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ORIGINAL ARTICLE

Comparison of long-term prognosis between non-obese and obese patients with non-alcoholic fatty liver disease

Michihiro Iwaki,*,† Takaomi Kessoku,*,† Kosuke Tanaka,*,† Anna Ozaki,* Yuki Kasai,* Atsushi Yamamoto,* Kota Takahashi,* Takashi Kobayashi,* Asako Nogami,* Yasushi Honda,* Yuji Ogawa,‡ Kento Imajo,§ Shunsuke Oyamada,¶ Noritoshi Kobayashi,‖ Shinichi Aishima,** Satoru Saito,* Atsushi Nakajima* and Masato Yoneda*

*Department of Gastroenterology and Hepatology, Yokohama City University School of Medicine, Graduate School of Medicine, Departments of †Palliative Medicine, ‖Oncology, Yokohama City University Hospital, ‡Department of Gastroenterology, National Hospital Organization Yokohama Medical Center, Yokohama, §Department of Gastroenterology, Shinyurigaoka General Hospital, Kawasaki, ¶Department of Biostatistics, JORTC Data Center, Tokyo and **Department of Pathology and Microbiology, Faculty of Medicine, Saga University, Saga, Japan

Key words
clinical events, non-alcoholic fatty liver disease, non-obese non-alcoholic fatty liver disease.

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Correspondence
Masato Yoneda, Department of Gastroenterology and Hepatology, Yokohama City University Graduate School of Medicine, 3-9 Fukuura, Kanazawa-ku, Yokohama 236-0004, Japan.
Email: yoneda@yokohama-cu.ac.jp

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Author contribution: Michihiro Iwaki, Takaomi Kessoku, Atsushi Nakajima, and Masato Yoneda participated in the study design. Michihiro Iwaki, Takaomi Kessoku, Kosuke Tanaka, Anna Ozaki, Yuki Kasai, Atsushi Yamamoto, Kota Takahashi, Takashi Kobayashi, Asako Nogami, Yasushi Honda, Masato Yoneda, and Satoru Saito were responsible for data collection. Liver biopsies were performed by Yasushi Honda, Yuji Ogawa, and Kento Imajo. Shinichi Aishima was responsible for the pathological evaluation. Michihiro Iwaki, Takaomi Kessoku, Shunsuke Oyamada, Atsushi Nakajima, and Masato Yoneda were involved in the data analysis. Michihiro Iwaki, Takaomi Kessoku, and Atsushi Nakajima were responsible for preparing the tables and figures. All authors contributed to the manuscript review and writing and have approved the final draft. All authors approved the final version of the article, including the authorship list.

Abstract

Background and Aim: Non-alcoholic fatty liver disease (NAFLD) can progress in non-obese patients as in obese patients. Reports on long-term prognosis in non-obese NAFLD patients are controversial. Therefore, we aimed to examine the long-term prognosis of non-obese patients with NAFLD.

Methods: This single-center, retrospective cohort study enrolled biopsy-proven non-obese and obese NAFLD patients between January 2002 and December 2011 and followed them up until 31 March 2021, for death and clinical events (cardiovascular and liver-related events and extrahepatic cancers).

Results: Of the 223 NAFLD patients, 58 (26.0%) were non-obese. Compared with obese patients, they had a lower fibrosis stage (0.8 ± 0.80 *vs* 1.2 ± 0.91; $P = 0.004$), milder lobular inflammation (0.9 ± 0.7 *vs* 1.1 ± 0.7; $P = 0.02$), and significantly lower serum creatinine, total bilirubin, ferritin, and type IV collagen 7S and higher high-density lipoprotein levels. After a median follow-up of 8.9 years, no significant difference was noted in mortality between the two groups (2 [3.4%] non-obese *vs* 5 [3.0%] obese; log-rank test, $P = 0.63$). Twelve patients (20.7%) in the non-obese group and 32 (19.4%) in the obese group had clinical events. Although the obese group had a higher incidence of clinical events during the first 10 years of follow-up, the non-obese group had a higher incidence after that (log-rank test, $P = 0.67$). The non-obese group had a high incidence of malignancy (9 [15.5%] non-obese *vs* 14 [8.3%] obese; $P = 0.13$).

Conclusion: Non-obese NAFLD does not necessarily have a good prognosis, and some cases have a poor prognosis such as extrahepatic cancers. Further validation is required in the future.

Introduction

Non-alcoholic fatty liver disease (NAFLD) is characterized by hepatic steatosis in the absence of viral hepatitis, medications, or significant alcohol consumption. NAFLD is the hepatic phenotype of lifestyle-related diseases and has become a relevant liver disease in recent years. NAFLD can be classified as non-alcoholic fatty liver (NAFL) and non-alcoholic steatohepatitis (NASH). NAFL is generally considered to have a good

prognosis; however, NASH, which is considered to be a progressive condition with liver inflammation and fibrosis, may lead to cirrhosis and the development of hepatocellular carcinoma. Additionally, studies have shown that the risk of cardiovascular event is increased in NAFLD.[1] NAFLD patients are reported to have a higher mortality rate than the general population, and the common causes of death are cardiovascular events, malignancies other than hepatocellular carcinoma, and liver disease-related deaths.[2,3]

The prevalence of NAFLD is high in individuals with obesity. However, NAFLD can progress in non-obese individuals; this condition is called "non-obese NAFLD" or "lean NAFLD." Non-obese NAFLD patients account for 12–20% of all patients with NAFLD.[4–7] In Asia, the commonly used criterion for non-obese NAFLD is NAFLD in patients with a body mass index (BMI) <25 kg/m.[2,8] According to previous reports, the pathological profile of non-obese NAFLD patients is relatively favorable.[9] With regard to long-term prognosis, non-obese NAFLD patients have no increased risk of overall mortality and lower NAFLD activity score (NAS) but an increased risk of developing severe liver disease.[10] Another report showed that there is a trend toward less severe disease in non-obese patients.[11] There is no unified view of the long-term prognosis. Therefore, we aimed to examine the long-term prognosis of non-obese patients with NAFLD.

Methods

Ethical approval. This clinical study was conducted in accordance with the principles of the Declaration of Helsinki and was approved by the local ethics committee of the Yokohama City University Hospital. Informed consent was obtained from all participants prior to enrollment. The study was registered as UMIN 000046497 (University Hospital Medical Information Network).

Study participants. This was a single-center, retrospective cohort study. We enrolled patients diagnosed with NAFLD by liver biopsy between 1 January 2000 and 31 December 2011. The lower limit for age was 20 years. The cases were followed up until 31 March 2021, for death and clinical events.

The exclusion criteria were other hepatic diseases, such as hepatitis C, hepatitis B, autoimmune hepatitis, primary biliary cirrhosis, sclerosing cholangitis, Wilson's disease, hemochromatosis, α1-antitrypsin deficiency, drug-induced steatohepatitis, and current or past history of significant alcohol consumption (>30 and >20 g/day of ethanol in men and women, respectively). Moreover, patients with coexisting malignancies and pregnant women were excluded. Patients were categorized into two groups: non-obese NAFLD ($n = 58$) and obese NAFLD ($n = 165$). We defined NAFLD with BMI <25 kg/m^2 as non-obese NAFLD and NAFLD with BMI ≥25 kg/m^2 as obese NAFLD.

Patients and public involvement. Patients were involved in the conduct of the study, especially the development of the research question was based on patients' experiences. The results of this study will be disseminated by an international report to the patients and medical staff.

Clinical and laboratory evaluation. We obtained a detailed history of all NAFLD patients, and detailed medical records were assigned to all participants. Herein, we retrospectively investigated the risk of clinical events and mortality. Clinical events included cardiovascular events, liver-related events (composite endpoint of gastroesophageal varices/bleeding, hepatocellular carcinoma, or decompensated cirrhosis), and extrahepatic cancers. Laboratory tests were performed using the standard techniques. Diabetes mellitus, hypertension, and dyslipidemia were diagnosed according to the standard criteria.[12–14]

DNA preparation and single-nucleotide polymorphisms genotyping. Genetic information was collected only from cases in which rs738409 (patatin-like phospholipase domain-containing protein 3 [*PNPLA3*]) had been previously determined by single-nucleotide polymorphisms (SNP) genotyping. No additional genotyping was performed for this study. Genomic DNA was extracted using Genomix (Talent, Trieste, Italy) from blood samples. Invader probe (Third Wave Technologies, Madison, WI, USA) was constructed for rs738409 and genotyped. The success rate of this assay was >99.0%.

Pathological evaluation. Liver biopsy samples were collected from all patients with NAFLD using a 16- or 18-G needle. The biopsy specimens were stained by HE staining and with Masson's trichrome stain and analyzed by an experienced pathologist (S. Aishima). An appropriate liver sample was defined as one measuring >16 mm in length or containing >10 portal tracts. Fatty liver was defined as >5% fat deposition in the sections. Grading and staging were performed according to Brunt *et al.* and Kleiner *et al.*, and NAS was evaluated as described previously.[15,16]

Follow-up evaluation. The follow-up period began on the date of biopsy and ended on the date of the last visit, death, or transplantation. Patients were followed up at 3- to 12-month intervals after the diagnosis of NAFLD. Clinical events were recorded during each visit, and anthropometric measurements and metabolic assessments were repeated. If the patient died, the date and cause of death were recorded. The follow-up duration was calculated as the time between the biopsy date and the most recent follow-up.

Statistical analysis. We analyzed the data using JMP version 14.0 (SAS Institute Inc., Cary, NC, USA) and Bell Curve for Excel (Social Survey Research Information Co., Ltd., Tokyo, Japan). Continuous and ordinal variables are expressed as mean (SD) or median and interquartile range (IQR) and compared using the unpaired *t*-test. Comparisons were made using a two-sided chi-square test for categorical variables; $P < 0.05$ was considered statistically significant. Survival probability and clinical outcomes were illustrated by Kaplan–Meier curves and compared between non-obese and obese patients using the log-rank test. Univariate and multivariate Cox regression models were used to identify the factors associated with the outcome in non-obese NAFLD patients. Variables selected for inclusion were based on statistical significance in the univariate model ($P < 0.05$). To determine whether individual risk factors could predict the occurrence of clinical events, receiver operating characteristic (ROC)

curves were drawn using logistic regression analysis. The ROC curve was used to determine the area under the curve (AUC) and cutoff values.

Results

Patient characteristics. As illustrated in Figure S1, Supporting information, 223 participants diagnosed with NAFLD on liver biopsy were enrolled in the study. Of the 223 patients with NAFLD, 58 (26.0%) were non-obese, and 165 (74.0%) were obese. The baseline characteristics of the study participants are presented in Table 1. The non-obese patients with NAFLD had significantly lower serum creatinine (0.67 *vs* 0.74 mg/dL; $P = 0.027$), total bilirubin (0.67 *vs* 0.81 mg/dL; $P = 0.009$), ferritin (211.5 *vs* 281.6 ng/mL; $P = 0.049$), and type IV collagen 7S (4.3 *vs* 5.0 ng/mL; $P < 0.001$) levels compared with obese patients with NAFLD. High-density lipoprotein cholesterol levels were significantly higher in the non-obese group than that in the obese group (57.8 *vs* 51.4 mg/dL; $P = 0.005$). In the non-obese group, dyslipidemia was seen in fewer patients than in the obese group (14.3% *vs* 32.3%; $P = 0.01$). As for rs738409 (*PNPLA3*), 55.3% of the non-obese group and 39.8% of the obese group had the *GG* genotype ($P = 0.10$). Compared with obese patients, non-obese patients had a lower grade of lobular inflammation (0.9 ± 0.7 *vs* 1.1 ± 0.7; $P = 0.02$) and a lower stage of fibrosis (0.8 ± 0.8 *vs* 1.2 ± 0.9; $P = 0.001$). There was no difference in the pathological findings of steatosis (1.3 ± 0.8 *vs* 1.3 ± 0.7; $P = 0.63$), ballooning (0.5 ± 0.8 *vs* 0.7 ± 0.7; $P = 0.09$), and NAS (2.7 ± 1.5 *vs* 3.0 ± 1.3; $P = 0.11$) (Table 2).

Table 2 Pathological characteristics of patients with non-obese non-alcoholic fatty liver disease (NAFLD) and obese NAFLD

Histology	Non-obese	Obese	*P*-value
NAS (0–8) mean (SD)	2.7 (1.5)	3.0 (1.3)	0.11
Median (IQR)	3 (2–4)	3 (2–4)	
Steatosis (0–3) mean (SD)	1.3 (0.8)	1.3 (0.7)	0.63
Median (IQR)	1 (1–2)	1 (1–1)	
Lobular inflammation (0–3) mean (SD)	0.9 (0.7)	1.1 (0.7)	0.02
Median (IQR)	1 (0–1)	1 (1–1)	
Ballooning (0–2) mean (SD)	0.5 (0.8)	0.7 (0.7)	0.09
Median (IQR)	0 (0–1)	1 (0–1)	
Stage of fibrosis (0–4) mean (SD)	0.8 (0.8)	1.2 (0.9)	0.001
Median (IQR)	1 (0–1)	1 (1–2)	

Pathological findings were assessed using the Brunt classification and NAS.
IQR, interquartile range; NAS, NAFLD activity score.

Table 1 Clinical, biochemical characteristics of patients with non-obese non-alcoholic fatty liver disease (NAFLD) and obese NAFLD

Characteristics	Non-obese	Obese	*P*-value
n	58	165	
Age (years, SD)	54.9 (13.5)	51.2 (11.6)	0.06
Male sex (*n*, %)	25 (43)	89 (54)	0.21
BMI (kg/m^2, SD)	22.4 (2.6)	29.8 (3.7)	<0.001
Systolic blood pressure (mmHg, SD)	128.8 (15.0)	132.2 (15.5)	0.42
Diastolic blood pressure (mmHg, SD)	77.3 (10.6)	80.6 (12.5)	0.27
Creatinine (mg/dL, SD)	0.67 (0.20)	0.74 (0.17)	0.027
Platelet count (/103 μL, SD)	23.9 (6.6)	23.3 (7.0)	0.59
Total bilirubin (mg/dL, SD)	0.67 (0.28)	0.81 (0.44)	0.009
Alkaline phosphatase (IU/L, SD)	283.3 (140.9)	279 (107.7)	0.84
γ-Glutamyltranspeptidase (IU/L, SD)	85.7 (87.7)	92.5 (126.5)	0.65
Alanine aminotransferase (IU/L, SD)	66.4 (53.3)	76.7 (59.1)	0.22
Aspartate aminotransferase (IU/L, SD)	46.9 (37.6)	50.1 (39.7)	0.58
Albumin (g/dL, SD)	4.5 (0.3)	4.5 (0.37)	0.58
Fasting glucose (mmol/L, SD)	110.3 (27.0)	119.2 (37.1)	0.055
Hemoglobin A_{1c} (%, SD)	6.02 (1.24)	6.25 (1.23)	0.26
Total cholesterol (mg/dL, SD)	207.8 (36.4)	201 (42.5)	0.22
HDL-cholesterol (mg/dL, SD)	57.8 (13.8)	51.4 (15.2)	0.005
LDL-cholesterol (mg/dL, SD)	127.6 (31.7)	130.1 (37.6)	0.89
Triglycerides (mg/dL, SD)	148.1 (78.3)	164.8 (93.0)	0.08
Ferritin (ng/mL, SD)	211.5 (201.2)	281.6 (260.9)	0.049
Type IV collagen 7S (ng/mL, SD)	4.2 (0.9)	5.0 (1.8)	<0.001
Hemoglobin (g/dL, SD)	14 (1.5)	14.5 (1.5)	0.06
FIB4-index (SD)	1.6 (1.2)	1.5 (1.3)	0.85
History of diabetes mellitus (*n*, %)	8 (14.0)	43 (26.7)	0.08
History of hypertension (*n*, %)	9 (16.4)	46 (28.6)	0.11
History of dyslipidemia (*n*, %)	8 (14.3)	52 (32.3)	0.01
PNPLA3 rs738409, *n*	38	108	
GG/CC + CG (*n*, %)	21/17 (55.3/44.7)	43/65 (39.8/60.2)	0.10

Data are presented as mean values with SDs for continuous parameters.
BMI, body mass index; HDL, high-density lipoprotein; LDL, low-density lipoprotein.

Table 3 Summary of clinical events during follow-up

Histology	Non-obese ($n = 58$)	Obese ($n = 165$)	*P*-value
Death (*n*, %)	2 (3.4%) (cancer 2)	5 (3.0%) (liver insufficiency 4, unknown 1)	0.87
Cardiovascular events (*n*, %)	2 (3.4%)	10 (6.1%)	0.44
Stroke (*n*, %)	2 (3.4%)	4 (2.4%)	NA
Myocardial infarction (*n*, %)	0	6 (3.6%)	NA
Liver-related events (*n*, %)	1 (1.7%)	8 (4.8%)	0.29
Other cancer (*n*, %)	9 (15.5%)	14 (8.5%)	0.13
Pancreas (*n*, %)	1 (1.7%)	0 (0%)	NA
Colon (*n*, %)	2 (3.4%)	1 (0.6%)	NA
Anal canal (*n*, %)	1 (1.7%)	0 (0%)	NA
Gastric (*n*, %)	0 (0%)	2 (1.2%)	NA
Duodenal (*n*, %)	0 (0%)	1 (0.6%)	NA
Biliary (*n*, %)	2 (3.4%)	0 (0%)	NA
Lung (*n*, %)	1 (1.7%)	2 (1.2%)	NA
Tongue (*n*, %)	1 (1.7%)	1 (0.6%)	NA
Thyroid (*n*, %)	0 (0%)	1 (0.6%)	NA
Laryngeal (*n*, %)	0 (0%)	1 (0.6%)	NA
Renal pelvis (*n*, %)	1 (1.7%)	0 (0%)	NA
Prost (*n*, %)	0 (0%)	1 (0.6%)	NA
Bladder (*n*, %)	0 (0%)	1 (0.6%)	NA
Lymphoma (*n*, %)	0 (0%)	2 (1.2%)	NA
Multiple myeloma (*n*, %)	0 (0%)	1 (0.6%)	NA

Prognosis of non-obese NAFLD. After a median follow-up of 8.9 years, two patients (3.4%) in the non-obese group and five (3.0%) in the obese group died. Both the non-obese patients died of cancer (Table 3). Among the obese patients, four died of liver failure and one died of an unknown cause. As shown in Figure 1, the survival probability was not significantly different between the two groups (log-rank test, $P = 0.63$).

A total of 33 patients experienced clinical events during the follow-up period, of which 12 (20.1%) belonged to the non-obese NAFLD group and 32 (19.4%) to the obese NAFLD group. No significant difference was noted in the rate of events between the two groups (log-rank test, $P = 0.67$) (Fig. 2a).

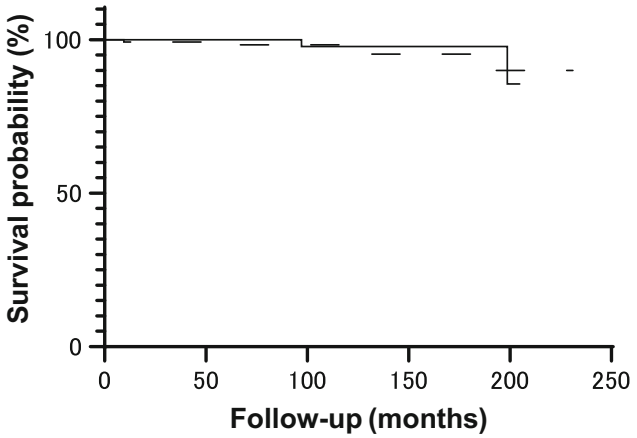

Figure 1 Kaplan–Meier analysis of survival probability in non-obese patients with non-alcoholic fatty liver disease (NAFLD) compared with that in obese patients. Non-obese NAFLD group ($n = 58$) and obese NAFLD group ($n = 165$). Survival probability was illustrated by Kaplan–Meier curves and compared between non-obese and obese patients using the log-rank test. No significant difference was noted between the groups (log-rank test, $P = 0.63$). (), Non-obese; (), obese.

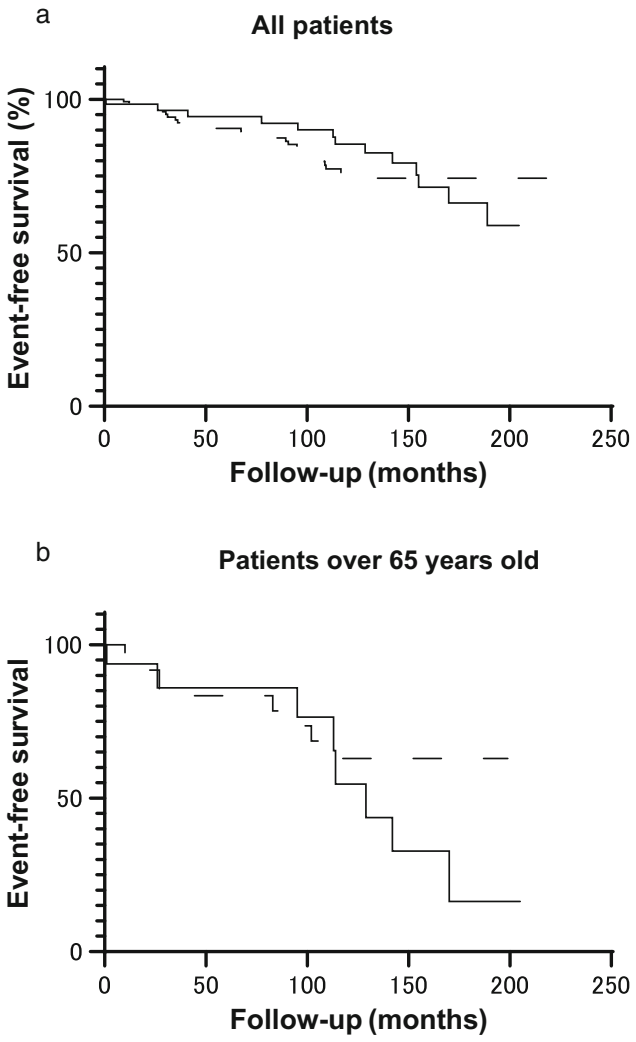

Figure 2 (a) Kaplan–Meier analysis of clinical event-free survival in non-obese patients with non-alcoholic fatty liver disease (NAFLD) compared with that in obese patients. Non-obese NAFLD group ($n = 58$) and obese NAFLD group ($n = 165$). Clinical outcomes were illustrated by Kaplan–Meier curves and compared between non-obese and obese patients using the log-rank test. No significant difference was found between the groups (log-rank test, $P = 0.67$). (b) Kaplan–Meier analysis of clinical event-free survival in non-obese NAFLD patients over 65 years compared with obese patients. Non-obese NAFLD group ($n = 16$) and obese NAFLD group ($n = 27$). Clinical outcomes were illustrated by Kaplan–Meier curves and compared between non-obese and obese patients using the log-rank test. (), Non-obese; (), obese.

During the first 10 years, the clinical event rate tended to be higher in the obese group; however, thereafter, it tended to be higher in the non-obese group.

The non-obese group was characterized by a higher incidence of malignancy compared with the obese group (9 (15.5%) non-obese *vs* 14 (8.3%) obese; $P = 0.13$) (Table 3). Regarding the type of cancer, there were single cases of pancreatic, lung, kidney pelvis, anal canal, and tongue cancer each and two cases of colonic and biliary tract cancer each. A trend could not be ascertained due to the low absolute number of individual cancers. In the non-obese and obese groups, 2 (3.4%) and 10 (6.1%) patients developed cardiovascular events, respectively ($P = 0.44$). In addition, one (1.7%) in the non-obese group and eight (4.8%) in the obese group developed liver-related events ($P = 0.29$). The incidence of cardiovascular events and liver-related events was lower in the non-obese group than in the obese group.

Risk factors for clinical events in the non-obese NAFLD. The Cox hazard model was used to examine the factors associated with clinical events in non-obese NAFLD patients. High age, low serum albumin levels, high type IV collagen 7S levels, and high FIB4-index were associated with clinical event rates in the univariable analysis (age–hazard ratio, 1.12; 95% CI, 1.05–1.21, $P = 0.0002$; albumin–hazard ratio, 0.09; 95% CI, 0.01–0.95, $P = 0.04$; type IV collagen 7S–hazard ratio, 2.28; 95% CI, 1.1–4.41, $P = 0.03$; FIB4-index–hazard ratio, 1.36, 95% CI, 0.96–1.78, $P = 0.046$). In a multivariate analysis using these factors, high age, low albumin, and high type IV collagen 7S were found to be independent factors significantly associated with clinical event rates (Table 4).

High age, low albumin, and high type IV collagen 7S predicted the occurrence of clinical events in non-obese NAFLD, with AUCs of 0.78, 0.68, and 0.63, respectively. The cutoff values for the occurrence of clinical events were as follows: age, 65 years; albumin, 4.4 g/dL; and type IV collagen 7S, 5.0 ng/mL. Using each cutoff value, Kaplan–Meier curves were drawn for cases with a high risk of clinical events. In particular, patients aged ≥65 years tended to have a higher incidence of clinical events in the non-obese group than in the obese group, although the difference was not statistically significant ($P = 0.25$, log-rank test) (Fig. 2b).

Table 4 Background factors associated with clinical events

	Univariate analysis		Multivariate analysis	
Factors	Hazard ratio (95% CI)	*P*-value	Hazard ratio (95% CI)	*P*-value
Age (years)	1.12 (1.05–1.21)	0.0002	1.16 (1.03–1.35)	0.03
Male sex	0.87 (0.26–2.90)	0.82	NA	NA
BMI	1.26 (0.91–2.08)	0.22	NA	NA
Creatinine (mmol)	1.44 (0.04–41.1)	0.83	NA	NA
Platelet count (/103 μL)	0.96 (0.87–1.05)	0.4	NA	NA
Alanine aminotransferase (IU/L)	1 (0.99–1.00)	0.92	NA	NA
Total bilirubin (mmol/L)	0.1 (0.002–2.28)	0.16	NA	NA
Alkaline phosphatase (IU/L)	1 (0.99–1.00)	0.94	NA	NA
γ-Glutamyltranspeptidase (IU/L)	1.0 (0.98–1.00)	0.39	NA	NA
Albumin (g/dL)	0.09 (0.01–0.95)	0.04	0.004 ($3.1e^{-5}$–0.12)	0.007
Fasting plasma glucose (mmol/L)	1 (0.99–1.02)	0.55	NA	NA
Hemoglobin A_{1c} (%)	1.18 (0.78–1.65)	0.39	NA	NA
Total cholesterol (mmol/L)	1.00 (0.98–1.03)	0.74	NA	NA
HDL-cholesterol (mmol/L)	1.03 (0.99–1.08)	0.14	NA	NA
LDL-cholesterol (mmol/L)	0.99 (0.97–1.01)	0.43	NA	NA
Triglycerides (mmol/L)	1.00 (0.99–1.01)	0.52	NA	NA
Ferritin (ng/mL)	1.00 (0.99–1.00)	0.14	NA	NA
Type IV collagen 7S (ng/mL)	2.28 (1.1–4.41)	0.03	4.07 (1.36–14.7)	0.02
Hemoglobin (g/dL)	0.95 (0.64–1.32)	0.77	NA	NA
FIB4-index	1.36 (0.96–1.78)	0.046	0.91 (0.31–2.20)	0.83
PNPLA3 rs738409 *GG*	1.43 (0.28–7.2)	0.66	NA	NA
History of diabetes mellitus	2.87 (0.84–9.89)	0.09	NA	NA
History of hypertension	0.37 (0.05–2.95)	0.35	NA	NA
History of dyslipidemia	0.68 (0.18–2.59)	0.57	NA	NA
NAS (0–8)	1.02 (0.69–1.48)	0.9	NA	NA
Steatosis (grade 2–3/0–1)	0.58 (0.16–2.14)	0.41	NA	NA
Lobular inflammation (grade 2–3/0–1)	1.48 (0.40–5.48)	0.56	NA	NA
Ballooning (grade 2–3/0–1)	0.87 (0.19–4.03)	0.86	NA	NA
Stage of fibrosis (stage 3–4/0–2)	1.37 (0.37–5.08)	0.64	NA	NA

Univariate and multivariate Cox regression models were used to identify factors associated with the outcome.
BMI, body mass index; CI, Confidence interval; HDL, high-density lipoprotein; LDL, low-density lipoprotein; NAS, non-alcoholic fatty liver disease activity score.

Discussion

Previous reports have shown that non-obese NAFLD patients have lower glucose tolerance and higher triglyceride and total cholesterol levels than non-obese controls. Most non-obese NAFLD patients will have some degree of metabolic abnormality.[17,18] The presence of NAFLD in lean individuals is said to be associated with overall and cardiovascular mortality, though no difference was found in the risk of carcinogenicity when compared with healthy individuals.[19] This means that the prognosis for non-obese NAFLD patients is worse than that for healthy individuals. However, the prognosis of non-obese NAFLD compared with that of obese NAFLD is debatable. In this study, the incidence of clinical events was higher in obese NAFLD patients for the first 10 years, consistent with two previous reports (with a median follow-up of 49 months and a median observation period of 4.6 years).[10,11] Interestingly, non-obese NAFLD tended to have a higher incidence of clinical events when the follow-up period was longer than 10 years. Although obesity is considered a risk factor for carcinogenesis,[20] the finding of a higher incidence of extrahepatic cancer in non-obese NAFLD patients in this study is highly novel. Compared with the obese group, the non-obese NAFLD group was more likely to develop extrahepatic cancers and had fewer cardiovascular and liver-related events. These results suggest that the non-obese group does not necessarily have a good prognosis and that there are some cases with a poor prognosis such as those with extrahepatic cancers, especially in the elderly. The longer observation period than that in previous reports may have contributed to these results.

Regional differences may also be a reason for the difference in prognosis from previous reports. A previous report showed that the prevalence of non-obese NAFLD exhibited regional variation. Europe had the highest (about 50%) while eastern Asia had the lowest prevalence of non-obese NAFLD among the NAFLD population.[21] This may be related to diagnostic methods, BMI cutoff values, lifestyle, dietary customs, and gut microbiota.[22] The racial differences in prognosis are not yet known, and further accumulation of cases is needed.

Recent reports have shown that NAFLD is associated with an increased risk of breast cancer in women and colorectal cancer in men.[23] In another review, NAFLD was implicated in the development of malignancies of the digestive organs (liver, colon, esophagus, stomach, and pancreas) and non-gastrointestinal sites (kidney in men and breast in women).[24] However, in this study, we could not show specific trends for the type of cancer. The mechanism by which NAFLD and NASH lead to extrahepatic carcinogenesis has not yet been elucidated. However, it has been suggested that dysbiosis of gut microbiota, insulin resistance, and the associated chronic inflammation create a microenvironment suitable for cancer development.[24]

In a previous report, noninvasive fibrosis markers, such as the FIB4 index and NFS (NAFLD fibrosis score), were reported to be risk factors not only for liver cancer but also for all cancers.[18] Older age, obesity, and the presence of diabetes mellitus are also risk factors for both extrahepatic carcinogenesis and hepatocellular carcinoma.[25,26] In non-obese NAFLD, advanced fibrosis and older age are reported to be associated with mortality.[10] In this study, older age, low albumin, and high type IV collagen 7S were found to be factors involved in clinical events (12 patients), including nine patients with extrahepatic cancers in the non-obese NAFLD group. Since old age is known to be a risk factor for carcinogenesis and cardiovascular events, it is not surprising that it was cited as a risk factor for clinical events. Increased risk of extrahepatic cancers in patients with low albumin and high type IV collagen 7S is a novel finding. Albumin is an indicator of liver function, and type IV collagen 7S is an indicator of liver fibrosis. Considering that there was only one liver-related event, we suspect that low albumin levels and high type IV collagen 7S concentrations are associated with the development of extrahepatic cancers in such patients. The results of this study suggest that non-obese patients with NAFLD who are older and have low albumin and high type IV collagen 7S should be carefully followed up. The FIB4 index, a common marker of liver fibrosis, has been reported to correlate with mortality and liver-related events in NAFLD.[27] In our study, the mean FIB4 index in the non-obese group was not high, and if they had been followed up by their general practitioners, they would have been discontinued from follow-up in many cases. However, we believe that regular follow-up is also necessary for patients older than 65 years, with hypoalbuminemia below 4.4 g/dL, and high type IV collagen7s levels above 5.0 ng/mL. There is still no consensus on what screening methods are the best and what cases should be followed up. The ideal screening and follow-up method will be proven in a prospective multicenter study that we are considering in the future.

It has been reported that non-obese NAFLD patients are more likely to have the rs738409 (*PNPLA3*) *GG* genotype.[28] The same trend was observed in the present study ($P = 0.10$). Although the *PNPLA3* risk allele is known to be associated with hepatocarcinogenesis,[29,30] its involvement in extrahepatic carcinogenesis is unknown. In this study, there was no association between *PNPLA3* risk allele and the occurrence of clinical events.

The obese group had a lower incidence of extrahepatic malignancy than the non-obese group, but a higher average incidence of all events. As the risk of cardiovascular events is higher in patients with obesity, dyslipidemia, and impaired glucose tolerance,[31–39] we speculate that the high incidence of complications, such as dyslipidemia, hypertension, and diabetes associated with obesity may be responsible for the high incidence of cardiovascular events. The higher incidence of liver-related events in the obese group can also be attributed to the degree of fibrosis and lobular inflammation seen in this study.

This study had several strengths. First, owing to the liver biopsies conducted, our participants could be histopathologically well characterized as patients with NAFLD. Second, the histopathological results were judged by one pathologist. Third, it was a long-term follow-up study with a median follow-up of 8.9 years.

Nonetheless, this study had several limitations. First, this was a retrospective study, and some patients were not followed up or had missing laboratory values. In addition, it was difficult to establish causal relationships. Second, this was a single-center analysis, and selection bias may have occurred. Moreover, the patients were enrolled at a tertiary care center in Japan, where liver biopsies are conducted for NAFLD patients with more severe liver conditions. Therefore, further validation is required. Third, due to fewer clinical events recorded over the course of

the study, it did not have sufficient statistical power. Therefore, studies with a larger population size should be conducted for further validation. Fourth, subgroups of patients with varying prognoses may have been present in the study population. Subgroup analysis failed to find subgroups with a statistically significant correlation to the occurrence of clinical events. Fifth, the *PNPLA3* gene polymorphism, which is associated with the development and progression of NAFLD/NASH, was not investigated in all patients. Finally, data on sarcopenia were not collected in this study. Sarcopenia has been also reported to be associated with the incidence of clinical events in NALFD patients.[40] For our future study, we will collect data on skeletal muscle mass and skeletal muscle index, which are necessary to assess sarcopenia.

In conclusion, long-term follow-up of more than 10 years suggests that non-obese NAFLD patients may have a higher risk of developing clinical events, mainly extrahepatic cancers. Although not statistically significant, the incidence of cardiovascular and liver-related events was higher in the obese group than in the non-obese group. The findings of this single-center analysis should be validated in prospective multi-institutional studies.

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Data availability statement. The supporting data are available from the corresponding author upon reasonable request.

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Supporting information

Additional supporting information may be found in the online version of this article at the publisher's website:

Figure S1. Flow of patients through the study.