Supplementary Methods

Inclusion and exclusion criteria

Patients ≥ 18 years of age with biopsy-proven NAFLD and written informed consent were included in the study. For this study, patients were included if they had a subsequent liver biopsy. Patients meeting any of the following criteria were excluded from the study: significant alcohol consumption (defined as ≥14 drinks/week for men or ≥7 drinks/week for women) within the previous 2-year period; underlying liver disease including hepatitis B, hepatitis C, hemochromatosis, Wilson's disease, alpha-1 antitrypsin deficiency, glycogen storage disease, autoimmune hepatitis, and cholestatic or vascular liver disease; clinical or laboratory evidence of secondary causes or chronic conditions associated with hepatic steatosis including nutritional disorders, human immunodeficiency virus infection, and use of steatogenic drugs such as amiodarone, glucocorticoids, methotrexate, l-asparaginase, and valproic acid; major systemic illnesses; decompensated liver disease (defined as Child-Pugh score >10 points); contraindications to magnetic resonance imaging (MRI) including metallic implants, claustrophobia, and body circumference exceeding the imaging chamber capacity; pregnancy or attempting to be pregnant.

Histological evaluation

All patients underwent a baseline liver biopsy, followed by a second liver biopsy. Histologic assessments of liver biopsies were systematically assessed by an experienced liver pathologist blinded to clinical data. Biopsy results were scored using NASH CRN

histologic scoring system. Fibrosis was scored from 0 to 4, with stage 4 fibrosis defined as cirrhosis. Hepatic steatosis and lobular inflammation was scored from 0 to 3. Hepatocyte ballooning was scored from 0 to 2. Hepatic steatosis, lobular inflammation, hepatocyte ballooning were combined to obtain NAS ranging from 0 to 8.

Sample size estimation

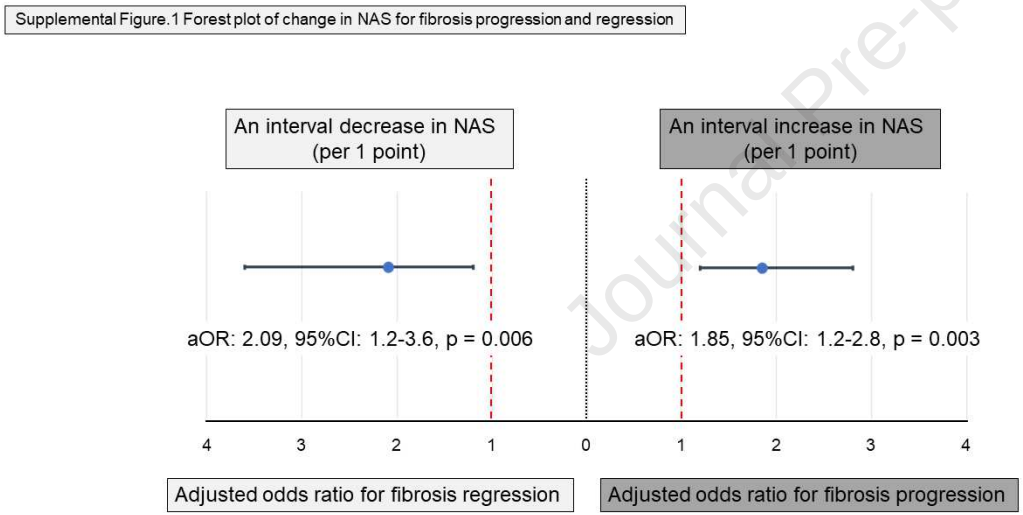
A previous study had suggested that the rate of fibrosis progression is 35% among patients with NAFL and NASH. We estimated NAS increase is associated with fibrosis progression with an odds ratio of 1.5. To obtain a power of 0.80 with an alpha of 0.05, we projected a sample size of 101 to obtain statistical significance. Therefore, we had adequate power to detect fibrosis progression associated with NAS increase in the study.

Statistical analysis

The association between change in NAS and change in fibrosis between two biopsies was examined using Fisher's exact test. Univariable and multivariable logistic regression analysis were performed for factors associated with fibrosis progression and regression. For fibrosis regression assessment, patients with stage 0 fibrosis at baseline were excluded from the analysis. Similarly, for fibrosis progression assessment, patients with stage 4 fibrosis at baseline were excluded from the analysis. Fibrosis regression was defined as ≥1 stage decrease in fibrosis and fibrosis progression was defined as ≥1 stage increase in fibrosis using the NASH CRN Histological Scoring, respectively. Factors with $p < 0.05$ on univariable logistic regression and age, sex, race/ethnicity, and diabetes

status were used for multivariable-adjusted logistic regression analysis. Statistical significance was defined as $p < 0.05$. All statistical analyses were performed using EZR (Saitama Medical center, Jichi Medical University, Saitama, Japan) and a graphical user interface for R (The R Foundation for Statistical Computing, Vienna, Austria).

Supplemental Figure.1 Forest plot of change in NAS for fibrosis progression and regression



In multivariable-adjusted logistic regression analysis for fibrosis progression, age, sex, race/ethnicity, diabetes status, and change in AST were adjusted. Similarly, in multivariable-adjusted logistic regression analysis for fibrosis regression, age, sex, race/ethnicity, diabetes status, GGT at baseline, and change in platelet counts were adjusted. Plot shows the adjusted odds ratio and the line indicates 95% CI.

CI, confidence interval; NAS, Nonalcoholic fatty liver disease Activity Score; AST, aspartate aminotransferase; GGT, gamma-glutamyl transferase

Supplemental Table.1 Patient characteristics

	All patients (n = 123)	Patients with no change in fibrosis (n = 69)	Patients with fibrosis progression (n = 30)	Patients with fibrosis regression (n = 24)	p value
Age (years)	54 (44-60)	54(43-61)	49(42-57)	59(49-63)	0.1
BMI (kg/m^2)	31.8(29-36)	31.6(28-36)	31.6(29-36)	33.2(30-37)	0.4
Female, n (%)	77 (62.6%)	41 (59.4%)	20 (66.7%)	17 (68.0%)	0.7
Race/Ethnicity, n (%)					0.4
White	54 (43.9%)	29 (42.0%)	15 (50.0%)	10 (41.7%)	
Hispanic	47 (38.2%)	29 (42.0%)	9 (30.0%)	9 (37.5%)	
Other	22 (17.9%)	11 (16.0%)	6 (20.0%)	5 (20.8%)	

Diabetes mellitus, n (%)	60 (48.8%)	30 (43.5%)	16 (53.3%)	14 (58.3%)	0.4
Hypertension, n (%)	64 (52.0%)	37 (53.6%)	16 (53.3%)	11 (45.8%)	0.9
Dyslipidemia, n (%)	51 (41.5%)	29 (42.0%)	12 (40.0%)	10 (41.7%)	1
Biochemical data					
AST (IU/L)	35(26-59)	38(28-64)	33(26-52)	29(23-55)	0.4
ALT (IU/L)	47(32-78)	48(37-81)	48(39-67)	38(26-92)	0.3
ALP (IU/L)	74(62-89)	73(65-92	77(61-86)	75(64-86)	0.6
GGT (IU/L)	39(26-62)	38(25-71)	42(32-60)	31(24-49)	0.2
Bilirubin (mg/dl)	0.5(0.3-0.6)	0.5(0.3-0.6	0.4(0.3-0.5)	0.4(0.3-0.6)	0.4
Total cholesterol (mg/dl)	182(156-207)	182(157-200)	182(156-212)	181(148-221)	0.9
HDL (mg/dl)	43(36-54)	42(36-54)	46(40-52)	42(34-58)	0.6
LDL (mg/dl)	100(83-125)	100(83-127)	102(79-120)	101(87-126)	0.9
TG (mg/dl)	142(110-196)	144(110-196)	125(95-178)	147(118-198)	0.5
Platelet count (10^9/L)	246(199-275)	246(198-278)	241(209-276)	247(195-270)	0.8
HbA1c (%)	6.0(5.6-6.7)	6.0(5.7-6.6)	6.2(5.5-6.9)	5.9(5.6-6.7)	0.8
Histological findings*					
Fibrosis stage, n (%)					0.04
0	34 (27.6%)	22 (31.9%)	12 (40.0%)	–	
1	44 (35.8%)	21 (30.4%)	10 (33.3%)	13 (54.2%)	
2	15 (12.2%)	3 (4.4%)	5 (16.7%)	7 (29.2%)	
3	19 (15.5%)	14 (20.3%)	3 (10.0%)	2 (8.3%)	
4	11 (8.9%)	9 (13.0%)	–	2 (8.3%)	
Steatosis grade (0/1/2/3)					0.1
0	1 (0.8%)	0 (0%)	0 (0%)	1 (4.2%)	
1	40 (32.5%)	23 (33.3%)	8 (26.6%)	9 (37.5%)	
2	49 (39.9%)	26 (37.7%)	11 (36.7%)	12 (50.0%)	
3	33 (26.8%)	20 (29.0%)	11 (36.7%)	2 (8.3%)	
Lobular inflammation (0/1/2/3)					0.4
0	0 (0%)	0 (0%)	0 (0%)	0 (0%)	
1	45 (36.6%)	28 (40.6%)	11 (36.7%)	6 (25.0%)	
2	71 (57.7%)	36 (52.2%)	17 (56.7%)	18 (75.0%)	

3	7 (5.7%)	5 (7.2%)	2 (6.6%)	0 (0%)	
Ballooning grade (0/1/2)					0.7
0	12 (9.8%)	9 (13.0%)	1 (3.3%)	2 (8.3%)	
1	70 (56.9%)	38 (55.1%)	19 (63.4%)	13 (54.2%)	
2	41 (33.3%)	22 (31.9%)	10 (33.3%)	9 (37.5%)	
NAS	5(4-6)	5(4-6)	5(5-6)	5(4-6)	0.8

Data are shown in median (interquartile range)

* NASH CRN histology scoring system was used

≥1 point increase or ≥1 point decrease in fibrosis stage at 2nd biopsy was defined as fibrosis progression or regression

P value indicates the difference between patients with no change in fibrosis, fibrosis progression, and fibrosis regression

BMI, body mass index; AST, aspartate aminotransferase; ALT, alanine aminotransferase; ALP, alkaline phosphatase, GGT, gamma glutamyl transferase; HDL, high-density lipoprotein; low-density lipoprotein; LDL, low-density lipoprotein; TG, triglycerides NAS, nonalcoholic fatty liver disease activity score