Observational Study

Medicine®

OPEN

Nonalcoholic non-virus-related hepatocellular carcinoma arising from nonsteatotic liver

Clinical and pathological features

Yasuyuki Shigematsu, MD[a,b], Hiroaki Kanda, MD, PhD[c], Gulanbar Amori, PhD[a,b], Yu Takahashi, MD[d], Yutaka Takazawa, MD, PhD[e], Kentaro Inamura, MD, PhD[a,b,*]

Abstract

Nonalcoholic non-virus-related hepatocellular carcinoma (NANV-HCC) is considered to occur in steatotic livers; however, emerging evidence indicates that a subset of NANV-HCC occurs in nonsteatotic livers. Currently, little information is available regarding this subset. This study sought to provide the clinical and pathological features of NANV-HCC in nonsteatotic livers.

We retrospectively investigated the clinicopathological features of 101 consecutive patients with NANV-HCC treated with a curative-intent hepatectomy. A background liver with <5% steatosis by area was regarded as a nonsteatotic liver. Survivals of patient subgroups were estimated using the Kaplan–Meier method, and log-rank tests were conducted to assess the survival difference. Multivariate analysis was performed with the Cox proportional hazards method.

Overall, 34 of 101 patients with NANV-HCC were found to have a nonsteatotic liver. Vascular invasion of the tumor was more frequently observed in patients with a nonsteatotic liver than in those with a steatotic liver ($P = .03$). The extent of lobular inflammation and fibrosis did not differ between patients with and without steatosis in the liver. NANV-HCC with a nonsteatotic liver was independently associated with a shorter disease-free survival (DFS) (hazard ratio [HR] 2.14; 95% confidence interval [CI] 1.21–3.80; $P = .009$) and a shorter overall survival (OS) (HR 2.79; 95% CI 1.27–6.16; $P = .01$) than NANV-HCC with a steatotic liver.

The absence of steatosis in the liver is independently associated with shorter DFS and OS in patients with NANV-HCC. Our findings indicate that nonsteatotic liver can be a surrogate phenotype of aggressive NANV-HCC.

Abbreviations: BMI = body mass index, CI = confidence interval, DFS = disease-free survival, DL = dyslipidemia, DM = diabetes mellitus, HBV = hepatitis B virus, HCC = hepatocellular carcinoma, HCV = hepatitis C virus, HR = hazard ratio, HT = hypertension, NANV-HCC = nonalcoholic non-virus-related HCC, NASH = nonalcoholic steatohepatitis, OS = overall survival.

Keywords: disease-free survival, hepatocellular carcinoma, hepatectomy, overall survival, steatosis

1. Introduction

Hepatocellular carcinoma (HCC) is the fifth most frequently diagnosed cancer and the fourth leading cause of cancer-related death in the world.[1,2] Chronic liver inflammation caused by hepatitis B virus (HBV) infection or/and hepatitis C virus (HCV) infection is known to be the primary cause of HCC. Recent epidemiological studies confirm that the incidence of hepatitis virus-related HCC has decreased in Japan due to advances in HBV and HCV treatment and the prevention of virus transmission. However, the incidence of non-virus-related HCC has increased.[3]

The pathogenesis of HCC in the context of the absence of HBV or HCV infection is not fully understood. Although chronic

Editor: Min Xu.

The study was approved by the Ethics Committee of the Cancer Institute Hospital and the committee's approval reference number is 2017-1136. Informed patient consent specific to this study was waived for this retrospective study.

The authors have no funding and conflicts of interests to disclose.

Supplemental Digital Content is available for this article.

The datasets generated during and/or analyzed during the current study are not publicly available, but are available from the corresponding author on reasonable request.

[a] Department of Pathology, The Cancer Institute Hospital, Japanese Foundation for Cancer Research, 3-8-31 Ariake, Koto, Tokyo, Japan, [b] Division of Pathology, The Cancer Institute, Japanese Foundation for Cancer Research, 3-8-31 Ariake, Koto, Tokyo, Japan, [c] Department of Pathology, Saitama Cancer Center, 780 Komuro, Ina, Kita-adachi-gun, Saitama, Japan, [d] Division of Hepatobiliary and Pancreatic Surgery, The Cancer Institute Hospital, Japanese Foundation for Cancer Research, 3-8-31 Ariake, Koto, Tokyo, Japan, [e] Department of Pathology, Toranomon Hospital, 2-2-2 Toranomon Minato, Tokyo, Japan.

[*] Correspondence: Kentaro Inamura, Division of Pathology, The Cancer Institute, Japanese Foundation for Cancer Research, 3-8-31 Ariake, Koto, Tokyo 135-8550, Japan (e-mail: kentaro.inamura@jfcr.or.jp).

How to cite this article: Shigematsu Y, Kanda H, Amori G, Takahashi Y, Takazawa Y, Inamura K. Nonalcoholic non-virus-related hepatocellular carcinoma arising from nonsteatotic liver: clinical and pathological features. Medicine 2022;101:5(e28746).

Received: 26 April 2021 / Received in final form: 2 December 2021 / Accepted: 7 January 2022

http://dx.doi.org/10.1097/MD.0000000000028746

alcohol intake is a well-known classical risk factor,[4] more recently, metabolic syndrome and its related disorders have been recognized to increase the risk of non-virus-related HCC.[5–7] HCC related to metabolic syndrome has been considered to be derived from a steatotic liver.[8–10] However, it can also develop in a nonsteatotic liver,[11] and hepatic steatosis is not always present. Several studies regarding nonalcoholic non-virus-related HCC (NANV-HCC) with a steatotic liver have been reported; however, few reports are focusing on NANV-HCC with a nonsteatotic liver. Therefore, the differences in clinical and pathological features between NANV-HCC in nonsteatotic livers and that in steatotic livers are poorly understood.

With the progression of steatohepatitis, the degree of steatosis and inflammatory cell infiltration is known to decrease in the background liver.[12] Thus, in nonsteatotic livers, it is difficult to distinguish whether there has been no steatohepatitis from the beginning or if the initial presence of steatohepatitis has disappeared over time.[13] Steatohepatitis eventually progresses to cirrhosis with little steatosis, which can lead to a failure to identify steatohepatitis as the cause of cirrhosis. The majority of recognized cryptogenic cirrhosis, in which 30% to 40% of patients develop HCC,[14] is considered to be derived from steatohepatitis due to the similarity of clinical features.[13,15] Therefore, nonsteatotic liver includes a wide range of clinical variation.

Regardless of the etiology, surgical resection is the preferred treatment for localized HCC in patients without liver dysfunction.[16,17] However, a high recurrence rate after surgical resection is a constant source of concern. Most recurrences of HCC are intrahepatic, and the likelihood of recurrence is associated with the condition of the liver. The presence of fibrosis, especially cirrhosis, is a major prognostic factor of recurrence after a curative-intent hepatectomy for virus-related HCC.[18] However, in patients without alcoholic abuse and hepatic virus infection, HCC often develops in non-cirrhotic liver,[19] and the fibrotic condition of the liver has little prognostic impact. Histological features to contribute to the development of a risk stratification system for recurrence in patients with NANV-HCC are needed.

This retrospective study investigated the impact of the condition of liver steatosis on surgical outcomes and its association with comorbidity of metabolic syndrome and pathological features of NANV-HCC.

2. Materials and methods

2.1. Patients

A total of 415 adult HCC patients underwent R0 resection at the Cancer Institute Hospital of Japanese Foundation for Cancer Research (JFCR) in Tokyo, Japan, between January 1, 2005, and December 31, 2016. Patients who did not have prior treatment for HCC, histories of HBV or HCV infection, and alcohol abuse were eligible. To minimize the effect of pre-existing liver disease, we excluded patients with congenital and chronic liver diseases, such as inherited enzyme deficiency diseases, primary biliary cholangitis, autoimmune hepatitis, primary biliary cholangitis-autoimmune hepatitis overlap syndrome, and hemochromatosis. The remaining 101 were included. The protocol of this retrospective study was approved by the ethical committee of JFCR (approval number 2017–1136). Informed patient consent specific to this study was waived for this retrospective study.

2.2. Data collection

Clinical records of all the patients were re-evaluated, and the following data were collected:

1. Basic characteristics including height, body weight, and sex at the preoperative assessment.
2. Daily alcoholic consumption, and history of disease including diabetes mellitus (DM), dyslipidemia (DL) (hypercholesterolemia and hypertriglyceridemia), hypertension (HT), and alcoholic hepatitis. Alcohol abuse required the consumption of 30 g/day by men and 20 g/day by women or a past medical history of chronic liver injury due to alcohol. Drug treatment for DM, DL, and HT was an alternative indicator.
3. Current medication for those diseases.
4. Preoperative serum markers including HBV surface antigen, HBV surface antibody, HBV core antibody and/or HBV DNA, HCV antibody and/or HCV-RNA, aspartate aminotransferase, alanine aminotransferase, platelets, and alpha-fetoprotein.

The presence of DM, DL, HT, and alcoholic hepatitis was confirmed by patient self-reporting system and reviewing patient referral documents. Body mass index (BMI) was calculated based on kg/m^2 and obesity was defined as a BMI $\geqq 25\,kg/m^2$.

2.3. Pathological evaluation

The pathological evaluation was conducted using hematoxylin and eosin-stained 4 μm serial sections of formalin-fixed and paraffin-embedded tissue. The diagnosis of HCC followed the fourth edition of the World Health Organization criteria.[20] For the evaluation of the background liver, liver tissue sections that do not contain tumors were used to minimize any influences from them. Steatotic condition, lobular inflammation, and hepatocyte ballooning were evaluated based on Kleiner criteria.[21] Fibrosis was evaluated based on the Brunt criteria.[22] The presence of fatty change, glycogen production, bile production, and intracytoplasmic inclusion bodies in tumor tissue required more than 5% of cancer cells involved.

2.4. Patient outcome

Survival was assessed until 31 December 2017. The median follow-up was 51 months (range, 4–135 months). The primary endpoint was overall survival (OS), which is the time from the date of HCC resection to death. The secondary endpoint was disease-free survival (DFS), which is the time from resection to either the first recurrence or death. Contrast-enhanced computed tomography and magnetic resonance imaging were used to evaluate recurrence; the recurrence date was defined as the inspection date obtained using the abovementioned imaging modalities. In patients without recurrence, the latest imaging inspection date was recorded as the censored data. The inspection was carried out every 3 months after the surgery for 1 year and every 6 months thereafter.

2.5. Statistical analysis

Statistical analyses were conducted using R version 3.3.3 (R Foundation for Statistical Computing, Vienna, Austria). The values of the continuous variable were reported as means and standard deviations. Categorical values were reported as

numbers and percentages. Between-group differences in continuous variables were compared by either the Mann–Whitney *U* test or Student *t* test and values of categorical variables were compared with Fisher exact test. The cumulative OS and DFS were estimated by the Kaplan–Meier method and compared by log-rank tests. Multivariate analyses were performed using the Cox proportional hazards method. The hazard ratio (HR) and its 95% confidence interval (CI) were calculated. Two-tailed *P* values <.05 were considered to indicate a statistical significance.

3. Results

3.1. Clinical and pathological characteristics in patients with NANV-HCC in nonsteatotic and steatotic liver

The patient baseline clinical and pathological characteristics are shown in Table 1. Of 415 HCC patients treated at our hospital, 101 patients (24.3%) had NANV-HCC. Of the 101 patients with NANV-HCC, 34 (33.6%) had a nonsteatotic liver (Fig. 1). Grade 1 steatosis (5%–33% of hepatocytes involved) was found in 39 patients, grade 2 steatosis (33%–66% of hepatocytes involved) in 21 patients, and grade 3 steatosis (>66% of hepatocytes involved) in 7 patients. Both the steatotic and nonsteatotic liver groups included more men than women, but the difference was not significant (*P* = .13). The mean preoperative BMI was 24.7 kg/m^2, and 61.4% of patients were not obese. All patients with a BMI < 18.5 were included in the nonsteatosis group (*P* < .001). Thus, emaciation is significantly associated with nonsteatotic liver.

The mean of the values of aspartate aminotransferase, alanine aminotransferase, and platelets in patients with NANV-HCC were within the range of reference values, and there was no significant difference between the two groups. Patients in the nonsteatosis group had higher alpha-fetoprotein levels than those in the steatosis group; however, the difference was not significant (*P* = .08). These results show that a steatosis condition in the background liver had a small impact on the levels of serological markers.

No association between the presence or absence of steatosis in the liver and preoperative liver function status (as measured using the preoperative indocyanine green elimination rate) was observed. The amount of blood loss and patients' transfusion requirements were similar between the two groups, and surgical technique did not affect the rate of surgical complications. The resected area in the nonsteatosis group was significantly larger than that in the steatosis group (*P* = .005).

Tumor size, the number of tumors, and the TNM stage were not significantly different between the two groups. Vascular invasion was observed more frequently in the nonsteatosis group than in the steatosis group (*P* = .03), whereas tumor differentiation was similar between the two groups. Ballooning degeneration was rarely observed in the nonsteatosis group, but was frequently observed in the steatosis group (*P* < .001). NANV-HCC with a nonsteatotic liver was more frequently observed in the left lobe compared to the steatotic liver (*P* = .03). There was no significant association between vascular invasion and tumor location (*P* = .8; Supplementary Digital Content Table S1, http://links.lww.com/MD2/A878). The extent of fibrosis and the degree of lobular inflammation were not significantly different between the two groups.

3.2. Survival impact of the absence of steatosis in the background liver in patients with NANV-HCC

Table 2 presents the results of the univariate and multivariate analyses of the DFS. In the univariate analysis, HR of nonsteatotic liver to steatotic liver was 2.22 (95% confidence interval [CI], 1.28–3.85), and a nonsteatotic liver was significantly associated with a shorter DFS (*P* = .004) (Fig. 2A). Tumor size of less than 5 cm had a HR of 0.54 (95% CI, 0.32–0.93) compared to a tumor size of equal to or more than 5 cm, and the tumor size was also significantly associated with DFS (*P* = .03). Of note, in our study, comparison of the advanced fibrosis (Stage 3–4) with the nonadvanced fibrosis group (Stage 0–2) showed no obvious significant difference between the two groups (*P* = .31). For vascular invasion, no significant difference was observed, although a tendency toward a shorter DFS was shown (*P* = .50). In the multivariate analysis including the number of tumors, tumor size, tumor differentiation, vascular invasion, steatosis condition, and fibrosis in the liver, steatotic condition was the only remaining variable, and a nonsteatotic liver was significantly associated with a shorter DFS (multivariate HR = 2.14, 95% CI, 1.21–3.80; *P* = .009). These data indicate that the absence of steatosis was an independent risk factor for recurrence.

Among the 101 patients with NANV-HCC, 29 (28.7%) died during the follow-up period. Twenty two (75.9%) of the deaths were due to HCC and 7 (24.1%) were due to other causes, including nasopharyngeal cancer, colon cancer, lung cancer, prostate cancer, acute myeloleukemia, and acute myocardial infarction. The OS results of the univariate and multivariate analyses are shown in Table 3. In the univariate analysis, patients with a nonsteatotic liver had a significantly shorter OS than those with a steatotic liver (*P* = .006; Fig. 2B). Although the area of the resected liver was significantly larger in the nonsteatosis group than in the steatosis group, no significant association was found between the extent of resection and OS. Thus, the area of the resected liver had little impact on patient survival outcomes in our study. In the multivariate analysis including the number of tumors, tumor size, tumor differentiation, vascular invasion, steatosis condition, and fibrosis in the liver, steatotic condition of the liver was only a remaining variable, and the nonsteatotic liver was significantly associated with shorter OS (multivariate HR = 2.79, 95% CI, 1.27–6.16; *P* = .01).

3.3. Cytological features of NANV-HCC in nonsteatotic and steatotic background liver

An association between steatotic liver and fatty degeneration of cancer cells has been reported in not only virus-related HCC but also non-virus-related HCC.[23,24] Thus, we investigated the cytological features in HCC, including fatty degeneration, glycogen production, bile production, and the presence of intracytoplasmic inclusion bodies of cancer cells. Fatty degeneration of cancer cells was present in more than half of the nonsteatotic livers; however, the difference between nonsteatotic and steatotic liver groups was not significant (*P* = .52) (Supplementary Digital Content Table S2, http://links.lww.com/MD2/A879). Similarly, glycogen and bile production and the presence of intracytoplasmic inclusion bodies in cancer cells in the two groups were not statistically different. Thus, the presence or absence of steatosis in the liver did not significantly affect the cytological characteristics in NANV-HCC in our study.

Table 1

Baseline clinical and pathological characteristics of NANV-HCC.

Variable	Total (n = 101)	Background liver steatosis		*P* value
		Absent (n = 34)	Present (n = 67)	
Age (yr)*				1.00
<65	28 (27.7)	9 (26.5)	19 (28.4)	
>=65	73 (72.3)	25 (73.5)	48 (71.6)	
Sex*				.13
Female	23 (22.8)	11 (32.4)	12 (17.9)	
Male	78 (77.2)	23 (67.6)	55 (82.1)	
Body mass index (kg/m^2)*				<.001
<18.5	8 (7.9)	8 (23.5)	0 (0)	
18.5–24.9	54 (53.5)	17 (50.0)	37 (55.2)	
>= 25	39 (38.6)	9 (26.5)	30 (44.8)	
Serological markers†				
AST (U/L)	38.0 (± 21.6)	40.7 (± 28.0)	36.7 (± 17.5)	.45
ALT (U/L)	34.2 (±21.1)	31.9 (± 26.0)	35.3 (± 18.3)	.49
PLT (x10^4/mL)	20.2 (± 8.1)	20.9 (± 10.5)	19.9 (± 6.7)	.58
AFP (ng/mL)	15.5 (± 54.9)	31.8 (± 73.3)	7.2 (± 41.0)	.08
ICG-K†	0.143 (± 0.036)	0.141 (± 0.039)	0.144 (± 0.035)	.73
Amount of blood loss (mL)†	527.2 (± 595.1)	500.4 (± 513.8)	540.7 (± 635.7)	.75
Blood transfusion*				1.00
No	94 (93.1)	32 (94.1)	62 (92.5)	
Yes	7 (6.9)	2 (5.9)	5 (7.5)	
Surgical procedure*				.005
Partial resection	28 (27.7)	8 (23.5)	20 (29.9)	
Subsegmentectomy Couinaud	21 (20.8)	2 (5.9)	19 (28.4)	
Segmentectomy	24 (23.8)	8 (23.5)	16 (23.9)	
Lobectomy	25 (24.8)	15 (44.1)	10 (14.9)	
Extended lobectomy	3 (2.9)	1 (3.0)	2 (2.9)	
TNM stage*				.41
IA	12 (11.9)	5 (14.7)	7 (10.4)	
IB	44 (43.6)	11 (32.4)	33 (49.3)	
II	38 (37.6)	15 (44.1)	23 (34.3)	
IIIA	7 (6.9)	3 (8.8)	4 (6)	
Number of tumors*				.45
1	81 (80.2)	26 (76.5)	55 (82.1)	
2–3	17 (16.8)	6 (17.6)	11 (16.4)	
>3	3 (3.0)	2 (5.9)	1 (1.5)	
Tumor size (cm)*				.19
<5	66 (65.3)	19 (55.9)	47 (70.1)	
>= 5	35 (34.7)	15 (44.1)	20 (29.9)	
Tumor differentiation*				.73
Well	14 (13.9)	4 (11.8)	10 (14.9)	
Moderate	66 (65.3)	24 (70.8)	42 (62.7)	
Poor	21 (20.8)	6 (17.6)	15 (22.4)	
Vascular invasion*				.03
Absence	63 (62.4)	16 (47.1)	47 (70.1)	
Presence	38 (37.6)	18 (52.9)	20 (19.8)	
Lobular inflammation*				.06
Score 0–1	54 (53.5)	23 (67.7)	31 (46.3)	
Score 2–3	47 (46.5)	11 (32.3)	36 (53.7)	
Ballooning*				<.001
Score 0	59 (58.4)	33 (97.1)	26 (38.8)	
Score 1	20 (19.8)	0	20 (29.9)	
Score 2	22 (21.8)	1 (2.9)	21 (31.3)	
Fibrosis*				.06
Stage 0–2	73 (72.3)	29 (85.3)	44 (65.7)	
Stage 3–4	28 (27.7)	5 (14.7)	23 (34.3)	
Tumor location*				.03
Right lobe	62 (61.4)	15 (44.1)	47 (70.2)	
Left lobe	24 (23.8)	13 (38.2)	11 (16.4)	
Both lobes	15 (14.9)	6 (17.7)	9 (13.4)	

AFP = alpha-fetoprotein, ALT = alanine aminotransferase, AST = aspartate aminotransferase, ICG-K = indocyanine green elimination rate, NANV-HCC = nonalcoholic non-virus-related hepatocellular carcinoma, PLT = platelets.

* Data presented as n (%).

† Data presented as mean ± standard deviation.

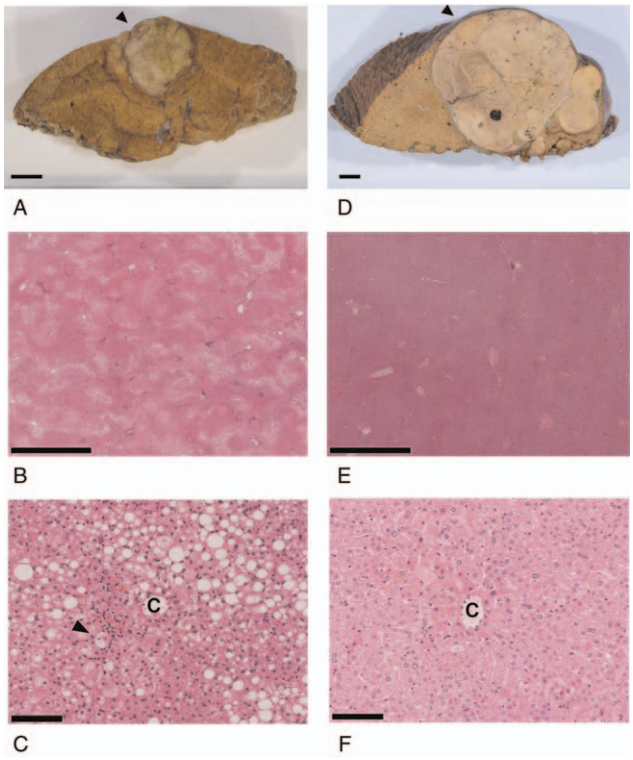

Figure 1. Representative nonalcoholic non-virus-related hepatocellular carcinoma (NANV-HCC). (A) Macro image of NANV-HCC (black arrowhead) in the steatotic liver (scale bar = 10 mm); (B) Low-power microscopic image of the steatotic background liver (scale bar = 2.5 mm); (C) High-power microscopic image of the steatotic background. A central vein and hepatocytes with lipids and intracytoplasmic inclusion bodies (black arrowhead) are visible. A character "c" represents a central vein (scale bar = 100 μm); (D) Macro image of NANV-HCC (black arrowhead) in the nonsteatotic liver (scale bar = 10 mm); (E) Low-power microscopic image of the nonsteatotic background liver (scale bar = 10 mm); (F) High-power microscopic image of the nonsteatotic background liver. A character "c" represents a central vein (scale bar = 100 μm).

3.4. Metabolic disorders in patients with and without steatosis in the liver

Metabolic disorders reflecting insulin resistance including DM and obesity have been recognized as risk factors for HCC.[25,26] Steatosis in the liver is considered to be a hepatic phenotype of metabolic disorders,[27] and to assess the possibility that the steatotic condition in the liver could be associated with metabolic disorders, we investigated the complication rate of the metabolic disorders, including DM, HT, DL, and obesity (Supplementary Digital Content Table S3, http://links.lww.com/MD2/A880). Overall, 50.5% of patients with NANV-HCC had DM, although the rate of DM was not significantly different between the two groups ($P = .09$). Interestingly, even in the nonsteatosis group, the prevalence of DM was 38.2%, which is 2 to 3 times higher than the previously reported prevalence of DM in virus-related HCC.[15,28] There was no significant difference in the rate of HT, DL, and obesity between the two groups. However, the presence of any metabolic disorders was significantly higher in the steatosis group (94.0%) than in the nonsteatosis group (70.6%) ($P = .004$). Although the steatosis group is more likely to have metabolic disorders than the nonsteatosis group, the nonsteatosis group also shows a high complication rate of metabolic disorders. These data indicate NANV-HCC is deeply associated with metabolic disorders regardless of steatotic condition in the liver.

4. Discussion

The clinical and pathological features of NANV-HCC in nonsteatotic livers are not well understood. This study found that NANV-HCC in nonsteatotic livers is associated with a higher frequency of vascular invasion than that in steatotic livers. The absence of steatosis was associated with early recurrence and poor prognosis after curative-intent hepatectomy. These results indicate that the steatosis condition of the liver reflects the pathological and clinical behavior of NANV-HCC. In addition, patients with NANV-HCC in the nonsteatotic liver showed a

Table 2

DFS analysis in patients with NANV-HCC.

		Univariate analysis			Multivariate analysis		
Variable	**MDT**	**HR**	**95% CI**	***P* value**	**HR**	**95% CI**	***P* value**
Age (yr)							
<65	1431	0.83	(0.46–1.50)	.53			
>= 65	1280		Reference				
Sex							
Female	723	1.53	(0.83–2.82)	.17			
Male	1507		Reference				
Body mass index (kg/m^2)							
<18.5	478	2.43	(0.97–6.15)	.06			
18.5–24.9	1438	0.99	(0.57–1.75)	.99			
> = 25	1431		Reference				
Surgical procedure							
Partial resection	991	1.19	(0.27–5.23)	.81			
Subsegmentectomy Couinaud	1661	0.64	(0.14–2.97)	.57			
Segmentectomy	1897	0.68	(0.15–3.06)	.61			
Lobectomy	525	1.54	(0.35–6.71)	.56			
Extended lobectomy	1431		Reference				

(*continued*)

Table 2

(continued).

Variable	Univariate analysis MDT	HR	95% CI	*P* value	Multivariate analysis HR	95% CI	*P* value
Number of tumor							
Single	1431	0.61	(0.33–1.12)	.11	0.67	(0.35–1.31)	.25
Multiple	520		Reference			Reference	
Tumor size (cm)							
< 5	1507	0.54	(0.32–0.93)	.03	0.57	(0.31–1.02)	.06
>=5	520		Reference			Reference	
Tumor differentiation							
Well-Moderate	1262	1.31	(0.68–2.53)	.43	1.04	(0.52–2.05)	.91
Poor	1644		Reference			Reference	
Vascular invasion							
Absent	1312	0.83	(0.48–1.43)	.50	0.97	(0.53–1.79)	.93
Present	1262		Reference			Reference	
Steatosis							
Absent	525	2.22	(1.28–3.85)	.004	2.14	(1.21–3.80)	.009
Present	1661		Reference			Reference	
Lobular inflammation							
Score 0–1	1438	0.97	(0.57–1.65)	.92			
Score 2–3	1280		Reference				
Ballooning							
Score 0	1507	1.06	(0.62–1.79)	.84			
Score 1–2	1266		Reference				
Fibrosis							
Stage 0–2	1507	0.74	(0.42–1.32)	.31	1.56	(0.80–3.05)	.19
Stage 3–4	992		Reference			Reference	

CI = confidence interval, DFS = disease-free survival, HR = hazard ratio, MDT = median disease-free survival time, NANV-HCC = nonalcoholic non-virus-related hepatocellular carcinoma.

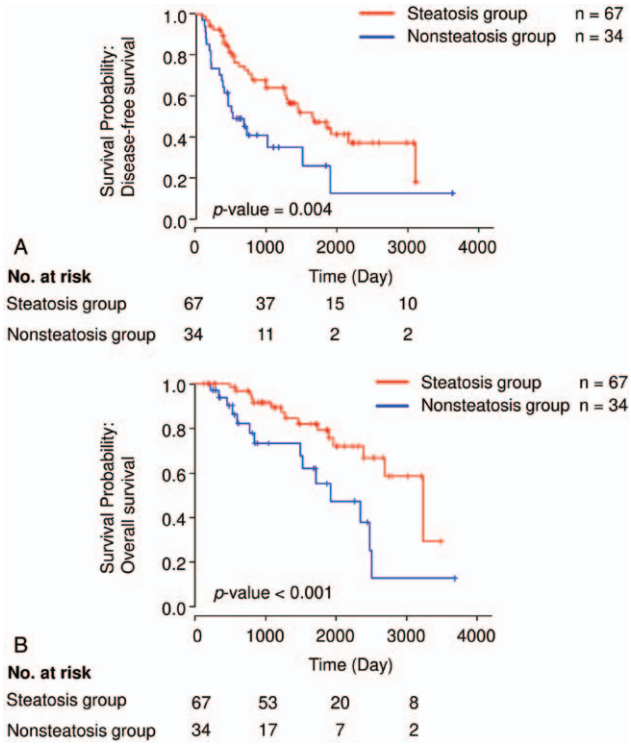

Figure 2. Kaplan–Meier curves of (A) disease-free survival and (B) overall survival after curative-intent resection in patients with nonalcoholic non-virus-related hepatocellular carcinoma. Red lines represent the survival probability of steatosis group and blue lines represent that of nonsteatosis group.

high prevalence rate of metabolic disorders, suggesting that they may associate with carcinogenesis.

NANV-HCC is associated with metabolic syndrome.[5,29–31] Indeed, in this study, 86.1% of the patients had metabolic disorders. Generally, metabolic disorders are considered to result in liver cell damage causing steatosis in the liver. Steatosis is the basis of nonalcoholic steatohepatitis (NASH)[32,33] leading to liver cirrhosis and HCC.[34,35] Thus, NANV-HCC is considered to be derived from steatotic livers. However, NANV-HCC also occurs in nonsteatotic livers. One possible explanation of the cause of NANV-HCC in nonsteatotic livers is metabolic disorders, which are considered to play a crucial role in carcinogenesis.[5,36] The degree of steatosis, which is initially induced by the metabolic disorders, may be reduced as the risk of carcinogenesis increases with repeated hepatocyte turnover due to steatohepatitis, while hepatic fibrosis remains relatively constant. However, we cannot rule out the existence of a direct pathway of carcinogenesis that does not involve hepatic steatosis nor the possibility of the presence of unknown infectious agents.

In NASH, steatosis may disappear as liver fibrosis progresses, and the end-stage cirrhotic NASH is called burnt-out NASH, which is characterized by massive fibrosis, but does not have specific pathological findings.[12,35] Without past medical records that confirm the presence of steatohepatitis, burnt-out NASH results in a diagnosis of cryptogenic cirrhosis. The absence of steatosis in the liver at the time of surgery does not always indicate that patients have never experienced NASH. However, in this study, the degree of fibrosis was grade 1 or 2 in the majority of patients with nonsteatotic livers, and the difference in the degree of fibrosis between the groups was not significantly different. As massive fibrosis is a key characteristic of burnt-out

Table 3

OS analysis in patients with NANV-HCC.

Variable	Univariate analysis				Multivariate analysis		
	MST	HR	95% CI	*P* value	HR	95% CI	*P* value
Age (yr)							
<65	2652	0.82	(0.37–1.84)	0.64			
>=65	2438		Reference				
Sex							
Female	2315	2.25	(0.96–5.29)	0.06			
Male	2652		Reference				
Body mass index (kg/m^2)							
<18.5	2469	1.29	(0.58–2.88)	0.52			
18.5–24.9	2350	1.92	(0.52–7.10)	0.33			
>=25	3180		Reference				
Surgical procedure							
Partial resection	1937	1.49	(0.19–11.7)	0.73			
Subsegmentectomy Couinaud	n.r.	0.61	(0.06–5.95)	0.91			
Segmentectomy	n.r.	0.88	(0.11–7.35)	0.67			
Lobectomy	2469	1.43	(0.18–11.6)	0.70			
Extended lobectomy	n.r.		Reference				
Number of tumor							
Single	2652	0.54	(0.24–1.25)	0.15	0.59	(0.22–1.57)	.29
Multiple	2438		Reference			Reference	
Tumor size (cm)							
<5	2438	0.83	(0.39–1.78)	0.64	1.80	(0.68–4.80)	.24
>=5	2652		Reference			Reference	
Tumor differentiation							
Well-Moderate	2469	2.07	(0.72–6.00)	0.17	1.59	(0.52–4.86)	.41
Poor	n.r.		Reference			Reference	
Vascular invasion							
Absent	3180	0.55	(0.26–1.17)	0.12	0.47	(0.19–1.21)	.11
Present	2469		Reference			Reference	
Steatosis							
Absent	1897	2.78	(1.33–5.80)	0.006	2.79	(1.27–6.16)	.01
Present	3180		Reference			Reference	
Lobular inflammation							
Score 0–1	2350	1.04	(0.49–2.20)	0.91			
Score 2–3	2469		Reference				
Ballooning							
Score 0	2438	1.32	(0.63–2.77)	0.46			
Score 1–2	2652		Reference				
Fibrosis							
Stage 0–2	2469	1.20	(0.51–2.81)	0.68	0.76	(0.25–2.31)	.63
Stage 3–4	3180		Reference			Reference	

CI = confidence interval, HR = hazard ratio, MST = median survival time, n.r. = not reached, NANV-HCC = nonalcoholic non-virus-related hepatocellular carcinoma, OS = overall survival.

NASH, it is not likely that the majority of nonsteatotic livers are due to burnt-out NASH in this study.

We found that the absence of steatosis is an independent risk factor for recurrence, which is consistent with the results of a previous study.[28] However, the previous study included patients with alcohol-related HCC, and overall survival was not evaluated in terms of the absence of steatosis in the liver. In this study, patients with alcohol-related HCC were excluded and we found that the absence of background steatosis was not only associated with early recurrence, but also with poor OS. The steatosis condition of the liver may reflect the aggressiveness of NANV-HCC. These findings can be used by physicians to determine the appropriate follow-up interval and to identify patients who would benefit from intensive adjuvant therapy.

This study has several limitations that deserve attention. First, due to the retrospective nature of this study, there is a potential for selection bias. All patients included in this study underwent hepatectomy with curative-intent, and no patients had advanced-stage disease. To minimize the risk of selection bias, consecutive patients treated between 2005 and 2016 were included in this study. Second, the evaluation of the liver was based on focal observation of the noncancerous region of the resected liver and did not represent the entire liver. Third, there is potential for a lead-time bias of the screening interval before the diagnosis of HCC. Fourth, the alcohol intake was self-reported and may have been inaccurate. Fifth, the nonsteatosis group had fewer patients than the steatosis group. This imbalance may obscure the clinicopathological differences between the nonsteatosis and steatosis groups. To obtain detailed features of the nonsteatosis group, studies with a larger patient population would be needed.

In conclusion, our study demonstrated that the absence of steatosis in the liver is associated with a shorter DFS and OS after curative-intent hepatectomy for NANV-HCC. Our findings

suggest that nonsteatotic liver can be a surrogate phenotype of aggressive NANV-HCC and a high-risk group for recurrence.

Acknowledgments

We thank Mr. Motoyoshi Iwakoshi and Ms. Tomoyo Kakita for their kind assistance in sample preparation.

Author contributions

Conceptualization: Yasuyuki Shigematsu, Hiroaki Kanda, Kentaro Inamura.
Data curation: Yasuyuki Shigematsu.
Formal analysis: Yasuyuki Shigematsu, Gulanbar Amori.
Methodology: Yasuyuki Shigematsu, Hiroaki Kanda, Gulanbar Amori, Yu Takahashi, Yutaka Takazawa, Kentaro Inamura.
Project administration: Yasuyuki Shigematsu, Kentaro Inamura.
Resources: Hiroaki Kanda, Yu Takahashi, Yutaka Takazawa, Kentaro Inamura.
Supervision: Kentaro Inamura.
Writing – original draft: Yasuyuki Shigematsu, Kentaro Inamura.
Writing – review & editing: Yasuyuki Shigematsu, Hiroaki Kanda, Gulanbar Amori, Yu Takahashi, Yutaka Takazawa, Kentaro Inamura.

References

[1] Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin 2018;68:394–424.
[2] Akinyemiju T, Abera S, Ahmed M, et al. Collaboration, GBoDLCThe burden of primary liver cancer and underlying etiologies from 1990 to 2015 at the global, regional, and national level: results from the global burden of disease study 2015. JAMA Oncol 2017;3:1683–91.
[3] Tateishi R, Uchino K, Fujiwara N, et al. A nationwide survey on non-B, non-C hepatocellular carcinoma in Japan: 2011-2015 update. J Gastroenterol 2019;54:367–76.
[4] Lieber CS. Alcohol and the liver: 1994 update. Gastroenterology 1994;106:1085–105.
[5] El-Serag HB, Tran T, Everhart JE. Diabetes increases the risk of chronic liver disease and hepatocellular carcinoma. Gastroenterology 2004; 126:460–8.
[6] Lai MS, Hsieh MS, Chiu YH, Chen TH. Type 2 diabetes and hepatocellular carcinoma: a cohort study in high prevalence area of hepatitis virus infection. Hepatology 2006;43:1295–302.
[7] Yu MC, Tong MJ, Govindarajan S, Henderson BE. Nonviral risk factors for hepatocellular carcinoma in a low-risk population, the non-Asians of Los Angeles County, California. J Natl Cancer Inst 1991;83:1820–6.
[8] Alexander M, Loomis AK, van der Lei J, et al. Risks and clinical predictors of cirrhosis and hepatocellular carcinoma diagnoses in adults with diagnosed NAFLD: real-world study of 18 million patients in four European cohorts. BMC Med 2019;17:95.
[9] Anstee QM, Reeves HL, Kotsiliti E, Govaere O, Heikenwalder M. From NASH to HCC: current concepts and future challenges. Nat Rev Gastroenterol Hepatol 2019;16:411–28.
[10] Said A, Ghufran A. Epidemic of non-alcoholic fatty liver disease and hepatocellular carcinoma. World J Clin Oncol 2017;8:429–36.
[11] Kanwal F, Kramer JR, Mapakshi S, et al. Risk of hepatocellular cancer in patients with non-alcoholic fatty liver disease. Gastroenterology 2018; 155:1828–37.e1822.
[12] Yoshioka Y, Hashimoto E, Yatsuji S, et al. Nonalcoholic steatohepatitis: cirrhosis, hepatocellular carcinoma, and burnt-out NASH. J Gastroenterol 2004;39:1215–8.
[13] Poonawala A, Nair SP, Thuluvath PJ. Prevalence of obesity and diabetes in patients with cryptogenic cirrhosis: a case-control study. Hepatology 2000;32(4 Pt 1):689–92.
[14] Kulik L, El-Serag HB. Epidemiology and management of hepatocellular carcinoma. Gastroenterology 2019;156:477–91.e471.
[15] Regimbeau JM, Colombat M, Mognol P, et al. Obesity and diabetes as a risk factor for hepatocellular carcinoma. Liver Transpl 2004;10:S69–73.
[16] Bruix J, Sherman M. Practice Guidelines Committee AAftSoLDManagement of hepatocellular carcinoma. Hepatology 2005;42:1208–36.
[17] European Association For The Study Of The, L., European Organisation For, R., Treatment Of CEASL-EORTC clinical practice guidelines: management of hepatocellular carcinoma. J Hepatol 2012;56:908–43.
[18] Sasaki K, Shindoh J, Margonis GA, et al. Effect of background liver cirrhosis on outcomes of hepatectomy for hepatocellular carcinoma. JAMA Surg 2017;152:e165059.
[19] Kawamura Y, Arase Y, Ikeda K, et al. Large-scale long-term follow-up study of Japanese patients with non-alcoholic Fatty liver disease for the onset of hepatocellular carcinoma. Am J Gastroenterol 2012;107: 253–61.
[20] Bosman FT, Carneiro F, Hruban RH, Theise ND. WHO classification of tumours of the digestive system. 4th Edition 2010;3:196–261.
[21] Kleiner DE, Brunt EM, Van Natta M, et al. Design and validation of a histological scoring system for nonalcoholic fatty liver disease. Hepatology 2005;41:1313–21.
[22] Brunt EM, Tiniakos DG. Histopathology of nonalcoholic fatty liver disease. World J Gastroenterol 2010;16:5286–96.
[23] Shibahara J, Ando S, Sakamoto Y, Kokudo N, Fukayama M. Hepatocellular carcinoma with steatohepatitic features: a clinicopathological study of Japanese patients. Histopathology 2014;64:951–62.
[24] Salomao M, Remotti H, Vaughan R, Siegel AB, Lefkowitch JH, Moreira RK. The steatohepatitic variant of hepatocellular carcinoma and its association with underlying steatohepatitis. Hum Pathol 2012;43: 737–46.
[25] El-Serag HB, Hampel H, Javadi F. The association between diabetes and hepatocellular carcinoma: a systematic review of epidemiologic evidence. Clin Gastroenterol Hepatol 2006;4:369–80.
[26] Polesel J, Zucchetto A, Montella M, et al. The impact of obesity and diabetes mellitus on the risk of hepatocellular carcinoma. Ann Oncol 2009;20:353–7.
[27] Paradis V, Zalinski S, Chelbi E, et al. Hepatocellular carcinomas in patients with metabolic syndrome often develop without significant liver fibrosis: a pathological analysis. Hepatology 2009;49:851–9.
[28] Nishio T, Hatano E, Sakurai T, et al. Impact of hepatic steatosis on disease-free survival in patients with non-B non-C hepatocellular carcinoma undergoing hepatic resection. Ann Surg Oncol 2015; 22:2226–34.
[29] Tokushige K, Hashimoto E, Kodama K. Hepatocarcinogenesis in nonalcoholic fatty liver disease in Japan. J Gastroenterol Hepatol 2013; 28:88–92.
[30] Calle EE, Rodriguez C, Walker-Thurmond K, Thun MJ. Overweight, obesity, and mortality from cancer in a prospectively studied cohort of U. S. adults. N Engl J Med 2003;348:1625–38.
[31] Ertle J, Dechene A, Sowa JP, et al. Non-alcoholic fatty liver disease progresses to hepatocellular carcinoma in the absence of apparent cirrhosis. Int J Cancer 2011;128:2436–43.
[32] Pais R, Pascale A, Fedchuck L, Charlotte F, Poynard T, Ratziu V. Progression from isolated steatosis to steatohepatitis and fibrosis in nonalcoholic fatty liver disease. Clin Res Hepatol Gastroenterol 2011;35:23–8.
[33] Tilg H, Moschen AR. Evolution of inflammation in nonalcoholic fatty liver disease: the multiple parallel hits hypothesis. Hepatology 2010;52:1836–46.
[34] Adams LA, Lymp JF, St Sauver J, et al. The natural history of nonalcoholic fatty liver disease: a population-based cohort study. Gastroenterology 2005;129:113–21.
[35] Powell EE, Cooksley WG, Hanson R, Searle J, Halliday JW, Powell LW. The natural history of nonalcoholic steatohepatitis: a follow-up study of forty-two patients for up to 21 years. Hepatology 1990;11:74–80.
[36] Cox AJ, West NP, Cripps AW. Obesity, inflammation, and the gut microbiota. Lancet Diabetes Endocrinol 2015;3:207–15.