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ORIGINAL ARTICLE

Outcomes of fatty liver disease with and without metabolic comorbidities and risk factors for mortality

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Abstract

Background and Aim: As the clinical course of metabolic-associated fatty liver disease (MAFLD) is unclear, we compared the clinical courses of MAFLD and non-alcoholic FLD (NAFLD).

Methods: Asian FLD patients ($n = 987$) from 1991 to 2021 (biopsy-proven in 939) were enrolled. The patients were divided into NAFLD (N-alone, $n = 92$), both MAFLD and N (M&N, $n = 785$), and M-alone ($n = 90$) groups. Clinical features, complications, and survival rates were compared among the three groups. Risk factors of mortality were subjected to Cox regression analysis.

Results: The N-alone group patients were significantly younger (N alone, M&N, and M alone: 50, 53, and 57 years, respectively), more frequently male (54.3%, 52.6%, and 37.8%), and had a low body mass index (BMI, 23.1, 27.1, and 26.7 kg/m^2) and FIB-4 index (1.20, 1.46, and 2.10). Hypopituitarism (5.4%) and hypothyroidism (7.6%) were significantly observed in the N-alone group. Hepatocellular carcinoma (HCC) developed in 0.0%, 4.2%, and 3.5% of the cases, and extrahepatic malignancies in 6.8%, 8.4%, and 4.7% of the cases, respectively, with no significant differences. The cardiovascular event rate was significantly higher in the M-alone group (1, 37, and 11 cases, $P < 0.01$). Survival rates were similar among the three groups. Risk factors for mortality were age and BMI in the N-alone group; age, HCC, alanine transaminase, and FIB-4 in the M&N group; and FIB-4 in the M-alone group.

Conclusion: Different risk factors for mortality may exist among the FLD groups.

Introduction

In patients with fatty liver disease (FLD), metabolic factors are associated with its development.[1–3] The concept and diagnostic criteria of metabolic (dysfunction)-associated fatty liver disease (MAFLD) have been proposed.[4] MAFLD includes overweight/obesity, type 2 diabetes, and metabolic dysfunction. Moreover, it could include other etiologies of chronic liver diseases.[5] Conversely, non-alcoholic fatty liver disease (NAFLD) excludes patients with moderate or heavy alcohol intake and other etiologies of chronic liver diseases, and the presence of metabolic dysfunction is not required.[5] MAFLD does not require pathological diagnosis, but MAFLD can diagnose more advanced fibrosis cases.[6,7]

Bianco *et al.*[8] showed that non-MAFLD and NAFLD were present in 4.7% of the general population and that the clinical features were younger age, lower body mass index (BMI), better metabolic profile, and less fibrosis. The clinical features of the two categories of FLD, namely MAFLD and NAFLD, frequently overlap. In the general population, nearly 3700 cases have been found to fulfill the criteria for both MAFLD and NAFLD.[8] The clinical features of MAFLD and NAFLD are reportedly older age, higher BMI, insulin

resistance, and metabolic comorbidities.[8] Patients with MAFLD but not NAFLD represent 2.6% of the general population and are predominantly male and have a better metabolic profile and more severe fibrosis.[8]

Regarding hepatocellular carcinoma (HCC) development in FLD, the prevalence of NAFLD-HCC was 0.44/1000 person-years and 5.29/1000 person-years in non-alcoholic steatohepatitis (NASH) patients in a global study.[9] Although the rate of HCC development is low, the prevalence of NASH-HCC is increasing. Therefore, high-risk patients need to be selected. Metabolic dysfunction has a significant impact on hepatocarcinogenesis in patients with FLD.[10]

In a large NAFLD cohort study, the liver-related death rate was found to be 0.77/1000 man-years and the overall mortality was 15.44/1000 man-years.[9] Eguchi *et al*. reported that HCC was a prognostic factor and the mortality rate of NASH-HCC was 40.0% over 2.7 years.[11] It showed higher mortality in F3/4 patients (F3/F4; 25.0%, F0/2; 0.0%). MAFLD increases all-cause mortality.[12] However, the clinical course of MAFLD and the difference between MAFLD and NAFLD are unclear.

A previous study evaluated the natural history of NAFLD via serial biopsy[13] and found that 9.3% of the patients developed end-stage liver disease and 34% had advanced fibrosis at about 10 years. However, there were no reports of baseline clinical, histological, or biochemical variables associated with disease progression.

We investigated the clinical course, HCC, extrahepatic malignancies, cardiovascular disease (CVD) events, progression of fibrosis, differences in the speed of the progression, and survival rates in Asian patients with MAFLD and NAFLD. We also identified the risk factors for mortality.

Methods

Patients and study design. This observational, single-center study was performed at the Tokyo Women's Medical University Hospital and enrolled 987 Asian NAFLD and/or MAFLD patients (diagnosed from 1991 to 2021). NAFLD was diagnosed according to evidence-based clinical practice guidelines,[2,3] and ≥95% of cases were biopsy-confirmed ($n = 939$). We rechecked the diagnoses of all patients. The pathological stage of NAFLD was evaluated in accordance with the classification system of Brunt *et al.*[14] MAFLD was diagnosed according to established criteria,[4] namely radiologically diagnosed hepatic steatosis and the presence of any one of the following three conditions: overweight/obesity, presence of diabetes mellitus, or evidence of metabolic dysregulation. Metabolic dysregulation was defined as the presence of two or more of the following conditions: waist circumference ≥90 in men and 80 cm in women, hypertension, dyslipidemia, prediabetes (i.e., fasting glucose levels 100–125 mg/dl, or HbA1c 5.7%–6.4%, homeostasis model assessment-insulin resistance (HOMA-IR) score[15] ≥2.5). In the analysis of HOMA-IR, insulin users were excluded.

Study 1. We classified the patients into the non-MAFLD/NAFLD (N-alone, $n = 92$), both MAFLD &N (M&N, $n = 785$), and M-alone ($n = 90$) groups. Non-M/non-N ($n = 20$) patients were excluded.

The three groups were compared in terms of age, proportion of males, BMI (kg/m^2), and comorbid lifestyle-related diseases (%). Laboratory parameters were collected at the time of biopsy. HbA1c (%) was evaluated according to the National Glycohemoglobin Standardization Program (NGSP), and the Japan Diabetes Society (JDS) value was converted to NGSP by JDS + 0.4. The FIB-4 index was defined as (age [years] × aspartate aminotransferase; AST [IU/L])/(platelet count [10^9/L] × √alanine transaminase; ALT [IU/L]).[16] Any underlying liver disease was detected by evaluating serum markers of hepatitis viruses, namely immunoglobulin and autoantibodies, imaging, and pathological findings. The criteria of secondary FLD of drug-induced FLD and moderate alcohol intake (30–60 g of ethanol) were included in MAFLD but excluded from NAFLD. Patients with heavy alcohol intake (60 g ethanol) were excluded.

Hypopituitarism, hypothyroidism, polycystic ovary syndrome, Turner syndrome, and inflammatory bowel disease were diagnosed on the basis of clinical data and criteria.[17–21]

HCC development, extrahepatic malignancies, CVD events, and survival rates were compared among patients followed up for ≥6 months in the N-alone ($n = 74$), M&N ($n = 667$), and M-alone ($n = 85$) groups. Risk factors for mortality were subjected to Cox regression analysis.

HCC diagnosis. Patients with NAFLD were followed up at 1- to 3-month intervals at our outpatient clinic. HCC was screened by images every 6 months and was diagnosed histologically or based on imaging findings consistent with the diagnosis, using at least two of the following modalities, in accordance with clinical guidelines: abdominal ultrasound, computed tomography, and/or magnetic resonance imaging.[22,23]

Study 2. A second liver biopsy was performed in 121 cases during the 3.3 (0.5–14.2)-year follow-up period. Compared to the previous stage, fibrosis was classified as progressed, stable, or improved. In cases of progressed fibrosis, the clinical features were compared between those with early (<4 years) and late (≥4 years) progression based on the median observation period.

This study was conducted in accordance with the principles of the Declaration of Helsinki and the ethical guidelines of the Tokyo Women's Medical University Hospital (Approved No. 4646, No. 4914). The Institutional Review Board of Tokyo Women's Medical University Hospital approved the study protocol. Comprehensive consent for using medical information from medical examination were obtained.

Statistical analysis. Data are presented as medians and ranges at baseline. Differences were assessed by the Kruskal–Wallis test or χ^2 test using SPSS software (version 25.0; IBM Corp., Armonk, NY, USA). A value of $P < 0.05$ was considered to indicate statistical significance. Cumulative curves for survival rates and new onset of HCC were constructed by the Kaplan–Meier method. The statistical significance of differences in survival among the three groups was evaluated by log-rank test. Survival rates were compared with those for HCC, extrahepatic malignancies, and cardiovascular events. Furthermore, we confirmed the events by the person-year

method using STATA 15 (StataCorp LLC, College Station, TX, USA). Risk factors for mortality were evaluated by Cox regression analysis. Hazard ratios (HRs) and 95% confidence interval (CI) were assessed. The factors included age, BMI, ALT, new onset of HCC, extrahepatic malignancies, CVD events, and profiles of N-alone, M&N, and M-alone groups. In study 2, fibrosis change was considered, and pathologically progressed cases of <4 and ≥4 years were compared with the Mann–Whitney *U*-test.

Results

Demographics and complication status of patients with NAFLD. A total of 987 Asian patients were diagnosed with FLD (939 biopsy-confirmed) (Fig. 1a). After excluding non-M/non-N cases ($n = 20$), we enrolled 967 patients (497 males, 51.4%) with a median age of 53 years (range 18–89 years). The demographics of the patients at the time of liver biopsy showed that the N-alone group had a median age of

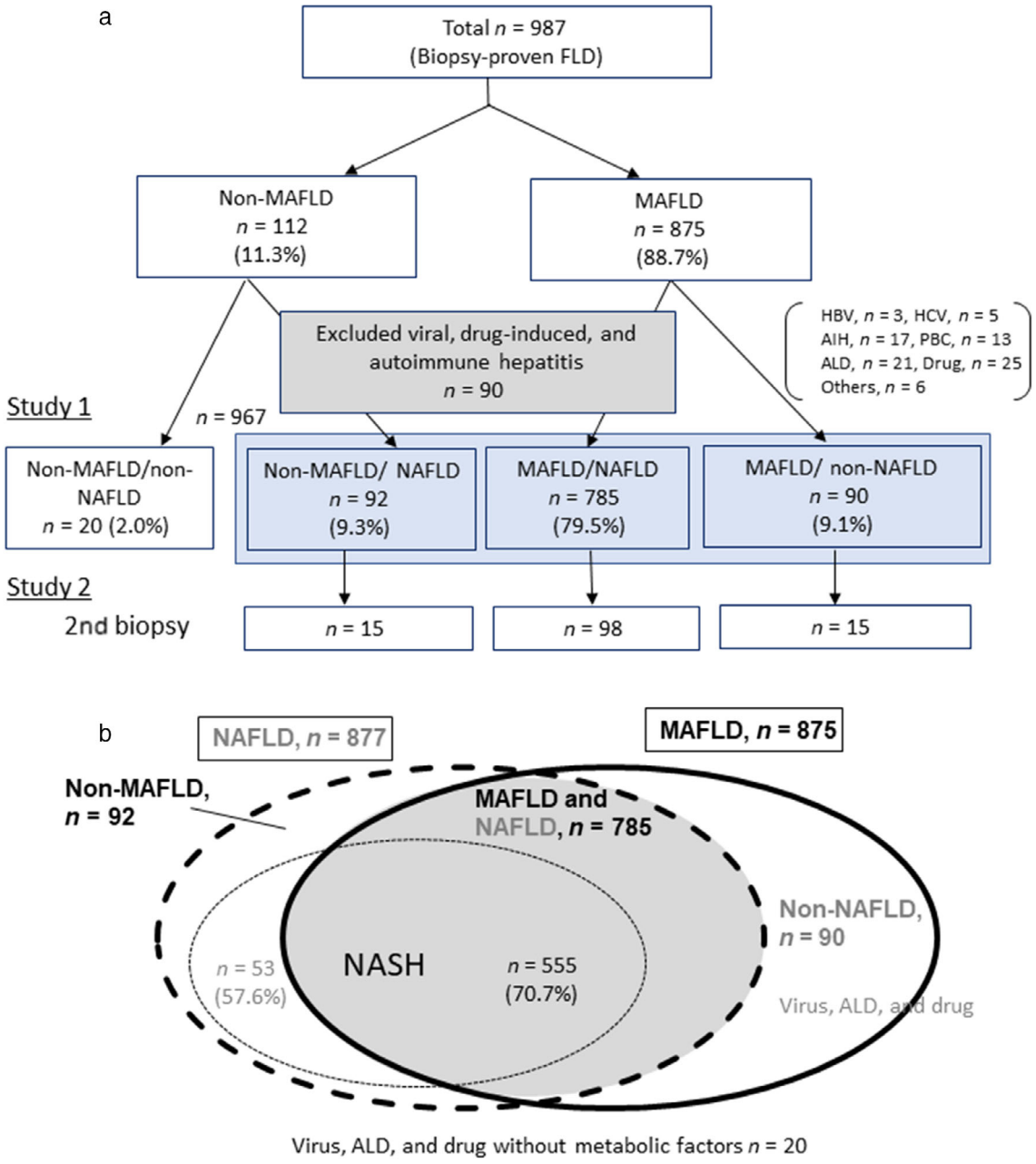

Figure 1 Flow diagram of the enrollment of patients with FLD diagnosed by liver biopsy. (a) Overall, 987 biopsy-proven FLD cases were enrolled and 112 cases of non-MAFLD and 875 cases of MAFLD were diagnosed. (b) NASH was diagnosed in 555 cases (70.7%). FLD, fatty liver disease; NASH, non-alcoholic steatohepatitis.

TABLE 1 Characteristics of patients with metabolic-associated fatty liver disease (MAFLD) and non-alcoholic fatty liver disease (NAFLD)

Variable	Total ($n = 967$)	NAFLD alone ($n = 92$)	MAFLD and NAFLD ($n = 785$)	MAFLD alone ($n = 90$)	*P*-value*
Age (years)	53 (18–89)	50 (21–77)	53 (18–89)	57 (31–75)	<0.01
Male sex (%)	497 (51.4%)	50 (54.3%)	413 (52.6%)	34 (37.8%)	0.02
BMI (kg/m^2)	26.6 (14.0–61.0)	23.1 (14.0–24.9)	27.1 (15.9–61.0)	26.7 (17.2–40.8)	<0.01
Waist circumference (cm)	91 (60–110)	89 (70–106)	93 (60–140)	87 (65–131)	<0.01
Dyslipidemia (%)	609 (63.0%)	32 (34.8%)	515 (65.6%)	62 (68.9%)	<0.01
Hypertension (%)	452 (46.7%)	9 (9.8%)	388 (49.4%)	55 (61.1%)	<0.01
Diabetes mellitus (%)	449 (46.4%)	0 (0.0%)	404 (51.5%)	45 (50.0%)	<0.01
30–60 g ethanol/day intake	21 (2.2%)	0 (0.0%)	0 (0.0%)	21 (23.3%)	—
Laboratory data					
Albumin (g/dl)	4.3 (1.8–5.5)	4.4 (2.8–5.5)	4.3 (1.8–5.5)	4.2 (1.9–5.2)	<0.01
Total bilirubin (mg/dl)	0.7 (0.1–33.7)	0.8 (0.1–7.9)	0.7 (0.2–33.7)	0.6 (0.3–6.3)	0.62
Aspartate aminotransferase (U/L)	48 (6–435)	37 (16–401)	49 (6–435)	58 (15–339)	<0.01
Alanine transaminase (U/L)	68 (5–911)	55 (11–408)	72 (5–911)	68 (10–556)	0.04
γ-Glutamyl transferase (U/L)	71 (2–1543)	70 (10–1543)	69 (2–912)	109 (15–1204)	<0.01
Fasting blood glucose (mg/dl)	104 (10–433)	94 (66–148)	106 (10–433)	105 (77–293)	<0.01
Hemoglobin $_{A1c}$ (%)	5.8 (3.7–13.4)	5.2 (3.7–6.8)	5.9 (4.0–13.4)	5.9 (4.1–9.6)	<0.01
IRI (μU/ml)	12.3 (0.7–181.0)	6.3 (2.0–102.0)	12.8 (0.7–181.0)	9.2 (3.0–75.0)	<0.01
HOMA-IR	3.34 (0.34–47.37)	1.44 (0.48–23.17)	3.52 (0.34–47.37)	3.00 (0.63–33.70)	<0.01
Triglycerides (mg/dl)	136 (28–833)	114 (35–433)	140 (28–809)	121 (53–833)	<0.01
Total cholesterol (mg/dl)	198 (63–482)	201 (63–380)	198 (75–482)	195 (91–342)	0.83
Ferritin (ng/ml)	206 (4–7656)	136 (4–1095)	214 (4–7656)	184 (6–2906)	0.15
Platelet counts (× 10^4/μl)	20.9 (3.7–96.0)	21.0 (4.2–45.1)	21.0 (3.7–96.0)	19.7 (4.7–39.4)	0.41
Prothrombin time (%)	96.0 (9.6–120.0)	98.7 (11.8–109.0)	96.2 (9.6–120.0)	92.7 (14.1–100.0)	0.31
AFP (ng/ml)	4 (1–1974)	4 (1–13)	4 (1–1974)	4 (1–14)	0.76
FIB-4 index	1.47 (0.10–17.78)	1.20 (0.28–16.71)	1.46 (0.10–17.78)	2.10 (0.12–8.29)	<0.01
Pathological findings					
Fibrosis; F3-4 (%)	282/939 (30.0%)	12/89 (13.5%)	244/764 (31.9%)	26/86 (30.2%)	<0.01
Inflammation; A2-3 (%)	652/922 (70.7%)	43/87 (49.4%)	547/750 (72.9%)	62/85 (72.9%)	<0.01
Steatosis; S2-3 (%)	688/922 (74.6%)	52/88 (59.1%)	576/750 (76.8%)	60/84 (71.4%)	<0.01

*Comparison between the NAFLD/non-MAFLD, NAFLD/MAFLD, and non-NAFLD/MAFLD groups by Kruskal–Wallis test.
AFP, alpha-fetoprotein; BMI, body mass index; FIB-4, fibrosis-4; IRI, immunoreactive insulin.

50 (range, 21–77) years, which was significantly less than that of the M&N (53 [18–89] years) and M-alone groups (57 [31–75] years; $P < 0.01$] (Table 1). The N-alone group included 50 (54.3%) males, compared to 413 (52.6%) in the M&N group and 34 (37.8%) in the M-alone group. The rate of male was significantly lower in the M-alone group ($P = 0.02$). The median BMI was 23.1, 27.1, and

Table 2 Complications of patients with different metabolic profiles and events after liver biopsy

Variable	Total ($n = 967$)	NAFLD alone ($n = 92$)	MAFLD and NAFLD ($n = 785$)	MAFLD alone ($n = 90$)	*P*-value*
Hypopituitarism (%)	21 (2.2%)	5 (5.4%)	16 (2.0%)	0 (0.0%)	0.04
Hypothyroidism (%)	14 (1.4%)	7 (7.6%)	6 (0.8%)	1 (1.1%)	<0.01
PCOS (%)	1 (0.1%)	0 (0.0%)	1 (0.1%)	0 (0.0%)	0.89
Turner syndrome (%)	5 (0.5%)	0 (0.0%)	5 (0.6%)	0 (0.0%)	0.56
Pancreatic resection (%)	5 (0.5%)	0 (0.0%)	5 (0.6%)	0 (0.0%)	0.56
Short bowel syndrome or IBD (%)	2 (0.2%)	1 (1.1%)	1 (0.1%)	0 (0.0%)	0.14
HCC at diagnosis of FLD (%)	67 (6.9%)	2 (2.2%)	62 (7.9%)	3 (3.3%)	0.05
HCC post biopsy (%)*	31/826 (3.8%)	0/74 (0.0%)	28/667 (4.2%)	3/85 (3.5%)	0.20
Extrahepatic malignancies (post biopsy, %)*	65/826 (7.9%)	5/74 (6.8%)	56/667 (8.4%)	3/85 (4.7%)	0.49
CVD event (%)*	49/826 (5.9%)	1/74 (1.4%)	37/667 (5.5%)	11/85 (12.9%)	<0.01
Death (liver-related /non-liver-related)	45/35	1/ 6	42/24	2/5	0.01

*Analysis of follow-up cases for more than half a year.
CVD, cardiovascular disease; HCC, hepatocellular carcinoma; IBD, Inflammatory bowel disease; MAFLD, metabolic-associated fatty liver disease; NAFLD, non-alcoholic fatty liver disease; PCOS, polycystic ovarian syndrome.

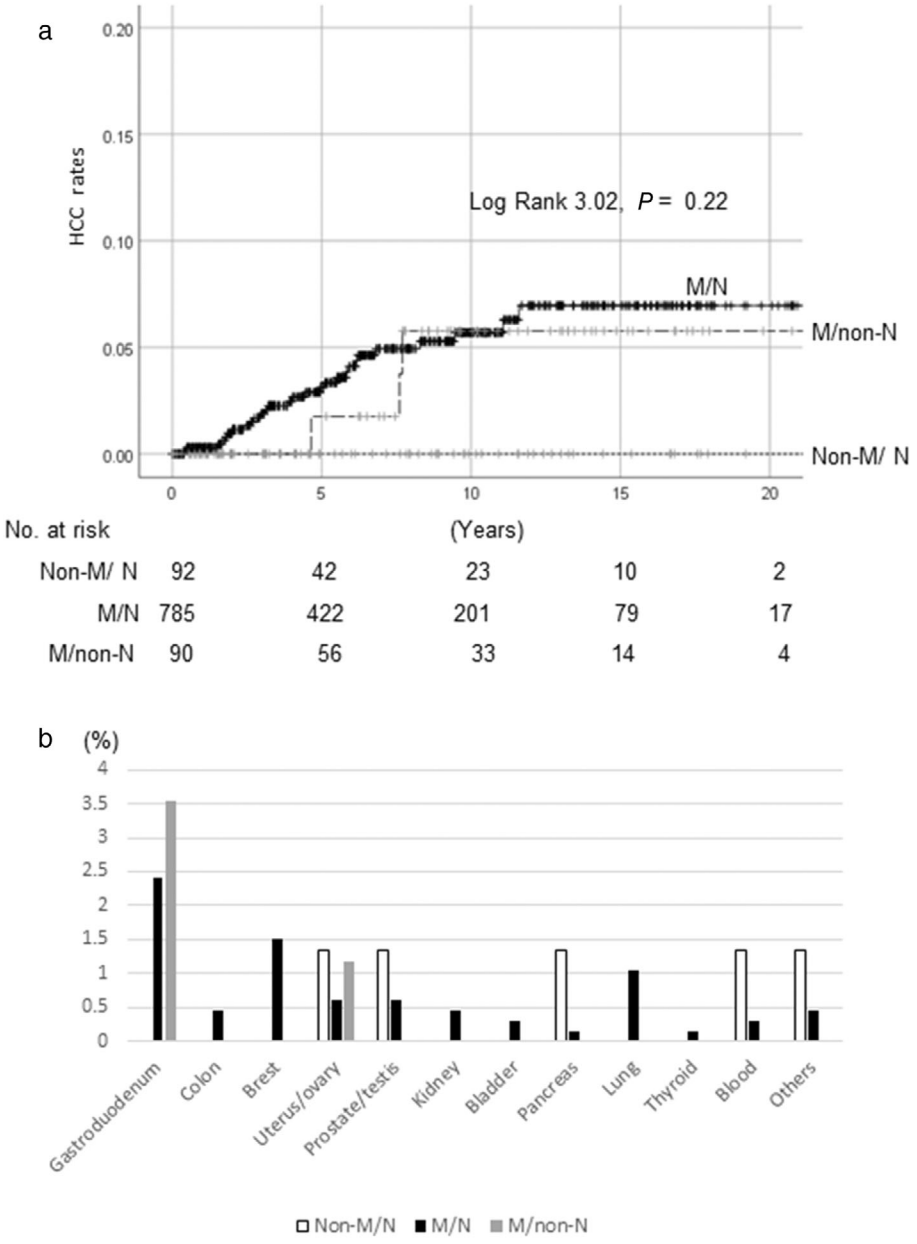

Figure 2 HCC development by metabolic profile and extrahepatic malignancies in patients with fatty liver disease. (a) HCC development and (b) extrahepatic malignancies. (a) There was no significant difference in HCC development among the N-alone, M&N, and M-alone groups ($P = 0.22$). (b) Extrahepatic malignancies were observed in 5, 56, and 3 cases and the percentages of all cancers are shown in the N-alone, M&N, and M-alone groups during the 5.7 (0.5–25.8)-year follow-up. M, metabolic-associated fatty liver disease; N, non-alcoholic fatty liver disease.

26.7 kg/m^2 in the N-alone, M&N, and M-alone groups, respectively. BMI was significantly lower in the N-alone group ($P < 0.01$). Complications in the N-alone group included dyslipidemia ($n = 32$) and hypertension ($n = 9$). These numbers were significantly lower in the N-alone group because these complications are components of the definition of MAFLD. In the M-alone group, 21 patients had moderate alcohol intake (30–60 g of ethanol).

Among the three groups, AST (37, 49, and 58 U/L), fasting blood sugar (94, 106, and 105 mg/dl), HbA1c (5.2, 5.9, and 5.9%), immunoreactive insulin (IRI; 6.3, 12.8, and 9.2 μU/mL), HOMA-IR (1.44, 3.52, and 3.00) (all $P < 0.01$) and ALT (55, 72, and 68 U/L, $P = 0.04$) were significantly lower in the N-alone group. The FIB-4 index was lower in the N-alone group (1.20, 1.46, and 2.10, $P < 0.01$). The high FIB-4 index in the M-alone group might be due to the age and alcohol intake.

Regarding complications (Table 2), the prevalence of hypopituitarism was 5.4%, 2.0%, and 0.0%, respectively, and that of hypothyroidism was 7.6%, 0.8%, and 1.1%, respectively. The prevalence of hypopituitarism and hypothyroidism cases was significantly higher in the N-alone group ($P = 0.04$ and $P < 0.01$).

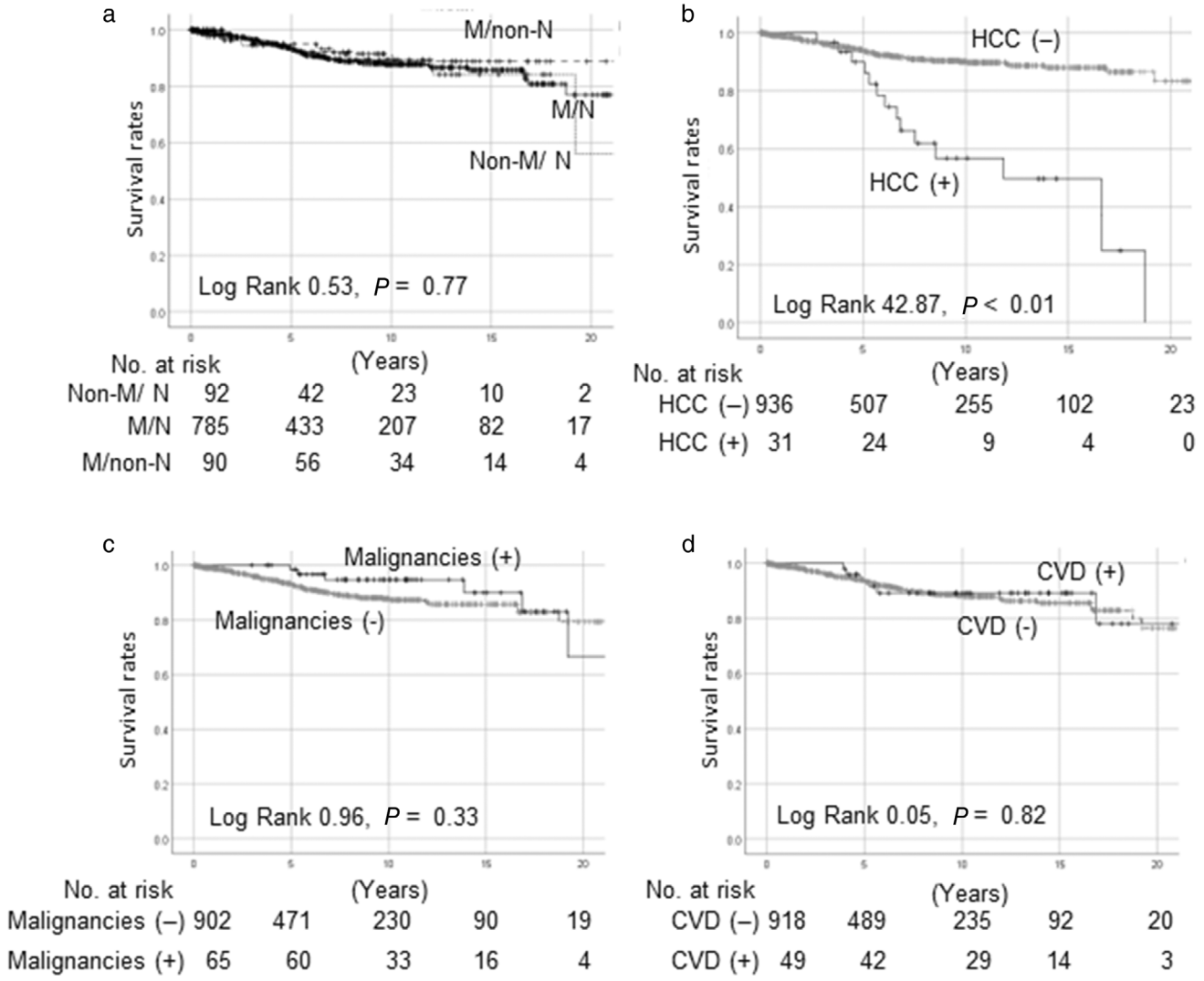

Figure 3 Survival rates of patients with fatty liver disease according to complications. (a) Metabolic profiles, (b) HCC development, (c) extrahepatic malignancies, and (d) cardiovascular events. Survival rates were estimated by Kaplan–Meier curves. The survival rates were not significantly different between the N-alone, M&N, and M-alone groups ($P = 0.77$, (a)). HCC cases had a significantly lower survival rate than non-HCC cases ([$P < 0.01$, (b))]. There was no significant difference according to extrahepatic malignancies and cardiovascular events ($P = 0.33$ and $P = 0.82$, (c and d)). M, metabolic-associated fatty liver disease; N, non-alcoholic fatty liver disease.

Pathological findings of the liver and NASH frequencies. Only 13.5% of the N-alone group had advanced fibrosis, 49.4% had A2-3 activity, and 59.1% had S2-3 steatosis. Advanced cases were less frequent in the N-alone group than in the M&N and M-alone groups. Overall, 606 FLD patients showed ballooning, and NASH was diagnosed in 555 cases (70.7%) after excluding other liver diseases (Fig. 1b). NASH was diagnosed in 53 (57.6%) N-alone patients. In the N-alone cases, NASH was relatively frequently diagnosed.

Events after liver biopsy. HCC was present in 2.2%, 7.9%, and 3.3% in the N-alone, M&N, and M-alone groups, respectively, at the time of diagnosis. The incidence of HCC tended to be higher in M&N patients ($P = 0.05$). During the follow-up period of 5.7 (0.5–25.8) years after liver biopsy, HCC was observed in 0.0%, 4.2%, and 3.5%, respectively ($P = 0.20$). The Kaplan–Meier curve shows the HCC rates (Fig. 2a). In the M&N and M-alone groups, HCC developed in 3.1% and 1.8% at 5 years and in 5.7% and 5.8% at 10 years, respectively. Extrahepatic malignancies during follow up were detected in 6.8%, 8.4%, and 4.7%, respectively. Figure 2b shows the percentages of cancers. Cancers in the M&N group comprised gastrointestinal cancers ($n = 19$, 2.8%, gastroduodenum and colon), gynecologic cancers ($n = 14$, 2.1%, breast and uterine cancers), urinary cancers ($n = 9$, 1.3%, prostate, kidney, and bladder), and others ($n = 14$). One case was complicated with two cancers. One each of pancreatic, maxillary gingival, testicular, and uterine cancer, and leukemia was observed in the N-alone group. Three gastrointestinal cancers and one uterine cancer were detected in the M-alone group. The rates of extrahepatic malignancies were similar in the three groups ($P = 0.49$).

Table 3 Pathological changes of paired biopsy cases

	Total ($n = 121$)	Improved* ($n = 17$)	Stable* ($n = 38$)	Progressed* ($n = 49$)	*P*-value
Age (first/second biopsy, years)	55 (16–78)/59 (19–84)	53 (18–68)/55 (20–73)	50 (18–77)/52 (20–82)	58 (16–78)/62 (19–80)	0.13/0.09
Period between two biopsies (years)	3.3 (0.5–14.2)	2.3 (0.6–14.2)	2.7 (0.5–9.6)	3.7 (0.6–13.9)	0.23
BMI (kg/m^2, Δ second-first/biopsy)	−0.4 (−2 to −3.7)	1.8 (1 to −3.3)	−0.5 (−0.7–1.5)	−0.1 (−2 to −4.2)	0.29
Dyslipidemia (%)	78 (64.5%)	9 (52.9%)	28 (73.7%)	33 (67.3%)	0.32
Hypertension (%)	68 (56.2%)	10 (58.8%)	17 (44.7%)	29 (59.2%)	0.43
Diabetes mellitus (%)	53 (43.8%)	10 (58.8%)	13 (34.2%)	21 (42.9%)	0.19
Fibrosis stage; F3-4 (%)	43 (35.5%)	7 (41.2%)	8 (21.1%)	11 (22.4%)	0.25
NASH (%)	74 (61.2%)	12 (70.6%)	25 (65.8%)	32 (65.3%)	0.92
NAFLD alone/MAFLD and NAFLD/MAFLD alone	13/94/14	2/13/2	5/29/4	6/37/6	0.97

*F4 cases ($n = 17$) were excluded.
BMI, body mass index; MAFLD, metabolic-associated fatty liver disease; NAFLD, non-alcoholic fatty liver disease, NASH, non-alcoholic steatohepatitis.

During this period, 1 (1.4%), 37 (5.5%, 16 cardiovascular and 21 cerebrovascular events), and 11 (12.9%, 7 cardiovascular and 5 cerebrovascular events) CVD events occurred, respectively. The number of CVD events was significantly higher in the M-alone group than in the other two groups ($P < 0.01$).

Survival rates of MAFLD and NAFLD. FLD survival rates were estimated by generating Kaplan–Meier curves (Fig. 3a). The 5-year survival rate was 92.2%, 93.4%, and 95.0% and that at 10 years was 89.4%, 87.8%, and 91.5%, respectively; the differences were not significant ($P = 0.77$). Incident rate of death by person-year method was 0.0125894, 0.0125306, and 0.0092116, respectively ($P = 0.53$). HCC cases had a significantly lower survival rate than non-HCC cases ($P < 0.01$, Fig. 3b). Incident rate by person-year method was 0.0102891 in patients without HCC and 0.0564567 in patients with HCC ($P < 0.01$). Survival was not significantly affected by extrahepatic malignancies and CVD events ($P = 0.33$ and $P = 0.82$, Fig. 3c,d). Incident rate by person-year method was 0.0126391 in patients without extrahepatic malignancies and 0.0082394 in patients with extrahepatic malignancies ($P = 0.31$), and 0.012298 in patients without CVD and 0.0106041 in patients with CVD ($P = 0.73$).

Fibrosis changes. A second liver biopsy was performed in 121 cases during the 3.3 (0.5–14.2)-year follow-up period (Table 3). After excluding 17 cases of liver cirrhosis, 17 cases showed improvement, 38 cases were stable, and 49 cases had progressed fibrosis. NASH was observed in 12 (70.6%), 25 (65.8%), and 32 (65.3%) cases in the improved, stable, and progressed cases of fibrosis, respectively. No significant differences were observed. No significant difference in progression was detected between the three FLD groups ($P = 0.97$; Fig. S1).

The first BMI and the serum level of ALT (both $P = 0.02$) were higher in early (<4 years, $n = 28$) and late (≥4 years, $n = 21$) progression cases. Hypertension was frequent in early progression cases ($P = 0.03$). There was no significant difference in the rates of type 2 diabetes ($P = 0.89$) and dyslipidemia ($P = 0.44$) in relation to the categories of N alone, M&N, and M alone ($P = 0.57$).

Risk factors for mortality. The risk factors for mortality by Cox regression analysis were age and BMI (HR 1.109, 95% CI: 1.032–1.192; HR 0.586, 95% CI: 0.409–0.840, both $P < 0.01$, respectively) in the N-alone group; age, HCC, ALT, and FIB-4 index (HR 1.037, 95% CI: 1.009–1.053; HR 0.992, 95% CI: 0.985–0.998; HR 1.180, 95% CI: 1.104–1.262; and HR 2.293, 95% CI: 1.244–4.227, all $P < 0.01$) in the M&N group; and FIB-4 index (HR 1.461, 95% CI: 1.028–2.076, $P = 0.04$) in the M-alone group (Table 4).

Discussion

We assessed FLD with different metabolic profiles, given that FLD has shown differences in relation to racial, demographic, complications, and outcome categories. We analyzed the characteristics of ≥95% of biopsy-proven FLD patients. N-alone patients were younger and had a higher rate of hormonal disorders and a lower rate of HCC than MAFLD cases; however, the rates of extrahepatic malignancies were similar to those in the MAFLD cases. CVD events were significantly observed in the M-alone group. Moreover, although pathological fibrosis was mild, NASH was diagnosed in about half of the cases. The survival and fibrosis progression rates

Table 4 Multivariate analysis of death according to metabolic-associated fatty liver disease (MAFLD) and non-alcoholic fatty liver disease (NAFLD) profiles

	Hazard ratio	95% confidence interval	*P*-value
NAFLD alone			
Age	1.109	1.032–1.192	<0.01
BMI	0.586	0.409–0.840	<0.01
MAFLD and NAFLD			
Age	1.037	1.009–1.053	<0.01
ALT (U/L)	0.992	0.985–0.998	<0.01
FIB-4 index	1.180	1.104–1.262	<0.01
HCC complication	2.293	1.244–4.227	<0.01
MAFLD alone			
FIB-4 index	1.461	1.028–2.076	0.04

ALT, alanine transaminase; BMI, body mass index; FIB-4, fibrosis-4; HCC, hepatocellular carcinoma.

were not significantly different among the three groups. The risk factors for FLD differed according to the metabolic profile.

In a large study, 7761 community patients aged 20–74 years in the United States included 2702 patients diagnosed with FLD by abdominal ultrasonography.[12] In that cohort, 75.6% fulfilled both the MAFLD and NAFLD definitions, 7.8% had MAFLD, 14.6% had NAFLD, and 1.9% did not have either. In this study, about 10% of FLD cases had either NAFLD or MAFLD. In the M-alone group, the number of females was significantly higher than in the other three groups; by contrast, other studies have reported a higher rate for males. This is likely due to the small number of patients with ALD, which can be diagnosed without liver biopsy.

NAFLD patients were younger and MAFLD patients were older in the three groups, in agreement with Bianco *et al*.[8] Secondary causes of NAFLD were included in the M-alone group if there were metabolic complications. Primary NAFLD is reportedly a more severe type of liver disease—in terms of pathological features and fibrosis stage—than secondary NAFLD.[24]

BMI was significantly lower in the N-alone group. This group had significantly higher rates of hypopituitarism and hypothyroidism than the other groups, as well as pathological findings of mild fibrosis, mild inflammation, and mild steatosis. In patients with hypopituitarism, FLD reportedly improves after hormone replacement therapy.[25,26] Therefore, we should consider evaluating hormone levels in these patients.

The rate of HCC was highest in the M&N group. The risk factors for HCC in NAFLD are reportedly male gender and diabetes mellitus.[27] The incidence of HCC at 10 years was 20.1% in patients with advanced fibrosis, and the mortality rate was higher in patients who were older, had a history of HCC, a lower serum level of albumin, and a higher level of γ-glutamyl transferase (GGT).[27] In this study, the new-onset incidence of HCC was highest in the M&N group (5.7% at 10 years). HCC rate in the N-alone group did not increase because of the younger age and the absence of diabetes. Hepatitis virus and alcohol intake may contribute to carcinogenesis; however, in the M-alone group, female cases predominated and therefore the frequency of developing HCC might be low despite the older age.

Regarding extrahepatic malignancies, colorectal cancers, breast cancers, and prostate cancers have been reported 1.4, 1.2, and 1.3 per 1000 person-years, respectively.[28] In this study, gastrointestinal cancers, gynecological cancers, and urinary cancers were detected in the M&N group, non-gastrointestinal cancers were detected in the N-alone group, and gastrointestinal cancers were detected in the M-alone group. The incidence of extrahepatic malignancies was non-significantly lower in the N-alone and M-alone groups ($P = 0.49$).

MAFLD patients reportedly have a high mortality rate. Patients with MAFLD had a 17% higher risk of all-cause mortality (HR 1.17).[12] MAFLD was associated with a higher risk of cardiovascular mortality. In a prior work, the overall 10-year survival rate was found to be 86.2%.[27] In this study, the survival rate was 89.4%, 87.8%, and 91.5% at 10 years in the three groups, respectively; the differences were not significant. The results were confirmed by person-year method, and significance was not evident. Conversely, the rate of CVD events was increased in the M&N and M-alone groups. Therefore, MAFLD is associated with an increased risk of CVD events.

There was early progression of fibrosis in patients with high BMI and serum ALT level. The complications associated with lifestyle-related diseases without hypertension were not significantly different. Regardless of the classification of NAFLD or MAFLD, fibrosis significantly developed in cases with a high BMI and severe hepatic inflammation.

Outcome of NAFLD/NASH demonstrated that fibrosis was the strongest predictor of death.[29–31] Our previous study showed that mortality was high in the patients with advanced fibrosis.[27] In a multicenter national study in Japan,[32] liver-related mortality was the leading cause of mortality, and fibrosis stage was independently associated. These results were consistent with reports from Europe and the United States. In a prospective study of NAFLD,[33] advanced fibrosis (F3 and F4) was associated with increased risks of liver-related complications and death. The results of the retrospective study were also reproduced in the prospective study. In our cohort, regarding risk factors for mortality, a low BMI was associated with a poor outcome in the N-alone group. In the M&N group, HCC development and fibrosis progression were risk factors for mortality. In the M-alone group, fibrosis progression was a risk factor for mortality. HCC did not remain a risk factor for survival in the N-alone and M-alone groups, probably because the incidence of HCC was low. We speculate that age and sarcopenia or the progression of fibrosis were strongly associated with survival in those groups. The FIB-4 index was not a risk factor for mortality in the N-alone group because more younger patients were observed in this group. In the M-alone group, CVD events showed an increase; however, it was not associated with the survival rate. This in agreement with the multicenter study in Japan.[32]

This study had several limitations. It was a single-center, observational design. And we could not measure high-sensitivity C- reactive protein (CRP) for the diagnosis of MAFLD. The majority of the cases underwent liver biopsy. Therefore, selection bias might have existed. Consideration of FLD without histological examination was necessary. Moreover, the number of sequential biopsy cases was small and the interval of liver biopsies was variable. We compared the cases with median interval periods of 4 years, <4 years, and >4 years to determine the difference. Additionally, almost the cases underwent liver biopsy for clinical indications for the evaluation of liver enzyme increase. It potentially biases toward the histological results. Furthermore, we observed few cases of death or events. But the results were compatible with those of previous studies. The risk factors of these events should be analyzed by increasing the number of cases, and this will be the subject of our next analysis.

Conclusions

The survival and progression rates were not significantly different among the three FLD groups. A high BMI and serum ALT level were implicated in hepatic fibrosis progression. The risk factors for mortality in FLD differ depending on the metabolic complications.

Data availability statement. The datasets used and/or analyzed in this study 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. Changes in fibrosis in sequential biopsy cases.
A second liver biopsy was performed in 121 cases during the 3.3 (0.5–14.2)-year follow-up period. After excluding 17 cases of liver cirrhosis, 17 cases had improved, 38 cases were stable, and 49 cases had progressed fibrosis. No significant differences in progression were detected between the N-alone, M&N, and M-alone groups ($P = 0.97$).
M, metabolic-associated fatty liver disease; N, non-alcoholic fatty liver disease.