembolization.

Table 1. Demographic and Laboratory Data for 13 Patients with Unresectable Hepatocellular Carcinoma Who Underwent Blood Sampling from the Hepatic and Peripheral Veins for the Measurement of the Cisplatin Concentration after Transcatheter Arterial Chemotherapy Using Cisplatin

Parameter	Group A (n=3)	Group B (n=5)	Group C (n=5)
Patient characteristics			
Gender, male:female	2:1	4:1	5:0
Age, yr*	58 (46-73)	67 (57-87)	69 (63-77)
Backgrounds of liver disease			
Hepatitis B surface antigen positive	2	1	1
Anti-HCV antibody positive	1	4	4
Both negative	0	0	0
Liver function status			
Child-Pugh classification, A/B	2/1	5/0	5/0
Laboratory data			
Albumin, g/dL*	3.4 (2.8-3.7)	3.1 (2.9-4.0)	3.7 (3.2-3.9)
Bilirubin, mg/dL*	0.6 (0.4-1.8)	0.7 (0.5-1.4)	1.0 (0.5-1.1)
Prothrombin time, %*	94.7 (72.6-100.3)	86.8 (72.2-97.3)	82.1 (63.0-89.5)
AFP, μg/L*	55.9 (31.7-114,560.0)	1,664.0 (38.9-98,200.0)	116.0 (6.8-3,702.0)
DCP, AU/L*	3,065.0 (2,139.0-12,391.0)	141.5 (32.0-137,420.0)	98.5 (14.0-190.0)

*Data are presented as median (range).
HCV, hepatitis C virus; AFP, α-fetoprotein; DCP, des-γ carboxyprothrombin.

Table 2. Profiles of 13 Patients with Unresectable Hepatocellular Carcinoma Who Underwent Blood Sampling from the Hepatic and Peripheral Veins for the Measurement of the Cisplatin Concentration after Transcatheter Arterial Chemotherapy Using Cisplatin

Profiles of liver cancer	Group A (n=3)	Group B (n=5)	Group C (n=5)
Tumor size, median (range), mm	139 (79-187)	65 (16-140)	26 (5-76)
Intrahepatic multiplicity			
Solitary	0	0	1
Multiple, localized to one segment	0	0	0
Multiple, localized to one lobe	0	2	0
Multiple, extended to both lobes	3	3	4
Portal vein invasion, no/yes	1/2	3/2	4/1

by embolization using 1-mm GCs; and in group C embolization was performed using 1-mm GCs after injection of suspended lipiodol with cisplatin powder. In patients treated with lipiodol, its volume ranged from 2.0 to 5.0 mL, with the dose being determined according to tumor size and degree of liver dysfunction.

3. Method of drug and pharmacokinetic analyses

A pharmacokinetic study of cisplatin was performed after transcatheter arterial chemotherapy on day 1. After administration of cisplatin, blood samples were collected from the hepatic and peripheral veins. Total and free platinum concentration was measured in each sample, with the detailed pharmacokinetic study being performed only on the hepatic vein samples. The time the arterial infusion finished represented the observation starting point (0 minute), with blood samples collected at 5, 10, and 30 minutes. A sample was also collected from a peripheral vein 120 minutes after the completion of cisplatin infusion (Fig. 2). The blood samples were collected into heparinized syringes for measurement of plasma ultrafilterable platinum levels. Each sample was centrifuged at 3,000 rpm for 10 minutes and the plasma then placed in an ultrafiltration kit (Contrifree, MMPS-3; Amicon Inc., Tokyo, Japan), followed by centrifugation at 1,700×g for 20 minutes. This plasma ultrafiltrate was frozen immediately and stored at <-20°C. Platinum concentrations were analyzed using flameless atomic absorption spectrophotometry using a Hitachi polarized Zeeman atomic absorption spectrometer (Model Z-8000 with graphite furnace, temperature controller and autosampler; Hitachi Factor, Tokyo, Japan). The sample volumes were 10 μL. The oven was programmed using the following steps: 1) drying, 40 seconds at 80°C to 100°C; 2) drying, 50 seconds at 100°C to 130°C; 3) drying, 15 seconds at 130°C to 600°C; 4) charring, 15 seconds at 1,800°C; 5) atomization, 10 seconds at 3,000°C; 6) cleaning, 3 seconds at 3,000°C.

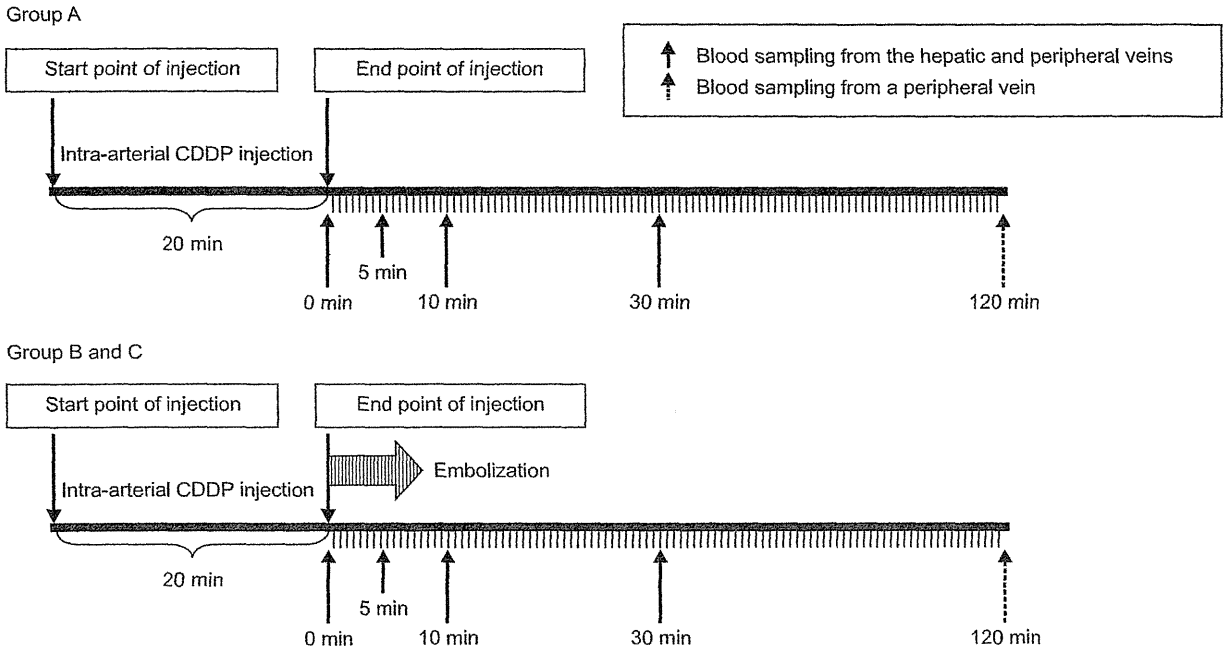

Fig. 2. Study protocol of cisplatin injection and blood sampling.
CDDP, cisplatin.

The absorbance of the samples was then measured at 265.9 nm. Standardization was performed using cisplatin saline solutions up to 1 μg/mL, with a detection limit of 10 ng/mL. Using this ultrafiltration kit almost all protein-bound cisplatin was eliminated and only free cisplatin (protein-unbound) could be measured. The measurement of cisplatin was carried out by NAC Co., Ltd., Tokyo, Japan. The AUC of total and free-cisplatin was calculated by the Automated Pharmacokinetic Analysis System computer program.[20]

4. Toxicity evaluation

Treatment-related toxicity was assessed using the National Cancer Institute Common Terminology Criteria version 4.0. The following toxicity evaluations were made within the 2 week period before treatment was started, and 3 to 7 days (three times during this period) and 2 weeks after treatment was started: hematological (leukocyte and thrombocyte counts) and clinical chemistry assessments (serum aspartate aminotransferase [AST], serum alanine aminotransferase [ALT], total bilirubin, and serum creatine).

RESULTS

1. Total and free-cisplatin concentration in hepatic vein samples following each treatment procedure

The mean±SD total and free-cisplatin concentrations in hepatic vein samples were 68.08±30.30 and 48.58±41.56 μg/mL at 0 minute, 8.18±0.92 and 7.31±1.46 μg/mL at 5 minutes, 6.48±1.95 and 5.70±1.65 μg/mL at 10 minutes, and 9.46±8.59 and 7.15±7.12 μg/mL at 30 minutes in patients in group A; 10.35±4.89 and 8.66±5.36 μg/mL at 0 minute, 5.35±1.04 and 4.23±1.39 μg/mL at 5 minutes, 5.23±1.79 and 3.22±0.91 μg/mL at 10 minutes, and 3.36±0.67 and 1.65±0.33 μg/mL at 30 minutes in patients in group B; and 5.54±5.21 and 4.81±4.95 μg/mL at 0 minute, 3.30±1.28 and 2.61±1.19 μg/mL at 5 minutes, 3.75±1.97 and 2.52±1.13 μg/mL at 10 minutes, and 2.55±1.37 and 1.75±1.05 μg/mL at 30 minutes in patients in group C (Fig. 3).

With the exception of the 30 minutes time point, free-cisplatin concentration and the mean concentration of total and free-cisplatin were higher in the order of group A, B, and C at each measurement point.

2. Total and free-cisplatin concentration from a peripheral vein following each treatment procedure

Mean±SD total and free-cisplatin concentration of samples collected from a peripheral vein were 12.35±3.01 and 11.94±2.67 μg/mL at 0 minute, 6.75±1.00 and 5.87±0.35 μg/mL at 5 minutes, 5.54±1.01 and 4.92±0.61 μg/mL at 10 minutes, 3.91±1.40 and 2.69±0.68 μg/mL at 30 minutes, and 1.59±0.76 and 0.66±0.23 μg/mL at 120 minutes in patients in group A; 5.54±1.47 and 3.80±0.68 μg/mL at 0 minute, 4.31±0.55 and 3.04±0.51 μg/mL at 5 minutes, 4.33±1.08 and 2.65±0.45 μg/mL at 10 minutes, 3.34±0.76 and 1.66±0.17 μg/mL at 30 minutes, and 2.48±0.54 and 0.39±0.15 μg/mL at 120 minutes in patients in group B; and 2.30±0.88 and 1.70±0.95 μg/mL at 0 minutes, 2.49±0.68 and 1.93±0.58 μg/mL at 5 minutes, 2.21±0.93 and 1.58±0.51 μg/mL at 10 minutes, 1.85±0.77 and 1.07±0.40 μg/

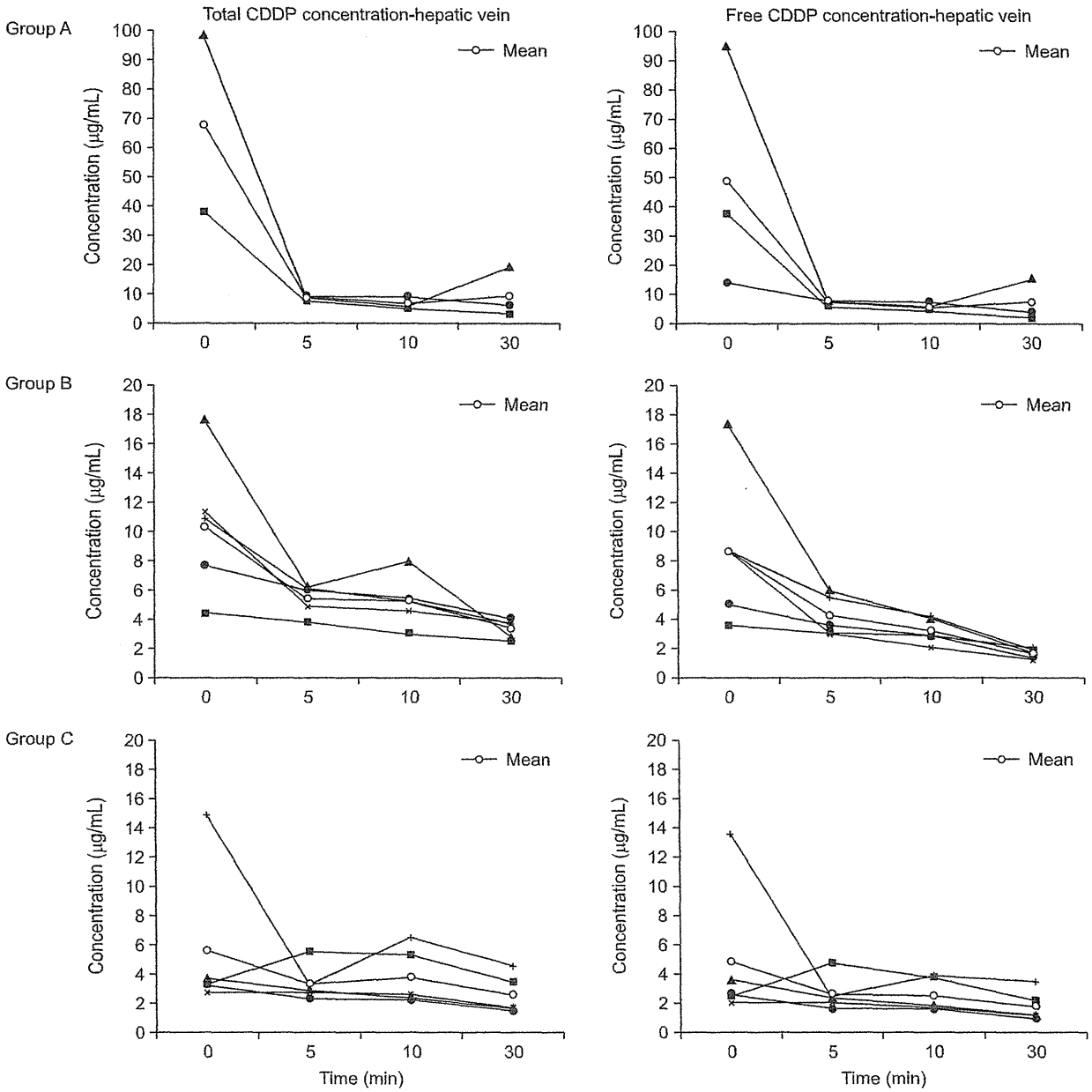

Fig. 3. Total and free cisplatin concentrations in samples collected from the hepatic vein after injection.
CDDP, cisplatin.

mL at 30 minutes, and 1.37±0.75 and 0.46±0.47 μg/mL at 120 minutes in patients in group C (Fig. 4).

The mean concentrations of total and free-CDDP were higher in the order of group A, B, and C at each measurement point, with the exception of the 120 time point.

3. Pharmacokinetic analysis of total cisplatin concentration in samples from the hepatic vein, following each treatment procedure

The pharmacokinetic analysis showed mean±SD maximum concentration (Cmax) of cisplatin in hepatic vein samples was 68.08±30.30 μg/mL in group A, 10.35±4.89 μg/mL in group B, and 5.99±5.06 μg/mL in group C.

Mean±SD $AUC_{0\text{-last}}$ was 6.43±2.70 μg/mL in group A, 2.52±0.65 μg/mL in group B, and 1.71±0.87 μg/mL in group C, while mean±SD $AUC_{0\text{-infinity}}$ was 11.84±6.16 μg/mL in group A, 5.93±2.00 μg/mL in group B, and 3.77±1.73 μg/mL in group C. The mean Cmax, $AUC_{0\text{-last}}$, and $AUC_{0\text{-infinity}}$ of total and free-cisplatin concentration were all higher in the order of group A, B, and C at each measurement point. The mean±SD of terminal half-life ($t_{1/2}Z$) was 0.53±0.17 hours in group A, 0.68±0.33 hours in group B, and 0.59±0.13 hours in group C (Table 3).

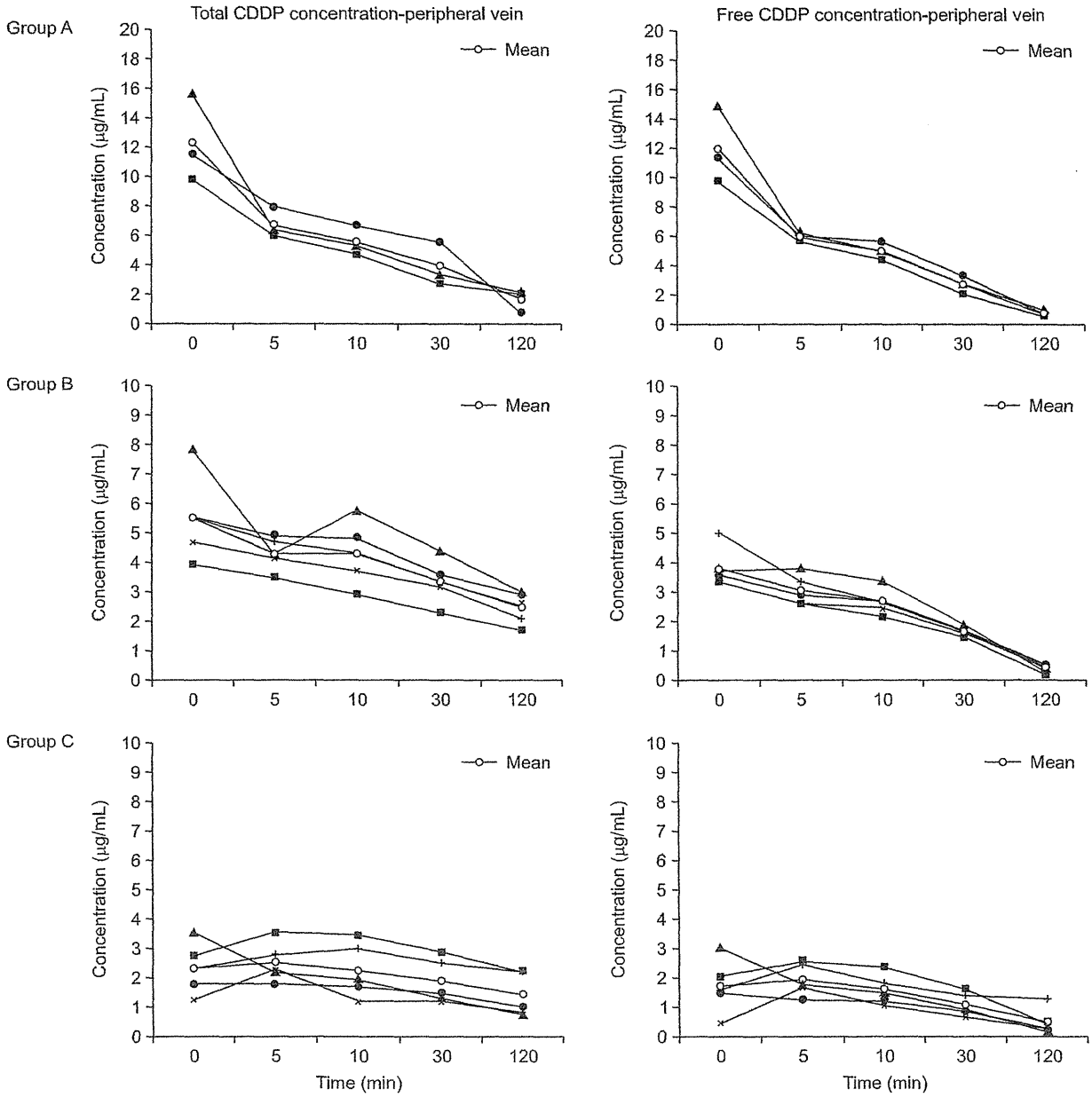

Fig. 4. Total and free cisplatin concentrations in samples collected from the peripheral vein after injection.
CDDP, cisplatin.

4. Pharmacokinetic analysis of free cisplatin concentration in samples from the hepatic vein following each treatment procedure

Pharmacokinetic analysis of free cisplatin in hepatic vein samples showed mean±SD Cmax was 48.58±41.56 μg/mL in group A, 8.66±5.36 μg/mL in group B, and 5.27±4.77 μg/mL in group C; $AUC_{0\text{-last}}$ was 5.01±2.81 μg/mL in group A, 1.66±0.51 μg/mL in group B, and 1.24±0.61 μg/mL in group C, and $AUC_{0\text{-Infinity}}$ was 7.80±4.96 μg/mL in group A, 2.48±0.53 μg/mL in group B, and 2.27±1.10 μg/mL in group C. The means of Cmax, $AUC^{0\text{-last}}$, and $AUC_{0\text{-infinity}}$ for total and free cisplatin concentration was higher in the order of group A, B, and C at each measurement point. The mean±SD $t_{1/2}Z$ was 0.36±0.05 hours in group A, 0.35±0.10 hours in group B, and 0.45±0.06 hours in group C (Table 4).

5. Toxic effects

In this study, grade 4 side effects were not observed, although the following grade 3 events were observed: decreased hemo-

Table 3. Pharmacokinetic Parameters of Total Cisplatin

Parameter	Group A (n=3)	Group B (n=5)	Group C (n=5)
Hepatic vein			
Cmax, μg/mL*	68.08±30.30	10.35±4.89	5.99±5.06
$t_{1/2}Z$, hr†	0.53±0.17	0.68±0.33	0.59±0.13
$AUC_{0\text{-last}}$, μg/hr/mL‡	6.43±2.70	2.52±0.65	1.71±0.87
$AUC_{0\text{-infinity}}$, μg/hr/mL§	11.84±6.16	5.93±2.00	3.77±1.73

Data are presented as mean±SD.
*Cmax, maximum concentration (units, μg equation of cisplatin/mL); †$t_{1/2}Z$, terminal half-life (units, hour); ‡$AUC_{0\text{-last}}$, area under the curve from zero to the last measurable time point (units, μg equation of cisplatin/hr/mL); and §$AUC_{0\text{-infinity}}$, area under the curve from zero to infinity (units, μg equation of cisplatin/hr/mL).

Table 4. Pharmacokinetic Parameters of Free Cisplatin

	Group A (n=3)	Group B (n=5)	Group C (n=5)
Hepatic vein			
Cmax, μg/mL*	48.58±41.56	8.66±5.36	5.27±4.77
$t_{1/2}Z$, hr†	0.36±0.05	0.35±0.10	0.45±0.06
$AUC_{0\text{-last}}$, μg/hr/mL‡	5.01±2.81	1.66±0.51	1.24±0.61
$AUC_{0\text{-infinity}}$, μg/hr/mL§	7.80±4.96	2.48±0.53	2.27±1.10

Data are presented as mean±SD.
*Cmax, maximum concentration (units, μg equation of cisplatin/mL); †$t_{1/2}Z$, terminal half-life (units, hour); ‡$AUC_{0\text{-last}}$, area under the curve from zero to the last measurable time point (units, μg equation of cisplatin/hr/mL); and §$AUC_{0\text{-infinity}}$, area under the curve from zero to infinity (units, μg equation of cisplatin/hr/mL).

globin level in one patient (8%), decreased platelet counts in one patient (8%), increased AST in five patients (38%), increased ALT in two patients (15%), and increased bilirubin level in two patients (15%). All these abnormalities resolved within two weeks. In this study group, no other serious complications or treatment-related deaths were observed after administration of cisplatin.

DISCUSSION

Cisplatin is one of the effective carcinostatic agents for HCC. When HCC is treated using transcatheter chemotherapy we usually use a combination of lipiodol and carcinostatics. TACE is now established as a method for administering chemotherapy in cases of HCC. Lipiodol has the characteristic of accumulating in a tumor vessel of HCC, and therefore carcinostatics are usually used in combination with lipiodol when performing TACE. It has been reported that water in an oil type emulsion is useful for steady accumulation and sustained release of carcinostatics.[21,22] However, cisplatin was prepared conventionally for use in intravenous drips using dosage increases in small steps, making preparation of the suspended injection with lipiodol difficult.

Until recently, mutual injections of cisplatin and lipiodol were used as one of the methods for administrating cisplatin in HCC patients. This method was reported previously as "sandwich therapy."[13] Now, "IA-call" which is a preparation of fine cisplatin powder, has been developed for use as an intrahepatic artery injection, with the fine powder being added easily to lipiodol to make a suspension.

In the present study we measured the concentration of total and free-cisplatin in hepatic vein and peripheral vein samples, and determined whether the treatment procedure influenced delay of drug delivery. Our data showed both total and free-cisplatin concentration increased in the order of group A, B, and C. These results may indirectly indicate that cisplatin was slowly released from liver tissue and decreased in the order of group A, B, and C. Regarding these results, we interpreted that lipiodol mainly affected the slow elution of cisplatin, and GCs augmented drug retention in the liver and tumor tissues by a temporary shut off of arterial blood flow. However, in this study, we could not directly investigate cisplatin concentrations in liver and tumor tissues. Although, one recent animal experimental study reported that suspended lipiodol with cisplatin powder mostly retained the cisplatin concentration as compared to other treatment methods (HAI and combined use of GCs without lipiodol) in VX-2 tumor tissues of rabbits.[23] Thus, we will need additional studies on human liver and tumor tissues. At this time, in order to retain cisplatin in liver tissue for a long duration, it was useful that the methods for administering cisplatin, lipiodol, and embolization also affected cisplatin concentration in liver and tumor tissue, and in the case of HCC patients treated with cisplatin, the use of TACE using Lipiodol and small-GCs provided additional benefits based on this study and previously reported experimental study results.[23] In recent years, we have used third-generation platinum compounds that do not have cross-resistance to cisplatin. Repeated use of cisplatin often causes drug resistance and allergic reactions such as anaphylaxis. The risk of allergic reactions increases from the third session of TACE with cisplatin,[24] and therefore, miriplatin can be considered as a second-line chemoembolization agent in patients who exhibit hypersensitivity or resistance to cisplatin. On the other hand, the development of drug-eluting microspheres (DEMs) provides a new treatment method for drug delivery.

Preclinical and clinical studies on TACE using DEM have demonstrated greater and longer retention times of drug within tumors and a lower systemic concentration compared with conventional TACE using lipiodol.[25-27]

To date, two types of microspheres capable of being loaded with a drug are commercially available: superabsorbent polymer microspheres (HepaSphere; Merit Medical Systems, Salt Lake City, UT, USA) and polyvinyl alcohol-based microspheres (DC

Bead; Biocompatibles, Farnham, UK). HepaSphere has a reservoir effect after loading with some chemotherapeutic agents, with two *in vitro* studies confirming that it efficiently loads and elutes doxorubicin, irinotecan, and cisplatin.[28,29]

In accordance with our previous report,[19] Seki and Hori[30] reported it was useful to switch anticancer therapy from epirubicin to cisplatin for treatment of HCC that had become refractory to TACE using epirubicin-loaded microspheres.

We therefore consider that it is necessary for future studies to carry out additional investigations on DEM.

Finally, this study had several limitations. First, the study sample size was too small and we could not examine liver and tumor tissues. In addition, tumor characteristics were different for each treatment method. Therefore, tumor characteristics (i.e., portal vein invasion, severe arterioportal) may have greatly affected the cisplatin concentrations in the hepatic and peripheral veins in group A. Regarding this point, we intend to investigate the same study protocol for patients with similar tumor characteristics in the future. Second, we only investigated useful drug delivery methods under single session transcatheter therapy. Therefore, we did not investigate continuous hepatic arterial infusional chemotherapy (i.e., combined use of cisplatin and 5-fluorouracil [5-FU]).

Primarily in Asian countries, many patients in group A are selected for continuous hepatic arterial infusional chemotherapy if they have adequate liver function. Therefore, additional studies will be needed under continuous hepatic arterial infusional chemotherapy with or without lipiodol. Also, we usually use epirubicin for first line treatment of HCC by TACE in Japan. Therefore, it is difficult to compare the actual efficacy of each anticancer drug (i.e., mitomycin-C, 5-FU) at the same level. Thus, we will need a prospective study to investigate this.

In conclusion, combined use of lipiodol and small-GCs clearly reduced the $AUC_{0\text{-infinity}}$ of total and free cisplatin concentrations in samples collected from the hepatic vein. In other words, free cisplatin concentrations in the liver were retained to a greater extent in the patient group administered lipiodol and small-GCs together. We consider that these results strongly support the combined use of embolization for treatment of HCC without advanced portal vein invasion or with severe arterioportal shunt that uses cisplatin at the time of injection into the hepatic artery.

CONFLICTS OF INTEREST

No potential conflict of interest relevant to this article was reported.

ACKNOWLEDGEMENTS

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REFERENCES

1. The Liver Cancer Study Group of Japan. Primary liver cancer in Japan. Sixth report. Cancer 1987;60:1400-1411.
2. Liver Cancer Study Group of Japan. Primary liver cancer in Japan. Clinicopathologic features and results of surgical treatment. Ann Surg 1990;211:277-287.
3. Wheeler PG, Melia W, Dubbins P, et al. Non-operative arterial embolisation in primary liver tumours. Br Med J 1979;2:242-244.
4. Chuang VP, Wallace S. Hepatic artery embolization in the treatment of hepatic neoplasms. Radiology 1981;140:51-58.
5. Okamura J, Horikawa S, Fujiyama T, et al. An appraisal of transcatheter arterial embolization combined with transcatheter arterial infusion of chemotherapeutic agent for hepatic malignancies. World J Surg 1982;6:352-357.
6. Yamada R, Sato M, Kawabata M, Nakatsuka H, Nakamura K, Takashima S. Hepatic artery embolization in 120 patients with unresectable hepatoma. Radiology 1983;148:397-401.
7. Lin DY, Liaw YF, Lee TY, Lai CM. Hepatic arterial embolization in patients with unresectable hepatocellular carcinoma: a randomized controlled trial. Gastroenterology 1988;94:453-456.
8. Ikeda K, Kumada H, Saitoh S, Arase Y, Chayama K. Effect of repeated transcatheter arterial embolization on the survival time in patients with hepatocellular carcinoma. An analysis by the Cox proportional hazard model. Cancer 1991;68:2150-2154.
9. Llovet JM, Real MI, Montaña X, et al. Arterial embolisation or chemoembolisation versus symptomatic treatment in patients with unresectable hepatocellular carcinoma: a randomised controlled trial. Lancet 2002;359:1734-1739.
10. Lo CM, Ngan H, Tso WK, et al. Randomized controlled trial of transarterial lipiodol chemoembolization for unresectable hepatocellular carcinoma. Hepatology 2002;35:1164-1171.
11. Shibata J, Fujiyama S, Sato T, Kishimoto S, Fukushima S, Nakano M. Hepatic arterial injection chemotherapy with cisplatin suspended in an oily lymphographic agent for hepatocellular carcinoma. Cancer 1989;64:1586-1594.
12. Sasaki Y, Imaoka S, Kasugai H, et al. A new approach to chemoembolization therapy for hepatoma using ethiodized oil, cisplatin, and gelatin sponge. Cancer 1987;60:1194-1203.
13. Imaoka S, Sasaki Y, Shibata T, et al. A pre-operative chemoembolization therapy using lipiodol, cisplatin and gelatin sponge for hepatocellular carcinoma. Cancer Chemother Pharmacol 1989;23 Suppl:S126-S128.
14. Yamamoto K, Shimizu T, Narabayashi I. Intraarterial infusion chemotherapy with lipiodol-CDDP suspension for hepatocellular carcinoma. Cardiovasc Intervent Radiol 2000;23:26-39.
15. Maeda S, Shibata J, Fujiyama S, et al. Long-term follow-up of hepatic arterial chemoembolization with cisplatin suspended in iodized oil for hepatocellular carcinoma. Hepatogastroenterology 2003;50:809-813.

16. Ikeda M, Maeda S, Shibata J, et al. Transcatheter arterial chemotherapy with and without embolization in patients with hepatocellular carcinoma. Oncology 2004;66:24-31.
17. Ando E, Tanaka M, Yamashita F, et al. Hepatic arterial infusion chemotherapy for advanced hepatocellular carcinoma with portal vein tumor thrombosis: analysis of 48 cases. Cancer 2002;95:588-595.
18. Kaneko S, Urabe T, Kobayashi K. Combination chemotherapy for advanced hepatocellular carcinoma complicated by major portal vein thrombosis. Oncology 2002;62 Suppl 1:69-73.
19. Kawamura Y, Ikeda K, Hirakawa M, et al. Efficacy of platinum analogue for advanced hepatocellular carcinoma unresponsive to transcatheter arterial chemoembolization with epirubicin. Hepatol Res 2009;39:346-354.
20. Yamaoka K. Automated pharmacokinetic analysis system. Tokyo: Nankodo, 1984.
21. Yi SW, Kim YH, Kwon IC, et al. Stable lipiodolized emulsions for hepatoma targeting and treatment by transcatheter arterial chemoembolization. J Control Release 1998;50:135-143.
22. Demachi H, Matsui O, Abo H, Tatsu H. Simulation model based on non-newtonian fluid mechanics applied to the evaluation of the embolic effect of emulsions of iodized oil and anticancer drug. Cardiovasc Intervent Radiol 2000;23:285-290.
23. Morimoto K, Sakaguchi H, Tanaka T, et al. Transarterial chemoembolization using cisplatin powder in a rabbit model of liver cancer. Cardiovasc Intervent Radiol 2008;31:981-985.
24. Kawaoka T, Aikata H, Katamura Y, et al. Hypersensitivity reactions to transcatheter chemoembolization with cisplatin and Lipiodol suspension for unresectable hepatocellular carcinoma. J Vasc Interv Radiol 2010;21:1219-1225.
25. Varela M, Real MI, Burrel M, et al. Chemoembolization of hepatocellular carcinoma with drug eluting beads: efficacy and doxorubicin pharmacokinetics. J Hepatol 2007;46:474-481.
26. Hong K, Khwaja A, Liapi E, Torbenson MS, Georgiades CS, Geschwind JF. New intra-arterial drug delivery system for the treatment of liver cancer: preclinical assessment in a rabbit model of liver cancer. Clin Cancer Res 2006;12:2563-2567.
27. Lewis AL, Gonzalez MV, Lloyd AW, et al. DC bead: in vitro characterization of a drug-delivery device for transarterial chemoembolization. J Vasc Interv Radiol 2006;17(2 Pt 1):335-342.
28. Jordan O, Denys A, De Baere T, Boulens N, Doelker E. Comparative study of chemoembolization loadable beads: in vitro drug release and physical properties of DC bead and hepasphere loaded with doxorubicin and irinotecan. J Vasc Interv Radiol 2010;21:1084-1090.
29. Maeda N, Osuga K, Higashihara H, et al. In vitro characterization of cisplatin-loaded superabsorbent polymer microspheres designed for chemoembolization. J Vasc Interv Radiol 2010;21:877-881.
30. Seki A, Hori S. Switching the loaded agent from epirubicin to cisplatin: salvage transcatheter arterial chemoembolization with drug-eluting microspheres for unresectable hepatocellular carcinoma. Cardiovasc Intervent Radiol 2012;35:555-562.

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Original Article

Discrimination of fibrotic staging of chronic hepatitis C using multiple fibrotic markers

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Aim: In order to evaluate and judge a fibrotic stage of patients with chronic hepatitis C, multivariate regression analysis was performed using multiple fibrotic markers.

Methods: A total of 581 patients from eight hepatology units and institutes were diagnosed by needle biopsy as having chronic liver disease caused by hepatitis C virus. Twenty-three variables and their natural logarithmic transformation were employed in the multivariate analysis.

Results: Multivariate regression analysis finally obtained the following function: $z = 2.89 \times ln$ (type IV collagen 7S) (ng/mL) $- 0.011 \times$ (platelet count) ($\times 10^3/mm^3$) $+ 0.79 \times ln$ (total bilirubin) (mg/dL) $+ 0.39 \times ln$ (hyaluronic acid) (μg/L) $- 1.87$. Median values of the fibrotic score of F1 ($n = 172$), F2 ($n = 80$), F3 ($n = 37$) and F4 ($n = 16$) were calculated as 1.00, 1.45, 2.82 and 3.83, respectively. Multiple regression coefficient and coefficient of determination were 0.56 and 0.320, respectively. Validation with patient data from other institutions demonstrated good reproducibility of the fibrotic score for hepatitis C (FSC), showing 1.10 in F1 ($n = 156$), 2.35 in F2 ($n = 73$), 3.16 in F3 ($n = 36$) and 3.58 in F4 ($n = 11$).

Conclusion: A concise multiple regression function using four laboratory parameters successfully predicted pathological fibrotic stage of patients with hepatitis C virus infection.

Key words: chronic hepatitis, hepatitis C virus, liver cirrhosis, liver fibrosis, multiple regression analysis, stage

INTRODUCTION

WHEN HEPATITIS C virus (HCV)-related chronic liver disease was found by biochemical and virological examination, peritoneoscopy and/or liver biopsy can establish the definitive diagnosis of chronic hepatitis and liver cirrhosis. Although these pathological procedures are reliable and informative both in diagnosis and treatment, they sometimes require medical invasion and financial costs, including the risk of bleeding from needle puncture, some pain experienced during the examination, medical expenses and hospitalization for a few days. The pathological examination is, therefore, rarely performed repeatedly in a short period of time, even when disease activity is severe and progression of liver disease is highly suspected. Recently, many authors described the usefulness of ultrasonographic elastography and magnetic resonance imaging technology in the estimation of staging of chronic hepatitis and cirrhosis.[1–4] These ways of estimation using the imaging apparatuses seem truly useful for current patients, but it cannot evaluate and compare with past fibrotic states of patients retrospectively. Moreover, the same apparatus for elastometry will not be available for repeated measurement for a follow-up examination, several years later for example.

In spite of the accuracy of biopsy and of convenience of elastography in chronic liver disease, clinical diagnosis based on biochemistry and hematology is still indispensable for the daily practice of many patients with

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HCV-related liver disease. Recently, several studies were published about estimation of hepatitis stages, using one or more serum biomarkers. Discriminant functions or multivariate analyses demonstrated that approximately 60–90% of patients with chronic hepatitis C were correctly classified as mild hepatitis and severe hepatitis with advanced fibrosis.[5–16] The usefulness of the discriminant functions was, however, less valuable up to the present time for a few reasons. First, these functions were made for the purpose of discrimination of severe hepatic fibrosis from mild fibrosis, and four histological classifications (F1, F2, F3 and F4) were selected in almost of the studies. Second, some studies analyzed both hepatitis B virus and HCV infection, although the significance and actual values of each liver function test in the evaluation of the severity of liver disease were not similar among each viral hepatitis and alcoholic liver disease. Third, biochemical markers for liver fibrosis (e.g. hyaluronic acid, type IV collagen, procollagen III peptide)[17–19] were not always included in those previous studies.

We tried to generate a function estimating fibrotic stages of HCV-related chronic hepatitis, which were objectively diagnosed by liver biopsy. The purpose of this study is, therefore, to make a reliable multiple regression function and to obtain practical coefficients for significant variables also using fibrotic markers.

METHODS

Patients

A TOTAL OF 605 Japanese patients with chronic hepatitis C were recruited for the study from eight hospitals in Japan: Toranomon Hospital, Hiroshima University Hospital (K. Chayama, M.D.), Ehime University Hospital (M. Onji, M.D.), Musashino Red Cross Hospital (N. Izumi, M.D.), Shishu University Hospital (E. Tanaka, M.D.), Showa University Hospital (M. Imawari, M.D.), Osaka University Hospital (T. Takehara, M.D.) and Kagoshima University Hospital (H. Tsubouchi, M.D.). Inclusion criteria for this study were: (i) positive HCV antibody for more than 6 months; (ii) persistent or intermittent elevation in aspartate aminotransferase (AST)/alanine aminotransferase (ALT) levels; and (iii) liver biopsy showing chronic hepatitis (F1, F2, F3 or F4). We excluded those patients with overt alcoholic liver disease or fatty liver, association of other types of liver disease (e.g. hepatitis B, primary biliary cirrhosis, autoimmune hepatitis), or those associated with hepatocellular carcinoma or other malignancy. Among the patients, 603 fulfilled the conditions for the study: complete demographic data, basic laboratory data of hematology and biochemistry, required liver biopsy specimens, and sufficient amount of frozen sera. We also excluded an additional 22 patients with eventual histological diagnosis of F0 stage.

Finally, a total of 581 patients who were diagnosed as having chronic hepatitis or cirrhosis (F1, F2, F3 or F4) were analyzed for the following hematological, biochemical and histopathological examination. There were 305 males and 276 females aged 15–78 with a median of 55 years.

All the patients presented written informed consent in individual hospitals and medical centers, and the study was approved by each ethical committee.

Hematological and biochemical examination

Hematological and standard biochemical evaluation had been performed in each medical institution: white blood cell, red blood cell count, hemoglobin, platelet count, total bilirubin, AST, ALT, AST/ALT ratio (AAR), γ-glutamyltransferase (GGT), total protein, albumin and γ-globulin.

Special biochemical examinations including fibrotic markers were carried out using stored frozen sera at −20°C or lower: α2-macroglobulin, haptoglobin concentration, haptoglobin typing, apolipoprotein A1, hyaluronic acid, tissue inhibitor of matrix metalloproteinase (TIMP)-1, TIMP-2, procollagen III peptide and type IV collagen 7S.

Histological diagnosis of chronic hepatitis and cirrhosis

All of the 581 cases fulfilled required standards of histological evaluation: sufficient length of specimen, hematoxylin–eosin staining and at least one specimen with fiber staining. Four independent pathologists (Y. T., J. F., F. K. and T. F.), who were not informed of patients' background and laboratory features except for age and sex, evaluated the 581 specimens regarding the stages of fibrosis and activity. Pathological classification of chronic hepatitis staging was based on Desmet *et al.*[20]

Before judgment of histological staging of individual specimens, the pathologists discussed objective and reproducible judgment of pathological diagnosis of hepatitis. They made a panel for obvious criteria using typical microscopic pictures for each stage, and it was always referred to during the procedure of pathological judgment. When inconsistent results were found in the diagnosis of stage of hepatitis among the pathologists, the final judgment was accepted as the majority rule among them.

Statistical analysis

Non-parametric procedures were employed for the analysis of background characteristics and laboratory data among patients in each stage, including Mann–Whitney *U*-test, Kruskal–Wallis test and χ^2-test.

The normality of the distribution of the data was evaluated by Kolmogorov–Smirnov one-sample test. Because certain variables partly did not conform to a normal distribution, natural logarithmic transformation of bilirubin, AST, ALT, GGT, α2-macroglobulin, hyaluronic acid, type IV collagen 7S and TIMP-2 were also analyzed in the following calculation. The natural logarithmic transformation of the results yielded a normal distribution or symmetrical distribution for all the analyzed factors. After the procedures, the following multiple regression analysis became rationally robust against deviations from normal distribution. In order to avoid introducing into the model any variables that were mutually correlated, we checked the interaction between all pairs of the variables by calculating variance of inflation factors. Of the highly correlated variables, less significant factors were removed from the viewpoint of multicollinearity.

Multivariate regression analysis was performed using 305 patient data from Toranomon Hospital (training dataset), to generate training data of predicting function. We used a stepwise method for selection of informative subsets of explanatory variables in the model. Multiple regression coefficient and coefficient of determination are also taken into account in the selection of variables. Next, we validated the obtained predictive function using the remaining 276 patient data from the other seven liver institutions (validation dataset).

A *P*-value of less than 0.05 with two-tailed test was considered to be significant. Data analysis was performed using the computer program SPSS version 19.[21]

For evaluation of the efficiency and usefulness of obtained function for estimation of fibrosis, we compared various fibrotic scores for hepatitis C, including AAR,[8] AST-to-platelet ratio index (APRI),[12] FIB-4[13] and FibroTest.[9]

RESULTS

Pathological diagnosis

FOUR PATHOLOGISTS INDEPENDENTLY judged the fibrotic stages and inflammatory activity for 581 specimens of chronic hepatitis/cirrhosis caused by HCV. A total of 328 patients (56.5%) had a fibrotic stage of F1, 153 (26.3%) F2, 73 (12.6%) F3 and 27 (4.6%) F4. In the training subgroup ($n = 305$), judgment of F1 was made in 172, F2 in 80, F3 in 37 and F4 in 16. In the validation group ($n = 276$), judgment as F1 was made in 156, F2 in 73, F3 in 36 and F4 in 11.

According to hepatitis activity classification, A0 was found in nine patients (1.52%), A1 in 350 (60.2%), A2 in 198 (34.1%) and A3 in 24 (4.1%).

Laboratory data of each hepatitis stage in training group

There were 161 males and 144 females with a median age of 54 years (range, 22–69). Laboratory data of the 305 patients in the training group are shown in Table 1. Although several individual items were well correlated with the severity of hepatic fibrosis, significant overlap values were noted among F1 to F4 stages: platelet count, GGT, γ-globulin, hyaluronic acid and type IV collagen 7S.

Regression function generated from training patient group

After stepwise variable selection, multivariate regression analysis finally obtained the following function: $z = 2.89 \times ln$ (type IV collagen 7S) (ng/mL) $- 0.011 \times$ (platelet count) ($\times 10^3$/mm^3) $+ 0.79 \times ln$ (total bilirubin) (ng/mL) $+ 0.39 \times ln$ (hyaluronic acid) (μm/L) $-$ 1.87. Median values of the fibrotic score of F1 ($n = 172$), F2 ($n = 80$), F3 ($n = 37$) and F4 stages ($n = 16$) were calculated as 1.00, 1.45, 2.82 and 3.83, respectively (Fig. 1). The multiple regression coefficient and coefficient of determination were 0.56 and 0.32, respectively.

A 55-year-old man with F1 fibrotic stage (Fig. 2a) showed serum type IV collagen concentration as 3.8 ng/mL, platelet as 152×10^3 count/mm^3, total bilirubin as 0.8 mg/dL and hyaluronic acid as 16 μg/L. The regression function provided his fibrotic score as 1.16. Another man aged 43 years had F3 fibrosis with severe hepatitis activity of A3 on histological examination (Fig. 2b). His type IV collagen was 11.0 ng/mL, platelet 162×10^3 count/mm^3, total bilirubin 0.7 mg/dL and hyaluronic acid 189 μg/L, and regression function calculated his fibrotic score as 4.98.

Validation of discriminant function

Validation data of 276 patients (Table 2) were collected from the other seven institutions in Japan. When applying the regression function for the validation set, the fibrotic score for hepatitis C (FSC) demonstrated good reproducibility, showing 1.10 in patients with chronic hepatitis of F1 ($n = 156$), 2.35 in F2 ($n = 73$), 3.16 in F3 ($n = 36$) and 3.58 in F4 ($n = 11$) (Fig. 3). Although F4

Table 1 Demography and laboratory data of 305 patients in training group

	F1 ($n = 172$)	F2 ($n = 80$)	F3 ($n = 37$)	F4 ($n = 16$)
Demography				
Males : females	97:75	38:42	20:17	6:10
Age (median, range)	51 (22–69)	55 (29–68)	55 (27–69)	56.5 (29–65)
Laboratory data (median, range)				
WBC ($\times 10^3/mm^3$)	4.7 (2.0–10.1)	4.3 (2.3–8.5)	4.5 (2.9–6.8)	4.7 (3.3–6.9)
Hemoglobin(g/dL)	14.6 (11.0–18.2)	14.4 (9.3–17.4)	14.6 (11.5–17.7)	14.55 (12.1–16.5)
Platelet ($\times 10^3/mm^3$)	183 (52–364)	161 (82–387)	131 (74–237)	124 (7.7–191)
Albumin (g/dL)	4.1 (2.3–4.9)	4.0 (3.5–4.6)	3.9 (3.1–4.6)	3.8 (3.3–4.3)
Bilirubin (mg/dL)	0.8 (0.2–1.9)	0.7 (0.3–1.7)	0.9 (0.4–7.5)	0.8 (0.5–7.4)
AST (IU/L)	42 (16–386)	61 (16–332)	63 (13–238)	71 (30–160)
ALT (IU/L)	60.5 (12–1664)	84.5 (10–647)	108 (27–415)	90.5 (36–264)
γ-GTP (IU/L)	40 (7–383)	48 (10–262)	54 (13–209)	58 (21–195)
γ-Globulin (g/dL)	1.47 (0.58–3.40)	1.61 (1.02–2.41)	1.69 (0.66–2.64)	1.79 (1.22–2.73)
γ-Globulin (%)	19.4 (10.0–40.5)	20.9 (14.0–28.3)	21.3 (8.1–30.4)	22.7 (16.5–36.9)
α2-Macroglobulin (mg/dL)	269 (123–505)	335 (154–551)	369 (183–627)	317 (207–511)
Haptoglobin (mg/dL)	94.5 (<5–265)	75.5 (<5–263)	56 (<5–2031)	75 (30–142)
Apolipoprotein A1 (mg/dL)	132 (71–209)	131 (73–207)	124 (98–166)	121 (83–153)
Hyaluronic acid (μg/L)	25 (<5–407)	41.5 (<5–263)	71 (<5–326)	89.5 (5–246)
TIMP-1 (ng/mL)	165 (73–291)	173 (97–302)	182 (126–308)	192.5 (128–260)
TIMP-2 (ng/mL)	77.5 (31–210)	80 (34–307)	76 (46–143)	78 (58–110)
Procollagen III peptide (U/mL)	0.75 (0.47–1.50)	0.805 (0.61–1.70)	0.86 (0.53–1.50)	1.05 (0.66–1.60)
Type IV collagen 7S (ng/mL)	4.0 (1.7–73)	4.3 (2.1–11.0)	5.2 (3.2–11.0)	5.8 (4.3–9.4)

γ-GTP, γ-glutamyl transpeptidase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; TIMP, tissue inhibitor of matrix metalloproteinase; WBC, white blood cell.

fibrotic stage consisted of only 11 patients and the score 3.58 was regarded as a rather low value, the scores of other stages of fibrosis were concordant with histological fibrosis.

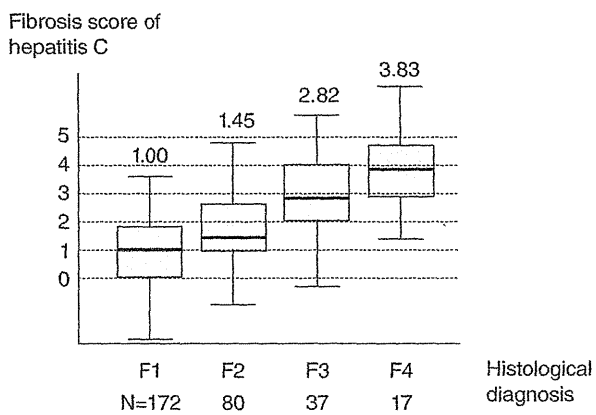

Figure 1 Box and whisker plots of fibrotic score of each group of histological fibrosis in the training dataset. Fibrotic score of hepatitis C (FSC) was generated by the function, $z = 2.89 \times ln$ (type IV collagen 7S) (ng/mL) $- 0.011 \times$ (platelet count) ($\times 10^3/mm^3$) $+ 0.79 \times ln$ (total bilirubin) (mg/dL) $+ 0.39 \times ln$ (hyaluronic acid) (μg/L) $- 1.87$.

Comparisons of efficacy with various fibrotic scores (Fig. 4)

In order to evaluate the efficacy and usefulness of the obtained FSC, we compared with previously reported fibrotic scores using training data. AAR, APRI, FIB-4 and FibroTest showed only slight correlation with actual histological stage. APRI and FIB-4 demonstrated increasing trends of the score associated with histological fibrosis, but significant overlapping scores were found through F1 to F4. Spearman's correlation coefficients of AAR, APRI, FIB-4 and FibroTest were 0.021 ($P = 0.707$), 0.462 ($P < 0.001$), 0.440 ($P < 0.001$) and 0.415 ($P < 0.001$), respectively. Our FSC showed Spearman's correlation coefficient of 0.572 ($P < 0.001$), and was of much higher value than the others.

DISCUSSION

RECOGNITION OF SEVERITY of chronic hepatitis is essential in managing patients with chronic HCV infection: estimation of length of infection, existence of any previous hepatitis activity, presumption of current fibrotic stage, and prediction of future fibrotic progression and hepatocarcinogenesis. Differential diagnosis of cirrhosis from chronic hepatitis is especially important

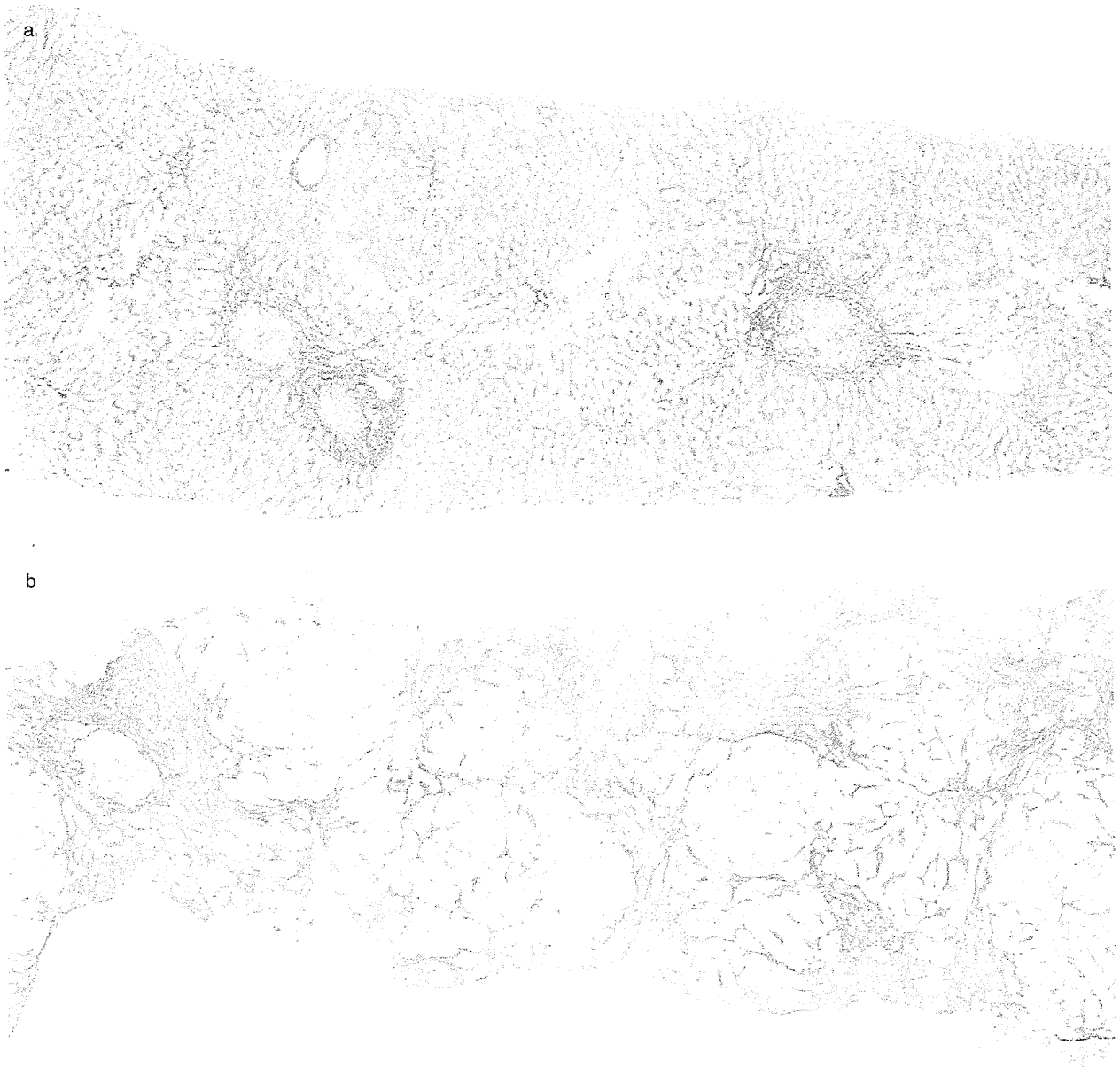

Figure 2 Case presentations of the training set. (a) A 55-year-old man with F1 fibrosis. Final regression function provided his fibrotic score as 1.16. (b) A 43-year-old man with F3 fibrosis with severe hepatitis activity. His regression coefficient was calculated as 4.98 (silver stain, ×40).

in the evaluation of chronic HCV infection. Identification of liver cirrhosis often leads to an important change in management of the patients: needs for fiberscopic examination for esophageal varices, ultrasonographic exploration for the association of liver cancer, and prediction of hepatic decompensation.

Recently, non-invasive estimation of severity of liver fibrosis has been reported in patients with HCV-related chronic hepatitis.[6–14] However, these studies were principally aimed at differentiation of advanced fibrotic stages of F3 or F4 from mild fibrotic stages of F1 or F2. Those discriminative functions were insufficient to

Table 2 Demography and laboratory data of 276 patients in validation group

	F1 ($n = 156$)	F2 ($n = 73$)	F3 ($n = 36$)	F4 ($n = 11$)
Demography				
Males : females	83:73	42:31	13:23	6:5
Age (median, range)	55 (15–74)	58 (32–77)	62.5 (30–78)	51 (38–73)
Laboratory data (median, range)				
WBC ($\times 10^3/mm^3$)	5.1 (2.1–10.5)	4.8 (2.6–9.0)	4.85 (2.3–14.2)	3.9 (3.2–6.0)
Hemoglobin (g/dL)	14.2 (8.9–17.7)	14.4 (11.8–17.4)	14.1 (10.1–16.4)	13.6 (8.9–16.3)
Platelet ($\times 10^3/mm^3$)	183 (59–440)	153 (80–265)	136 (64–348)	135 (79–153)
Albumin (g/dL)	4.3 (3.1–5.3)	4.3 (3.3–5.2)	4.05 (3.0–5.5)	3.9 (3.0–4.7)
Bilirubin (mg/dL)	0.7 (0.2–8.7)	0.7 (0.2–1.7)	0.8 (0.2–2.5)	0.8 (0.4–11.0)
AST (IU/L)	35 (11–1390)	49 (19–183)	80 (20–190)	96 (29–257)
ALT (IU/L)	49 (11–1635)	62 (12–575)	84 (14–218)	115 (29–303)
γ-GTP (IU/L)	35 (11–600)	52 (10–497)	51 (14–236)	112 (17–312)
γ-Globulin (g/dL)	1.47 (0.70–2.14)	1.60 (0.80–2.37)	1.71 (0.63–2.62)	2.19 (1.70–2.82)
γ-Globulin (%)	19.5 (9.2–26.4)	20.8 (10.8–30.8)	22.4 (9.5–29.9)	27.4 (21.8–35.3)
α2-Macroglobulin (mg/dL)	271.5 (126–572)	381 (172–573)	405.5 (196–594)	468 (242–655)
Haptoglobin (mg/dL)	95 (<5–305)	80 (<5–223)	63.5 (<5–192)	65 (<5–130)
Apolipoprotein A1 (mg/dL)	126 (45–198)	127 (63–191)	116 (46–172)	108 (62–171)
Hyaluronic acid (μg/L)	37.5 (<5–1260)	68 (5–1000)	140.5 (23–2610)	159 (33–364)
TIMP-1 (ng/mL)	157.5 (77–301)	172 (89–355)	188.5 (99–430)	192 (112–320)
TIMP-2 (ng/mL)	70 (21–294)	73 (21–207)	89 (27–280)	76 (36–120)
Procollagen III peptide (U/mL)	0.73 (0.52–8.30)	0.81 (0.53–1.60)	1.00 (0.63–1.90)	1.00 (0.68–1.60)
Type IV collagen 7S (ng/mL)	3.9 (1.2–12.0)	4.5 (2.3–9.9)	5.8 (2.8–16.0)	6.1 (4.6–10.0)

γ-GTP, γ-glutamyl transpeptidase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; TIMP, tissue inhibitor of matrix metalloproteinase; WBC, white blood cell.

recognize the stepwise progression of viral hepatitis from F1 through F4. This dichotomy (mild or severe) of chronic hepatitis C seemed less valuable in the study of disease progression, disease control abilities of antiviral drugs and estimation of histological improvement after anti-inflammatory drugs. A histology-oriented, practical and reliable formula is therefore required for the diagnosis and investigation of chronic hepatitis C.

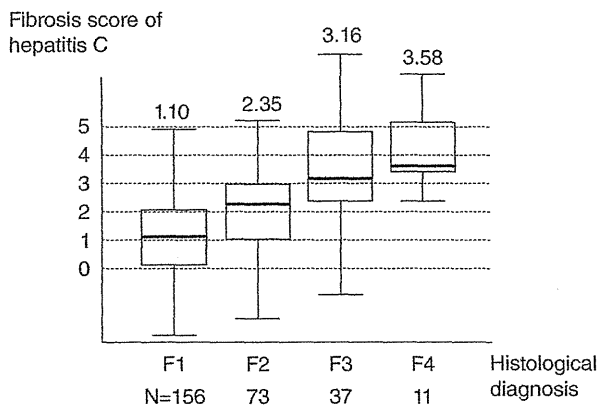

Figure 3 Box and whisker plots of fibrotic score of each group of histological fibrosis in the validation dataset. Fibrotic score of hepatitis C (FSC) was generated by the function, $z = 2.89 \times ln$ (type IV collagen 7S) (ng/mL) $- 0.011 \times$ (platelet count) ($\times 10^3/mm^3$) $+ 0.79 \times ln$ (total bilirubin) (ng/mL) $+ 0.39 \times ln$ (hyaluronic acid) (μg/L) $- 1.87$.

This study was aimed to establish non-invasive evaluation and calculation of liver fibrosis for patients with chronic HCV infection. Although it was retrospectively performed as a multicenter study of eight institutions, judgment of histological diagnosis was independently performed by four pathologists in the other hospital, informed of nothing except for the patient's age, sex and positive HCV infection. Objective judgment of the histological staging and grading in sufficient biopsy specimens could be obtained.

As many as 581 patients with chronic hepatitis C were analyzed in this study, who had been diagnosed as having chronic hepatitis or cirrhosis by liver biopsy performed in experienced liver units in Japan. To obtain the most suitable equation approximating histological fibrotic stage, multivariate analysis was performed using two demographic parameters (age and sex) and 21 hematological and biochemical markers with or without logarithmic transformation. They included many kinds of fibrotic markers: α2-macroglobulin, haptoglobin concentration, haptoglobin typing, apolipo-

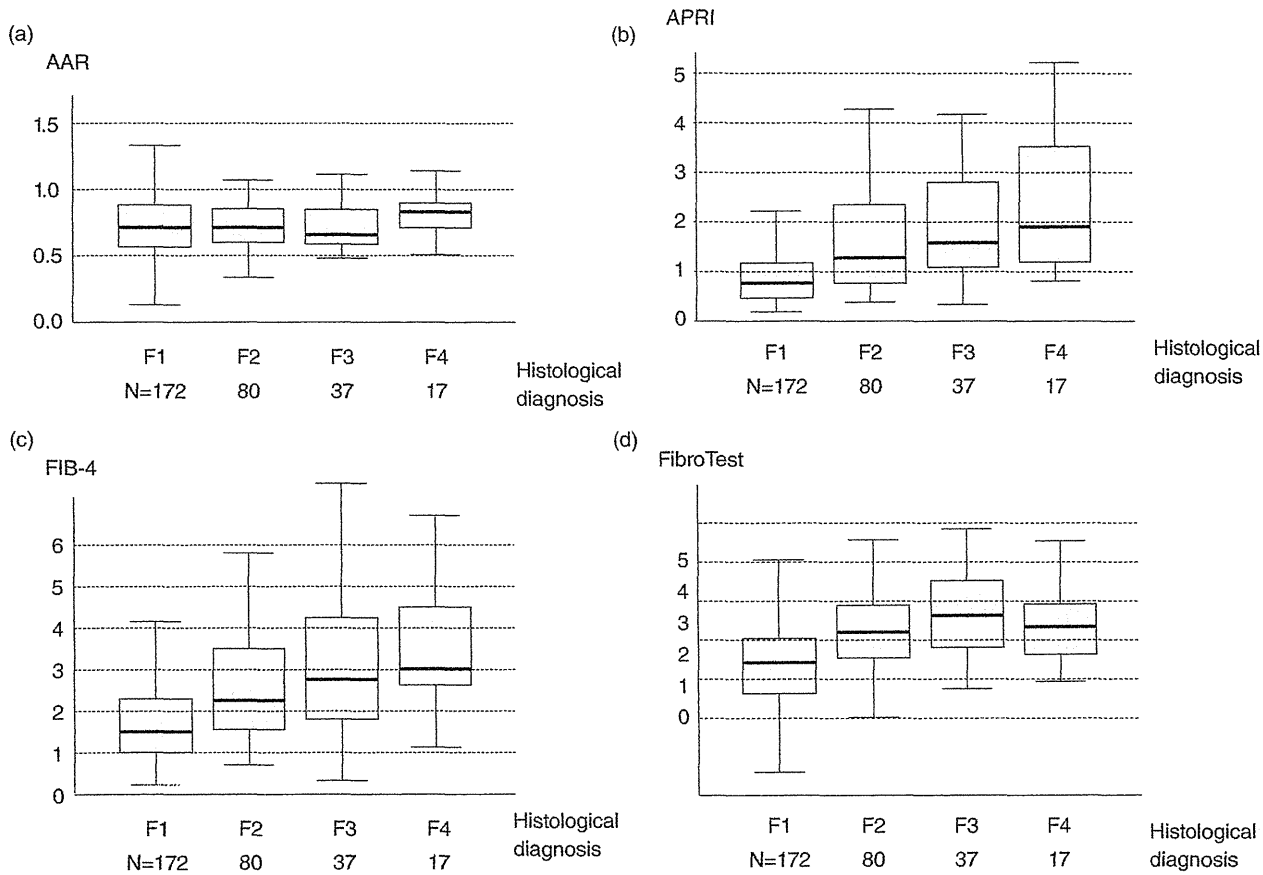

Figure 4 Previously published fibrotic scores: (a) aspartate aminotransferase (AST)/alanine aminotransferase (ALT) ratio (AAR),[8] (b) AST-to-platelet ratio index (APRI), calculated by AST / (upper limit of normal of AST) / (platelet count [$\times 10^9$/L]) $\times$ 100.[12] (c) FIB-4 score, calculated by age $\times$ AST [IU/L] / (platelet count [$\times 10^9$/L] $\times$ ALT [IU/L]$^{0.5}$).[13] (d) FibroTest score regression coefficient was: $Z = 4.467 \times \log^{10}$ (α2-macroglobulin [g/L]) $- 1.357 \times \log^{10}$ (haptoglobin [g/L]) $+ 1.017 \times \log^{10}$ [γ-glutamyltransferase [GGT] [IU/L]) $+ 0.0281 \times$ (age [years]) $+ 1.737 \times \log^{10}$ (bilirubin [μm/L]) $- 1.184 \times \log^{10}$ (apolipoprotein A1 [g/L]) $+ 0.301 \times$ (sex [female = 0, male = 1]) $- 5.54$.[9]

protein A1, hyaluronic acid, TIMP-1, TIMP-2, procollagen III peptide and type IV collagen 7S. Multiple regression analysis finally generated a first-degree polynomial function consisting of four variables: type IV collagen 7S, platelet count, bilirubin and hyaluronic acid. A constant numeral (−1.87) was finally adjusted in the regression equation in order to obtain fitted figures for fibrotic stages of F1, F2, F3 and F4. From the magnitude of the standardized partial regression coefficient of individual variable in the function, *ln* (type IV collagen 7S) demonstrated the most potent contribution toward the prediction of liver fibrosis. Platelet count and *ln* (bilirubin) proved to be the second and third distinctive power in the model, respectively.

The obtained figure of FSC was generated to imitate actual "F factor" of histological staging. FSC was sufficiently fitted to actual fibrotic stages with certain overlapping as was usually found in histological ambiguity judged by pathologists. Because judgment of fibrosis in chronic hepatitis often shows a transitional histological staging, pathological examination could not always achieve a clear-cut diagnosis discriminating F1, F2, F3 or F4. Considering the limitation of pathological difficulty in differentiation of the four continuous disease entities, the obtained regression function showed satisfactory high accuracy rates in the prediction of liver disease severity. FSC can provide one or two decimal places (e.g. 2.4 or 2.46) and the utility of the score is possibly higher

than mere histological staging of F1, F2, F3 or F4. The reproducibility was confirmed by the remaining 276 patients' data obtained from the other seven hospitals. Although the validation data were collected from different geographic area and different chronologic situation, FSC showed similar results in prediction of histological staging.

Fibrotic score for hepatitis C seemed a very useful quantitative marker in evaluating severity of fibrotic severity of hepatitis C patients without invasive procedures and without any specialized ultrasonography or magnetic resonance imaging. FSC also has an advantage of measurement, in which old blood samples are available for retrospective assessment of varied clinical settings: old sera from 20 years ago at the time of initial liver biopsy, or paired sera before and after a long-term anti-inflammatory therapy, for example. These kinds of retrospective assessments of fibrotic staging will be valuable in estimating a long-term progression of liver disease, in evaluating efficacy of a long-term medication or other medical intervention, or in making a political judgment from the viewpoint of socioeconomic efficacy.

The score can be calculated for any patients with chronic HCV infection. Although this multiple regression model dealt with appropriate logarithmic transformation for non-normal distribution parameters, the regression analysis was based on a linear regression model. Very slight fibrosis can be calculated as less than 1.00, which is commonly found with a slight degree of chronic hepatitis with a tiny fibrotic change as F0. Very severe fibrosis may be calculated as more than 4.00, which is an imaginable and nonsense number in the scoring system of fibrosis. FSC is, however, very useful and valuable in real clinical setting. Estimation of severity of liver fibrosis in outpatient clinics, evaluation of natural progression of patients' fibrosis over 10 years, and assessment of a long-term administration of interferon in patients with chronic hepatitis C from the viewpoint of fibrotic change. In this study, because certain patients actually had a history of interferon administration, regression of liver fibrosis during and after the treatment could be assessed when prior sera were available for serial evaluation of FSC. We can also expect the usefulness of evaluation of carcinogenic risk after sustained virological response, and stage progression with alcohol intake or obesity-induced steatosis. Recent development of new directly acting antiviral agents require evaluation for long-term histological advantage, for aggravation of hepatitis stage during viral and biochemical breakthrough caused by HCV mutation, estimation of future carcinogenic risk, and even for the best way of management of patients with chronic hepatitis C. FSC seems one of the ideal methods of approximation for fibrotic stage of chronic hepatitis C. Repeated measurement is quite suitable for patients with an unestablished treatment or trial, every 1 or 2 years, for example. Because the current regression function was generated from the data of HCV-related chronic liver disease, this equation would not be suitable for the recognition of HBV-related chronic liver disease,[22] alcoholic liver disease and other congenital or autoimmune liver diseases. To recognize the latter diseases, other studies about individual diseases must be performed.

We compared the usefulness of the FSC with that of other fibrotic scores.[8,9,12,13] More simple and inexpensive AAR or APRI could not well estimate fibrotic stages with poor correlation coefficients of 0.021 and 0.462, which were much lower than the coefficient of FSC of 0.572. FibroTest, which contained three costly fibrotic markers (α2-macroglobulin, haptoglobin and apolipoprotein A1), also showed a low correlation coefficient of 0.415, suggesting that the usefulness was limited in HCV positive Asian patients. Although FIB-4 demonstrated the best coefficient of 0.440 among the fibrotic scores, significant overlaps were found between neighboring stages and obtained scores were not coordinated for rea17l histological classification. Because this study also measured those special markers included in FibroTest, the ability of discrimination of fibrotic stages could be compared among the five fibrotic scoring systems.

In conclusion, FSC was a useful and reliable biomarker for prediction of liver fibrosis in patients with chronic HCV infection. FSC is expected to be introduced and utilized in varied kinds of studies and trials. Its accuracy and reproducibility require further validation using more numbers of patients in several countries other than Japan.

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REFERENCES

1 Sandrin L, Fourquet B, Hasquenoph JM *et al.* Transient elastography: a new noninvasive method for assessment of hepatic fibrosis. *Ultrasound Med Biol* 2003; 29: 1705–13.
2 Hanna RF, Kased N, Kwan SW *et al.* Double-contrast MRI for accurate staging of hepatocellular carcinoma in patients with cirrhosis. *AJR Am J Roentgenol* 2008; 190: 47–57.

3 Hagiwara M, Rusinek H, Lee VS *et al.* Advanced liver fibrosis: diagnosis with 3D whole-liver perfusion MR imaging – initial experience. *Radiology* 2008; **246:** 926–34.
4 Taouli B, Chouli M, Martin AJ, Qayyum A, Coakley FV, Vilgrain V. Chronic hepatitis: role of diffusion-weighted imaging and diffusion tensor imaging for the diagnosis of liver fibrosis and inflammation. *J Magn Reson Imaging* 2008; **28:** 89–95.
5 Williams AL, Hoofnagle JH. Ratio of serum aspartate to alanine aminotransferase in chronic hepatitis. Relationship to cirrhosis. *Gastroenterology* 1988; **95:** 734–9.
6 Poynard T, Bedossa P. Age and platelet count: a simple index for predicting the presence of histological lesions in patients with antibodies to hepatitis C virus. METAVIR and CLINIVIR Cooperative Study Groups. *J Viral Hepat* 1997; **4:** 199–208.
7 Sheth SG, Flamm SL, Gordon FD, Chopra S. AST/ALT ratio predicts cirrhosis in patients with chronic hepatitis C virus infection. *Am J Gastroenterol* 1998; **93:** 44–8.
8 Giannini E, Botta F, Fasoli A *et al.* Progressive liver functional impairment is associated with an increase in AST/ALT ratio. *Dig Dis Sci* 1999; **44:** 1249–53.
9 Imbert-Bismut F, Ratziu V, Pieroni L, Charlotte F, Benhamou Y, Poynard T, MULTIVIRC Group. Biochemical imarkers of liver fibrosis in patients with hepatitis C virus infection: a prospective study. *Lancet* 2001; **357:** 1069–75.
10 Wai CT, Greenson JK, Fontana RJ *et al.* A simple noninvasive index can predict both significant fibrosis and cirrhosis in patients with chronic hepatitis C. *Hepatology* 2003; **38:** 518–26.
11 Benlloch S, Berenguer M, Prieto M, Rayón JM, Aguilera V, Berenguer J. Prediction of fibrosis in HCV-infected liver transplant recipients with a simple noninvasive index. *Liver Transpl* 2005; **11:** 456–62.
12 Chrysanthos NV, Papatheodoridis GV, Savvas S *et al.* Aspartate aminotransferase to platelet ratio index for fibrosis evaluation in chronic viral hepatitis. *Eur J Gastroenterol Hepatol* 2006; **18:** 389–96.
13 Sterling RK, Lissen E, Clumeck N *et al.* Develoment of a simple noninvasive index to predict significant fibrosis in patients with HIV/HCV coinfection. *Hepatology* 2006; **43:** 1317–25.
14 Alsatie M, Kwo PY, Gingerich JR *et al.* A multivariable model of clinical variables predicts advanced fibrosis in chronic hepatitis C. *J Clin Gastroenterol* 2007; **41:** 416–21.
15 Attallah AM, Abdallah SO, El Sayed AS *et al.* Non-invasive predictive score of fibrosis stages in chronic hepatitis C patients based on epithelial membrane antigen in the blood in combination with routine laboratory markers. *Hepatol Res* 2011; **41:** 1075–84.
16 Kalantari H, Hoseini H, Babak A, Yaran M. Validation of hepascore as a predictor of liver fibrosis in patients with chronic hepatitis C infection. *Hepat Res Treat* 2011; **2011:** 972759.
17 Engstrom-Laurent A, Loof L, Nyberg A, Schroder T. Increased serum levels of hyaluronate in liver disease. *Hepatology* 1985; **5:** 638–42.
18 Murawaki Y, Ikuta Y, Koda M, Kawasaki H. Serum type III procollagen peptide, type IV collagen 7S domain, central triple-helix of type IV collagen and tissue inhibitor of metalloproteinases in patients with chronic viral liver disease: relationship to liver histology. *Hepatology* 1994; **20:** 780–7.
19 Fabris C, Falleti E, Federico E, Toniutto P, Pirisi M. A comparison of four serum markers of fibrosis in the diagnosis of cirrhosis. *Ann Clin Biochem* 1997; **34:** 151–5.
20 Desmet VJ, Gerber M, Hoofnagle JH, Manns M, Sheuer PJ. Classification of chronic hepatitis: diagnosis, grading, and staging. *Hepatology* 1994; **19:** 1513–20.
21 IBM SPSS Inc. IBM SPSS for Windows. Version 19.0 manual. SPSS Japan Inc., an IBM company. Armonk NY, USA, 2009.
22 Ikeda K, Izumi N, Tanaka E *et al.* Fibrosis score consisting of four serum markers successfully predicts pathological fibrotic stages of chronic hepatitis B. *Hepatol Res* 2012; **43:** 596–604.

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A Pilot Study of Triple Therapy With Telaprevir, Peginterferon and Ribavirin for Elderly Patients With Genotype 1 Chronic Hepatitis C

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The prevalence of hepatitis C virus (HCV) infection in elderly patients has been increasing in Japan. However, there are no reports on the safety and efficacy of the triple therapy of telaprevir, peginterferon, and ribavirin for elderly patients with chronic HCV infection. This study evaluated the safety and efficacy of triple therapy [12 weeks of telaprevir 1,500 mg/day, reduction dose, and 24 weeks of peginterferon and ribavirin] in 18 elderly Japanese patients aged >65 years, with chronic infection with HCV genotype 1b. Four patients received triple therapy with telaprevir 2,250 mg/day and the other 14 patients received telaprevir 1,500 mg/day. Sustained virological response-12 (HCV RNA negativity at 12 weeks after completion of therapy) was 50% (9 of 18 patients); while 4 of 18 (22%) patients discontinued triple therapy due to adverse events (skin rashes, anemia, poor appetite). The dose of telaprevir did not affect HCV RNA clearance rates. Regardless of the dose, 50% of the treated patients achieved sustained virological response-12, evaluated by intention-to-treat analysis. Furthermore, the fall in hemoglobin and the rise in serum creatinine were significantly milder in the telaprevir 1,500 mg group than the telaprevir 2,250 mg/day group. Further analysis showed that 67% (6 of 9 elderly patients) with IL28B gene (rs8099917) genotype TT, treated with telaprevir 1,500 mg, achieved sustained virological response-12. These results suggest that 24-week triple therapy with telaprevir 1,500 mg seems safe and efficacious for elderly Japanese patients infected with HCV genotype 1b. ***J. Med. Virol. 85:1746–1753, 2013.*** © 2013 Wiley Periodicals, Inc.

KEY WORDS: HCV; telaprevir; peginterferon; ribavirin; elderly patient

INTRODUCTION

Hepatitis C virus (HCV) often causes chronic liver infection, and can potentially cause liver cirrhosis and hepatocellular carcinoma (HCC) [Niederau et al., 1998; Kenny-Walsh, 1999]. There is a growing need for treatment of chronic HCV in elderly patients with increased proportion of such patients in the last few decades. This is important since Japanese patients infected with HCV are much older than Western patients due to the widespread HCV infection that affected Japan about 20 years ago [Yoshizawa et al., 2006].

Sustained virological responders who are negative for serum HCV RNA at 24 weeks after the completion of interferon therapy are likely to remain in virological and biochemical remission and show histological improvement [Marcellin et al., 1997; Shiratori et al., 2000]. In addition, interferon therapy reduces the risk of HCC in virological or biochemical responders [Imai et al., 1998; Ikeda et al., 1999; Yoshida et al., 1999]. Especially, HCV in elderly patients is associated with hepatocarcinogenesis and poor survival [Ikeda et al., 2009], and sustained virological response to interferon therapy is associated with improved clinical outcome [Asahina et al., 2010]. However, the sustained virological response rate tends to be lower in elderly patients with chronic hepatitis C, due in part to less tolerability and efficacy of interferon (IFN) combination therapy compared with adult patients [Iwasaki et al., 2006; Honda et al., 2010].

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Several direct acting antiviral agents have been designed and developed recently, represented by NS3/4A or NS5A protease inhibitors and NS5B polymerase or NS5A inhibitors [Asselah and Marcellin, 2011]. Among them, telaprevir has shown more effective results when combined with peginterferon and ribavirin in the treatment of chronic hepatitis C than peginterferon and ribavirin combination therapy [McHutchison et al., 2009, 2010; Hézode et al., 2010; Kumada et al., 2011]. However, there are no reports about the safety and efficacy of the triple therapy, which are combined with telaprevir, peginterferon, and ribavirin for elderly patients with chronic HCV infection. Clinically, it is important to determine whether elderly patients with HCV infection can be treated with triple therapy of telaprevir, peginterferon, and ribavirin.

The aim of this pilot study was to evaluate the safety and efficacy of triple therapy with telaprevir, peginterferon, and ribavirin for elderly patients with chronic HCV infection genotype 1b.

PATIENTS AND METHODS

Study Population

From May 2008 through November 2012, 297 patients with chronic hepatitis C were selected for treatment with telaprevir, peginterferon, and ribavirin at the Department of Hepatology, Toranomon Hospital (located in metropolitan Tokyo). Subsequently, 18 of these patients received the triple therapy based on the following inclusion criteria: (1) diagnosis of chronic HCV infection; (2) infection with HCV genotype 1b confirmed by sequence analysis in the NS5B region; (3) HCV RNA levels >5.0 $\log_{10}$ IU/ml, determined by the COBAS TaqMan HCV test (Roche Diagnostics, Tokyo, Japan); (4) Japanese aged ≥66 years at the start of treatment; (5) agreed to be treated with telaprevir, peginterferon, and ribavirin; (6) no evidence of liver cirrhosis; (7) no evidence of HCC; (8) negative for hepatitis B surface antigen; (9) no evidence of human immunodeficiency virus infection; (10) no evidence of autoimmune hepatitis, alcoholic liver disease, hemochromatosis or chronic liver disease other than chronic HCV infection; and (11) no history of cardiac disease, cerebral disorder, and pulmonary disease. The study protocol was in compliance with the Good Clinical Practice Guidelines and the 1975 Declaration of Helsinki, and was approved by the institutional review board. Each patient gave an informed consent before participating in this trial.

Table I summarizes the profiles and laboratory data of the 18 patients at the time of commencement of treatment. Treatment efficacy was evaluated by intention-to-treat analysis classified as treatment failure in patients who could not complete the treatment regimen. HCV RNA levels and hemoglobin were monitored at baseline and weeks 1, 2, 4, 8, 12, 16, 20, and 24 during treatment.

Four patients were treated with telaprevir 750 mg every 8-hr (q8h) (2,250 mg/day group), while the other 14 patients were treated with telaprevir 750 mg twice daily at 12-hr interval (q12h) (1,500 mg/day group). Peginterferon-α-2b was injected subcutaneously at a median dose of 1.5 μg/kg

TABLE I. Characteristics of Patients at Baseline

Number of patients	18
Age (years)*	68 (66–73)
Male/female	10/8
Body mass index (kg/m^2)*	22.8 (18.9–26.3)
Viral load of HCV ($\log_{10}$ IU/ml)	6.5 (5.1–7.3)
Serum aspartate aminotransferase (IU/L)	36 (11–95)
Serum alanine aminotransferase (IU/L)	38 (19–80)
Serum albumin (g/dl)	3.8 (3.3–4.1)
Gamma-glutamyl transpeptidase (IU/L)	27 (10–62)
Leukocyte count (/mm^3)	4,000 (2,500–7,300)
Hemoglobin (g/dl)	14.0 (12.5–16.1)
Platelet count (×104/mm^3)	15.5 (9.6–21.4)
Alpha-fetoprotein (μg/L)	4 (1–18)
Treatment	
Peginterferon α-2b dose (μg/kg)*	1.5 (1.0–1.8)
Ribavirin dose (mg/kg)*	7.7 (5.8–13.2)
Telaprevir dose(1,500/2,250 mg/day)	14/4
Amino acid substitutions in the HCV genotype 1b	
Core aa 70 (arginine/glutamine)	10/8
Core aa 91 (leucine/methionine)	11/7
ISDR of NS5A (wild-type/non wild-type/ND)	17/0/1
Genetic variation near IL28B gene rs8099917 genotype (TT/TG/GG)	11/6/1
Past history of interferon therapy Treatment-naïve/relapsers to previous treatment/nonresponders to previous treatment	3/10/5
Comorbidities[a]	
Diabetes mellitus	3 (17%)
Hypertension	9 (50%)

Data are numbers (percentages) of patients, except those denoted by *, which represent the median (range) values.
[a]All patients were not on medications.