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Citation	Journal of Gastroenterology and Hepatology. 37(12); 2313-2320
Issue Date	2022-12-08
Type	Journal Article
Textversion	Author
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DOI	10.1111/jgh.16019

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Original Article

Age-dependent effects of diabetes and obesity on liver-related events in nonalcoholic fatty liver disease: Subanalysis of CLIONE in Asia

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This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1111/jgh.16019

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Funding statement: No funding support to this study.

Conflict of interest disclosure: The authors do not have any disclosures to report.

Ethics approval statement: This multicenter registry-based historical cohort study was approved by the institutional review board of Saga University Hospital, Saga, Japan (approval no. 2020-04-R-02; June 30, 2020), which waived the requirement for informed consent due to the use of pre-existing data.

ABSTRACT

Background& Aims: Older age, type 2 diabetes mellitus (T2DM), and obesity are known risk factors for liver-related events (LRE). We investigated the impacts of T2DM and obesity on LRE according to age in Japanese patients with nonalcoholic fatty liver disease (NAFLD).

Methods: We performed a subanalysis of a retrospective cohort study (CLIONE in Asia), including 1,395 patients with biopsy-proven NAFLD. The median follow-up was 4.6 years.

Results: The median age was 57 years, and 36.2% had T2DM. The median body mass index (BMI) was 27.4, and 28.5% were severely obese (BMI ≥ 30). During follow-up, 37 patients developed hepatocellular carcinoma (HCC), and 58 patients developed LRE. In patients younger than 65 years, advanced fibrosis (hazard ratio [HR] 7.69, $p < 0.001$) and T2DM (HR 3.37, $p = 0.017$) were HCC risk factors, and advanced fibrosis (HR 9.40, $p < 0.001$) and T2DM (HR 2.51, $p = 0.016$) were LRE risk factors. In patients 65 years and older, advanced fibrosis (HR 4.24, $p = 0.010$) and obesity (HR 4.60, $p = 0.006$) were HCC risk factors, and advanced fibrosis (HR 4.22, $p = 0.002$) and obesity (HR 4.22, $p = 0.002$) were LRE risk factors.

Conclusion: T2DM and obesity contributed to LRE in younger and older patients, respectively, along with advanced fibrosis. Therefore, controlling T2DM in patients younger than 65 years and controlling weight in patients 65 years and older could prevent LRE. The development of age-dependent screening and management strategies is necessary for patients with NAFLD.

Keywords: nonalcoholic fatty liver disease, nonalcoholic steatohepatitis, hepatocellular carcinoma, liver-related events, fibrosis, Age, type 2 diabetes mellitus, obesity, BMI, CLIONE in Asia

Introduction

Nonalcoholic fatty liver disease (NAFLD) and nonalcoholic steatohepatitis (NASH) are common causes of chronic liver disease that lead to liver cirrhosis, hepatic failure, and hepatocellular carcinoma (HCC) in the absence of significant alcohol consumption [1, 2]. NASH is also linked to metabolic disorders, such as obesity, type 2 diabetes mellitus (T2DM), and dyslipidemia [3]. A prospective cohort study conducted by the NASH Clinical Research Network reported that the all-cause mortality was higher among patients with NAFLD than among the general population [4]. Another large cohort study identified hepatic fibrosis as the only predictive factor for liver-related events (LRE) which includes HCC and decompensation, and mortality in patients with NAFLD [5,6]. Furthermore, mortality and the incidence of hepatic decompensation events increase with increasing fibrosis stages, and patients with advanced fibrosis are at higher risks of HCC and LRE [4].

According to a large retrospective multicenter cohort study conducted in Japan, the proportion of patients with nonviral HCC etiologies increased from 10.0% in 1991 to 32.5% in 2015 [7]. This increase may be associated with the rising prevalence of metabolic syndrome, as obesity and diabetes are highly associated with several cancers, including HCC [8]. The characteristics of Japanese patients with NAFLD differ from those of patients with NAFLD in many Western countries. A systematic review revealed that NAFLD was less prevalent in Japan (25.5%) than in other Asian countries, the US, and Europe and that only one-quarter (26%) of Japanese patients with NAFLD were obese (BMI>27.5), whereas 21% were lean (BMI<23) and 53% were overweight (BMI 23-27.5) [9]. The review also found that lean patients with NASH in Japan had a low prevalence of metabolic syndrome and tended to be older than those patients with NASH who were overweight or obese [9]. Genetic factors and the gut macrobiotic profiles of Japanese patients with NAFLD may contribute to these characteristics. Recently, we performed a multicenter, registry-based, retrospective cohort study—CLIONE in Asia—of 1,398 patients with biopsy-proven NAFLD [10]. We clarified that extrahepatic cancer, not cardiovascular events, was the leading cause of mortality in Japanese patients with NAFLD and hepatic fibrosis was significantly associated with LRE but not with mortality.

In addition to advanced fibrosis, older age is a risk factor for LRE, although some people develop LRE at a younger age. Considering the difference in backgrounds of NAFLD patients by age, it is assumed that the risk of LRE varies with age. Therefore, this study aimed to identify

the risk factors for LRE and how they vary with age in Japanese patients with biopsy-proven NAFLD.

Materials and Methods

Patients

This study was performed as a subanalysis of our previous study [10]. After excluding 3 patients with missing body mass index (BMI) data, we enrolled 1,395 Japanese patients diagnosed with NAFLD by liver biopsy and selected between December 1994 and December 2020 from the) and the following 15 hospitals in Japan participating in the Japan Study Group of Nonalcoholic Fatty Liver disease (JSG-NAFLD). We defined NAFLD as steatosis in ≥5% of hepatocytes [11] in the absence of other liver diseases, such as viral hepatitis, autoimmune hepatitis, and drug-induced liver disease. We excluded patients with daily alcohol consumption >30 g for men and >20 g for women. Each patient underwent imaging by ultrasound, computed tomography, or magnetic resonance imaging every 6–12 months to monitor HCC development. LRE were defined as any events requiring hospitalization, such as hepatic ascites, gastroesophageal varices, and hepatic encephalopathy in addition to HCC. This multicenter registry-based historical cohort study was approved by the institutional review board of Saga University Hospital, Saga, Japan (approval no. 2020-04-R-02; June 30, 2020), which waived the requirement for informed consent due to the use of pre-existing data. All patients provided written informed consent at the time of liver biopsy, and the study was conducted in accordance with the Declaration of Helsinki (2013).

Physical examination and laboratory and clinical parameters

We used standard clinical laboratory assays to measure blood cell counts and serum concentrations of albumin, aspartate aminotransferase, alanine aminotransferase, gamma-glutamyl transpeptidase, total cholesterol, triglycerides, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, fasting plasma glucose, hemoglobin A1c, and the 7S domain of type IV collagen. BMI was calculated as weight in kilograms divided by the square of height in meters.

Patients taking oral hypoglycemic agents and those with a fasting glucose concentrations ≥ 126 mg/dL or non-fasting glucose concentrations ≥ 200 mg/dL were diagnosed with T2DM. Patients with serum levels of low-density lipoprotein cholesterol ≥ 140 mg/dL, triglycerides ≥ 150 mg/dL, or high-density lipoprotein cholesterol < 40 mg/dL, and receiving treatment for

dyslipidemia were diagnosed with dyslipidemia. Patients with a systolic blood pressure of ≥140 mmHg, diastolic blood pressure of ≥90 mmHg, or receiving treatment for hypertension were diagnosed with hypertension.

Liver histology

We assessed liver pathology as previously described [10]. Digital images of biopsy samples were obtained using a batch slide scanner (NanoZoomer 3.2.15; Hamamatsu Photonics KK, Hamamatsu, Japan). Digital images were read and scored by an experienced pathologist (SA) at Saga University, who was blinded to the patients' clinical and laboratory data. Grading and staging were performed as described by Kleiner et al. [11] Brunt. Advanced fibrosis was defined as fibrosis stages 3 and 4.

Follow-up evaluation

The follow-up period was defined starting from the date of biopsy until the date of the last visit or death. Patient follow-up was conducted at 3- to 12-month intervals after NAFLD diagnosis, and anthropometric measurements and metabolic assessments were repeated at each visit. Only the first occurrence of LRE (the composite endpoint of gastroesophageal varices and bleeding, HCC, or decompensation) after liver biopsy was considered.

Statistical analysis

Patient characteristics were assessed using the Chi-square test or the Mann–Whitney U test, depending on the distribution of the data. We performed multivariate logistic regression analysis to identify factors associated with advanced fibrosis by calculating adjusted odds ratios (aORs) and 95% CIs after adjusting for sex, age, BMI, and presence or absence of T2DM and hypertension. Multivariate cox regression analysis was used to determine risk factors for LRE by calculating hazard ratios (HRs) after adjusting for sex, age, BMI, presence or absence of T2DM, hypertension, and fibrosis stage (0-2, 3-4). To identify the risk factor for LRE among each age group, we performed multivariate cox regression analysis adjusted with sex, BMI, presence or absence of T2DM, hypertension, and fibrosis stage (0-2, 3-4). All statistical analyses were performed using SPSS version 22 (SPSS Inc., Chicago, IL). All *p*-values less than 0.05 obtained from two-tailed tests were considered significant.

Results

Patient characteristics

Table 1 summarizes the demographic profiles and laboratory and histological data of the study patients. The 1,395 patients had a median age of 57 years and a median BMI of 27.4, and 598 (42.9%) were men. At biopsy, 1,005 (72%) patients were younger than 65 years. Among all patients, 935 (67%) had NASH (based on the fatty liver inhibition of progression algorithm) and 239 (17.1%), 539 (38.6%), 393 (28.2%), 198 (14.2%), and 26 (1.9%) were classified as presenting with hepatic fibrosis stages 0, 1, 2, 3, and 4 (cirrhosis), respectively. The group of patients 65 years and older included more women (72.8%) with a lower BMI and higher prevalences of T2DM and hypertension than the group younger than 65 years. Patients 65 years and older also had significantly higher aspartate aminotransferase and fasting plasma glucose levels and fibrosis-4 index scores. Patients younger than 65 years had significantly higher platelet counts and albumin, alanine aminotransferase, and gamma-glutamyl transpeptidase levels. The prevalence of advanced hepatic fibrosis was significantly higher in the older group (22.1%) than in the younger group (13.7%, $p < 0.001$).

Factors associated with advanced fibrosis

We performed multivariate logistic regression analysis to evaluate the associations of sex, age, BMI, T2DM, and hypertension with advanced fibrosis. Among all patients with NAFLD, advanced fibrosis was significantly associated with age (per 1 year; aOR 1.05, 95% CI 1.03–1.06, $p < 0.001$), BMI (per 1 kg/m^2; aOR 1.09, 95% CI 1.06–1.13, $p < 0.001$), and T2DM (aOR 2.40, 95% CI 1.77–3.27, $p < 0.001$) (Table 2). Significant risk factors for advanced fibrosis among patients younger than 65 years included sex (male; aOR 1.72, 95% CI 1.15–2.57, $p = 0.01$), age (per 1 year; aOR 1.08, 95% CI 1.05–1.11, $p < 0.001$), BMI (per 1 kg/m^2; aOR 1.11, 95% CI 1.07–1.16, $p < 0.001$), and T2DM (aOR 2.42, 95% CI 1.64–3.59, $p < 0.001$). Significant risk factors for advanced fibrosis among patients 65 years and older included sex (male; aOR 0.43, 95% CI 0.23–0.81, $p = 0.01$) and T2DM (aOR 2.30, 95% CI 1.38–3.82, $p = 0.001$) (Table 2).

Incidence of hepatocellular carcinoma and liver-related events

The median duration from the time of liver biopsy to the last follow-up visit was 4.6 years (range, 0.3–21.6 years). The median follow-up duration for patients younger than 65 years (4.9 years) was significantly longer than the follow-up duration for patients 65 years and older (3.9 years) ($p<0.001$). Of the 1,395 patients with NAFLD, 37 developed HCC (4.2 per thousand

person-years) and 58 developed LRE (6.6 per thousand person-years) during the follow-up period. Following biopsy, the cumulative HCC incidence was 1.9% after 5 years and 5.1% after 10 years (Figure 1a), whereas the cumulative incidence of LRE was 3.3% after 5 years and 7.2% after 10 years (Figure 1b). Among the 37 patients who developed HCC, 21 were younger than 65 years, and 16 were 65 years and older at the time of biopsy. The cumulative hepatocarcinogenesis rate was significantly higher in the older group than in the younger group ($p < 0.001$). Among the 58 patients who developed LRE, 35 were younger than 65 years, and 23 were 65 years and older at the time of biopsy. The cumulative incidence of LRE was also significantly higher in the older group than in the younger group ($p = 0.002$).

Factors associated with hepatocellular carcinoma and liver-related events by age

Table 3 shows the risk factors for HCC and LRE that we identified in this study. The multivariate analysis considered sex, age, T2DM, hypertension, BMI, and fibrosis stage. Among all patients with NAFLD, the significant risk factors for LRE were age (≥65 years; HR 2.57, $p = 0.002$), T2DM (HR 2.02, $p = 0.014$), and fibrosis stage (stages 3 and 4; HR 6.30, $p < 0.01$). The significant risk factors for HCC development were sex (male; HR 2.16, $p = 0.025$), age (≥ 65 years; HR 2.96, $p = 0.004$), T2DM (HR 2.02, $p = 0.047$), and fibrosis stage (stages 3 and 4; HR 5.43, $p < 0.01$).

Next, we evaluated risk factors for LRE and HCC according to age. Among patients younger than 65 years, we identified T2DM (HR 2.51 for LRE; HR 3.37 for HCC) and advanced fibrosis (stages 3 and 4; HR 9.40 for LRE; HR 7.69 for HCC) as risk factors for both LRE and HCC. Among patients 65 years and older, BMI (≥30; HR 4.22, $p = 0.002$) and advanced fibrosis (stages 3 and 4; HR 4.22, $p = 0.002$) were identified as significant risk factors for LRE, and sex (male; HR 3.13, $p = 0.026$), BMI (≥30; HR 4.60, $p = 0.006$), and advanced fibrosis (stages 3 and 4; HR 4.24, $p = 0.010$) were identified as significant risk factors for HCC (Table 4). Among patients younger than 65 years, those with T2DM showed significantly higher LRE and HCC development rates than those without T2DM ($p < 0.01$ and $p < 0.01$, respectively); however, this trend was not observed among patients 65 years and older ($p = 0.209$ and $p = 0.745$, respectively) (Figures 2, 3).

Discussion

We found that the risk factors for HCC development and LRE differed according to age in this large cohort of Japanese patients with NAFLD. To our knowledge, this is the first report to

assess age-related differences in risk factors among patients with biopsy-proven NAFLD. Advanced fibrosis and T2DM were risk factors for HCC and LRE in patients with NAFLD younger than 65 years, whereas advanced fibrosis and obesity were risk factors for HCC and LRE in patients with NAFLD 65 years and older.

The combination of NAFLD and NASH was identified as the second most common cause of HCC among waitlisted liver transplant candidates in 2017 [12], and Younossi et al. [3] reported annual HCC rates of 0.44 and 5.29 per thousand person-years among patients with NAFLD and NASH, respectively. Liver fibrosis is the most important predictor of mortality in patients with NAFLD [5, 13]. In a meta-analysis of fibrosis stage–specific data pooled from 5 multinational NAFLD cohorts, Dulai et al. [14] reported that all-cause and liver-related mortality increased exponentially with increasing fibrosis stage and that patients with NAFLD were at increased risk, even during the early stages of fibrosis. Though HCC is not the most common cause of death in patients with NAFLD, advanced hepatic fibrosis is a well-known risk factor for HCC and LRE. We previously performed a large-scale cohort study of Japanese patients with biopsy-proven NAFLD (CLIONE in Asia) [10] and found that fibrosis stage was significantly associated with LRE but not with overall mortality. In the present study, the annual rates of HCC development and LRE during the follow-up period were 4.2 and 6.6 per thousand person-years, respectively, which are higher than those reported in previous studies. This difference may be due to our hospital-based cohort including a larger proportion of NASH patients than other studies.

In addition to advanced fibrosis, we found that T2DM was a risk factor for LRE and HCC development among patients younger than 65 years. T2DM has been reported as a risk factor for NAFLD, HCC, and progression to advanced fibrosis [15]. Another study showed that insulin increases the risk of various cancers by stimulating cell growth through insulin receptors [16]. In many cases, T2DM is difficult to cure. Although the prevalence of T2DM was significantly lower in the younger group than in the older group in our study, younger patients with NAFLD had a longer disease duration, which may be associated with an increase in exposure to carcinogenic factors, including hyperinsulinemia [17].

We identified obesity (BMI $\geq$ 30) as a risk factor for LRE in patients with NAFLD 65 years and older. Meta-analyses have shown that overweight individuals have a 50% to 85% increased risk of incident HCC compared with nonobese individuals [18, 19], and a review reported that

obesity (BMI ≥ 30) doubles the risk of HCC [20]. Several hypotheses exist regarding the mechanisms through which overweight may contribute to carcinogenesis. In one hypothesis, adipose tissue remodeling in obese patients leads to chronic inflammation and the modification of adipokine secretion from adipocytes and macrophages. Adipose-derived proinflammatory molecules, such as interleukin-6 and tumor necrosis factor α, may activate oncogenic pathways in the liver [21]. Leptin, an adipokine with proinflammatory and profibrogenic effects, promotes growth by activating the Janus kinase/signal transducer and activator of transcription, phosphoinositide 3-kinase/Akt, and extracellular signal–regulated kinase signaling pathways [22]. Secretion of both leptin and tumor necrosis factor α from adipocytes increases in obese patients [23]. Furthermore, adiponectin activates 5′-AMP-activated protein kinase, which suppresses tumor growth and promotes cell apoptosis by regulating the mammalian target of rapamycin and c-Jun N-terminal kinase/caspase-3 signaling pathways, but its use is inhibited during obesity [24]. These pro-oncogenic molecular pathways are enhanced independently of liver fibrosis in obese patients.

Asian populations, including the population of Japan, experience lower obesity prevalence rates—especially for severe obesity—than Western populations [25]. The prevalence of obesity in Japan also decreases with age [9]. In this study, the prevalences of overweight (BMI ≥ 25) and obesity (BMI ≥ 30) were significantly lower for the older group (62.3% and 13.6%, respectively) than for the younger group (78.2% and 34.2%, respectively), which may indicate that severely obese patients 65 years and older are at an increased risk of hepatocarcinogenesis due to the persistence of the obesity-to-carcinogenesis mechanism. Asian people are at a higher risk of HCC development than White people because Asian people have more abdominal visceral fat relative to abdominal subcutaneous fat than Caucasian [26]. Further research on the mechanisms through which obesity contributes to HCC development in older patients is needed.

This study had strengths, including a large cohort that included many older subjects from multiple institutions, which allowed us to more accurately measure risk factors for LRE by age among patients with NAFLD. In addition, confirmation of the NAFLD diagnosis by liver biopsy allowed more precise analysis of histological findings and avoided the uncertainty associated with NAFLD diagnosis performed using ultrasonography. One pathologist performed all diagnoses to reduce the potential for variation in diagnoses between pathologists. This study also had some limitations. It was a hospital-based study with a retrospective cohort

and could have been subject to selection bias. Additionally, the follow-up period was not long enough to draw firm conclusions, especially for the older group of patients.

In conclusion, the results of this cohort study suggest that the previously identified risk factors of HCC and other LRE such as T2DM and obesity differ with age among Japanese patients with NAFLD. Adequate control of T2DM decreases the risk of HCC by approximately 30% [27]. Thus, the successful control of T2DM at a younger age and during earlier stages of NAFLD might reduce the overall incidence of LRE. Though sarcopenia must be considered, weight reduction in older Asian patients is also an important consideration for preventing LRE. As individualized treatment of NAFLD becomes more important in the future, it is important to evaluate risk factors by the cluster to which the patient belongs, including age and race. The results of this study will be useful for establishing an age-dependent screening strategy and treatment goals for patients with NAFLD to prevent LRE.

ACKNOWLEDGMENTS

The authors are grateful to Ms. Keiko Ota and Ms. Masayo Kitano (Osaka City University) for their technical assistance in creating this REDCap project. The authors are also grateful to Ms. Kozue Tashiro and Ms. Maki Miyahara (Saga University Hospital) and Ms. Miki Noguchi (Kawasaki Medical School) for their technical assistance in creating virtual slides.

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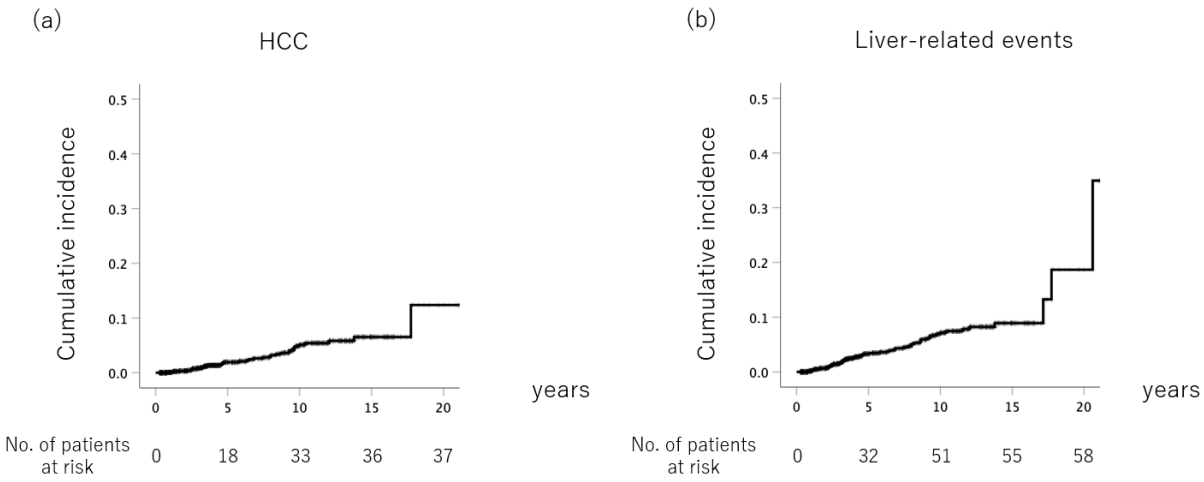

Figure 1. Cumulative incidence of (a) hepatocellular carcinoma (HCC) and (b) liver-related events in this study.

Figure 2

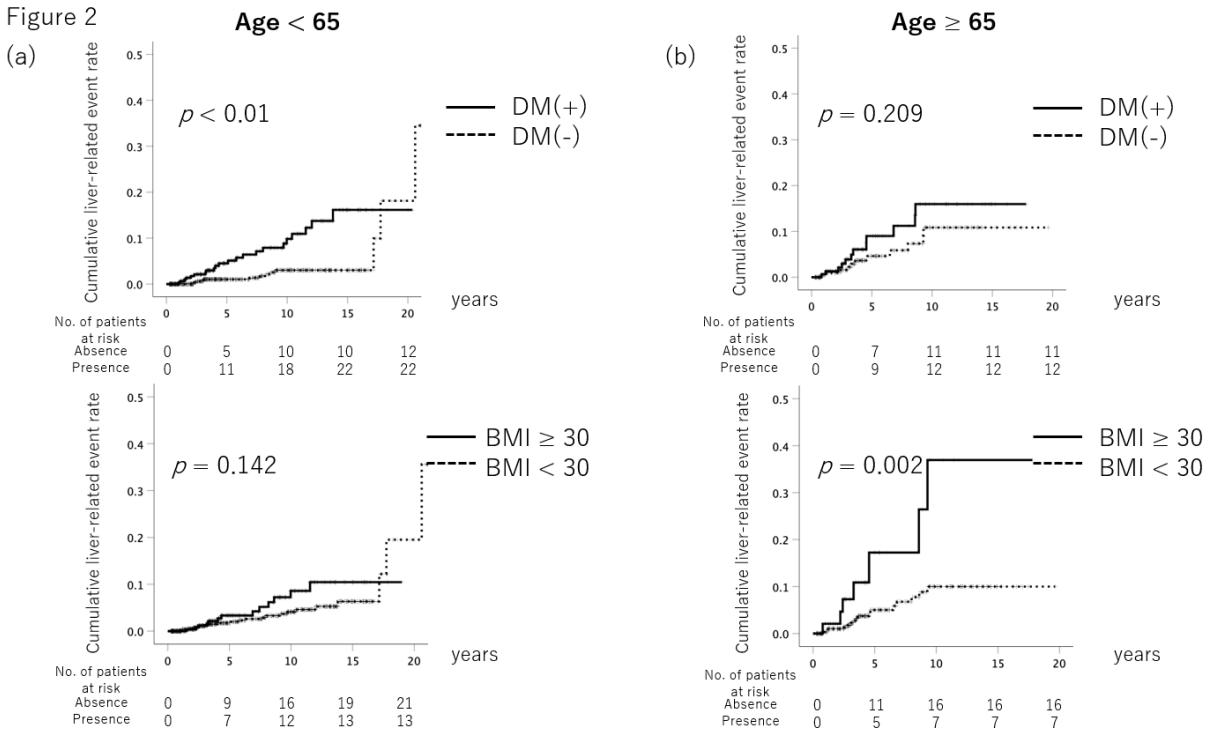

Figure 2. Cumulative incidence of liver-related events in patients (a) younger than 65 years and (b) those 65 years and older relative to the incidence of diabetes mellitus (DM) and body mass index (BMI).

Figure 3

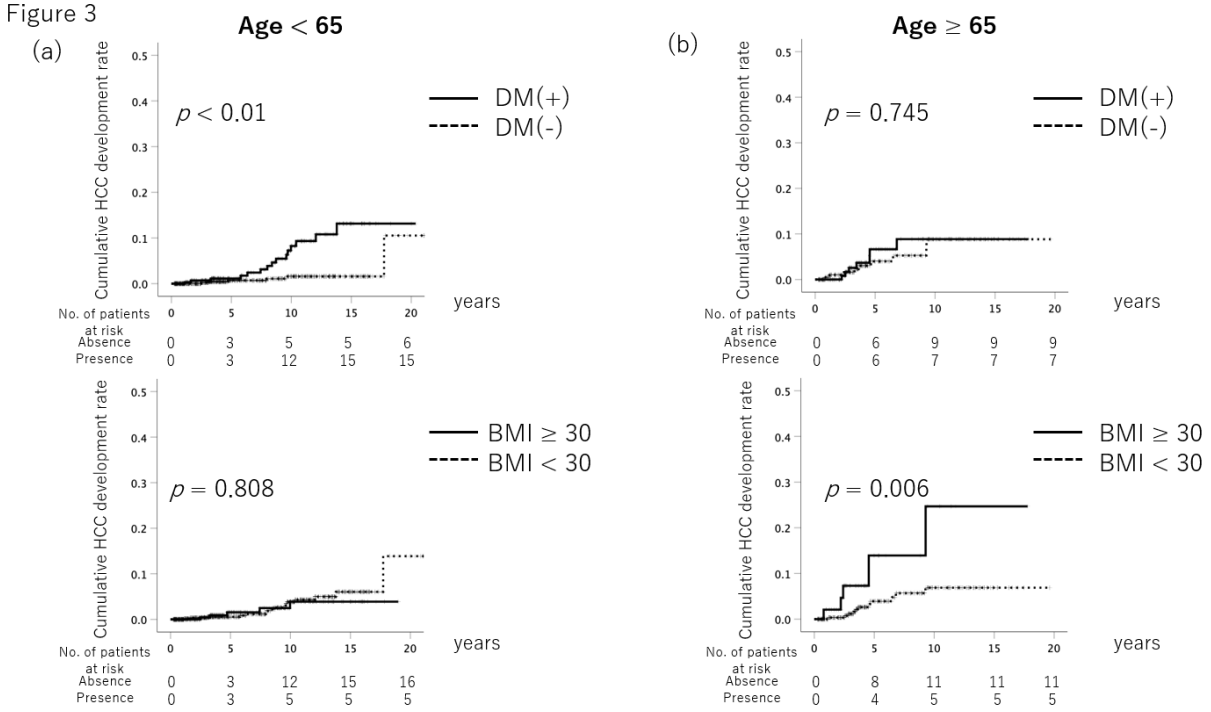

Figure 3. Cumulative incidence of hepatocellular carcinoma (HCC) in patients (a) younger than 65 years and (b) those 65 years and older relative to the incidence of diabetes mellitus (DM) and body mass index (BMI).

Table 1 Characteristics of patients with nonalcoholic fatty liver disease according to age.

Variable	Total n = 1,395	Age < 65 years n = 1,005	Age ≥ 65 years n = 390	*p*-value
Sex, male/female	598/797	492/513	106/284	<0.001
Age, years	57 (17–86)			
BMI, kg/m^2	27.4 (10.3–53.3)	28.6 (10.3–53.3)	26.3 (17.5–43.2)	<0.001
Hypertension	586 (42.0%)	351 (34.9%)	235 (60.3%)	<0.001
T2DM	505 (36.2%)	343 (34.1%)	162 (41.5%)	0.011
Hyperlipidemia	804 (44.0%)	565 (56.2%)	239 (61.3%)	0.091
Albumin, g/dL	4.4 (2.8–5.4)	4.4 (2.8–5.4)	4.2 (2.8–5.1)	<0.001
AST, U/L	51 (11–414)	50 (11–259)	54 (16–414)	0.02
ALT, U/L	73 (10–523)	81 (10–523)	58 (10–433)	<0.001
GGT, U/L	60.5 (9–1447)	64.5 (9–1447)	55 (12–511)	<0.001
Platelet count, ×10^3/μL	214 (34–637)	225 (34–470)	188 (56–637)	<0.001
Total cholesterol, mg/dL	198 (83–336)	200 (77–482)	191 (95–350)	<0.001
LDL cholesterol, mg/dL	126 (11–357)	129 (11–357)	117 (45–281)	<0.001
HDL cholesterol, mg/dL	47 (14–147)	47 (14–147)	50 (20–117)	0.002
TG, mg/dL	137 (41–851)	142 (43–851)	125 (41–523)	<0.001
FPG, mg/dL	104 (63–598)	103 (72–598)	109 (63–231)	<0.001
HbA1c, %	5.9 (4.2-12.9)	5.9 (4.2-12.9)	6.1 (4.4-12.6)	0.001
Type IV collagen 7s, ng/mL	4.4 (2.3-15.0)	4.4 (2.3-15.0)	5.3 (2.6-12.0)	<0.001
FIB-4 index	1.56 (0.09–12.56)	1.18 (0.09–12.48)	2.73 (0.50–12.56)	<0.001
Liver histology				
Fibrosis stage, 0/1/2/3/4	239/539/393/198/26	201/408/258/122/16	38/131/135/76/10	<0.001
Steatosis score, 0/1/2/3	8/980/271/136	6/648/227/124	2/332/44/12	<0.001
Inflammation score, 0/1/2/3	69/880/365/81	58/669/237/41	11/211/128/40	<0.001
Ballooning score, 0/1/2	452/614/329	373/440/192	79/174/137	<0.001

Results are presented as n (%) for qualitative data and median (range) for quantitative data. Abbreviations: BMI, body mass index; T2DM, type 2 diabetes mellitus; AST, aspartate aminotransferase; ALT, alanine aminotransferase; GGT, gamma-glutamyl; TG, triglycerides; FPG, fasting plasma glucose; FIB-4, fibrosis-4.

Table 2 Factors associated with advanced fibrosis in patients with nonalcoholic fatty liver disease.

	Multivariate analysis	
	aOR (95% CI) [a]	*p*-value
Sex (male)	1.05 (0.76–1.45)	0.78
Age (per 1 year)	1.05 (1.03–1.06)	<0.001
BMI (per 1 kg/m^2)	1.09 (1.06–1.13)	<0.001
T2DM (positive)	2.40 (1.77–3.27)	<0.001
Hypertension (positive)	1.36 (0.98–1.87)	0.06
Age < 65 years		
Sex (male)	1.72 (1.15–2.57)	0.01
Age (per 1 year)	1.08 (1.05–1.11)	<0.001
BMI (per 1 kg/m^2)	1.11 (1.07–1.16)	<0.001
T2DM (positive)	2.42 (1.64–3.59)	<0.001
Hypertension (positive)	1.20 (0.80–1.79)	0.39
Age ≥ 65 years		
Sex (male)	0.43 (0.23–0.81)	0.01
Age (per 1 year)	1.05 (0.99–1.12)	0.07
BMI (per 1 kg/m^2)	1.06 (0.99–1.14)	0.08
T2DM (positive)	2.30 (1.38–3.82)	0.001
Hypertension (positive)	1.67 (0.96–2.90)	0.07

Abbreviations: aOR, adjusted odds ratio; BMI, body mass index; T2DM, type 2 diabetes mellitus.
[a] Estimated using multivariate logistic regression analysis.

Table 3 Factors associated with the development of liver-related events and hepatocellular carcinoma in patients with nonalcoholic fatty liver disease.

Variable	Category	Liver-related events		HCC	
		HR (95% CI) [a]	*p*-value	HR (95% CI) [a]	*p*-value
Sex	1: female	1		1	
	2: male	1.40 (0.81–2.41)	0.23	2.16 (1.10–4.22)	0.025
Age (years)	1: <65	1		1	
	2: ≥65	2.57 (1.41–4.68)	0.002	2.96 (1.42–6.20)	0.004
T2DM	1: No	1		1	
	2: Yes	2.02 (1.15–3.53)	0.014	2.02 (1.01–4.04)	0.047
Hypertension	1: No	1		1	
	2: Yes	0.74 (0.42–1.30)	0.30	0.91 (0.45–1.83)	0.78
BMI (kg/m^2)	1: <30	1		1	
	2: ≥30	1.53 (0.84–2.78)	0.16	1.02 (0.46–2.25)	0.97
Fibrosis stage	1: 0,1,2	1		1	
	2: 3,4	6.30 (3.63–10.92)	<0.001	5.43 (2.70–10.94)	<0.001

Abbreviations: HCC, hepatocellular carcinoma; HR: hazard ratio; T2DM, type 2 diabetes mellitus; BMI, body mass index.
[a] Estimated using multivariate Cox regression analysis.

Table 4 Factors associated with the development of liver-related events and hepatocellular carcinoma in patients with nonalcoholic fatty liver disease according to age.

Variable	Category	Liver-related events		HCC	
		HR (95% CI) [a]	*p*-value	HR (95% CI) [a]	*p*-value
Age < 65 years					
Sex	1: female	1		1	
	2: male	1.22 (0.61–2.43)	0.58	1.88 (0.78–4.56)	0.16
T2DM	1: No	1		1	
	2: Yes	2.51 (1.19–5.30)	0.016	3.37 (1.24–9.17)	0.017
Hypertension	1: No	1		1	
	2: Yes	1.22 (0.60–2.48)	0.58	1.76 (0.71–4.32)	0.22
BMI (kg/m^2)	1: <30	1		1	
	2: ≥30	0.89 (0.42–1.87)	0.76	0.40 (0.14–1.18)	0.096
Fibrosis stage	1: 0,1,2	1		1	
	2: 3,4	9.40 (4.55–19.40)	<0.001	7.69 (3.03–19.56)	<0.001
Age ≥ 65 years					
Sex	1: female	1		1	
	2: male	2.03 (0.86–4.81)	0.11	3.13 (1.14–8.54)	0.026
T2DM	1: No	1		1	
	2: Yes	1.57 (0.66–3.73)	0.30	1.11 (0.39–3.17)	0.84
Hypertension	1: No	1		1	
	2: Yes	0.66 (0.15–1.87)	0.23	0.38 (0.13–1.10)	0.075
BMI (kg/m^2)	1: <30	1		1	
	2: ≥30	4.22 (1.70–10.47)	0.002	4.60 (1.55–13.68)	0.006
Fibrosis stage	1: 0,1,2	1		1	
	2: 3,4	4.22 (1.67–10.63)	0.002	4.24 (1.41–12.73)	0.010

Abbreviations: HCC, Hepatocellular carcinoma; HR: hazard ratio; T2DM, type 2 diabetes mellitus; BMI, body mass index.

[a] Estimated using multivariate Cox regression analysis