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Original Article

A Prospective Study of Long-term Outcomes in Female Patients with Nonalcoholic Steatohepatitis Using Age- and Body Mass Index-matched Cohorts

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In patients with nonalcoholic steatohepatitis (NASH), the prevalence of cirrhosis is higher among women than men, and hepatocellular carcinoma (HCC) develops mainly in the cirrhotic stage among women. However, the long-term outcomes in female patients with NASH have not been fully elucidated, and age, gender and BMI were not simultaneously adjusted in previous studies on the prognosis of NASH. To elucidate the outcomes in female patients with NASH, we prospectively compared NASH patients with advanced fibrosis (advanced NASH) with hepatitis C virus-related advanced fibrosis (advanced CHC) patients and NASH patients with mild fibrosis (mild NASH) using study cohorts that were adjusted for body mass index (BMI) in addition to age. The median follow-up period was 92.5 months. Liver-related complication-free survival was significantly reduced in the advanced NASH group compared to the mild NASH group. No liver-related complications developed in the mild NASH group. The overall survival, liver-related complication- and cardiovascular/cerebrovascular disease-free survival were not significantly different between the advanced NASH and CHC groups. Female patients with NASH and advanced fibrosis may have a less favorable prognosis for liver-related complications than the matched cohorts with NASH and mild fibrosis, but may have a similar prognosis to the matched cohorts with CHC.

Key words: nonalcoholic steatohepatitis, chronic hepatitis C, prognosis, female

Non-alcoholic fatty liver disease (NAFLD), which includes a wide spectrum of histopathology, is the most common etiology worldwide for chronic liver disease [1–4]. The prevalence of NAFLD in the general population varies from 9% to 37% worldwide [5–12]. The prevalence of nonalcoholic steatohepatitis (NASH), the more aggressive form of NAFLD, is increasing with the growing epidemics of diabetes and obesity. The incidence of NASH is estimated to be 1–3% among the adult Japanese population [13] and 5.7% of the general population in the Western world [1]. Obesity, diabetes mellitus, hyperlipidemia, the metabolic syndrome and insulin resistance have been established as risk factors for primary NAFLD [1, 14–18]. Its prevalence and related complications are predicted to increase [1].

Race or ethnicity affects the prevalence of NASH [2]. In addition, the clinicopathological features of NASH depend on age and gender, with female patients becoming significantly more prevalent with age in the Japanese population [13]. Age, advanced fibrosis, cirrhosis, diabetes mellitus, obesity and iron deposi-

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tion are considered to be established independent risk factors for hepatocellular carcinoma (HCC) in NASH patients [19]. Obesity in particular has been established as a significant risk factor for developing various malignancies, including liver cancers [20–25]. Body mass index (BMI) and diabetes have been found to be independent risk factors associated with the progression of fibrosis [26]. At the same time, all-cause mortality is higher in obese people, primarily due to increased cardiovascular disease mortality and increased obesity-related cancer (colon, breast, uterine, ovarian, renal and pancreatic) mortality [27]. Obesity is common in Japanese patients with nonvalvular atrial fibrillation, which is closely associated with a history of stroke [28]. Thus, age, gender, obesity and fibrosis are closely related to the prognosis for NASH in the Japanese population.

In patients with nonalcoholic steatohepatitis (NASH), the prevalence in cirrhosis, the most severe fibrosis grade, is higher among women than among men [29] and HCC develops mainly in the cirrhotic stage among women [30], although HCC is reportedly less prevalent among women than men [22, 30, 31]. However, long-term outcomes in female patients with NASH have not been fully elucidated. Thus, the aim of this study is to elucidate the long-term outcomes in such patients. Several studies have compared long-term outcomes between NASH and chronic hepatitis C (CHC) patients or between NASH patients graded by fibrosis severity. However, most of these studies adjusted only for age and sex between the study cohorts. Thus, we adjusted for the important clinicopathological factors of age and BMI in addition to gender between the study cohorts, and compared the long-term outcomes of NASH with advanced fibrosis (advanced NASH), hepatitis C virus (HCV)-related advanced fibrosis (advanced CHC) and NASH with mild fibrosis (mild NASH) in a Japanese population.

Materials and Methods

Study population. Surveys of the natural history of NASH and CHC were started in 2003. NASH was proved histologically. CHC patients were positive for HCV viral RNA by a quantitative polymerase chain reaction assay. Written informed consent was obtained from all NASH and CHC patients in our university hospital and affiliated hospitals, and their clinicopathological data were prospectively recorded for the study.

We recruited 20 consecutive female patients with severe fibrosis defined as more severe than or equal to bridging fibrosis (a group of advanced NASH) and 19 consecutive female patients with mild fibrosis defined as perivenular and pericellular fibrosis (a group of mild NASH) who presented to our hospital. The female CHC patients were selected to match the age, BMI and degree of liver-fibrosis of the advanced NASH patients based on the new Inuyama classification [32]. Twenty female patients with HCV-related advanced fibrosis (a group of advanced CHC) were selected from among those patients without a history of interferon (IFN)-based therapy (such as IFN plus ribavirin), who were also not receiving IFN-based therapy during the observation period. Except for a history of IFN-based therapy, all the patients were selected in a blinded fashion on the basis of age, BMI and degree of liver fibrosis, without knowledge of the clinical outcomes. The advanced CHC group consisted of 20 patients. The details of these groups are shown in Tables 1 and 2. Two independent hepatopathologists assessed the pathological findings according to the scoring system of Brunt *et al.* [33], without knowledge of each patient's clinical and biochemical data.

Other liver disorders causing steatohepatitis or liver disease and HBc-Ab positive patients were excluded. To reduce any lead-time bias in the survival analysis, we excluded patients with a hepatic mass, history of CVD and cancer at the time of the liver biopsy. The details on alcohol consumption were collected independently by at least 2 physicians and were confirmed by close family members. All of the study participants had current and past daily alcohol intakes of less than not only 40 g per week but also 20 g per day and were non-smokers.

Diabetic type was defined when fasting plasma glucose was 126 mg/dL or higher, and/or plasma glucose 2 h after 75 g glucose load was 200 mg/dL or higher. Casual plasma glucose higher than 200 mg/dL was also regarded as indicating diabetic type. Diabetes mellitus was diagnosed when the patient met the hyperglycemia criteria for "diabetic type" more than twice in separate examinations conducted on separate days. The diagnosis of diabetes could be made by a single plasma glucose test meeting the criteria for

Table 1 Comparison of the baseline characteristics of the advanced NASH and advanced CHC groups

	Advanced NASH (n = 20)	Advanced CHC (n = 20)	*P*
Age* (years)	69, 55–81	69.5, 57–85	0.201
Body mass index* (kg/m^2)	27.4, 22.9–35.2	27.0, 24.2–35.8	0.730
Diabetes mellitus	12 (60%)	7 (35%)	0.205
Hyperlipidemia	8 (40%)	2 (10%)	0.065
Hypertension	7 (35%)	10 (50%)	0.523
Liver fibrosis (F3/F4)	2/18	0/20	0.487
Platelet count* ($\times 10^4$/uL)	10.0, 5.3–19.7	6.8, 4.9–11.4	0.010
Prothrombin time* (%)	73, 47–98	79.5, 60–118	0.217
AST* (IU/L)	49, 30–126	64, 30–101	0.787
ALT* (IU/L)	42, 16–188	41, 21–81	0.527
γ-GTP* (IU/L)	54, 14–361	29, 12–50	0.050
Albumin* (g/dL)	3.9, 3.3–4.7	3.5, 3.2–4.0	0.028
Total bilirubin* (mg/dL)	0.9, 0.4–2.0	1.1, 0.6–1.9	0.778
Total cholesterol* (mg/dL)	159, 113–242	137, 91–177	0.022
Triglyceride* (mg/dL)	91, 30–222	72, 45–129	0.449
Fasting blood sugar* (mg/dL)	100, 80–152	119, 84–214	0.035
HbA1c* (%)	5.2, 4.1–6.5	5.6, 4.5–8.5	0.394

*Values are median, range. Advanced NASH, nonalcoholic steatohepatitis with advanced fibrosis; Advanced CHC, chronic hepatitis C with advanced fibrosis; AST, aspartate aminotransferase; ALT, alanine aminotransferase; γ-GTP, gamma-glutamyltranspeptidase; HbA1c, hemoglobin A1c.

Table 2 Comparison of the baseline characteristics of the advanced NASH and mild NASH groups

	Advanced NASH (n = 20)	Mild NASH (n = 19)	*P*
Age* (years)	69, 55–81	67, 52–78	0.260
Body mass index* (kg/m^2)	27.4, 22.9–35.2	24.8, 20.5–31.2	0.271
Diabetes mellitus	12 (60%)	12 (63.2%)	0.899
Hyperlipidemia	8 (40%)	8 (42.1%)	0.848
Hypertension	7 (35%)	14 (73.7%)	0.025
Liver fibrosis (F1/F2/F3/F4)	0/0/2/18	7/12/0/0	$<$0.001
Platelet count* ($\times 10^4$/uL)	10.0, 5.3–19.7	20.1, 13.3–24.8	$<$0.001
Prothrombin time* (%)	73, 47–98	82, 75–108	$<$0.001
AST* (IU/L)	49, 30–126	59, 29–186	0.316
ALT* (IU/L)	42, 16–188	61, 33–291	0.038
γ-GTP* (IU/L)	54, 14–361	50, 23–214	0.301
Albumin* (g/dL)	3.9, 3.3–4.7	4.5, 3.7–4.9	0.061
Total bilirubin* (mg/dL)	0.9, 0.4–2.0	0.7, 0.3–1.3	0.048
Total cholesterol* (mg/dL)	159, 113–242	189, 132–248	0.034
Triglyceride* (mg/dL)	91, 30–222	109, 50–283	0.099
Fasting blood sugar* (mg/dL)	100, 80–152	101, 85–174	0.119
HOMA-IR*	3.01, 1.56–6.50	3.15, 0.51–6.96	0.116
HbA1c* (%)	5.2, 4.1–6.5	6.0, 5.0–8.1	0.142

*Values are median, range. Advanced NASH, nonalcoholic steatohepatitis with advanced fibrosis; Mild NASH, nonalcoholic steatohepatitis with mild fibrosis; AST, aspartate aminotransferase; ALT, alanine aminotransferase; γ-GTP, gamma-glutamyltranspeptidase; HOMA-IR, homeostasis model assessment-insulin resistance; HbA1c, hemoglobin A1c.

"diabetic type" when one of the following 3 conditions existed: (1) the subject had typical symptoms of diabetes mellitus (thirst, polyuria, polydipsia, weight loss), (2) Hemoglobin A1c was 6.5% or higher, and (3) unequivocal diabetic retinopathy was detected. Hyperlipidemia was diagnosed when the serum triglyceride concentrations were above 150 mg/dL and/or the serum cholesterol concentrations were above 220 mg/dL on at least 3 occasions or lipid-lowering drugs were prescribed. Hypertension was diagnosed when blood pressure was greater than 140/90 mmHg on at least 3 occasions or antihypertensive drugs were prescribed.

We prospectively compared the long-term outcomes of the advanced NASH group with those of the advanced CHC and mild NASH groups. The study protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki.

Data collection. Age, height and weight were documented at the time of the liver biopsy. None of the patients had ascites at the liver biopsy. The type 2 diabetes mellitus, hypertriglycemia, arterial hypertension and CVD histories were ascertained at this time, and any subsequent developments in these diseases were noted during the follow-up and confirmed by the treating physician. The laboratory data were also obtained at the time of the liver biopsy.

Assessment of outcomes. The patients were monitored clinically at least every 3 months until the data analysis. None of the patients were lost to follow-up. The following outcomes were evaluated: (1) overall survival; (2) liver-related complication-free survival; (3) HCC-free survival; (4) CVD complication-free survival; and (5) the causes of death, which were determined from the hospital medical records and death certificate. Liver-related complications included ascites, hepatic encephalopathy, variceal bleeding and HCC. HCC was diagnosed if the following conditions were met: (1) pathological features consistent with HCC were identified by histological examination of liver tissue obtained by needle biopsy or other procedures; or (2) 1 or more hepatic space-occupying lesions were detected by ultrasonography or computed tomography and were shown to have vascular patterns typical of HCC by angiography, triple-phase spiral computed tomography or contrast-enhanced magnetic resonance imaging. CVD complications include acute coronary syndrome, cerebral infarction and cerebral hemorrhage which were diagnosed by CT or coronary angiography.

Statistical methods. Descriptive statistics were computed for all the variables, including medians and ranges for the continuous variables and frequencies for the categorical factors. The presence of specific categorical features across different subsets was compared using Fisher's exact test or G-test. The across-group comparisons of the numerical data were performed using the Student's *t*-test for normally distributed data and the Mann-Whitney U-test for non-normally distributed data.

The start date for the purposes of the analyses was the date of the biopsy. The patients were followed until they either underwent liver transplantation or died. The termination date for analysis was the date of liver transplantation or death. The patients were censored at the time of transplantation or at the last follow-up visit. A time-to-failure analysis (Kaplan-Meier) was performed, and the log-rank test was used for across-group comparisons.

Results

Characteristics of the Patients with advanced NASH, advanced CHC and mild NASH. The median age in the advanced NASH patients was 69 (range, 55–81) years (Table 1). The median BMI was 27.4 (range, 22.9–35.2). Twelve patients (60%) had diabetes mellitus, 8 (40%) had hyperlipidemia and 7 (35%) had hypertension.

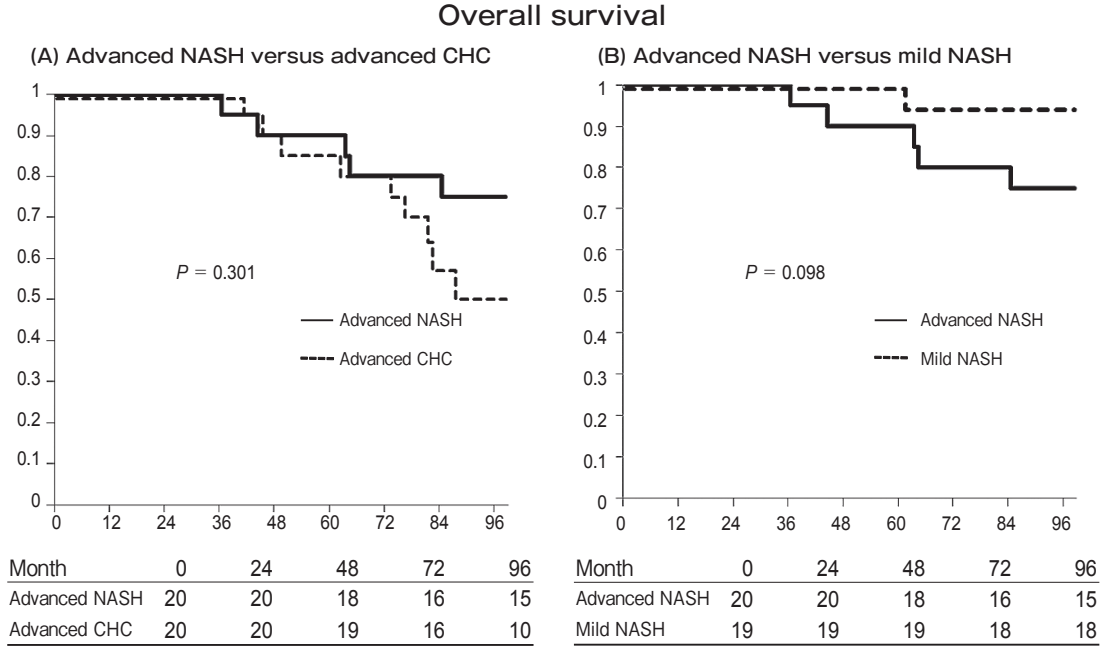
The median age in the advanced CHC patients was 69.5 (range, 57–85) years (Table 1). The median BMI was 27.0 (range, 24.2–35.8). Seven patients (35%) had diabetes mellitus, 2 (10%) had hyperlipidemia and 10 (50%) had hypertension. The patients with advanced NASH had higher platelet counts ($p = 0.010$), higher serum gamma-glutamyl transpeptidase levels ($p = 0.050$), higher albumin levels ($p = 0.028$), higher total cholesterol levels ($p = 0.022$) and lower fasting blood sugar levels ($p = 0.035$) than those with advanced CHC (Table 1).

Of the 19 mild NASH patients, the median age was 67 (range, 52–78) years (Table 2). The median BMI was 24.8 (range, 20.5–31.2). Twelve patients (63.2%) had diabetes mellitus, 8 (42.1%) had hyperlipidemia and 14 (73.7%) had hypertension. Hypertension was more common in the mild NASH patients than in the

advanced NASH patients ($p = 0.025$) (Table 2). The advanced NASH patients had lower platelet counts ($p < 0.001$), lower prothrombin times ($p < 0.001$), higher total bilirubin levels ($p = 0.048$), lower total cholesterol levels ($p = 0.034$) and lower alanine aminotransferase levels ($p = 0.038$) than those with mild NASH (Table 2).

Comparisons of overall survival in the advanced NASH group with advanced CHC and mild NASH groups. During a mean follow-up period of 85.6 months (median, 92.5 months; range, 36–98 months), 5 patients (25.0%) died in the advanced NASH group. The causes of death were HCC (2 patients), liver failure (2 patients) and cerebral infarction (1 patient) in the advanced NASH group. During a mean follow-up period of 81.3 months (median, 87.5 months; range, 41–97 months), 10 patients (50.0%) died in the advanced CHC group. The causes of death were HCC (7 patients) and liver failure (3 patients) in the advanced CHC group. During a mean follow-up period of 90.8 months (median, 92 months; range, 61–98 months), only 1 patient died of acute myocardial infarction in the mild NASH group. The overall survival in the advanced NASH group was not significantly different compared to those in the advanced CHC (Fig. 1A) or mild NASH (Fig. 1B) groups. There was a statistical trend towards association between the severity of NASH stage and overall survival, but the trend did not reach statistical significance ($p = 0.098$).

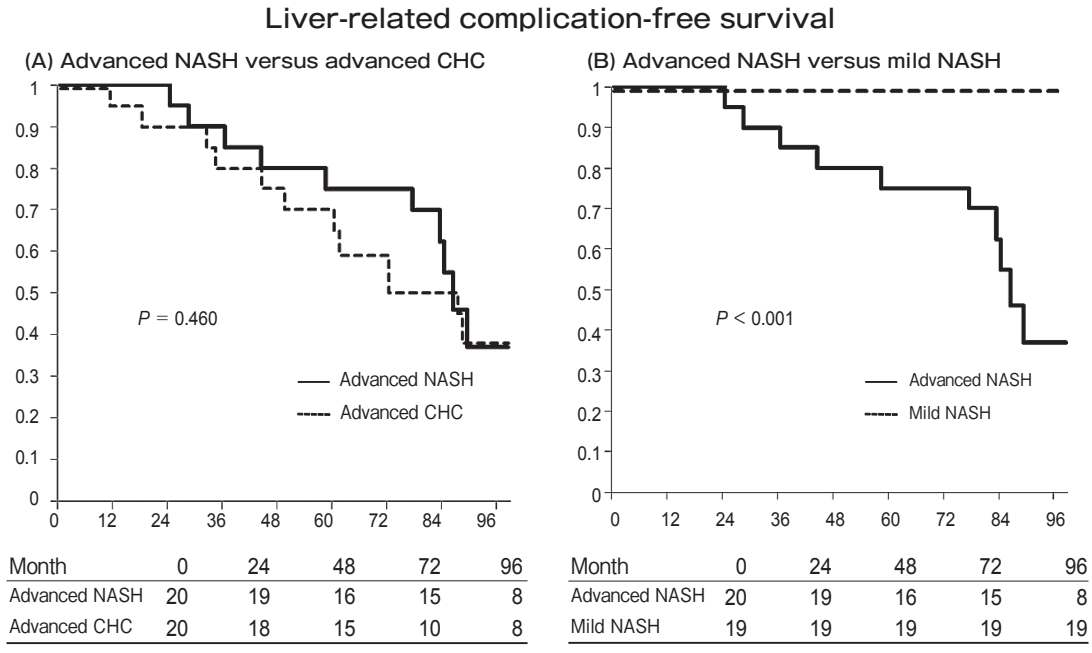
Comparisons of liver-related complications- and HCC-free survival in the advanced NASH with advanced CHC and mild NASH groups. During the follow-up period, 8 patients (40%) developed HCC and 4 (20%) developed liver failure in the advanced NASH group. In the advanced CHC group, 8 patients (40%) developed HCC and 4 (20%) developed liver failure. Neither HCC nor liver failure was observed in the mild NASH group. Liver-related complications- and HCC-free survival were not significantly different between the advanced NASH and advanced CHC groups (Figs. 2A and 3A). However, liver-related complications- and HCC-free survival were significantly reduced in the advanced NASH



Month	0	24	48	72	96
Advanced NASH	20	20	18	16	15
Advanced CHC	20	20	19	16	10

Month	0	24	48	72	96
Advanced NASH	20	20	18	16	15
Mild NASH	19	19	19	18	18

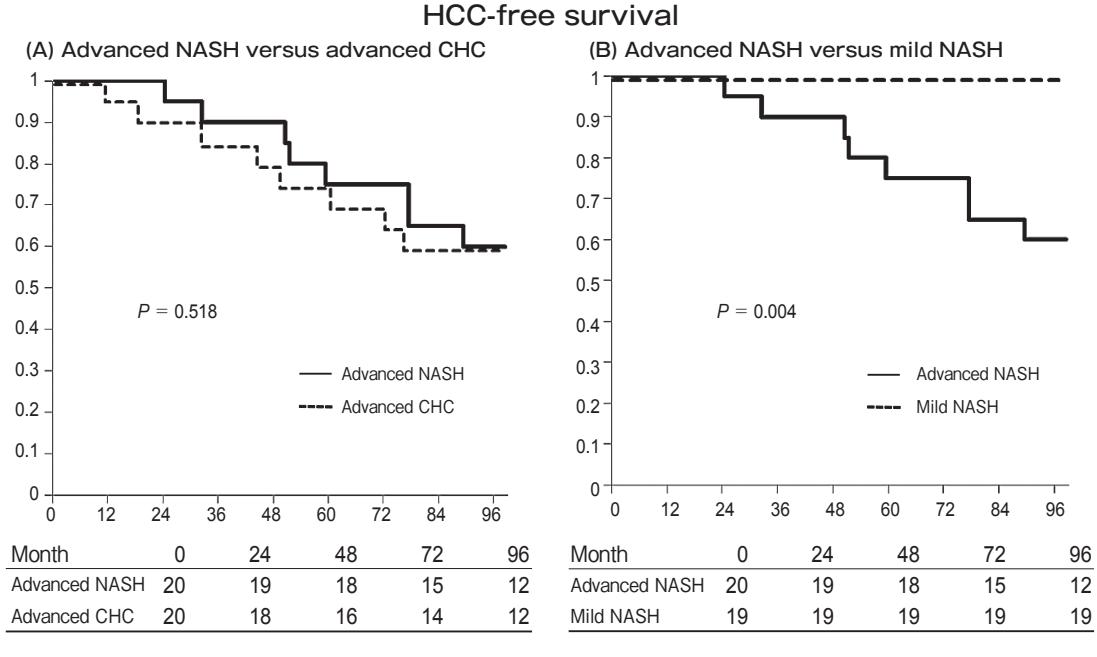
Fig. 1 **A**, The cumulative probabilities of overall survival for the NASH with advanced fibrosis group (advanced NASH) (solid line) and for the matched hepatitis C virus (HCV)-related advanced fibrosis group (advanced CHC) (dotted line). There was no significant difference in overall survival between the 2 groups ($p = 0.301$); **B**, The cumulative probabilities of overall survival for the advanced NASH group (solid line) and the matched NASH with mild fibrosis group (mild NASH) (dotted line). There was a statistical trend towards association between the severity of NASH stage and overall survival, but the trend did not reach statistical significance ($p = 0.098$).



Month	0	24	48	72	96
Advanced NASH	20	19	16	15	8
Advanced CHC	20	18	15	10	8

Month	0	24	48	72	96
Advanced NASH	20	19	16	15	8
Mild NASH	19	19	19	19	19

Fig. 2 **A**, The cumulative probabilities of liver-related complication-free survival for the advanced NASH group (solid line) and the matched advanced CHC group (dotted line). There was no significant difference in liver-related complication-free survival between the 2 groups ($p = 0.460$); **B**, The cumulative probabilities of liver-related complication-free survival in the advanced NASH group (solid line) and the mild NASH group (dotted line). Liver-related complication-free survival was significantly reduced in the advanced NASH group compared to the mild NASH group ($p < 0.001$).



Month	0	24	48	72	96
Advanced NASH	20	19	18	15	12
Advanced CHC	20	18	16	14	12

Month	0	24	48	72	96
Advanced NASH	20	19	18	15	12
Mild NASH	19	19	19	19	19

Fig. 3 **A**, The cumulative probabilities of HCC-free survival for the advanced NASH group (solid line) and the matched advanced CHC group (dotted line). There was no significant difference in HCC-free survival between the 2 groups ($p = 0.518$); **B**, The cumulative probabilities of HCC-free survival in the advanced NASH group (solid line) and the mild NASH group (dotted line). HCC-free survival was significantly reduced in the advanced NASH group compared to the mild NASH group ($p = 0.004$).

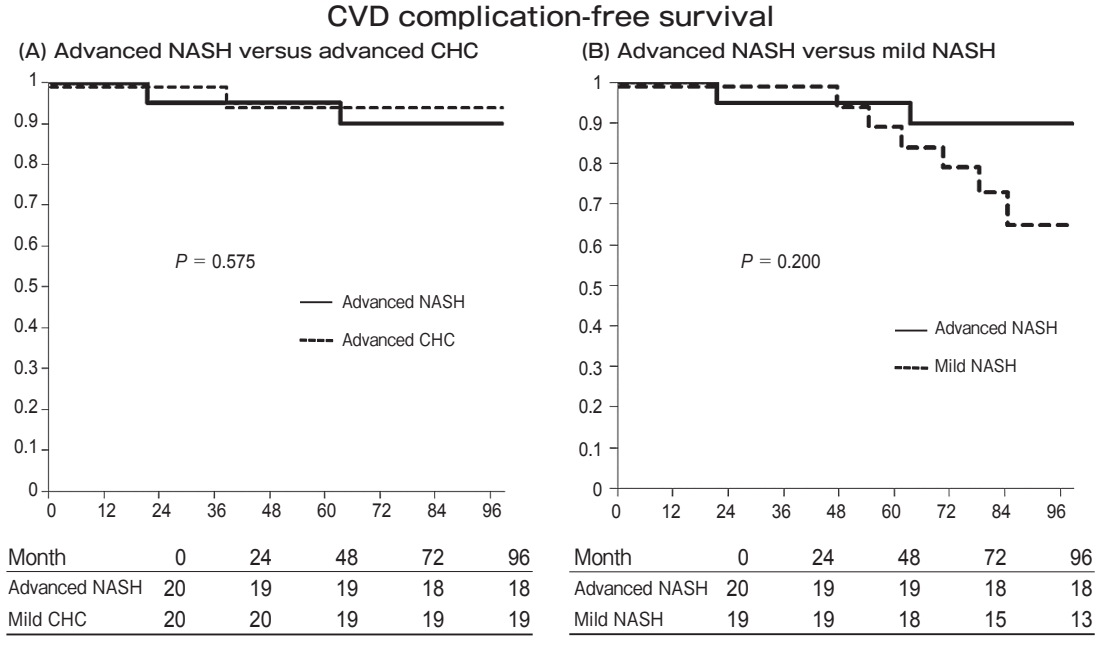


Fig. 4 **A**, The cumulative probabilities of cardiovascular/cerebrovascular disease (CVD) complications for the advanced fibrosis NASH group (solid line) and the group of matched advanced fibrosis CHC group (dotted line). There was no significant difference in the CVD complication-free survival between the 2 groups ($p = 0.575$); **B**, The cumulative probabilities of CVD complication-free survival for the advanced fibrosis NASH group (solid line) and the matched mild NASH group (dotted line). There was no significant difference in CVD complication-free survival between the 2 groups ($p = 0.200$).

group compared to the mild NASH group (Figs. 2B and 3B).

Comparisons of CVD complication-free survival in the advanced NASH group with advanced CHC and mild NASH groups. During the follow-up period, 2 patients (10%) developed cerebral infarctions in the advanced NASH group. One cerebral infarction was observed in the advanced CHC group. Two cerebral infarctions (10.5%) and 4 acute coronary syndromes (21.1%) were observed in the mild NASH group. The CVD complication-free survival in the advanced NASH group was not significantly different compared to those in the advanced CHC (Fig. 4A) or mild NASH (Fig. 4B) groups.

Discussion

The major finding of our study is that the outcomes of NASH with advanced fibrosis may be worse than those of NASH with mild fibrosis regarding liver-related complications, including HCC development in female patients. Thus, although more attention has been paid to male patients because male gender has been reported to be an HCC risk factor in NASH patients [22, 30, 31], female NASH patients should also be recognized as a high-risk population that, if their histological stage is high, are more likely to suffer liver-related complications. Another distinctive aspect of our study is that we focused on female patients, and age and BMI were adjusted between each study group, while previous studies comparing NASH and CHC cirrhosis adjusted for age and sex only [29, 34, 35]. As mentioned above, age, gender, obesity and fibrosis are closely related to the prognosis of NASH in the Japanese population. In addition, obesity and diabetes mellitus are recognized as risk factors for HCC and cardiovascular disease [31, 36–38]. Thus, our study has an advantage in that age, gender and BMI were adjusted between each study group.

Our finding that the reduced liver-related complication-free survival in the group of advanced NASH patients compared to the mild NASH group is consistent with several studies [39, 40], although these

study cohorts were not age-, sex- and BMI-matched. Our study is also consistent with prior studies [22, 41] showing that advanced fibrosis is one of the risk factors for developing HCC in NASH patients. Notably, our study showed that advanced fibrosis may be independent of age, BMI and diabetes mellitus as risk factors for developing HCC in NASH patients.

The similar overall survival rates we observed for NASH-associated and HCV-associated cirrhosis are consistent with the results of several previous reports [29, 34], but are in contrast to those of Sanyal *et al.* [35] that found NASH-associated cirrhosis to have a lower mortality rate than HCV-associated cirrhosis. A possible reason for this inconsistency is that the large sample size and long-term (more than 20 years) follow-up in Sanyal's study provided sufficient power to reach statistical significance. Another possible reason is racial difference, given that our results are similar to those of Yatsuji *et al.* [29] in a Japanese population, which showed no significant differences in the overall survival rates between the NASH and HCV groups. Furthermore, our finding of no significant difference in the liver-related complication-free survival between the NASH and HCV groups is consistent with the results of Yatsuji *et al.* [29] but in contrast to those of Sanyal *et al.* [35]. The possible reasons for the inconsistency are the same as those mentioned above.

Yatsuji *et al.* [29] showed that the 5-year overall survival rate of NASH-associated cirrhosis was 75.2%, while it was almost 90% in our study, although both study populations were Japanese. A possible reason for this inconsistency is the difference in the frequency of diabetes mellitus and gender composition between the studies. Our study population was comprised of female patients, and the frequency of diabetes mellitus in this population was relatively low compared to the study population in Yatsuji *et al.* [29], which might have influenced our findings of a lower frequency of CVD-related or liver-related complications. Another possible reason is that the liver function based on serum albumin level and total bilirubin level in our study population was relatively good compared to the study population in Yatsuji *et al.* [29], which might have led to better overall survival in our study.

Because of the small sample size of our study cohorts, we could not use a Cox proportional-hazards regression model in our study population. In addition, some of the baseline characteristics were significantly different between the advanced NASH, advanced CHC and mild NASH groups.

In conclusion, female patients with NASH and advanced fibrosis may have a less favorable prognosis than age- and BMI-matched cohorts with NASH and mild fibrosis regarding liver-related complications, including HCC development. In contrast, the prognosis of female patients with NASH and advanced fibrosis may be similar to that of age-, BMI- and fibrosis grade-matched cohorts with CHC, although the results were preliminary. Larger cohort studies with much longer follow-ups are needed to confirm our data.

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