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ORIGINAL ARTICLE—LIVER, PANCREAS, AND BILIARY TRACT

Clinical characteristics, treatment, and prognosis of non-B, non-C hepatocellular carcinoma: a large retrospective multicenter cohort study

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Abstract
Background The number of hepatocellular carcinoma (HCC) patients with non-viral etiologies is increasing in Japan. We conducted a nation-wide survey to examine the characteristics of those patients.
Methods After we assessed the trend of patients who were first diagnosed with HCC at 53 tertiary care centers in Japan from 1991 to 2010, we collected detailed data of 5326 patients with non-viral etiology. The etiologies were categorized as autoimmune hepatitis, primary biliary cirrhosis, alcoholic liver disease (ALD), non-alcoholic fatty liver disease (NAFLD), unclassified, and other. Baseline characteristics at initial diagnosis, the modality of the initial treatment, and survival status were collected via a website. Survival of the patients was assessed by the Kaplan–Meier method and Cox proportional hazard regression.
Results The proportion of patients with non-viral etiologies increased from 10.0 % in 1991 to 24.1 % in 2010. Of the patients, 92 % were categorized as ALD, NAFLD, or unclassified. Body mass index (BMI) was $\geq$ 25 kg/m^2 in 39 %. Diabetes was most prevalent in NAFLD (63 %), followed by unclassified etiology (46 %) and ALD (45 %). Approximately 80 % of patients underwent radical therapy, including resection, ablation, or transarterial chemoembolization. Survival rates at 3, 5, 10, 15, and 20 years were 58.2, 42.6, 21.5, 15.2, and 15.2 %, respectively. Multivariate analysis revealed that patients with BMI $>$ 22 and $\leq$ 25 kg/m^2 showed the best prognosis versus other BMI categories, after adjusting by age, gender, tumor-related factors, and Child-Pugh score.
Conclusions Most cases of non-B, non-C HCC are related to lifestyle factors, including obesity and diabetes. Slightly overweight patients showed the best prognosis.

Keywords Hepatocellular carcinoma · Non-alcoholic fatty liver disease · Non-alcoholic steatohepatitis · Alcoholic liver disease · Retrospective study

For the INUYAMA NOBLESSE Study group. Members of the INUYAMA NOBLESSE Study group are listed in "Appendix".

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Introduction

Hepatocellular carcinoma (HCC) is a typical example of an infection-associated malignancy [1]. The geographical

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distribution of the highly endemic area of HCC overlaps that of chronic hepatitis B and C [2]. Rigorous efforts to control horizontal transmission of hepatitis B virus (HBV) by vaccination since the mid-1980s succeeded in reducing hepatitis B-related HCC in children [3]. Screening for hepatitis C virus (HCV) and the ending of paid blood donations markedly reduced the incidence of transfusion-associated hepatitis [4]. In those with active chronic hepatitis B, long-term suppression using nucleotide analogs may reduce the incidence of HBV-related HCC [5, 6], and the eradication of HCV by interferon-based therapy can reduce HCV-related HCC [7, 8]. It can reasonably be concluded that hepatitis virus-related HCC will continue to decrease in the future [9, 10].

While HCC is a typical example of a virus-related cancer, it is also well known to be strongly related to life style. Chronic alcoholism is a classical risk factor [11]; more recently, obesity has been recognized to strongly affect HCC development in males, versus various other malignancies [12]. There is also growing evidence suggesting that type 2 diabetes increases the incidence of HCC [13, 14]. Due to the globally increasing proportion of the obese population over the past 30 years [15], obesity-related HCC will likely continue to increase.

Unlike virus-related HCC, in which the high-risk populations and surveillance programs are well established, little is known about the characteristics of virus-unrelated HCC. To reduce the forthcoming global burden of obesity-related HCC, to clarify its clinical features is quite important. The Non-B, Non-C Liver Cancer, Etiology, Prognosis and Treatment (NOBLESSE) study was conducted as a special project of the Inuyama Symposium, an assembly of 56 gastroenterology and hepatology units in university hospitals and tertiary care hospitals in Japan, to investigate the characteristics of non-B, non-C HCC patients.

Patients and methods

Patients

This retrospective study complied with the ethical guidelines for epidemiological research designed by the Japanese Ministry of Education, Culture, Sports, Science and Technology and Ministry of Health, Labour, and Welfare. The study protocol was approved by the University of Tokyo Medical Research Center Ethics Committee (approval number 3710) and the Institutional Review Board or Ethics Committee of each participating institution. Informed consent was waved because of the retrospective design. This study was registered with the University Hospital Medical Information Network (UMIN) Clinical Trial Registry (UMIN-CTR000007570).

First we collected the number of patients with HCC who were first diagnosed with HCC in the participating hospitals from 1991 to 2010 and categorized them as HBV-related, HCV-related, both HBV and HCV-related, and non-B, non-C to assess trends in the proportion of background etiologies. Next we collected detailed data of non-B, non-C HCC patients defined as negative for both hepatitis B surface antigen (HBsAg) and anti-HCV antibody. Patients who lost HBsAg before the diagnosis of HCC or who were positive for HBV DNA were excluded.

Diagnosis of HCC

The diagnosis of HCC was made by dynamic computed tomography (CT) or dynamic magnetic resonance imaging (MRI) with consideration of hyperattenuation in the arterial phase, with washout in the late phase as a definite sign of this disease [16] or pathology. In years when dynamic CT was not available, the diagnosis was also made by angiography.

Data collection

The patients were registered via a website specially designed by the investigators. The following characteristics at diagnosis were collected: age, gender, body height, body weight, etiology of background liver disease, daily alcohol consumption; comorbidities including liver cirrhosis, fatty liver by ultrasonography, hypertension, dyslipidemia, and diabetes; tumor factors including tumor size of the maximal nodule, number of tumor nodules, the presence of vascular invasion, and extrahepatic metastasis; symptoms including ascites and hepatic encephalopathy, laboratory data, including serum albumin, total bilirubin, aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), gamma-glutamyl transpeptidase (GGT), platelet count, prothrombin activity, alpha-fetoprotein (AFP), des-gamma-carboxyprothrombin (DCP), and lens culinaris agglutinin-reactive fraction of AFP (AFP-L3); and treatment modality for the first time, including hepatic resection, liver transplantation, ablation, transarterial chemoembolization (TACE), transarterial chemotherapy, systemic chemotherapy, radiation therapy, and supportive therapy. Body mass index (BMI), Child–Turcotte–Pugh (CTP) score, and Barcelona-Clinic–Liver-Cancer (BCLC) stages were calculated automatically using the data obtained above.

The etiology of background liver diseases was categorized as follows: autoimmune hepatitis (AIH), primary biliary cirrhosis (PBC), AIH–PBC overlap syndrome, alcoholic liver disease, non-alcoholic fatty liver disease (NAFLD), Budd-Chiari syndrome, hemochromatosis, Wilson disease, and others. The diagnosis of the

background liver disease, hypertension, dyslipidemia, and diabetes was made by the attending physician, based on the Japanese clinical guidelines for each disease. Daily alcohol consumption was calculated from forms of alcohol and frequency. Alcoholic liver disease was defined as chronic liver injury with daily alcohol consumption $\geq$ 80 g/day without another definite etiology. NAFLD was defined as a history of fatty liver or who were diagnosed with fatty liver, radiologically or pathologically, with alcohol consumption $\leq$ 20 g/day. Those with cryptogenic chronic liver disease who did not meet the criteria described above for alcoholic liver disease or NAFLD were categorized as unclassified.

Patient survival status was also registered. Status was defined as alive, dead, or lost to follow-up. Observations were censored on 31 December 2011. In diseased patients, the cause of death was categorized according to the criteria of the Liver Cancer Study Group of Japan [17], as follows: liver cancer progression, liver failure, gastrointestinal bleeding, gastro-esophageal varices rupture, rupture of liver cancer, operative death, other, and unknown.

Statistical analysis

Data are expressed as medians with 25th to 75th percentiles, unless otherwise indicated. Numbers and percentages were used for qualitative variables. Student's t test was used for comparisons of two continuous variables. Differences among groups were assessed with one-way analysis of variance (ANOVA) for continuous data, and with the Chi squared test for categorical data. The Cochran–Armitage trend test was used to evaluate increasing or decreasing trends in etiology. Survival time was defined as the interval between the day of the first diagnosis and death or the last visit to the hospital until 31 December 2011. Cumulative survival curves were constructed with the Kaplan–Meier method and compared with the log-rank test. To assess the hazard ratios of various factors on overall survival, the Cox proportional hazard model was used.

Statistical analyses were performed using the 'R' software (ver. 2.13.0; http://www.R-project.org). All tests were two-sided, and p values < 0.05 were considered to indicate statistical significance.

Results

Patient profiles

Of 33,782 patients who were first diagnosed with HCC at the 53 participating hospitals from 1991 to 2010, 5326 (15.8 %) were categorized as non-B, non-C. A marked increase in the proportion of patients categorized as non-B, non-C was observed ($p < 0.001$ by Cochran–Armitage test; Fig. 1). The proportion of non-B, non-C patients was 24.1 % in 2010, whereas it was only 10.0 % in 1991. The distribution of background liver diseases among non-B, non-C patients was as follows: AIH in 161 (3.0 %), PBC in 164 (3.1 %), AIH–PBC overlap syndrome in 18 (0.3 %), alcoholic liver disease in 1423 (26.7 %), NAFLD in 596 (11.2 %), Budd-Chiari Syndrome in 20 (0.4 %), hemochromatosis in 9 (0.2 %), Wilson's disease in 5 (0.1 %), unclassified in 2875 (54.0 %), and other in 53 (1.0 %). 'Other' included schistosomiasis japonica, suspicion of autoimmune liver diseases, and normal liver. As few patients were categorized as AIH–PBC overlap syndrome, Budd-Chiari syndrome, hemochromatosis and Wilson's disease, they were combined with 'others' in Table 1. Among non-B, non-C patients, 31 and 10 % were diagnosed as HCC at the department of gastroenterology or hepatology and other department in the participating hospital, respectively. The remaining 59 % were diagnosed at other hospitals and referred to the participating hospitals. Forty-one percent of patients were followed by imaging modalities before the diagnosis of HCC.

The median [interquartile range (IQR)] age in the entire cohort was 70.0 (63.0–75.0) years and approximately three-quarters were males. Patients with alcoholic liver disease were significantly younger than other etiologies ($p < 0.001$). The male to female ratio was different among the etiologies: females predominated in autoimmune liver diseases. The vast majority were non drinkers or light drinkers, except for those with alcoholic liver disease or unclassified etiology. Among those judged as unclassified, 41 % were moderate drinkers.

The distribution of BMI varied across the etiologies and gender. The median BMI was the highest in those with

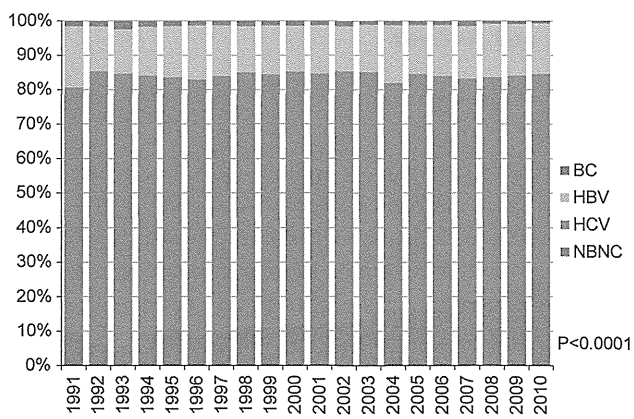

Fig. 1 Trend in background liver disease in hepatocellular carcinoma in Japan. A marked increase in the proportion of patients categorized as non-B, non-C in the participating hospitals was observed ($p < 0.001$ by Cochran–Armitage test)

Table 1 Baseline characteristics of the HCC patients analyzed in this study (n = 5,326)

	ALL	AIH	PBC	Alcoholic liver disease	NAFLD	Unclassified	Others
Number of patients	5,326	161	166	1,423	596	2,875	105
Age (year)							
Median	70.0	70.0	71.5	66.0	72.0	71.0	70.0
IQR	63.0–75.0	66.0–76.0	66.0–77.0	60.0–72.0	66.0–77.0	64.0–76.0	58.0–76.0
Male gender [*n* (%)]	4,022 (75.5)	43 (26.7)	52 (31.3)	1,327 (93.3)	348 (58.4)	2,188 (76.1)	64 (61.0)
Alcohol consumption (g/day)[a]							
≤ 20 [*n* (%)]	2623 (50.9)	144 (90.0)	146 (90.7)		596 (100.0)	1661 (59.0)	80 (86.0)
21–79 [*n* (%)]	1179 (22.9)	9 (5.6)	9 (5.6)			1154 (41.0)	7 (7.5)
≥ 80 [*n* (%)]	1351 (26.2)	7 (3.7)	6 (3.7)	1423 (100.0)			6 (6.5)
Diabetes [*n* (%)][b]	2345 (46.1)	48 (30.6)	27 (17.0)	621 (45.2)	359 (62.7)	1264 (46.4)	26 (27.1)
Hypertension [*n* (%)][c]	2063 (42.7)	51 (35.4)	42 (26.8)	493 (38.0)	313 (55.5)	1135 (44.1)	29 (31.9)
Dyslipidemia [*n* (%)][d]	720 (14.6)	26 (17.1)	12 (7.6)	171 (12.7)	125 (22.9)	374 (14.2)	12 (12.6)
Fatty liver [*n* (%)][e]	936 (24.0)	18 (15.5)	7 (5.5)	219 (20.7)	280 (64.4)	403 (19.3)	9 (13.4)
Liver Cirrhosis [*n* (%)][f]	3439 (67.0)	127 (80.9)	145 (87.9)	1115 (80.2)	368 (63.4)	1619 (59.0)	65 (67.0)
Anti-HBcAb positive [*n* (%)][g]	1501 (40.3)	27 (23.5)	35 (31.3)	410 (40.8)	159 (34.6)	837 (43.0)	33 (40.7)
ALT (U/L)							
Median	32	29	29	33	33	32	29
IQR	22–50	20–44	20–41.3	22–50	22–51	22–51	20–54
Platelet count ($\times 10^9/\mu L$)[h]							
Median	135	105	103	123	138	148	124
IQR	90–193	72–166	74–139	84–173	94–189	97–205	81–183
Child-Pugh class[i]							
A [*n* (%)]	3500 (69.0)	89 (57.4)	83 (52.9)	843 (62.1)	439 (76.5)	1976 (72.4)	70 (72.2)
B [*n* (%)]	1231 (24.3)	54 (34.8)	57 (36.3)	383 (28.2)	120 (20.9)	595 (21.8)	22 (22.7)
C [*n* (%)]	338 (6.7)	12 (7.7)	17 (10.8)	131 (9.7)	15 (2.6)	158 (5.8)	5 (5.2)
Tumor characteristics							
Maximal tumor size (cm)[j]							
Median	3.2	3.0	2.8	3.0	3.0	3.5	3.0
IQR	2.0–6.0	2.0–4.3	1.7–3.5	2.0–5.0	2.0–5.0	2.2–7.0	2.0–5.1
Diffuse type [*n* (%)]	209 (4.0)	6 (3.7)	1 (0.6)	62 (4.4)	17 (2.9)	119 (4.2)	4 (3.8)
Number of nodules[k]							
Single [*n* (%)]	2700 (51.1)	87 (54.0)	110 (66.3)	664 (46.8)	340 (57.0)	1443 (50.8)	56 (53.8)
2–3 [*n* (%)]	1368 (25.9)	46 (28.6)	40 (24.1)	402 (28.3)	156 (26.2)	697 (24.5)	27 (26.0)
> 3 [*n* (%)]	1220 (23.1)	28 (17.4)	16 (9.6)	353 (24.9)	100 (16.8)	702 (24.7)	21 (20.2)
Vascular invasion [*n* (%)][l]	187 (3.5)	3 (1.9)	1 (0.6)	52 (3.7)	13 (2.2)	116 (4.1)	2 (1.9)
Extrahepatic metastasis [*n* (%)][m]	401 (7.6)	8 (5.0)	2 (1.2)	114 (8.0)	26 (4.4)	244 (8.6)	7 (6.7)
AFP (ng/mL)[n]							
≤20 [*n* (%)]	2908 (59.4)	80 (54.1)	71 (51.4)	827 (62.4)	361 (63.1)	1515 (58.0)	54 (55.7)
21–200 [*n* (%)]	820 (16.8)	33 (22.3)	29 (21.0)	229 (17.3)	92 (16.1)	423 (16.2)	14 (14.4)
>200 [*n* (%)]	1164 (23.8)	35 (23.6)	38 (27.5)	270 (20.4)	119 (20.8)	673 (25.8)	29 (29.9)
DCP (mAU/mL)[o]							
≤100 [*n* (%)]	2121 (45.8)	75 (53.6)	81 (59.1)	593 (46.8)	299 (53.9)	1032 (42.1)	41 (47.7)
101–400 [*n* (%)]	787 (17.0)	23 (16.4)	25 (18.2)	227 (17.9)	95 (17.1)	400 (16.3)	17 (19.8)
>400 [*n* (%)]	1727 (37.3)	42 (30.0)	31 (22.6)	448 (35.3)	161 (29.0)	1017 (41.5)	28 (32.6)
AFP-L3 (%)[p]							
≤10 [*n* (%)]	1765 (67.7)	53 (64.6)	39 (55.7)	498 (69.6)	263 (73.5)	881 (66.1)	31 (66.0)
10.1–15 [*n* (%)]	74 (2.8)	3 (3.7)	4 (5.7)	17 (2.4)	7 (2.0)	43 (3.2)	0 (0)
>15 [*n* (%)]	767 (29.4)	26 (31.7)	27 (38.6)	201 (28.1)	88 (24.6)	409 (30.7)	16 (34.0)

As few patients were categorized as having the AIH–PBC overlap syndrome, Budd-Chiari syndrome, hemochromatosis or Wilson's disease, they were combined with 'others'. Data were missing in [a]173, [b]241, [c]498, [d]388, [e]1434, [f]193, [g]1606, [h]61, [i]257, [j]42, [k]38, [l]28, [m]26, [n]434, [o]691, and [p]3677 patients

AFP alpha-fetoprotein, *AFP-L3* lens culinaris agglutinin-reactive fraction of AFP, *ALT* alanine aminotransferase, *Anti-HBcAb* anti-hepatitis B core antibody, *DCP* des-gamma-carboxy prothrombin, *IQR* interquartile range

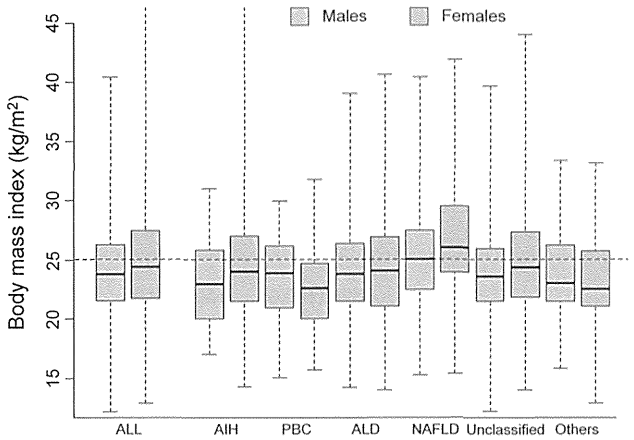

Fig. 2 Body mass index according to background liver disease. Median (25th–75th percentiles) BMI values in all categories were 23.8 (21.6–26.3) kg/m^2 in males and 24.4 (21.8–27.5) kg/m^2 in females. *Box plot 'whiskers'* show the minimum and maximum values; the *horizontal line* in each box plot shows the median, and the *colored* segment shows the interquartile range. *AIH* autoimmune hepatitis, *PBC* primary biliary cirrhosis, *ALD* alcoholic liver disease, *NAFLD* non-alcoholic fatty liver disease

NAFLD. Females had significantly higher BMI than males in NAFLD and those unclassified ($p = 0.01$ and <0.001, respectively; Fig. 2).

Nearly half of the patients were complicated with diabetes (Table 1, Supplementary Fig. 1). The proportion of those with diabetes was highest in NAFLD patients. A similar trend was observed in the proportions of hypertension and dyslipidemia. The presence of fatty liver, judged by ultrasonography at the diagnosis of HCC, varied across the etiologies. The proportion was approximately 20 % in alcoholic liver disease and unclassified etiology, while it was lower in autoimmune liver diseases, especially PBC. It was also suggested that fatty liver could not be detected by ultrasonography in approximately 30 % at the diagnosis of HCC in NAFLD.

Approximately two-thirds of the patients were complicated with cirrhosis. The proportion of those with cirrhosis was lower in those with NAFLD and unclassified etiology compared with other etiologies ($p < 0.001$). Reflecting the proportion of cirrhosis, platelet counts were highest in those with unclassified etiology, followed by those with NAFLD.

Regarding the diagnosis process, 30.3 % of the patients had their tumor pointed out for the first time in the participating department, 10.6 % in another department of the same hospital, and 59.1 % at other hospitals. Patients were diagnosed at more advanced stages in those with unclassified etiology; the tumor size was the largest and the proportion of patients with vascular invasion and extrahepatic metastasis was also the largest. The sensitivity of DCP was superior to that of AFP (54.2 vs. 40.6 % with cutoff values of 100 mAU/mL and 20 ng/mL, respectively).

Treatment and survival

Among 5058 patients in whom BCLC staging could be determined, 2533 (50.1 %), 1913 (37.8 %), 283 (5.6 %), and 329 (6.5 %) were categorized as stages A, B, C, and D, respectively (Table 2). The distribution of the initial treatment was as follows: resection in 1073 (20.3 %), ablation in 1060 (20.0 %), TACE + ablation in 470 (8.9 %), TACE in 1590 (30.1 %), transarterial chemotherapy with one-shot and continuous infusion in 99 (1.9 %), systemic therapy in 20 (0.3 %), radiation therapy in 20 (0.4 %), liver transplantation in 17, others in 30 (0.6 %), and supportive care in 429 (8.1 %).

During the mean follow-up period of 2.6 years, 2225 patients died and 670 patients were lost to follow-up. The causes of death were cancer progression in 1411 (58.0 %), liver failure in 359 (14.8 %), gastrointestinal bleeding, including varices rupture, in 87 (3.6 %), tumor rupture in 71 (2.9 %), operative death in 13 (0.5 %), and other in 284 (11.7 %). The cause of death was unspecified in 206 (8.5 %). Median survival time [95 % confidence interval (CI)] after the initial diagnosis of HCC was 4.03 (3.82–4.20) years. Overall survival rates at 1, 3, 5, 7, 10, 15, and 20 years were 80.1, 58.2, 42.6, 32.2, 21.5, 15.2,

Table 2 Distribution of treatments according to BCLC stage

	A	B	C	D
Number of patients	2533	1913	283	329
Hepatic resection [*n* (%)]	616 (24.3)	398 (20.8)	30 (10.6)	3 (0.9)
Ablation [*n* (%)]	887 (35.0)	81 (4.2)	4 (1.4)	52 (15.8)
TACE + ablation [*n* (%)]	335 (13.2)	116 (6.1)	3 (1.1)	4 (1.2)
TACE [*n* (%)]	517 (17.1)	840 (43.9)	78 (27.6)	83 (25.2)
Transarterial chemotherapy [*n* (%)]	83 (3.2)	278 (14.5)	87 (30.7)	27 (8.2)
Systemic therapy [*n* (%)]	5 (0.2)	50 (2.6)	25 (8.8)	7 (2.1)
Radiation therapy [*n* (%)]	5 (0.2)	4 (0.2)	3 (1.1)	5 (1.5)
Liver transplantation [*n* (%)]	11 (0.4)	6 (0.3)	0(0.0)	0 (0.0)
Others [*n* (%)]	12 (0.5)	5 (0.3)	2 (0.7)	4 (1.2)
Supportive therapy [*n* (%)]	64 (2.5)	135 (7.1)	51 (18.0)	144 (43.8)

BCLC stage could not be determined in 268 patients

TACE transarterial chemoembolization

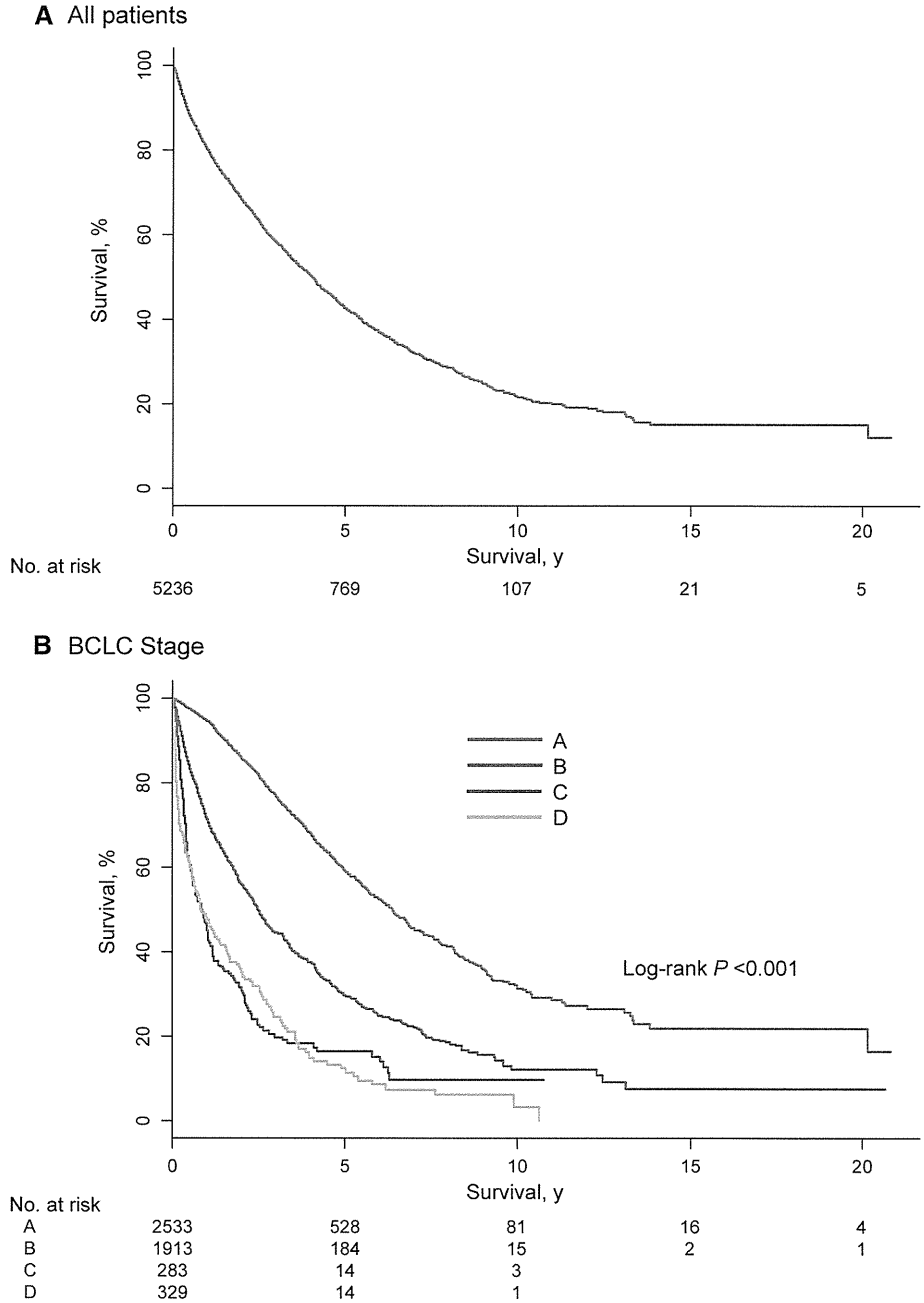

Fig. 3 Overall survival. **A** Overall survival of the entire patient cohort. Overall survival rates at 1, 3, 5, 7, 10, 15, and 20 years were 80.1, 58.2, 42.6, 32.2, 21.5, 15.2, and 15.2 %, respectively. **B** Overall survival according to BCLC stage. Survival rates at 1, 3, 5, 7, 10, 15, and 20 years were 94.5, 76.4, 58.7, 44.7, 30.7, 21.9, and 21.9 % in stage A, 71.1, 44.1, 29.1, 22.2, 13.0, 9.0, and 9.0 % in stage B, 44.6, 18.8, 15.5, 9.3, and 9.3 % in Stage C, and 48.0, 24.4, 12.3, 7.3, 3.1 %, respectively, in Stage D

and 15.2 %, respectively (Fig. 3a). When stratified by BCLC stage, the median (95 % CI) survival times were 6.39 (5.96–6.85), 2.48 (2.34–2.68), 0.83 (0.61–1.03), and 0.80 (0.64–1.23) years in BCLC stages A, B, C, and D, respectively. There was a significant difference in survival among the stages (Fig. 3b, $p < 0.001$).

Univariate Cox regression analysis revealed that the following factors were significantly related to poor survival: old age ($p < 0.001$), male gender ($p = 0.003$), alcohol consumption $\geq$80 g/day ($p < 0.001$), BMI ($p = 0.001$), Child-Pugh score ($p < 0.001$), maximal tumor size ($p < 0.001$), number of nodules ($p < 0.001$), the presence of vascular invasion ($p < 0.001$), the presence of extrahepatic metastasis ($p < 0.001$), AFP ($p < 0.001$), DCP ($p < 0.001$), and AFP-L3 ($p < 0.001$). The presence of diabetes was indicated as a better prognosis factor, though with marginal significance (hazard ratio, 0.93; 95 % CI, 0.86–1.01; $p = 0.08$). BMI showed a V-shaped hazard distribution: those with BMIs of 22.1–25 kg/m^2 had the best outcomes, whereas those with higher and lower BMI showed worse prognoses. We plotted relative hazard against BMI using cubic splines. The V-shape hazard distribution was also observed in the plot (Supplementary Fig. 2).

We performed a multivariate analysis using the variables above, except that AFP-L3 was excluded because of missing values. The results showed that age, BMI, alcohol consumption, Child-Pugh score, tumor size, number of tumor nodules, extrahepatic metastasis, AFP, and DCP were significant factors related to poor prognosis (Fig. 4). The presence of diabetes again showed no statistical significance.

Discussion

In the present study, a rapidly increasing proportion of HCC patients with non-viral etiologies was found. A similar trend was reported in a national survey by the Liver Cancer Study Group of Japan [18]. As the number of newly diagnosed HCC cases in Japan was almost at a plateau throughout the study period [19], not only the proportion,

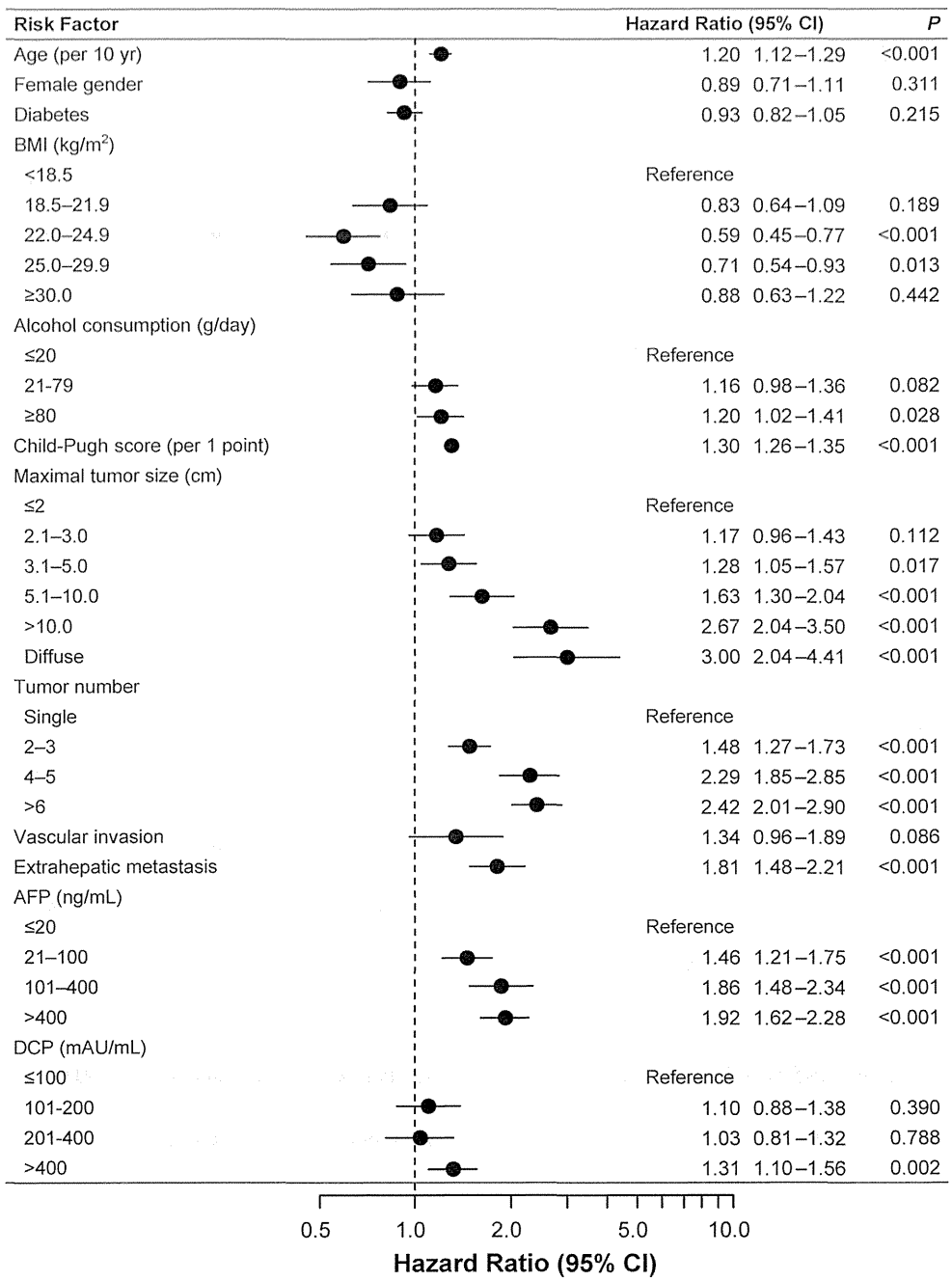

Fig. 4 Multivariate Cox proportional hazard regression analysis of survival. *AFP* alpha-fetoprotein, *AFP-L3* lens culinaris agglutinin-reactive fraction of AFP, *DCP* des-gamma-carboxy prothrombin *AFP* alpha-fetoprotein, *AFP-L3* lens culinaris agglutinin-reactive fraction of AFP, *ALT* alanine aminotransferase, *Anti-HBcAb* anti-hepatitis B core antibody, *DCP* des-gamma-carboxy prothrombin, *IQR* interquartile range

but also the number, of patients with non-viral etiologies was increasing. As a risk factor of HCC, alcohol consumption has not increased over the last two decades in Japan according to statistics from the Ministry Labour and Welfare in Japan [20]. In contrast, the size of the obese population is increasing rapidly due to changes in the diet in Japan. The proportion of patients with diabetes has also increased in the past three decades [21]. It seems reasonable that the rapidly increasing number of HCC patients with non-viral etiologies was largely due to the rapidly increasing obese population.

Among non-viral chronic liver diseases, the natural history of AIH, PBC, and alcoholic liver disease are well known compared with that of NAFLD. In these three, HCC ordinarily arises through cirrhosis after long-lasting chronic inflammation in the liver [22–24]. Indeed, liver cirrhosis was a complication in more than 80 % of those patients. In contrast, the proportion of cirrhosis was smaller and platelet counts were higher in NAFLD patients than those with AIH, PBC, and alcoholic liver disease. It has been reported that a significant proportion of patients (41.7 %) with both NAFLD and HCC are not complicated with cirrhosis [25]. That a significant proportion of patients with NAFLD or unclassified etiology were not complicated with cirrhosis suggests that to characterize a high-risk population within them would be difficult.

In this study, almost half of the patients were complicated with diabetes mellitus. According to a systematic review investigating the relationship between diabetes and HCC, the presence of diabetes is an approximately 2.5-fold risk of HCC [26]. Judging from the wide variation in the proportion of patients with diabetes among the etiologies, it seems that diabetes correlates more strongly with hepatocarcinogenesis in some chronic liver diseases, such as NAFLD, than others.

In this study, we defined NAFLD as a history of fatty liver and alcohol consumption of no more than 20 g/day. As shown in Table 1, fatty liver was not diagnosed by ultrasonography at the diagnosis of HCC in approximately 30 % of patients with NAFLD-related HCC. Those patients would be categorized as unclassified when a history of fatty liver was not confirmed. That is, a significant proportion of those categorized as unclassified could be burn-out non-alcoholic steatohepatitis (NASH). Similarly, alcohol-related HCC could be included in unclassified patients because approximately 40 % of the patients in the category were moderate drinkers. In the first place, it might be unreasonable to categorize those patients clearly, because moderate alcohol intake, obesity, and fatty liver are mutually correlated and may have a synergistic effect on hepatocarcinogenesis.

Occult infection with HBV represented by the presence of antibody to hepatitis B core antigen (anti-HBc) has been considered as a risk factor of non-B, non-C HCC defined as negative for both HBsAg and anti-HCV antibody [27, 28]. Indeed the prevalence of anti-HBc antibody was higher in this study compared to a previous report in blood donors [29]. It is also to be noted that those with anti-HBc antibody may include chronic HBV carriers with HBsAg loss before the diagnosis of HCC, who had significant risk for HCC [30].

Patients were diagnosed at less-advanced stages than we expected. This is partly because all participating hospitals were tertiary care centers. Those with terminal stages diagnosed in primary or secondary hospitals were unlikely to be referred to the participating hospitals. In addition, 41 % of patients were followed by imaging modalities before the diagnosis of HCC. As a result, a large majority of patients underwent radical therapies, such as hepatic resection, ablation, or TACE, as the initial treatment.

Prognostic factors for HCC have been investigated fully in previous studies [31]. However, to our knowledge, this is the first report of the detailed relationship between BMI and survival in HCC patients. Indeed, BMI showed a V-shaped hazard function for death. It is well known that the relationship between BMI and all-cause mortality is V-shaped, with a BMI around 22 kg/m^2 showing the best prognosis. However in this study, the lowest relative hazard was observed at a slightly overweight BMI. We had expected that the best BMI would be around 22 kg/m^2, because obesity is thought to affect hepatocarcinogenesis in this cohort and may affect recurrence after treatment. This would be because the relatively underweight patients included those with more advanced disease. However, the trend remained after adjustment for other significant factors, including those related to the tumor. Overweight patients may have some advantage versus underweight patients that we did not investigate.

The presence of diabetes did not affect survival in this study. One meta-analysis reported that the presence of diabetes increased the risk of all-cause mortality in HCC patients by 1.38-fold (95 % CI, 1.13–1.68) [32]. It is quite reasonable that those with diabetes had additional risk for death from cardiovascular, cerebrovascular, infectious or renal diseases. Some kind of biases might exist behind the fact that the presence of diabetes did not worsen the patients' survival, which needs further investigation.

Most of the major limitations of this study relate to its retrospective design.

(1) Because the major data source was a database maintained by each participating hospital, some data were missing. Patients who were not registered in the database could not be entered into this study. However, the proportion of patients with missing data on important items, such as alcohol consumption, was less than 5 %; this would not affect the overall results. (2) As the amount of daily

alcohol intake was self-reported, some patients might have underreported their alcohol intakes. Some should possibly have been categorized as having alcoholic liver disease. (3) Similarly, because the diagnosis of NAFLD was based on a past history or ultrasound examination at the diagnosis of HCC, undiagnosed burn-out NASH patients were included in those unclassified, especially when not followed in clinics or hospitals. Based on the high proportion of those with lifestyle diseases and moderate drinkers, at least a majority of those unclassified would be related to chronic alcoholism, obesity, or both.

In conclusion, the proportion of HCC patients without chronic viral hepatitis in Japan is increasing rapidly. Most had lifestyle disease-related backgrounds, especially related to obesity. Narrowing down a high-risk population would be difficult because one-third of the patients were non-cirrhotic, and obesity, fatty liver, and diabetes are prevalent in Japan.

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

Appendix

The following investigators enrolled patients in the Inuyama NOBLESSE Study: Joji Toyota, Yoshiyasu Karino (Hokkaido P.W.F.A.C Sapporo-Kosei General Hospital, Sapporo); Kazuyuki Suzuki, Hidekatsu Kuroda (Iwate Medical University, Iwate); Yoshiyuki Ueno, Hisayoshi Watanabe (Yamagata University Faculty of Medicine, Yamagata); Yutaka Aoyagi, Hirokazu Kawai (Niigata University Graduate School of Medical and Dental Science, Niigata); Eiji Tanaka, Takefumi Kimura (Shinshu University School of Medicine, Matsumoto); Kendo Kiyosawa, Hiromitsu Mori (Nagano Red Cross Hospital, Nagano); Nobuyuki Enomoto (University of Yamanashi Faculty of Medicine, Chuo); Masao Omata, Hitoshi Mochizuki (Yamanashi Central Hospital, Kofu); Satoshi Mochida, Mie Inao (Saitama Medical University, Irumagun); Kunihiko Hino, Hiromi Hoshino (Delta Clinic, Tokorozawa); Masashi Mizokami, Kazumoto Murata (Kohnodai Hospital, National Center for Global Health and Medicine, Ichikawa); Osamu Yokosuka, Fumihiko Kanai (Chiba University Graduate School of Medicine, Chiba); Ryosuke Tateishi, Kenichiro Enooku, Koji Uchino, Masaya Sato, Shintaro Kayaki, Tatsuya Minami, Shintaro Mikami, Naoto Fujiwara (University of Tokyo Graduate School of Medicine, Tokyo); Sumio Watanabe, Kazuyoshi Kon (Juntendo University, Tokyo); Michio Imawari, Junichi Eguchi (Showa University, Tokyo); Hajime Takikawa, Masaki Mikami (Teikyo University, Tokyo); Shunji Mishiro, Masahiro Arai (Toshiba General Hospital, Tokyo); Hiromitsu Kumada, Yusuke Kawamura (Department of Hepatology, Toranomon Hospital, Kawasaki); Namiki Izumi, Takanori Hosokawa (Musashino Red-Cross Hospital, Musashino); Mitsuhiko Moriyama, Jumpei Hayashi (Nihon University School of Medicine, Tokyo); Michihiro Suzuki, Kotaro Matsunaga (Kawasaki City Tama Hospital, Kawasaki); Katsuaki Tanaka, Manabu Morimoto (Yokohama City University Medical Center, Yokohama); Takafumi Ichida, Katsuharu Hirano (Juntendo University Shizuoka Hospital, Izunokuni); Yasuhito Tanaka, Kei Fujiwara (Nagoya City University Graduate School of Medical Sciences, Nagoya); Takashi Kumada (Ogaki Municipal Hospital, Ogaki); Hisataka Moriwaki, Koji Takai (Gifu University Graduate School of Medicine, Gifu); Shuichi Kaneko, Masaaki Kitahara (Kanazawa University Graduate School of Medical Sciences, Kanazawa); Hiroshi Fukui, Masao Fujimoto (Nara Medical University, Kashihara); Yukio Osaki, Akihiro Nasu (Osaka Red Cross Hospital, Osaka); Takeshi Okanoue, Toshihide Shima (Saiseikai Suita Hospital, Suita); Toshihito Seki, Rinako Kawanura (Kansai Medical University Takii Hospital, Moriguchi); Masatoshi Kudo, Yasunori Minami (Kinki University School of Medicine, Osaka-Sayama); Tetsuo Takehara, Takayuki Yakushijin (Osaka University Graduate School of Medicine, Suita); Michio Kato, Seiji Morioka (Minami Wakayama Medical Center, Tanabe); Shuhei Nishiguchi, Hironori Tanaka (Hyogo College of Medicine, Nishinomiya); Keisuke Hino, Yasuyuki Tomiyama (Kawasaki Medical School, Kurashiki); Kazuhide Yamamoto, Kazuhiro Nouso (Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, Okayama); Kazuaki Chayama, Hiroshi Aikata (Hisoshima University Graduate School of Biomedical Sciences, Hiroshima); Isao Sakaida, Makoto Segawa (Yamaguchi University Graduate School of Medicine, Ube); Kiwamu Okita, Akira Kato (Shimonoseki Kohsei Hospital, Shimonoseki); Yoshikazu Murawaki, Naoto Maeda (Tottori University Faculty of Medicine, Yonago); Morikazu Onji, Yoichi Hiasa (Ehime University Graduate School of Medicine, Tōon); Michio Sata, Takumi Kawaguchi (Kurume University School of Medicine, Kurume); Masaru Harada, Michihiko Shibata (University of Occupational and Environmental Health, Kitakyushu); Hideyuki Nomura (Shin-Kokura Hospital, Kokura); Shotaro

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Simple scoring system for predicting cirrhosis in nonalcoholic fatty liver disease

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Abstract

AIM: To investigate a simple noninvasive scoring system for predicting liver cirrhosis in nonalcoholic fatty liver disease (NAFLD) patients.

METHODS: A total of 1048 patients with liver-biopsy-confirmed NAFLD were enrolled from nine hepatology centers in Japan (stage 0, 216; stage 1, 334; stage 2, 270; stage 3, 190; stage 4, 38). The weight and height of the patients were measured using a calibrated scale after requesting the patients to remove their shoes and any heavy clothing. Venous blood samples were obtained in the morning after the patients had fasted overnight for 12 h. Laboratory evaluation was performed in all patients. Statistical analysis was conducted using SPSS version 12.0. Continuous variables were expressed as mean ± SD.

RESULTS: The optimal cutoff value of platelet count, serum albumin, and aminotransferase/alanine aminotransferase ratio (AAR) was set at < 15.3 $10^4/\mu L$, < 4.0 g/dL, and > 0.9, respectively, by the receiver operating characteristic curve. These three variables were combined in an unweighted sum (platelet count = 1 point, serum albumin = 1 point, AAR = 1 point) to form an easily calculated composite score for predicting cirrhosis in NAFLD patients, called the PLALA (platelet, albumin, AAR) score. The diagnosis of PLALA $\geqslant$ 2 had sufficient accuracy for detecting liver cirrhosis in NAFLD patients.

CONCLUSION: The PLALA score may be an ideal scoring system for detecting cirrhosis in NAFLD patients with sufficient accuracy and simplicity to be considered for clinical use.

Key words: Nonalcoholic fatty liver disease; Cirrhosis; Fibrosis; Platelet; Albumin; Alanine aminotransferase ratio

Core tip: Nonalcoholic fatty liver disease (NAFLD) is an important cause of chronic and progressive liver injury. We aimed to develop a simple noninvasive scoring system for predicting liver cirrhosis in NAFLD patients. These three variables were combined in an unweighted sum [platelet count = 1 point, serum albumin = 1 point, aminotransferase (AST)/alanine aminotransferase (ALT) ratio = 1 point] to form an easily calculated composite score, called the PLALA (platelet, albumin, AST/ALT ratio) score. The diagnosis of PLALA $\geqslant$ 2 had sufficient accuracy for detecting liver cirrhosis in NAFLD patients. The PLALA score may be an ideal scoring system for detecting cirrhosis in NAFLD patients.

Kessoku T, Ogawa Y, Yoneda M, Imajo K, Sumida Y, Eguchi Y, Fujii H, Hyogo H, Ono M, Suzuki Y, Kawaguchi T, Chayama K, Tanaka S, Fujimoto K, Anzai K, Saibara T, Sata M, Itoh Y, Nakajima A, Okanoue T; Japan Study Group of NAFLD (JSG-NAFLD). Simple scoring system for predicting cirrhosis in nonalcoholic fatty liver disease. *World J Gastroenterol* 2014; 20(29): 10108-10114 Available from: URL: http://www.wjgnet.com/1007-9327/full/v20/i29/10108.htm DOI: http://dx.doi.org/10.3748/wjg.v20.i29.10108

INTRODUCTION

Nonalcoholic fatty liver disease (NAFLD) is an important cause of chronic liver injury in many countries[1,2]. NAFLD represents a spectrum of conditions that are characterized histologically by macrovesicular hepatic steatosis, and the diagnosis is made after excluding a history of consumption of alcohol in amounts sufficient to be considered harmful to the liver. NAFLD range over a wide spectrum, extending from simple steatosis, which is generally benign, through to nonalcoholic steatohepatitis (NASH) to liver cirrhosis, end-stage liver disease, and even hepatocellular carcinoma despite the absence of significant alcohol consumption[3-7]. The probability of developing advanced fibrosis and hepatocellular carcinoma is significantly greater in individuals with steatohepatitis than in those with simple steatosis. Data collected from the United States have shown that the prevalence of NAFLD has increased steady in recently years, despite other diseases remaining at steady states. Natural history studies suggest that fibrosis progression occurs in 32%-37% of patients over 3-6 years[8-10], and up to 12% of patients will progress to cirrhosis over 8-10 year[11]. If these patients with NAFLD progress liver cirrhosis, they need to be kept under the surveillance data for early detection of HCC and gastroesophageal varices, similar to the case, such as hepatitis C[12-14].

Liver biopsy as a confirmation tool of NASH can reveal the histologic activity of steatosis, inflammation, and fibrosis. It is frequently used for diagnosis as the gold standard tool in patients with NASH[1,3,15]. However, it is difficult to perform liver biopsy for every patient with NAFLD to ascertain the presence of NASH and determine the stage and grade of the disease[16]. The estimated number of patients with NAFLD has reached 80-100 million in the United States, and the corresponding number of patients in Japan has been estimated at 10-20 million. The prevalence of NAFLD and nonalcoholic steatohepatitis (NASH) is increasing and is becoming a major target disease not only in Western countries, but also in Japan. Therefore, alternative diagnostic methods, noninvasive procedures such as transient elastography, have recently been developed. However, these are not appropriate for health check-ups because they cannot be used in patient with ascites, thick subcutaneous fat, narrow intercostal spaces, and hepatic atrophy.

Therefore, the aim of this study was to develop a mass screening system for general physicians, which can be used for predicting liver cirrhosis in NAFLD patients, using routine laboratory parameters.

MATERIALS AND METHODS

Patients

1048 NAFLD patients who underwent liver biopsy were enrolled between 2002 and 2011 from institutes affiliated with the Japan Study Group of NAFLD (JSG-NAFLD), represented by the following 10 hepatology centers: Yokohama City University, Asahikawa Medical College, Kurume University, Nara City Hospital, Hiroshima University, Saga Medical School, Osaka City University, Kyoto Prefectural University of Medicine, Kochi Medical School, and Saiseikai Suita Hospital. The study was conducted with the approval of the Ethics Committee of all hepatology centers. Liver biopsy was available in all NAFLD patients for the purpose of diagnosis and staging of NASH. Macrovesicular steatosis affecting at least 5% of the hepatocytes was observed in all the cases, with displacement of the nuclei to the edges of the cells[17]. The exclusion criteria included history of hepatic disease such as chronic hepatitis C or concurrent active hepatitis B (seropositive for hepatitis B surface antigen); autoimmune hepatitis; primary biliary cirrhosis; Wilson disease; hemochromatosis; α1-antitrypsin deficiency; sclerosing cholangitis; hepatic injury caused by substance abuse, or current or past consumption of > 20 g of alcohol daily. Informed consent for evaluation of liver histology was obtained from all the enrolled patients, and the present study was performed in accordance and compliance with the Ethic Principles of the 1975 Declaration of Helsinki.

Table 1 Characteristics of patients in the estimation and validation groups

Variables	Fibrosis stages 0-3 (non-cirrhosis)[1]	Fibrosis stage 4 (cirrhosis)[1]	*P* value[2]
Age (yr)	51.1 ± 15.0	63.9 ± 9.6	< 0.0010
n	1010	38	-
BMI (kg/m^2)	27.8 ± 4.9	28.6 ± 3.9	0.3648
AST (IU/L)	58.3 ± 38.2	70.2 ± 74.7	0.0721
ALT (IU/L)	92.2 ± 64.4	67.5 ± 65.6	0.0208
AAR	0.71 ± 0.29	1.15 ± 0.41	< 0.0010
ALP (IU/L)	258.8 ± 94.9	312.2 ± 155.6	0.0012
GGT (IU/L)	88.2 ± 93.8	95.4 ± 73.0	0.6408
ChE (IU/L)	383.0 ± 95.3	298.7 ± 127.0	< 0.0010
Albumin (g/dL)	4.42 ± 0.41	3.69 ± 0.47	< 0.0010
Ferritin (ng/mL)	255.3 ± 249.8	227.5 ± 198.0	0.5677
Fasting glucose (mg/dL)	113.3 ± 38.9	124.0 ± 57.2	0.1116
Fasting insulin (μU/mL)	14.8 ± 13.8	18.4 ± 10.5	0.1938
HOMA-IR	4.31 ± 5.10	6.05 ± 5.64	0.1015
Hemoglobin (g/dL)	14.5 ± 1.6	13.2 ± 1.5	< 0.0010
Platelet (× 10^4/μL)	22.6 ± 6.53	12.08 ± 4.40	< 0.0010
Hyaluronan (ng/mL)	54.5 ± 81.0	250.7 ± 191.0	< 0.0010
Collagen Ⅳ (ng/mL)	4.70 ± 3.60	8.14 ± 1.80	< 0.0010

[1]Results are presented as numbers for qualitative data or as mean ± SD for quantitative data; [2]*P* values were calculated by the *t* test or the χ^2 test. ALP: Alkaline phosphatase; ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; AAR: AST/ALT ratio; BMI: Body mass index; ChE: Cholinesterase; GGT: γ-glutamyl transpeptidase; HOMA-IR: Homeostasis model assessment-insulin resistance.

Anthropometric and biochemical measurements

Body mass index (BMI) was calculated as body weight (kg) divided by height (m^2). Fasted Human blood was collected from all biopsy-proven patients in the morning after overnight for 12 h. In patients with NAFLD, the blood cell counts and the serum levels of aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), γ-glutamyl transpeptidase, albumin, ferritin, cholinesterase (ChE), fasting plasma glucose, fasting immunoreactive insulin, hyaluronan, and collagen Ⅳ were measured consecutively in the each hospital's laboratory. Homeostasis model assessment of insulin resistance (HOMA-IR) was calculated for NAFLD patients as using the following formula: fasting insulin (μU/mL) × fasting plasma glucose (mg/dL)/405.

Histological evaluation

All patients had undergone percutaneous liver biopsy under ultrasound guidance. Liver biopsies were obtained with 16 or 18 gauge needle biopsy apparatus. The number of biopsy specimen fragments was one or two. Liver tissue specimens were fixed in formalin, embedded in paraffin and stained, and analyzed independently by an expert pathologist who was blinded to the clinical data. Fatty liver was defined as the presence of > 5% steatosis. In addition to steatosis, the diagnosis of NASH was histologically confirmed as the presence of lobular inflammation, hepatocyte injures including hepatocyte ballooning cells or perisinusoidal/pericellular fibrosis in zone 3 of the hepatic acini[4,18,19]. The grading and staging of NASH was assessed by Brunt's modified semi-quantitative system, which classifies inflammatory activity into 3 grades [grade 1, mild; grade 2, moderate; grade 3, severe, and the stage of fibrosis into a 4 stages (stage 1, zone3 perivenular and/or perisinusoidal fibrosis; stage 2, stage 1 with periportal fibrosis; stage 3, bridging fibrosis; and stage 4, cirrhosis)]. The individual parameters of fibrosis were scored independently according to the NASH Clinical Research Network (CRN) scoring system developed by the NASH CRN[20].

Statistical analysis

Statistical analysis was performed by using SPSS software version 12.0 for windows (SPSS, Chicago, IL, United States). Continuous variables were expressed as mean ± SD. Qualitative data were represented as numbers, with the percentages indicated within parentheses. The statistical significance of differences between the two groups in the quantitative data were assessed using the *t*-test or the χ^2 test. Data sets involving more than two independent groups were assessed by Kruskal-Wallis test, because the variables were often not normally distributed. The diagnostic performance was assessed by analysis of receiver-operating characteristic (ROC) curves, and the ROC curve was a plot of sensitivity versus (1-specificity) for all possible cutoff values. The probabilities of a true-positive (sensitivity) and true-negative (specificity) were determined for selected cutoff values, and the area under the ROC curve (AUROC) was calculated for each index. Statistical significance was defined as a $P < 0.05$.

RESULTS

Patient and laboratory characteristics of enrolled subjects

Using a multicenter database, 1048 biopsy-proven cases of NAFLD were investigated (fibrosis stage 0, 216; stage 1, 334; stage 2, 270; stage 3, 190; stage 4, 38). The clinical laboratory data and liver biopsy specimens with characteristics of individuals with fibrosis stages 0-3 and stage 4 (cirrhosis) are shown in Table 1. The age, AST/ALT ratio (AAR), ALP, hyaluronan, and collagen Ⅳ were significantly higher, and ChE, albumin, hemoglobin, and platelet were significantly decreased in NAFLD patients with liver cirrhosis (fibrosis stage 4), compared with those with no cirrhosis (fibrosis stages 0-3).

Multiple logistic regression analysis of factors related to fibrosis: Fibrosis stage 0-3 vs fibrosis stage 4 (cirrhosis)

Multiple logistic regression analysis is performed by using age, AAR, serum ChE, albumin, hemoglobin, platelet, hyaluronan, and collagen Ⅳ, which were significantly increased or decreased in NAFLD patients with liver cirrhosis (fibrosis stage 4) compared with NAFLD patients without cirrhosis (fibrosis stages 0-3), by univariate analysis ($P < 0.0001$) (Table 2). On the factors using multiple logistic regression analysis associated with fibrosis stage 0-3 compared with stage 4, AAR ($P = 0.0427$), serum albumin level ($P < 0.001$), and platelet ($P < 0.001$) were the

Table 2 Multiple logistic regression analysis of factors associated with stage 0-3 compared with stage 4 (cirrhosis)

Variables	OR	95%CI	*P* value
Age (yr)	0.976	0.935-1.018	0.2610
AAR	0.122	0.054-0.279	0.0427
Cholinesterase (IU/L)	1.000	0.995-1.005	0.9200
Albumin (g/dL)	5.977	2.430-14.703	< 0.0001
Hemoglobin (g/dL)	0.988	0.761-1.283	0.9263
Platelet ($\times 10^4/\mu L$)	1.282	1.169-1.405	< 0.0001
Hyaluronan (ng/mL)	1.000	0.997-1.003	0.8879
Type IV collagen 7s	0.941	0.883-1.004	0.0671

ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; AAR: AST/ALT ratio.

factors related to progression to cirrhosis.

ROC curve for differentiating stage 4 fibrosis based on albumin, platelet, and AAR

We performed ROC curve analysis for differentiating fibrosis stage 4 (cirrhosis) based on albumin, platelet, and AAR (Figure 1). For detecting cirrhosis (fibrosis stage 4) compared with non-cirrhosis (fibrosis stage 0-3), the AUROC for AAR, albumin, and platelet was 0.843, 0.898, and 0.918, respectively. Based on the ROC curve, the cut-off level for the diagnosis of AAR, albumin, and platelet was set at ≥ 0.9, ≤ 4.0 g/dL, and $\leq 15.3 \times 10^4$/mL, respectively. Sensitivity, specificity, negative predictive value (NPV), and positive predictive value (PPV) of AAR, albumin, and platelet were 76.3%/84.2%/81.6% (sensitivity), 82.9%/84.6%/88.6% (specificity), 98.9%/99.3%/99.2% (NPV), and 13.9%/17.0%/21.2% (PPV), respectively.

PLALA score (platelet, albumin, AAR)

By multiple logistic regression analysis, three variables remained significant, including platelet, albumin, and AAR. Thus, these three variables, platelet $< 15.3 \times 10^4/\mu L$, albumin < 4.0 g/dL, and AAR > 0.9, were combined to form an easily calculated composite score for predicting NAFLD with cirrhosis, called the PLALA score. The three variables were given a score of 1 point each (Figure 1), and a score of 0-3 was calculated. Figure 2 shows the percentage of patients with cirrhosis (fibrosis stage 4) with a platelet $< 15.3 \times 10^4/\mu L$, albumin < 4.0 g/dL, and AAR > 0.9.

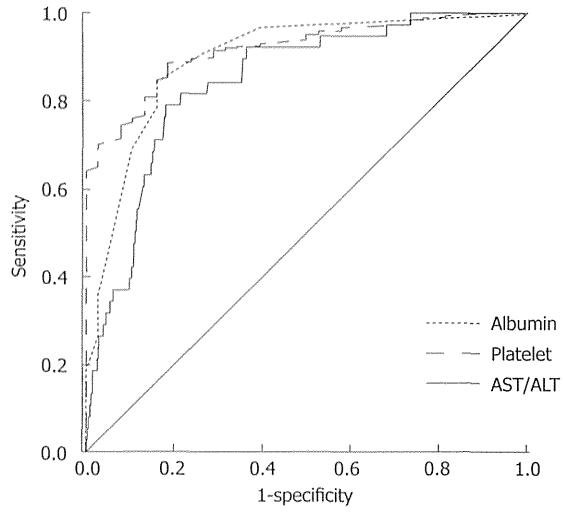
The diagnostic accuracy of the scoring system in distinguishing patients with and without cirrhosis was confirmed in 1048 patients. The percentage of patients with cirrhosis (fibrosis stage 4) with a PLALA score of 0, 1, 2, and 3 was 0%, 13%, 29%, and 58%, respectively (Figure 3). When using a PLALA score of 2 as a cutoff, the sensitivity, specificity, NPV, and PPV were 86.8%, 90.8%, 99.5%, and 26.2%, respectively. All of these data were superior to those of platelet, albumin, and AAR.

DISCUSSION

We developed a simple scoring system to differentiate cirrhosis from non-cirrhosis in NAFLD patients. The three variables platelet, albumin, and AAR were combined in an unweighted sum (platelet $< 15.3 \times 10^4/\mu L$; 1 point, serum albumin < 4.0 g/dL; 1 point, and AAR > 0.9; 1 point) and formed an easily calculated composite score for predicting cirrhosis in NAFLD patients, called the PLALA score. A PLALA score (2 and 3) was useful for detecting liver cirrhosis in NAFLD patients (sensitivity, 86.8%; specificity, 90.8%; NPV, 99.5%; PPV, 26.2%).

Bhala *et al*[21] reported that 2.4% of patients with NAFLD followed up for approximately 85.6 ± 54.5 mo developed HCC, and 66.7% of the patients with NAFLD-associated HCC had cirrhosis (fibrosis stage 4). Hashimoto *et al*[22] reported that 88% of patients with NASH-associated HCC had advanced fibrosis (stage 3 or 4). Therefore, advanced fibrosis was recognized as an important risk factor for HCC. Furthermore, HCC was the major cause of mortality in NASH patients with advanced fibrosis[22]. It is important to closely follow cirrhosis patients with NASH. In the field of NAFLD, various scoring systems have been reported, for example NAFIC[23], FIB4 index[24], HAIR[25], BAAT[26], BARD[13], NAFLD fibrosis score[27], and N score (Nippon)[28]. These scoring systems can differentiate NASH from NAFLD or differentiate advanced fibrosis (stages 3 or 4) from mild fibrosis (stage 0-2). However, the PLALA score developed in our study, with the three variables platelet, albumin, and AAR, differentiates cirrhosis (stage 4) from non-cirrhosis in the NAFLD patients (stages 0-3), and it is easy to calculate.

The platelet is one of the most commonly reported parameters associated with clinically significant portal



Variables	Cutoff values	Score values
Platelet ($\times 10^4/\mu L$)	$< 15.3 \times 10^4$/mL	1 point
Albumin (mg/dL)	< 4.0 g/dL	1 point
AAR	> 0.9	1 point

Figure 1 Receiver operating characteristic curve. Receiver operating characteristic curve for differentiating fibrosis stage 4 based on albumin, platelet, and AAR; aspartate aminotransferase (AST)/alanine aminotransferase (ALT) ratio. PLALA score (platelet, albumin, AAR).

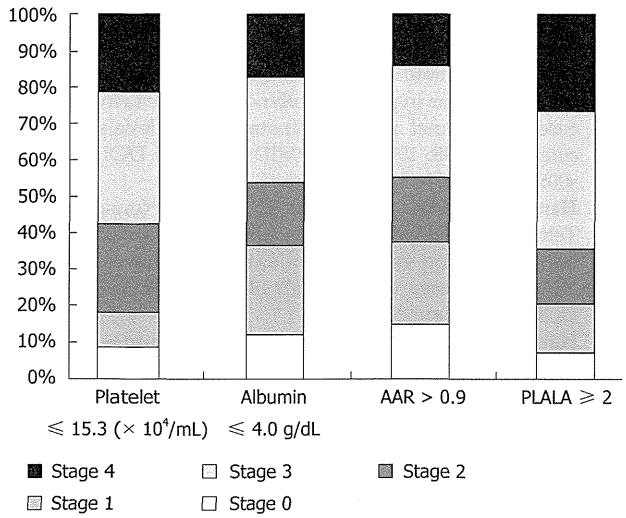


Figure 2 The percentage of patients with cirrhosis. The percentage of patients with cirrhosis (stage 4) with a platelet < 15.3 $\times 10^4/\mu L$, albumin < 4.0 g/dL, and alanine aminotransferase ratio; aspartate aminotransferase (AST)/alanine aminotransferase (ALT) ratio (AAR) > 0.9.

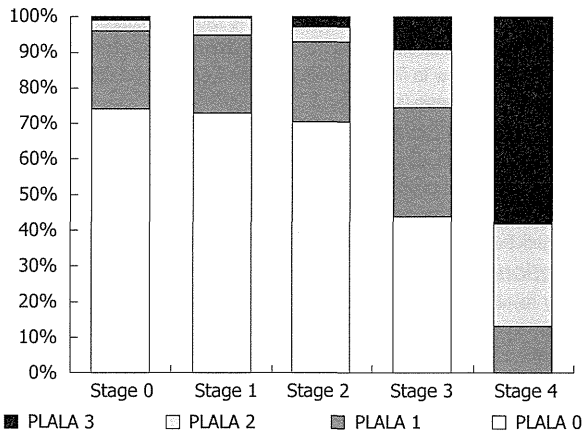


Figure 3 PLALA score and fibrosis stage of 1048 biopsy-proven nonalcoholic fatty liver disease patients.

hypertension in compensated cirrhosis patients[29]. The levels of serum albumin reflect the protein-synthesizing capacity of the liver. Patients with advanced cirrhosis almost always have hypoalbuminemia caused by decreased protein synthesis in the hepatocytes. AAR reflects fibrosis of the liver[30,31]. When only one of three values is positive, factors that may cause false-positive low cutoff values for platelet are older age (decreased platelet production), idiopathic thrombocytopenic purpura, idiopathic portal hypertension, and drugs. Factors that may cause false-positive low levels of serum albumin are loss of urinary albumin due to renal dysfunction (*e.g.*, nephritic syndrome and diabetic nephropathy), severe burns, and inadequate protein intake. The possibility of false-positive values for AAR > 0.9 is within the normal range of AST and ALT.

Liver cirrhosis simultaneously induces liver fibrosis, portal hypertension, and decreased production of albumin. Thus, PLALA score includes: platelet, which reflects portal hypertension; albumin, which reflects protein production; and AAR, which reflects liver fibrosis. A PLALA score of 2 or 3 points is highly diagnostic for liver cirrhosis in patients with false-positive results for NAFLD. If these NAFLD patients with liver cirrhosis have early detection of HCC and portal hypertension, such as gastroesophageal varices, it is important for them to need to be kept under surveillance.

This study had several limitations. The study had a largely retrospective design. The proportion of patients with advanced fibrosis was small. Therefore, in contrast to the NPVs, the PPVs did not have sufficient accuracy for the diagnosis of advanced fibrosis. Therefore, it would seem appropriate to consider liver biopsy in all patients with values above the cutoff of the selected index, PLALA (2 and 3). We previously reported, possibly for the first time, that transient elastography and acoustic radiation force impulse elastography can be used to measure the severity of fibrosis in patients with NAFLD[32,33]. It is possible that a combination of transient elastography and the aforementioned scoring systems may provide better performance than each of them used alone, although this needs to be verified. The patients were recruited from multiple hepatology centers in Japan with a particular interest in the study of NAFLD; therefore, the possibility of some referral bias cannot be ruled out. Patient selection bias could also have existed, because liver biopsy might have been considered for NAFLD patients who were likely to have NASH and progression of fibrosis. Thus, the findings may not represent NAFLD patients in the community at large. We also acknowledge that the pathological diagnosis was mainly determined using liver tissues derived from percutaneous liver biopsies, which are prone to sampling errors and/or inter-observer variability[34,35]. There is a possibility that our results might not be adaptable for NAFLD patients of other races, because all participants were Japanese. Because of these limitations, the present results need to be validated in independent populations by other investigators.

In conclusions, the PLALA score may be an ideal scoring system for detecting cirrhosis in NAFLD patients, because it is easy to use, cost effective, and accurate. Therefore, we consider that this scoring system is useful for mass screening by general physicians, using routine laboratory parameters.

COMMENTS

Background

Nonalcoholic fatty liver disease (NAFLD) is one of the most common causes of chronic liver damage in many countries around the world. Although liver biopsy is useful as the gold standard method for diagnosis of nonalcoholic steatohepatitis (NASH) with cirrhosis, it is difficult to perform liver biopsy for every patient with NAFLD. However, noninvasive markers for predicting cirrhosis in NAFLD patients have not yet been well established.

Research frontiers

In the field of NAFLD, various scoring systems related liver fibrosis have been reported, for example FIB4 index, NAFLD fibrosis score. These scoring systems can differentiate advanced fibrosis (stage 3 or 4) from mild fibrosis (stage

0-2). However, NASH-related cirrhosis (stage 4) is cause of most complication, especially hepatocellular carcinoma and portal hypertension. Therefore, the research hotspot is how to find noninvasive scoring systems for detecting with NASH-associated cirrhosis (stage 4).

Innovations and breakthroughs

The PLALA score developed with the three variables, differentiates cirrhosis (stage 4) from non-cirrhosis in the NAFLD patients (stages 0-3). When using a PLALA score of 2 as a cutoff, the sensitivity, specificity, negative predictive value, and positive predictive value were 86.8%, 90.8%, 99.5%, and 26.2%, respectively.

Applications

The study results suggest that PLALA score is to develop a mass screening system for general physicians, which can be used for predicting liver cirrhosis in NAFLD patients, using routine laboratory parameters.

Terminology

NAFLD is mainly represents a spectrum of liver disease from simple steatosis to nonalcoholic fatty steatohepatitis, which can progress to cirrhosis and hepatocellular carcinoma, despite the absence of significant alcohol consumption. PLALA score is constructed from platelet, Alb, AAR. These three variables were combined to form an easily calculated composite score for predicting NAFLD with cirrhosis.

Peer review

The manuscript aimed to develop a simple noninvasive scoring system for predicting liver cirrhosis in nonalcoholic fatty liver disease patients by using early available clinical and biochemical variables. This article is interesting, original and well written, and gives good clues to the readers.

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ORIGINAL ARTICLE—LIVER, PANCREAS, AND BILIARY TRACT

Bofutsushosan, a Japanese herbal (Kampo) medicine, attenuates progression of nonalcoholic steatohepatitis in mice

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Abstract

Background Obesity-induced liver disease (nonalcoholic fatty liver disease, NAFLD) is now the commonest cause of chronic liver disease in affluent nations. There are presently no proven treatments for NAFLD or its more severe stage, nonalcoholic steatohepatitis (NASH). Bofutsushosan (BTS), a Japanese herbal (Kampo) medicine, long used as an anti-obesity medicine in Japan and other Asian countries, has been shown to reduce body weight and improve insulin resistance (IR) and hepatic steatosis. The precise mechanism of action of BTS, however, remains unclear. To evaluate the ability of BTS to prevent the development of NASH, and determine the mediators and pathways involved.

Methods C57BL/6 mice were injected intra-peritoneally with gold-thioglucose and fed a high-fat diet (HF) or HF diet admixed with either 2 or 5 % BTS for 12 weeks. The effectiveness of BTS in attenuating features of NASH and the mechanisms through which BTS attenuated NASH were then assayed through an assessment of the anthropometric, radiological, biochemical and histological parameters.

Results BTS attenuated the progression of NASH through induction of adiponectin and its receptors along with an induction of PPAR-α and PPAR-γ, decreased expression of SREBP-1c, increased hepatic fatty acid oxidation and increased hepatic export of triglycerides. BTS moreover, reduced IR through phosphorylation of the protein kinase, Akt.

Conclusions BTS through induction of adiponectin signaling and Akt attenuated development of NASH. Identification of the active entity in BTS should allow development of novel treatments for NASH.

Keywords NAFLD · Adiponectin · Bofutsushosan · Kampo medicine

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Abbreviations

NAFLD	Nonalcoholic fatty liver disease
NASH	Nonalcoholic steatohepatitis
GTG	Gold-thioglucose
HF	High-fat-diet
BTS	Bofutsushosan
IR	Insulin resistance
IGT	Impaired glucose tolerance
GTT	Glucose tolerance test