World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/1007-9327office
wjg@wjgnet.com
doi:10.3748/wjg.v17.i7.914

World J Gastroenterol 2011 February 21; 17(7): 914-921
ISSN 1007-9327 (print) ISSN 2219-2840 (online)

ORIGINAL ARTICLE

Body mass index is associated with age-at-onset of HCV-infected hepatocellular carcinoma patients

Takumi Akiyama, Toshihiko Mizuta, Seiji Kawazoe, Yuichiro Eguchi, Yasunori Kawaguchi, Hirokazu Takahashi, Iwata Ozaki, Kazuma Fujimoto

Takumi Akiyama, Toshihiko Mizuta, Yuichiro Eguchi, Yasunori Kawaguchi, Hirokazu Takahashi, Iwata Ozaki, Kazuma Fujimoto, Department of Internal Medicine, Saga Medical School, Saga 8498501, Japan
Takumi Akiyama, Seiji Kawazoe, Department of Hepatology, Saga Prefectural Hospital, Saga 8408571, Japan
Author contributions: Akiyama T and Mizuta T designed research and analyzed data; Kawazoe S, Eguchi Y, Kawaguchi Y, Takahashi H and Ozaki I provided the patient data; Fujimoto K reviewed the manuscript; Akiyama T wrote the paper; Mizuta T reviewed and edited the manuscript.
Correspondence to: Toshihiko Mizuta, MD, PhD, Department of Internal Medicine, Saga Medical School, 5-1-1 Nabeshima, Saga 8498501, Japan. mizutat@med.saga-u.ac.jp
Telephone: +81-952-342362 Fax: +81-952-342017
Received: August 10, 2010 Revised: September 29, 2010
Accepted: October 6, 2010
Published online: February 21, 2011

Abstract

AIM: To identify factors associated with the age at onset of hepatitis C virus (HCV)-related hepatocellular carcinoma (HCC).

METHODS: Five hundred and fifty-six consecutive patients positive for HCV antibody and treatment-naïve HCC diagnosed between 1995 and 2004 were analyzed. Patients were classified into three groups according to age at HCC onset: < 60 years (n = 79), 60-79 years (n = 439), or ⩾ 80 years (n = 38). Differences among groups in terms of sex, body mass index (BMI), lifestyle characteristics, and liver function were assessed. Factors associated with HCC onset in patients < 60 or ⩾ 80 years were analyzed by logistic regression analysis.

RESULTS: Significant differences emerged for sex, BMI, degree of smoking and alcohol consumption, mean bilirubin, alanine aminotransferase (ALT), and γ-glutamyl transpeptidase (GGT) levels, prothrombin activity, and platelet counts. The mean BMI values of male patients > 60 years old were lower and mean BMI values of female patients < 60 years old were higher than those of the general Japanese population. BMI > 25 kg/m^2 [hazard ratio (HR), 1.8, P = 0.045], excessive alcohol consumption (HR, 2.5, P = 0.024), male sex (HR, 3.6, P = 0.002), and GGT levels > 50 IU/L (HR, 2.4, P = 0.014) were independently associated with HCC onset in patients < 60 years. Low ALT level was the only factor associated with HCC onset in patients aged ⩾ 80 years.

CONCLUSION: Increased BMI is associated with increased risk for early HCC development in HCV-infected patients. Achieving recommended BMI and reducing alcohol intake could help prevent hepatic carcinogenesis.

Key words: Age-at-onset; Hepatocellular carcinoma; Hepatitis C virus; Body mass index; Alcohol consumption; Sex difference

Peer reviewers: Heitor Rosa, Professor, Department of Gastroenterology and Hepatology, Federal University School of Medicine, Rua 126 n.21, Goiania - GO 74093-080, Brazil; Jian Wu, Associate Professor of Medicine, Internal Medicine/Transplant Research Program, University of California, Davis Medical Center, 4635 2nd Ave. Suite 1001, Sacramento, CA 95817, United States

Akiyama T, Mizuta T, Kawazoe S, Eguchi Y, Kawaguchi Y, Takahashi H, Ozaki I, Fujimoto K. Body mass index is associated with age-at-onset of HCV-infected hepatocellular carcinoma patients. *World J Gastroenterol* 2011; 17(7): 914-921 Available from: URL: http://www.wjgnet.com/1007-9327/full/v17/i7/914.htm DOI: http://dx.doi.org/10.3748/wjg.v17.i7.914

INTRODUCTION

Hepatocellular carcinoma (HCC) is the fifth most com-

mon cancer in men and the eighth most common cancer in women worldwide. The incidence and mortality associated with HCC have been reported to be increasing in countries in North America, Europe and Asia. Infection with hepatitis C virus (HCV) infection is likely to play an important role in the pathogenesis of HCC[1-3]. In Japan, over 70% of cases of HCC diagnosed in the last 20 years are related to HCV infection[3].

One report estimates that 3%-35% of patients progress to cirrhosis 25 years after infection with HCV and 1%-3% progress to HCC 30 years after infection[4]. However, the factors that influence the development of HCC in patients infected with HCV remain largely unknown. Previous studies have suggested that host factors, such as sex, alcohol consumption, smoking, diabetes mellitus, and obesity, are important risk factors for HCC[5-11]. In addition, recent studies have suggested that HCV infection causes insulin resistance and leads to oxidative stress, potentiating fibrosis and hepatic carcinogenesis[12-14].

Therefore, we hypothesized that obesity influences the time to onset of HCC related to HCV infection, which is reflected in the patient's age at onset. To test this hypothesis, we investigated the relationship between body mass index (BMI) and lifestyle factors and age at onset of HCC in HCV-infected patients.

MATERIALS AND METHODS

Study participants

The study was conducted in accordance with the Helsinki Declaration. Written informed consent on the use of clinical records for research purposes was obtained from all subjects.

From January 1995 to December 2004, 656 consecutive patients positive for HCV antibodies and diagnosed with HCC for the first time at Saga Medical School Hospital and Saga Prefectural Hospital, without prior HCC treatment, were recruited for this study. Patients were excluded from the study if they were positive for hepatitis B surface antigen (n = 8), were previously treated with interferon (n = 23), had uncontrolled ascites (n = 27), or had an advanced tumor stage accompanied by tumor thrombus in portal tract or extrahepatic metastasis (n = 42). The remaining 556 patients (351 men, 205 women), with a median age at HCC onset of 67.8 years (range, 41-92 years) were enrolled in this study.

Diagnosis and staging of HCC

Diagnosis of HCC was confirmed by combined ultrasonography and dynamic computed tomography (CT), dynamic magnetic resonance imaging, or CT during angiography, demonstrating a hypervascular contrast pattern of the nodule in the arterial phase and a hypovascular pattern in the portal phase. If the nodule contrast patterns were not consistent with those typical for HCC, a needle biopsy of the tumor was taken for pathological diagnosis.

Tumor stage was classified according to the 5th Edition of the General Rules for the Clinical and Pathological Study of Primary Liver Cancer, 2008, published by the Liver Cancer Study Group of Japan[15]. This classification system assumes three conditions: (1) tumor diameter of $\leq$ 2 cm; (2) a single tumor is present; and (3) no vascular invasion of the tumor. If all three conditions are met, the tumor is classified as stage I; if two conditions are met, it is classified as stage II; if only one condition is met, it is classified as stage III; and if none of the conditions are met, it is classified as stage IV.

Exposure and laboratory data

At the time of HCC diagnosis, blood tests were performed and BMI was calculated as weight in kilograms divided by the square of the height in meters (kg/m^2). Prothrombin activity and serum albumin and total bilirubin levels were measured and used to determine the Child-Pugh status. Blood samples were also used to measure alanine aminotransferase (ALT) and γ-glutamyl transpeptidase (GGT) levels and other liver function tests.

Patients were classified according to the World Health Organization (WHO) BMI criteria: underweight, BMI < 18.5 kg/m^2; normal weight, BMI 18.5-25 kg/m^2; overweight, BMI 25-30 kg/m^2; and obese, BMI $\geq$ 30 kg/m^2[16]. Diagnosis of diabetes mellitus was made either by reviewing medical history or by assessing glucose levels with fasting plasma glucose level of $\geq$ 7.0 mmol/L or a 2-h plasma glucose level of $\geq$ 11.1 mmol/L[17]. Patients were questioned by nurses about their smoking and drinking habits during the last 10 years. We defined heavy drinking as > 60 g of alcohol consumed per day and habitual smoking as > 20 pack years.

Statistical analysis

To identify factors associated with age at onset of HCC in patients with chronic hepatitis C, we compared clinical factors in two groups of patients; those aged < 60 years at HCC onset and those aged $\geq$ 80 years. We then analyzed risk factors affecting earlier (onset age < 60 years) and later (onset age $\geq$ 80 years) development of HCC in patients with chronic HCV.

We used the Kruskal-Wallis test or the χ^2 test to compare clinicopathological variables between three groups of patients. The differences in age at onset of HCC between the two groups stratified by BMI were analyzed by the Tukey-Kramer method. Univariate and multivariate logistic regression analyses were performed to identify factors associated with earlier or later onset of HCC.

Data processing and analysis were performed by using the SAS (SAS Institute Inc.). Two-tailed P values of < 0.05 were considered significant.

RESULTS

Patient characteristics

A histogram showing age at onset of HCC in 556 HCV-infected patients is depicted in Figure 1. The median age of patients was 67.8 years, with a nearly normal age distribution for the study population.

The clinical characteristics were categorized into three groups according to age at onset of HCC; < 60 years

Table 1 Clinical characteristics of patients classified with hepatocellular carcinoma occurrence age

Factors	Occurrence age of HCC (years old)			*P*
	< 60 (*n* = 79)	60-80 (*n* = 439)	≥ 80 (*n* = 38)	
Sex				
Male/Female, *n*	70/9	264/175	17/21	< 0.0001[a]
BMI (kg/m^2)	23.8 ± 3.4	22.9 ± 3.4	21.8 ± 3.3	0.02[b]
< 25/25 <, *n*	50/29	325/114	32/6	0.039[a]
Diabetes mellitus				
With/without, *n*	16/63	86/353	2/36	0.088[a]
Smoking (pack years)				
< 20/≥ 20, *n*	43/36	137/302	9/29	0.0001[a]
Alcohol consumption (g/d)				
< 60/≥ 60, *n*	65/14	416/23	36/2	< 0.0001[a]
Tumor stage				
Ⅰ/Ⅱ/Ⅲ, *n*	21/33/25	116/196/127	11/16/11	0.981[a]
Child-Pugh class				
A/B/C, *n*	55/23/1	349/87/3	34/4/0	0.145[a]
Albumin (g/dL)	3.57 ± 0.53	3.64 ± 0.50	3.63 ± 0.41	0.586[b]
< 3.5/≥ 3.5, *n*	30/49	151/288	14/24	0.433[a]
Total bilirubin (mg/dL)	1.23 ± 0.62	1.05 ± 0.55	0.80 ± 0.30	0.0002[b]
< 2.0/≥ 2.0, *n*	70/9	413/26	38/0	0.047[a]
Prothrombin activity (%)	76.1 ± 16.6	79.9 ± 15.1	89.1 ± 12.2	0.0002[b]
< 70/≥ 70, *n*	26/53	98/341	3/35	0.003[a]
Platelet count (× 10^4/μL)	10.4 ± 7.8	11.0 ± 5.5	13.1 ± 5.8	0.005[b]
< 10/≥ 10, *n*	45/34	223/216	14/24	0.125[a]
ALT (IU/L)	78.3 ± 39.3	71.8 ± 44.1	41.7 ± 19.3	< 0.0001[b]
< 80/≥ 80, *n*	47/32	288/151	36/2	0.0004[a]
GGT (IU/L)	123.7 ± 102.5	86.0 ± 86.6	54.6 ± 36.1	< 0.0001[b]
< 50/≥ 50, *n*	15/64	173/266	20/18	0.0002[a]

Continuous variables are expressed as mean ± standard deviation. Statistical analysis was done using a: the χ^2 test or b: the Turkey-Kramer test. HCC: Hepatocellular carcinoma; BMI: Body mass index; ALT: Alanine aminotransferase; GGT: γ-glutamyl transpeptidase.

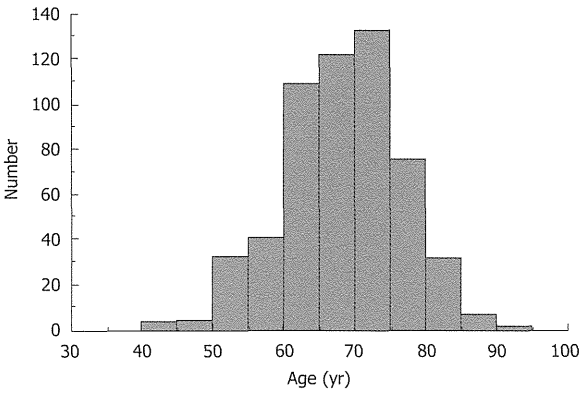

Figure 1 Histogram showing age at onset of hepatocellular carcinoma in hepatitis C virus-infected patients (*n* = 556). Median age, 67.8 years; range, 41-92 years.

(*n* = 79), 60-79 years (*n* = 439), and ≥ 80 years (*n* = 38) (Table 1). Of those aged < 60 years, 88.6% were men, a much higher percentage than in those aged 60-79 years (60.1%) and those aged ≥ 80 years (44.7%). In terms of BMI, the mean value increased, and the percentage of patients with BMI < 25 kg/m^2 decreased while that of patients with BMI > 25 increased with decreasing age at onset of HCC. However, this is a normal phenomenon in the general population. Therefore, we compared the mean BMI values according to the age at onset of HCC for patients in this study with BMI values of the general Japanese population in 2005 and 2006, which were published by the Ministry of Health, Labour and Welfare, Japan (http://www.mhlw.go.jp/). The mean BMI of male HCC patients aged > 60 years was lower whereas that of female HCC patients aged < 60 years was higher than those of the general population (Figure 2). This indicates that the association between BMI and age at onset of HCC observed in this study was affected by factors independent of natural aging. We found that there were significantly more heavy drinkers ($P < 0.0001$) and habitual smokers ($P = 0.0001$) among patients aged < 60 years, compared with the other two age groups. Although the three groups did not differ in terms of Child-Pugh status, total bilirubin, ALT, and GGT levels were higher, and prothrombin activity and platelet counts were lower in patients aged < 60 years at HCC onset. No differences emerged in terms of the prevalence of diabetes mellitus or the distribution of tumor stage among the three groups.

Factors associated with the development of HCC at < 60 years of age

We investigated risk factors associated with the development of HCC at a younger age (i.e. < 60 years of age) (Table 2). In univariate analysis, the following were found to be significant risk factors for earlier age at onset of HCC: male sex [hazard ratio (HR), 5.4; 95% CI, 2.65-11.12; $P < 0.0001$], BMI > 25 kg/m^2 (HR, 1.7; 95% CI, 1.04-2.85;

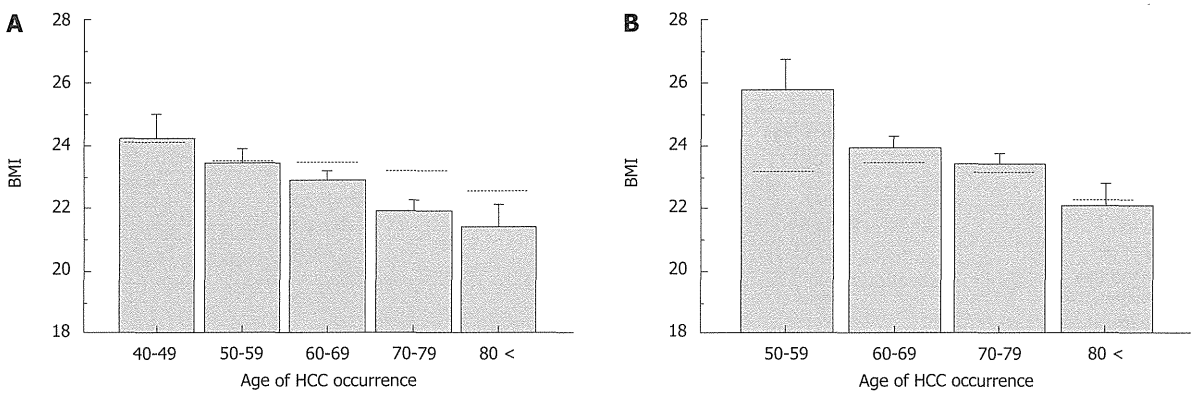

Figure 2 Mean body mass index in each age group at onset of hepatocellular carcinoma (A: Men; B: Women). The bars show the mean body mass index (BMI) ± SD in patients with hepatocellular carcinoma (HCC). The dashed lines show the mean BMI for the general Japanese population in 2005 and 2006, which was surveyed by the Ministry of Health, Labour and Welfare, Japan.

Table 2 Analysis of factors affecting development of hepatocellular carcinoma at younger age (under 60 yr old)

Variables	Univariate analysis			Multivariate analysis		
	HR	95% CI	*P*	HR	95% CI	*P*
Sex						
Female	1			1		
Male	5.43	2.647-11.120	< 0.0001	3.58	1.580-8.133	0.002
BMI						
< 25	1			1		
≥ 25	1.73	1.044-2.851	0.033	1.82	1.015-3.270	0.045
Diabetes mellitus						
Without	1			1		
With	1.12	0.619-2.037	0.703	1.00	0.516-1.952	0.991
Smoking (packs year)						
< 20	1			1		
≥ 20	2.71	1.669-4.393	< 0.0001	1.64	1.904-2.991	0.104
Alcohol (g/d)						
< 60	1			1		
≥ 60	3.89	1.926-7.874	0.0002	2.51	1.130-5.563	0.024
Total bilirubin (mg/dL)						
< 2.0	1			1		
≥ 2.0	2.23	1.003-4.958	0.049	2.33	0.898-6.033	0.082
Prothrombin activity (%)						
≥ 70	1			1		
< 70	1.91	1.111-3.262	0.019	1.60	0.859-2.987	0.139
Platelet ($\times 10^4/\mu L$)						
≥ 10	1			1		
< 10	1.34	0.829-2.166	0.232	1.60	0.877-2.886	0.118
ALT (IU/L)						
< 80	1			1		
≥ 80	1.44	0.884-2.350	0.142	1.17	0.656-2.090	0.542
GGT (IU/L)						
< 50	1			1		
≥ 50	3.24	1.731-6.053	0.0002	2.38	1.194-4.727	0.014

HR: Hazard ratio; BMI: Body mass index; ALT: Alanine aminotransferase; GGT: γ-glutamyl transpeptidase.

$P = 0.033$), habitual smoking (HR, 2.7; 95% CI, 1.67-4.39; $P < 0.0001$), heavy drinking (HR, 3.9; 95% CI, 1.93-7.87; $P = 0.0002$), total bilirubin > 2.0 mg/dL (HR, 2.2; 95% CI, 1.00-4.96; $P = 0.049$), prothrombin activity > 70% (HR, 1.9; 95% CI, 1.11-3.26; $P = 0.019$), and GGT level > 50 IU/L (HR, 3.2; 95% CI, 1.73-6.05; $P = 0.0002$). In multivariate analysis, independent risk factors for earlier age at onset of HCC were male sex (HR, 3.6; 95% CI, 1.58-8.13; $P = 0.002$), BMI > 25 kg/m^2 (HR, 1.8; 95% CI, 1.015-3.270; $P = 0.045$), heavy drinking (HR, 2.5; 95% CI, 1.13-5.56; $P = 0.024$), and GGT > 50 IU/L (HR, 2.4; 95% CI, 1.19-4.73; $P = 0.014$).

Factors associated with the development of HCC at ≥ 80 years of age

We also investigated factors associated with the develop-

Table 3 Analysis of factors affecting development of hepatocellular carcinoma at older age (over 80 yr old)

Variables	Univariate analysis			Multivariate analysis		
	HR	95% CI	*P*	HR	95% CI	*P*
Sex						
Female	1			1		
Male	0.45	0.229-0.867	0.017	0.47	0.200-1.119	0.089
BMI						
<25	1			1		
≥ 25	0.49	0.201-1.201	0.119	0.48	0.174-1.321	0.155
Diabetes mellitus						
Without	1			1		
With	0.23	0.054-0.957	0.043	0.32	0.074-1.412	0.133
Smoking (packs year)						
< 20	1			1		
≥ 20	0.58	0.270-1.258	0.169	0.81	0.306-2.164	0.680
Alcohol (g/d)						
< 60	1			1		
≥ 60	0.72	0.167-3.118	0.663	0.45	0.056-3.606	0.451
Total bilirubin (mg/dL)						
< 2.0	1			1		
≥ 2.0	1.00	-	0.97	1.00	-	0.98
Prothrombin activity (%)						
≥ 70	1			1		
< 70	0.10	0.014-0.755	0.025	0.15	0.020-1.166	0.07
Platelet (× 10^4/μL)						
≥ 10	1			1		
< 10	0.54	0.275-1.076	0.080	0.62	0.287-1.360	0.236
ALT (IU/L)						
< 80	1			1		
≥ 80	0.10	0.024-0.427	0.002	0.13	0.030-0.569	0.007
GGT (IU/L)						
< 50	1			1		
≥ 50	0.51	0.262-0.984	0.045	1.01	0.479-2.146	0.971

HR: Hazard ratio; BMI: Body mass index; ALT: Alanine aminotransferase; GGT: γ-glutamyl transpeptidase.

ment of HCC at an older age (i.e. ≥ 80 years of age) (Table 3). In univariate analysis, the following were significantly and negatively associated with age at onset of HCC ≥ 80 years: male sex (HR, 0.45; 95% CI, 0.23-0.87; $P = 0.017$), diabetes mellitus (HR, 0.23; 95% CI, 0.05-0.96; $P = 0.043$), prothrombin activity < 70% (HR, 0.1; 95% CI, 0.01-0.76; $P = 0.025$), ALT > 80 IU/L (HR, 0.1; 95% CI, 0.02-0.43; $P = 0.002$), and GGT > 50 IU/L (HR, 0.51; 95% CI, 0.26-0.98; $P = 0.045$). In multivariate analysis, ALT > 80 IU/L was the only independent factor associated with age at onset of HCC ≥ 80 years (HR, 0.13; 95% CI, 0.03-0.57; $P = 0.007$).

Age at onset of HCC stratified by BMI in relation to sex or alcohol consumption

Differences in age at onset of HCC stratified by BMI were assessed in relation to sex or alcohol consumption. In men, age at onset decreased significantly with increasing BMI (mean age ± SD; underweight, 71.1 ± 7.4 years; normal weight, 67.0 ± 8.5 years; overweight, 63.6 ± 8.1 years; obese, 57.0 ± 7.0 years) (Figure 3A). Although a similar trend was noted in women, this was not significant (underweight, 73.6 ± 7.8 years; normal weight, 70.4 ± 7.0 years; overweight, 68.9 ± 6.4 years; obese, 67.0 ± 7.5 years) (Figure 3B).

Although an association between BMI and age at onset of HCC was found among non-heavy drinkers (Figure 4A), no association was found among heavy drinkers (Figure 4B).

DISCUSSION

The results of this study revealed that higher BMI, heavy alcohol consumption, male sex, and high GGT levels are independent risk factors for younger age at onset of HCC in patients with chronic HCV infection. This study confirms the previously reported risk factors for HCC and is the first to investigate the relationship between age and HCC development.

It seems plausible that the duration of HCV infection plays a role in the age at which cirrhosis progresses to HCC. However, Hamada *et al*[18] reported a significant negative correlation between the time from HCV infection to onset of HCC and the patient's age at the time of infection, and as a result, the onset of HCC was considered to occur in patients during their 60 s regardless of their age at time of infection. This indicates that factors other than duration of HCV infection may be associated with the age at onset of HCC in HCV-infected patients.

Recent studies have shown that HCV proteins, such

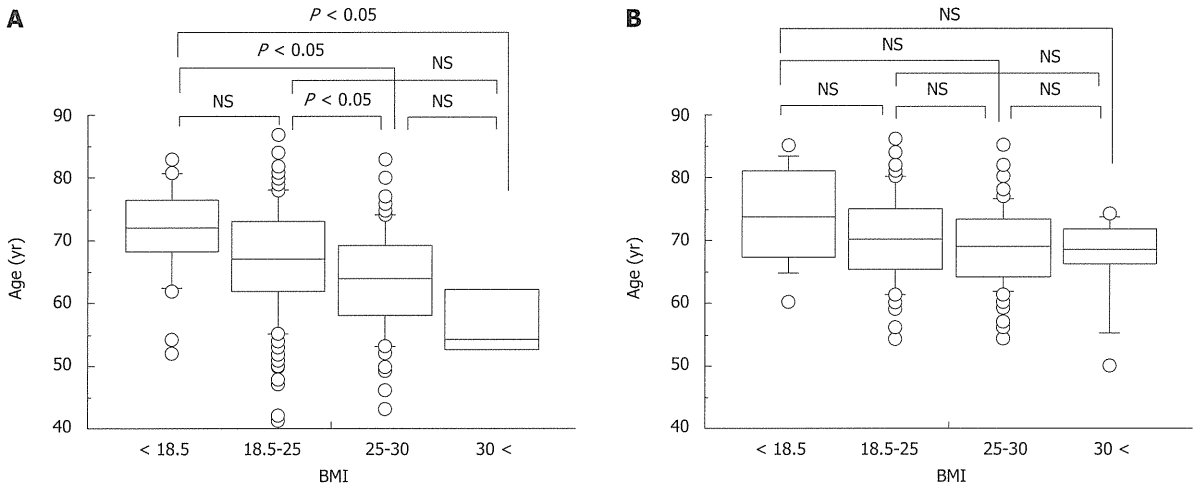

Figure 3 Differences in age at onset of hepatocellular carcinoma stratified by body mass index according to sex (A: Men; B: Women). Statistical analysis was performed using the Tukey-Kramer method. NS: Not significant; BMI: Body mass index.

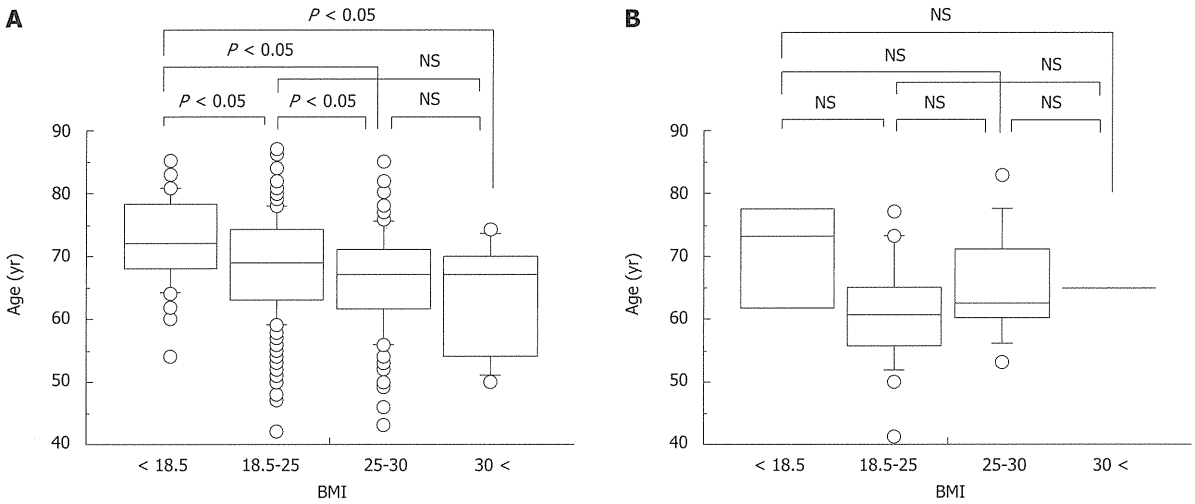

Figure 4 Differences in age at onset of hepatocellular carcinoma stratified by body mass index according to degree of alcohol consumption (A: Non-heavy drinkers < 60 g/d; B: Heavy drinkers ⩾ 60 g/d). Statistical analysis was performed using the Tukey-Kramer method. NS: Not significant; BMI: Body mass index.

as the core protein, cause oxidative damage by exposing the endoplasmic reticulum to oxidative stress[19-21]. Hepatic oxidative stress is strongly associated with increased risk for HCC in patients with chronic HCV[22]. Because oxidative stress is also caused by various host-related factors, it is expected to be influenced more strongly by host-related factors in HCV-infected patients than in those with HCV-negative liver disease. Indeed, we have previously reported that visceral fat accumulation was associated with greater insulin resistance in chronic HCV patients than in those with non-alcoholic fatty liver disease[23]. Therefore, it is plausible that the association between earlier onset of HCC and increased BMI is due to the generation of hepatic oxidative stress.

An interesting aspect of our results is that underweight patients, defined as those with a BMI of < 18.5 kg/m^2, tended to be older at HCC onset than patients within the normal weight range (BMI 18.5-25 kg/m^2). Recently, Ohki *et al*[11] reported that patients with a BMI < 18.5 kg/m^2 had the lowest risk of developing HCC due to chronic HCV infection among all BMI groups. In general, the mortality rate associated with cardiovascular disease or cancer is higher in underweight patients than in normal weight patients[24,25]. Clearly, a larger cohort study is needed to investigate whether leanness confers a protective effect against hepatocarcinogenesis in HCV-infected patients.

Excessive alcohol consumption is also known to exacerbate hepatic oxidative stress and evoke liver fibrosis or HCC[20,26]. In this study, there was no association between BMI and age at onset of HCC in heavy drinkers. We speculate that this group may include some patients who are malnourished and possibly losing weight.

Sex modulates the natural history of chronic liver disease. Previous studies have suggested that chronic HCV

infection progresses more rapidly in men than women, and that cirrhosis is predominately a disease of men and postmenopausal women[27]. Shimizu et al suggested that estrogens protect against oxidative stress in liver injury and hepatic fibrosis[28]. In this study, the effect of BMI on age at onset of HCC was more remarkable in men than women. We speculate two mechanisms to account for this difference: (1) estrogens mitigate oxidative stress or insulin resistance associated with obesity; and (2) subcutaneous fat accumulation is more dominant in obese women than visceral fat, which is known to produce several adipokines that cause insulin resistance[29].

In addition, we examined factors associated with onset of HCC at an older age ($\geqslant$ 80 years). In this analysis, ALT level was the only independent factor associated with hepatocarcinogenesis in HCV-infected patients at an age $\geqslant$ 80 years. It is well known that ALT levels are associated with liver inflammation and fibrosis progression, and Ishiguro et al recently reported that elevated ALT levels were strongly associated with the incidence of HCC, regardless of hepatitis virus positivity, in a large population-based cohort study[30]. Therefore, lower ALT levels might indicate a slow course of progression of hepatic fibrosis or carcinogenesis.

A limitation of this study is that it was a cross-sectional observation, rather than a cohort follow-up study. Further studies are needed to confirm our results.

In conclusion, the results of the present study indicate that higher BMI, excessive alcohol consumption, and male sex are independent risk factors for onset of HCV-related HCC at an age of < 60 years. These results suggest that interventions to promote changes in the lifestyle of patients with chronic HCV may slow the progression of HCV infection to HCC.

ACKNOWLEDGMENTS

The authors would like to thank Yukie Watanabe and Chieko Ogawa for their assistance.

COMMENTS

Background

The incidence and mortality associated with hepatocellular carcinoma (HCC) have been increasing worldwide, and hepatitis C virus (HCV) infection plays an important role in the pathogenesis of HCC. However, the factors that influence the development of HCC in HCV-infected patients remain largely unknown. Previous studies have suggested that host factors, such as sex, alcohol consumption, smoking, diabetes mellitus, and obesity, are important risk factors for HCC. Meanwhile, it has been reported that HCV infection causes insulin resistance and leads to oxidative stress, potentiating fibrosis and hepatic carcinogenesis. Therefore, we hypothesized that body mass index (BMI) influences the onset age of HCC related to HCV infection.

Research frontiers

Many studies have indicated that obesity is an independent and a significant risk factor for HCC occurrence. Recently, several metabolic markers have been implicated in the development and progression of HCC.

Innovations and breakthroughs

This study indicated that higher BMI, heavy alcohol consumption, male sex, and high γ-glutamyl transpeptidase levels are independent risk factors for younger age at onset of HCV-related HCC. Interestingly, the underweight patients (BMI < 18.5 kg/m^2), tended to be older at HCC onset than patients within the normal weight range (BMI 18.5-25 kg/m^2).

Applications

The results of this study suggest that achieving an adequate body weight along with a reduction of alcohol intake in patients with chronic hepatitis C could help prevent hepatic carcinogenesis.

Peer review

The study was reasonably designed and well conducted, and the data support their conclusions.

REFERENCES

1 **El-Serag HB**. Epidemiology of hepatocellular carcinoma in USA. *Hepatol Res* 2007; **37** Suppl 2: S88-S94
2 **El-Serag HB**, Rudolph KL. Hepatocellular carcinoma: epidemiology and molecular carcinogenesis. *Gastroenterology* 2007; **132**: 2557-2576
3 **Umemura T**, Ichijo T, Yoshizawa K, Tanaka E, Kiyosawa K. Epidemiology of hepatocellular carcinoma in Japan. *J Gastroenterol* 2009; **44** Suppl 19: 102-107
4 **El-Serag HB**. Hepatocellular carcinoma and hepatitis C in the United States. *Hepatology* 2002; **36**: S74-S83
5 **Fattovich G**, Stroffolini T, Zagni I, Donato F. Hepatocellular carcinoma in cirrhosis: incidence and risk factors. *Gastroenterology* 2004; **127**: S35-S50
6 **Sinn DH**, Paik SW, Kang P, Kil JS, Park SU, Lee SY, Song SM, Gwak GY, Choi MS, Lee JH, Koh KC, Yoo BC. Disease progression and the risk factor analysis for chronic hepatitis C. *Liver Int* 2008; **28**: 1363-1369
7 **Marrero JA**, Fontana RJ, Fu S, Conjeevaram HS, Su GL, Lok AS. Alcohol, tobacco and obesity are synergistic risk factors for hepatocellular carcinoma. *J Hepatol* 2005; **42**: 218-224
8 **Hara M**, Tanaka K, Sakamoto T, Higaki Y, Mizuta T, Eguchi Y, Yasutake T, Ozaki I, Yamamoto K, Onohara S, Kawazoe S, Shigematsu H, Koizumi S. Case-control study on cigarette smoking and the risk of hepatocellular carcinoma among Japanese. *Cancer Sci* 2008; **99**: 93-97
9 **Polesel J**, Zucchetto A, Montella M, Dal Maso L, Crispo A, La Vecchia C, Serraino D, Franceschi S, Talamini R. The impact of obesity and diabetes mellitus on the risk of hepatocellular carcinoma. *Ann Oncol* 2009; **20**: 353-357
10 **Aizawa Y**, Shibamoto Y, Takagi I, Zeniya M, Toda G. Analysis of factors affecting the appearance of hepatocellular carcinoma in patients with chronic hepatitis C. A long term follow-up study after histologic diagnosis. *Cancer* 2000; **89**: 53-59
11 **Ohki T**, Tateishi R, Sato T, Masuzaki R, Imamura J, Goto T, Yamashiki N, Yoshida H, Kanai F, Kato N, Shiina S, Yoshida H, Kawabe T, Omata M. Obesity is an independent risk factor for hepatocellular carcinoma development in chronic hepatitis C patients. *Clin Gastroenterol Hepatol* 2008; **6**: 459-464
12 **Vidali M**, Tripodi MF, Ivaldi A, Zampino R, Occhino G, Restivo L, Sutti S, Marrone A, Ruggiero G, Albano E, Adinolfi LE. Interplay between oxidative stress and hepatic steatosis in the progression of chronic hepatitis C. *J Hepatol* 2008; **48**: 399-406
13 **Koike K**. Steatosis, liver injury, and hepatocarcinogenesis in hepatitis C viral infection. *J Gastroenterol* 2009; **44** Suppl 19: 82-88
14 **Sheikh MY**, Choi J, Qadri I, Friedman JE, Sanyal AJ. Hepatitis C virus infection: molecular pathways to metabolic syndrome. *Hepatology* 2008; **47**: 2127-2133
15 **Liver Cancer Study Group**. The General Rules for the Clinical and Pathological Study of Primary Liver Cancer. 5th ed. Japan, 2008: 24-28
16 **World Health Organization**. Obesity: Preventing and managing the global epidemic; Report of a WHO Consultation on Obesity, Geneva, 3-5, June 1997. Geneva: WHO, 1998
17 **Report of a WHO Consultation**. Definition, diagnosis and classification of diabetes mellitus and its complication:

Part1. Diagnosis and classification of diabetes mellitus. World Health Organization, Department of Noncommunicable Disease Surveillance, Geneva, 1999
18 **Hamada H**, Yatsuhashi H, Yano K, Daikoku M, Arisawa K, Inoue O, Koga M, Nakata K, Eguchi K, Yano M. Impact of aging on the development of hepatocellular carcinoma in patients with posttransfusion chronic hepatitis C. *Cancer* 2002; **95**: 331-339
19 **Tardif KD**, Waris G, Siddiqui A. Hepatitis C virus, ER stress, and oxidative stress. *Trends Microbiol* 2005; **13**: 159-163
20 **Ji C**, Kaplowitz N. ER stress: can the liver cope? *J Hepatol* 2006; **45**: 321-333
21 **Ciccaglione AR**, Costantino A, Tritarelli E, Marcantonio C, Equestre M, Marziliano N, Rapicetta M. Activation of endoplasmic reticulum stress response by hepatitis C virus proteins. *Arch Virol* 2005; **150**: 1339-1356
22 **Tanaka H**, Fujita N, Sugimoto R, Urawa N, Horiike S, Kobayashi Y, Iwasa M, Ma N, Kawanishi S, Watanabe S, Kaito M, Takei Y. Hepatic oxidative DNA damage is associated with increased risk for hepatocellular carcinoma in chronic hepatitis C. *Br J Cancer* 2008; **98**: 580-586
23 **Eguchi Y**, Mizuta T, Ishibashi E, Kitajima Y, Oza N, Nakashita S, Hara M, Iwane S, Takahashi H, Akiyama T, Ario K, Kawaguchi Y, Yasutake T, Iwakiri R, Ozaki I, Hisatomi A, Eguchi T, Ono N, Fujimoto K. Hepatitis C virus infection enhances insulin resistance induced by visceral fat accumulation. *Liver Int* 2009; **29**: 213-220
24 **Whitlock G**, Lewington S, Sherliker P, Clarke R, Emberson J, Halsey J, Qizilbash N, Collins R, Peto R. Body-mass index and cause-specific mortality in 900 000 adults: collaborative analyses of 57 prospective studies. *Lancet* 2009; **373**: 1083-1096
25 **Calle EE**, Thun MJ, Petrelli JM, Rodriguez C, Heath CW Jr. Body-mass index and mortality in a prospective cohort of U.S. adults. *N Engl J Med* 1999; **341**: 1097-1105
26 **de la Monte SM**, Yeon JE, Tong M, Longato L, Chaudhry R, Pang MY, Duan K, Wands JR. Insulin resistance in experimental alcohol-induced liver disease. *J Gastroenterol Hepatol* 2008; **23**: e477-e486
27 **Farinati F**, Sergio A, Giacomin A, Di Nolfo MA, Del Poggio P, Benvegnù L, Rapaccini G, Zoli M, Borzio F, Giannini EG, Caturelli E, Trevisani F. Is female sex a significant favorable prognostic factor in hepatocellular carcinoma? *Eur J Gastroenterol Hepatol* 2009; **21**: 1212-1218
28 **Shimizu I**, Ito S. Protection of estrogens against the progression of chronic liver disease. *Hepatol Res* 2007; **37**: 239-247
29 **Geer EB**, Shen W. Gender differences in insulin resistance, body composition, and energy balance. *Gend Med* 2009; **6** Suppl 1: 60-75
30 **Ishiguro S**, Inoue M, Tanaka Y, Mizokami M, Iwasaki M, Tsugane S. Serum aminotransferase level and the risk of hepatocellular carcinoma: a population-based cohort study in Japan. *Eur J Cancer Prev* 2009; **18**: 26-32

S- Editor Sun H **L- Editor** O'Neill M **E- Editor** Ma WH

J Gastroenterol (2011) 46:529–535
DOI 10.1007/s00535-010-0338-x

ORIGINAL ARTICLE—LIVER, PANCREAS, AND BILIARY TRACT

Metabolic factors are associated with serum alanine aminotransferase levels in patients with chronic hepatitis C

Yumi Kobayashi · Yasunori Kawaguchi · Toshihiko Mizuta · Takuya Kuwashiro · Satoshi Oeda · Noriko Oza · Hirokazu Takahashi · Shinji Iwane · Yuichiro Eguchi · Keizo Anzai · Iwata Ozaki · Kazuma Fujimoto

Received: 24 March 2010 / Accepted: 11 October 2010 / Published online: 3 November 2010

Abstract
Background Although serum alanine aminotransferase (ALT) activity is an important marker for the management of chronic hepatitis C (CHC), the factors associated with serum ALT levels remain to be fully understood. This study aimed to clarify the association between serum ALT levels and clinical, histological, and virological factors in patients with CHC.
Methods We retrospectively analyzed 256 patients with CHC who underwent liver biopsy, and classified them into three groups according to serum ALT levels: normal to minimal (<40 IU/L), mild (40–80 IU/L), and moderate to severe elevation (≥80 IU/L). All demographic and laboratory data were collected at the time of liver biopsy. All biopsies were evaluated for fibrosis, inflammation, and steatosis. Glucose metabolism was assessed by various indices derived from oral glucose tolerance tests, including the homeostasis model assessment for insulin resistance (HOMA-IR). In 180 patients, visceral fat area was measured at the umbilical level by abdominal computed tomography.
Results Ordered logistic regression analysis showed that higher serum ALT levels were significantly associated with male sex, lower high-density lipoprotein cholesterol (HDL-C), higher HOMA-IR, and higher grades of histological inflammation and steatosis. HOMA-IR, HDL-C, and hepatic steatosis were associated with visceral fat accumulation.
Conclusions Metabolic factors, as well as sex and hepatic inflammation, are independent risk factors for serum ALT elevation in hepatititis C virus (HCV)-infected patients. Metabolic factors may offer targets to decrease serum ALT levels.

Keywords Alanine aminotransferase · Chronic hepatitis C · Hepatic steatosis · High-density lipoprotein cholesterol · HOMA-IR

Y. Kobayashi · Y. Kawaguchi · T. Mizuta (✉) · T. Kuwashiro · S. Oeda · N. Oza · H. Takahashi · S. Iwane · Y. Eguchi · K. Anzai · I. Ozaki · K. Fujimoto
Department of Internal Medicine, Saga Medical School, 5-1-1 Nabeshima, Saga, Japan
e-mail: mizutat@cc.saga-u.ac.jp

Introduction

Approximately 170 million people worldwide are chronically infected with hepatitis C virus (HCV), which is responsible for a range of diseases including minimal to severe chronic hepatitis, cirrhosis, and hepatocellular carcinoma (HCC) [1–3]. To date, many studies have investigated the factors associated with the heterogeneous clinical course of HCV infection.

Major factors associated with the progression of liver fibrosis in chronic hepatitis C (CHC) patients include older age at infection, excessive alcohol consumption, male sex, histological inflammation, and elevated serum alanine aminotransferase (ALT) levels [4–6]. Moreover, recent studies have shown that metabolic abnormalities such as hepatic steatosis, obesity, and diabetes can worsen the course of CHC [5, 7–10]. Insulin resistance (IR) in non-diabetic HCV-infected patients is also related to hepatic steatosis and fibrosis, and is often established early in the course of CHC [11, 12]. Meanwhile, we have shown that eradication of HCV by interferon (IFN) therapy can improve whole-body IR in HCV-infected patients [13]. On the other hand, we have also reported that visceral fat accumulation is more strongly associated with IR in

patients with CHC than in patients with non-alcoholic fatty liver disease [14]. Although HCV viral load and genotype do not seem to significantly influence the rate of progression [4, 5, 9, 15], genotype 3 is strongly associated with hepatic steatosis [8, 10]. Furthermore, experimental studies using an HCV core transgenic mouse model have revealed the roles of HCV in the development of hepatic steatosis [16], IR [17], and HCC [18]. However, the relationships described above are extremely complicated and remain to be fully understood.

Serum ALT activity is an important marker for the management of CHC [19] and for the diagnosis of liver diseases [20, 21], and it can be easily and repeatedly measured within daily clinical practice. Therefore, clarifying the associations between serum ALT levels and viral and host factors, including metabolic factors, would be useful to better understand the pathogenesis and natural course of CHC. Here, we retrospectively investigated the clinical, histological, and virological characteristics of HCV-infected patients to identify factors associated with serum ALT levels.

Patients and methods

Patients

A total of 372 consecutive patients with CHC who visited Saga Medical School Hospital for IFN therapy between January 2002 and April 2009 were included in this retrospective study. To investigate the association between serum ALT levels and viral and host factors, including the metabolic characteristics, of HCV-infected patients, patients were selected based on the following criteria: (1) serum HCV-RNA positivity, (2) serum hepatitis B surface antigen negativity, (3) histological evaluation of a liver biopsy, and (4) recent oral glucose tolerance test (OGTT). Patients who had received any liver supportive therapy within 1 month before liver biopsy were excluded. We also excluded patients taking medications for diabetes mellitus (DM) or dyslipidemia, because these medications could influence glucose and lipid metabolism, masking the association with HCV. We finally analyzed data from 256 patients with CHC.

Clinical and laboratory assessments

All demographic and laboratory data were collected at the time of liver biopsy. The demographic data included age, sex, and alcohol use. Average alcohol intake (g/day) was evaluated by interview. Venous blood samples were taken after a 12-h overnight fast to determine the platelet count, and to determine the levels of aspartate aminotransferase (AST; IU/L), ALT (IU/L), γ-glutamyl transpeptidase (γ-GTP; IU/L), total cholesterol (TC; mg/dL), triglyceride (TG; mg/dL), and high-density lipoprotein cholesterol (HDL-C; mg/dL). Patients were classified into three groups according to serum ALT levels: normal to minimal (<40 IU/L), mild (40–80 IU/L), and moderate to severe elevation (≥80 IU/L). For the OGTT, patients ingested a solution containing 75 g glucose, and venous blood samples were collected at 0, 30, 60, 90, and 120 min for the measurement of plasma glucose (PG) and serum insulin (SI) levels. PG levels were determined using a glucokinase method, and serum immunoreactive insulin levels were measured using a chemiluminescent enzyme immunoassay kit (Abbott Japan, Tokyo, Japan). Glucose tolerance was evaluated according to the criteria of the World Health Organization (WHO) [22]: normal glucose tolerance (NGT) as fasting PG (FPG) <110 mg/dL and 2-h PG < 140 mg/dL; impaired fasting glycemia (IFG) as FPG 110–126 mg/dL and 2-h PG < 140 mg/dL; impaired glucose tolerance (IGT) as FPG < 126 mg/dL and 2-h PG 140–200 mg/dL; and DM as FPG ≥ 126 mg/dL or 2-h PG ≥ 200 mg/dL. Although this study included patients who met the criteria for DM, their FPG levels were less than 126 mg/dL. The indices of basal insulin secretion and insulin sensitivity were evaluated by the homeostasis model assessment (HOMA) method [23] and calculated as follows:

$$\beta \text{ cell function (HOMA} - \beta) = \text{fasting SI (FSI; } \mu\text{U/mL)} \times 360/[\text{FPG(mg/dL)} - 63]$$

$$\text{Insulin resistance (HOMA} - \text{IR)} = \text{FPG} \times \text{FSI}/405$$

The insulinogenic index (II) [24], a marker of early-phase insulin secretion, was calculated as $(SI_{30} - FSI)/(PG_{30} - FPG)$. The whole-body insulin sensitivity index (WBISI) [25] was calculated as $10{,}000/(FPG \times FSI \times \text{mean PG } 0 - 120 \times \text{mean SI } 0 - 120)^{0.5}$. Body mass index (BMI) was calculated as weight (kg) divided by height (m) squared. We determined serum HCV-RNA levels using quantitative polymerase chain reaction (PCR) assays (COBAS Amplicore HCV Monitor test v2.0, original method or high-range method; Roche Diagnostics, Tokyo, Japan) until November 2007 and by real-time PCR assays (COBAS TaqMan HCV test; Roche Diagnostics) from December 2007. The HCV genotype was determined on the basis of the sequence of the core region [26].

In 180 patients who underwent abdominal computed tomography, visceral fat area (VFA; cm^2) was measured at the umbilical level and calculated with Fat Scan software (N2 Systems, Osaka, Japan) [27].

Liver histology

Percutaneous liver biopsy was performed using a Super-Core™ Biopsy Instrument (Medical Device Technologies,

Gainesville, FL, USA) under ultrasound guidance. For each patient, a 15-mm-long liver biopsy specimen was fixed in 10% formalin, embedded in paraffin, sectioned, and stained with hematoxylin–eosin and Azan for histological evaluation. Histological hepatic fibrosis and inflammation were scored using the METAVIR scoring system [28]. Based on the degree of lymphocyte infiltration and hepatocyte necrosis, inflammation was classified with scores of A0 to A3, with higher scores indicating more severe inflammation. Fibrosis was graded from F0 to F4, as follows: F0, no fibrosis; F1, portal fibrosis without septa; F2, portal fibrosis with rare septa; F3, numerous septa without cirrhosis; and F4, cirrhosis. Steatosis was quantified as the percentage of hepatocytes that contained fat droplets, and was classified into three groups: <5, 5–30, and ≥30%.

Statistical analysis

Continuous variables are summarized as means ± standard deviation. Differences in trends among the elevated serum ALT groups were assessed using the Jonckheere–Terpstra test for continuous variables, the Cochran–Armitage test for binomial variables, and Somers's D for ordered categorical variables. Ordered logistic regression was used to assess the association of selected dichotomized variables and elevated serum ALT categories. P values of <0.05 were considered statistically significant.

Results

Patient characteristics

The baseline characteristics of the 256 patients are presented in Table 1. Only 9% of the patients showed excessive alcohol intake of >50 g/day. In terms of serum ALT levels, 86 patients (33.6%) showed normal to minimal elevation, 96 patients (37.5%) showed mild elevation, and 74 patients (28.9%) showed moderate to severe elevation. About one-third of the patients had abnormal glucose tolerance, with 66.4, 26.2, and 7.4% of patients having NGT, IGT, and DM, respectively. None of the patients met the criteria for IFG. In terms of histological evaluation, more than half of the patients had moderate to severe inflammation (A2, A3), F4 (cirrhosis) was observed in 12 patients (4.7%), and moderate to severe steatosis (≥30%) was observed in 17 patients (6.6%). Of the 180 patients in whom VFA was measured, 42 (23.3%) had visceral obesity (VFA ≥100 cm^2).

Characteristics of the patients according to serum ALT levels

Table 2 shows the characteristics of the patients stratified according to serum ALT levels. In terms of demographic and laboratory data, the ratio of males to females ($P = 0.0003$), BMI ($P = 0.0015$), FSI ($P < 0.0001$), HOMA-β ($P < 0.0001$), and HOMA-IR ($P < 0.0001$) were positively correlated with serum ALT, while platelet count ($P = 0.0009$), HCV-RNA load ($P = 0.0478$), WBISI ($P < 0.0001$), and serum HDL-C levels ($P < 0.0001$) were negatively correlated with serum ALT. In terms of histological findings, the grades of inflammation ($P < 0.0001$), fibrosis ($P < 0.0001$), and steatosis ($P = 0.0001$) were

Table 1 Baseline characteristics of 256 patients with chronic hepatitis C

Variables	All patients
Age (years)	55.8 ± 10.6 (24–74)
Sex (males/females)	135/121
BMI (kg/m^2)	23.5 ± 2.9 (16.9–38.3)
<22/22–25/≥25	74/113/69
Alcohol consumption (g/day)	
<20/20–50/≥50	191/42/23
Platelets ($\times 10^4/mm^3$)	16.0 ± 5.5 (5.5–35.8)
AST (IU/L)	57.6 ± 36.4 (11–233)
ALT (IU/L)	71.8 ± 60.6 (7–395)
<40/40–80/≥80	86/96/74
γ-GTP (IU/L)	61.9 ± 76.4 (11–708)
HCV RNA load (log IU/mL)	6.07 ± 0.67 (3.87–7.40)
<5/5–6/≥6	21/80/155
HCV genotype: 1a/1b/2a/2b	1/190/46/19
Glucose tolerance: NGT/IGT/DM	170/67/19
Fasting plasma glucose (mg/dL)	87.4 ± 9.6 (65–125)
Fasting serum insulin (μU/mL)	9.1 ± 5.3 (1.6–33.9)
HOMA-β	153 ± 128 (33–1530)
HOMA-IR	1.99 ± 1.23 (0.31–7.95)
Insulinogenic index	1.08 ± 1.32 (−0.31–14.9)
WBISI	5.23 ± 3.24 (1.01–23.29)
Total cholesterol (mg/dL)	169.9 ± 32.1 (83–279)
Triglyceride (mg/dL)	101.6 ± 53.7 (33–607)
HDL-C (mg/dL)[a]	50.1 ± 15.3 (23–108)
Histological findings	
Inflammation: A1/A2/A3	111/115/30
Fibrosis: F0/F1/F2/F3/F4	5/121/73/45/12
Steatosis (%): <5/5–30/≥30	189/50/17
Visceral fat area (cm^2)[b]	73.6 ± 39.6 (15.2–220.7)
<50/50–100/100–150/≥150	59/79/33/9

Data are expressed as means ± SD (ranges) or as numbers of patients

BMI body mass index, *AST* aspartate aminotransferase, *ALT* alanine aminotransferase, *γ-GTP* γ-glutamyl transpeptidase, *HCV* hepatitis C virus, *NGT* normal glucose tolerance, *IGT* impaired glucose tolerance, *DM* diabetes mellitus, *HOMA-β* homeostasis model assessment for β-cell function, *HOMA-IR* homeostasis model assessment for insulin resistance, *WBISI* whole-body insulin sensitivity index, *HDL-C* high-density lipoprotein cholesterol

[a] $n = 248$, [b] $n = 180$

Table 2 Patients' characteristics stratified by serum ALT level

Variables	Serum ALT level (IU/L)			
	<40 ($n = 86$)	40–80 ($n = 96$)	≥80 ($n = 74$)	*P*
Age (years)	56.0 ± 10.4	56.9 ± 11.0	54.2 ± 10.3	0.4876
Sex (male, %)	31 (36.0)	55 (57.3)	49 (66.2)	0.0003
BMI (kg/m^2)	22.7 ± 2.7	23.8 ± 2.8	24.1 ± 3.1	0.0015
<22/22–25/≥25	36/33/17	24/42/30	14/38/22	0.0033
Alcohol consumption (g/day)				
<20/20–50/≥50	67/11/8	73/18/5	51/13/10	0.2161
Platelets (× 10^4/mm^3)	17.9 ± 5.9	15.1 ± 5.4	14.8 ± 4.7	0.0009
HCV RNA load (logIU/mL)	6.20 ± 0.63	6.00 ± 0.69	6.00 ± 0.69	0.0478
<5/5–6/≥6	5/19/62	10/33/53	6/28/40	0.0185
HCV genotype 1 (%)	61 (70.9)	79 (82.3)	51 (68.9)	0.3220
Glucose tolerance: NGT/IGT/DM	66/17/3	58/30/8	46/20/8	0.0523
Fasting plasma glucose (mg/dL)	85.6 ± 8.0	88.7 ± 9.7	87.9 ± 11.1	0.3747
Fasting serum insulin (μU/mL)	7.01 ± 3.95	8.93 ± 4.06	11.83 ± 6.84	<0.0001
HOMA-β	121 ± 72	140 ± 73	207 ± 201	<0.0001
HOMA-IR	1.50 ± 0.88	1.97 ± 0.94	2.61 ± 1.60	<0.0001
Insulinogenic index	0.92 ± 0.63	1.02 ± 1.65	1.36 ± 1.39	0.3746
WBISI	6.72 ± 3.95	4.84 ± 2.45	4.00 ± 2.53	<0.0001
Total cholesterol (mg/dL)	173.1 ± 32.2	166.1 ± 33.0	171.0 ± 30.7	0.3952
Triglyceride (mg/dL)	98.1 ± 46.8	98.2 ± 38.2	110.2 ± 74.2	0.1393
HDL-C (mg/dL)[a]	56.1 ± 17.8	48.3 ± 13.7	45.3 ± 11.4	<0.0001
Histological findings				
Inflammation: A1/A2/A3	63/20/3	27/57/12	21/38/15	<0.0001
Fibrosis: F0–F1/F2/F3–F4	63/14/9	38/30/28	25/29/20	<0.0001
Steatosis (%): <5/5–30/≥30	74/11/1	73/18/5	42/21/11	0.0001
Visceral fat area (cm^2)[b]	64.0 ± 38.6	75.3 ± 36.7	83.6 ± 42.0	0.0047
<50/50–100/100–150/≥150	29/26/8/3	17/29/14/2	13/24/11/4	0.0169

Data are expressed as means ± SD or as numbers of patients

ALT alanine aminotransferase, *BMI* body mass index, *NGT* normal glucose tolerance, *IGT* impaired glucose tolerance, *DM* diabetes mellitus, *HOMA-β* homeostasis model assessment for β-cell function, *HOMA-IR* homeostasis model assessment for insulin resistance, *WBISI* whole-body insulin sensitivity index, *HDL-C* high-density lipoprotein cholesterol

[a] $n = 248$, [b] $n = 180$

positively associated with serum ALT. No differences according to serum ALT levels were found in mean age, alcohol consumption, viral genotype, glucose tolerance, FPG levels, the II, or the serum levels of TC and TG.

We selected variables with $P < 0.01$ for ordered logistic regression analysis. However, FSI, HOMA-β, and WBISI were excluded from this analysis because these parameters were highly confounded by HOMA-IR, which was more strongly associated with serum ALT levels than these factors. VFA was also excluded from this analysis because of missing data for a number of patients. Ordered logistic regression analysis (Table 3) showed that gradual serum ALT elevation was significantly associated with male sex [odds ratio (OR) 2.136, 95% confidence interval (CI) 1.273–3.585, $P = 0.004$], HOMA-IR ≥ 2 (OR 2.396, 95% CI 1.360–4.220, $P = 0.002$), HDL-C ≥ 49 mg/dL (OR 0.529, 95% CI 0.315–0.887, $P = 0.016$), hepatic inflammation ≥ A2 (OR 3.138, 95% CI 1.773–5.554, $P < 0.001$), and hepatic steatosis ≥ 5% (OR 1.875, 95% CI 1.027–3.424, $P = 0.041$).

Associations between visceral fat accumulation and serum ALT levels

Serum ALT levels were positively correlated with VFA ($P = 0.0047$) (Table 2). Although VFA was excluded from the ordered logistic regression analysis because of the limited number of examined patients, factors such as

Table 3 Ordered logistic regression model to identify clinical factors associated with serum ALT

Variables	Odds ratio	95% CI	*P*
Sex: male	2.136	1.273–3.585	0.004
BMI (kg/m^2): ≥25	1.041	0.592–1.830	0.888
Platelets (×10^4/mm^3): ≥15	0.653	0.391–1.092	0.104
HOMA-IR: ≥2	2.396	1.360–4.220	0.002
HDL-C (mg/dL): ≥49	0.529	0.315–0.887	0.016
Inflammation: A2–A3	3.138	1.773–5.554	<0.001
Fibrosis: F3–F4	0.785	0.410–1.503	0.465
Steatosis (%): ≥5	1.875	1.027–3.424	0.041

ALT alanine aminotransferase,*CI* confidence interval, *BMI* body mass index, *HOMA-IR* homeostasis model assessment for insulin resistance, *HDL-C* high-density lipoprotein cholesterol ($n = 248$)

HOMA-IR, serum HDL-C levels, and hepatic steatosis, which were independently associated with serum ALT levels, were strongly associated with VFA (Fig. 1).

Discussion

This study identified several factors associated with serum ALT levels according to the level of elevation, i.e., mild (40–80 IU/L) and moderate to severe elevation (≥80 IU/L). Serum ALT activity has long been used as a marker of hepatic inflammation to assess liver disease [20, 21]. Many reports have also shown that serum ALT activity is higher in males than in females [4, 5, 29–31]. Here, we found that elevated serum ALT levels in HCV-infected patients were strongly associated with severe hepatic inflammation and male sex, in addition to showing an association with higher HOMA-IR values, lower HDL-C levels, and a higher grade of hepatic steatosis, factors which were also associated with visceral fat accumulation. Considering these results, it seems that metabolic factors enhance the liver damage caused by immunologic reactions to HCV.

The mechanisms responsible for the strong association between male sex and serum ALT activity have not been fully elucidated, although some reports have speculated on the possible roles of sex hormones and alcohol consumption [4, 30]. In the present study, alcohol consumption was significantly greater in males than in females ($P < 0.0001$ by χ^2 test), but there was no difference in alcohol consumption level according to the level of ALT activity. Future studies should also measure sex hormones to help clarify these differences between sexes.

Although large cohort studies have revealed the importance of metabolic factors influencing serum ALT activity in blood donors [29] and in HCV-infected patients [30], to our knowledge, our study is the first to show that hepatic inflammation and metabolic factors are associated

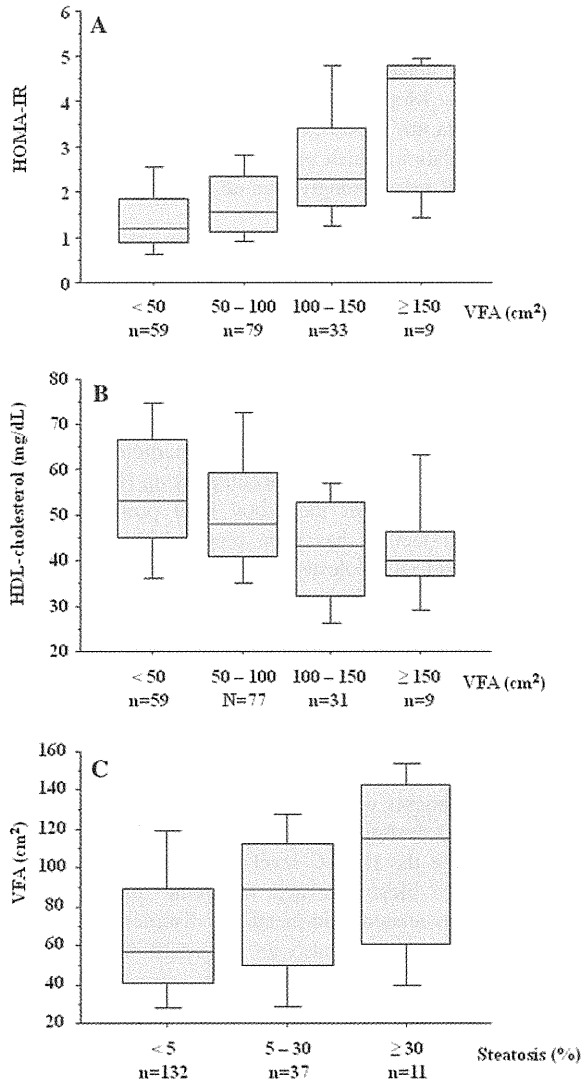

Fig. 1 Correlations of HOMA-IR, HDL-cholesterol, and hepatic steatosis with VFA. **a** HOMA-IR ($n = 180$, $P < 0.0001$), **b** HDL-cholesterol ($n = 176$, $P < 0.0001$), and **c** hepatic steatosis ($n = 180$, $P = 0.001$) were significantly associated with VFA (Jonckheere–Terpstra test). *VFA* Visceral fat area, *HOMA-IR* homeostasis model assessment for insulin resistance, *HDL* high-density lipoprotein

with elevated serum ALT levels in patients with CHC. In particular, we believe that the metabolic factors identified in the present ordered logistic regression analysis might originate from the host rather than from the virus. Meanwhile, experimental studies have shown that the HCV core protein itself might induce hepatic steatosis [16] and IR [17]. Similarly, clinical studies have shown that genotype 3 is associated with hepatic steatosis [8] and elevated ALT [30], and that HCV infection is associated with IR, regardless of the genotype [11–13]. Although only HCV

genotypes 1 and 2 were included in the present study, viral factors such as genotype and viral load were not associated with either hepatic steatosis (data not shown) or serum ALT levels. Moreover, IR was not associated with viral factors (data not shown), but was associated with visceral fat accumulation, which means that the host factors might influence IR more strongly than do the viral factors, as we previously reported [14].

It is notable that lower serum HDL-C was also detected as an independent risk factor for elevated serum ALT levels. It is well known that lipids play important roles in HCV infection. Endocytosis of HCV occurs via the low-density lipoprotein (LDL) receptor and could be promoted by complexing of the virus to LDL or very low-density lipoprotein [32]. In hepatocytes, lipid droplets are required for the formation of infectious virus particles [33]. Such changes in lipid metabolism accompanying HCV infection are expected to alter the blood lipid profiles in CHC patients. In fact, it has been reported that serum TC, HDL-C, and LDL-cholesterol (LDL-C) were lower in CHC patients than in normal subjects [34]. Prati et al. [30] showed that the serum ALT activity in CHC was inversely associated with serum TC and TG levels, but their data did not include HDL-C. In our study, while there were no significant differences in serum TC or TG levels among the three ALT categories, serum HDL-C was significantly negatively correlated with serum ALT activity. As the lipid profile in patients with ALT elevation is a marker of lifestyle-related dyslipidemia, we speculate that the association between the HDL-C level and ALT activity can be attributed to lifestyle-related metabolic disorders rather than to virus-related lipid metabolic disorders.

Visceral obesity is also an important risk factor for metabolic syndrome, cardiovascular disease, and non-alcoholic fatty liver disease [35, 36]. In the present study, VFA was strongly associated with HOMA-IR, serum HDL-C levels, and hepatic steatosis. Of note, the magnitude of the correlations between serum ALT categories and VFA was weaker than that of the correlations with other metabolic factors, which may reflect the notion that visceral adiposity is a primary cause of most metabolic disorders. Based on these results, we speculate that visceral obesity may indirectly contribute to elevated serum ALT levels via changes in glucose and lipid metabolism.

The limitation of our study is that it was a retrospective, cross-sectional study. Because of this design, the history of alcohol intake, which could affect liver function tests, might have been inaccurate. Moreover, because ALT was only measured once, the ALT data do not reflect fluctuations in the ALT level. Furthermore, because metabolic factors also fluctuate according to lifestyle and/or medication, a prospective longitudinal study is needed to clarify the association between fluctuations in metabolic factors and those in serum ALT levels. Although we could not determine serum iron and ferritin levels in this retrospective study, many reports have shown that iron overload in HCV infection is associated with higher ALT levels [37, 38], and that iron reduction therapy can lower serum ALT levels [39–42]. Therefore, iron metabolism is an important factor in regard to the serum ALT activity in HCV-infected patients. Further studies on glucose and lipid metabolism, as well as iron-related factors, should be conducted to elucidate the complicated mechanism responsible for the changes in serum ALT activity in HCV-infected patients.

In conclusion, the present study has shown that metabolic factors, in addition to male sex and hepatic inflammation, are independent risk factors for elevated serum ALT levels in HCV-infected patients. Our results suggest that metabolic factors may offer new targets to decrease serum ALT levels. Further prospective studies are needed to determine whether correcting these metabolic factors through lifestyle modifications and/or medications for the treatment of metabolic diseases could lower the serum ALT levels and thus improve the clinical course in patients with CHC.

References

1. Lauer G, Walker BD. Hepatitis C virus infection. N Engl J Med. 2001;345:1–52.
2. Alberti A, Noventa F, Benvegnu L, Boccato S, Gatta A. Prevalence of liver disease in a population of asymptomatic persons with hepatitis C virus infection. Ann Intern Med. 2002;137:961–4.
3. Poynard T, Yuen MF, Ratziu V, Lai CL. Viral hepatitis C. Lancet. 2003;362:2095–100.
4. Poynard T, Bedossa P, Opolon P, for the OBSVIRC, METAVIR, CLINIVIR, and DOSVIRC groups. Natural history of liver fibrosis progression in patients with chronic hepatitis C. Lancet. 1997;349:825–32.
5. Marcellin P, Asselah T, Boyer N. Fibrosis and disease progression in hepatitis C. Hepatology. 2002;36:S47–56.
6. Fontaine H, Nalpas B, Poulet B, Carnot F, Zylberberg H, Brechot C, et al. Hepatitis activity index is a key factor in determining the natural history of chronic hepatitis C. Hum Pathol. 2001;32:904–9.
7. Hourigan LF, Macdonald GA, Purdie D, Whitehall VH, Shorthouse C, Clouston A, et al. Fibrosis in chronic hepatitis C correlates significantly with body mass index and steatosis. Hepatology. 1999;29:1251–9.
8. Adinolfi LE, Gambardella M, Andreana A, Tripodi MF, Utili R, Ruggiero G. Steatosis accelerates the progression of liver damage of chronic hepatitis C patients and correlates with specific HCV genotype and visceral obesity. Hepatology. 2001;33:1358–64.
9. Fartoux L, Chazouillères O, Wendum D, Poupon R, Serfaty L. Impact of steatosis on progression of fibrosis in patients with mild hepatitis C. Hepatology. 2005;41:82–7.
10. Leandro G, Mangia A, Hui J, Fabris P, Rubbia-Brandt L, Colloredo G, HCV meta-analysis (on) Individual Patients' Data Study Group, et al. Relationship between steatosis, inflammation,

and fibrosis in chronic hepatitis C: a meta-analysis of individual patient data. Gastroenterology. 2006;130:1636–42.
11. Petit JM, Bour JB, Galland-Jos C, Minello A, Verges B, Guiguet M, et al. Risk factors for diabetes mellitus and early insulin resistance in chronic hepatitis C. J Hepatol. 2001;35:279–83.
12. Cammà C, Bruno S, Di Marco V, Di Bona D, Rumi M, Vinci M, et al. Insulin resistance is associated with steatosis in nondiabetic patients with genotype 1 chronic hepatitis. Hepatology. 2006;43: 64–71.
13. Kawaguchi Y, Mizuta T, Oza N, Takahashi H, Ario K, Yoshimura T, et al. Eradication of hepatitis C virus by interferon improves whole-body insulin resistance and hyperinsulinemia in patients with chronic hepatitis C. Liver Int. 2009;29:871–7.
14. Eguchi Y, Mizuta T, Ishibashi E, Kitajima Y, Oza N, Nakashita S, et al. Hepatitis C virus infection enhances insulin resistance induced by visceral fat accumulation. Liver Int. 2009;29:213–20.
15. Benvegnù L, Pontisso P, Cavalletto D, Noventa F, Chemello L, Alberti A. Lack of correlation between hepatitis C virus genotypes and clinical course of hepatitis C virus-related cirrhosis. Hepatology. 1997;25:211–5.
16. Moriya K, Yotsuyanagi H, Shintani Y, Fujie H, Ishibashi K, Matsuura Y, et al. Hepatitis C virus core protein induces hepatic steatosis in transgenic mice. J Gen Virol. 1997;78:1527–31.
17. Shintani Y, Fujie H, Miyoshi H, Tsutsumi T, Tsukamoto K, Kimura S, et al. Hepatic C virus infection and diabetes: direct involvement of the virus in the development of insulin resistance. Gastroenterology. 2004;126:840–8.
18. Moriya K, Fujie H, Shintani Y, Yotsuyanagi H, Tsutsumi T, Ishibashi K, et al. The core protein of hepatitis C virus induces hepatocellular carcinoma in transgenic mice. Nat Med. 1998;4: 1065–7.
19. Alberti A. Towards more individualized management of hepatitis C virus patients with initially or persistently normal alanine aminotransferase levels. J Hepatol. 2005;42:266–74.
20. Pratt DS, Kaplan MM. Evaluation of abnormal liver-enzyme results in asymptomatic patients. N Engl J Med. 2000;342: 1266–71.
21. Craxì A, Almasio P. Diagnostic approach to liver enzyme elevation. J Hepatol. 1996;25(Suppl 1):47–51.
22. Alberti KG, Zimmet PZ. Definition, diagnosis and classification of diabetes mellitus and its complications. Part 1: Diagnosis and classification of diabetes mellitus provisional report of a WHO consultation. Diabet Med. 1998;15:539–53.
23. Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia. 1985;28:412–9.
24. Seltzer HS, Allen EW, Herron AL Jr, Brennan MT. Insulin secretion in response to glycemic stimulus: relation of delayed initial release to carbohydrate intolerance in mild diabetes mellitus. J Clin Invest. 1967;46:323–35.
25. Matsuda M, DeFronzo RA. Insulin sensitivity indices obtained from oral glucose tolerance testing. Comparison with the euglycemic insulin clamp. Diabetes Care. 1999;22:1462–70.
26. Ohno T, Mizokami M, Wu RR, Saleh MG, Ohba K, Orito E, et al. New hepatitis C virus (HCV) genotyping system that allows for identification of HCV genotypes 1a, 1b, 2a, 2b, 3a, 3b, 4, 5a, and 6a. J Clin Microbiol. 1997;35:201–7.
27. Yoshizumi T, Nakamura T, Yamane M, Islam AH, Menju M, Yamasaki K, et al. Abdominal fat: standardized technique for measurement at CT. Radiology. 1999;211:283–6.
28. Bedossa P, Poynard T, for the METAVIR Cooperative Study Group. An algorithm for the grading of activity in chronic hepatitis C. Hepatology. 1996;24:289–93.
29. Prati D, Taioli E, Zanella A, Della Torre E, Butelli S, Del Vecchio E, et al. Updated definitions of healthy ranges for serum alanine aminotransferase levels. Ann Intern Med. 2002;137:1–10.
30. Prati D, Shiffman ML, Diago M, Gane E, Rajender Reddy K, Pockros P, et al. Viral and metabolic factors influencing alanine aminotransferase activity in patients with chronic hepatitis C. J Hepatol. 2006;44:679–85.
31. Piton A, Poynard T, Imbert-Bismut F, Khalil L, Delattre J, Pelissier E, et al. Factors associated with serum alanine transaminase activity in healthy subjects: consequences for the definition of normal values, for selection of blood donors, and for patients with chronic hepatitis C. MULTIVIRC Group. Hepatology. 1998;27: 1213–9.
32. Agnello V, Abel G, Elfahal M, Knight GB, Zhang QX. Hepatitis C virus and other flaviviridae viruses enter cells via low density lipoprotein receptor. Proc Natl Acad Sci USA. 1999;96: 12766–71.
33. Miyanari Y, Atsuzawa K, Usuda N, Watashi K, Hishiki T, Zayas M, et al. The lipid droplet is an important organelle for hepatitis C virus production. Nat Cell Biol. 2007;9:1089–97. (Erratum in: Nat Cell Biol. 2007; 9:1216).
34. Siagris D, Christofidou M, Theocharis GJ, Pagoni N, Papadimitriou C, Lekkou A, et al. Serum lipid pattern in chronic hepatitis C: histological and virological correlations. J Viral Hepat. 2006;13:56–61.
35. Bergman RN, Kim SP, Hsu IR, Catalano KJ, Chiu JD, Kabir M, et al. Abdominal obesity: role in the pathophysiology of metabolic disease and cardiovascular risk. Am J Med. 2007;120:S3–8.
36. Eguchi Y, Eguchi T, Mizuta T, Ide Y, Yasutake T, Iwakiri R, et al. Visceral fat accumulation and insulin resistance are important factors in nonalcoholic fatty liver disease. J Gastroenterol. 2006;41:462–9.
37. Di Bisceglie AM, Axiotis CA, Hoofnagle JH, Bacon BR. Measurements of iron status in patients with chronic hepatitis. Gastroenterology. 1992;102:2108–13.
38. Bonkovsky H, Banner BF, Rothman AL. Iron and chronic viral hepatitis. Hepatology. 1997;25:759–68.
39. Hayashi H, Takikawa T, Nishimura N, Yano M, Isomura T, Sakamoto N. Improvement of serum aminotransferase levels after phlebotomy in patients with chronic active hepatitis C and excess hepatic iron. Am J Gastroenterol. 1994;89:986–8.
40. Yano M, Hayashi H, Yoshioka K, Kohgo Y, Saito H, Niitsu Y, et al. A significant reduction in serum alanine aminotransferase levels after 3-month iron reduction therapy for chronic hepatitis C: a multicenter, prospective, randomized, controlled trial in Japan. J Gastroenterol. 2004;39:570–4.
41. Tanaka N, Horiuchi A, Yamaura T, Komatsu M, Tanaka E, Kiyosawa K. Efficacy and safety of 6-month iron reduction therapy in patients with hepatitis C virus-related cirrhosis: a pilot study. J Gastroenterol. 2007;42:49–55.
42. Tanaka N, Horiuchi A, Yamaura T, Komatsu M, Yokoyama T, Okaniwa S, et al. Efficacy and safety of addition of minor bloodletting (petit phlebotomy) in hepatitis C virus-infected patients receiving regular glycyrrhizin injections. J Gastroenterol. 2009;44:577–82.

Hepatology Research

Hepatology Research 2011; **41**: 564–571 doi: 10.1111/j.1872-034X.2011.00799.x

Original Article

Data mining reveals complex interactions of risk factors and clinical feature profiling associated with the staging of non-hepatitis B virus/non-hepatitis C virus-related hepatocellular carcinoma

Takumi Kawaguchi,[1] Tatsuyuki Kakuma,[2] Hiroshi Yatsuhashi,[3] Hiroshi Watanabe,[4] Hideki Saitsu,[5] Kazuhiko Nakao,[6] Akinobu Taketomi,[7] Satoshi Ohta,[8] Akinari Tabaru,[9] Kenji Takenaka,[10] Toshihiko Mizuta,[11] Kenji Nagata,[12] Yasuji Komorizono,[13] Kunitaka Fukuizumi,[14] Masataka Seike,[15] Shuichi Matsumoto,[16] Tatsuji Maeshiro,[17] Hirohito Tsubouchi,[18] Toyokichi Muro,[19] Osami Inoue,[20] Motoo Akahoshi[21] and Michio Sata:[1] The Liver Cancer Study Group of Kyushu

[1]Department of Digestive Disease Information and Research and Department of Medicine, Kurume University School of Medicine, [2]The Biostatistics Center, Medical School, Kurume University, Kurume, [3]Department of Therapeutic Research, Clinical Research Center, National Hospital Organization Nagasaki Medical Center, Omura, [4]Hepatology Division, Fukuoka Red Cross Hospital, [5]Department of Surgery, Center for Liver Diseases, National Hospital Organization Kyushu Medical Center, Fukuoka, [6]Department of Gastroenterology and Hepatology, Graduate School of Biomedical Sciences, Nagasaki University, Nagasaki, [7]Department of Surgery and Science, Kyushu University, [8]Division of Gastroenterology, National Kyushu Cancer Center, Fukuoka, [9]Third Department of Internal Medicine, University of Occupational and Environmental Health, Japan, School of Medicine, Kitakyushu, [10]Fukuoka City Hospital, Fukuoka, [11]Department of Internal Medicine, Saga University, Saga, [12]Gastroenterology and Hematology, Internal Medicine, Faculty of Medicine, University of Miyazaki, Miyazaki, [13]Hepatology, Nanpuh Hospital, Kagoshima, [14]Department of Gastroenterology, National Hospital Organization Kyushu Medical Center, Fukuoka, [15]Department of Internal Medicine I, Faculty of Medicine, Oita University, Yufu, [16]Department of Internal Medicine, Fukuoka Tokushukai Medical Center, Fukuoka, [17]Department of Infections, Respiratory, and Digestive Medicine Control and Prevention of Infectious Disease Faculty of Medicine, University of the Ryukyus, Okinawa, [18]Department of Digestive and Life-style Related Disease, Kagoshima University Graduate School of Medical and Dental Scienses, Kagoshima, [19]Department of Gastroenterology, National Hospital Organization Oita Medical Center, Oita, [20]Digestive Organ Center, Nagasaki Labour Welfare Hospital, Sasebo, and [21]Department of Internal Medicine, Nishinihon Hospital, Kumamoto, Japan

Aim: Non-hepatitis B virus/non-hepatitis C virus-related hepatocellular carcinoma (NBNC-HCC) is often detected at an advanced stage, and the pathology associated with the staging of NBNC-HCC remains unclear. Data mining is a set of statistical techniques which uncovers interactions and meaningful patterns of factors from a large data collection. The aims of this study were to reveal complex interactions of the risk factors and clinical feature profiling associated with the staging of NBNC-HCC using data mining techniques.

Methods: A database was created from 663 patients with NBNC-HCC at 20 institutions. The Milan criteria were used as staging of HCC. Complex associations of variables and clinical feature profiling with the Milan criteria were analyzed by graphical modeling and decision tree algorithm methods, respectively.

Results: Graphical modeling identified six factors independently associated with the Milan criteria: diagnostic year of HCC; diagnosis of liver cirrhosis; serum aspartate aminotransferase (AST); alanine aminotransferase (ALT); α-fetoprotein (AFP); and des-γ-carboxy prothrombin (DCP) levels. The decision trees were created with five variables to classify six groups of patients. Sixty-nine percent of the patients were

Correspondence: Dr Takumi Kawaguchi, Department of Digestive Disease Information and Research, Kurume University School of Medicine, 67 Asahi-machi, Kurume 830-0011, Japan. Email: takumi@med.kurume-u.ac.jp
Received 19 December 2010; revision 6 February 2011; accepted 22February 2011.

within the Milan criteria, when patients showed an AFP level of 200 ng/mL or less, diagnosis of liver cirrhosis and an AST level of less than 93 IU/mL. On the other hand, 18% of the patients were within the Milan criteria, when patients showed an AFP level of more than 200 ng/mL and ALT level of 20 IU/mL or more.

Conclusion: Data mining disclosed complex interactions of the risk factors and clinical feature profiling associated with the staging of NBNC-HCC.

Key words: data mining, disease progression, hepatoma, non-viral hepatitis, tumor marker

INTRODUCTION

HEPATOCELLULAR CARCINOMA (HCC) is the fifth most common cancer and the third most common cause of cancer-related deaths worldwide.[1–3] Chronic infection with hepatitis B virus (HBV) or hepatitis C virus (HCV) is a risk factor for HCC. Recent developments in the management of patients with viral hepatitis have resulted in early detection of HCC and improvement of prognosis.[4–8]

The number of patients with non-HBV/non-HCV-related HCC (NBNC-HCC) has been increasing, and NBNC-HCC now accounts for 12–16% of all the HCC cases in Japan.[8,9] A variety of factors are involved in the development and progression of this cancer including age, sex, alcoholic liver disease and diabetes mellitus.[10–12] Therefore, neither early detection nor improved prognosis has been achieved in NBNC-HCC.[6] Radical treatment is applicable to patients with NBNC-HCC who meet the Milan criteria;[13] however, this cancer is often detected at an advanced stage. For earlier detection, it is important to understand the complex interactions of the risk factors and clinical feature profiling associated with the Milan criteria, a staging system for NBNC-HCC.

Data mining, a set of statistical techniques, uncovers meaningful patterns and interactions of variables from a large data collection even when there is no a priori hypothesis imposed.[13] Graphical modeling is an exploratory multivariate analysis of data mining that reveals complex associations between variables.[14] This analysis assumes that the response variable is influenced by multiple factors.[15] Therefore, different from results of univariate analysis, an association between a risk factor and an outcome variable may disappear or appear because of the effects of another set of variables known as "confounding factors".[16,17] Furthermore, its findings are visualized as a graph, which provides an idea of how variables interact and denotes the conditional independence structure between random variables.[15] Therefore, graphical modeling is now identified as a new approach to model clinical data.[18]

Decision tree making is another exploratory technique of data mining that represents a series of rules for classification by identifying priorities.[19–21] It is an explicit, quantitative and systematic approach to decision-making under conditions of uncertainty and allows clinicians to choose an option that maximizes the net benefit to the patient.[22] Recently, decision trees were used to reveal the clinical feature profiling for staging of pancreatic cancer[23] and ovarian cancer.[24] However, decision trees have never been applied to identify the clinical feature profiling associated with the staging of NBNC-HCC.

The aims of this study were to reveal complex interactions of the risk factors and clinical feature profiling associated with the staging of NBNC-HCC using data mining techniques.

METHODS

Patient database

BETWEEN 1995 AND 2006, a total of 10 133 patients were diagnosed with HCC at 23 institutions located in Kyushu, a high morbidity area of HCC in Japan. Among them, 1363 patients were diagnosed with NBNC-HCC according to the negative results of both serum hepatitis B surface antigen and serum anti-HCV antibody or HCV RNA.

In order to examine the clinical variables associated with the staging of NBNC-HCC, a database of 663 patients with NBNC-HCC at 20 institutions was created on the basis of the following variables: diagnostic year of HCC; age; sex; family history of liver disease; past history of blood transfusion; alcohol intake; diagnosis of liver cirrhosis; diagnosis of liver disease; diagnosis of diabetes mellitus; serum aspartate aminotransferase (AST) level; serum alanine aminotransferase (ALT) level; serum α-fetoprotein (AFP) level; serum des-γ-carboxy prothrombin (DCP) level; size of HCC; and number of HCC.

For practical use, alcohol intake, serum AFP level and serum DCP level were categorized as follows. Alcohol intake: none; 60 g/day or less; 60–100 g/day; or more than 100 g/day. AFP level: 20 ng/mL or less; 20–200 ng/mL; or more than 200 ng/mL. DCP level: 40 mAU/mL or less; 40–100 mAU/mL; or more than 100 mAU/mL.

The study protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki, as reflected by the approval of the Ethics Committee of the Kurume University School of Medicine.

Diagnosis and staging of HCC

The diagnosis of HCC was based on the clinical practice manual proposed by the Japan Society of Hepatology,[25] by using serum AFP and DCP levels and imaging techniques including ultrasonography, computerized tomography, magnetic resonance imaging, hepatic angiography and/or tumor biopsy. The Milan criteria (single nodule ≤5 cm or three nodules <3 cm) were used for the staging of HCC.[26]

Data mining

An association between the Milan criteria and each risk factor was examined by Student's *t*-test and χ^2-test. Because of the insufficient scientific evidence for testing specific clinical hypotheses, graphical modeling and decision trees were employed to explore complex associations between the Milan criteria and a set of risk factors.

MIM software (http://www.hypergraph.dk/) was used for graphical modeling. R package rpart (recursive partitioning and regression trees by Terry Therneau and Beth Atkinson; http://www.mayo.edu/biostatistics) was used to construct a decision tree algorithm. In order to evaluate the prediction error, the original data ($n = 663$) were randomly divided into a training dataset ($n = 442$) and a test dataset ($n = 221$). Ten-hold cross-validation was conducted to construct the initial tree on the basis of the training dataset; then, the optimal-size tree was constructed by examining a set of cost–complexity parameters. The overall prediction error rate as well as the sensitivity and specificity were calculated by applying the results of the decision tree algorithm to the test dataset.

RESULTS

Characteristics of patients with NBNC-HCC

THE PATIENTS' CHARACTERISTICS are summarized in Table 1. Family history of liver disease and history of blood transfusion were not noted in more than 80% of the patients. Approximately 40% of the patients did not have any etiology of chronic liver disease.

Univariate analysis of variables associated with the Milan criteria

Univariate analysis showed that diagnosis of liver cirrhosis, serum AST level, serum ALT level, serum AFP level and serum DCP level were significantly associated with the Milan criteria (Table 2).

Table 1 Characteristics of all patients

Variable	
n	663
Diagnostic year of HCC (years)	2002 ± 3
Age (years)	68.1 ± 9.9
Male/female	480/183
Family history of liver disease (yes/no/unclear)	79/547/37
History of blood transfusion (no/before 1989/after 1989/unclear)	584/29/22/28
Daily alcohol intake (none/<60 g/60–100 g/>100 g)	254/183/141/85
Etiology of chronic liver disease (none/alcohol/others)	296/188/179
Diagnosis of liver cirrhosis (yes/no)	260/403
Diagnosis of diabetes mellitus (no/yes without medication/yes with medication)	396/109/158
Serum AST level (U/L)	53.3 ± 51.3
Serum ALT level (U/L)	51.8 ± 49.9
Serum AFP level (ng/mL)	9397 ± 71066
Serum DCP level (mAU/mL)	8003 ± 37377
Size of HCC (cm)	5.0 ± 3.4
Number of HCC	2.8 ± 2.9

Data are expressed as the mean ± standard deviation or the number of patients.
AFP, α-fetoprotein; ALT, alanine aminotransferase; AST, aspartate aminotransferase; DCP, des-γ-carboxy prothrombin; HCC, hepatocellular carcinoma.

Graphical modeling

Complex interactions of the risk factors associated with the Milan criteria were visualized graphically (Fig. 1). Graphical modeling identified six independent factors directly associated with the Milan criteria: diagnostic year of HCC; diagnosis of liver cirrhosis; serum AST level; serum ALT level; serum AFP level; and serum DCP level (Fig. 1). Although alcohol intake, diagnosis of liver disease and diagnosis of diabetes mellitus were not directly associated with the Milan criteria, they were associated with the Milan criteria through diagnosis of liver cirrhosis (Fig. 1).

Decision tree algorithm

With the training dataset ($n = 442$), a decision tree algorithm was created by using five variables to classify six groups of patients (Fig. 2). A serum AFP level of 200 ng/mL or less was the cut-off value for the initial

Table 2 Univariate analysis of the variables associated with the Milan criteria

Variable	Statistical method	Test statistics	Degree of freedom (df)	*P*
Diagnostic year of HCC (years)	χ^2	13.4013	11	0.2679
Age (years)	Pooled	−1.07	661	0.2843
Sex	χ^2	0.2975	1	0.5854
Family history of liver disease	χ^2	1.7412	1	0.187
History of blood transfusion	χ^2	4.9527	2	0.084
Daily alcohol intake	χ^2	2.4158	3	0.4907
Liver cirrhosis	χ^2	28.9521	1	<0.0001
Diabetes mellitus	χ^2	0.926	2	0.6294
AST level (U/L)	Satterthwaite	3.06	387.51	0.0023
ALT level (U/L)	Satterthwaite	4.79	546.95	<0.0001
AFP level (ng/mL)	χ^2	63.1357	2	<0.0001
DCP level (mAU/mL)	χ^2	47.7161	2	<0.0001

Associations between the variables and the Milan criteria were analyzed by the indicated statistical methods. $P < 0.05$ was considered significant.
AFP, α-fetoprotein; ALT, alanine aminotransferase; AST, aspartate aminotransferase; DCP, des-γ-carboxy prothrombin; HCC, hepatocellular carcinoma.

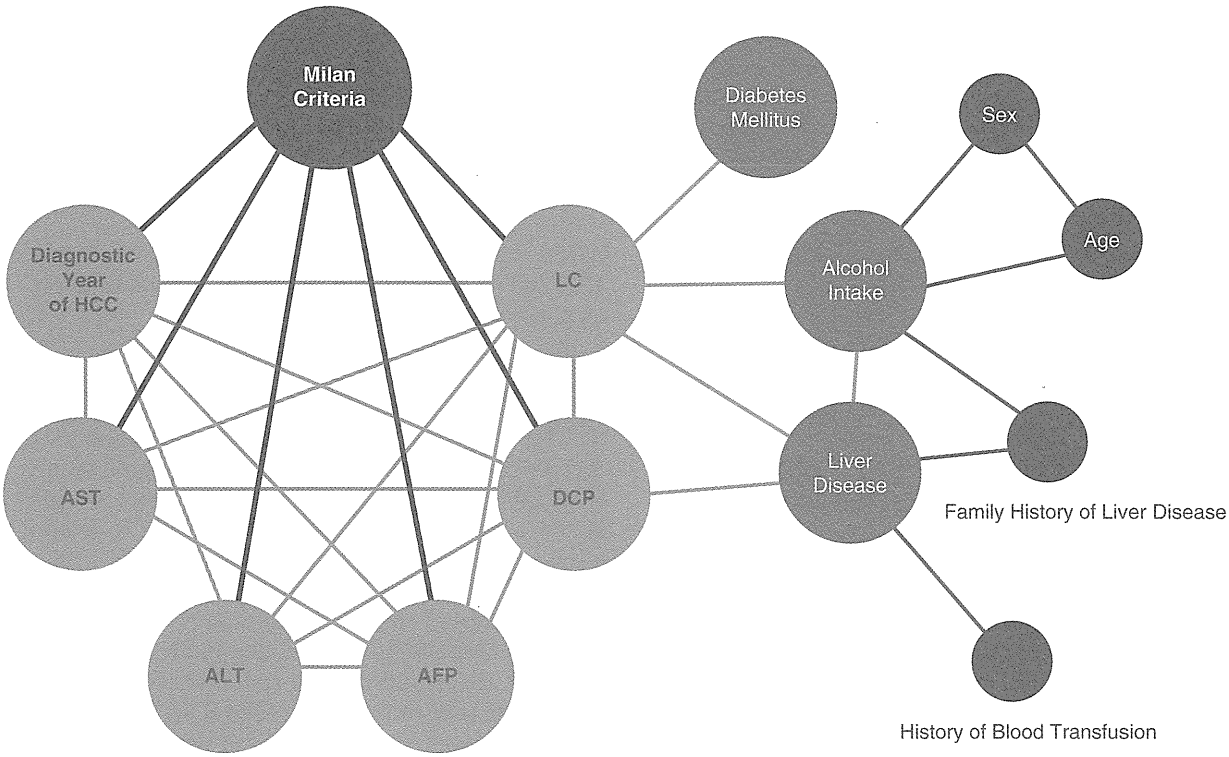

Figure 1 Graphical modeling of the interactions of the risk factors associated with the Milan criteria. AFP, α-fetoprotein; ALT, alanine aminotransferase; AST, aspartate aminotransferase; DCP, des-γ-carboxy prothrombin; HCC, hepatocellular carcinoma; LC, liver cirrhosis.

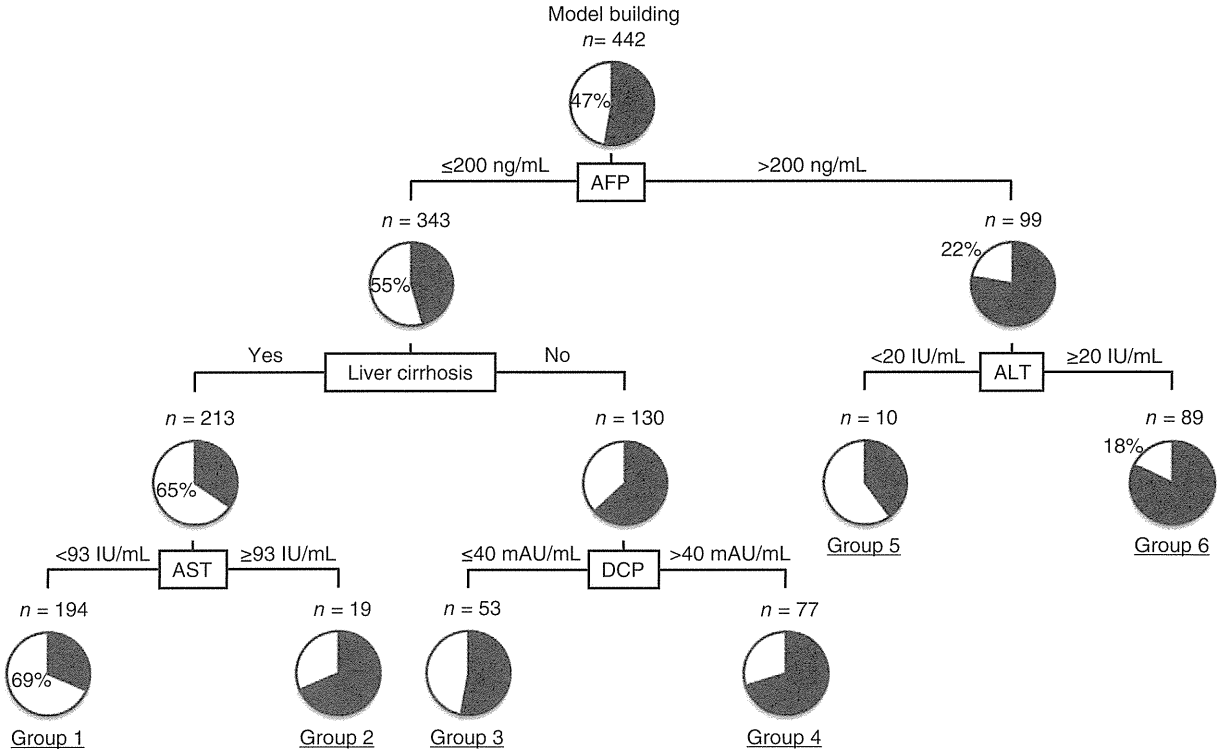

Figure 2 Decision tree algorithm of the variables associated with the Milan criteria. The patients were classified according to the indicated cut-off values of the variables. The pie graphs indicate the percentage of patients with HCC within (white)/beyond the Milan criteria in each group. AFP, α-fetoprotein; ALT, alanine aminotransferase; AST, aspartate aminotransferase; DCP, des-γ-carboxy prothrombin; HCC, hepatocellular carcinoma.

classification. Among the patients with an AFP level of 200 ng/mL or less, diagnosis of liver cirrhosis was used as the variable for the second division. Among the patients with liver cirrhosis, a serum AST level of less than 93 IU/mL was the cut-off value for the third division. Thus, 69% of the patients were within the Milan criteria, when the patients met all of the following conditions: AFP of 200 ng/mL or less; diagnosis of liver cirrhosis; and AST of less than 93 IU/mL (group 1; Fig. 2). On the other hand, only 18% of the patients were within the Milan criteria, when patients showed an AFP level of more than 200 ng/mL and an ALT level of 20 IU/mL or more (group 6; Fig. 2).

There were no significant differences in the patients' characteristics between the training dataset and the test dataset. Prediction error was obtained by applying the results of the decision tree algorithm to the test dataset. The sensitivity (proportion of patients with HCC correctly classified as beyond the Milan criteria) and specificity (proportion of patients with HCC correctly classified as within the Milan criteria) were 72.1% (75/104) and 68.4% (80/117), respectively; the overall prediction error rate was 29.8% (66/221).

DISCUSSION

IN THIS STUDY, we revealed the complex interactions of the risk factors associated with staging of NBNC-HCC using graphical modeling. In addition, we presented a decision tree algorithm to identify clinical feature profiling associated with the staging of NBNC-HCC.

Various factors seem to be intricately related to the progression of NBNC-HCC. In this study, by graphical modeling, we identified six variables directly associated with the Milan criteria: serum AST level; serum ALT level; serum AFP level; serum DCP level; diagnosis of liver cirrhosis; and diagnostic year of HCC. Chronic hepatic inflammation modulates many of the signaling cascades involved in cell proliferation, survival and invasion of