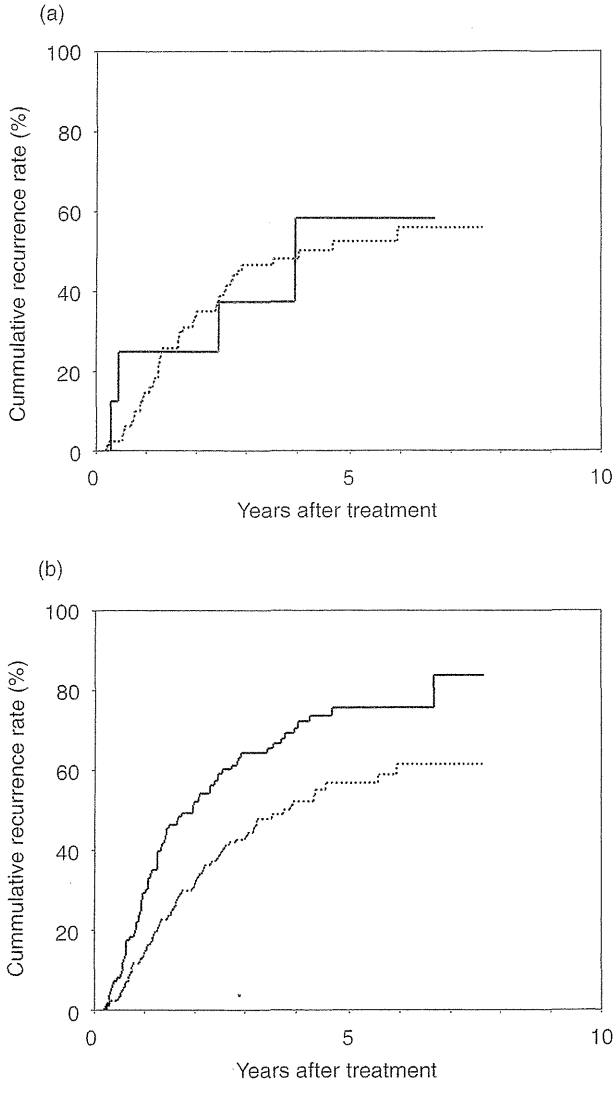

Figure 3 Cumulative recurrence rate of hepatocellular carcinoma (HCC) for postoperative α-fetoprotein (AFP) and AFP-L3% in resected patients. (a) Recurrence rate for postoperative AFP: solid line, recurrence rate in patients with AFP ≥ 20 ng/mL; broken line, recurrence rate in patients with AFP < 20 ng/mL. (b) Recurrence rate for postoperative AFP-L3%: solid line, recurrence rate in patients with AFP-L3 ≥ 5%; broken line, recurrence rate in patients with AFP < 5%.

Prognostic factors for HCC recurrence

Factors related to HCC recurrence were analyzed by the Kaplan–Meier method and multivariate analysis (Table 4). Potential risk factors for recurrence included the following 15 variables: age, gender, etiology of background liver disease, amount of alcohol intake, albumin, bilirubin, aspartate aminotransferase (AST), platelet count (PLT), prothrombin time (PT), preoperative AFP, AFP-L3%, DCP, tumor size, tumor number, and treatment procedure (resection or ablation). In all of the patients ($n = 250$), factors that were significantly related to HCC recurrence were RFA therapy, multiple tumors, albumin <3.5 g/dL, AST ≥ 50 IU/L, platelets <10 × 104/μL, prothrombin time <80%, preoperative AFP-L3% ≥ 5%, and preoperative DCP ≥ 40 mAU/mL by the Kaplan–Meier method (Table 4A). On multivariate analysis, the following were significant prognostic factors: multiple tumors ($P = 0.004$), preoperative AFP-L3% ≥ 5% ($P = 0.003$), albumin <3.5 g/dL ($P = 0.008$), and RFA ($P = 0.003$) (Table 4B).

In the 93 resected patients, on multivariate analysis, factors contributing to HCC recurrence were tumor number and preoperative AFP-L3% ($P = 0.003$ and 0.019, respectively). In the 157 RFA patients, similarly the four factors of age, preoperative AFP, AFP-L3%, and albumin were identified ($P = 0.003$, 0.006, 0.009, and 0.011, respectively) (data not shown).

Histological features and serum AFP, AFP-L3%, and DCP levels

From the 93 patients who underwent resection, we were able to obtain 85 specimens and assess their histological features. Ten nodules were well-differentiated HCCs; 69, moderately differentiated HCCs; and the remaining six, poorly differentiated HCCs. The nodules were macroscopically classified: four nodules were of small nodular type with indistinct margin (SNIM); 50, of simple nodular type (SN); 24, of simple nodular type with extranodular growth (SNEG); and seven, of confluent multinodular type (CM). Microscopic vascular invasion was observed in 14 (16.5%) nodules, and microscopic intrahepatic metastasis was observed in four (4.7%) nodules.

The median (25–75 percentile) preoperative DCP level in moderately/poorly differentiated HCCs was 25 (15–113) AU/L, whereas that of the well-differentiated HCCs was 18 (14–20) AU/L, and this difference was statistically significant ($P = 0.041$). Similarly, a significant difference was observed in the preoperative AFP-L3% between groups: the median AFP-L3% in the SNEG/CM group was 6.4 (2.5–18.9), whereas in the SNIM/SN group, it was 2.5 (≤0.5–7.4) ($P = 0.032$).

DISCUSSION

IN THE PRESENT study, AFP-L3% assayed by the μTAS method was detected with high clinical sensitivity

Table 4 Prognostic factors of hepatocellular carcinoma (HCC) recurrence. (A) Cumulative recurrence rate by variable and (B) Multivariate analysis

(A) Cumulative recurrence rate by variable

Variables	*n*	3-year Recurrence (%)	*P*-value
Treatment			
Resection	93	45.9	0.003
RFA	157	58.0	
Tumor number			
Single	193	50.8	0.003
Multiple	57	62.9	
Albumin			
<3.5 g/dL	105	64.9	0.001
≥3.5 g/dL	145	45.2	
AST			
<50 IU/L	131	48.3	0.009
≥50 IU/L	119	58.7	
PLT			
$<10 \times 10^4/mm^3$	87	65.4	0.024
$\geq 10 \times 10^4/mm^3$	163	47.4	
PT			
<80%	51	74.7	0.001
≥80%	199	48.1	
Preoperative AFP-L3%			
<5%	132	42.7	0.001
≥5%	118	65.5	
Preoperative DCP			
<40 mAU/mL	194	49.6	0.025
≥40 mAU/mL	56	67.0	

(B) Multivariate analysis

Variables		Hazard ratio (95% CI)	*P*-value
Tumor number	(multiple/single)	1.70 (1.19–2.43)	0.004
Preoperative AFP-L3%	(≥5%/<5%)	1.63 (1.18–2.26)	0.003
Albumin	(<3.5/≥3.5 g/dL)	1.55 (1.12–2.14)	0.008
Treatment	(RFA/resection)	1.09 (1.03–1.16)	0.003

AST, aspartate aminotransferase; CI, confidence interval; PLT, platelet count; PT, prothrombin time; RFA, radiofrequency ablation.

even in cases of HCC at a relatively early stage, which can be potentially cured by hepatic resection or RFA. It is worth noting that the sensitivity for HCC was as high as 47.2% when the cutoff value of AFP-L3% was set to 5%, compared to the sensitivity of 38.0% for total AFP. In addition, using a cutoff value of 10%, the sensitivity was 18.8%, which is comparable to that reported with the conventional method in patients whose HCC was curatively treated.[17–19]

One of the advantages of the highly sensitive μTAS method is measurement of AFP at low concentrations. Previously, the conventional method was unable to accurately determine AFP-L3% when total AFP concentration was less than 20 ng/mL, while in the present study detection of AFP-L3% was possible in 40.3%, 24.0%, and 12.3% of patients with AFP values less than 20 ng/mL when using the cutoff value for the AFP-L3% was set to 5%, 7%, and 10%, respectively. In our previous study of prognostic factors in patients that underwent hepatic resection or RFA with HCC of size less than 3 cm and not more than three tumors, it was reported that DCP was a significant prognostic factor in RFA

patients, while both AFP and DCP were not in resected patients.[27] During that study, we could not measure the highly sensitive AFP-L3%, and we measured the conventional AFP-L3% in only about half the patients. Therefore, we did not include the results of the AFP-L3% levels in that study. In the present study using the highly sensitive μTAS method to assay AFP-L3%, multivariate analysis revealed the AFP-L3% is a predictive factor for HCC recurrence with statistical significance both in the group of overall study population and surgically resected patients. These results showed that this highly sensitive assay method can increase clinical sensitivity and predict recurrence, suggesting that it is of additional clinical utility.

Toyoda *et al.*[24] assayed AFP-L3% in 270 patients with AFP less than 20 ng/mL and 396 patients with chronic liver diseases using the same μTAS method as in the present study, and reported that the AFP-L3% assayed by this method was useful for differential diagnosis of HCC and benign liver diseases with a sensitivity of 41.5% and specificity of 85.1% with the AFP-L3% cutoff value of 5%. He also found AFP-L3% to be related to survival rate. In the present study, the sensitivity was similar to that reported by Toyoda *et al.*,[24] although it was not possible to compare specificity, since in this study we included only HCC patients.

Similarly, Tamura *et al.*[25] reported a sensitivity of 60%, specificity of 90.3%, accuracy of 76.4%, positive predictive value (PPV) of 83.9%, and negative predictive value (NPV) of 72.8% at a cutoff value of 7% in 295 HCC patients and 350 patients with benign liver diseases. Comparison of cutoff values showed that the 7% was most clinically useful. Compared with the sensitivity of 60% reported by Tamura *et al.*, the sensitivity at 31.6% was relatively low in the present study with cutoff value at 7%. This appears to reflect differences in some fundamental patient characteristics between the two studies: for example, Stage III and IV HCC accounted for 50.2% of patients (148 of 295) in the report by Tamura *et al.* and 10.8% (27 of 250) in the present study.

The optimal cutoff value of a marker depends on the target disease under study and its intended use. We believed that the cutoff value for differential diagnosis between HCC and benign liver disease should achieve high specificity, preferably using receiver-operating characteristic (ROC) curve analysis. The purpose of the present study was to identify recurrence-predictive factors in a patient population with curatively treatable HCC at a relatively early stage; we determined that 5% AFP-L3% was most useful.

The relationships of postoperative AFP and AFP-L3% with HCC recurrence were also investigated in the present study. Notably, postoperative AFP-L3% remaining elevated greater than 5% was indicative of risk of HCC recurrence. Furthermore, it is noted that total AFP turned negative in 78.4% of patients after curative treatment, while AFP-L3% did in only 38.1% of patients (5% cutoff). Included in the present study of recurrence were all resected patients in whom radical cure was histologically confirmed. Therefore, all remnants of HCC should have been surgically removed. We speculate that lack of reduction in AFP-L3% after curative treatment appears was due to intra-hepatic multi-centric carcinogenesis or intra-hepatic micrometastasis. Miyaaki *et al.*,[28] who assayed AFP-L3% and protein induced by vitamin K absence-II (PIVKA-II), also known as DCP, by the conventional method in 110 resected patients, reported more cases of infiltrative growth-type HCC and poorly differentiated-type HCC in patients with postoperative AFP-L3% greater than 10%. Tada *et al.*[29] also reported a high rate of infiltrative growth, capsule infiltration, septum formation, portal vein invasion, and hepatic invasion in 111 patients with HCC with a high level of AFP-L3%. Regrettably, however, subsequent HCC recurrence was not followed. In our patients, the preoperative DCP level was related to the histological grade of the tumor, and a preoperative AFP-L3% greater than 5% was related to the macroscopic type of the nodule. In contrast, no relationship was observed between the postoperative markers and histological features in the current study. Unfortunately, we cannot clearly explain the discrepancies between the results of Tada *et al.* and this study; further examination with a larger number of patients is required to determine the relationship between highly sensitive AFP-L3% and the histological features of the tumors. In any case, patients with high level of AFP-L3% either before or after curative treatment should be followed closely.

The present study shows the high clinical sensitivity in diagnosis of HCC using μTAS AFP-L3% in patients with curative treatment of HCC. With a cutoff value of 5%, sensitivity was optimal in AFP less than 20 ng/mL where the conventional method was unable to determine the AFP-L3% value. Furthermore, both pre- and postoperative AFP-L3% were determined as prognostic factors of HCC recurrence. Since the high recurrence rate of HCC after even curative treatment is reported, it is of great importance to be able to predict such recurrence. Our study showed that the highly sensitive AFP-L3% is expected to be of clinical utility in predicting recurrence after curative treatments.

REFERENCES

1 Jemal A, Siegel R, Ward E *et al.* Cancer statistics, 2008. *CA Cancer J Clin* 2008; **58**: 71–96.
2 Oka H, Tamori A, Kuroki T, Kobayashi K, Yamamoto S. Prospective study of alpha-fetoprotein in cirrhotic patients monitored for development of hepatocellular carcinoma. *Hepatology* 1994; **19**: 61–6.
3 Daniele B, Bencivenga A, Megna AS, Tinessa V. Alpha-fetoprotein and ultrasonography screening for hepatocellular carcinoma. *Gastroenterology* 2004; **127**: S108–12.
4 Marrero JA, Feng Z, Wang Y *et al.* Alpha-fetoprotein, des-gamma carboxyprothrombin, and lectin-bound alpha-fetoprotein in early hepatocellular carcinoma. *Gastroenterology* 2009; **137**: 110–18.
5 Saitoh S, Ikeda K, Koida I *et al.* Diagnosis of hepatocellular carcinoma by concanavalin A affinity electrophoresis of serum alpha-fetoprotein. *Cancer* 1995; **76**: 1139–44.
6 Bruix J, Sherman M, Llovet JM *et al.* Clinical management of hepatocellular carcinoma. Conclusions of the Barcelona-2000 EASL conference. *J Hepatol* 2001; **35**: 421–30.
7 Omata M, Lesmana LA, Tateishi R *et al.* Asian Pacific Association for the Study of the Liver consensus recommendations on hepatocellular carcinoma. *Hepatol Int* 2010; **4**: 439–74.
8 Makuuchi M, Kokudo N. Surveillance algorithm and diagnostic algorithm for hepatocellular carcinoma. *Hepatology Research* 2010; **40**: 6–7.
9 Bruix J, Sherman M. Management of hepatocellular carcinoma: an update. *Hepatology* 2011; **53**: 1020–2.
10 Peng SY, Chen WJ, Lai PL, Jeng YM, Sheu JC, Hsu HC. High alpha-fetoprotein level correlates with high stage, early recurrence and poor prognosis of hepatocellular carcinoma: significance of hepatitis virus infection, age, p53 and beta-catenin mutations. *Int J Cancer* 2004; **112**: 44–50.
11 Imamura H, Matsuyama Y, Miyagawa Y *et al.* Prognostic significance of anatomical resection and des-gamma-carboxy prothrombin in patients with hepatocellular carcinoma. *Br J Surg* 1999; **86**: 1032–8.
12 Di Bisceglie AM, Hoofnagle JH. Elevations in serum alpha-fetoprotein levels in patients with chronic hepatitis B. *Cancer* 1989; **64**: 2117–20.
13 Liaw YF, Tai DI, Chen TJ, Chu CM, Huang MJ. Alpha-fetoprotein changes in the course of chronic hepatitis: relation to bridging hepatic necrosis and hepatocellular carcinoma. *Liver* 1986; **6**: 133–7.
14 Chu CW, Hwang SJ, Luo JC *et al.* Clinical, virologic, and pathologic significance of elevated serum alpha-fetoprotein levels in patients with chronic hepatitis C. *J Clin Gastroenterol* 2001; **32**: 240–4.
15 Aoyagi Y, Isemura M, Suzuki Y *et al.* Fucosylated alpha-fetoprotein as marker of early hepatocellular carcinoma. *Lancet* 1985; **2**: 1353–4.
16 Taketa K, Sekiya C, Namiki M *et al.* Lectin-reactive profiles of alpha-fetoprotein characterizing hepatocellular carcinoma and related conditions. *Gastroenterology* 1990; **99**: 508–18.
17 Toyoda H, Kumada T, Kaneoka Y *et al.* Prognostic value of pretreatment levels of tumor markers for hepatocellular carcinoma on survival after curative treatment of patients with HCC. *J Hepatol* 2008; **49**: 223–32.
18 Tamura Y, Igarashi M, Suda T *et al.* Fucosylated fraction of alpha-fetoprotein as a predictor of prognosis in patients with hepatocellular carcinoma after curative treatment. *Dig Dis Sci* 2010; **55**: 2095–101.
19 Tateishi R, Shiina S, Yoshida H *et al.* Prediction of recurrence of hepatocellular carcinoma after curative ablation using three tumor markers. *Hepatology* 2006; **44**: 1518–27.
20 Sterling RK, Jeffers L, Gordon F *et al.* Clinical utility of AFP-L3% measurement in North American patients with HCV-related cirrhosis. *Am J Gastroenterol* 2007; **102**: 2196–205.
21 Shimizu K, Taniichi T, Satomura S, Matsuura S, Taga H, Taketa K. Establishment of assay kits for the determination of microheterogeneities of alpha-fetoprotein using lectin-affinity electrophoresis. *Clin Chim Acta* 1993; **214**: 3–12.
22 Yamagata Y, Katoh H, Nakamura K, Tanaka T, Satomura S, Matsuura S. Determination of alpha-fetoprotein concentration based on liquid-phase binding assay using anion exchange chromatography and sulfated peptide introduced antibody. *J Immunol Methods* 1998; **212**: 161–8.
23 Kagebayashi C, Yamaguchi I, Akinaga A *et al.* Automated immunoassay system for AFP-L3% using on-chip electrokinetic reaction and separation by affinity electrophoresis. *Anal Biochem* 2009; **388**: 306–11.
24 Toyoda H, Kumada T, Tada T *et al.* Clinical utility of high sensitive lens culinaris agglutinin-reactive alpha-fetoprotein in hepatocellular carcinoma patients with alpha-fetoprotein less than 20 ng/mL. *Cancer Sci* 2011; **102**: 1025–31. [Epub ahead of print].
25 Tamura Y, Igarashi M, Kawai H, Suda T, Satomura S, Aoyagi Y. Clinical advantage of highly sensitive on-chip immunoassay for fucosylated fraction of alpha-fetoprotein in patients with hepatocellular carcinoma. *Dig Dis Sci* 2010; **55**: 3576–83.
26 Liver Cancer Study Group of Japan. *The General Rules for the Clinical and Pathological Study of Primary Liver Cancer. English Edn.* Tokyo: Kanehara, 2003.
27 Kobayashi M, Ikeda K, Kawamura Y *et al.* High serum des-gamma-carboxy prothrombin level predicts poor prognosis after radiofrequency ablation of hepatocellular carcinoma. *Cancer* 2009; **115**: 571–80.

28 Miyaaki H, Nakashima O, Kurogi M, Eguchi K, Kojiro M. Lens culinaris agglutinin-reactive alpha-fetoprotein and protein induced by vitamin K absence II are potential indicators of a poor prognosis: a histopathological study of surgically resected hepatocellular carcinoma. *J Gastroenterol* 2007; 42: 962–8.

29 Tada T, Kumada T, Toyoda H *et al.* Relationship between Lens culinaris agglutinin-reactive alpha-fetoprotein and pathologic features of hepatocellular carcinoma. *Liver Int* 2005; 25: 848–53.

Hepatology Research

Hepatology Research 2011; **41**: 946–954 doi: 10.1111/j.1872-034X.2011.00845.x

Original Article

Development rate of chronic kidney disease in hepatitis C virus patients with advanced fibrosis after interferon therapy

Yasuji Arase, Fumitaka Suzuki, Yusuke Kawamura, Yoshiyuki Suzuki, Masahiro Kobayashi, Naoki Matsumoto, Norio Akuta, Hitomi Sezaki, Tetsuya Hosaka, Kyoko Ogawa, Norihiro Imai, Yuya Seko, Satoshi Saito, Kenji Ikeda, Mariko Kobayashi and Hiromitsu Kumada

Department of Hepatology and Okinaka Memorial Institute for Medical Research, Toranomon Hospital, Tokyo, Japan

Aim: The aim of this retrospective cohort study is to assess the development incidence and predictive factors for chronic kidney disease (CKD) after the termination of interferon therapy in hepatitis C virus (HCV) positive Japanese patients with liver cirrhosis.

Methods: A total of 650 HCV positive, liver cirrhotic patients who were treated with interferon and showed an estimated glomerular filtration rate (eGFR) of ≥60 mL/min per 1.73 m^2 after the termination of interferon therapy were enrolled. CKD was defined as an eGFR of <60 mL/min per 1.73 m^2. End-stage-CKD was defined as an eGFR of <15 mL/min/1.73 m^2. The primary goal is the new development of CKD and end-stage-CKD.

Results: Eighty-five patients developed CKD, and six patients progressed to end-stage-CKD. The development rate of CKD was 5.2% at the 5th year, 14.5% at the 10th year and 30.6% at the 15th year. Multivariate Cox proportional hazards analysis showed that CKD occurred when patients had age increments of 10 years (hazard ratio: 2.32; 95% confidence interval [CI] 1.61–3.35; $P < 0.001$), eGFR decrements of 10 mL/min per 1.73 m^2 (hazard ratio: 1.66; 95% CI 1.27–2.16; $P < 0.001$), hypertension (hazard ratio: 2.00; 95% CI 1.13–3.53; $P = 0.017$), diabetes (hazard ratio: 1.79; 95% CI 1.02–3.14; $P = 0.042$), and non-clearance of HCV (hazard ratio: 2.67; 95% CI 1.34–5.32; $P = 0.005$). The development rate of end-stage-CKD was 0.4% at the 5th year, 1.6% at the 10th year and 2.8% at the 15th year.

Conclusions: The annual incidence for CKD among cirrhotic patients with HCV was determined to be about 1.0–1.5%. In addition, the annual incidence for end-stage-CKD is one order of magnitude lower than that of CKD.

Key words: chronic kidney disease, hepatitis C virus, liver cirrhosis

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; CKD, chronic kidney disease; CI, confidence interval; eGFR, estimated glomerular filtration rate; HCV, hepatitis C virus; IFN, interferon; SVR, Sustained virological response.

INTRODUCTION

HEPATITIS C VIRUS (HCV) is a major risk for hepatocellular carcinoma (HCC).[1–4] In addition, chronic HCV infection has been associated with a variety of extrahepatic complications such as essential mixed cryoglobulinemia, lymphoproliferative disorders, autoimmune thyroiditis, sialadenitis, cardiomyopathy, and diabetes.[5–8]

Data supporting a link between hepatitis C infection and chronic kidney disease (CKD) have been reported.[9–15] CKD, a disease entity including mild to end-stage renal diseases due to any etiology, was recently defined as an estimated glomerular filtration rate (eGFR) <60 mL/min per 1.73 m^2 and/or the presence of proteinuria.[16] CKD is currently considered a serious worldwide public health problem.[16,17] Tsuji *et al.* have reported that HCV infection enhance the onset of end-stage renal disease.[18,19] Dalrymple *et al.* have

Correspondence: Dr Yasuji Arase, Department of Hepatology, Toranomon Hospital, 2-2-2, Toranomon, Minato-ku, Tokyo 105-8470, Japan. Email: es9y-ars@asahi-net.or.jp
Received 6 May 2011; revision 19 May 2011; accepted 22 May 2011.

showed that HCV-positive patients had a 40% higher likelihood for developing renal insufficiency compared with seronegative subjects.[20] We had reported that patients with severe fibrosis had high possibility of progressed kidney damage.[11,12] Although there is growing evidence to support the concept that HCV infection is a risk factor for CKD, there have been a few interventional studies confirming this issue. This issue needs to be confirmed with a long-term follow-up of patients.

With this background in mind, the retrospective cohort study was initiated to investigate the cumulative incidence and risk factors of aggravation of renal function after prolonged follow-up in HCV-infected and cirrhotic patients treated with interferon (IFN) monotherapy or combination therapy of IFN and ribavirin. The strengths of the current study are the large numbers of patients included and the long-term follow-up of patients.

METHODS

Patients

A TOTAL OF 982 HCV positive and cirrhotic patients with infection were treated with IFN monotherapy or combination therapy of IFN and ribavirin between September 1990 and December 2007 in the Department of Hepatology, Toranomon Hospital, Tokyo, Japan. Out of 982 patients, 650 satisfied the following criteria: (i) an estimated glomerular filtration rate (eGFR) of ≥60 (mL/min per 1.73 m^2); (ii) features of cirrhosis diagnosed by laparoscopy and/or liver biopsy before the initiation of IFN therapy; (iii) positivity for serum HCV-RNA before the initiation of IFN therapy; (iv) age of ≥40 years; (v) period of ≤ 1year on IFN therapy; (vi) negativity for hepatitis B surface antigen (HBsAg), antinuclear antibodies, or antimitochondrial antibodies in serum, as determined by radioimmunoassay or indirect immunofluorescence assay; (vii) no evidence of HCC nodules as shown by ultrasonography and/or computed tomography; and (viii) no underlying systemic disease, such as systemic lupus erythmatosus, rheumatic arthritis. Next, we excluded from the study all the patients with a history of alcohol abuse or advanced liver cirrhosis of encephalopathy, bleeding esophageal varices, or ascites.

Alcohol abuse is a pattern of drinking that involves one or more of the following problems within a one-year period: (i) failure to carry out major responsibilities at work, school, or home; (ii) drinking in physically dangerous situations, such as while driving; (iii) legal problems related to using alcohol; and (iv) continued drinking despite ongoing problems in relationships with other people that are related to alcohol use.[21]

The primary outcome was the new development of CKD and/or end-stage CKD. CKD was defined as the first time when eGFR of <60 mL/min per 1.73 m^2 persisted for up to 3 months. End-stage CKD was defined as the first time when eGFR of <15 mL/min per 1.73 m^2 persisted for up to 3 months. Serum creatinine level was also measured using an enzymatical method, and the eGFR was estimated from the Japanese Society of Nephrology CKD Practice Guide: eGFR (mL/min per 1.73 m^2) = 194 × (serum creatinine level [mg/dL])$^{-1.094}$ × (age [y])$^{-0.287}$ The product of this equation was multiplied by a correction factor of 0.739 for women. CKD's stages were defined from estimated eGFR of <60 mL/min per 1.73 m^2 or dipstick proteinuria (≥+1) as follows: stage 1, eGFR ≥90 and proteinuria (≥+1); stage 2, 90 > eGFR ≥60 and proteinuria (≥+1); stage 3, 60 > eGFR ≥ 30; stage 4, 30 > eGFR ≥ 15; and stage 5, eGFR of <15. In the present study, patients with stage 3–5 were regarded as having CKD regardless of the absence of other markers of kidney damage.[22,23]

The physicians in charge explained the methods and side effects of IFN therapy, the storage of serum samples, and the use of stored serum samples to each patient and/or patient's family before IFN therapy. Informed consent was obtained from 650 patients before the initiation of IFN therapy. All of the studies were performed retrospectively by collecting and analyzing data from the patient records. This study had been approved by the Institutional Review Board of our hospital.

Laboratory investigation

Anti-HCV was detected using a second-generation enzyme-linked immunosorbent assay (ELISA II) (Abbott Laboratories, North Chicago, IL, USA). HCV-RNA was determined by the Amplicor method (Cobas Amplicor HCV Monitor Test, version 2.0; Roche, Tokyo, Japan). HBsAg was tested by radioimmunoassay (Abbott Laboratories, Detroit, MI, USA). Diagnosis of HCV infection was based on detection of serum HCV antibody and positive HCV RNA. HCV genotype and HCV RNA level were determined by the serum samples stored at −80°C before the initiation of IFN therapy.

Height and weight were recorded at baseline and the body mass index was calculated as weight (in kg)/height (in m^2). The criteria for the diagnosis of diabetes include: (i) casual plasma glucose ≥200 mg/dL; (ii) fasting plasma glucose (FPG) ≥126 mg/dL; and (iii) 2 h post-glucose (oral glucose tolerance test) ≥200 mg/dL.[24]

Patients were regarded as hypertension by the confirmation of blood pressure ≥140 mmHg systolic and/or ≥90 mmHg diastolic on at least three visits. Blood pressure was measured by a physician with a mercury sphygmomanometer, with subjects sitting and relaxed for at least 10 min.

Evaluation of liver cirrhosis

Liver status of the 650 patients was determined on the basis of peritoneoscopy and/or liver biopsy. Liver biopsy specimens were obtained using a modified Vim Silverman needle with an internal diameter of 2 mm (Tohoku University style, Kakinuma Factory, Tokyo, Japan), fixed in 10% formalin, and stained with hematoxylin-eosin, Masson's trichrome, silver impregnation, and periodic acid-Schiff after diastase digestion. The size of specimens for examination was more than six portal areas.[25]

Follow-up

The starting time of follow up was 3 months after the termination of IFN therapy. After that, patients were followed-up monthly to tri-monthly in our hospital. Physical examination and biochemical tests were conducted at each examination together with regular check-ups. Blood samples were taken for routine analyses. These included transaminase activities, total cholesterol, uric acid, glucose, complete blood cell count, serum HCV RNA, and creatinine level. Fifty-seven patients were lost to follow-up. Because the appearance of worsening renal function was not identified in the 57 patients, they were considered as censored data in statistical analysis.[26] Moreover, patients retreated with antiviral agents were regarded as withdrawals at the time of starting the retreatment of antiviral agents.

Statistical analysis

Clinical differences between sustained virological response (SVR) group and non-SVR group were evaluated by Wilcoxon rank sum test or Fisher's exact test. The cumulative development rate of CKD and end-stage CKD was calculated from 3 months after the termination of IFN treatment using the Kaplan–Meier method. Independent factors associated with the development rate of CKD and end-stage CKD were analyzed by the Cox proportional hazard model. The following 17 variables were analyzed for potential covariates for incidence of aggravation of renal function: age, sex, body mass index, eGFR, HCV RNA level, HCV genotype, alanine aminotransferase, aspartate aminotransferase, platelet count, type of IFN, combination of ribavirin, efficacy of IFN therapy, triglyceride, total cholesterol, uric acid, hypertension, diabetes, and frequencies of using contrast medium in computed tomography. HCV RNA level and HCV genotype were measured by the serum samples stored −80 °C before the initiation of IFN therapy. Yearly frequencies of using contrast medium in computed tomography were determined by clinical records. The remaining 15 variables were determined at the starting time of follow up after IFN therapy. A *P*-value of less than 0.05 was considered significant. Data analysis was performed using SPSS 11.5 for Windows (SPSS, Chicago, IL, USA).

RESULTS

Patients' characteristics

TABLE 1 SHOWS the characteristics of the 650 HCV-positive and cirrhotic patients treated with IFN monotherapy or combination therapy of IFN and ribavirin. There were several differences in clinical backgrounds between the SVR group and the non-SVR group. However, there was no significant difference in eGFR between SVR group and non-SVR group. The sustained virological response (SVR) rate was 30.6% (169/553) in IFN monotherapy and 42.2% (41/97) in combination therapy of IFN and ribavirin. Thus, the number of patients with SVR was 210. The mean follow-up period after the termination of anti-virus drugs was 6.5 years.

Incidence of CKD in cirrhotic patients with HCV

A total of 85 subjects (56 men and 29 women) developed CKD during the follow-up period. Of these, 14 were SVR and 67 were non-SVR. The cumulative development rate of CKD was determined to be 4.9% at the 5th year, 14.5% at the 10th year and 30.6% at the 15th year by the use of the Kaplan–Meier method (Fig. 1).

The factors associated with the development of CKD in all 650 patients treated with IFN are shown in Table 2. Multivariate Cox proportional hazards analysis showed that CKD development after the termination of IFN therapy occurred when patients had age increments of 10 years (hazard ratio: 2.32; 95% confidence interval [CI] 1.61–3.35; $P < 0.001$), eGFR decrements of 10 mL/min pe 1.73 m^2 (hazard ratio: 1.66; 95% CI 1.27–2.16; $P < 0.001$), hypertension (hazard ratio: 2.00; 95% CI 1.13–3.53; $P = 0.017$), diabetes (hazard ratio: 1.79; 95% CI 1.02–3.14; $P = 0.042$), and non-SVR (hazard ratio:

Table 1 Patients characteristics

Characteristic	Total	SVR	Non-SVR	*P**
n	650	210	440	
Sex (male/female)	405/245	134/76	271/169	0.604
Age (years)	57.4 ± 11.7	57.0 ± 11.9	57.6 ± 12.8	0.185
Height (cm)	162.8 ± 9.1	163.3 ± 9.2	162.1 ± 9.1	0.270
Body weight (kg)	63.1 ± 13.7	63.6 ± 13.9	62.1 ± 13.7	0.387
Body mass index	23.6 ± 3.1	23.7 ± 3.2	23.6 ± 3.2	0.654
Blood pressure (systolic, mmHg)	132 ± 17	130 ± 17	133 ± 18	0.334
Blood pressure (diastolic, mmHg)	78 ± 12	78 ± 11	79 ± 11	0.929
Hypertension (+/−)	152/498	48/162	104/336	0.844
HCV-genotype (1b/2a/2b/others)	389/159/56/46	92/84/19/15	297/75/37/31	<0.001
HCV RNA level (KIU/mL)	659 ± 508	435 ± 476	728 ± 532	<0.001
eGFR	85.2 ± 15.5	86.2 ± 15.9	84.7 ± 15.7	0.141
Fasting plasma glucose (mg/dL)	100 ± 31	99 ± 25	102 ± 34	0.888
Diabetes	149/501	42/168	107/333	0.232
Total cholesterol (g/dL)	156 ± 30	158 ± 38	154 ± 30	0.486
Triglyceride (mg/dL)	104 ± 46	108 ± 56	102 ± 45	0.764
Uric Acid (mg/dL)	5.6 ± 2.1	5.5 ± 2.1	5.7 ± 2.2	0.433
AST (IU/L)	62 ± 50	39 ± 19	73 ± 55	<0.001
ALT (IU/L)	68 ± 72	36 ± 20	80 ± 80	<0.001
Platelet ($\times 10^4/mm^3$)	11.6 ± 4.7	12.2 ± 5.0	11.3 ± 4.5	0.040
Frequencies of contrast imaging per year (≥1/<1)	252/398	28/182	224/216	<0.001
IFN monotherapy†/combination therapy‡	553/97	169/41	384/56	0.026

*Clinical differences between SVR group and Non-SVR group were evaluated by Wilcoxon rank sum test or Fisher's exact test.
†Outbreak of IFN monotherapy: recombinant IFNα 2a, 73 cases; recombinant IFNα 2b, 52 cases; natural IFNα, 278 cases; natural IFNβ, 150 cases; total dose of IFN = 572 ± 165 megaunit.
‡Outbreak of combination therapy: recombinant IFNα 2b+ribavirin, 29 cases, total dose of IFN = 502 ± 182 megaunit, total dose of ribavirin = 160 ± 68 g; peg IFNα 2b+ribavirin, 68 cases, total dose of peg IFN = 4.10 ± 1.08 mg, total dose of ribavirin = 202 ± 56 g.
Data are number of patients, median (range) or mean ± standard deviation.
ALT, alanine aminotransferase; AST, aspartate aminotransferase; CKD, chronic kidney disease; eGFR, estimated glomerular filtration rate; HCV, hepatitis C virus; IFN, interferon; SVR, sustained virological response.

2.67; 95% CI 1.34–5.32; $P. = 0.005$). The cumulative development rate for CKD based on difference of efficacy of the IFN therapy is shown in Figure 2. In addition to non-SVR, the four factors of aging, low eGFR, hypertension, and diabetes are high risk of developing the CKD. The development rates for CKD based on difference of age, eGFR, blood pressure, and blood glucose level at the starting time of follow-up are shown in Figure 3.

Incidence of end-stage CKD in cirrhotic patients with HCV

A total of six subjects (five male and one female) developed end-stage CKD during the follow-up period. The cumulative development rate of end-stage CKD was determined to be 0.4% at the 5th year, 1.6% at the 10th year and 2.8% at the 15th year by the use of the Kaplan–

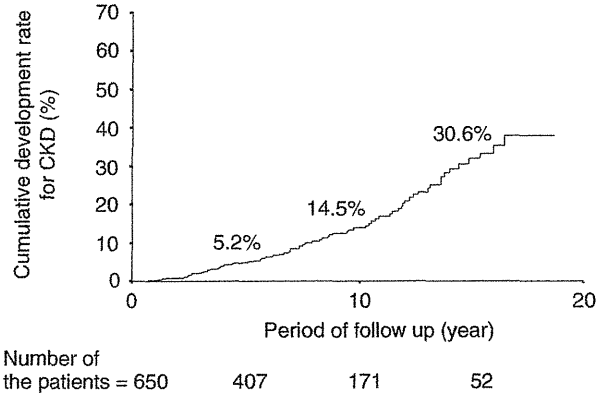

Figure 1 Cumulative development rate for chronic kidney disease (CKD) in hepatitis C virus (HCV) positive and cirrhotic patients treated with interferon.

Table 2 Predictive factors for chronic kidney disease (CKD) development

Variables	Univariate analysis		Cox-regression	
	HR (95% CI)	*P*	HR (95% CI)	*P*
Age, per 10 years	2.30 (1.72–3.12)	<0.001	2.32 (1.61–3.35)	<0.001
Sex (female/male)	0.90 (0.57–1.40)	0.628		
Body mass index (≥25/<25)	1.35 (0.72–2.50)	0.347		
HCV load(KIU/mL, ≥1000/<1000)	1.39 (0.80–2.38)	0.173		
Genotype (1/2)	1.19 (0.78–1.89)	0.436		
AST (IU/L, ≥50/<50)	1.63 (0.92–2.94)	0.097		
ALT (IU/L, ≥50/<50)	2.01 (1.13–3.57)	0.016		
Platelet ($\times 10^4/mm^3$, ≥15/<15)	0.70 (0.25–1.94)	0.487		
eGFR, per decrease of 10 mL/min/1.73 m^2	2.00 (1.56–2.56)	<0.001	1.66 (1.27–2.16)	<0.001
Uric acid (mg/dL, ≥7.0/<7.0)	1.43 (0.81–2.47)	0.225		
Triglyceride (mg/dL, ≥150/<150)	1.61 (0.62–3.70)	0.336		
Cholesterol (mg/dL, ≥220/<220)	1.22 (0.48–3.12)	0.678		
Diabetes (+/–)	2.76 (1.79–4.22)	0.001	1.79 (1.02–3.14)	0.042
Hypertension (+/–)	2.82 (1.80–4.39)	<0.001	2.00 (1.13–3.53)	0.017
Combination of ribavirin (+/–)	0.75 (0.36–1.58)	0.453		
Kind of IFN (beta/alpha)	0.91 (0.53–1.57)	0.729		
Efficacy (non-SVR/SVR)	2.10 (1.21–3.58)	0.008	2.67 (1.34–5.32)	0.005
Frequencies of contrast imaging per year (≥1/<1)	1.83 (1.17–2.87)	0.009		

ALT, alanine aminotransferase; AST, aspartate aminotransferase; CKD, chronic kidney disease; eGFR, estimated glomerular filtration rate; HCV, hepatitis C virus; HR, hazards ratio; IFN, interferon; SVR, sustained virological response.

Meier method (Fig. 4). The factors associated with the incidence of end-stage CKD in all 650 patients are shown in Table 3. There were no significant factors associated with the incidence of end-stage CKD as shown in Table 3.

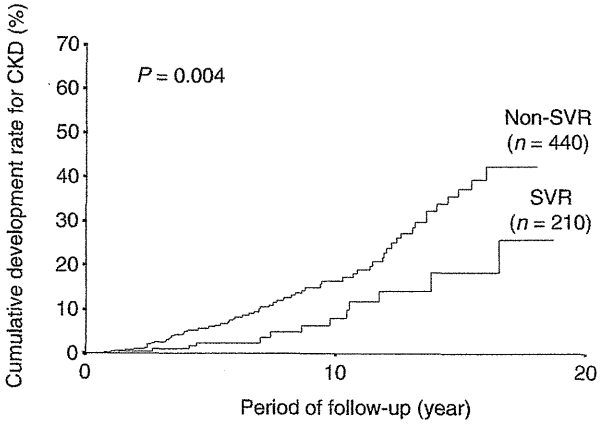

Figure 2 Cumulative development rate for chronic kidney disease (CKD) based on the difference of efficacy in hepatitis C virus (HCV) positive and cirrhotic patients treated with interferon.

DISCUSSION

WE HAVE DESCRIBED the development incidence for CKD and end-stage CKD after the termination of IFN therapy in HCV positive and liver cirrhotic patients treated with IFN. In the present study, the liver cirrhotic patients were enrolled to evaluate the new onset of CKD or end-stage CKD. Moreover, kidney damage has been reported in patients treated with IFN.[27] To exclude kidney damage originated from IFN-related side effects, patients with eGFR of ≥60 (mL/min per 1.73 m^2) for 3 months after the termination of IFN were enrolled in the present study. Our results indicate that the annual incidence for CKD as defined by a GFR of less than 60 mL/min per 1.73 m^2 for a prolonged follow-up after the termination of IFN therapy in HCV positive and cirrhotic patients is about 1.0–1.5% based on the development incidence for CKD at the 5th year and the 10th year. In addition, the annual incidence for end-stage CKD is one order of magnitude lower than that of a total of CKD.

Imai *et al.* have reported that about 20% of the Japanese adult population have stage 3 to 5 CKD by the use of database for 527 594 (male, 211 034; female, 316 560) participants obtained from the general adult population aged over 20 years who received annual

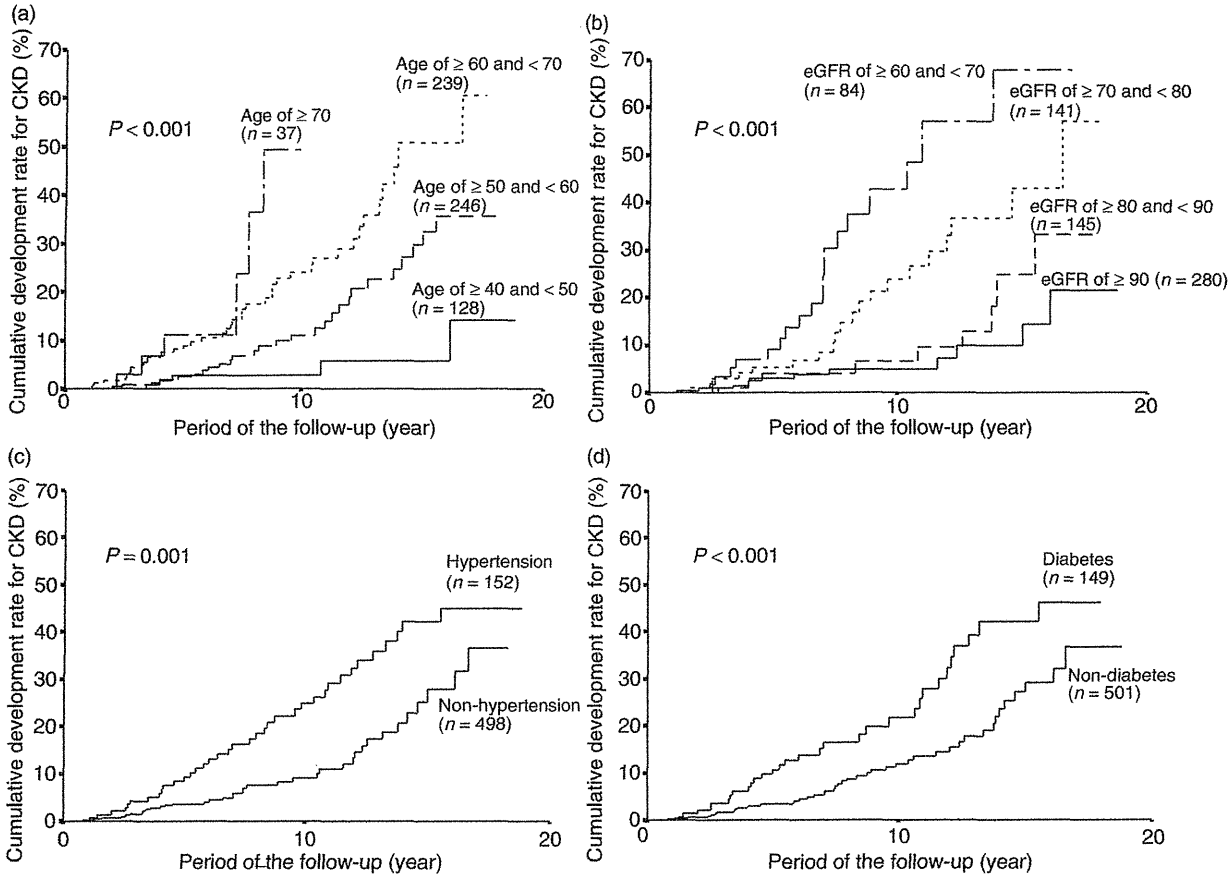

Figure 3 Cumulative development rate for chronic kidney disease (CKD) in hepatitis C virus (HCV) positive and cirrhotic patients treated with interferon: (a) Cumulative development rate for CKD based on difference of age; (b) Cumulative development rate for CKD based on the difference of estimated glomerular filtration rate (eGFR); (c) Cumulative development rate for CKD based on the difference of blood pressure; (d) Cumulative development rate for CKD based on the difference of glucose level.

health check programs in 2000–2004, from seven different prefectures in Japan. Next, the prevalence of CKD stage 3 in the study population, stratified by age groups of 20–29, 30–39, 40–49, 50–59, 60–69, 70–79, and 80–89 years, were 1.4%, 3.6%, 10.8%, 15.9%, 31.8%, 44.0%, and 59.1%, respectively. Moreover, they provided that the prevalence of stage 4+5 was <0.2%. Our results agreed with Imai's report in the fact that end-stage CKD patients were few.

The present study was limited by a retrospective cohort trial. This cohort is over 10 years; hence, many patients had complications, such as diabetes and hypertension. However, the development of CKD was mainly evaluated based on the clinical characteristics at the initiation of follow-up. Second limitation of the study was that we defined CKD according to eGFR alone. Gener-

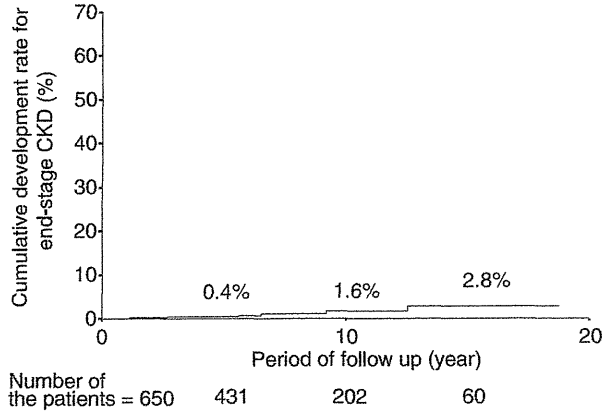

Figure 4 Cumulative development rate for end-stage chronic kidney disease (CKD) based on the difference of efficacy in hepatitis C virus (HCV) positive and cirrhotic patients treated with interferon.

Table 3 Predictive factors for end-stage chronic kidney disease (CKD) development

Variables	Univariate analysis	
	HR (95%CI)	*P*
Age, per 10 years	2.13 (0.86–5.30)	0.104
Sex (female/male)	0.24 (0.03–1.92)	0.182
Body mass index (≥25/<25)	0.80 (0.16–4.10)	0.782
HCV load (KIU/mL, ≥1000/<1000)	1.58 (0.37–6.67)	0.535
Genotype (1/2)	2.74 (0.66–11.50)	0.167
AST (IU/L, ≥50/<50)	1.45 (0.18–11.76)	0.730
ALT (IU/L, ≥50/<50)	1.89 (0.45–7.93)	0.382
Platelet ($\times 10^4$/mm^3, ≥15/<15)	0.67 (0.16–2.86)	0.586
eGFR, per decrease of 10 mL/min/1.73 m^2	1.70 (0.89–3.23)	0.105
Uric acid (mg/dL, ≥7.0/<7.0)	1.27 (0.23–6.96)	0.784
Triglyceride (mg/dL, ≥150/<150)	1.33 (0.15–11.87)	0.802
Cholesterol (mg/dL, ≥220/<220)	1.03 (0.12–8.67)	0.980
Diabetes (+/−)	1.89 (0.45–7.93)	0.382
Hypertension (+/−)	2.83 (0.70–11.41)	0.143
Combination of ribavirin (+/−)	0.88 (0.10–7.66)	0.908
Kind of IFN (beta/alpha)	2.08 (0.52–8.37)	0.300
Efficacy (non-SVR/SVR)	3.25 (0.40–26.4)	0.269
Frequencies of contrast imaging per year (≥1/<1)	3.72 (0.70–19.72)	0.123

ALT, alanine aminotransferase; AST, aspartate aminotransferase; CKD, chronic kidney disease; eGFR, estimated glomerular filtration rate; HCV, hepatitis C virus; HR, hazards ratio; IFN, interferon; SVR, sustained virological response.

ally, a recent definition of CKD also includes proteinuria.[28,29] Although the use of both eGFR and proteinuria might lead to a more accurate classification of CKD, we could not assess proteinuria in this study. Third, prescribed agents during the follow-up were not considered in the present study. However, therapy intervention is very important for protecting new development for CKD. In future, the intervention therapy for protecting the development of CKD should be evaluated. Finally, in the present study, patients were treated with different types of antivirus therapy (IFN monotherapy or combination therapy of IFN and ribavirin) for different durations (4 weeks to 52 weeks). This heterogeneity makes it slightly difficult to interpret the results of the study. On the other hand, the strengths of the present study are a long-term follow-up in the large numbers of patients included.

The present study shows several findings with regard to development incidence for CKD or end-stage CKD after the termination of IFN therapy for HCV positive and cirrhotic patients. First, SVR is effective for protecting the development incidence for CKD in HCV patients with liver cirrhosis. Though the role of HCV in the pathogenesis of aggravation of renal function remains speculative, the following possible mechanism have been reported: (i) systemic immune response to HCV infection mediated by cryoglobulins, HCV-antibody immune complexes, or amyloid deposition;[8,30,31] (ii) toll-like receptors increased expression in glomeruli induce immune response;[32] and (iii) insulin resistance and hyperinsulinemia cause excess intrarenal production of insulin-like growth factor-1 and transforming growth factor β, thus induce oxidative stress.[33] In addition, patients with liver cirrhosis might have the possibility of kidney damage such as hypovolemia due to fluid loss or hemorrhage, hepatorenal syndrome, and drug-induced renal failure. Second, in addition to non-SVR, the present study suggests that aging, low eGFR, hypertension, and diabetes enhanced the development of worsening renal function in cirrhotic patients with HCV infection after the termination of IFN. The repeated use of contrast imaging of computed tomography might worsen renal function. However, in the present study, SVR, aging, low eGFR, hypertension, and diabetes were the main predictive factors for the development of CKD compared to the repeated use of contrast imaging of computed tomography. The result that aging, hypertension and diabetes were associated with the development of worsening renal function agreed with several studies.[16–19]

In the present study, the predictive factors for end-stage CKD (stage 5) were not similar to those for CKD 3–5. The possible reason for this discrepancy is as follows. First, the number of patients who had progressed to end-stage CKD was six. Because of so few patients, we could not show the statistical significance in the predictive factors for end-stage CKD. Second, development of end-stage CKD might be robust to the several factors at the initiation of the follow-up. Development of end-stage CKD might be associated with the accidents during follow-up, such as the repeated use of contrast medium and hypovolemia due to bleeding. In fact, four of six patients who progressed to end-stage CKD had been given the repeated use of contrast medium. Next, whether HCV eradication in patients whose renal function progressed to stage of CKD 3–5 improves the mortality due to cardiovascular disease and stroke is a very important issue. However, this problem was not evaluated in the present study. This should be clarified by further examination.

In conclusion, our study suggests that the annual incidence for CKD among cirrhotic patients with HCV was determined to be about 1.0–1.5%. In addition, the annual incidence for end-stage CKD is one order of magnitude lower than that of CKD.

ACKNOWLEDGMENTS

THE AUTHORS ACKNOWLEDGE the editorial assistance of Thomas Hughes.

REFERENCES

1 Colombo M, Kuo G, Choo QL *et al.* Prevalence of antibodies to hepatitis C virus in Italian patients with hepatocellular carcinoma. *Lancet* 1989; **2**: 1006–8.
2 Hasan F, Jeffers LJ, De Medina M *et al.* Hepatitis C-associated hepatocellular carcinoma. *Hepatology* 1990; **12**: 589–91.
3 Tsukuma H, Hiyama T, Tanaka S *et al.* Risk factors for hepatocellular carcinoma among patients with chronic liver disease. *N Engl J Med* 1993; **328**: 1797–801.
4 Ikeda K, Saitoh S, Koida I *et al.* A multivariate analysis of risk factors for hepatocellular carcinogenesis: a prospective observation of 795 patients with viral and alcoholic cirrhosis. *Hepatology* 1993; **18**: 47–53.
5 Gumber SC, Chopra S. Hepatitis C: a multifaceted disease-review of extrahepatic manifestations. *Ann Intern Med* 1995; **123**: 615–20.
6 Pawlotsky JM, Roudot-Thoraval F, Simmonds P *et al.* Extrahepatic immunologic manifestations in chronic hepatitis C and hepatitis C virus serotypes. *Ann Intern Med* 1995; **122**: 169–73.
7 Arase Y, Suzuki F, Suzuki Y *et al.* Sustained virological response reduces incidence of onset of type 2 diabetes in chronic hepatitis C. *Hepatology* 2009; **49**: 739–44.
8 Agnello V, Chung RT, Kaplan LM. A role for hepatitis C virus infection in type II cryoglobulinemia. *N Engl J Med* 1992; **327**: 1490–5.
9 Johnson RJ, Gretch DR, Yamabe H *et al.* Membranoproliferative glomerulonephritis associated with hepatitis C virus infection. *N Engl J Med* 1993; **328**: 465–70.
10 Yamabe H, Johnson RJ, Gretch DR *et al.* Membranoproliferative glomerulonephritis associated with hepatitis C virus infection responsive to interferon-alpha. *Am J Kidney Dis* 1995; **25**: 67–9.
11 Arase Y, Ikeda K, Murashima N *et al.* Glomerulonephritis in autopsy cases with hepatitis C virus infection. *Int Med* 1998; **37**: 836–40.
12 Arase Y, Ikeda K, Tubota A *et al.* Serum levels of γ-globulin and total bilirubin influence the prevalence of multiple extrahepatic complication in patients with hepatitis C virus infection. *Hepatol Res* 2003; **25**: 14–21.
13 Fabrizi F, Lunghi G, Messa P, Martin P. Therapy of hepatitis C virus-associated glomerulonephritis: current approaches. *J Nephrol* 2008; **21**: 813–25.
14 Martin P, Fabrizi F. Hepatitis C virus and kidney disease. *J Hepatol* 2008; **49**: 613–24.
15 Ahmed MS, Wong CF, Shawki H, Kapoor N, Pandya BK. Rapidly deteriorating renal function with membranoproliferative glomerulonephritis Type 1 associated with hepatitis C treated successfully with steroids and antiviral therapy: a case report and review of literature. *Clin Nephrol* 2008; **69**: 298–301.
16 National Kidney Foundation. K/DOQI clinical practice guidelines for chronic kidney disease: evaluation, classification, and stratification. *Am J Kidney Dis* 2002; **39**: S1–S266.
17 Coresh J, Selvin E, Stevens LA *et al.* Prevalence of chronic kidney disease awareness, prevalence, and trends among U.S. adults 1999 to 2000. *JAMA* 2007; **298**: 2038–47.
18 Tsui JI, Vittinghoff E, Shlipak MG *et al.* Association of hepatitis C seropositivity with increased risk for developing end-stage renal disease. *Arch Intern Med* 2007; **167**: 1271–6.
19 Tsui JI, Vittinghoff E, Shlipak MG, O'Hare AM. Relationship between hepatitis C and chronic kidney disease: results from the Third National Health and Nutrition Examination Survey. *J Am Soc Nephrol* 2006; **17**: 1168–74.
20 Dalrymple LS, Koepsell T, Sampson J *et al.* Hepatitis C virus infection and the prevalence of renal insufficiency. *Clin J Am Soc Nephrol* 2007; **2**: 715–21.
21 Babor TF, Caetano R. The trouble with alcohol abuse: What are we trying to measure, diagnose, count and prevent? *Addiction* 2008; **103**: 1057–105.
22 Imai E, Horio M, Nitta K *et al.* Estimation of glomerular filtration rate by the MDRD study equation modified for Japanese patients with chronic kidney disease. *Clin Exp Nephrol* 2007; **11**: 41–50.
23 Imai E, Horio M, Iseki K *et al.* Prevalence of chronic kidney disease (CKD) in the Japanese general population predicted by the MDRD equation modified by a Japanese coefficient. *Clin Exp Nephrol* 2007; **11**: 156–63.
24 Genuth S, Alberti KG, Bennett P *et al.* Expert Committee on the Diagnosis and Classification of Diabetes Mellitus. Follow-up report on the diagnosis of diabetes mellitus. *Diabetes Care* 2003; **26**: 3160–7.
25 Desmet VJ, Gerber M, Hoofnagle JH, Manns M, Sheuer PJ. Classification of chronic hepatitis: diagnosis, grading and staging. *Hepatology* 1994; **19**: 1513–20.
26 Fleming TR, Harrington DP, O'Brien PC. Designs for group sequential tests. *Control Clin Trials* 1984; **5**: 348–61.
27 Raanani P, Ben-Bassat I. Immune-mediated complications during interferon therapy in hematological patients. *Acta Haematol* 2002; **107**: 133–44.
28 Lhotta K. Beyond hepatorenal syndrome: glomerulonephritis in patients with liver disease. *Semin Nephrol* 2002; **22**: 302–8.

29 Alpers CE, Kowalewska J. Emerging paradigms in the renal pathology of viral diseases. *Clin J Am Soc Nephrol* 2007; 2 (Suppl 1): S6–S12.
30 Wörnle M, Schmid H, Banas B *et al.* Novel role of toll-like receptor 3 in hepatitis C-associated glomerulonephritis. *Am J Pathol* 2006; **168**: 370–85.
31 Shintani Y, Fujie H, Miyoshi H *et al.* Hepatitis C virus infection and diabetes: direct involvement of the virus in the development of insulin resistance. *Gastroenterology* 2004; **126**: 840–8.
32 Sarafidis PA, Ruilope LM. Insulin resistance, hyperinsulinemia, and renal injury: mechanisms and implications. *Am J Nephrol* 2006; **26**: 232–44.
33 Ishizaka N, Ishizaka Y, Seki G, Nagai R, Yamakado M, Koike K. Association between hepatitis B/C viral infection, chronic kidney disease and insulin resistance in individuals undergoing general health screening. *Hepatol Res* 2008; **38**: 775–83.

Clinical Study

Oncology

Oncology 2011;80:188–194
DOI: 10.1159/000328749

Received: April 4, 2011
Accepted: April 4, 2011
Published online: June 27, 2011

Previous Chemoembolization Response after Transcatheter Arterial Chemoembolization (TACE) Can Predict the Anti-Tumor Effect of Subsequent TACE with Miriplatin in Patients with Recurrent Hepatocellular Carcinoma

Norihiro Imai Kenji Ikeda Yuya Seko Yusuke Kawamura Hitomi Sezaki
Tetsuya Hosaka Norio Akuta Masahiro Kobayashi Satoshi Saitoh
Fumitaka Suzuki Yoshiyuki Suzuki Yasuji Arase Hiromitsu Kumada

Department of Hepatology, Toranomon Hospital, Tokyo, Japan

Key Words
Hepatocellular carcinoma · Miriplatin · Transcatheter arterial chemoembolization

Abstract
Aim: The purpose of this retrospective study was to evaluate the efficacy and safety of transcatheter arterial chemoembolization (TACE) with miriplatin in patients with unresectable hepatocellular carcinoma (HCC). ***Methods:*** From 2007 to 2010, 122 consecutive patients with unresectable HCC were treated by TACE with miriplatin-lipiodol suspension in our institute. Twenty-two patients (18%) had a solitary nodule and 100 patients (82%) had multiple nodules. Ninety-eight patients (80%) had a history of TACE. ***Results:*** Thirty-five of the 122 treated patients (29%) showed complete response (CR). And no serious complications were observed. Patients who had shown CR after previous TACE (pre-CR) were significantly more likely to show CR in the current study compared with patients who had shown less successful responses after previous TACE (56 vs. 20%, p = 0.003). Multivariate analysis revealed that response after previous TACE (pre-CR, risk ratio: 4.76; p = 0.035), tumor multiplicity (solitary, risk ratio: 9.69; p = 0.003), and injection artery (peripheral to segmental hepatic artery, risk ratio: 5.28; p = 0.040) were significant independent predictors associated with CR after TACE using miriplatin. ***Conclusion:*** In repetition of TACE treatment, switching the TACE agent from epirubicin or cisplatin to miriplatin offered a favorable treatment effect, especially in patients who had shown a CR after previous TACE.

Introduction

Hepatocellular carcinoma (HCC) is one of the most common malignant diseases worldwide [1]. Since it is well known that more than 80% of HCC cases are associated with liver cirrhosis, a routine check-up including ultrasound for cirrhotic patients could potentially lead to the detection of early HCC [2–4]. Since curative therapies, including resection, liver transplantation, and percutaneous ablation (percutaneous ethanol injection and radiofrequency ablation) are applicable in only 30–40% of

KARGER
Fax +41 61 306 12 34
E-Mail karger@karger.ch
www.karger.com

0030–2414/11/0804–0188$38.00/0

Accessible online at:
www.karger.com/ocl

Norihiro Imai
Department of Hepatology
Toranomon Hospital
2-2-2 Toranomon, Minato-ku, Tokyo 105-8470 (Japan)
Tel. +81 3 3588 1111, E-Mail norihiro.imai@gmail.com

HCC patients, transcatheter arterial chemoembolization (TACE) has been recognized as an effective palliative treatment option for patients with advanced HCC [5–12].

Although many chemotherapeutic agents (e.g. doxorubicin, epirubicin, mitomycin C, and cisplatin) are used with the ethyl ester of iodized fatty acids from poppy-seed oil (Lipiodol Ultra-fluide; Laboratoire Guerbet, Aulnay-Sous-Bois, France) in TACE, the best choices for first- and second-line drugs remain uncertain [13–15]. Miriplatin (cis-[((1R,2R)-1,2-cyclohexanediamine-N,N′)bis(myristato)]-platinum(II)monohydrate; Dainippon Sumitomo Pharma Co., Osaka, Japan) is a novel lipophilic cisplatin derivative that can be suspended in lipiodol, a lipid lymphographic agent [16–19]. When lipiodol is injected into an artery feeding HCC nodules, it selectively accumulates in the tumor. Accordingly, a miriplatin-lipiodol emulsion is deposited within the HCC nodules and gradually releases active platinum compounds into tumor tissues. Clinical trials have demonstrated that miriplatin is effective in the treatment of HCC, but the efficacy of TACE using miriplatin for patients with recurrent HCC after TACE has not been evaluated [20, 21]. The purpose of this retrospective study was to evaluate the efficacy and safety of TACE using miriplatin for patients with HCC.

Patients and Methods

Study Population

From December 2007 to December 2010, 122 consecutive patients with unresectable HCC were treated by TACE with a miriplatin-lipiodol suspension at the Department of Hepatology, Toranomon Hospital, Tokyo, Japan. The study group consisted of 79 men and 43 women ranging in age from 48 to 87 years (median, 72 years). They included 11 patients (9%) positive for HBs-Ag, 103 patients (84%) positive for HCV antibody, and 8 patients (7%) negative for both. At the time of the miriplatin administration, median values were as follows: total bilirubin level = 1.1 mg/dl; serum albumin concentration = 3.3 g/dl; indocyanin-green retention rate at 15 min = 29%; prothrombin activity = 82.5%; alpha-fetoprotein (AFP) concentration = 31.2 ng/ml; and des-gamma-carboxyprothrombin (DCP) concentration = 53 AU/l. As for Child-Pugh classification, 92 patients (75%) were Class A and 30 patients (25%) were Class B. The clinical characteristics of the study group are summarized in table 1. The study protocol was approved by the ethics committee of our hospital, and written informed consent was obtained from all participating patients.

Hepatocellular Carcinoma

Before treatment with miriplatin, all patients underwent a comprehensive evaluation consisting of medical history, physical examination, measurement of tumor size, performance status, chest radiograph, liver-imaging studies (dynamic computerized tomography [dynamic CT], ultrasonography [US], digital-subtraction angiography [DSA]), complete blood count, and blood chemistry. Diagnosis of HCC was established based on the findings of dynamic CT, US and DSA. Patients who had extrahepatic metastasis of HCC or other malignancies were excluded.

Tumor profiles and TACE treatment history for the study group are summarized in table 2. Twenty-two patients (18%) had a solitary nodule and 100 patients (82%) had multiple nodules. The median diameter of the largest tumor was 20 mm (range 10–100 mm). Ninety-eight patients (80%) had a history of TACE. Thirty-seven patients had received cisplatin, and 80 patients had received epirubicin. Among these patients, the median number of

Table 1. Demographic characteristics and pretreatment assessments of 122 patients who underwent TACE using a miriplatin/lipiodol suspension for unresectable HCC

Number of cases	122
Age, years	72 (48–87)
Gender, male	65%
Etiology, HCV/HBV/others	103/11/8
Child-Pugh Class, A/B/C	92/30/0
ICG-R15, %	29 (4–78)
Albumin, g/dl	3.3 (2.0–4.2)
Total bilirubin, mg/dl	1.1 (0.4–4.9)
Prothrombin activity, %	82.5 (45.7–123.1)
Platelet, $\times 10^3/\mu l$	93 (29–282)
AFP, ng/ml	31.2 (1.8–152,800)
DCP, AU/l	53 (6–65,290)

HCV = Hepatitis C virus; HBV = hepatitis B virus; ICG-R15 = indocyanine-green retention rate at 15 min.

Variables are expressed as medians with ranges in parentheses.

Table 2. Tumor profiles, treatment history, and study drug dosages of 122 patients who underwent TACE using miriplatin for unresectable HCC

Tumor size, mm	20 (10–100)
Intrahepatic multiplicity, solitary	22 (18%)
Number of tumors	4 (1–100)
Presence of portal vein invasion	3 (2%)
History of TACE	98 (80%)
History of TACE with epirubicin	80 (66%)
History of TACE with cisplatin	37 (30%)
Median interval between previous TACE and miriplatin administration, months	4 (1–41)
Dosage of miriplatin, mg	80 (20–120)
Dosage of lipiodol, ml	3 (1–6)
Injection from peripheral to segmental branch of the hepatic artery	22 (18%)

Variables are expressed as medians with ranges in parentheses or number of cases.

TACE procedures was four (range 1–13), and the median interval between previous TACE and miriplatin administration was 4 months (range 1–41 months).

Treatment Protocol

Patients were hydrated through a peripheral line. The femoral artery was catheterized under local anesthesia, and the catheter was inserted superselectively into the hepatic artery that supplied the target tumor for injection of the miriplatin-lipiodol suspension and 1-mm gelatin cubes (Gelpart; Nippon Kayaku, Tokyo). The miriplatin-lipiodol suspension was administrated slowly under careful fluoroscopic guidance. The dose of miriplatin/lipiodol was determined according to tumor size and the degree of liver dysfunction.

Assessment of Therapeutic Effects

The effect of chemotherapy was evaluated by dynamic CT 1 to 3 months after TACE with miriplatin, and was based on the change in the maximum diameter of the viable target lesions (i.e. showing enhancement in the arterial phase). Response categories, according to the criteria of Modified Response Evaluation Criteria in Solid Tumors (mRECIST) [22], are as follows: complete response (CR) = disappearance of any intratumoral arterial enhancement in all target lesions; partial response (PR) = at least a 30% decrease in the sum of diameters of viable target lesions; stable disease (SD) = any cases that do not qualify for either PR or progressive disease; and progressive disease (PD) = an increase of at least 20% in the sum of the diameters of viable target lesions.

Toxicity Evaluation

Treatment-related toxicity was assessed using the National Cancer Institute Common Terminology Criteria (version 4.0). Within 2 weeks before TACE with miriplatin, and at 3 to 7 days (three times during this period) and at 1 month afterward, the following toxicity evaluations were made: hematological assessments (i.e. leukocyte and thrombocyte counts) and clinical chemistry assessments (i.e. serum aspartate aminotransferase [AST], serum alanine aminotransferase [ALT], albumin, total bilirubin, serum creatine, and prothrombin activity).

Statistical Analysis

The distribution of subject characteristics was assessed by the chi-square test or the Mann-Whitney's U test, as appropriate. Multivariate logistic regression analysis was used to evaluate significant factors for CR by TACE with miriplatin. All variables are expressed as mean (range). All tests were 2-sided, and p values less than 0.05 were considered statistically significant. Statistical analyses were performed using SPSS, version 13.0 (SPSS Inc., IBM, Somers, N.Y., USA).

Results

Dosing of Study Drugs

Table 2 summarizes the profiles and study drug data of 122 HCC patients who were treated with miriplatin. The median dosage of miriplatin was 80 mg (range 20–120 mg), and the median dosage of lipiodol was 3 ml (range 1–6 ml). Twenty-two patients (18%) were injected with the miriplatin-lipiodol suspension from the peripheral to the segmental branch of the hepatic artery. Thirty patients (25%) were injected with the miriplatin-lipiodol suspension from the anterior or posterior segmental branch of the right hepatic artery. Sixty-six patients (54%) were injected with the miriplatin-lipiodol suspension from the right or left branch of the hepatic artery. And 4 patients (3%) were injected with the miriplatin-lipiodol suspension from the proper hepatic artery.

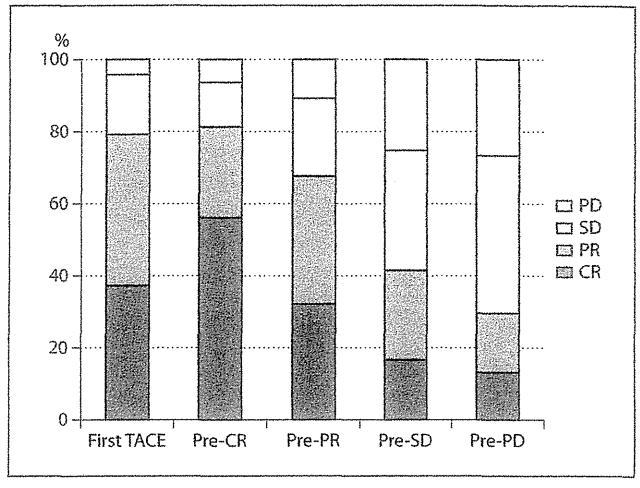

Fig. 1. The efficacy of TACE using miriplatin in patients with HCC according to response to previous TACE. Abbreviations used in the figure: CR = complete response; PR = partial response; SD = stable disease; PD = progressive disease. First TACE group (n = 24): patients who received TACE for the first time. pre-CR group (n = 16): patients who showed CR after previous TACE. pre-PR group (n = 28): patients who showed PR after previous TACE. pre-SD group (n = 24): patients who showed SD after previous TACE. pre-PD group (n = 30): patients who showed PD after previous TACE.

Treatment Effects

Thirty-five of the 122 treated patients (29%) showed CR, 35 patients (29%) showed PR, 33 patients (27%) showed SD, and 19 patients (15%) showed PD. Overall, 58% of patients showed an objective response (i.e. CR or PR).

Treatment Effects according to Previous TACE Effect

The efficacy of TACE using miriplatin according to the treatment effect of previous TACE was as follows (and is illustrated in fig. 1). For the first TACE group (patients who received TACE for the first time), 9 of 24 patients (38%) showed CR; for the pre-CR group (patients who

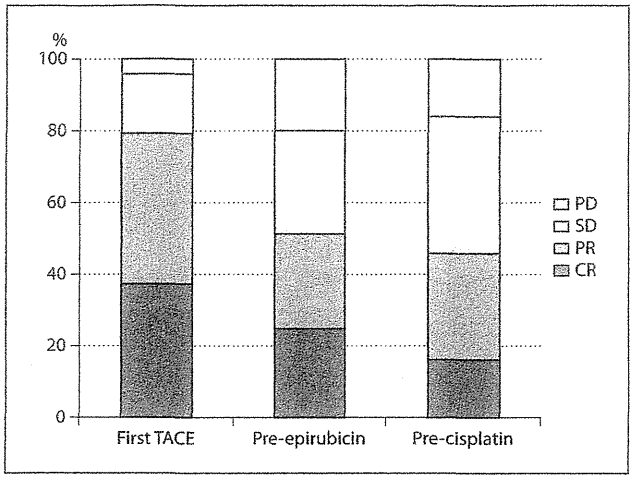

Fig. 2. The efficacy of TACE using miriplatin in patients with HCC according to previous TACE agent. Abbreviations used in the figure: CD = complete response; PR = partial response; SD = stable disease; PD = progressive disease. First TACE group (n = 24): patients who received TACE for the first time. Pre-cisplatin group (n = 37): patients who had received TACE using cisplatin. Pre-epirubicin group (n = 80): patients who had received TACE using epirubicin.

showed CR response after previous TACE), 9 of 16 patients (56%) showed CR; for the pre-PR group (patients who showed PR response after previous TACE), 9 of 28 patients (32%) showed CR; for the pre-SD group (patients who showed SD response after previous TACE), 4 of 24 patients (17%) showed CR; and for the pre-PD group (patients who showed PD response after previous TACE), 4 of 30 patients (13%) showed CR.

Treatment Effects according to Previous TACE Agent

In patients who had received TACE using epirubicin, 20 of 80 patients (25%) showed CR and 21 of 80 patients (26%) showed PR. In patients who had received TACE using cisplatin, 6 of 37 patients (16%) showed CR and 11 of 37 patients (30%) showed PR. In each of the above groups, the objective response rate (sum of CR and PR) was significantly lower than that in patients who received their first TACE ($p = 0.015$ and $p = 0.010$, respectively), as illustrated in figure 2.

Univariate analysis identified the following six factors as influencing the rate of CR: response after previous TACE (pre-CR group vs. other groups, $p = 0.005$), tumor multiplicity (solitary vs. multiple, $p < 0.0001$), gamma-GTP concentration (≤40 vs. >40 IU/l, $p = 0.037$), AFP concentration (≤40 vs. >40 ng/ml, $p = 0.042$), DCP concentration (≤50 vs. >50 AU/l, $p = 0.003$), and injection artery (peripheral to segmental hepatic artery vs. right or left hepatic artery and proper hepatic artery, $p = 0.001$). These parameters were entered into multivariate logistic regression analysis, which revealed that response after previous TACE (pre-CR group vs. other groups, risk ratio: 4.76; 95% CI: 1.11–20.37; $p = 0.035$), tumor multiplicity (solitary vs. multiple, risk ratio: 9.69; 95% CI: 2.18–42.92; $p = 0.003$), and injection artery (peripheral to segmental hepatic artery vs. right or left hepatic artery and proper hepatic artery, risk ratio: 5.28; 95% CI: 1.07–25.95; $p = 0.040$) were significant independent predictors associated with CR after TACE using miriplatin (table 3).

Adverse Effects

Fever, anorexia, and elevation of serum transaminase levels were observed in most patients after miriplatin administration (table 4). The following Grade 4 events were observed: decreased neutrophil count in 1 patient (1%), increased AST in 4 patients (3%), and increased ALT in 1 patient (1%); all these cases resolved within 2 weeks. In this study group, no vascular complications of the hepatic artery were observed. No other serious complications or treatment-related deaths were observed after miriplatin administration.

Discussion

TACE is most widely performed in patients with HCC who are not eligible for curative therapy. The survival benefit of TACE has been confirmed by randomized controlled trials and meta-analyses. Various anti-cancer drugs, such as doxorubicin, epirubicin, mytomycin C, cisplatin, and neocarzinostatin, have been used as TACE agents for the treatment of HCC. However, the most effective and least toxic TACE protocol for HCC has yet to be identified [13–15].

Although TACE can be repeated in most patients, good therapeutic efficacy cannot be expected when the same anti-cancer drug is used more than once since various types of resistance to therapy can develop during repetition of TACE. Platinum derivatives are frequently administered to patients with advanced HCC that is unresponsive to anthracycline and antibiotic drugs [23, 24]. Miriplatin was developed as a lipophilic platinum complex in an effort to produce a superior anti-tumor effect in HCC with lower toxicity compared with cisplatin [16–

Table 3. Univariate and multivariate analysis of predictors of complete necrosis (logistic regression analysis)

Category	Univariate		Multivariate	
	Hazard ratio (95% CI)	p value	Hazard ratio (95% CI)	p value
Tumor multiplicity, solitary vs. multiple	8.57 (3.08–23.8)	<0.0001	9.69 (2.19–42.9)	0.003
Response by pre-TACE, pre-CR vs. others	4.91 (1.59–15.1)	0.005	4.76 (1.11–20.3)	0.035
Injection artery, peripheral to segmental hepatic artery vs. others	2.50 (0.96–6.48)	0.001	5.28 (1.07–25.9)	0.040
DCP, ≤50 vs. >50 AU/l	4.04 (1.61–10.13)	0.003	3.55 (0.99–12.6)	0.051
gamma-GTP, ≤40 vs. >40 IU/l	2.39 (1.05–5.44)	0.037		
AFP, ≤40 vs. >40 ng/ml	2.50 (1.03–6.06)	0.042		

Table 4. Adverse effects after miriplatin administration

Grade:	1	2	3	4
White blood cell decreased	1 (1%)	27 (22%)	7 (6%)	0
Neutrophil count decreased	2 (2%)	21 (17%)	5 (4%)	1 (1%)
Anemia	40 (33%)	21 (17%)	3 (2%)	0
Platelet count decreased	72 (59%)	21 (17%)	11 (9%)	0
AST increased	55 (45%)	23 (19%)	30 (25%)	4 (3%)
ALT increased	54 (44%)	12 (10%)	19 (16%)	1 (1%)
Fever	67 (55%)	14 (11%)	0	0
Anorexia	56 (46%)	1 (1%)	0	0
Nausea	23 (19%)	0	0	0
Abdominal pain	22 (18%)	4 (3%)	0	0
Hepatic infection	0	0	1 (1%)	0

Values denote numbers of subjects. Treatment-related toxicity was assessed using the National Cancer Institute Common Terminology Criteria version 4.0.

19]. Miriplatin-lipiodol suspension is a stable colloidal emulsion that is deposited within HCC tumors, where it gradually releases active derivatives of miriplatin.

According to pharmacokinetic studies, the plasma concentration of total platinum is much lower in patients treated with miriplatin compared with that in patients treated with intra-arterial cisplatin: the Cmax is approximately 300-fold lower and the Tmax roughly 500-fold longer for miriplatin than the corresponding values for intra-arterial cisplatin.

Miriplatin/lipiodol releases 1,2-diaminocyclohexane platinum (II) dichloride (DPC) as its active platinum compound, which binds to nuclear DNA and mediates miriplatin/lipiodol cytotoxicity. In a cisplatin-resistant rat hepatoma cell-line model, cross-resistance to DPC was not observed [25].

Prior to the current study, clinical trials have shown that miriplatin is effective for the treatment of HCC, but the efficacy of switching the TACE anti-cancer drug from epirubicin or cisplatin to miriplatin for a repeat TACE had not been evaluated.

In the present study, having a low number of tumors (solitary vs. multiple), receiving the treatment injection in the peripheral to segmental hepatic artery, and having shown complete tumor necrosis after prior TACE (pre-CR group) were highly correlated with complete tumor necrosis after TACE with miriplatin. A previous CR may be a surrogate marker for other factors, such as tumor sensitivity to anti-cancer agents and intra-hepatic metastasis. Among the 54 patients in this study who had shown no change or disease progression after previous TACE (pre-SD and pre-PD groups), 19 patients (35%) showed an

objective response by switching the TACE agent from epirubicin or cisplatin to miriplatin.

In repetition of TACE, vascular complications can cause development of parasitic feeding arteries for liver cancers leading to insufficient tumor embolization; rapid tumor growth may follow. In the present study, no vascular complications or other serious adverse events were observed. These results suggest that miriplatin may be used effectively and safely as a second-line TACE drug for recurrent HCC after TACE.

Previous studies reported that complete tumor necrosis after TACE offered favorable long-term survival outcomes to HCC patients [7, 26]. In the current study, miriplatin administration was associated with a beneficial tumor response even in recurrent HCC after TACE. These results suggest that miriplatin administration may offer a favorable prognosis for recurrent HCC after TACE.

Conclusion

In repetition of TACE in HCC patients, switching the TACE agent from epirubicin or cisplatin to miriplatin offered a favorable treatment effect, especially in patients who had shown CR after previous TACE. These results suggest that miriplatin may be used effectively and safely as a second-line TACE drug for recurrent HCC after TACE.

Disclosure Statement

The following authors have received honoraria (lecture fee) from Dainippon Sumitomo Pharma Co., Ltd, Osaka, Japan: Hiromitsu Kumada, MD; Kenji Ikeda, MD; Yasuji Arase, MD; Yoshiyuki Suzuki, MD; Fumitaka Suzuki, MD; and Norio Akuta, MD.

References

1 Parkin DM, Bray F, Ferlay J, Pisani P: Global cancer statistics, 2002. CA Cancer J Clin 2005;55:74–108.
2 Bosch X, Ribes J, Borras J: Epidemiology of primary liver cancer. Semin Liver Dis 1999; 19:271–285.
3 Okuda K, Fujimoto I, Hanai A, Urano Y: Changing incidence of hepatocellular carcinoma in Japan. Cancer Res 1987;47:4967–4972.
4 Kudo M: Early detection and curative treatment of early-stage hepatocellular carcinoma. Clin Gastroenterol Hepatol 2005; 3:S144–S148.
5 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.
6 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.
7 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. Cancer 1991;68:2150–2154.
8 Llovet JM, Real MI, Montaña X, Planas R, Coll S, Aponte J, Ayuso C, Sala M, Muchart J, Solà R, Rodés J, Bruix J: Barcelona Liver Cancer Group. Arterial embolisation or chemoembolisation versus symptomatic treatment in patients with unresectable hepatocellular carcinoma: a randomised controlled trial. Lancet 2002;359:1734–1739.
9 Lo CM, Ngan H, Tso WK, Liu CL, Lam CM, Poon RT, Fan ST, Wong J: Randomized controlled trial of transarterial lipiodol chemoembolization for unresectable hepatocellular carcinoma. Hepatology 2002;35:1164–1171.
10 Camma C, Schepis F, Orlando A, Albanese M, Shahied L, Trevisani F, Andreone P, Craxi A, Cottone M: Transarterial chemoembolization for unresectable hepatocellular carcinoma: Meta-analysis of randomized controlled trials. Radiology 2002;224:47–54.
11 Ikeda M, Maeda S, Shibata J, Muta R, Ashihara H, Tanaka M, Fujiyama S, Tomita K: Transcatheter arterial chemotherapy with and without embolization in patients with hepatocellular carcinoma. Oncology 2004; 66:24–31.
12 Takayasu K, Arii S, Ikai I, Omata M, Okita K, Ichida T, Matsuyama Y, Nakanuma Y, Kojiro M, Makuuchi M, Yamaoka Y; Liver Cancer Study Group of Japan. Prospective cohort study of transarterial chemoembolization for unresectable hepatocellular carcinoma in 8510 patients. Gastroenterology 2006;131: 461–469.
13 Marelli L, Stigliano R, Triantos C, Senzolo M, Cholongitas E, Davies N, Tibballs J, Meyer T, Patch DW, Burroughs AK: Transarterial therapy for hepatocellular carcinoma: which technique is more effective? A systematic review of cohort and randomized studies. Cardiovasc Intervent Radiol 2007;30:6–25.
14 Kamada K, Nakanishi T, Kitamoto M, Aikata H, Kawakami Y, Ito K, Asahara T, Kajiyama G: Long-term prognosis of patients undergoing transcatheter arterial chemoembolization for unresectable hepatocellular carcinoma: comparison of cisplatin lipiodol suspension and doxorubicin hydrochloride emulsion. J Vasc Interv Radiol 2001;12:847–854.
15 Ikeda M, Maeda S, Ashihara H, Nagahama H, Tanaka M, Sasaki Y: Transcatheter arterial infusion chemotherapy with cisplatin-lipiodol suspension in patients with hepatocellular carcinoma. J Gastroenterol 2010;45: 60–67.
16 Maeda M, Uchida NA, Sasaki T: Liposoluble platinum (II) complexes with antitumor activity. Jpn J cancer Res 1986;77:523–525.
17 Kishimoto S, Ohtani A, Fukuda H, Fukushima S, Takeuchi Y: Relation between intracellular accumulation and cytotoxic activity of cis[(1R,2R)-1,2-cyclohexanediamine-N,N′)bis(myristato)]platinum(II) suspended in lipiodol. Biol Pharm Bull 2003;26:683–686.
18 Hanada M, Baba A, Tsutsumishita Y, Noguchi T, Yamaoka T: Intra-hepatic arterial administration with miriplatin suspended in an oily lymphographic agent inhibits the growth of human hepatoma cells orthotopically implanted in nude rats. Cancer Sci 2009;100:189–194.
19 Hanada M, Baba A, Tsutsumishita Y, Noguchi T, Yamaoka T, Chiba N, Nishikaku F: Intra-hepatic arterial administration with miriplatin suspended in an oily lymphographic agent inhibits the growth of tumors implanted in rat livers by inducing platinum-DNA adducts to form and massive apoptosis. Cancer Chemother Pharmacol 2009;64:473–483.