

**Table 1** Patient characteristics at the starting time of follow up†

	NAFLD group	HCV group	P-value
<i>n</i>	1600	1600	
Age (years)	62.5 ± 9.5	62.6 ± 8.7	0.936
Gender (male/female)	1200/400	1200/400	1.000
Body mass index	25.1 ± 2.6	21.8 ± 4.0	<0.001
Blood pressure			
(systolic, mmHg)	132 ± 17	133 ± 18	0.972
(diastolic, mmHg)	76 ± 11	77 ± 12	0.937
Hypertension (+/-)	279/1321	306/1294	0.252
Smoking (+/-)	421/1179	396/1141	0.807
AST (IU/L)	29 ± 15	77 ± 64	<0.001
ALT (IU/L)	37 ± 25	104 ± 97	<0.001
GGT (IU/L)	73 ± 79	83 ± 97	0.196
Albumin (g/dL)	4.2 ± 0.3	4.1 ± 0.4	0.883
Triglyceride (mg/dL)	161 ± 105	99 ± 51	<0.001
Total cholesterol (mg/dL)	211 ± 33	176 ± 38	<0.001
FPG (mg/dL)	104.1 ± 10.5	95.8 ± 9.3	<0.001
FPG (DM/pre-DM /normal)	208/330/1062	184/276/1140	<0.001
Platelet (×10 <sup>4</sup> /mm <sup>3</sup> )	22.1 ± 6.5	15.8 ± 5.8	<0.001
AFP (ng/mL)	3.4 ± 2.4	10.8 ± 10.0	<0.001
Follow-up period (year)	8.2 ± 3.8	8.2 ± 3.9	0.928

†Data are number of patients or mean ± standard deviation.

AFP,  $\alpha$ -fetoprotein; ALT, alanine aminotransferase; AST, aspartate aminotransferase; DM, diabetes mellitus, FPG, fasting plasma glucose; GGT, gamma-glutamyltransferase; HCV, hepatitis C virus; NAFLD, non-alcoholic fatty liver disease.

NAFLD group and the HCV group in both males and females.

$P = 0.002$ ), male (HR: 1.49; 95%CI = 1.16–1.94;  $P = 0.002$ ), and thrombocytopenia (HR: 1.49; 95%CI = 1.14–1.96;  $P = 0.002$ ).

### Predictive factors for the development of malignancies

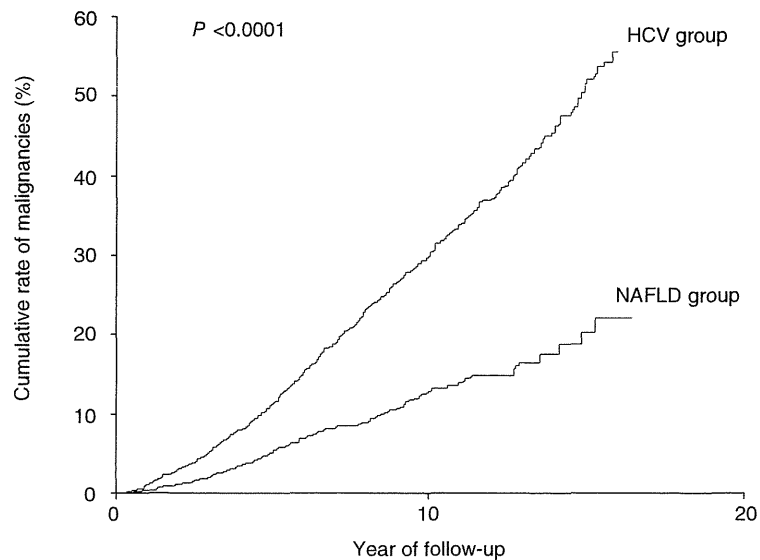
The factors associated with the development of malignancies in the NAFLD group and HCV group are shown in Tables 4 and 5. In the NAFLD group, multivariate Cox proportional hazards analysis shows that malignancies occurred when patients had an age of  $\geq 70$  years (hazard ratio [HR]: 2.10; 95%CI = 1.38–3.17;  $P < 0.001$ ), current smoking (HR: 1.64; 95%CI = 1.18–2.27;  $P = 0.003$ ), and elevated glucose level (HR: 1.32; 95%CI = 1.08–1.61;  $P = 0.007$ ).

On the other hand, in HCV group, multivariate Cox proportional hazards analysis shows that malignancies development rate was high with statistical significance when patients had elevated AFP (HR: 2.52; 95%CI = 1.94–3.44;  $P < 0.001$ ), elevated glucose level (HR: 1.35; 95%CI = 1.18–1.59;  $P < 0.001$ ), elevated AST level (HR: 1.75; 95%CI = 1.13–2.70;  $P = 0.010$ ), hypoalbuminemia (HR: 1.51; 95%CI = 1.15–1.97;

### DISCUSSION

THE DEVELOPMENT INCIDENCE of malignancies in elderly patients with NAFLD or HCV has been described in the present study. The reason for selecting elderly patients is that development of malignancies in patients with age of  $\geq 60$  years occur frequently compared with young patients. Thus, it is likely that the difference between NAFLD and HCV patients tends to become clear.

The present study shows several findings with regard to the development of malignancies in elderly Japanese patients with NAFLD or HCV. First, HCC in the NAFLD group accounted for approximately 6% of the cause of malignancies. The four malignancies of the stomach, colon, prostate, and lung accounted for about 60% in the NAFLD group. Matsuda *et al.* have reported the cancer incidence in Japan.<sup>32</sup> According to their report, the outbreak of malignancies in a Japanese male popu-



No. patients				
NAFLD	1600	1028	583	92
HCV	1600	1040	598	104

**Figure 1** Cumulative development rate of malignancies in non-alcoholic hepatic diseases (NAFLD) or hepatitis C virus (HCV) patients.

lation was observed in the following order in 2005: gastric cancer 20.4% > colon cancer 16.0% > lung cancer 15.4% > prostatic cancer 10.9% > HCC 7.4%. On the other hand, the outbreak of malignancies in a Japanese female population was observed in the following order in 2005: mammary cancer 18.0% > colon

cancer 16.2% > gastric cancer 13.6% > lung cancer 9.3% > uterine cancer 6.8%. The incidence of prostate cancer in NAFLD was greater than that in a total Japanese population. Renehan *et al.* showed that body mass index is connected with prostate carcinogenesis relative to other tumours.<sup>33</sup> NAFLD patients might tend to have

**Table 2** Development rate of each malignancy in the non-alcoholic fatty liver disease (NAFLD) group and the hepatitis C virus (HCV) group†

Malignancies	NAFLD group		HCV group		P‡
	n (%)†	1000 person years	n (%)†	1000 person years	
Total	167 (100%)	12.96	395 (100%)	30.88	<0.001
Hepatocellular carcinoma	10 (6.0%)	0.78	267 (67.9%)	20.86	<0.001
Gastric cancer	34 (20.4%)	2.66	28 (7.1%)	2.19	0.522
Colon cancer	31 (18.6%)	2.42	26 (6.6%)	2.03	0.593
Prostate cancer	21 (12.6%)	1.64	14 (3.5%)	1.10	0.308
Lung cancer	17 (10.2%)	1.33	13 (3.3%)	1.02	0.583
Malignant lymphoma	1 (0.6%)	0.08	9 (2.3%)	0.70	0.021
Other cause	46 (27.5%)	3.59	31 (7.8%)	2.43	0.106
Unknown origin	6 (3.6%)	0.46	7 (1.8%)	0.55	1.000

†Data are number of patients (%) and development rates of each malignancy per 1000 person years. ‡Comparison of new development in each malignancy between both groups by log rank test.

**Table 3** Development rate of Each Malignancy between the non-alcoholic fatty liver disease (NAFLD) group and the hepatitis C virus (HCV) group based on the difference of gender†

Malignancies	Male		P‡	Female		P‡
	NAFLD (n = 1200)	HCV (n = 1200)		NAFLD (n = 400)	HCV (n = 400)	
Total	13.96	34.17	<0.001	10.31	20.93	<0.001
Hepatocellular carcinoma	0.83	23.75	<0.001	0.63	10.83	<0.001
Gastric cancer	2.91	2.40	0.571	1.88	1.39	1.000
Colon cancer	2.42	2.19	0.655	1.88	1.39	1.000
Lung cancer	1.33	1.05	0.676	1.25	0.93	1.000
Malignant lymphoma	0.08	0.63	0.124	0.00	0.93	0.577
Prostate cancer	1.64	1.10	0.306			
Breast cancer				1.81	1.41	1.000
Other cause	3.59	4.38	0.604	2.43	1.71	0.577
Unknown origin	0.46	0.52	1.000	0.30	0.62	1.000

†Data are development rates of each malignancy per 1000 person years. ‡Comparison of new development in each malignancy between NAFLD group and HCV group based on the difference of gender by log rank test

carcinogenesis of prostate based on obesity. Our results show that physicians in charge of NAFLD patients should pay attention to the malignancies of stomach, colon, prostate, and lung in addition to development of HCC. Moreover, aging, hyperglycemia, and smoking were dominating factors to enhance the development of malignancies in NAFLD group.

Second, HCC in the HCV group accounted for about two-thirds of the outbreak of malignancies. In the

present study, the development rates of HCC and malignant lymphoma in the HCV group were statistically higher than those in the NAFLD group. The high incidences of HCC and malignant lymphoma have been reported by many researchers.<sup>15–19,34</sup> Male, hyperglycemia, elevated AST, hypoalbuminemia, thrombocytopenia, and elevated AFP were dominating factors to enhance the development of malignancies in the HCV group. Hypoalbuminemia, thrombocytopenia,

**Table 4** Predictive factors for malignancies in the non-alcoholic fatty liver disease (NAFLD) group†

Variables	Univariate analysis		Cox-regression	
	HR (95% CI)	P-value	HR (95% CI)	P-value
Age (years, ≥70/<70)	2.34 (1.60–3.44)	<0.001	2.09 (1.42–3.07)	<0.001
Gender (M/F)	1.11 (0.76–1.60)	0.631		
BMI (≥25/<25)	0.74 (0.52–1.04)	0.079		
Hypertension (-/+)	1.27 (0.88–1.84)	0.197		
Smoking (+/-)	1.62 (1.18–2.24)	0.003	1.64 (1.18–2.27)	0.003
AST (IU/L, ≥34/<34)	1.03 (0.62–1.70)	0.973		
ALT (IU/L, ≥36/<36)	1.27 (0.76–2.08)	0.357		
GGT (IU/L, ≥109/<109)	1.26 (0.79–2.01)	0.350		
Albumin (g/dL, <3.9/≥3.9)	1.41 (0.90–2.04)	0.145		
Triglyceride (mg/dL, ≥150/<150)	1.20 (0.85–1.69)	0.282		
Total cholesterol (mg/dL, ≥220/<220)	1.39 (0.87–2.23)	0.170		
Glucose (DM/ pre-DM/non-DM)	1.39 (1.14–1.69)	0.001	1.32 (1.08–1.61)	0.007
Platelet (×10 <sup>3</sup> /mm <sup>3</sup> , <15/≥15)	1.41 (1.02–1.96)	0.036		
AFP (ng/mL, ≥10/<10)	1.11 (0.35–3.48)	0.338		

†Data are number of patients or mean ± standard deviation.

AFP,  $\alpha$ -fetoprotein; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; DM, diabetes mellitus, FPG, fasting plasma glucose; GGT, gamma-glutamyltransferase.

**Table 5** Predictive factors for malignancies in the hepatitis C virus (HCV) group†

Variables	Univariate analysis		Cox-regression	
	HR (95% CI)	P-value	HR (95% CI)	P-value
Age (years, $\geq 70$ / $< 70$ )	1.41 (1.11–1.78)	0.003		
Gender (M/F)	1.78 (1.4692.10)	<0.001	1.49 (1.16–1.94)	0.002
BMI ( $\geq 25$ / $< 25$ )	1.85 (0.71–4.85)	0.201		
Hypertension (+/–)	1.20 (1.01–1.44)	0.045		
Smoking (+/–)	1.71 (1.43–2.10)	<0.001		
AST (IU/L, $\geq 36$ / $< 36$ )	2.26 (1.73–3.01)	<0.001	1.75 (1.13–2.70)	0.010
ALT (IU/L, $\geq 30$ / $< 30$ )	1.69 (1.33–2.16)	<0.001		
GGT (IU/L, $\geq 109$ / $< 109$ )	1.99 (1.53–2.58)	0.014		
Albumin (g/dL, $< 3.9$ / $\geq 3.9$ )	2.07 (1.65–2.56)	<0.001	1.51 (1.15–1.97)	0.002
Triglyceride (mg/dL, $\geq 150$ / $< 150$ )	1.15 (0.56–2.41)	0.789		
Total cholesterol (mg/dL, $\geq 220$ / $< 220$ )	0.51 (0.19–1.35)	0.159		
Glucose (DM/pre-DM/non-DM)	1.37 (1.23–1.55)	<0.001	1.35 (1.18–1.59)	<0.001
Platelet ( $\times 10^3$ /mm <sup>3</sup> , $< 15$ / $\geq 15$ )	2.28 (1.81–2.92)	<0.001	1.49 (1.14–1.96)	0.002
AFP (ng/mL, $\geq 10$ / $< 10$ )	3.10 (2.46–4.11)	<0.001	2.50 (1.94–3.44)	<0.001

†Data are number of patients or mean  $\pm$  standard deviation.

AFP,  $\alpha$ -fetoprotein; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; DM, diabetes mellitus, GGT, gamma-glutamyltransferase.

and elevated AFP indicate the advanced liver fibrosis: it is probable that these factors enhance the HCC development as reported before.<sup>35</sup> Our result shows that HCV positive males with hyperglycemia, hypoalbuminemia, elevated AST, thrombocytopenia, and elevated AFP should be carefully checked for HCC.

Third, there were no significant differences in the development of each malignancy between males and females in the NAFLD group. On the other hand, rare development of HCC in males was statistically higher than that of females. However, there are no significant differences in the development of each malignancy except for HCC between males and females in the HCV group. This result suggests that development differences based on gender except for HCC in HCV group might be not important.

Cirrhotic NASH enhances the liver-related events such as HCC and liver failure. However, most patients with NAFLD do not have NASH. According to Japanese annual health check reports, 9–30% of Japanese adults demonstrate evidence of NAFLD by US. Since it is known that about 10% of individuals with NAFLD have NASH, the prevalence of NASH is estimated to be 1–3% of the adult Japanese population.<sup>14</sup> In patients with cirrhotic NASH, HCC and liver failure are the main causes of morbidity and mortality (5-year cumulative HCC development rate 11.3%, 5-year survival rate 75.2%, respectively). However, in the present study, most NAFLD was thought to be non-NASH. Our results

suggest that patients with NAFLD before progression to NASH should be followed up to closely check the malignancies other than HCC in addition to HCC. On the other hand, patients with HCV should be followed up to take care to check liver-related disease containing HCC.

The present study was limited that most of the NAFLD patients were not undergoing histological or morphological assessment by peritoneoscopy or liver biopsy before the starting time of follow up owing to their advanced age on the day of the first consulting or normal transaminase. Another limitation was that there are several differences in clinical background such as liver fibrosis between the NAFLD and HCV groups. 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 with a large number of patients included.

Our results indicate the following: (i) Physicians in charge of NAFLD patients should pay attention to the carcinogenesis development of stomach, colon, prostate, and lung containing HCC; and (ii) physicians in charge of HCV patients should closely check for HCC.

#### ACKNOWLEDGMENTS

THE PRESENT WORK was supported in part by grants-in-aid from Okinaka memorial institute for medical research and Japanese Ministry of Health, Labour and Welfare.

## REFERENCES

- 1 Angulo P. Nonalcoholic fatty liver disease. *N Engl J Med* 2002; 346: 1221–31.
- 2 Williams R. Global changes in liver disease. *Hepatology* 2006; 44: 521–6.
- 3 Torres DM, Harrison SA. Diagnosis and therapy of nonalcoholic steatohepatitis. *Gastroenterology* 2008; 134: 1682–98.
- 4 Vuppalanchi R, Chalasani N. Nonalcoholic fatty liver disease and nonalcoholic steatohepatitis: selected practical issues in their evaluation and management. *Hepatology* 2009; 49: 306–17.
- 5 Fan JC, Farrell GC. Epidemiology of non-alcoholic fatty liver disease in China. *J Hepatol* 2009; 50: 204–10.
- 6 Watanabe S, Yaginuma R, Ikejima K, Miyazaki A. Liver diseases and metabolic syndrome. *J Gastroenterol* 2008; 43: 509–18.
- 7 Vega GL, Chandalia M, Szczepaniak LS, Grundy SM. Metabolic correlates of nonalcoholic fatty liver in women and men. *Hepatology* 2007; 46: 716–22.
- 8 van der Poorten D, Milner KL, Hui J *et al.* Visceral fat a key mediator of steatohepatitis in metabolic liver disease. *Hepatology* 2008; 48: 449–57.
- 9 Angulo P, Keach JC, Batts KP, Lindor KD. Independent predictors of liver fibrosis in patients with nonalcoholic steatohepatitis. *Hepatology* 1999; 30: 1356–62.
- 10 Stern SE, Williams K, Ferrannini E, DeFronzo RA, Bogardus C, Stern MP. Identification of individuals with insulin resistance using routine clinical measurements. *Diabetes* 2005; 54: 333–9.
- 11 Adams LA, Feldstein A, Lindor KD, Angulo P. Nonalcoholic fatty liver disease among patients with hypothalamic and pituitary dysfunction. *Hepatology* 2004; 39: 909–14.
- 12 Kheirandish-Gozal L, Sans Capdevila O, Kheirandish E, Gozal D. Elevated serum aminotransferase levels in children at risk for obstructive sleep apnea. *Chest* 2008; 133: 92–9.
- 13 Arase Y, Suzuki F, Ikeda K, Kumada H, Tsuji H, Kobayashi T. Multivariate analysis of risk factors for the development of type 2 diabetes in nonalcoholic fatty liver disease. *J Gastroenterol* 2009; 44: 1064–70.
- 14 Hashimoto E, Tokushige K. Prevalence, gender, ethnic variations, and prognosis of NASH. *J Gastroenterol* 2011; 46 (Suppl 1): 63–9.
- 15 Simonetti RG, Camma C, Fiorello F *et al.* Hepatitis C virus infection as a risk factor for hepatocellular carcinoma in patients with cirrhosis. A case control study. *Ann Intern Med* 1992; 116: 97–102.
- 16 Kasahara A, Hayashi N, Mochizuki K *et al.* Risk factors for hepatocellular carcinoma and its incidence after interferon treatment in patients with chronic hepatitis C. Osaka Liver Disease Study Group. *Hepatology* 1998; 2: 1394–402.
- 17 Imai Y, Kawata S, Tamura S *et al.* Relation of interferon therapy and hepatocellular carcinoma in patients with chronic hepatitis C. Osaka Hepatocellular Carcinoma Prevention Study Group. *Ann Intern Med* 1998; 129: 94–9.
- 18 Okanoue T, Itoh Y, Minami M *et al.* Interferon therapy lowers the rate of progression to hepatocellular carcinoma in chronic hepatitis C but not significantly in an advanced stage: a retrospective study in 1148 patients. Viral Hepatitis Therapy Study Group. *J Hepatol* 1999; 30: 653–9.
- 19 Ikeda K, Saitoh S, Arase Y *et al.* Effect of interferon therapy on hepatocellular carcinogenesis in patients with chronic hepatitis type C; A long-term observation study of 1643 patients using statistical bias correction with proportional hazard analysis. *Hepatology* 1999; 29: 1124–30.
- 20 Yasuda K. Early gastric cancer: diagnosis, treatment techniques and outcomes. *Eur J Gastroenterol Hepatol* 2006; 18: 839–45.
- 21 Van Gossum A. Guidelines for colorectal cancer screening – a puzzle of tests and strategies. *Acta Clin Belg* 2010; 65: 433–6.
- 22 Tsukada K, Takada T, Miyazaki M *et al.* Japanese Association of Biliary Surgery; Japanese Society of Hepato-Biliary-Pancreatic Surgery; Japan Society of Clinical Oncology. Diagnosis of biliary tract and ampullary carcinomas. *J Hepatobiliary Pancreat Surg* 2008; 15: 31–40.
- 23 Cascinu S, Falconi M, Valentini V, S J, Guidelines ESMO. Working Group. Pancreatic cancer: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. *Ann Oncol* 2010; 21 (Suppl 5): v55–8.
- 24 Currie GP, Kennedy AM, Denison AR. Tools used in the diagnosis and staging of lung cancer: what's old and what's new? *QJM* 2009; 102: 443–8.
- 25 Maresh EL, Mah V, Alavi M *et al.* Differential expression of anterior gradient gene AGR2 in prostate cancer. *BMC Cancer* 2010; 10: 680–7.
- 26 Harris NL, Jaffe ES, Stein H *et al.* A revised European-American classification of lymphoid neoplasms: a proposal from the International Lymphoma Study Group. *Blood* 1994; 84: 1361–92.
- 27 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.
- 28 Lonardo A, Bellini M, Tartoni P, Tondelli E. The bright liver syndrome. Prevalence and determinants of a "bright" liver echopattern. *Ital J Gastroenterol Hepatol* 1997; 29: 351–6.
- 29 Harrington DP, Fleming TR. A class of rank test procedures for censored survival data. *Biometrika* 1983; 62: 205–9.
- 30 Kaplan EL, Meier P. Nonparametric estimation for incomplete observation. *J Am Stat Assoc* 1958; 53: 457–81.
- 31 DR Cox. Regression models and life tables. *J R Stat Soc* 1972; 34: 248–75.
- 32 Matsuda T, Marugame T, Kamo KI, Katanoda K, Ajiki W, Sobue T. The Japan Cancer Surveillance Research Group. Cancer incidence and incidence rates in Japan in 2005:

- based on data from 12 population-based cancer registries in the monitoring of cancer incidence in Japan (MCIJ) project. *Jpn J Clin Oncol* 2011; 41: 139–47.
- 33 Renehan AG, Tyson M, Egger M, Heller RF, Zwahlen M. Body-mass index and incidence of cancer: a systematic review and meta-analysis of prospective observational studies. *Lancet* 2008; 371: 569–78.
- 34 Kawamura Y, Ikeda K, Arase Y *et al.* Viral elimination reduces incidence of malignant lymphoma in patients with hepatitis C. *Am J Med* 2007; 120: 1034–41.
- 35 Ikeda K, Saitoh S, Suzuki Y *et al.* Disease progression and hepatocellular carcinogenesis in patients with chronic viral hepatitis: a prospective observation of 2215 patients. *J Hepatol* 1998; 28: 930–8.

## Risk factors for hepatocellular carcinoma in Japanese patients with autoimmune hepatitis type 1

Teruko Hino-Arinaga · Tatsuya Ide · Ryoko Kuromatsu · Ichiro Miyajima · Kei Ogata · Reiiichiro Kuwahara · Akiko Hisamochi · Takuji Torimura · Michio Sata · Autoimmune Hepatitis Study Group

Received: 15 July 2011 / Accepted: 23 November 2011 / Published online: 21 December 2011  
 © Springer 2011

### Abstract

**Background** Hepatocellular carcinoma (HCC) is occasionally seen even in patients with autoimmune hepatitis (AIH) without prior infection either with hepatitis C virus (HCV) or hepatitis B virus. The aim of this study was to identify the incidence of and risk factors for HCC with AIH in a large-scale population with a long-term follow-up in Japan.

**Methods** One hundred and eighty patients diagnosed with AIH were enrolled (F/M = 159/21; mean age, 59.9 years; mean observation period, 80.2 months). Patients with positive HCV antibody/serum HCV RNA and/or positive HBs Ag were excluded. Initial treatment included immunosuppressant therapy ( $n = 147$ ), other drugs ( $n = 28$ ), and no drug ( $n = 5$ ). Patients underwent abdominal ultrasonography at intervals of 3–6 months during observation. Patients' demographic factors, biochemical data, liver histology, medications, response to treatment, and complications were evaluated in relation to HCC.

**Results** During the observation period, six patients (3.3%) developed HCC. Univariate analysis showed that

risk factors for HCC were cirrhosis at diagnosis with AIH ( $p = 0.0002$ ), absence of a treatment response ( $p = 0.033$ ), abnormal alanine aminotransferase (ALT) at the final observation ( $p = 0.0002$ ), and diabetes ( $p = 0.0015$ ). Multivariate analysis showed that risk factors for HCC were cirrhosis at diagnosis of AIH (odds ratio 4.08) and abnormal ALT at final observation (odds ratio 3.66).

**Conclusion** This retrospective study showed that cirrhosis at diagnosis of AIH and abnormal ALT at final observation were independently associated with HCC development. It is important to pay attention to the presence of cirrhosis at diagnosis of AIH and to normalize ALT.

**Keywords** Autoimmune hepatitis · Hepatocellular carcinoma · Risk factors · Liver cirrhosis

### Introduction

The etiology and pathogenesis of autoimmune hepatitis (AIH) have not yet been clearly defined. However, the responses to prednisolone (PSL) and azathioprine (AZA) therapies are favorable, and the long-term survival rate ranges from 80 to 90% or more [1–3]. On the other hand, we previously reported that factors involved in the poor prognosis of AIH included an unfavorable response to initial treatment and the presence of liver cirrhosis at the time of diagnosis [4]. According to studies regarding the appearance of hepatocellular carcinoma (HCC) in patients with AIH, the background has changed over time, although the incidence is low [5–14]. Several studies have indicated that factors for the appearance of HCC include viral infection, the administration of immunosuppressive agents, and liver fibrosis. However, the detection of hepatitis C virus (HCV) and development of virological tests have

The members of the Autoimmune Hepatitis Study Group are listed in the Appendix.

T. Hino-Arinaga (✉) · T. Ide · R. Kuromatsu · I. Miyajima · K. Ogata · R. Kuwahara · A. Hisamochi · M. Sata  
 Division of Gastroenterology, Department of Medicine,  
 Kurume University School of Medicine, 67 Asahi-machi,  
 Kurume, Fukuoka 830-0011, Japan  
 e-mail: terukoh@med.kurume-u.ac.jp

T. Torimura  
 Division of Gastroenterology, Department of Medicine,  
 Research Center for Innovative Cancer Therapy,  
 Kurume University School of Medicine,  
 67 Asahi-machi, Kurume, Fukuoka 830-0011, Japan

markedly changed the clinical background of AIH and background factors for carcinogenesis. In 1993, international diagnostic criteria for AIH were newly prepared by the International Autoimmune Hepatitis Group (IAHG) [15]. Factors for viral infection were excluded by scoring. Studies regarding the development of HCC in AIH patients before 1995 reported the association between the development of HCC and HCV infection in some patients [5–7, 9]. However, Park et al. [8] indicated that HCC developed in 1 (0.5%) of 212 patients in the absence of viral infection.

On the other hand, recent studies have suggested the association of obesity, diabetes, hyperinsulinemia, and fatty liver with carcinogenesis [16–24].

In this retrospective analysis of prospectively acquired data, we identify the incidence of HCC in patients with AIH type-1, and define risk factors associated with the development of HCC in patients with AIH type-1 in a large-scale population with a long-term follow-up in Japan.

## Patients and methods

### Patients

One hundred and eighty patients who were diagnosed with AIH type-1 between November 1978 and April 2007 at Kurume University Hospital (Kurume, Japan) and affiliated hospitals and could be followed up until April 2008 were enrolled. The diagnosis of AIH was based on the IAHG criteria [15, 25]. Female/male = 159/21. The mean age was  $59.9 \pm 14.3$  years. The mean observation period was  $80.2 \pm 66.5$  months. Patients positive for HCV antibody, HCV RNA, and hepatitis B surface (HBs) antigen were excluded. One hundred and seventy-one patients had antinuclear antibodies (ANA), and 50 patients had smooth muscle antibodies (ASMA). In this survey, serum from all patients was tested and was negative for antibodies to liver kidney microsome type-1 antibody (LKM-1). All patients were diagnosed with type-1 AIH. One hundred and twenty-eight patients (71.1%) were diagnosed as “definite” and 52 patients (28.9%) as “probable” according to the “revised scoring system” of the IAHG criteria. Autoimmune disease-related complications consisted of chronic thyroiditis in 33 patients, rheumatoid arthritis (RA) in 12, Sjögren’s syndrome in 10, systemic lupus erythematosus (SLE) in 6, and primary biliary cirrhosis (PBC) in 4.

The study protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki, and comprehensive informed consent regarding the use of the data was obtained in writing from each subject. The committee of the institutional review board (IRB) of our university judged that this retrospective study did not need IRB approval.

### Clinical and laboratory assessments

The following factors were examined: gender, age, AIH score, alanine aminotransferase (ALT), immunoglobulin G (IgG), liver histology at the time of AIH diagnosis, treatments, normalization of serum ALT by treatment, serum ALT level at the final observation/at the onset of HCC, and complications, such as fatty liver, diabetes mellitus, obesity, and hepatitis B core (HBc) antibody. Patients with a bright liver, deep attenuation, and hepatorenal contrast on abdominal ultrasonography were diagnosed with fatty liver. A diagnosis of diabetes mellitus was made in accordance with the diagnostic criteria established by the Japan Diabetes Society [26]. Patients with a body mass index (BMI) of 25 or higher were regarded as obese. During follow-up, fatty liver was observed in 29 (16.3%) of 178 patients, diabetes mellitus in 27 (15.2%) of 178 patients, and obesity in 38 (39.2%) of 97 patients. Of 68 patients, 18 (26.4%) were positive for HBc antibody. These characteristics were evaluated in relation to HCC.

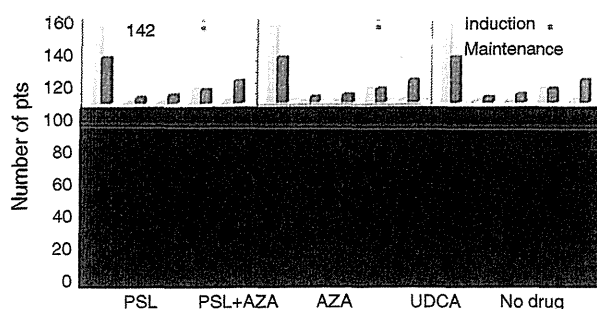
### Histological assessment

Liver biopsy was performed before the initial treatment in 155 (86.1%) of the 180 patients. Histological assessments were performed by two pathologists of the Department of Pathology at Kurume University School of Medicine. All patients had at least interface hepatitis on liver histology, and each satisfied the histological criteria for AIH. Staging and grading were evaluated according to the classification of Desmet et al. [27]. The diagnosis of liver cirrhosis required fibrosis with at least one regenerative nodule. In the absence of histological analysis, cirrhosis was diagnosed according to the presence of thrombocytopenia, low level of serum albumin, esophagogastric varices, ascites, and compatible imaging features on ultrasound sonography or computed tomography. A diagnosis of liver cirrhosis was established in 34 (18.9%) of the 180 patients.

### Definitions

The normalization of the ALT level during the course was defined as the presence of a treatment response. The absence of ALT normalization during the course was defined as the absence of a treatment response. Patients with an ALT level exceeding the upper limit of the normal range (ALT <35 U/L) were regarded as showing an abnormal ALT level. Patients with an additional increase in the ALT level after ALT normalization were regarded as showing relapse. Patients with the disappearance of symptoms and normalization of the serum ALT and  $\gamma$ -globulin levels were regarded as achieving complete remission. In patients with HCC, the ALT level on the final observation was regarded as that at the onset of HCC.





**Fig. 1** Induction and maintenance treatment regimens for AIH. PSL prednisolone, AZA azathioprine, UDCA ursodeoxycholic acid

### Treatment regimens

One hundred and forty-two patients (78.9%) were treated with PSL monotherapy (0.8–1.0 mg/kg/day), 3 patients (1.7%) were treated with PSL in combination with AZA (50 mg/day), 2 patients (1.1%) were treated with AZA monotherapy (50–100 mg/day), 27 patients (15%) were treated with ursodeoxycholic acid (UDCA) (300–600 mg/day), and 6 patients were not treated with any drug (Fig. 1). The PSL dose was individually tapered to the lowest dose required to maintain biochemical remission. If patients sustained complete biochemical and clinical remission, i.e., the disappearance of symptoms and normalization of the serum ALT and  $\gamma$ -globulin levels, for at least 1 year, they received maintenance treatment including drug-free therapy (23.3%). Few patients were treated with AZA, because AZA has yet to be approved in Japan. The majority of patients were treated with PSL (Fig. 1). Of the 180 patients, 154 (85.6%) responded to treatment. Of 145 patients treated with PSL, 131 (90.3%) responded. However, 14 (9.7%) did not respond. Of 131 patients in whom PSL therapy normalized the ALT level, relapse was detected in 55 (42.0%).

### Screening tests, diagnosis, and treatments of HCC

All patients underwent abdominal ultrasonography and serum alpha-fetoprotein ( $\alpha$ -FP) determination every 3–6 months. In patients with HCC, the stage was evaluated according to the tumor/node/metastasis (TNM) staging system. Treatment was selected in accordance with the algorithm for HCC treatment established by the Ministry of Health, Labour and Welfare study group [28, 29].

### Statistical analysis

The examined variables and age are expressed as mean  $\pm$  SD. Data analyses were performed using JMP software (version 6.0.3, SAS Inc., Cary, NC, USA). Uni- and multivariate analyses by logistic regression were used to determine independent factors associated with HCC

development. The cumulative probability of HCC development was estimated by employing the Kaplan–Meier method, and the difference between curves was evaluated using the log-rank (Mantel–Cox) and generalized Breslow–Gehan–Wilcoxon tests. *p* values less than 0.05 were considered significant.

### Results

#### Characteristics and outcomes of AIH patients with HCC

Among the 180 patients, 6 patients (3.3%) developed HCC. The mean observation period until HCC diagnosis was  $128.2 \pm 89.9$  months (range 17.7–252.0 months). Their average age was  $65.3 \pm 7.4$  years old at the time of HCC diagnosis. Table 1 shows the characteristics of patients with HCC. All of them were female, and 5 of 6 (83.3%) had liver cirrhosis at the time of AIH diagnosis. The remaining patient developed liver cirrhosis at the time of HCC diagnosis. Four patients received PSL therapy and 1 patient received AZA therapy, but these 5 patients showed the absence of a treatment response. The remaining patient did not receive therapy for AIH, because the ALT level was less than twice the upper limit of the normal range, and because ischemic heart disease was present. Four patients (66.7%) had diabetes at the development of HCC. Three patients were obese, with BMIs of 29.0, 31.4, and 31.4. Three of the 6 (50%) showed HBc antibody presence.

Table 2 shows the outcome of these patients. Five patients' HCCs were solitary, and their sizes were 17–50 mm. One patient had multifocal HCC. Six patients received medical treatment for HCC including transarterial chemoembolization (TACE), radio-frequency ablation (RFA), and percutaneous ethanol injection (PEI), excluding patient no. 2. Patient no. 2 could not receive chemotherapy because her hepatic reserve function was low, as her Child–Pugh score was 11 points. These patients were followed up for a mean of 26.7 months (range 18–48 months) after HCC diagnosis. Two of them died: tumor rupture at 19 months and liver failure at 30 months. The remaining 4 patients were still being seen on an outpatient follow-up basis at the final observation.

#### Comparison of AIH patients with and without HCC

Univariate analysis showed that the risk factors for the development of HCC in AIH patients were cirrhosis at diagnosis (83.3 vs. 16.6%, respectively, *p* = 0.0002), absence of treatment response (100 vs. 11.5%, respectively, *p* = 0.033), abnormal ALT at the final observation (100 vs. 21.3%, respectively, *p* = 0.0002), and diabetes (66.7 vs.

**Table 1** Characteristics of patients with HCC

Patient no.	At the diagnosis of AIH							Complications during observation				Observation period until Dx of HCC (months) <sup>a</sup>
	Age/sex	ALT (IU/ml)	IgG (mg/dl)	Liver stage	Hx stage/grade	Tx	Response to Tx	Fatty liver	Diabetes	BMI	HBcAb	
1	57/F	42	2,001	LC	F4/A2	AZA	None	+	+	31.4	–	17.7
2	69/F	40	2,500	LC	–	PSL	None	–	+	–	+	42.0
3	78/F	33	2,230	LC	F4/A2	None	–	–	–	31.4	–	117.5
4	65/F	46	3,581	LC	–	PSL	None	–	–	22.7	+	141.0
5	60/F	73	3,671	CH	F3/A2	PSL	None	–	+	29.0	+	199.6
6	63/F	55	1,740	LC	–	PSL	None	–	+	23.6	–	252.0

Staging and grading were evaluated according to the classification of Desmet et al. [27]

HCC hepatocellular carcinoma, ALT alanine aminotransferase, Hx histology, Tx treatment, BMI body mass index, HBcAb hepatitis B core antibody, Dx diagnosis, LC liver cirrhosis, CH chronic hepatitis, AZA azathioprine, PSL prednisolone

<sup>a</sup> Mean 128.2 months

**Table 2** Outcome of patients with HCC

	Patient no.	HCC			Outcome	Observation period after Dx of HCC (months) <sup>a</sup>
		Number of tumors	Size (mm)	Tx		
HCC hepatocellular carcinoma, Tx treatment, Dx diagnosis, TACE transarterial chemoembolization, RFA radio-frequency ablation, PEI percutaneous ethanol injection, ALT alanine aminotransferase, PSL prednisolone, AZA azathioprine	1	1	40	TACE + RFA	Recurrence	18
	2	1	23	None	Death (HCC rupture)	19
	3	Multifocal		Systemic chemo.	Death (hepatic failure)	30
	4	1	17	PEI	Recurrence	48
	5	1	50	TACE	Recurrence	24
	6	1	17	RFA	No recurrence ALT normalized (PSL–AZA)	21

<sup>a</sup> Mean 26.7 months

13.4%, respectively,  $p = 0.0015$ ) (Table 3). Multivariate analysis showed that liver cirrhosis at the time of AIH diagnosis ( $p = 0.0138$ , odds ratio 4.08) and abnormal ALT at the final observation ( $p = 0.0236$ , odds ratio 3.66) were independent risk factors for the development of HCC.

Kaplan–Meier analysis of HCC development in AIH patients with or without liver cirrhosis was performed (Fig. 2). The 10-year developmental probability of HCC was 15% and the 20-year probability was 23% in patients with liver cirrhosis at the time of AIH diagnosis. On the other hand, the 10-year developmental probability of HCC was 0% and the 20-year developmental probability of HCC was 16% in patients with non-liver cirrhosis at the time of AIH diagnosis. Patients with cirrhosis at the time of AIH diagnosis had a significantly higher risk of HCC than patients with non-cirrhosis at the time of AIH diagnosis ( $p = 0.004$ , log-rank test).

## Discussion

The scoring system established by the IAHG provided objective diagnostic criteria for AIH [15]. Since the criteria

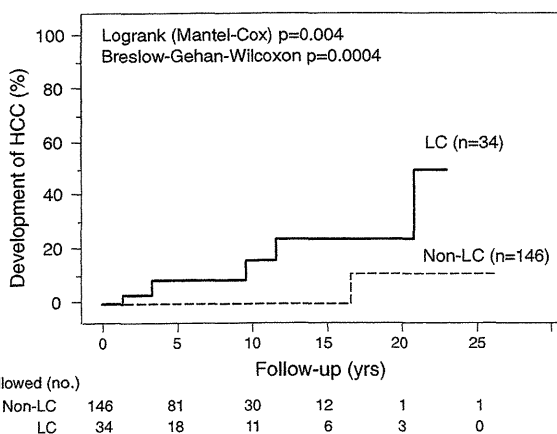
were revised in 1999 [25], more than 10 years have passed. In addition, it became possible to achieve a favorable long-term prognosis employing PSL and AZA therapies. In this study, we examined risk factors for HCC in AIH patients at this timing, leading to the elimination of viral factors such as HBV and HCV. This is very significant for achieving a long-term prognosis. In this study, we excluded patients who were positive for HCV antibody and RNA on an additional test. Concerning HBV, HBs antigen- and HBV DNA-positive patients were excluded. However, we did not exclude any patient with a history of an HBc antibody-positive reaction, and confirmed whether HBc antibody presence is a risk factor. On the other hand, recent studies showed that obesity, diabetes, hyperinsulinemia, and fatty liver were related to carcinogenesis [16–24]. In this study, hyperinsulinemia could not be examined, but diabetes and obesity were assessed, respectively. Thus, a univariate analysis of the data obtained in this study was conducted. The absence of treatment response (100 vs. 11.5%,  $p = 0.033$ ), abnormal ALT at the final observation (100 vs. 21.3%,  $p = 0.0002$ ), diabetes (66.7 vs. 13.4%,  $p = 0.0015$ ), and presence of liver cirrhosis at the time of

**Table 3** Comparison of patients with/without HCC

Univariate analysis	HCC (n = 6)	Non-HCC (n = 174)	p value
Age	65.3 ± 7.4	59.7 ± 14.4	ns
Sex (F/M)	6/0	153/21	ns
AIH score	15.5 ± 2.1	15.9 ± 2.9	ns
IgG (mg/dl) at AIH Dx	2,621.5 ± 818.8	2,746.3 ± 1,015.2	ns
LC (yes/no) at AIH Dx	5/1 (83.3%)	29/145 (16.6%)	0.0002
PSL or/and AZA (yes/no)	5/1 (83.3%)	140/34 (80.5%)	ns
Treatment response (yes/no)	0/6 (0.0%)	154/20 (88.5%)	0.033
ALT at final observation (normal/abnormal)	0/6 (0.0%)	137/37 (78.7%)	0.0002
Fatty liver (yes/no)	1/5 (16.7%)	28/144 (16.2%)	ns
Diabetes (yes/no)	4/2 (66.7%)	23/149 (13.4%)	0.0015
Obesity (yes/no)	3/2 (60.0%)	35/57 (38.0%)	ns
HBcAb (+/–)	3/3 (50.0%)	15/47 (24.2%)	ns
Multivariate analysis	p value	Odds ratio	95% CI
LC at AIH Dx	0.0138	4.08	1.54–18.32
Abnormal ALT at final observation	0.0236	3.66	1.38–16.42

Values are expressed as mean ± SD, or *n/n* (%) in which % is the percentage of yes or normal

HCC hepatocellular carcinoma, AIH autoimmune hepatitis, Dx diagnosis, LC liver cirrhosis, PSL prednisolone, AZA azathioprine, ALT alanine aminotransferase, HBcAb hepatitis B core antibody, ns not significant



**Fig. 2** Cumulative probability of HCC development in AIH patients with or without liver cirrhosis (Kaplan–Meier method). HCC hepatocellular carcinoma, LC liver cirrhosis, Pt patient

diagnosis (83.3 vs. 16.6%,  $p = 0.0002$ ) were significant factors for HCC development.

In patients with autoimmune diseases, an impaired immune function, which is characteristic of these diseases, may be involved in carcinogenesis. Several studies reported that the risk of hematologic malignant neoplasm was high in patients with rheumatoid arthritis (RA) or SLE [30–33]. In these, important risk factors for hematologic malignant neoplasm in RA patients included the disease course and immunosuppressive agents [30, 31]. Furthermore, the risk of onset differs among immunosuppressive agents. A study indicated that cyclophosphamide was a risk factor for hematologic malignant neoplasm and lymphoma [32]. On the other hand, another study suggested that the inflammation-related enhancement of disease activity is an important risk factor for lymphoma rather than treatment in RA patients [33]. In this study regarding the development of HCC in AIH patients, the absence of ALT normalization, i.e., persistent active hepatitis, was also a risk factor for the development of HCC. In 5 of 6 patients who developed HCC, treatment with PSL or AZA was performed. However, the 5 patients did not respond to these regimens, and the transaminase levels did not normalize. Miyake et al. [34] also reported that a persistent elevation of the serum ALT level led to poor survival and HCC development in type 1 AIH patients. When the maintenance dose of PSL was higher, the incidence of HCC was significantly higher. This reflects that ALT normalization was not achieved. However, neither PSL nor AZA therapies were significant factors for the development of HCC. There was no significant difference between the two therapies (data not shown). These results suggest that treatment with PSL or immunosuppressive agents is not a risk factor for the development of HCC in AIH patients; treatment with these agents should not be avoided. It is important to normalize the liver function regardless of treatment regimens, for the prevention of HCC.

Concerning AIH treatment, PSL is effective, and monotherapy with PSL or combination therapy with PSL and AZA is recommended in the American Association for the Study of Liver Diseases (AASLD) guidelines established in 2002 and 2010, considering complications and side effects [35, 36]. Previous randomized, controlled trials of AIH treatment were reviewed in the *Journal of Hepatology* in 2010 [37]. In this study, monotherapy with PSL was performed in 78.9% ( $n = 142$ ) of patients receiving induction therapy. For maintenance therapy, AZA was administered to 27 patients (15%) only; combination therapy with PSL and AZA or monotherapy with AZA. Few patients were treated with AZA, because this agent has yet to be approved in Japan. On the other hand, UDCA alone was selected without employing PSL or AZA as

induction therapy in 27 patients (15%). In 19 (70.4%) of the 27 patients, the liver function was normalized, but not in 8 (29.6%). One of the 8 patients died of liver failure. Regarding the effects of UDCA on AIH, a study reported that monotherapy with UDCA led to remission in some patients, and that combination therapy with PSL inhibited recrudescence related to PSL-dose reduction [38]. Another study indicated that UDCA improved not only the inflammatory response but also IgG level [39]. Furthermore, UDCA therapy is effective in elderly patients [40]. However, currently, the assessment of UDCA therapy and long-term prognosis remain to be clarified. Patients to be treated should be examined.

Over the past few years, many studies have reported that diabetes mellitus (DM) is a risk factor for HCC [17–19]. In Japan, HCV infection is a major risk factor for HCC [41]. Studies involving HCV-positive patients also showed that the incidence of HCC was markedly higher in patients with DM [42, 43]. Among those with alcoholic liver cirrhosis, the incidence of HCC also differed between patients with and without DM [44]. The results of this study also suggest that DM becomes a risk factor for the onset of HCC in patients with AIH. As PSL treatment is primarily performed in AIH patients, the concurrent development of DM must be considered. On the basis of these results, PSL treatment should be switched to combination therapy or monotherapy with AZA in patients undergoing long-term therapy. Articles published in 2009 indicated that obesity and DM were risk factors for HCC, excluding HBV and HCV [23, 24]. Several studies have reported the relationship of steatosis, non-alcoholic fatty liver disease (NAFLD), and obesity with HCC [18, 20–22]. However, in this study, fatty liver was not a significant factor. The proportions of obese and HBc antibody-positive patients were higher in the HCC group, although there were no significant differences.

In this study, uni- and multivariate analyses showed that liver cirrhosis ( $p = 0.0138$ , odds ratio 4.08) and abnormal ALT at the final observation ( $p = 0.0236$ , odds ratio 3.66) were risk factors for the development of HCC in AIH patients. Montano-Loza et al. [12] reported that not only liver cirrhosis but also the appearance of symptoms of portal hypertension, such as ascites, esophageal varices, and thrombopenia, was related to HCC. However, in 6 patients with HCC investigated in this study, liver cirrhosis was observed at the development of HCC, but neither ascites nor esophageal varices were noted. The risk of HCC in liver cirrhosis patients was reviewed in the journal *Gastroenterology* in 2004 [45]. The incidence of HCC in patients with HBV/HCV infection or alcoholic liver diseases was examined with respect to the presence or absence of liver cirrhosis. The presence of liver cirrhosis increased the incidence of HCC 2- to 6-fold. The molecular

mechanism of hepatocarcinogenesis in liver cirrhosis patients has been clarified [46]. The detailed mechanism should be further investigated in order to inhibit hepatocarcinogenesis as a result of liver cirrhosis.

With respect to gender, the incidence of HCC in males is higher than in females among patients with viral or alcoholic liver diseases. In this study, all HCC patients were female. As 80–90% of AIH patients were female, there was no significant gender difference in the incidence of HCC in AIH patients. Montano-Loza [12] reported that male gender was a risk factor for HCC in AIH patients. However, Yeoman et al. [11] indicated that there was no gender difference. As represented by race-related differences in the HLA-type of AIH, gender-related risks may also differ among races. Concerning this, data should be accumulated in the future.

In summary, 3.3% of AIH patients developed HCC. Risk factors for the development of HCC evaluated by univariate analysis were (1) liver cirrhosis at the time of AIH diagnosis, (2) absence of a treatment response, (3) abnormal ALT at the final observation, and (4) diabetes. Risk factors evaluated by multivariate analysis were liver cirrhosis at the time of AIH diagnosis and abnormal ALT at the final observation. In patients with AIH, it is important to normalize the liver function by positive therapy with PSL or AZA and prevent deterioration to liver cirrhosis. In patients with liver cirrhosis, the normalization of the liver function and early detection of HCC are important.

**Conflict of interest** The authors have declared that no conflict of interest exists.

## Appendix

The members of the Autoimmune Hepatitis Study Group include the following: Drs. Kazunori Noguchi and Hiroto Kumemura, Omuta City Hospital, Omuta, Fukuoka; Drs. Masatoshi Tanaka and Maisa Hori, Kurume University Medical Center, Kurume, Fukuoka; Dr. Kunitaka Fukuizumi, Kyusyu Medical Center, Fukuoka; Dr. Nobuyoshi Tajiri, Social Insurance Tagawa Hospital, Tagawa, Fukuoka; Dr. Kazuhiko Oho, Yanagawa Hospital, Yanagawa, Fukuoka; Dr. Terufumi Sakai, St. Mary's Hospital, Kurume, Fukuoka; Dr. Ryuichi Nouno, Kumamoto Central Hospital, Kikuchi, Kumamoto; Dr. Hirofumi Fukushima, Saiseikai Futsukaichi Hospital, Futsukaichi, Fukuoka; Dr. Yoichi Yano, Saga Social Insurance Hospital, Saga; Dr. Hiroshi Yoshida, Yame General Hospital, Yame, Fukuoka; Dr. Yasuyo Morita, Nagata Hospital, Yanagawa, Fukuoka; Dr. Kunihide Ishii, Fukuoka Asakura Medical Association Hospital, Asakura, Fukuoka, Dr. Miki Shirachi, Chikugo City Hospital, Chikugo, Fukuoka, Japan.

## References

- Roberts SK, Thorneau TM, Czaja AJ. Prognosis of histological cirrhosis in type 1 autoimmune hepatitis. *Gastroenterology*. 1996;110:848–57.
- Toda G, Zeniya M, Watanabe F, Imawari M, Kiyosawa K, Nishioka M, et al. Present status of autoimmune hepatitis in Japan—correlating the characteristics with international criteria in an area with a high rate of HCV infection. Japanese National Study Group of Autoimmune Hepatitis. *J Hepatol*. 1997;26:1207–12.
- Czaja AJ. Treatment of autoimmune hepatitis. *Semin Liver Dis*. 2002;22:365–78.
- Hino T, Kumashiro R, Ide T, Koga Y, Ishii K, Tanaka E, et al. Predictive factors for remission and death in 73 patients with autoimmune hepatitis in Japan. *Int J Mol Med*. 2003;11:749–55.
- Wang KK, Czaja AJ. Hepatocellular carcinoma in corticosteroid-treated severe autoimmune chronic active hepatitis. *Hepatology*. 1988;8:1679–83.
- Yousuf M, Kiyosawa K, Sodeyama T, Yoda H, Nakano Y, Furuta S. Development of hepatocellular carcinoma in a man with autoimmune chronic active hepatitis. *J Gastroenterol Hepatol*. 1992;7:66–9.
- Ryder SD, Koskinas J, Rizzi PM, McFarlane IG, Portmann BC, Naoumov NV, et al. Hepatocellular carcinoma complicating autoimmune hepatitis: role of hepatitis C virus. *Hepatology*. 1995;22:718–22.
- Park SZ, Nagorney DM, Czaja AZ. Hepatocellular carcinoma in autoimmune hepatitis. *Dig Dis Sci*. 2000;45:1944–8.
- Nishiyama R, Kanai T, Abe J, Hara R, Watahiki Y, Sakaguchi T, et al. Hepatocellular carcinoma associated with autoimmune hepatitis. *J Hepatobiliary Pancreat Surg*. 2004;11:215–9.
- Okino H, Satoh T, Watanabe J, Masumoto A, Takeda S. Hepatocellular carcinoma arising from autoimmune hepatitis: report of a case. *Surg Today*. 2007;37:716–8.
- Yeoman AD, Al-Chalabi T, Karani JB, Quaglia A, Devlin J, Mieli-Vergani G, et al. Evaluation of risk factors in the development of hepatocellular carcinoma in autoimmune hepatitis: implications for follow-up and screening. *Hepatology*. 2008;48:863–70.
- Montano-Loza AJ, Carpenter HA, Czaja AJ. Predictive factors for hepatocellular carcinoma in type 1 autoimmune hepatitis. *Am J Gastroenterol*. 2008;103:1944–51.
- Werner M, Almer S, Prytz H, Lindgren S, Wallerstedt S, Björnsson E, et al. Hepatic and extrahepatic malignancies in autoimmune hepatitis. A long-term follow-up in 473 Swedish patients. *J Hepatol*. 2009;50:388–93.
- Teufel A, Weinmann A, Centner C, Piendl A, Lohse AW, Galle PR, et al. Hepatocellular carcinoma in patients with autoimmune hepatitis. *World J Gastroenterol*. 2009;15:578–82.
- Johnson P, McFarlane I. Meeting report: International Autoimmune Hepatitis Group. *Hepatology*. 1993;18:993–1005.
- Calle EE, Rodriguez C, Walker-Thurmond K, Thun MJ. Overweight, obesity, and mortality from cancer in a prospectively studied cohort of US adults. *N Engl J Med*. 2003;348:1625–38.
- El-Serag HB, Richardson PA, Everhart JE. The role of diabetes in hepatocellular carcinoma: a case-control study among United States Veterans. *Am J Gastroenterol*. 2001;96:2462–7.
- Regimbeau JM, Colombat M, Mognol P, Durand F, Abdalla E, Degott C, et al. Obesity and diabetes as a risk factor for hepatocellular carcinoma. *Liver Transpl*. 2004;10:S69–73.
- Davila JA, Morgan RO, Shaib Y, McGlynn KA, El-Serag HB. Diabetes increases the risk of hepatocellular carcinoma in the United States: a population based case control study. *Gut*. 2005;54:533–9.
- Bugianesi E, Leone N, Vanni E, Marchesini G, Brunello F, Carucci P, et al. Expanding the natural history of nonalcoholic steatohepatitis: from cryptogenic cirrhosis to hepatocellular carcinoma. *Gastroenterology*. 2002;123:134–40.
- Marrero JA, Fontana RJ, Su GL, Conjeevaram HS, Emick DM, Lok AS. NAFLD may be a common underlying liver disease in patients with hepatocellular carcinoma in the United States. *Hepatology*. 2002;36:1349–54.
- Mori S, Yamasaki T, Sakaida I, Takami T, Sakaguchi E, Kimura T, et al. Hepatocellular carcinoma with nonalcoholic steatohepatitis. *J Gastroenterol*. 2004;39:391–6.
- Polesel J, Zucchetto A, Montella M, Dal Maso L, Crispo A, La Vecchia C, et al. The impact of obesity and diabetes mellitus on the risk of hepatocellular carcinoma. *Ann Oncol*. 2009;20:353–7.
- Gao C, Yao SK. Diabetes mellitus: a “true” independent risk factor for hepatocellular carcinoma? *Hepatobiliary Pancreat Dis Int*. 2009;8:465–73.
- Alvarez F, Berg PA, Bianchi FB, Bianchi L, Burroughs AK, Cancado EL, et al. International Autoimmune Hepatitis Group Report: review of criteria for diagnosis of autoimmune hepatitis. *J Hepatol*. 1999;31:929–38.
- Kuzuya T, Nakagawa S, Satoh J, Kanazawa Y, Iwamoto Y, Kobayashi M, et al. Committee of the Japan Diabetes Society on the diagnostic criteria of diabetes mellitus. Report of the committee on the classification and diagnostic criteria of diabetes mellitus. *Diabetes Res Clin Pract*. 2002;55:65–85.
- Desmet VJ, Gerber M, Hoofnagle JH, Manns M, Scheuer PJ. Classification of chronic hepatitis: diagnosis, grading and staging. *Hepatology*. 1994;19:1513–20.
- Group formed to establish the guidelines for evidence-based clinical practice for the treatment of liver cancer. Clinical practice guidelines for hepatocellular carcinoma. Tokyo: Kanehara & Co.; 2005.
- Kudo M, Okanoue T. Management of hepatocellular carcinoma in Japan: consensus-based clinical practice manual proposed by the Japan Society of Hepatology. *Oncology*. 2007;72:S2–15.
- Jones M, Symmons D, Finn J, Wolfe F. Does exposure to immunosuppressive therapy increase the 10 years malignancy and mortality risks in rheumatoid arthritis? A matched cohort study. *Br J Rheumatol*. 1996;35:738–45.
- Asten P, Barrett J, Symmons D. Risk of developing certain malignancies is related to duration of immunosuppressive drug exposure in patients with rheumatic diseases. *J Rheumatol*. 1999;26:1705–14.
- Bernatsky S, Clarke AE, Suissa S. Hematologic malignant neoplasms after drug exposure in rheumatoid arthritis. *Arch Intern Med*. 2008;168:378–81.
- Baecklund E, Iliadou A, Askling J, Ekbom A, Backlin C, Granath F, et al. Association of chronic inflammation, not its treatment, with increased lymphoma risk in rheumatoid arthritis. *Arthritis Rheum*. 2006;54:692–701.
- Miyake Y, Iwasaki Y, Terada R, Okamoto R, Ikeda H, Makino Y, et al. Persistent elevation of serum alanine aminotransferase levels leads to poor survival and hepatocellular carcinoma development in type 1 autoimmune hepatitis. *Aliment Pharmacol Ther*. 2006;24:1197–205.
- Manns MP, Czaja AJ, Gorham JD, Krawitt EL, Mieli-Vergani G, Vergani D, et al. American Association for the Study of Liver Diseases. Diagnosis and management of autoimmune hepatitis. *Hepatology*. 2010;51:2193–213.
- Czaja A, Freese D. Diagnosis and treatment of autoimmune hepatitis. *Hepatology*. 2002;36:479–97.
- Lamers MM, van Oijen MG, Pronk M, Drenth JP. Treatment options for autoimmune hepatitis: a systematic review of randomized controlled trials. *J Hepatol*. 2010;53:191–8.

38. Miyake Y, Iwasaki Y, Kobashi H, Yasunaka T, Ikeda F, Takaki A, et al. Efficacy of ursodeoxycholic acid for Japanese patients with autoimmune hepatitis. *Hepatol Int*. 2009;3:556–62.
39. Nakamura K, Yoneda M, Yokohama S, Tomori K, Sato Y, Aso K, et al. Efficacy of ursodeoxycholic acid in Japanese patients with type 1 autoimmune hepatitis. *J Gastroenterol Hepatol*. 1998;13:490–5.
40. Miyake T, Miyaoka H, Abe M, Furukawa S, Shigematsu S, Furukawa E, et al. Clinical characteristics of autoimmune hepatitis in older aged patients. *Hepatol Res*. 2006;36:139–42.
41. Umemura T, Ichijo T, Yoshizawa K, Tanaka E, Kiyosawa K. Epidemiology of hepatocellular carcinoma in Japan. *J Gastroenterol*. 2009;44:102–7.
42. Tazawa J, Maeda M, Nakagawa M, Ohbayashi H, Kusano F, Yamane M, et al. Diabetes mellitus may be associated with hepatocarcinogenesis in patients with chronic hepatitis C. *Dig Dis Sci*. 2002;47:710–5.
43. Veldt BJ, Chen W, Heathcote EJ, Wedemeyer H, Reichen J, Hofmann WP, et al. Increased risk of hepatocellular carcinoma among patients with hepatitis C cirrhosis and diabetes mellitus. *Hepatology*. 2008;47:1856–62.
44. Torisu Y, Ikeda K, Kobayashi M, Hosaka T, Sezaki H, Akuta N, et al. Diabetes mellitus increases the risk of hepatocarcinogenesis in patients with alcoholic cirrhosis: a preliminary report. *Hepatol Res*. 2007;37:517–23.
45. Fattovich G, Stroffolini T, Zagni I, Donato F. Hepatocellular carcinoma in cirrhosis: incidence and risk factors. *Gastroenterology*. 2004;127:S35–50.
46. El-Serag HB, Rudolph KL. Hepatocellular carcinoma: epidemiology and molecular carcinogenesis. *Gastroenterology*. 2007;132:2557–76.

## Serum vascular endothelial growth factor as a predictor of response and survival in patients with advanced hepatocellular carcinoma undergoing hepatic arterial infusion chemotherapy

Takashi Niizeki · Shuji Sumie · Takuji Torimura · Junichi Kurogi · Ryoko Kuromatsu · Hideki Iwamoto · Hajime Aino · Masahito Nakano · Atsushi Kawaguchi · Tatsuyuki Kakuma · Michio Sata

Received: 22 June 2011 / Accepted: 26 December 2011 / Published online: 1 March 2012  
 © Springer 2012

### Abstract

**Background** Hepatic arterial infusion chemotherapy (HAIC) has been recognized as a useful therapeutic modality for patients with advanced hepatocellular carcinoma (HCC). The aim of this study was to investigate the association between serum vascular endothelial growth factor (VEGF) levels and the therapeutic effect of HAIC and the survival of patients undergoing HAIC.

**Methods** Seventy-one patients with advanced HCC underwent HAIC through a subcutaneously implanted infusion port. One chemotherapy course consisted of low-dose cisplatin (10 mg/body on days 1–5) and 5-fluorouracil (250 mg/body on days 1–5), and 1 treatment cycle consisted of 2–3 courses of chemotherapy. Serum VEGF levels were measured with the Bio-Plex Suspension Array System (Bio-Rad Laboratories).

**Results** The median survival time (MST) of all patients was 10.2 months, and the 1-, 2-, 3-, and 5-year survival rates were 46.5, 21.9, 12.8, and 3.7%, respectively. Of the 71 patients, 3 achieved a complete response (CR) and 22 achieved a partial response (PR) [response rate (CR + PR/71) = 35%]. The serum VEGF level ( $\geq 100$  pg/mL,  $P = 0.026$ ) was an independent predictor of therapeutic effect, and was positively correlated with the platelet count ( $r = 0.569$ ,  $P < 0.001$ ) and tumor size ( $r = 0.543$ ,

$P < 0.001$ ). Child–Pugh class ( $P = 0.046$ ), serum VEGF level ( $P = 0.004$ ), and therapeutic effect ( $P = 0.005$ ) were identified by multivariate analysis as independent predictors of survival.

**Conclusions** These results demonstrate that the serum VEGF level in patients with advanced HCC undergoing HAIC is an important predictive factor for therapeutic effect and survival.

**Keywords** Vascular endothelial growth factor · Hepatocellular carcinoma · Hepatic arterial infusion chemotherapy · Platelet count · Macroscopic vascular invasion

### Introduction

Hepatocellular carcinoma (HCC) is one of the most common malignancies in the world. Recently, advanced imaging procedures have led to the increased detection of early-stage HCC and improved survival, because curative therapies, such as hepatic resection, liver transplantation, and radiofrequency ablation, are possible in patients with early-stage disease [1]. However, long-term survival is still unsatisfactory because of high recurrence rates, even after curative therapy [2]. The development of advanced HCC with macroscopic vascular invasion (MVI) especially hinders the use of additional curative therapies, and therefore contributes to poor survival. MVI, including the presence of a tumor thrombus in the major portal vein, is known to be the most important negative risk factor impacting survival after resection or liver transplantation for patients with HCC [3, 4]. The median survival time of HCC patients with MVI has been reported to be 2–3 months [5, 6].

T. Niizeki (✉) · S. Sumie · T. Torimura · J. Kurogi · R. Kuromatsu · H. Iwamoto · H. Aino · M. Nakano · M. Sata  
 Division of Gastroenterology, Department of Medicine,  
 Kurume University School of Medicine, 67 Asahi-machi,  
 Kurume, Fukuoka 830-0011, Japan  
 e-mail: niizeki\_takashi@kurume-u.ac.jp

A. Kawaguchi · T. Kakuma  
 Biostatistics Center, Kurume University School of Medicine,  
 67 Asahi-machi, Kurume, Fukuoka 830-0011, Japan

Hepatic arterial infusion chemotherapy (HAIC) via an implanted port system has been reported to be a useful therapeutic modality for patients with advanced HCC, especially for those with a major portal vein tumor thrombus [7–12]. Various chemotherapeutic regimens are used for HAIC, with the combination of cisplatin (CDDP) and 5-fluorouracil (5-FU) being one of the most common regimens [8–12]. Ando et al. first reported that repeated HAIC using low-dose CDDP and 5-FU was useful for patients with advanced HCC and tumor thrombus in the portal vein [7, 8]. Several other studies have also demonstrated the usefulness of HAIC using low-dose CDDP and 5-FU [13–15]. However, previous studies did not clearly determine the predictors of therapeutic effect and prognosis in patients undergoing HAIC.

Angiogenesis plays an important role in tumor proliferation, invasion, and metastasis. Tumor aggressiveness is mediated by angiogenic factors such as vascular endothelial growth factor (VEGF), which is a major factor involved in aggressive tumor behavior [16]. An elevated serum VEGF level has been demonstrated to be correlated with advanced stages of HCC, including vascular invasion and metastasis [17–20]. Previous studies have shown that high serum VEGF levels predicted poor overall and disease-free survival in HCC patients treated with hepatic resection [18], radiofrequency ablation [19], or transcatheter arterial chemoembolization [20]. However, the significance of serum VEGF as a predictor of treatment effect and survival is unclear in patients with advanced HCC undergoing HAIC.

In this study, we evaluated the association of serum VEGF with therapeutic effect in patients undergoing HAIC and identified the predictors of elevated serum VEGF levels. We also investigated the associations of several risk factors, including serum VEGF levels, with survival in patients with advanced HCC with MVI.

## Subjects and methods

### Subjects

Hepatocellular carcinoma and vascular invasion were diagnosed using a combination of contrast-enhanced computed tomography (CT), magnetic resonance imaging (MRI), ultrasonography (US), and digital subtraction angiography (DSA), in addition to the determination of alpha-fetoprotein (AFP) and des-gamma-carboxy prothrombin (DCP) serum levels, within 1 month before treatment. Hepatic functional reserve was evaluated before treatment using the Child–Pugh scoring system. From June 1996 to May 2003, 124 consecutive HCC patients with a tumor thrombus in the portal vein were referred to Kurume

University School of Medicine. Of these 124 patients, 92 patients underwent HAIC; 14 patients received other treatments, including hepatic resection, transcatheter arterial infusion chemoembolization (TACE), and systemic chemotherapy; and 18 patients received best supportive care. Eligibility criteria for this study were as follows: (1) Eastern Cooperative Oncology Group (ECOG) performance status of 0–2, (2) Child–Pugh class A or B, (3) serum bilirubin <3.0 mg/dL, (4) serum creatinine <1.5 mg/dL, (5) no extrahepatic metastasis, and (6) patients not previously treated with low-dose CDDP and 5-FU. Among the 92 patients undergoing HAIC, 71 patients met these criteria and were enrolled in the study. The study protocol (No. 2350) was approved by the Ethics Committee of Kurume University, and informed consent for participation in the study was obtained from each subject and conformed to the guidelines of the 1975 Declaration of Helsinki.

### Response assessment

To determine therapeutic effect, baseline tumor measurements were determined within 1 month before treatment by combining the largest diameters of selected target lesions in each patient as measured by CT or MRI. Four to 6 weeks after the initial treatment cycle and every 2–3 months thereafter, CT or MRI was performed. Therapeutic effect was determined according to the best overall response, which was defined by the Response Evaluation Criteria in Solid Tumors (RECIST) as follows: complete response (CR), disappearance of all measurable lesions for more than 4 weeks; partial response (PR), greater than 30% decrease in the sum of the largest target-lesion diameters and no development of a new lesion for more than 4 weeks; progressive disease (PD), greater than 25% increase in the sum of the largest target-lesion diameters or the appearance of a new lesion; and stable disease (SD), neither PR nor PD seen for more than 8 weeks [21].

### Implantation of arterial catheter

An indwelling catheter (5-Fr Anthon P-U Catheter; Toray Medical, Tokyo, Japan) was inserted through the femoral or brachial artery, with the distal end of the catheter extended into the hepatic artery or gastroduodenal artery, and the proximal end connected to the port system (P-U Celsite Port; Toray Medical), which was implanted subcutaneously. The right gastric, gastroduodenal, and posterior superior pancreaticoduodenal arteries were occluded with TRUFILL coils (Codman & Shurtleff, Raynham, MA, USA) to prevent gastroduodenal ulcers being caused by the anticancer agents. Of the 71 patients, 17 had replacement of the hepatic artery; 8 had replacement of the right hepatic



artery (RHA) from the superior mesenteric artery (SMA), 5 had replacement of the left hepatic artery (LHA) from the left gastric artery (LGA), 2 had replacement of the common hepatic artery from the SMA, and 2 had replacement of the LHA from the LGA and the RHA from the SMA. In 10 patients, the indwelling catheter was extended into the RHA or LHA alone; in 4 patients, two indwelling catheters were extended into the RHA and LHA, respectively, and in 3 patients, the RHA or LHA was embolized with a TRU-FILL coil and the catheter inserted as described above.

#### Therapeutic HAIC regimen

Chemotherapeutic agents were administered via a portable mechanical infusion pump. One course of chemotherapy consisted of the daily administration of CDDP (Nippon Kayaku, Tokyo, Japan) (10 mg/body, for 30 min, on days 1–5) followed by 5-FU (Kyowa Hakko Kogyo, Tokyo, Japan) (250 mg/body, for 3 h, on days 1–5). Days 6 and 7 were rest days. In principle, one cycle of treatment consisted of 2–3 weekly courses of HAIC (completion of protocol) and this cycle was performed as an inpatient procedure. This first cycle was followed by continuous HAIC chemotherapy with CDDP (20 mg/body) and 5-FU (250 mg/body), repeated every 2 weeks at the outpatient clinic. Treatments were discontinued if there was an occurrence of grade 3 or higher adverse effects according to the ECOG classification [22], with the exception of total bilirubin  $>3.0$  mg/dL, platelet count  $<25 \times 10^9/L$ , and leukocyte count  $<1500/mm^3$ . The patients received 1.0–5.0 (median 3.0) chemotherapy courses. More than 2 consecutive HAIC courses were delivered in 66 patients (completion of protocol). In 5 patients (including 4 patients with Child–Pugh class B), HAIC was stopped after less than 2 consecutive courses (protocol not completed).

#### Serum VEGF measurements

Blood samples for serum VEGF measurements were collected before HCC therapy was initiated. Fasting morning blood samples were obtained from all subjects and stored at  $-20^\circ\text{C}$  until analysis. Serum VEGF levels were measured using the Bio-Plex Suspension Array System (Bio-Rad Laboratories, Hercules, CA, USA).

#### Statistical analysis

Medians (ranges) were determined for the measured variables. Comparisons between 2 groups of patients, those achieving CR or PR and those with SD or PD, were performed using the Mann–Whitney *U*-test for continuous variables, and the  $\chi^2$  test for discrete variables. Only variables that demonstrated a *P* value of  $<0.2$  in the

univariate analysis were entered into a stepwise multiple logistic regression model to identify factors associated with therapeutic effect and serum VEGF level. Pearson correlation coefficients were calculated to examine the association of serum VEGF level with platelet count and tumor size. Kaplan–Meier survival curves were compared using the log-rank test. Univariate and multivariate Cox proportional hazards models were used to identify any independent variables that were related to survival. *P* values of  $<0.05$  were considered to be statistically significant. Statistical analysis was performed using SPSS software (SPSS, Chicago, IL, USA).

## Results

### Patient characteristics

Table 1 summarizes the clinical profiles of the 71 HCC patients enrolled in this study. There were 56 male and 15 female patients, with a median age of 65 years (range 44–85). With respect to viral markers, 42 patients were positive for hepatitis C virus (HCV) antibody, and 19 patients were positive for hepatitis B surface antigen. There were 43 and 28 patients classified as Child–Pugh class A and B, respectively. The median maximum tumor size was 90 mm (range 24–217). There were 22 patients with a portal vein tumor thrombus of the main trunk, and 15

**Table 1** Patients' baseline clinical characteristics

Patient characteristics	
Gender (male/female)	56/15
Age (years)	65 (44–85)
HCV infection (+/–)	42/29
HBV infection (+/–)	19/52
Child–Pugh class (A/B)	43/28
Platelet count ( $\times 10^9/L$ )	117 (33–275)
VEGF (pg/mL)	81.6 (3.4–884.2)
AFP (ng/mL) ( $<1000/\geq 1000$ )	35/36
DCP (AU/mL) ( $<1000/\geq 1000$ )	20/46
Previous treatment (yes/no)	26/45
Tumor characteristics	
Maximum tumor size (mm)	90 (24–217)
Macroscopic findings (nodular/infiltrative)	33/38
Tumor location (unilobular/bilobular)	47/24
Grade of portal vein invasion (trunk/first or second branch)	22/49
Hepatic vein invasion (present/absent)	15/56

Items in parentheses are ranges

HCV hepatitis C virus, HBV hepatitis B virus, VEGF vascular endothelial growth factor, AFP alpha-fetoprotein, DCP des-gamma-carboxy prothrombin

**Table 2** Comparison of patient characteristics based on therapeutic effect

	CR + PR group (n = 25)	SD + PD group (n = 46)	P value
Gender (male/female)	17/8	39/7	0.108
Age (years)	68 (50–81)	65 (44–85)	0.426
Child–Pugh class (A/B)	18/7	25/21	0.146
Platelet count ( $\times 10^9/L$ )	86 (33–253)	119 (33–275)	0.238
VEGF (pg/mL)	31.6 (3.4–884.2)	97.0 (4.1–657.4)	0.014
AFP (ng/mL) (<1000/ $\geq$ 1000)	10/15	25/21	0.248
DCP (AU/mL) (<1000/ $\geq$ 1000)	9/16	11/35	0.280
Previous treatment (yes/no)	9/16	17/29	0.936
Maximum tumor size (mm)	75 (30–149)	91 (24–217)	0.147
Macroscopic finding (nodular/infiltrative)	12/13	21/25	0.850
Tumor location (unilobular/bilobular)	11/14	13/33	0.181
Grade of portal vein invasion (main trunk/first or second branch)	7/18	15/31	0.688
Hepatic vein invasion (present /absent)	3/22	12/34	0.228

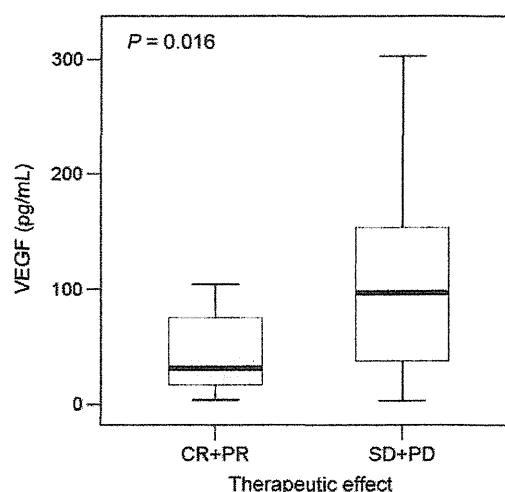
CR complete response, PR partial response, SD stable disease, PD progressive disease, VEGF vascular endothelial growth factor, AFP alpha-fetoprotein, DCP des-gamma-carboxy prothrombin

patients with a hepatic vein tumor thrombus. Twenty-six patients had received previous treatments for HCC, consisting of the following: hepatic resection (n = 4), TACE (n = 15), percutaneous ethanol injection (n = 13), and radiofrequency ablation (n = 6).

Therapeutic effect and predictors of response

Of the 71 patients, 3 (4%) patients achieved a CR, 22 (31%) patients achieved a PR, 25 (35%) patients had SD, and 21 (30%) patients had PD; the response rate (CR + PR/71) was 35%. The characteristics of the 2 patient response groups (the CR + PR [response to therapy] and SD + PD [without response to therapy] groups) are shown in Table 2. The median VEGF level in patients with response to therapy was significantly lower than the median VEGF level in patients without response to therapy (P = 0.014) (Fig. 1). There were no significant differences between other patient and tumor characteristics. In addition, multiple logistic regression analysis identified the serum VEGF level [ $\geq 100$  pg/mL, odds ratio (OR) 4.77, 95% confidence interval (CI) 1.21–18.90; P = 0.026] as an independent predictor of therapeutic effect.

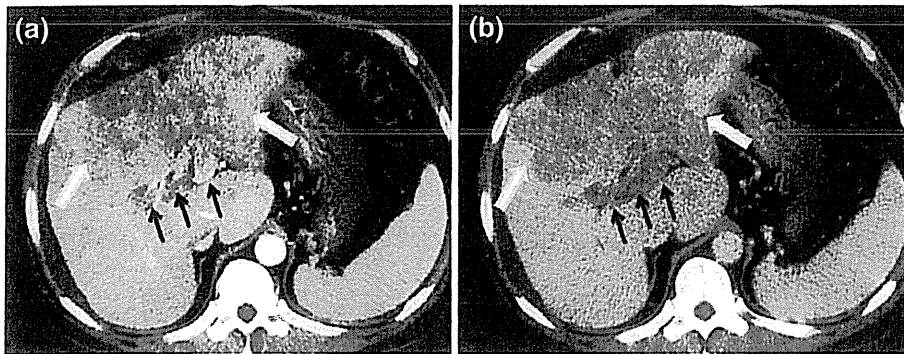
Figures 2 and 3 show CT scans of patients with high and low serum VEGF levels, respectively. Figure 2 shows contrast-enhanced CT scans of a patient who had a high serum VEGF level (566.0 pg/mL) and was classified as having PD. The scans show hypovascular and heterogeneous enhancement patterns of the main tumor and the tumor thrombus in the portal vein. Figure 3 shows contrast-enhanced CT scans of a patient who had a low serum VEGF level (9.5 pg/mL) and was classified as having PR. The scans show hypervascular and homogeneous enhancement patterns of the main tumor and multiple intrahepatic metastases.



**Fig. 1** Comparison of serum vascular endothelial growth factor (VEGF) levels according to therapeutic effect. The box indicates the 25th and 75th percentiles of the data and the middle line indicates the median. The median VEGF level was significantly lower in the complete response plus partial response (CR + PR) group (31.6 pg/mL) compared to the stable disease plus progressive disease (SD + PD) group (97.0 pg/mL) (P = 0.014)

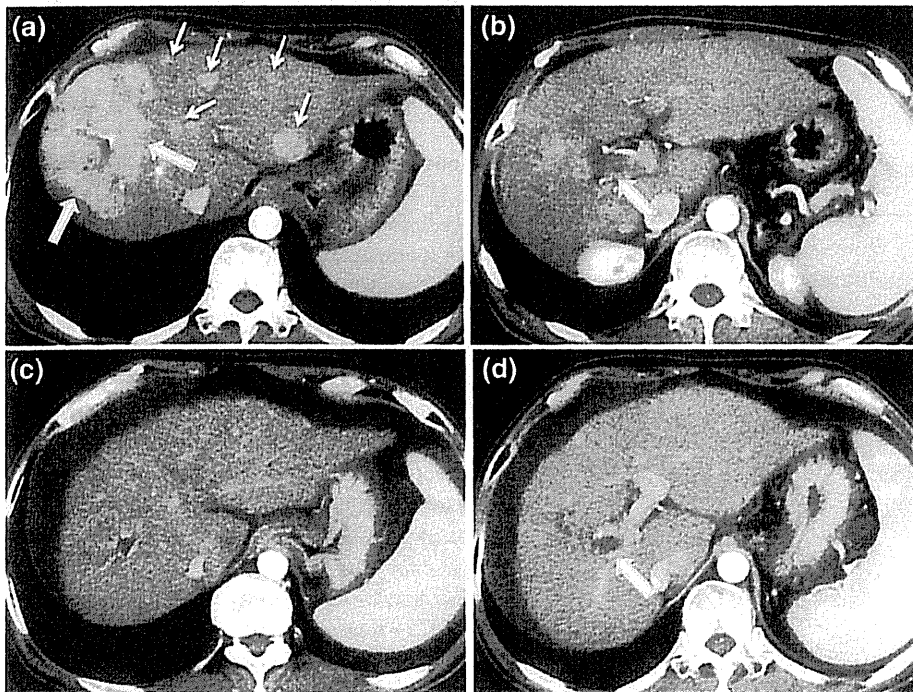
Serum VEGF level, platelet count, and tumor size

Multiple logistic regression analysis was performed to identify which variables were independently associated with the serum VEGF level (Table 3). Platelet count ( $\geq 120 \times 10^9/L$ , OR 9.85, 95% CI 2.38–40.77; P = 0.002), tumor size ( $\geq 100$  mm, OR 4.60, 95% CI 1.13–19.01; P = 0.034), and tumor location (bilobular, OR 8.01, 95% CI 1.28–50.30; P = 0.026) were independent predictors of the serum VEGF level. The serum VEGF level was positively correlated with platelet count [ $r = 0.569$ , P < 0.001 (Fig. 4a)] and tumor



**Fig. 2** Contrast-enhanced computed tomography scans obtained before therapy for a 48-year-old man with high serum vascular endothelial growth factor level and advanced hepatocellular carcinoma who was classified as having progressive disease after hepatic arterial infusion

chemotherapy. **a** Early-phase and **b** delayed-phase scans show the main tumor (*thick arrows*), 16.5 cm in diameter, in the left lobe and a tumor thrombus in the portal vein (*thin arrows*) in the bilateral first branches and main trunk



**Fig. 3** Contrast-enhanced computed tomography (CT) scans obtained before therapy (**a**, **b**) for a 57-year-old man with low serum vascular endothelial growth factor level and advanced hepatocellular carcinoma who was classified as having a partial response after hepatic arterial infusion chemotherapy. **a** Early-phase scans show the main tumor (*thick arrows*), 9.5 cm in diameter, in the right anterior segment, and multiple nodules (*thin arrows*) in both lobes and

**b** tumor thrombus in the portal vein (*thick arrows*) in the right first branch and main trunk. **c** Contrast-enhanced early-phase CT scan obtained after therapy shows that the main tumor and other nodules are markedly reduced in size. **d** Contrast-enhanced early-phase CT scan obtained after therapy shows that the tumor thrombus in the portal vein (*thick arrows*) is decreased in its extent and diameter

size [ $r = 0.543$ ,  $P < 0.001$  (Fig. 4b)] by Pearson correlation calculations.

#### Survival and predictors of outcome

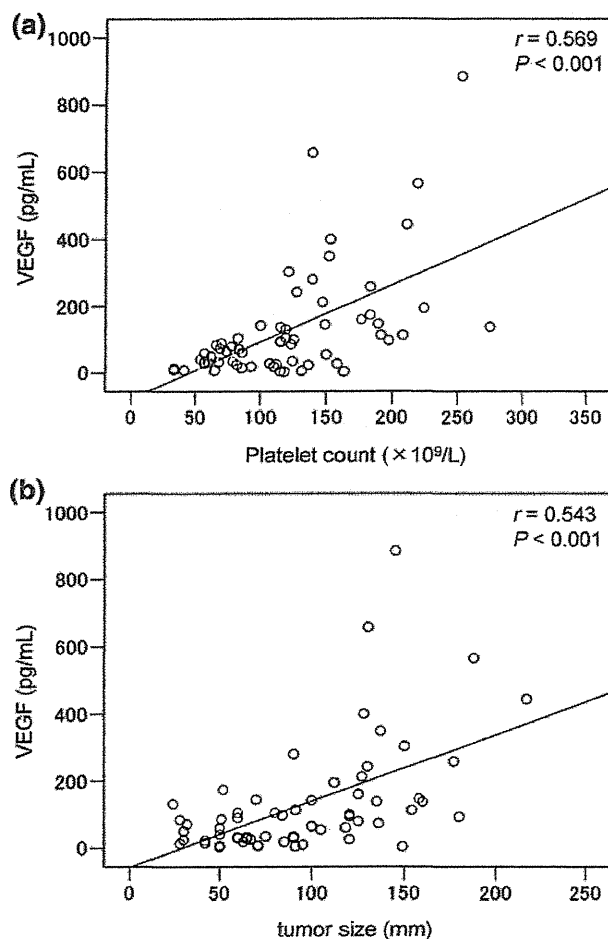
The cumulative survival curve of the 71 patients is shown in Fig. 5. The median survival time (MST) of these patients

was 10.2 months. The 1-, 2-, 3-, and 5-year survival rates were 46.5, 21.9, 12.8, and 3.7%, respectively. Cox proportional hazards regression analysis was performed to identify independent predictors of survival (Table 4). The results of univariate analysis showed that Child–Pugh class B ( $P = 0.002$ ), platelet count ( $\geq 120 \times 10^9/L$ ,  $P = 0.006$ ), serum VEGF level ( $\geq 100$  pg/mL,  $P < 0.001$ ), tumor size

**Table 3** Independent predictors of VEGF by multiple logistic regression analysis

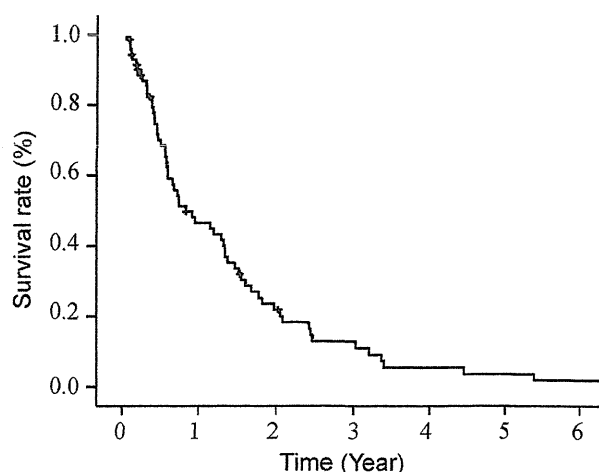
	OR (95% CI)	P value
Platelet count ( $\geq 120 \times 10^9/L$ )	9.85 (2.38–40.77)	0.002
Maximum tumor size ( $\geq 100$ mm)	4.60 (1.13–19.01)	0.034
Tumor location (bilobular)	8.01 (1.28–50.30)	0.026

VEGF vascular endothelial growth factor, 95% CI 95% confidence interval, OR odds ratio



**Fig. 4** Correlation between serum vascular endothelial growth factor (VEGF) level and platelet count ( $r = 0.569$ ,  $P < 0.001$ ) (a). Correlation between serum VEGF level and tumor size ( $r = 0.543$ ,  $P < 0.001$ ) (b)

( $>100$  mm,  $P = 0.002$ ), tumor location (bilobular,  $P = 0.023$ ), portal vein invasion (main trunk,  $P < 0.001$ ), and therapeutic effect (SD + PD,  $P < 0.001$ ) were significant risk factors adversely affecting survival. By multivariate analysis, Child–Pugh class B (hazard ratio [HR] 1.81, 95% CI 1.01–3.25;  $P = 0.046$ ), serum VEGF level ( $\geq 100$  pg/mL, HR 2.42, 95% CI 1.33–4.38;  $P = 0.004$ ),



**Fig. 5** Cumulative survival of 71 hepatocellular carcinoma patients with macroscopic vascular invasion treated with hepatic arterial infusion chemotherapy. The median survival time (MST) of these patients was 10.2 months. The 1-, 2-, 3-, and 5-year survival rates were 46.5, 21.9, 12.8, and 3.7%, respectively

and therapeutic effect (SD + PD, HR 2.46, 95% CI 1.31–4.62;  $P = 0.005$ ) were identified as independent predictors of survival. Cumulative survival curves plotted for therapeutic effects, serum VEGF level, and Child–Pugh class are shown in Fig. 6.

#### Adverse reactions and complications

Twenty-two of the 71 patients (31%) developed more than 1 adverse reaction or complication. The most common adverse reactions were mild liver dysfunction and gastrointestinal symptoms, which were primarily controlled by medical treatment and/or suspension of HAIC. Two patients with Child–Pugh class B developed liver dysfunction thought to be caused by either the progression of HCC or treatment-related toxicity, and HAIC was stopped. Brief and reversible grade 3 or 4 leukocytopenia developed in 9 (13%) patients. Mild thrombocytopenia or anemia occurred infrequently.

The complications that occurred were associated mainly with the implantation of the infusion catheter. Infection of the port system occurred in 5 patients. These infections were controlled by antibiotics and conservative care, but there were 2 patients with Child–Pugh class B who discontinued HAIC. Occlusion of the hepatic artery, which interfered with HAIC and caused its termination, occurred in 1 patient.

#### Discussion

Advanced HCC with MVI, especially for patients with a major portal vein tumor thrombus, still has a poor