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ORIGINAL ARTICLE
Coronary artery disease

High fibrosis-4 index predicts the new onset of ischaemic heart disease during a 10-year period in a general population

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Aims The fibrosis-4 (FIB-4) index, calculated using age, platelet count, and levels of aspartate aminotransferase and alanine aminotransferase, is a non-invasive indicator for the detection of liver fibrosis. Advanced hepatic fibrosis is associated with morbidity and mortality in patients with non-alcoholic fatty liver disease. However, the relationship between liver fibrosis and the development of ischaemic heart disease (IHD) has not fully been addressed.

Methods and results We investigated the association between the FIB-4 index and the new onset of IHD during a 10-year period in a general population of subjects who received annual health examinations ($n = 28\,990$). After exclusion of subjects with missing data and those with a history of IHD at baseline, a total of 13 448 subjects (men/women: 8774/4674, mean age: 48 years) were included. During the 10-year period, 378 men (4.3%) and 77 women (1.6%) had a new onset of IHD. Multivariable Cox proportional hazard models with a restricted cubic spline showed that hazard risk for the development of IHD increased with a higher FIB-4 index at baseline after adjustment of age, sex, fatty liver (FL) determined by ultrasonography, estimated glomerular filtration rate, habits of current smoking and alcohol drinking, family history of IHD, and diagnosis of hypertension, diabetes mellitus and dyslipidaemia. When divided by FL, the FIB-4 index becomes an independent predictor for the development of IHD in subjects with FL but not in those without FL. The addition of the FIB-4 index to traditional risk factors for IHD significantly improved the discriminatory capability.

Conclusion A high level of the FIB-4 index predicts the new onset of IHD during a 10-year period.

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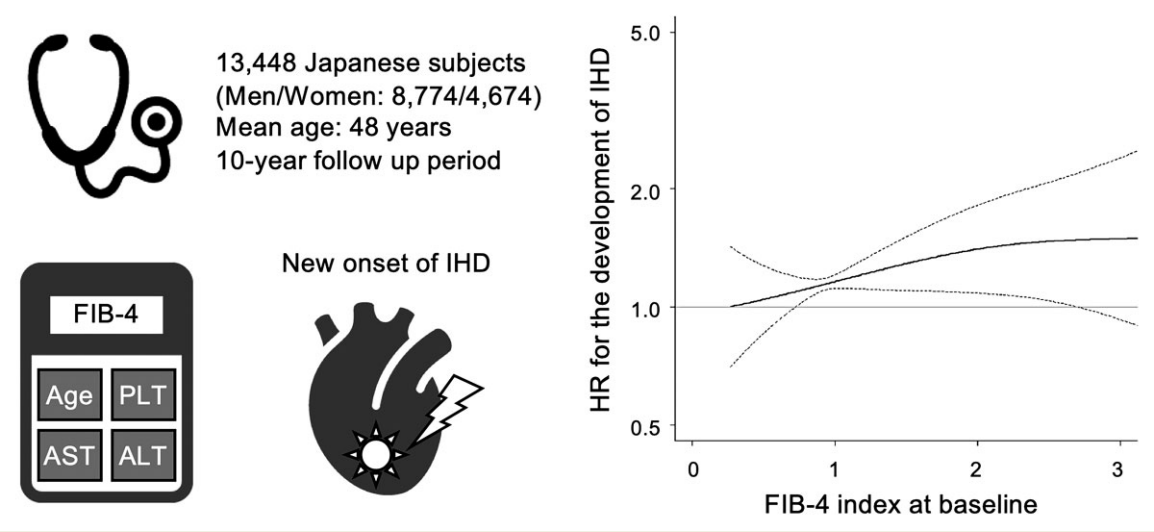
Graphical Abstract



High FIB-4 index predicts the development of ischemic heart disease (IHD).

Keywords Hepatic fibrosis • Coronary heart disease • Biomarker

Introduction

Non-alcoholic fatty liver disease (NAFLD) is a prominent cause of chronic liver disease,[1] and the prevalence of NAFLD has been dramatically increasing worldwide, leading to a major cause of liver-related morbidity and mortality.[2] NAFLD includes a spectrum of diseases ranging from simple fatty liver (FL) to non-alcoholic steatohepatitis and may progress to cirrhosis and hepatocellular carcinoma.[3] NAFLD is also associated with obesity, diabetes mellitus, dyslipidaemia, and hypertension [1,4] as well as cardiovascular disease including ischaemic heart disease (IHD).[5,6] It is noteworthy that a new concept of metabolic dysfunction-associated fatty liver disease (MAFLD) has recently been proposed regardless of alcohol consumption.[7]

Liver biopsy is considered as a reference method for assessing the severity of steatosis and inflammation and degree of fibrosis in patients with NAFLD.[8] However, liver biopsy is an invasive procedure that may cause several complications, and sampling errors and inter- and intra-observer variabilities can be major issues.[9,10] Because of these limitations, non-invasive methods for assessment of the severity of hepatic fibrosis have been developed using serum markers.[11] Among them, the fibrosis-4 (FIB-4) index, calculated using age, platelet count, and levels of aspartate aminotransferase (AST) and alanine aminotransferase (ALT), is a simple and non-invasive indicator for detection of liver impairment and fibrosis.[12] The FIB-4 index was validated to evaluate liver fibrosis in patients with liver diseases of various aetiologies including NAFLD and viral hepatitis.[12–14] A high FIB-4 index has also been reported to be associated with all-cause mortality in several chronic diseases including microscopic polyangiitis, rheumatoid arthritis, and heart failure.[15–17]

In addition to liver-related complications, subjects with NAFLD have an increased prevalence of cardiovascular events including myocardial infarction and stroke, which are even more common than liver-related complications.[18] It has also been shown that the FIB-4 index as a marker of advanced hepatic fibrosis is associated with coronary artery calcification and cardiovascular events including IHD, stroke, arrhythmia, and cardiac death in patients with NAFLD, type 2 diabetes mellitus, atrial fibrillation, or IHD [19–26] (see Supplementary material online, *Table S1*).

However, to the best of our knowledge, there has been only one study showing the association between the FIB-4 index and new onset of IHD, and it was performed in 1481 patients with type 2 diabetes mellitus.[26] Therefore, the relationship between liver fibrosis and the development of IHD has not been fully addressed, especially in a general population. The primary hypothesis is that the FIB-4 index, which is known as a marker of hepatic fibrosis, is an independent risk factor for the new onset of IHD. In the present study, we investigated the associations of the FIB-4 index as a continuous variable and categorized the subgroups of the FIB-4 index divided by cutoff values with new onset of IHD during a 10-year period in a Japanese general population using a large number of subjects who underwent annual health checkups. Since it has been reported that there is a sex difference in the prevalence of IHD[27] or FL[28] in Japan, we also performed *post hoc* analyses for the interaction between the FIB-4 index and sex for the risk of IHD. In addition, since liver fibrosis is a consequence of hepatosteatosis, subjects were also divided into two groups by the presence and absence of FL.

Methods

Study subjects

All subjects who received annual health examinations at Keijinkai Maruyama Clinic, Sapporo, Japan in 2006 were enrolled in this

registry ($n = 28\,990$).[29,30] A flow chart of the study participants is shown in *Figure 1*. Pre-specified exclusion criteria were the absence of data for estimated glomerular filtration rate (eGFR), the FIB-4 index, habits of current smoking and alcohol drinking, and history of IHD defined by a self-reported questionnaire survey at baseline. IHD was defined as angina pectoris, myocardial infarction or treatment with percutaneous coronary intervention and/or coronary artery bypass grafting, which were determined by a self-reported questionnaire survey. After exclusion, a total of 13 448 subjects (male/female: 8774/4674) who underwent abdominal ultrasonography in 2006 and received health examinations at least once in the period from 2007 to 2016 were included in the present study. The study conformed to the principles outlined in the Declaration of Helsinki and was performed with the approval of the institutional ethical committee of Sapporo Medical University (number: 30-2-32). Written informed consent was obtained from all of the subjects.

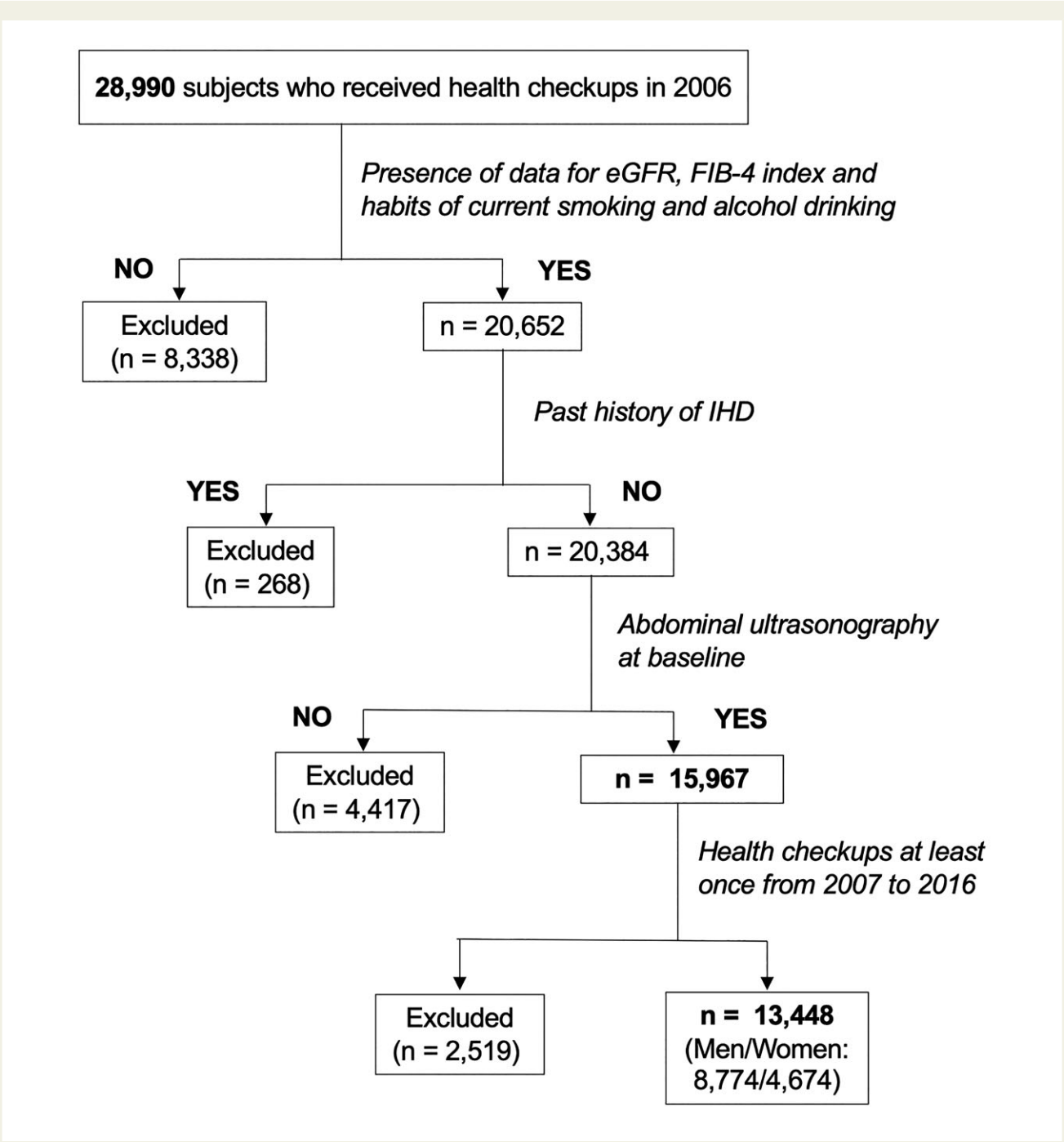


Figure 1 Flow chart of the selected study participants. Among 28 990 subjects enrolled in 2006, a total of 13 448 subjects (men/women: 8774/4674) were finally included for analyses in the present study. eGFR, estimated glomerular filtration rate; FIB-4, fibrosis-4; IHD, ischaemic heart disease.

Table 1 Characteristics of the recruited subjects at baseline ($n = 13\,448$)

	All $n = 13\,348$	Men $n = 8774$	Women $n = 4674$	*P*-value
Age (years)	48 ± 9	49 ± 9	47 ± 9	<0.001
Body mass index	23.2 ± 3.4	24.1 ± 3.2	21.7 ± 3.3	<0.001
Waist circumference (cm)	83.7 ± 9.3	86.3 ± 8.4	79.0 ± 9.1	<0.001
Systolic blood pressure (mmHg)	117 ± 16	120 ± 16	110 ± 16	<0.001
Diastolic blood pressure (mmHg)	75 ± 11	77 ± 11	70 ± 10	<0.001
Current smoking habit	4751 (35.3)	3913 (44.6)	838 (17.9)	<0.001
Alcohol drinking habit	6478 (48.2)	5210 (59.4)	1268 (27.1)	<0.001
Family history				
IHD	1305 (9.7)	805 (9.2)	500 (10.7)	0.005
Comorbidity				
Hypertension	2337 (17.4)	1864 (21.2)	473 (10.2)	<0.001
Diabetes mellitus	744 (5.5)	653 (7.4)	91 (2.0)	<0.001
Dyslipidaemia	3392 (25.2)	2206 (25.1)	1186 (25.4)	<0.001
Fatty liver	4734 (35.5)	4037 (46.0)	697 (14.9)	<0.001
Biochemical data				
Haemoglobin (g/dL)	14.3 ± 1.5	15.1 ± 1.1	12.9 ± 1.2	<0.001
Platelet (10^4/μL)	23.8 ± 5.2	23.4 ± 5.0	24.6 ± 5.5	<0.001
Albumin (g/dL)	4.4 ± 0.2	4.4 ± 0.2	4.3 ± 0.2	<0.001
Creatinine (mg/dL)	0.73 ± 0.25	0.81 ± 0.25	0.60 ± 0.19	<0.001
eGFR (mL/min/1.73 m^2)	84.6 ± 14.6	83.3 ± 14.1	87.0 ± 15.2	<0.001
Uric acid (mg/dL)	5.5 ± 1.4	6.1 ± 1.2	4.4 ± 1.0	<0.001
AST (U/L)	24 (18–26)	25 (19–28)	20 (16–22)	<0.001
ALT (U/L)	27 (15–31)	31 (18–37)	18 (12–20)	<0.001
GGT (U/L)	51 (19–58)	65 (27–74)	26 (14–27)	<0.001
FPG (mg/dL)	93 ± 19	96 ± 21	87 ± 14	<0.001
Haemoglobin A1c (%)	5.3 ± 0.7	5.4 ± 0.8	5.2 ± 0.5	<0.001
Total cholesterol (mg/dL)	205 ± 34	206 ± 34	204 ± 34	<0.001
LDL cholesterol (mg/dL)	123 ± 31	124 ± 31	120 ± 31	<0.001
HDL cholesterol (mg/dL)	60 ± 16	56 ± 14	69 ± 15	<0.001
Triglycerides (mg/dL)	115 (64–139)	134 (79–161)	78 (50–93)	<0.001
FIB-4 index	1.03 ± 0.49	1.04 ± 0.49	1.00 ± 0.48	<0.001

Variables are expressed as number (%), mean values ± SD or medians (interquartile ranges).
ALT, alanine aminotransferase; AST, aspartate aminotransferase; eGFR, estimated glomerular filtration rate; FIB-4, fibrosis-4; FPG, fasting plasma glucose; GGT, γ-glutamyl transpeptidase; HDL, high-density lipoprotein; IHD, ischaemic heart disease; LDL, low-density lipoprotein.

Measurements

Medical examinations and blood samplings were performed after an overnight fast. Blood pressure was measured twice consecutively on the upper arm using a sphygmomanometer (#601, Kenzmedico, Saitama, Japan), and average blood pressure was used for analysis. Body height and weight were measured in light clothing without shoes, and body mass index was calculated as body weight in kilograms divided by height in meters squared. eGFR was calculated by the following equation for Japanese people: eGFR (mL/min/1.73 m^2) = 194 × serum creatinine$^{(-1.094)}$ × age$^{(-0.287)}$ × 0.739 (if female).[31] The FIB-4 index, a marker of hepatic fibrosis, was calculated by the following formula: {age [years] × AST [U/L]/[platelet count (10^9/L) × ALT (U/L)$^{1/2}$]}.[12]

A self-administered questionnaire survey was performed to obtain information on habits of current smoking and alcohol drinking (≥3 times/week), history of IHD, family history of IHD, and use of drugs for hypertension, diabetes mellitus, and dyslipidaemia at baseline. Hypertension was diagnosed in accordance with the guideline of the Japanese Society of Hypertension:[32] systolic blood pressure ≥140 mmHg, diastolic blood pressure ≥90 mmHg or self-reported use of anti-hypertensive drugs. Diabetes mellitus was diagnosed as fasting plasma glucose ≥126 mg/dL, haemoglobin A1c ≥ 6.5%, or self-reported use of anti-diabetic drugs. Dyslipidaemia was diagnosed as low-density lipoprotein (LDL) cholesterol ≥ 140 mg/dL, high-density lipoprotein (HDL) cholesterol <40 mg/dL, triglycerides ≥ 150 mg/dL, or self-reported use of anti-dyslipidaemic drugs.

Abdominal ultrasonography

Abdominal ultrasonography was performed using SSA-250A or SSA-340A (Toshiba Medical, Otawara, Japan) by 10 well-experienced echographers with at least 5 years of experience who were trained by gastroenterologists with >5 years of experience.

FL was determined by any findings of high-intensity bright liver, hepato-renal contrast, vascular obscuration, and deep attenuation in the liver.[33,34] The images and the presence of hepatic steatosis were independently reviewed by certified gastroenterologists who were blinded to clinical data.

Statistical analysis

Numeric variables are expressed as mean values ± standard deviation (SD) for parameters with normal distributions and as medians (interquartile ranges) for parameters with skewed distributions. The distribution of each parameter was tested for its normality using the Shapiro-Wilk *W* test. Comparisons between two groups were performed by Student's *t*-test for parametric parameters and the Mann–Whitney *U* test for non-parametric parameters. Intergroup differences in percentages of demographic parameters were examined by the χ^2 test. The association between the FIB-4 index at baseline and the development of IHD was investigated by multivariable Cox proportional hazard models with a restricted cubic spline after adjustment of confounders including age, sex, presence of FL, eGFR, current smoking habit, alcohol drinking habit, family history of IHD and diagnosis of hypertension, diabetes mellitus and dyslipidaemia at baseline. Hazard ratios (HRs), 95% confidence intervals (CIs), and Akaike's information criterion (AIC) for the development of IHD by the FIB-4 index at baseline were calculated by adjustment of the confounders in several models. Parameters with a lower AIC score constitute a better-fit model. The ability of the FIB-4 index to predict new onset of IHD was investigated using receiver operating characteristic (ROC) curve analyses. The area under the curve (AUC) and 95% CI were calculated, and the cutoff values were obtained by Youden's index.[35] To compare the discrimination between the models adjusted for traditional risk factors for IHD with and without the FIB-4 index, C-statistics analogous to the AUC were estimated using the method of DeLong *et al.*[36] Moreover, the increased discriminatory value was examined by net reclassification improvement (NRI) and integrated discrimination improvement (IDI).[37] Since the FIB-4 index is calculated using age, age was excluded from the traditional risk factors for the development of IHD in analyses for discriminatory capacity because of multicollinearity. A *P* value of <0.05 was considered statistically significant. All data were analyzed using EZR[38] and R version 4.0.3 (R Core Team, R Foundation for Statistical Computing, Vienna, Austria, 2020, https://www.R-project.org).

Results

Characteristics of the study subjects

Basal characteristics of the included subjects are shown in *Table 1*. Mean age was 48 years, and 65.7% of subjects were men. The FIB-4 index was significantly higher in men than in women (mean values ± SD: 1.04 ± 0.49 vs. 1.00 ± 0.48). Prevalence of a family history of IHD was higher in women than in men. Prevalences of hypertension, diabetes mellitus, and dyslipidaemia in men/women were 21.2%/10.2%, 7.4%/2.0%, and 25.1%/25.4%, respectively. Prevalence of FL detected by abdominal ultrasonography was significantly higher in men ($n = 4037$, 46.0%) than in women ($n = 697$, 14.9%) (*Table 1*).

Table 2 Multivariable Cox proportional hazard analyses for the new onset of IHD ($n = 13,448$)

	HR (95% CI)	*P*-value
FIB-4 index	1.21 (1.03–1.41)	0.010
Age	1.05 (1.04–1.07)	<0.001
Sex (men)	2.18 (1.67–2.86)	<0.001
FL	1.22 (1.01–1.49)	0.043
eGFR	1.00 (0.99–1.01)	0.756
Current smoking habit	1.24 (1.01–1.51)	0.036
Alcohol drinking habit	0.77 (0.64–0.94)	0.009
Family history of IHD	1.63 (1.26–2.10)	<0.001
Hypertension	1.49 (1.21–1.85)	<0.001
Diabetes mellitus	1.68 (1.26–2.23)	<0.001
Dyslipidaemia	1.27 (1.04–1.55)	0.019
	(AIC = 8140)	
Interaction: FIB-4 index × sex		0.638
Interaction: FIB-4 index × FL		0.320

CI, confidence interval; eGFR, estimated glomerular filtration rate; FIB-4, fibrosis-4; FL, fatty liver; HR, hazard ratio; IHD, ischaemic heart disease.

Cumulative incidence for new onset of IHD during a follow-up period

The mean follow-up period was 6.5 years (range: 1–10 years), and follow-up summation was 92 682 (men/women: 60 230/32 452) person-years. Among the 13 348 included subjects (men/women: 8774/4674), 378 men (4.3%) and 77 women (1.6%) developed new onset of IHD during a 10-year period. The cumulative incidence rate of IHD was 47.9 (95% CI: 43.7–52.6) [men/women: 60.3 (54.5–66.7)/24.6 (19.6–30.9)] per 1000 person-years in all of the subjects.

Impact of the FIB-4 index at baseline on new onset of IHD during a follow-up period

Multivariable Cox proportional hazard analyses for new onset of IHD before adjustment (Model 1), after adjustment of age (Model 2) or sex (Model 3), and after adjustment of age, sex, presence of FL, current smoking habit and alcohol drinking habit (Model 4) showed that the FIB-4 index was an independent predictor for new onset of IHD except in Model 2 (see Supplementary material online, *Table S2*). However, Model 2 adjusted for only age had an issue about multicollinearity between the FIB-4 index and age. After adjustment of age, sex, presence of FL, current smoking habit, alcohol drinking habit, eGFR, family history of IHD and diagnosis of hypertension, diabetes mellitus and dyslipidaemia, the FIB-4 index remained as an independent determinant of the development of IHD with the minimum AIC among the models (AIC: 8140) (*Table 2*). The FIB-4 index [HR (95% CI): 1.21 (1.03–1.41), $P = 0.010$] as well as male sex [HR (95% CI): 2.18 (1.67–2.86), $P < 0.001$] and presence of FL [HR (95% CI): 1.22 (1.01–1.49), $P = 0.043$] were independently associated with new onset of IHD (*Table 2*). However, there was no significant interaction of the FIB-4 index with sex ($P = 0.638$) or

presence of FL ($P = 0.320$) for the adjusted HR for development of IHD.

Multivariable Cox proportional hazard models with a restricted cubic spline showed that HR for the development of IHD increased with a higher FIB-4 index at baseline after adjustment of age, sex, presence of FL, eGFR, habits of current smoking and alcohol drinking, family history of IHD, and diagnosis of hypertension, diabetes mellitus and dyslipidaemia at baseline (*Figure 2A*).

Impact of categorized subgroups of the FIB-4 index on the new onset of IHD

Multivariable Cox proportional hazard analyses showed that the categorized subgroup with the FIB-4 index >1.3, which has been reported to be a cutoff value for detection of hepatic fibrosis,[13,39] was independently associated with new onset of IHD after adjustment of age, sex, presence of FL, eGFR, habits of current smoking and alcohol drinking, family history of IHD, and diagnosis of hypertension, diabetes mellitus and dyslipidaemia at baseline (see Supplementary material online, *Table S3*). When the FIB-4 index was divided by quintiles, HRs tended to be high in a higher quintile, though those were not statistically significant (see Supplementary material online, *Table S4*).

ROC curve analyses showed that the cutoff value of the FIB-4 index for prediction of IHD was 1.21 [sensitivity/specificity: 40.0%/74.9%; AUC (95% CI): 0.59 (0.56–0.62)] (see Supplementary material online, *Figure S1*). When the FIB-4 index was divided by the cutoff value, the categorized subgroup with the FIB-4 index >1.21 was independently associated with new onset of IHD after adjustment of confounders (see Supplementary material online, *Table S5*).

Impact of the FIB-4 index on new onset of IHD divided by the presence of FL

Since liver fibrosis is a consequence of hepatosteatosis, subjects were divided into two groups by the presence and absence of FL. Basal characteristics of subjects with and those without FL, FL+ group and FL– group, respectively, are shown in *Table 3*. There was no significant difference in FIB-4 level between the FL+ group and FL– group. Prevalences of a family history of IHD were comparable in the FL+ and FL– groups. Subjects with the FL+ group were significantly older than subjects in the FL– group and there was a larger proportion of men in the FL+ group. Subjects with the FL+ group had larger body mass index and larger waist circumference, higher prevalences of current smoking and alcohol drinking habits, higher prevalences of hypertension, diabetes mellitus and dyslipidaemia, higher levels of systolic and diastolic blood pressures, haemoglobin, albumin, creatinine, uric acid, AST, ALT, γ-glutamyl transferase, fasting plasma glucose, haemoglobin A1c, total cholesterol, LDL cholesterol and triglycerides and lower levels of eGFR and HDL cholesterol than did subjects in the FL– group.

Multivariable Cox proportional hazard models with a restricted cubic spline showed that HR for the development of IHD increased with a higher FIB-4 level at baseline after adjustment of age, sex, eGFR, habits of current smoking and alcohol drinking, family history of IHD, and diagnosis of hypertension, diabetes mellitus and dyslipidaemia at baseline in the FL+ group (*Figure 2B*) but not in

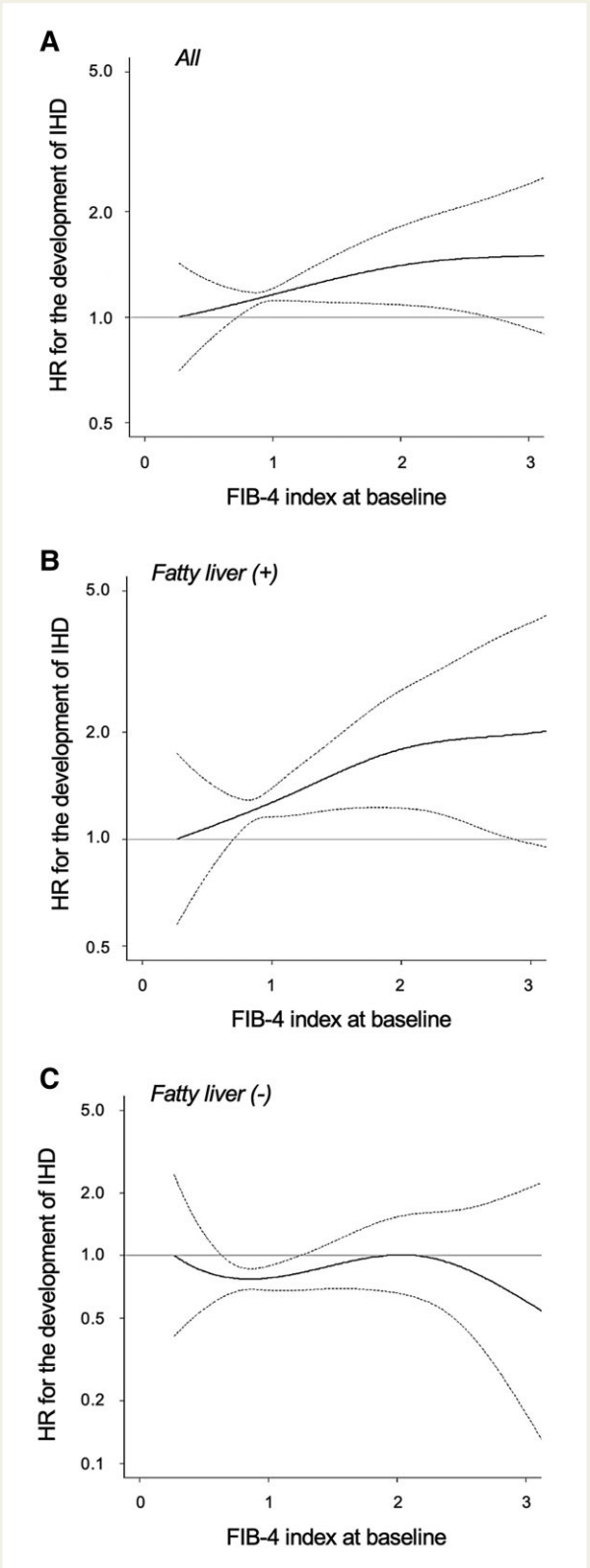


Figure 2 Hazard ratios for the development of ischaemic heart disease by the fibrosis-4 index at baseline. (*A–C*) Hazard ratios for the development of ischaemic heart disease by the fibrosis-4 index at baseline analyzed by multivariable Cox proportional hazard models with a restricted cubic spline after adjustment of age,

(Continued)

Figure 2 Continued
sex, presence of fatty liver, habits of current smoking and alcohol drinking, family history of ischaemic heart disease and diagnosis of diabetes mellitus, hypertension, and dyslipidaemia at baseline in all of the subjects (*A*) as well as in subjects with fatty liver (*B*) and those without fatty liver (*C*). Solid line: HR; dashed line: 95% confidence interval. The reference values of the fibrosis-4 index were 0.27 as minimum values.

the FL− group (*Figure 2C*). FIB-4 level was independently associated with new onset of IHD in the FL+ group [HR (95% CI): 1.42 (1.13–1.77), $P = 0.002$] but not in the FL− group [HR (95% CI): 1.04 (0.75–1.42), $P = 0.831$] (*Table 4*).

Discriminatory capacity of the addition of the FIB-4 index for predicting the development of IHD

The addition of the FIB-4 index to traditional risk factors for the development of IHD, including sex, presence of FL, habits of current smoking and alcohol drinking, family history of IHD and diagnosis of diabetes mellitus, hypertension and dyslipidaemia, significantly improved the discriminatory capacity assessed using AUC (0.661 vs. 0.667, $P = 0.033$), continuous NRI (value: 0.246, $P < 0.001$) and IDI (value: 0.0020, $P = 0.032$) (*Table 5*). Since the FIB-4 index is calculated using age, age was excluded from the traditional risk factors for the development of IHD in analyses for discriminatory capacity because of multicollinearity. As a note, when age was included as a traditional risk factor in analyses of discriminatory capacity, the addition of the

Table 3 Characteristics of the recruited subjects divided by FL ($n = 13\,448$)

	FL− $n = 8714$	FL+ $n = 4734$	*P*-value
Age (years)	47 ± 9	50 ± 9	<0.001
Men (%)	4737 (54.4)	4037 (85.3)	<0.001
Body mass index	21.9 ± 2.6	25.8 ± 3.2	<0.001
Waist circumference (cm)	79.9 ± 7.6	90.7 ± 8.0	<0.001
Systolic blood pressure (mmHg)	114 ± 16	122 ± 16	<0.001
Diastolic blood pressure (mmHg)	72 ± 11	79 ± 10	<0.001
Current smoking habit	2929 (33.6)	2140 (38.2)	<0.001
Alcohol drinking habit	4102 (47.1)	2376 (50.2)	<0.001
Family history			
IHD	839 (9.6)	466 (9.8)	0.692
Comorbidity			
Hypertension	1045 (12.0)	1292 (27.3)	<0.001
Diabetes mellitus	219 (2.5)	525 (11.1)	<0.001
Dyslipidaemia	1939 (22.3)	1453 (30.7)	<0.001
Biochemical data			
Haemoglobin (g/dL)	14.0 ± 1.5	15.0 ± 1.3	<0.001
Platelet (10^4/μL)	23.8 ± 5.1	23.9 ± 5.3	0.052
Albumin (g/dL)	4.3 ± 0.2	4.4 ± 0.2	<0.001
Creatinine (mg/dL)	0.71 ± 0.29	0.77 ± 0.15	<0.001
eGFR (mL/min/1.73 m^2)	85.3 ± 14.6	83.3 ± 14.5	<0.001
Uric acid (mg/dL)	5.2 ± 1.3	6.1 ± 1.3	<0.001
AST (U/L)	21 (17–23)	28 (20–31)	<0.001
ALT (U/L)	20 (13–24)	39 (22–46)	<0.001
GGT (U/L)	39 (16–42)	73 (31–84)	<0.001
FPG (mg/dL)	89 ± 15	100 ± 25	<0.001
Haemoglobin A1c (%)	5.2 ± 0.5	5.6 ± 0.9	<0.001
Total cholesterol (mg/dL)	201 ± 33	213 ± 34	<0.001
LDL cholesterol (mg/dL)	119 ± 30	130 ± 31	<0.001
HDL cholesterol (mg/dL)	64 ± 16	53 ± 13	<0.001
Triglycerides (mg/dL)	93 (56–110)	156 (94–184)	<0.001
FIB-4 index	1.03 ± 0.48	1.03 ± 0.51	0.989

Variables are expressed as number (%), mean values ± SD or medians (interquartile ranges).
ALT, alanine aminotransferase; AST, aspartate aminotransferase; eGFR, estimated glomerular filtration rate; FIB-4, fibrosis-4; FL, fatty liver; FPG, fasting plasma glucose; GGT, γ-glutamyl transpeptidase; HDL, high-density lipoprotein; IHD, ischaemic heart disease; LDL, low-density lipoprotein.

Table 4 Multivariable Cox proportional hazard analyses for the new onset of IHD

	FL− (*n* = 8714)		FL+ (*n* = 4734)	
	HR (95% CI)	***P*-value**	**HR (95% CI)**	***P*-value**
FIB-4 index	1.04 (0.75–1.42)	0.831	1.42 (1.13–1.77)	0.002
Age	1.06 (1.04–1.08)	<0.001	1.04 (1.02–1.06)	<0.001
Sex (Men)	2.69 (1.93–3.75)	<0.001	1.44 (0.92–2.27)	0.113
eGFR	1.00 (0.99–1.01)	0.967	1.00 (0.99–1.01)	0.816
Current smoking habit	0.96 (0.72–1.27)	0.757	1.58 (1.20–2.09)	0.001
Alcohol drinking habit	0.76 (0.58–1.01)	0.055	0.77 (0.58–1.01)	0.061
Family history of IHD	1.41 (0.97–2.06)	0.074	1.84 (1.31–2.59)	<0.001
Hypertension	1.18 (0.84–1.66)	0.335	1.77 (1.34–2.33)	<0.001
Diabetes mellitus	1.91 (1.15–3.16)	0.012	1.54 (1.09–2.17)	0.014
Dyslipidaemia	1.19 (0.88–1.61)	0.250	1.33 (1.02–1.76)	0.039

CI, confidence interval; eGFR, estimated glomerular filtration rate; FIB-4, fibrosis-4; FL, fatty liver; HR, hazard ratio; IHD, ischaemic heart disease.

Table 5 Discrimination of the addition of the FIB-4 index into traditional risk factors for IHD

	AUC		NRI		IDI	
	Value (95% CI)	***P*-value**	**Value (95% CI)**	***P*-value**	**Value (95% CI)**	***P*-value**
Traditional model[a]	0.661 (0.632–0.689)	–	–	–	–	–
Traditional model[a] + FIB-4 index	0.667 (0.638–0.695)	0.033	0.246 (0.140–0.351)	<0.001	0.0020 (0.0002–0.0039)	0.032

[a]Traditional model includes sex, the presence of fatty liver, estimated glomerular filtration rate, current smoking habit, alcohol drinking habit, family history of IHD, and diagnosis of hypertension, diabetes mellitus and dyslipidaemia.
AUC, area under the curve; CI, confidence interval; FIB-4, fibrosis-4; IDI, integrated discrimination improvement; IHD, ischaemic heart disease; NRI, net reclassification improvement.

FIB-4 index to traditional risk factors did not significantly improve the discriminatory capacity (data not shown), indicating that age as a risk factor is a strong component in the FIB-4 index for the risk of IHD.

A summary of reclassification for the addition of the FIB-4 index to traditional risk factors is shown in *Table 6*. When the traditional model with the FIB-4 index was used, 23 subjects were correctly reclassified into a higher risk category and 18 subjects were inappropriately reclassified into a lower risk category among subjects who had developed IHD. On the contrary, 709 subjects were correctly reclassified into a lower risk category and 518 subjects were inappropriately reclassified into a higher risk category among subjects who had not developed IHD.

Discussion

The present study showed an independent association between the FIB-4 index as an indicator of hepatic fibrosis and the risk for development of IHD during a 10-year period in a general population. Multivariable Cox proportional hazard models with a restricted cubic spline showed that HR for the development of IHD increased with a higher FIB-4 index at baseline after adjustment of multiple confounding factors. Furthermore, the addition of the FIB-4 index to traditional risk factors for IHD significantly improved the discriminatory capability, though the discriminatory power of the FIB-4 index for the development of IHD was relatively low. When divided by the presence and absence of FL, the FIB-4 index was an independent predictor for the development of IHD in the FL+ group but not in the FL− group. Since there was no significant interaction between the FIB-4 index and sex for the adjusted HR, the analyses were performed in total subjects, not in subjects divided by sex.

It has been shown in cross-sectional studies that several liver fibrosis scores including the FIB-4 index predict histological fibrosis with reasonable accuracy.[12–14] Furthermore, there have been several studies showing that severity of hepatic fibrosis estimated by the FIB-4 index is associated with cardiovascular disease [19–26] (see Supplementary material online, *Table S1*). However, most of the studies focused on coronary artery calcification and cardiovascular events including IHD, stroke, arrythmia, and cardiac death in patients with NAFLD, type 2 diabetes mellitus, atrial fibrillation, or IHD. A previous study showed that the FIB-4 index was independently associated with the development of IHD in 1481 patients (men/women: 706/775) with type 2 diabetes mellitus.[26] In the present study, we showed for the first time that the FIB-4 index is independently associated with the development of IHD during a 10-year period in a general population using a large number of Japanese subjects (*n* = 13 348, men/women: 8774/4674).

There are several non-invasive markers for predicting NAFLD and liver fibrosis.[11,40] The fatty liver index (FLI) is a non-invasive marker

Table 6 Reclassification for the absolute risk for development of IHD in all subjects

Number of subjects who developed IHD					
		Traditional model + FIB-4 index			
		<1.95%	**1.95–3.15%**	**3.15–4.4%**	**>4.4%**
Traditional model	<1.95%	35	2	0	0
	1.95–3.15%	1	60	10	0
	3.15–4.4%	0	9	72	11
	>4.4%	0	0	8	150
Number of subjects who did not develop IHD					
		Traditional model + FIB-4 index			
		<1.95%	**1.95–3.15%**	**3.15–4.4%**	**>4.4%**
Traditional model	<1.95%	2384	83	1	1
	1.95–3.15%	125	2076	224	8
	3.15–4.4%	0	378	1827	211
	>4.4%	0	0	206	2143

Traditional model includes sex, estimated glomerular filtration rate, current smoking habit, alcohol drinking habit, family history of IHD, and diagnosis of hypertension, diabetes mellitus and dyslipidaemia.
FIB-4, fibrosis-4; IHD, ischaemic heart disease.

calculated using body mass index, waist circumference and levels of γ-glutamyl transferase and triglycerides, and it has a high concordance with the histological criteria for NAFLD.[41,42] There have been several studies showing that the FLI is associated with the development of hypertension,[43] diabetes mellitus,[44] chronic kidney disease,[45] heart failure [46] and IHD [47] as well as fibroblast growth factor 21 (FGF21), a hepatokine,[48] and fatty acid binding-protein 4 (FABP4),[49] an atherogenic adipokine with possible therapeutic relevance.[50,51] On the contrary, NAFLD fibrosis score (NFS) is widely used to identify individuals with high probability of having advanced liver fibrosis and it is calculated by age, body mass index, presence of impaired fasting glucose or diabetes mellitus, and levels of AST, ALT, platelet count, and albumin.[52] Independent associations of cardiovascular events including IHD with NFS as well as the FIB-4 index have been shown in 898 subjects with NAFLD.[25] These results indicate relationships of IHD with liver steatosis and fibrosis estimated by blood markers, supporting the results of the present study.

Several underlying mechanisms by which NAFLD and its consecutive liver fibrosis increase the risk of cardiovascular disease have been proposed.[53] NAFLD and its consecutive liver fibrosis are strongly related to hepatic and adipose tissue insulin resistance, a synergistical risk factor for cardiovascular disease.[54] It has also been reported that patients with NAFLD and non-alcoholic steatohepatitis have an atherogenic condition of dyslipidaemia including abnormal elevations in triglycerides and LDL cholesterol and an abnormal decrease in HDL cholesterol.[55] Atherogenic cardiovascular disease has been reported to be linked to chronic inflammation.[56] In the Canakinumab Antiinflammatory Thrombosis Outcome Study (CANTOS) trial, canakinumab, a therapeutic monoclonal antibody targeting interleukin-1β, led to a significantly lower rate of recurrent cardiovascular events than placebo independent of lipid-level lowering therapy.[57] Several markers of chronic inflammation, including high-sensitivity C-reactive protein (hsCRP), interleukin 6, interleukin-1β, and tumor necrosis factor-α have been reported to be increased in patients with NAFLD.[53] It has been reported that the FIB-4 index and hsCRP are interrelated and that the FIB-4 index is significantly associated with presence of microvascular disease independent of hsCRP in patients with type 2 diabetes mellitus.[58] In the present study, we did not investigate inflammatory markers including hsCRP. Further investigation about the relationship of the FIB-4 index with hsCRP for the development of IHD is needed.

Systemic inflammation and oxidative stress may cause an increase in endothelial dysfunction, alteration of vascular tone, and promotion of plaque formation and coagulation in blood vessels.[56] It has been reported that plasma activity of xanthine oxidoreductase, a potential enhancer of reactive oxidative stress,[59] was strongly associated with liver dysfunction [60] and was independently associated with levels of FGF21, adiponectin and FABP4.[61] Dysregulation of adipokines including decreased adiponectin and increased FABP4 has also been reported to be observed in individuals with MAFLD,[49] and it may cause atherosclerosis and cardiovascular events in connection with metabolic inflammation.[50,51,62] Furthermore, intestinal dysbiosis has recently been proposed as an important regulator in NAFLD and its related cardiovascular disease via secondary bile acids, trimethylamine, and short-chain fatty acids.[63]

This study has several limitations. First, since the study subjects had a yearly health checkup at a single urban clinic, the possibility of sample selection bias cannot be ruled out. Second, although FL was determined by abdominal ultrasonography, the severity of hepatic steatosis and fibrosis was not taken into consideration. Third, accurate information on alcohol consumption was not obtained in the present study. Fourth, the presence of hepatitis B and hepatitis C was

unknown at baseline, though prevalences of hepatitis B (0.63%) and hepatitis C (0.49%) were reported to be relatively low in the Japanese population.[64] Fifth, it is possible that several conditions including pancreatobiliary diseases, cancers, certain medications, acute unwell states, and pregnancy affect components for calculation of the FIB-4 index including AST, ALT, and platelet counts. However, those conditions were not taken into consideration in the present study. Finally, IHD was determined by a self-reported questionnaire and was not confirmed by coronary artery angiography and/or other modalities. Therefore, since patients who died of myocardial infarction and those who had a severe outcome might not be able to receive an annual health check-up, the number of patients with IHD would have been underestimated.

Conclusions

In conclusion, a high level of the FIB-4 index is an independent predictor for new onset of IHD during a 10-year period. The addition of the FIB-4 index to traditional risk factors significantly improves the discriminatory capability.

Lead author biography

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Data availability

The data underlying this article will be shared on reasonable request to the corresponding author.

Supplementary material

Supplementary material is available at *European Heart Journal Open* online.

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