The impact of an increased Fibrosis-4 index and the severity of hepatic steatosis on mortality in diabetes patients

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Abstract

Background & aims: Data on the effects of liver fibrosis and hepatic steatosis on outcomes in diabetic patients are limited. Therefore, we investigated the predictive value of the fibrosis and the severity of hepatic steatosis for all-cause mortality in diabetes patients.

Methods: A total of 1,903 patients with diabetes from the Third National Health and Nutrition Examination Survey (NHANES III) dataset were enrolled. Presumed hepatic fibrosis was evaluated with Fibrosis-4 index (FIB-4). The mortality risk and corresponding hazard ratio (HR) were analyzed with the Kaplan-Meier method and multivariable Cox proportional hazard models.

Results: Over a median follow-up of 19.4 years, all-cause deaths occurred in 69.6%. An FIB-4 ≥1.3 was an independent predictor of mortality in diabetic patients (HR: 1.198, 95% confidence interval [CI]: 1.054-1.361, *p* =0.006). Overall, an FIB-4 ≥1.3 without moderate-severe steatosis increased the mortality risk (HR: 1.277; 95%CI: 1.077-1.513, *p* =0.005). The similar results were found in diabetes patients with metabolic dysfunction-associated fatty liver disease (MAFLD) (HR: 1.457; 95%CI: 1.045-2.032, *p* =0.027), metabolic syndrome (MetS) (HR: 1.343; 95%CI: 1.051-1.716, *p* =0.019) or abdominal obesity (HR: 1.325; 95%CI: 1.048-1.674, *p*=0.019).

Conclusions: Liver fibrosis, as estimated by FIB-4, may serve as a more reliable prognostic indicator for diabetic patients than hepatic steatosis. Diabetes patients with an FIB-4 ≥1.3 without moderate-severe steatosis had a significantly increased all-cause mortality risk. These findings highlight the importance of identifying and monitoring those patients, as they may benefit from further evaluation and risk stratification.

Introduction

Diabetes is a major public health issue with a prevalence of approximately 9%,[1] and it poses substantial economic and clinical burdens. [2, 3] It is well established that diabetes increases the risk of all-cause mortality. Compared with individuals with normal glucose tolerance, the adjusted all-cause mortality hazard ratio (HR) for patients with known and newly diagnosed diabetes are 2.3 and 1.3, respectively.[4]

Chronic liver diseases, such as hepatic steatosis and liver fibrosis, are emerging diabetes complications. The prevalence of hepatic steatosis is high in patients with diabetes, ranging from 52.39–70%.[5–7] Furthermore, diabetes predisposes patients to severe liver disease.[8, 9] It is estimated that 21%-24% of patients with type 2 diabetes mellitus (DM2) have liver fibrosis, mainly of metabolic origin and often undiagnosed.[6, 10]. Hence, the severity of liver disease burden in diabetic patients has attracted more attention.

Although some authors have examined the association between hepatic steatosis and the risk of mortality in diabetes patients, data remain to a degree inconsistent, partly due to the participants differ and the design of the studies employed. Hepatic fibrosis is regarded as the main prognostic determinant

of advanced disease,[11] and the mortality risk may increase with the stage of fibrosis.[12, 13] However, the association between the severity of hepatic steatosis or liver fibrosis and all-cause mortality in diabetes patients has not been well studied. Moreover, it is unclear which of these two factors has a more significant effect on diabetes prognosis. Because liver biopsy and transient elastography (TE) outcomes were not available in general population, it could be difficult to assess liver fibrosis in those patients. Hence, simple and noninvasive tests that rely on serum biomarkers to screen these high-risk patients may be more appropriate and useful. Therefore, we investigated the association between all-cause mortality and Fibrosis-4 index (FIB-4) as well as hepatic steatosis in patients with diabetes. We also analyzed the effects of these two factors in diabetic patients who also had metabolic dysfunction-associated fatty liver disease (MAFLD), metabolic syndrome (MetS) or abdominal obesity.

PATIENTS AND METHODS

Study population and study design

This study was a retrospective, observational analysis of longitudinal medical data from the Third National Health and Nutrition Examination Survey (NHANES III) conducted by the National Center for Health Statistics (NCHS) of the United States Centers for Disease Control and Prevention (CDC). The survey used a nationwide probability sample of the noninstitutionalized civilian population in the United States from 1988 to 1994 and utilized a complex, stratified, multistage probability sampling design. It collected information from participants using standardized household interviews, physical examinations, and biological sample testing. The NCHS of the CDC provided mortality information as of December 31, 2019, from the national death index. The study was conducted following the guidelines of the Declaration of Helsinki, and the National Center for Health Statistics Research Ethics Review Board approved the NHANES protocol.

Definitions and measurements

Diabetes was diagnosed according to the American Diabetes Association (ADA) Standards of Medical Care in Diabetes.[14] Participants with obesity were defined as those with a body mass index (BMI) of ≥ 30 kg/m^2, and those without obesity were defined as those with a BMI under 30 kg/m^2. MAFLD was defined as the presence of risk factors metabolic disorder in patients with hepatic steatosis.[15] In addition, The criteria provided by the Consensus Statement from the International Diabetes Federation (IDF) were used as the diagnostic criteria for MetS in patients with diabetes.[16]

The FIB-4 was calculated as initially proposed using data obtained at baseline. The FIB-4 formula included age, serum aspartate transaminase (AST), alanine transaminase (ALT), and platelets (PLT).[17] This study followed the cutoff value proposed in non-alcoholic fatty liver disease (NAFLD) and the recommendation of the Nonalcoholic Steatohepatitis (NASH) Council.[18, 19] Accordingly, the entire population was divided into two groups: a high FIB-4 index group (FIB-4 $\geq$ 1.3) and a low FIB-4 index group (FIB-4 < 1.3). However, to improve the accuracy of the FIB-4 index in older individuals (over 65 years

old), the cutoff value was adjusted as follows: an FIB-4 index < 2 was considered low, and an FIB-4 index ≥ 2 was considered high.[20] This adjustment ensures that older individuals receive more accurate diagnoses and appropriate treatment. To assess hepatic steatosis, videotapes from the NHANES III ultrasound examinations were digitized using a DVD–VHS videocassette recorder. The degree of hepatic steatosis was graded as normal, mild, moderate, or severe.

Physical and blood measurements, including demographic indicators (age, gender, and ethnicity), anthropometric measurements (BMI, blood pressure, and waist circumference), and serological parameters (ALT, AST, serum C-reactive protein [s-CRP], PLT, total cholesterol [TC], triglycerides [TGs], high-density lipoprotein cholesterol [HDL-C], albumin [ALB], and serum creatinine [SCr]), were extracted and analyzed.

Patients were divided into four groups according to their hepatic steatosis status and FIB-4 as follows: group 1 had an FIB-4 of < 1.3 without moderate-severe steatosis, group 2 had an FIB-4 of < 1.3 with moderate-severe steatosis, group 3 had an FIB-4 of ≥ 1.3 without moderate-severe steatosis, and group 4 had an FIB-4 of ≥ 1.3 with moderate-severe steatosis.

Quantification and statistical analysis

Statistical analyses were performed with SPSS Statistics version 22.0 (SPSS Inc., Chicago, IL, USA) and R 4.1.2 (http://www.R- project.org). Continuous data were presented as medians and interquartile ranges (IQR), and categorical data were presented as frequencies and percentages. Baseline characteristics were compared with the Mann-Whitney U test or Kruskal-Wallis H test (for continuous variables) and the Chi-square test or Fisher exact test (for categorical variables).

First, the all-cause mortality rate of patients was estimated with the Kaplan-Meier method. The cumulative survival by months of follow-up in each subgroup was plotted and compared with a log-rank test. Next, the all-cause mortality rates in each of the four groups were estimated and further stratified by abdominal obesity, MetS or MAFLD. Finally, univariable and multivariable Cox proportional hazards regressions were performed to estimate the HR and 95% CI for all-cause mortality. Moreover, we performed a sensitivity analysis by excluding participants younger than 35 years of age.

We employed a stepwise regression procedure to identify variables for inclusion in a parsimonious and clinically relevant prognostic model, while also evaluating the assumptions of the model by Schoenfeld residuals. To address the violation of the proportional hazards assumption, we have employed stratification by specifically stratifying the ALB variable, resulting in a more robust analytical framework (supplemental methods). The final models were adjusted for sex; age; ethnicity; hypertension; elevated TGs; s-CRP, or SCr; and diagnostic groups. A two-tailed *p*-value of < 0.05 was considered significant.

RESULTS

Study population and baseline characteristics

The flow chart of the inclusion of study participants is shown in Fig. 1. 1,903 patients with diabetes with graded ultrasound video images were included in the primary analyses. A total of 20.9% (398/1,903) of patients had an FIB-4 of ≥ 1.3 (or ≥ 2.0 if aged ≥ 65 years), of whom 43.7% (174/398) had moderate-severe steatosis. A total of 79.1% (1,505/1,903) of patients had an FIB-4 of < 1.3 (or < 2.0 if aged over 65 years), and 44.7% (672/1,505) of these patients had moderate-severe steatosis.

As demonstrated in Table 1, patients with diabetes had a high prevalence of MAFLD (59.3%), MetS (66.0%), and abdominal obesity (70.2%). The all-cause mortality rate was 69.6% (1,325/1,903), and the median follow-up time was 19.4 years. The differences in baseline characteristics between the surviving and nonsurviving populations are shown in Table 1. Among the nonsurviving population, 25.2% had an FIB-4 of ≥ 1.3, and 43.2% had moderate-severe steatosis. Compared with those who survived, the nonsurvivng population was older; mainly male; nonobese; and more likely to have hypertension, lower ALB levels, higher SCr levels, an FIB-4 of ≥ 1.3, elevated TGs levels, and Elevated TC. We did not observe a significant difference in the proportion of moderate-severe steatosis between the two groups (p = 0.108). **Supplementary Table 1** summarizes the baseline characteristics of participants in the four groups.

Table 1
Baseline characteristics of participants with diabetes.

	Overall (n = 1,903)	Non-survival group (n = 1,325)	Survival group (n = 578)	p value
Age at enrollment (yr), medians (IQR)	60 (48, 67)	64 (57, 69)	47 (38, 55)	< 0.001
< 65, n (%)	1226 (64.4)	690 (52.1)	536 (92.7)	< 0.001
≥ 65, n (%)	677 (35.6)	635 (47.9)	42 (7.3)	
Male,sex, n (%)	814 (42.8)	619 (46.7)	19 (33.7)	< 0.001
Ethnicity, n (%)				< 0.001
Non-Hispanic whites	632 (33.2)	498 (37.6)	134 (23.2)	
Non-Hispanic blacks	531 (27.9)	378 (28.5)	153 (26.5)	
Mexican Americans	660 (34.7)	413 (31.2)	247 (42.7)	
Others	80 (4.2)	36 (2.7)	44 (7.6)	
Obesity, n (%)	839 (44.1)	555 (41.9)	284 (49.1)	0.004
Abdominal obesity[a], n (%)	1284 (70.2)	884 (69.9)	400 (70.9)	0.653
Hypertension, n (%)	1314 (69.0)	1027 (77.5)	287 (49.7)	< 0.001
ALT (U/L), medians (IQR)	16 (11, 22)	15 (11, 21)	18 (13, 28)	< 0.001

Data are presented as the medians and IQR, or n and percentage.

p values: n (%) -chi-squared test or Fisher's exact test, median (IQR)-Mann-Whitney test.

Abdominal obesity, waist circumference > 102 cm for male and > 88 cm for female.

*FIB-4 < 2 for age≥65 years, FIB-4≥2 for age≥65 years.

[a]Data on waist circumference were available for 1,829 participants. [b]Data on HDL level were available for 1,871 participants.[c]Data on TGs level were available for 1,769 participants. [d]The presence or absence of MetS could be determined in 1,829 participants.

ALT, alanine transaminase; AST, aspartate transaminase; PLT, platelet; s-CRP, serum C-reactive protein; ALB, albumin; SCr, serum creatinine; HDL-C, high-density lipoprotein cholesterol; TGs, triglycerides; TC, total cholesterol; MetS, metabolic syndrome; FIB-4, Fibrosis-4; MAFLD, metabolic dysfunction-associated fatty liver disease.

	Overall (n = 1,903)	Non-survival group (n = 1,325)	Survival group (n = 578)	p value
AST (U/L),medians (IQR)	19 (16, 25)	19 (16, 25)	20 (16, 26)	0.116
PLT ($\times 10^9$/L), medians (IQR)	265.5 (223.0, 317.0)	260.5 (218.0, 313.0)	274.8 (231.8, 330.1)	< 0.001
s-CRP (mg/dL), medians (IQR)	0.33 (0.21, 0.80)	0.33 (0.21, 0.88)	0.30 (0.21, 0.70)	0.063
ALB (g/L), medians (IQR)	40 (38, 43)	40 (38, 43)	41 (39, 43)	0.004
SCr (mg/dL), medians (IQR)	1.0 (0.9, 1.2)	1.1 (0.9, 1.2)	1.0 (0.9, 1.1)	< 0.01
HDL-C[b]<40mg/dL (male), <50 mg/dL (female), n (%)	1006 (53.8)	686 (52.6)	320 (56.3)	0.141
TGs[c] ≥150 mg/dL, n (%)	1015 (57.4)	737 (55.7)	278 (48.2)	0.002
TC ≥ 5.2 mmol/L, n (%)	1250 (65.7)	898 (67.8)	352 (60.9)	0.004
MetS[d], n (%)	1207 (66.0)	845 (66.8)	362 (64.2)	0.276
Follow-up months, medians (IQR)	233 (129, 319)	170 (92, 243)	330 (318, 350)	< 0.001
FIB-4	1.09 (0.76)	1.23 (0.77)	0.80 (0.54)	< 0.001
FIB-4 Category, n (%)	1505 (79.1)	991 (74.8)	514 (88.9)	< 0.001
FIB-4 < 1.3*	1505 (79.1)	991 (74.8)	514 (88.9)	
FIB-4 ≥ 1.3*	398 (20.9)	334 (25.2)	64 (11.1)	

Data are presented as the medians and IQR, or n and percentage.

p values: n (%) -chi-squared test or Fisher's exact test, median (IQR)-Mann-Whitney test.

Abdominal obesity, waist circumference > 102 cm for male and > 88 cm for female.

*FIB-4 < 2 for age≥65 years, FIB-4≥2 for age≥65 years.

[a]Data on waist circumference were available for 1,829 participants. [b]Data on HDL level were available for 1,871 participants.[c]Data on TGs level were available for 1,769 participants. [d]The presence or absence of MetS could be determined in 1,829 participants.

ALT, alanine transaminase; AST, aspartate transaminase; PLT, platelet; s-CRP, serum C-reactive protein; ALB, albumin; SCr, serum creatinine; HDL-C, high-density lipoprotein cholesterol; TGs, triglycerides; TC, total cholesterol; MetS, metabolic syndrome; FIB-4, Fibrosis-4; MAFLD, metabolic dysfunction-associated fatty liver disease.

	Overall (n = 1,903)	Non-survival group (n = 1,325)	Survival group (n = 578)	p value
MAFLD, n (%)	1129 (59.3)	777 (58.6)	353 (61.1)	0.321
Steatosis Category, n (%)				0.108
No moderate- severe steatosis	1057 (55.5)	752 (56.8)	305 (52.8)	
Moderate- severe steatosis	846 (44.5)	573 (43.2)	273 (47.2)	
Groups according to hepatic steatosis status and FIB-4, n (%)				< 0.001
Group1: FIB-4 < 1.3*without moderate-severe steatosis	833 (43.8)	556 (42.0)	277 (47.9)	
Group2: FIB-4 < 1.3* with moderate-severe steatosis	672 (35.3)	435 (32.8)	237 (41.0)	
Group3: FIB-4 ≥ 1.3* without moderate-severe steatosis	224 (11.8)	196 (14.8)	28 (4.8)	
Group4: FIB-4 ≥ 1.3* with moderate-severe steatosis	174 (9.1)	138 (10.4)	36 (6.2)	
Data are presented as the medians and IQR, or n and percentage.				
p values: n (%) -chi-squared test or Fisher's exact test, median (IQR)-Mann-Whitney test.				
Abdominal obesity, waist circumference > 102 cm for male and > 88 cm for female.				
*FIB-4 < 2 for age≥65 years, FIB-4≥2 for age≥65 years.				
[a]Data on waist circumference were available for 1,829 participants. [b]Data on HDL level were available for 1,871 participants.[c]Data on TGs level were available for 1,769 participants. [d]The presence or absence of MetS could be determined in 1,829 participants.				
ALT, alanine transaminase; AST, aspartate transaminase; PLT, platelet; s-CRP, serum C-reactive protein; ALB, albumin; SCr, serum creatinine; HDL-C, high-density lipoprotein cholesterol; TGs, triglycerides; TC, total cholesterol; MetS, metabolic syndrome; FIB-4, Fibrosis-4; MAFLD, metabolic dysfunction-associated fatty liver disease.				

An FIB-4 ≥ 1.3 without moderate-severe steatosis was an independent risk factor for all-cause mortality in patients with diabetes

First, we investigated the effects of FIB-4 and the severity of steatosis on all-cause mortality in patients with diabetes. We found a higher cumulative all-cause mortality among participants with an FIB-4 ≥ 1.3 than in those with an FIB-4 < 1.3 group (log-rank $p < 0.001$). Similar trends were observed in patients with and without MAFLD (log-rank $p < 0.001$). Compared with the non-MAFLD subgroup, the MAFLD subgroup did not have an increased risk of all-cause mortality (log-rank $p = 0.29$). However, a significantly higher

cumulative all-cause mortality was observed among participants without moderate-severe steatosis than among those with moderate-severe steatosis (log-rank $p = 0.047$) (Fig. 2).

As shown in **Supplementary Table 2**, the univariate Cox regression analysis showed that an FIB-4 of ≥ 1.3 (vs. an FIB-4 of < 1.3) and the absence of moderate-severe steatosis (vs. the presence of moderate-severe steatosis) were associated with a higher all-cause mortality risk ($p < 0.05$). In the multivariable Cox regression model, an FIB-4 of ≥ 1.3 remained an independent risk factor for all-cause mortality (HR: 1.491, 95% CI 1.313–1.694, $p < 0.001$). However, no significant difference between the risk of all-cause mortality was observed between groups with and without moderate-severe steatosis ($p > 0.05$).

Due to the inconsistent effects of having an FIB-4 of ≥ 1.3 versus having moderate-severe steatosis on diabetes prognosis, we estimated all-cause mortality rates according to the FIB-4 in participants without moderate-severe steatosis and those with moderate-severe steatosis compared with an FIB-4 of < 1.3 without moderate-severe steatosis. Overall, the all-cause mortality risk was highest in group 3 (FIB-4 of ≥ 1.3 without moderate-severe steatosis); in comparison, it was lower in groups 4 (FIB-4 of ≥ 1.3 with moderate-severe steatosis) and 1 (FIB-4 of < 1.3 without moderate-severe steatosis) and lowest in group 2 (FIB-4 of < 1.3 with moderate-severe steatosis) (log-rank $p < 0.001$) (Fig. 3a).

In the populations with and without moderate-severe hepatic steatosis, participants with an FIB-4 ≥ 1.3 exhibited a higher cumulative all-cause mortality than those with an FIB-4 of < 1.3. These results were consistent with those in diabetes patients with MAFLD, MetS or abdominal obesity(Fig. 3). Furthermore, in subgroups with MAFLD, MetS or abdominal obesity, the risk of all-cause mortality was higher in patients with an FIB-4 ≥ 1.3 without moderate-severe steatosis compared with those in the other three groups.

The multivariate Cox regression analysis showed that male gender (HR: 1.352, 95% CI 1.201–1.522, $p < 0.001$), age (45–59 years) (HR: 2.155, 95% CI 1.716–2.707, $p < 0.001$), or age (over 60 years) (HR: 5.692, 95% CI 4.569–7.090, $p < 0.001$), hypertension (HR: 1.284, 95% CI 1.117–1.474, $p < 0.001$), elevated TGs levels (HR: 1.178, 95% CI 1.049–1.324, $p = 0.006$), increased s-CRP levels (HR: 1.060, 95% CI 1.016–1.107, $p = 0.007$), increased SCr levels (HR: 1.132, 95% CI 1.045–1.226, $p = 0.002$), patients of FIB-4 ≥ 1.3 without moderate-severe steatosis (HR: 1.277, 95% CI 1.077–1.513, $p = 0.005$) were independent risk factors for all-cause mortality (Table 2 and Fig. 4).

Table 2
Multivariate Cox regression analyses of risk factors for mortality in patients with diabetes.

	HR (95%CI)	P value
Male	1.352 (1.201–1.522)	< 0.001
age		
20–44 years	Reference	
45–59 years	2.155 (1.716–2.707)	< 0.001
≥ 60 years	5.692 (4.569–7.090)	< 0.001
Ethnicity		
Non-Hispanic whites	Reference	
Non-Hispanic blacks	1.026 (0.887–1.187)	0.729
Mexican Americans	0.784 (0.684–0.899)	0.001
Other	0.506 (0.359–0.713)	< 0.001
Hypertension	1.284 (1.117–1.474)	< 0.001
Elevated TGs	1.178 (1.049–1.324)	0.006
s-CRP	1.060 (1.016–1.107)	0.007
SCr	1.132 (1.045–1.226)	0.002
Groups according to hepatic steatosis status and FIB-4		
Group1: FIB-4 < 1.3* without moderate-severe steatosis	Reference	
Group2: FIB-4 < 1.3* with moderate-severe steatosis	0.954 (0.835–1.089)	0.485
Group3: FIB-4 ≥ 1.3* without moderate-severe steatosis	1.277 (1.077–1.513)	0.005
Group4: FIB-4 ≥ 1.3* with moderate-severe steatosis	1.051 (0.864–1.278)	0.619
*FIB-4 < 2 for age≥65 years, FIB-4 ≥ 2 for age≥65 years.		
The risk factors for all-cause mortality in patients with diabetes were estimated using the Cox test regression.		
HDL-C, high-density lipoprotein cholesterol; TGs, triglycerides; TC, total cholesterol; s-CRP, serum C-reactive protein; ALB, albumin; SCr, serum creatinine; FIB-4, Fibrosis-4 index; HR, hazard ratio; CI, confidence interval.		

Synergistic contribution of FIB-4 ≥ 1.3 without moderate-severe steatosis as a predictor of all-cause mortality in diabetes patients combined with MAFLD, MetS or abdominal obesity

Multivariate analysis revealed a positive association between an FIB-4 of ≥ 1.3 and all-cause mortality in non-MetS subgroup, but not in non-abdominal obesity subgroup (**Supplementary Fig. 1**). However, the association between an FIB-4 of ≥ 1.3 and all-cause mortality differed in subgroups with MAFLD, MetS or abdominal obesity. Notably, patients with an FIB-4 of ≥ 1.3 without moderate-severe steatosis had significantly higher all-cause mortality than patients in other groups (Fig. 4). After adjusting for confounding factors, the patients in the MAFLD subgroup with an FIB-4 ≥ 1.3 without moderate-severe steatosis had a significantly increased all-cause mortality risk (HR: 1.457, 95% CI 1.045–2.032, $p = 0.027$); however, this was not observed in the other three groups ($p > 0.05$). The same trend was observed in participants with abdominal obesity (HR: 1.325, 95% CI 1.048–1.674, $p = 0.019$) and MetS (HR: 1.343, 95% CI 1.051–1.716, $p = 0.019$).

We performed a sensitivity analysis by excluding participants with age < 35years. Kaplan-Meier estimation curves of cumulative all-cause mortality and multivariate Cox regression analysis for the four groups are shown in **Supplementary Fig. 2**. The result remained unaffected. More specifically, an FIB-4 ≥ 1.3 without moderate-severe steatosis had the highest risk of all-cause mortality (HR: 1.263, 95% CI 1.065–1.497, p = 0.007).

DISCUSSION

We conducted a longitudinal, multiethnic, US population-based study with a median follow-up period of 19.4 years and demonstrated that an FIB-4 ≥ 1.3 increased the all-cause mortality risk in patients with diabetes. However, moderate-severe steatosis was not independently associated with higher all-cause mortality in diabetes patients. Notably, the most significant predictor of all-cause mortality in all participants or in those with MAFLD, MetS or abdominal obesity was an FIB-4 ≥ 1.3 without moderate-severe steatosis.

Although FIB-4 is often used to screen the degree of fibrosis in patients with liver disease, FIB-4 can also be considered a moderate-to-high risk factor for liver fibrosis or cirrhosis in patients with diabetes.[21] The proportion of patients with an FIB-4 ≥ 1.3 in the current study was 20.9%, which was consistent with previous studies.[6, 10] In the current study, an FIB-4 ≥ 1.3 had prognostic value for patients with diabetes. This might be due to the reason that an FIB-4 ≥ 1.3 was associated with an increased risk of fibrosis in diabetes patients; and the risk of mortality and liver-related morbidity increased with advanced stages of liver fibrosis.[13] Therefore, it is crucial to evaluate the fibrotic burden and the effect of fibrosis on mortality in patients with diabetes. The FIB-4 could be used to screen patients at high risk for diabetes to significantly facilitate patient's risk stratification.

Interestingly, we found that compared with patients without MAFLD, those with MAFLD did not have an increased risk of mortality in diabetes patients. Research by Kim D et al. demonstrated that NAFLD alone was not associated with all-cause mortality in US adults after adjusting for metabolic risk factors.[22] In the current study, patients with diabetes who had an FIB-4 ≥ 1.3 but without moderate-severe steatosis had the highest all-cause mortality risk than any other groups in overall diabetes patients, as well as

diabetes patients with MAFLD, MetS or abdominal obesity. An FIB-4 ≥ 1.3 increased the all-cause mortality risk in patients without moderate-severe steatosis, suggesting that liver fibrosis may be a more significant risk factor for all-cause mortality than steatosis; furthermore, this finding indicated a complex interaction between hepatic steatosis and liver fibrosis in patients with diabetes. On the one hand, diabetes is a risk factor for significant or advanced liver fibrosis regardless of the presence of liver disease. [23–25] In turn, this also explains why liver fibrosis but not the severity of hepatic steatosis could be used to predict mortality risk in diabetes patients. On the other hand, previous studies have reported a high incidence of NASH in patients with DM2, and patients with burnt-out NASH no longer have excess hepatic fat.[26, 27] The theory of burnt-out NASH may help explain these results. From this perspective, the absence of moderate-severe steatosis may signify liver fibrosis. In fact, patients were more likely to have an FIB-4 < 1.3 than an FIB-4 ≥ 1.3 in the group without moderate-severe steatosis (78.8% vs. 21.2%) in our study. However, patients with an FIB-4 ≥ 1.3 did not have a lower prevalence of without moderate-severe steatosis (56.3%), indicating that liver fibrosis and steatosis in patients with diabetes are not parallel.

The Global NASH Council recommends that primary care physicians and diabetologists use clinical risk stratification to identify patients who are at risk for liver fibrosis. This involves screening patients with DM2 using the FIB-4 index and referring them to a specialist if their FIB-4 is ≥ 1.3 (or ≥ 2.0 in those 65 years and older), particularly if they have one additional component of MetS.[19] Our results further support this recommendation that more attention should be given to these patients; specifically those without moderate-severe steatosis, they should be referred to a specialist for further evaluation.

The association between an FIB-4 of ≥ 1.3 without moderate-severe steatosis and all-cause mortality differed in non-MetS subgroup and non-abdominal obesity subgroup. Evidence suggests that MetS or abdominal obesity is associated with liver fibrosis even exacerbate liver fibrosis in chronic hepatitis B (CHB) patients.[28–30] As such, we speculate that MetS or abdominal obesity played a similar role in diabetic patients. The coexistence of diabetes with MetS or abdominal obesity leads to severe fibrosis. This can be partially explain our results. But this hypothesis would need further mechanistic studies to explore.

The study has several limitations. First, NHANES III was carried out in the USA and omitted certain populations, including an oversampling of Asian Americans and participants with races and ethnicities other than white, black, and Hispanic. Therefore, these findings might not be generalizable to Asian Americans and different racial and ethnic groups. Second, covariates were only assessed at baseline, and these covariates could have changed over time. Third, we could not estimate the sensitivity of the FIB-4 for evaluating liver fibrosis in patients with diabetes. Instead, patients with an FIB-4 ≥ 1.3 were considered to have a moderate to high risk of liver fibrosis. Fourth, therapy for diabetes was not considered since evidence suggested that the diabetes treatment affected FIB-4. However, these above two limitations did unaffect the necessity and significance of applying FIB-4 for further evaluation and screening for these patients.

In conclusion, our research indicated that the FIB-4 was a reliable tool for assessing mortality risk in patients with diabetes. Additionally, our findings underscored that screening for the stage of liver fibrosis, rather than steatosis, was crucial in patients with diabetes; this is because an FIB-4 ≥ 1.3, but not the severity of steatosis, was identified as an independent risk factor for all-cause mortality in diabetes patients. An FIB-4 ≥ 1.3 without moderate-severe steatosis significantly increased all-cause mortality risk, especially in participants with MAFLD, MetS, abdominal obesity. In summary, our results may help identify high-risk patients with diabetes who need more specialized care.

Abbreviations

HR, hazard ratio; CI, confidence interval; CLD, chronic liver disease; DM2, type 2 diabetes mellitus; NAFLD, nonalcoholic fatty liver disease; MAFLD, metabolic dysfunction- associated fatty liver disease; NHANES III, Third National Health and Nutrition Examination Survey; NCHS, National Center for Health Statistics; CDC, Centers for Disease Control and Prevention; ADA, American Diabetes Association; BMI, body mass index; MS, metabolic syndrome; IDF, International Diabetes Federation; FIB-4, Fibrosis-4 index; AST, aspartate transaminase; ALT, alanine transaminase; PLT, platelet; NASH, nonalcoholic steatohepatitis; CRP, serum C-reactive protein; TC, total cholesterol; TGs, triglycerides; HDL, high- density lipoprotein cholesterol; ALB, albumin; SCr, serum creatinine; CHB, chronic hepatitis B; PCPs, primary care practitioners; TE, transient elastography.

Declarations

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Competing Interests

The authors declare that they have no conflict of interest.

Author Contributions

All authors contributed to the study conception and design. Study concept and design (Jie Li, Xiaoyan Ma); acquisition of data (Xiaoyan Ma,Yixuan Zhu, Yee Hui Yeo, Xiaoming Xu); statistical analysis and interpretation of data (Xiaoyan Ma, Yixuan Zhu, Yee Hui Yeo, Xiaoming Xu, Fajuan Rui, Wenjing Ni, Qi Gu, Xin Tong, Shengxia Yin, Xiaolong Qi); drafting of the manuscript (Xiaoyan Ma, Yixuan Zhu, Yee Hui Yeo,

Zhiwen Fan); study supervision(Xin Tong, Shengxia Yin, Xiaolong Qi, Jie Li, Yee Hui Yeo, Zhiwen Fan,Chao Wu); critical revision of the manuscript for important intellectual content (Jie Li, Chao Wu, Junping Shi). All authors were responsible for the interpretation of data, the drafting, critical revision of the manuscript for important intellectual content, and the final approval of the version to be submitted.

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Figures

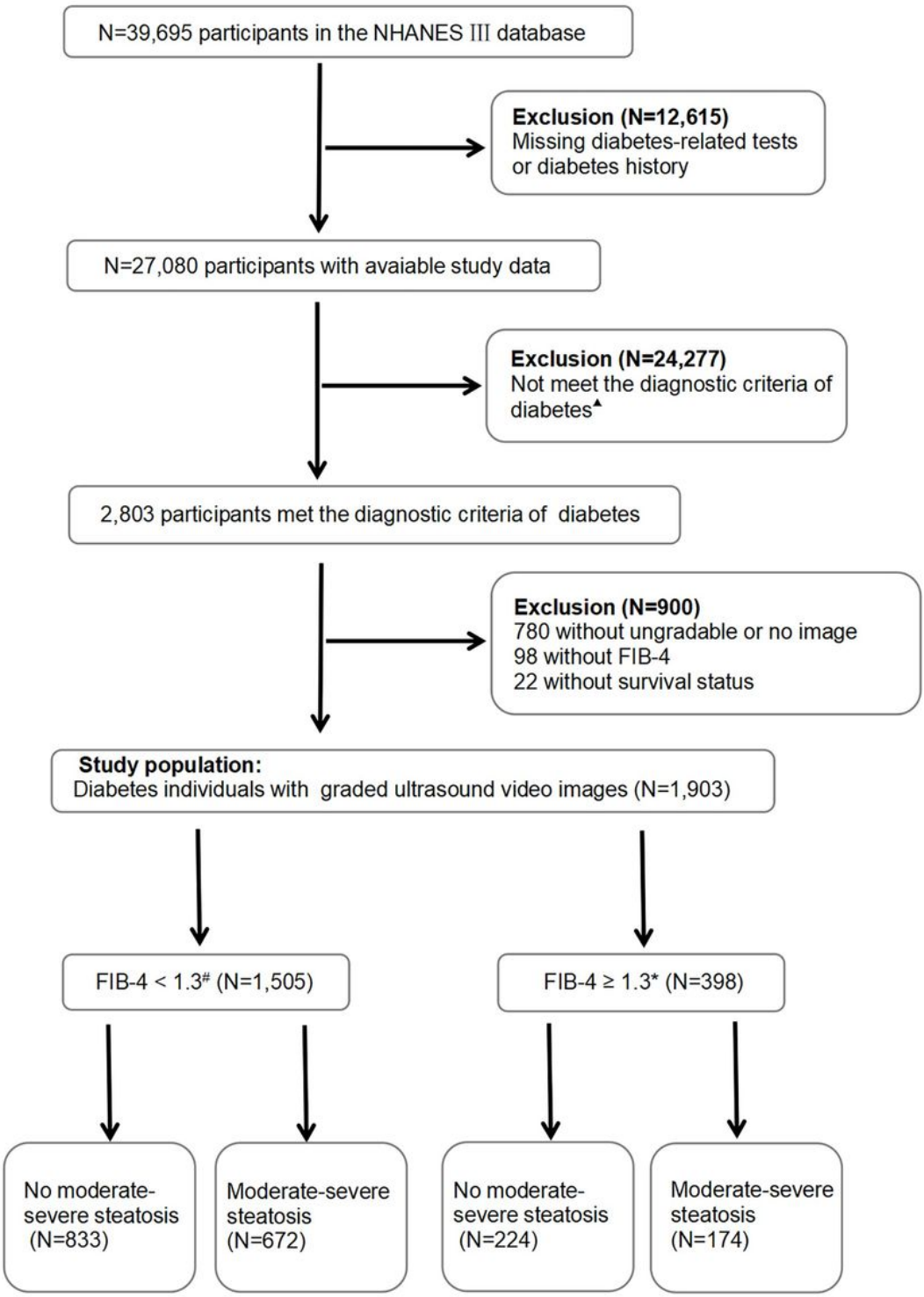

Figure 1

Flow chart of the study participants ▲Diabetes was defined according to the American Diabetes Association (ADA) "Standards of Medical Care in Diabetes". *FIB-4 < 2 for age ≥65 years, FIB-4 ≥2 for age ≥65 years. FIB-4, Fibrosis-4 index.

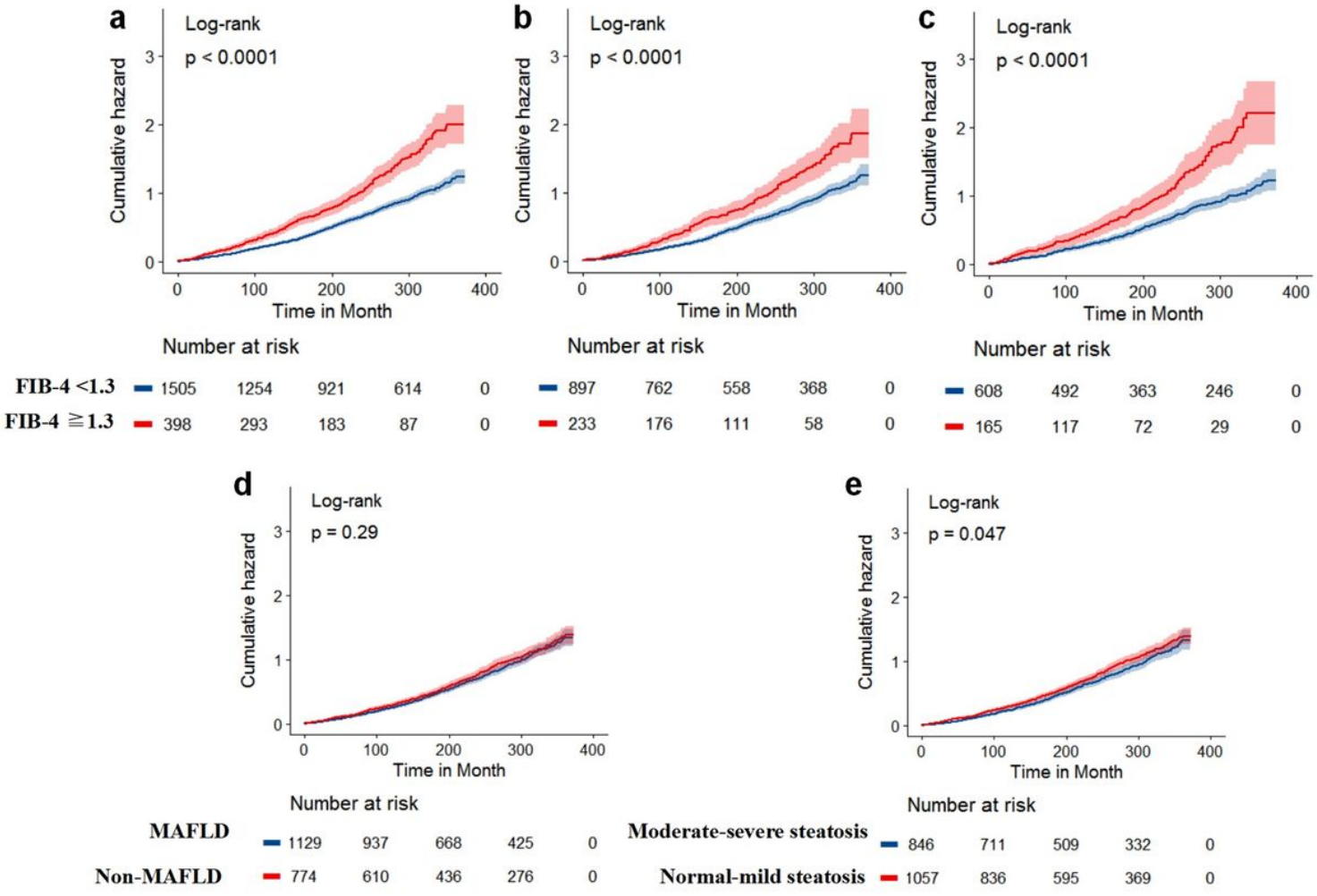

Figure 2

Kaplan-Meier estimation curves of cumulative all-cause mortality in patients with diabetes (a) Cumulative mortality by FIB-4 in overall subjects. (b)Cumulative mortality by FIB-4 in MAFLD subgroup. (c)Cumulative mortality by FIB-4 in Non-MAFLD subgroup. (d)Cumulative mortality by MAFLD in overall subjects. (e)Cumulative mortality by the severity of hepatic steatosis in overall subjects. FIB-4, Fibrosis-4 index; MAFLD, metabolic dysfunction-associated fatty liver disease.

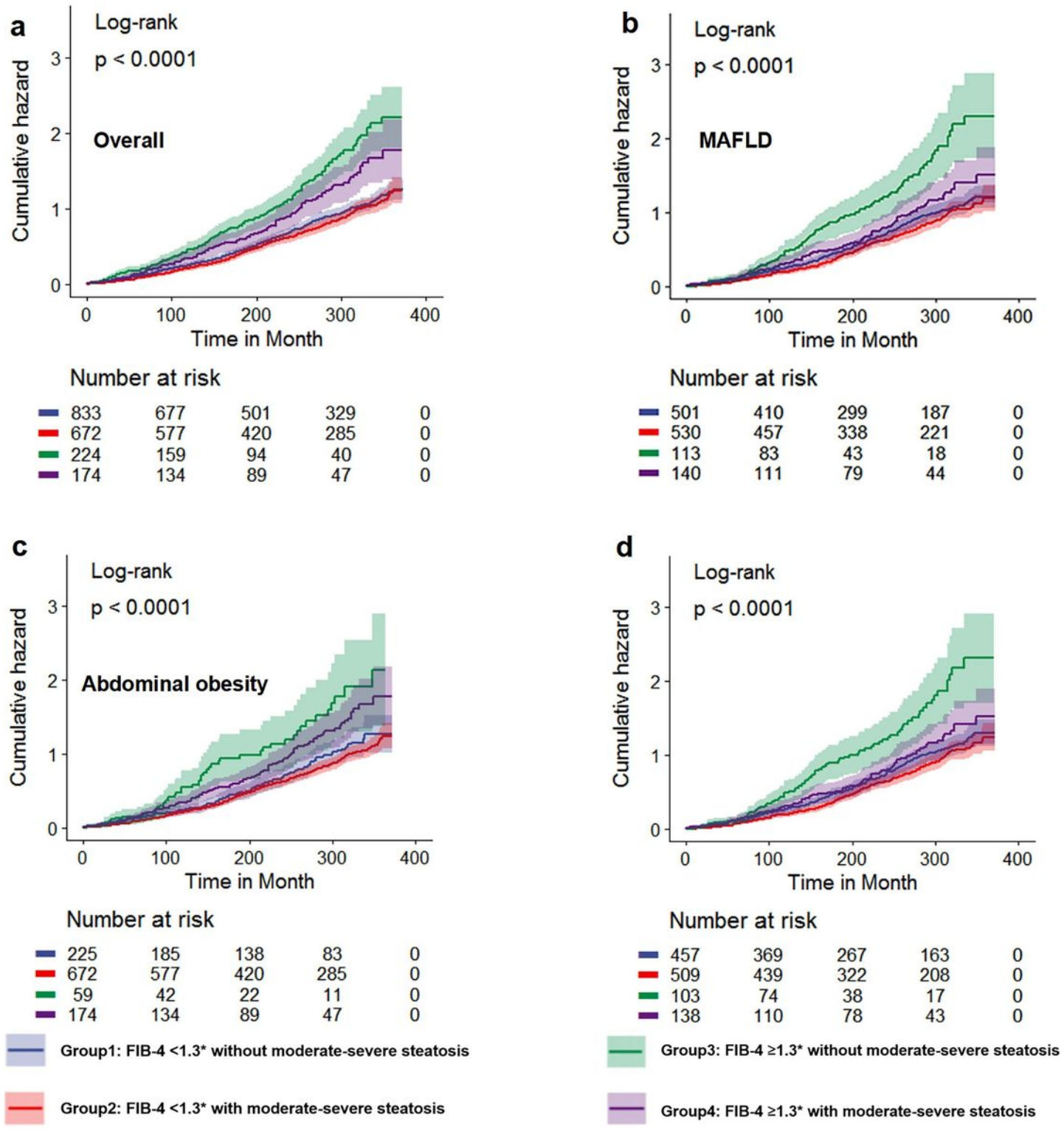

Figure 3

Kaplan-Meier estimation curves of cumulative all-cause mortality overall and in subgroups based on the four diagnostic groups in patients with diabetes (a) Overall. (b) MAFLD. (c) Abdominal obesity. (d) MetS. *FIB-4 < 2 for age ≥65 years, FIB-4 ≥ 2 for age ≥ 65 years. MAFLD, metabolic dysfunction-associated fatty liver disease; MetS, metabolic syndrome. FIB-4, Fibrosis-4 index.

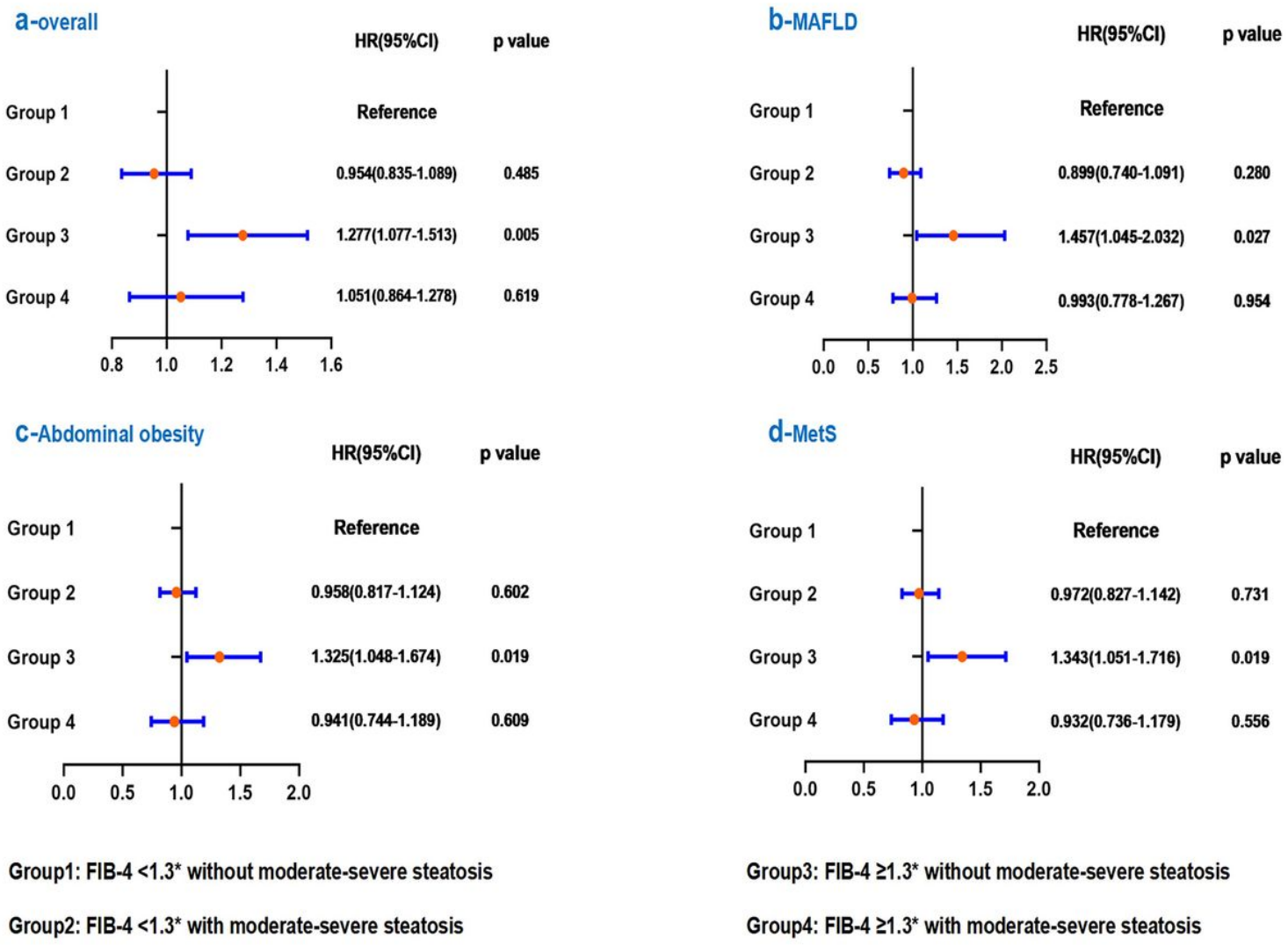

Figure 4

Multivariable analysis of mortality risk in the four groups of patients in the diabetes subgroup (a) Overall; (b) MAFLD; (c) Abdominal obesity; (d) MetS. MAFLD, metabolic dysfunction-associated fatty liver disease; MetS, metabolic syndrome. *FIB-4 < 2 for age ≥65 years, FIB-4 ≥ 2 for age ≥ 65 years. MAFLD, metabolic dysfunction-associated fatty liver disease; MetS, metabolic syndrome.

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