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ORIGINAL ARTICLE—LIVER, PANCREAS, AND BILIARY TRACT

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

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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.

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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 ± 14.3 years. The mean observation period was 80.2 ± 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 γ-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.

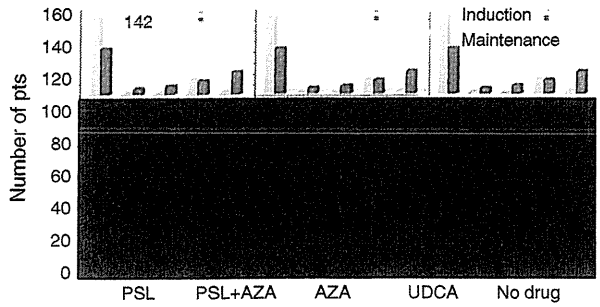

Fig. 1 Induction and maintenance treatment regimens for AIH. *PSL* prednisolone, *AZA* azathioprine, *UDCA* ursodeoxycholic acid

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 γ-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 (α-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 ± 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 ± 89.9 months (range 17.7–252.0 months). Their average age was 65.3 ± 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					Tx	Response to Tx	Complications during observation				Observation period until Dx of HCC (months)[a]
	Age/sex	ALT (IU/ml)	IgG (mg/dl)	Liver stage	Hx stage/grade			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

[a] Mean 128.2 months

Table 2 Outcome of patients with HCC

Patient no.	HCC			Outcome	Observation period after Dx of HCC (months)[a]
	Number of tumors	Size (mm)	Tx		
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

HCC hepatocellular carcinoma, *Tx* treatment, *Dx* diagnosis, *TACE* transarterial chemoembolization, *RFA* radiofrequency ablation, *PEI* percutaneous ethanol injection, *ALT* alanine aminotransferase, *PSL* prednisolone, *AZA* azathioprine

[a] 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

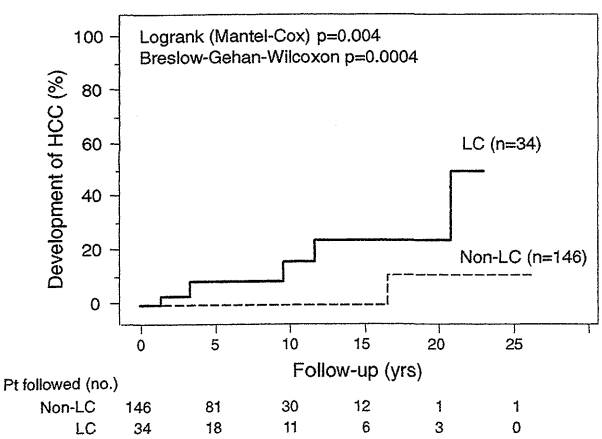

Fig. 2 Cumulative probability of HCC development in AIH patients with or without liver cirrhosis (Kaplan–Meier method). *HCC* hepatocellular carcinoma, *LC* liver cirrhosis, *Pt* patient

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.

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ORIGINAL ARTICLE—LIVER, PANCREAS, AND BILIARY TRACT

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

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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].

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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 Anthron 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°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 χ^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/≥1000)	35/36
DCP (AU/mL) (<1000/≥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/≥1000)	10/15	25/21	0.248
DCP (AU/mL) (<1000/≥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 [≥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.

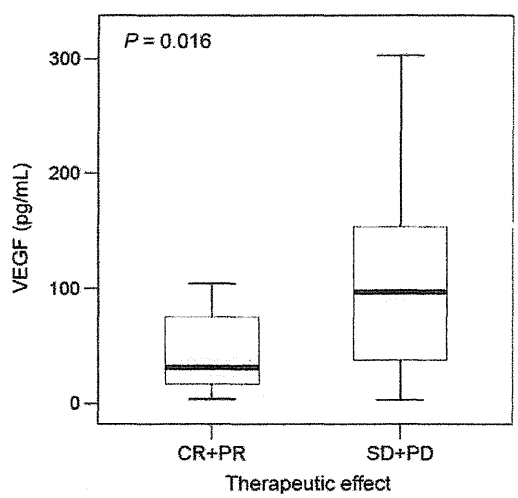

Fig. 1 Comparison of serum vascular endothelial growth factor (VEGF) levels according to therapeutic effect. The *box* indicates the 25th and 75th percentiles of the data and the *middle line* indicates the median. The median VEGF level was significantly lower in the complete response plus partial response (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 (≥120 × 10^9/L, OR 9.85, 95% CI 2.38–40.77; $P = 0.002$), tumor size (≥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

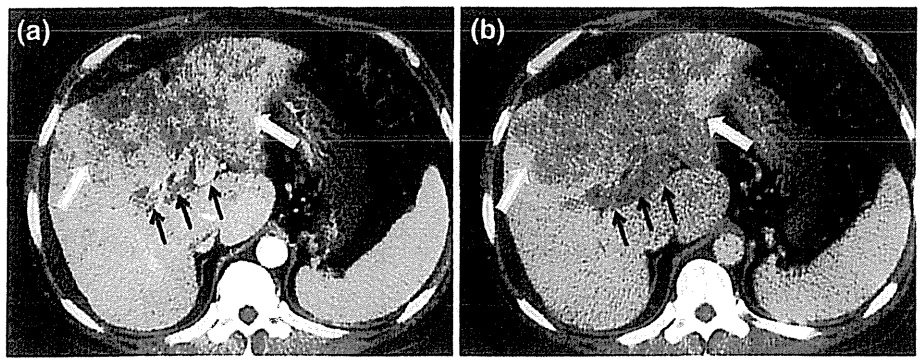

Fig. 2 Contrast-enhanced computed tomography scans obtained before therapy for a 48-year-old man with high serum vascular endothelial growth factor level and advanced hepatocellular carcinoma who was classified as having progressive disease after hepatic arterial infusion chemotherapy. **a** Early-phase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

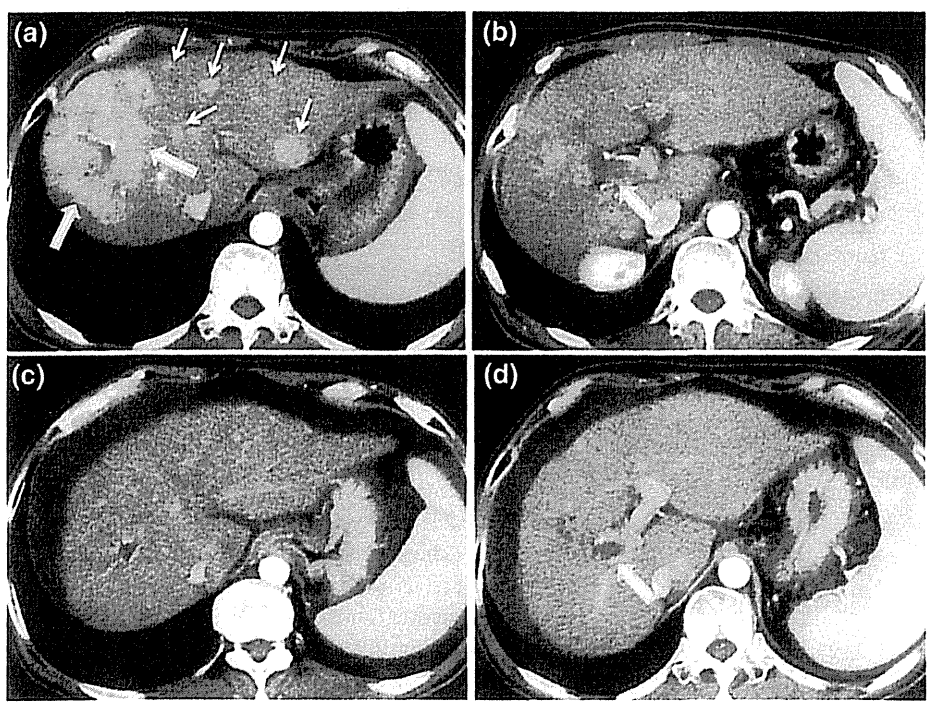

Fig. 3 Contrast-enhanced computed tomography (CT) scans obtained before therapy (**a**, **b**) for a 57-year-old man with low serum vascular endothelial growth factor level and advanced hepatocellular carcinoma who was classified as having a partial response after hepatic arterial infusion chemotherapy. **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 (≥ 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 (≥120 × 10^9/L)	9.85 (2.38–40.77)	0.002
Maximum tumor size (≥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

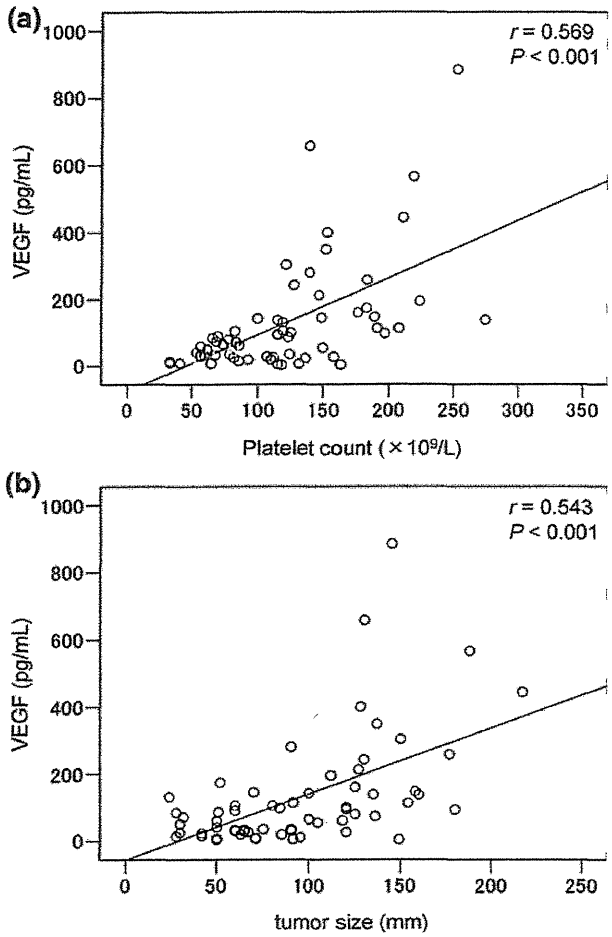

Fig. 4 Correlation between serum vascular endothelial growth factor (VEGF) level and platelet count ($r = 0.569$, $P < 0.001$) (**a**). Correlation between serum VEGF level and tumor size ($r = 0.543$, $P < 0.001$) (**b**)

(>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 (≥100 pg/mL, HR 2.42, 95% CI 1.33–4.38; $P = 0.004$),

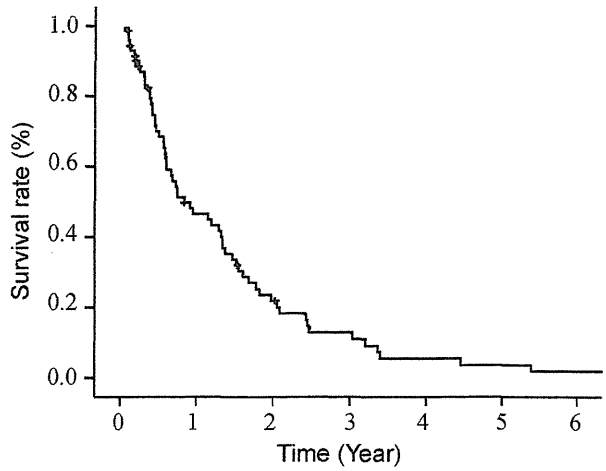

Fig. 5 Cumulative survival of 71 hepatocellular carcinoma patients with macroscopic vascular invasion treated with hepatic arterial infusion chemotherapy. The median survival time (MST) of these patients was 10.2 months. The 1-, 2-, 3-, and 5-year survival rates were 46.5, 21.9, 12.8, and 3.7%, respectively

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

Table 4 Univariate and multivariate analyses of survival in hepatocellular carcinoma patients

	Univariate		Multivariate	
	HR (95% CI)	*P* value	HR (95% CI)	*P* value
Gender (male)	1.42 (0.77–2.62)	0.272		
Age (>65 years)	0.83 (0.50–0.84)	0.497		
Child–Pugh class (B)	2.34 (1.37–4.01)	0.002	1.81 (1.01–3.25)	0.046
Platelet count ($\times 10^9$/L) (≥120)	2.08 (1.24–3.49)	0.006		
VEGF (≥100 pg/mL)	2.94 (1.63–5.30)	<0.001	2.42 (1.33–4.38)	0.004
AFP (ng/mL) (≥1000)	1.01 (0.60–1.68)	0.980		
DCP (AU/mL) (≥1000)	0.86 (0.49–1.50)	0.590		
Previous treatment (yes)	0.85 (0.49–1.48)	0.569		
Maximum tumor size (mm) (≥100)	2.25 (1.35–3.76)	0.002		
Macroscopic finding (infiltrative)	1.42 (0.85–2.38)	0.178		
Tumor location (bilobular)	1.89 (1.09–3.27)	0.023		
Grade of portal vein invasion (trunk)	2.87 (1.61–5.12)	<0.001		
Grade of hepatic vein invasion (present)	1.31 (0.72–2.39)	0.385		
Therapeutic effect (SD + PD)	2.93 (1.68–5.13)	<0.001	2.46 (1.31–4.62)	0.005

HR hazard ratio, *95% CI* 95% confidence interval, *SD* stable disease, *PD* progressive disease, *VEGF* vascular endothelial growth factor, *AFP* alpha-fetoprotein, *DCP* des-gamma-carboxy prothrombin

prognosis [5, 6]. HAIC using various chemotherapeutic regimens has been recognized to be a useful therapeutic modality for advanced HCC with MVI [7–12]. The combination of CDDP and 5-FU is one of the most common HAIC regimens and was used in the present study. Ando et al. and Lai et al. reported respective response rates of 48 and 33%, and MSTs of 10.2 and 9.5 months, from their HAIC studies that used low-dose CDDP and 5-FU [8, 13]. In the present study, patients with advanced HCC with MVI underwent short-term HAIC (2–3 weekly courses) using low-dose CDDP and 5-FU, and the response rate and MST were 35% and 10.2 months, respectively. Thus, our study demonstrated efficacy of HAIC similar to that shown by Ando et al. and Lai et al. [8, 13], although the treatment was short-term.

There have not been any previous studies that have clarified the predictors of therapeutic effect in patients with advanced HCC undergoing HAIC. In the present study, we evaluated various factors to determine which ones were predictive of a therapeutic effect following HAIC. By multiple logistic regression analysis, the serum VEGF level was found to be the only independent predictor of therapeutic effect. High VEGF levels have been recently shown to be independent markers for predicting poor response to chemotherapy in other studies of patients with various cancers [23–25]. Angiogenesis promotes the development and growth of tumors, including HCC, and VEGF is known to be the most important factor in tumor angiogenesis [16]. In contrast to a healthy vasculature, tumor vessels are known to be highly abnormal both structurally and functionally [26, 27]. These abnormal tumor vessels are characterized by irregular, disorganized, and tortuous architecture and are very dysfunctional. The network of abnormal tumor vasculature exhibits remarkable spatial and temporal heterogeneity. These abnormalities not only impair the delivery and perfusion of chemotherapeutic drugs, but also result in a hypoxic environment [28]. Hypoxia induces chemotherapy-resistant tumor cells [29]. Thus, an enlarged abnormal tumor vascular network caused by a high serum VEGF level may lead to resistance to HAIC. Moreover, we showed that in a patient with a high serum VEGF level and poor response to HAIC, the tumor tended to appear as hypovascular and heterogeneous on contrast-enhanced CT. This finding indicates the presence of an abnormal tumor vascular network in patients with high serum VEGF levels. Therefore, while patients with low serum VEGF levels are suitable for HAIC monotherapy, patients with high serum VEGF require more effective therapy, not only for the tumor cells, but also for disease with abnormal vasculature. A previous study has reported that antiangiogenic therapy can normalize tumor vasculature [26], which could, theoretically, lead to the increased delivery of oxygen and chemotherapeutic drugs. VEGF is an important molecular target of antiangiogenic therapy. VEGF-targeting agents such as the monoclonal antibody bevacizumab and the multikinase inhibitor sorafenib, which targets VEGF receptors 1, 2, and 3, have been recently approved for use in advanced HCC [30, 31]. Therefore, we suggest that in patients with high serum VEGF levels, the response to HAIC may be improved by adding anti-VEGF agents to the treatment regimen.

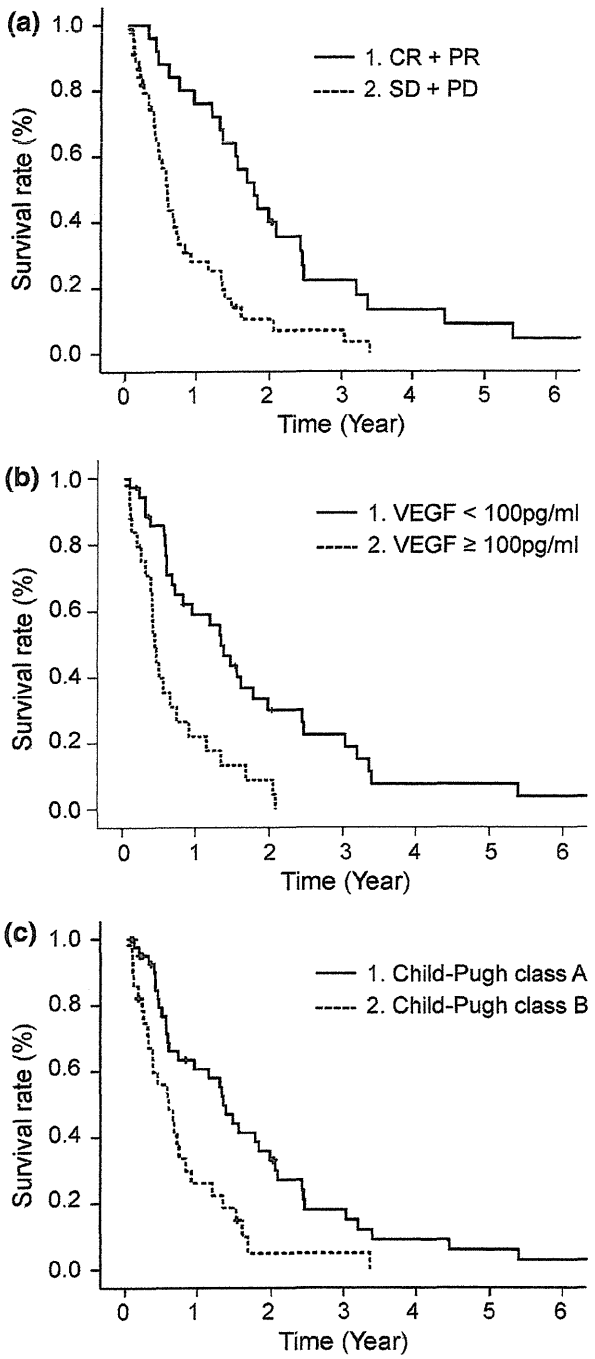

Fig. 6 **a** Cumulative survival of patients by therapeutic effect. The median survival times (MSTs) of responders [complete response (CR) + partial response (PR)] and nonresponders [stable disease (SD) + progressive disease (PD)] were 21.7 and 7.2 months, respectively ($P = 0.0001$). **b** Cumulative survival of patients by serum vascular endothelial growth factor (VEGF) level. The MSTs of patients with serum VEGF levels of <100 and ≥100 pg/mL were 16.8 and 5.8 months, respectively ($P = 0.0002$). **c** Cumulative survival of patients by Child–Pugh class. The MSTs of patients with Child–Pugh class A and B cirrhosis were 16.4 and 7.4 months, respectively ($P = 0.0014$)

We demonstrated that the serum VEGF level was positively correlated with platelet count and tumor size. Several studies have shown that the degree of elevation of serum VEGF level was positively correlated with tumor size and HCC tumor stage [17–20]. Poon et al. [32] demonstrated that there was significantly higher VEGF mRNA expression in tumors than in normal liver tissues, and there was a significant correlation of VEGF mRNA expression with VEGF protein expression in tumors. They also found that both tumor cytosolic VEGF protein and VEGF mRNA increased significantly with advancing tumor stage [32]. Platelets contain several angiogenic growth factors that are released by platelet activation, and these factors affect processes such as wound healing and tumor growth. Large amounts of VEGF are stored in platelet α-granules [33]. Previous studies have reported significantly elevated serum VEGF levels that correlated with platelet counts in HCC patients [19, 32]. Poon et al. [32] demonstrated that, when corrected for platelet count, the amount of serum VEGF per platelet indicated the release of VEGF by platelets, and the amount of serum VEGF per platelet was significantly correlated with tumor VEGF protein level. The increased serum VEGF level per platelet and increased serum VEGF level were also associated with advancing tumor stage [32]. These results suggest that VEGF released from tumor cells is stored and transported by platelets in the bloodstream, and that this reservoir of VEGF may have a role in tumor angiogenesis and progression. Moreover, these reports support our results indicating that platelet count and tumor size are simple and useful markers for identifying patients with high serum VEGF levels.

In the present study, multivariate analysis demonstrated that 3 factors, the therapeutic effect produced by HAIC, serum VEGF level, and Child–Pugh classification, were independent prognostic factors. Several studies have reported that the therapeutic effect was a significant prognostic factor in patients with advanced HCC who were treated using HAIC [8, 11–13]. The results of these studies demonstrated that the short-term reduction or disappearance of intrahepatic tumor, including MVI, and/or continuation of this state, were the main factors associated with prolonged survival of patients treated with HAIC. Our results demonstrating a significant association between the serum VEGF level and the therapeutic effect of HAIC suggest that high serum VEGF results in poor response to HAIC, active angiogenesis, rapidly progressive disease, and, ultimately, poor survival. Moreover, previous studies have shown that hepatic function was an independent prognostic factor in patients with HCC [6, 8, 11–13]. Hepatic reserve is important for the hepatic extraction and metabolism of HAIC agents. In the present study, liver dysfunction necessitating the suspension or discontinuation of HAIC occurred more frequently in patients with Child–

Pugh class B than in patients with Child–Pugh class A; therefore, we presume that liver function is an important predictor of survival.

In conclusion, this study demonstrated that the serum VEGF level was an important predictive factor of therapeutic effect and survival in patients with advanced HCC undergoing HAIC for the first time. Moreover, our results suggest that platelet count and tumor size are simple and useful markers for predicting the serum VEGF level. Based on these results, additional study evaluating VEGF and HAIC in the management of patients with advanced HCC with MVI is warranted.

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Conflict of interest The authors declare that they have no conflict of interest.

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