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ORIGINAL ARTICLE

Liver fibrosis markers and all cause mortality in people with type 2 diabetes: A population based study (The Ayrshire Diabetes Outcomes Cohort (ADOC) Study)

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Abstract

Aims: To describe the distribution of the biomarker scores Fibrosis-4 (FIB4), nonalcoholic fatty liver disease (NAFLD) fibrosis score (NFS), and aspartate aminotransferase to platelet ratio index (APRI), and the associations between risk categories and all-cause mortality.

Materials and Methods: This was a retrospective cohort study of 12 589 patients, with follow-up from January 2012 until November 2021. The cut-off points used to identify low risk were: FIB4 <1.3 if aged <65 years or <2.0 if aged ≥65 years; NFS < −1.455 if aged <65 years or <0.12 if aged ≥ 65 years; APRI <1 (independent of age). High-risk cut-off points were FIB4 >2.67, NFS >0.676 and APRI ≥1 (all independent of age). Multivariable Cox regression analysis was performed to assess the association between liver fibrosis scores and all-cause mortality.

Results: The mean ± standard deviation age was 65.2 ± 12.1 years, 54.5% were men and the median (interquartile range) diabetes duration was 5.8 (2.8–9.3) years. The prevalence of high-risk categories was 6.1% for FIB4, 23.5% for NFS and 1.6% for APRI. During a median follow-up of 9.8 years, 3925 patients (31.1%) died, resulting in a crude mortality rate of 40.4 per 1000 person-years. The overall adjusted all-cause mortality hazard ratios (95% confidence intervals [CIs]) in the high- compared with low-fibrosis-risk groups were 3.69 (1.95–2.75) for FIB4, 2.32 (2.88–4.70) for NFS, and 3.92 (2.88–5.34) for APRI. Stratified adjusted all-cause mortality hazard ratios for individuals under 65 years and people over 65 years of age at cohort entry were 3.89 (95% CI 2.99–5.05) and 1.44 (95% CI 1.28–1.61) for FIB4, 2.50 (95% CI 1.89–3.18) and 1.35 (95% CI 1.24–1.48) for NFS and 3.74 (95% CI 2.73–5.14) and 1.64 (95% CI 1.24–2.17) for APRI.

Conclusions: All three fibrosis risk scores were positively associated with all-cause mortality in people with type 2 diabetes, with higher relative risks in younger than older people. Effective interventions are required to minimize excess mortality in people at high risk of liver fibrosis.

KEYWORDS
cohort study, fatty liver disease, liver, observational study, real-world evidence, type 2 diabetes

1 | INTRODUCTION

Nonalcoholic fatty liver disease (NAFLD) is characterized by fat deposition in the liver in the absence of excessive alcohol consumption or other causes of liver disease[1] and is considered the hepatic manifestation of metabolic syndrome.[2] In developed countries, NAFLD is now the most common aetiology of chronic liver disease, affecting an estimated one-third of all adults and up to 70% of those with type 2 diabetes.[2,3] People with type 2 diabetes have a higher prevalence of advanced fibrosis and subsequent liver-related complications of NAFLD than people without diabetes.[3–5] Additionally, and importantly, people with type 2 diabetes and NAFLD also have an increased risk of cardiovascular morbidity and mortality that is independent of conventional cardiovascular risk factors, compared to people with type 2 diabetes who do not have NAFLD.[6–8]

The assessment of hepatic fibrosis stage is the cornerstone of current diagnostic and prognostic assessment of NAFLD, given its position as the strongest predictor for long-term liver outcomes.[9,10] Whilst liver biopsy remains the "gold standard" method for staging the degree of fibrosis, it is limited by cost, sampling variability, and risk of complications. Consequently, liver biopsy is not feasible in a condition with such a high prevalence in the population.[11] Several noninvasive risk scores have been developed to calculate the likelihood of liver fibrosis,[12] and these are recommended by international guidelines to screen for severe NAFLD in patients with type 2 diabetes.[13] Additionally, it is likely that the use of noninvasive liver fibrosis score thresholds in primary care, to identify patients who are eligible for vibration-controlled transient elastography of the liver, is likely to grow in the near future.[14,15] Of the available liver fibrosis biomarker scores, the Fibrosis-4 (FIB4) score[16] is readily available and recommended as the first-line screening tool. However, there are several other similar simple scores, such as the Enhanced Liver Fibrosis Score (ELF™, which is not commonly used despite National Institute for Health and Care Excellence [NICE] guidelines in the United Kingdom recommending its use),[17] the NAFLD fibrosis score (NFS), and the aspartate aminotransferase (AST) to platelet ratio index (APRI).[18–20]

In addition to their use in risk stratification for fibrosis, the biomarker scores are also positively associated with likelihood of progression to cirrhosis and end-stage liver disease, although their ability to predict overall mortality is less clear, particularly in patients with type 2 diabetes.[4,21–23] In this study, in addition to describing the distribution of FIB4, NFS and APRI (ELF™ scores were not available) in a cohort of individuals with type 2 diabetes, we sought to describe the association between risk score categories and all-cause mortality and to compare the strength of the associations between the different scores and all-cause mortality.

2 | MATERIALS AND METHODS

We performed a retrospective cohort study in patients identified from electronic primary care records for adults aged 18 years or older in 45 (out of 53) general practices in the Scottish region of Ayrshire and Arran (covering approximately 81% of a population of approximately 370 000). Data were extracted for all 13 561 patients with type 2 diabetes, defined using read codes,[24] who were registered with a participating practice on January 1, 2012. A total of 214 people with a diagnosis of alcoholic liver disease or viral hepatitis at baseline or during follow-up were excluded (Figure 1).

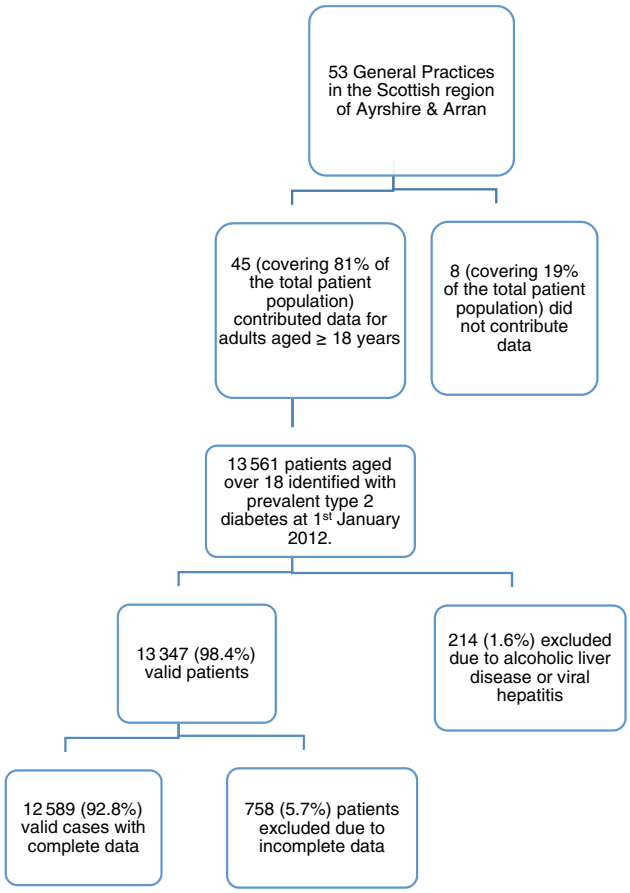

FIGURE 1 Flow diagram describing cohort selection

Data were available on age, sex, date of diabetes diagnosis, smoking status and presence of comorbidities (defined using read codes for mental illness, stroke/transient ischaemic attack [TIA], peripheral vascular disease, percutaneous coronary intervention [PCI]/coronary artery bypass graft, retinopathy, liver and colon cancer), factors in the FIB4, NFS and APRI fibrosis scores (body mass index [BMI], AST, alanine aminotransferase [ALT], platelet count and albumin levels), glycated haemoglobin (HbA1c), estimated glomerular filtration rate (eGFR), lipid levels and prescribing of statins and drugs used in diabetes. Abnormal eGFR was defined as <60 mL/min/1.73m^2. eGFR was treated as a categorical variable because a numerical value for eGFR is only provided by the laboratory if it is under 60 mL/min/1.73m^2, otherwise it is reported as ≥ 60 mL/min/1.73m^2. Measurements closest to cohort entry date were used. In sensitivity analysis a limit of measurements within 1 year of baseline was used.

Follow-up was measured in days from cohort entry on January 1, 2012 to the earliest of date of death, emigration or November 1, 2021.

The project was registered with the Clinical Governance Department, NHS Ayrshire and Arran, and Caldicott Guardian approval was obtained from each general practice. As all data were anonymized, individual patient consent was not required.

2.1 | Missing data

A total of 758 patients (5.7%) were excluded due to incomplete data. There were statistically significant differences between people with incomplete and complete data for only five baseline characteristics: duration of diabetes (median 6.7 vs. 5.8 years); albumin level (mean 4.3 vs. 4.2 g/L); prevalence of stroke/TIA (14.1% vs. 9.7%); retinopathy (38.8% vs. 44.5%); and abnormal eGFR (30.5% vs. 40.8%). There was no significant difference in prevalence of diabetes mellitus between practices that did and did not provide data (4.6% vs. 4.8%; $\chi^2 = 3.38$, $P = 0.07$).

2.2 | Liver fibrosis score calculations

The FIB4, NFS and APRI scores were calculated[16,18,19] using data measured as close to cohort entry as possible. The three scores were categorized into low, intermediate and high groups at recommended cut-off values[25,26]; the cut-off points indicating low probability of advanced liver fibrosis were: FIB4 <1.3 if aged < 65 years or <2.0 if aged ≥65 years; NFS < −1.455 if aged <65 years or <0.12 if aged ≥65 years; APRI <1 (independent of age). The upper cut-off points (indicating high probability of advanced liver fibrosis) were all independent of age: FIB4 > 2.67; NFS >0.676; APRI ≥1.

2.3 | Statistical analysis

Continuous data were described as means (standard deviation [SD]) or as medians (interquartile range [IQR]). Baseline characteristics of participants with low, intermediate and high FIB4 and NFS scores were compared by analysis of variance or Kruskal-Wallis tests (with post hoc Bonferroni correction for multiple comparisons) and by t-test or Mann-Whitney test for the two APRI categories. Categorical characteristics were compared across fibrosis risk categories by chi-squared test (or Fisher's exact test when appropriate), again with post-hoc Bonferroni correction.

Kaplan-Meier curves and log-rank tests were used to compare the cumulative hazard of crude all-cause mortality during follow-up between individuals with low, intermediate and high FIB4 scores/NFSs and between low and high APRI scores.

Multivariable Cox regression analysis was performed to assess the association of liver fibrosis scores with all-cause mortality after adjusting for confounding variables. All analyses were adjusted for the following covariates: age; sex; diabetes duration; smoking status; presence of comorbidities at baseline; eGFR; HbA1c level; cholesterol level; and prescription of statins and glucose-lowering drugs. AST and ALT levels were included as covariates in a sensitivity analysis. Age, diabetes duration, cholesterol levels and HbA1c levels were treated as continuous variables, with the others treated as categorical variables. Both analyses with continuous standardized scores (estimated for increments in 1-standard deviation [SD] of each fibrosis score) and with categorical scores (with the low-risk group as the reference category) were undertaken. These results are presented as hazard ratios for Cox regression models with their respective 95% confidence intervals (CIs). The proportional hazards assumption was checked using log minus log cumulative survival plots, which demonstrated that the assumption was not violated.

Potential interactions were tested between each liver fibrosis score and age (<65 vs. ≥65 years), sex, diabetes duration (<10 vs. ≥10 years), presence of comorbidities at baseline, and glycaemic control (HbA1c <58.5 mmol/mol [<7.5%] vs. ≥58.5 mmol/mol [≥7.5%]) and all-cause mortality.[27]

Interaction terms were added for the above variables to the Cox regression on the entire dataset. The statistically significant interactions between fibrosis risk category and age stratified at 65 years were retained in the model and additional stratified Cox regression models were run for each age stratum. In all analyses a two-tailed probability value <0.05 was considered statistically significant.

The relative ability of the different biomarker scores to discriminate between survival and mortality was assessed using the area under receiver-operating characteristic curves (AUROCs).

Analyses were performed using SPSS version 17.0 (SPSS Inc., Chicago, Illinois).

3 | RESULTS

3.1 | Liver fibrosis scores and baseline characteristics

A total of 12 589 people with complete data were included in the analysis. Table 1 gives the baseline characteristics of the cohort stratified by FIB4, NFS and APRI categories. The median (IQR) time from measurement of each of the liver fibrosis biomarkers to cohort entry was 8 (3–20) months for FIB4, 10 (5–22) for NFS and 7 (3–18) for APRI. The median (IQR) values of FIB4, NFS and APRI were 1.219 (0.883 to 1.690), −0.207 (−1.043 to 0.618) and 0.215 (0.158 to 0.303), respectively. Prevalence of high-risk categories was 6.1% for FIB4, 23.5% for NFS and 1.6% for APRI. Figure 2 demonstrates the overlap of the various categories as a Venn diagram. Of the 2964 cases in the NFS high category, 2266 (76%) were not in the high category for either FIB4 or APRI. Of the 762 cases in the FIB4 high category 694 (91%) were also in the NFS high category. Of the 196 cases in the APRI high category 141 (72%) were in both the FIB4 and NFS high categories.

3.2 | Mortality during follow-up

During a median follow-up of 9.8 years (total 97 055 person-years), 3925 patients (31.1% of the cohort) died and crude mortality was 40.4 per

TABLE 1 Baseline characteristics of study population stratified by Fibrosis-4 score, nonalcoholic fatty liver disease fibrosis score and aspartate aminotransferase to platelet ratio index categories

	Total (n = 12 589)	Low FIB4 (n = 9706)	Intermediate FIB4 (n = 2121)	High FIB4 (n = 762)	Low NFS (n = 4800)	Intermediate NFS (n = 4825)	High NFS (n = 2964)	Low APRI (n = 12 393)	High APRI (n = 196)
Age, years	65.2 (12.1)	64.2 (12.1)	66.8 (11.1)**	73.1 (10.2)**	66.0 (12.0)	60.4 (11.0)**	71.6 (10.4)**	65.2 (12.1)	62.7 (11.3)*
Sex: male, %	54.5	52.4	60.7††	63.0††	48.8	59.7††	55.1††	54.2	70.0**
Smoking status: current/ex, %	56.4	56.7	55.4	55.8	58.4	55.6‡	54.5†	56.5	54.1
Diabetes duration, years	5.8 (2.8–9.3)	5.9 (2.8–9.3)	5.4 (2.6–9.0)*	6.6 (3.4–9.7)*	5.9 (2.8–9.2)	5.2 (2.5–8.8)**	6.7 (3.3–10.1)**	5.9 (2.8–9.3)	5.4 (2.6–8.3)
BMI, kg/m^2	31.5 (6.8)	31.7 (6.8)	31.5 (6.8)	29.8 (6.1)**	28.7 (4.9)	32.5 (6.1)**	34.5 (8.4)**	31.5 (6.8)	31.3 (6.6)
Albumin, g/dL	4.3 (0.3)	4.3 (0.3)	4.3 (0.3)	4.2 (0.4)**	4.4 (0.3)	4.4 (0.3)	4.1 (0.4)**	4.3 (0.3)	4.1 (0.5)**
Platelet count, $\times 10^9$	249 (76)	269 (70)	194 (41)**	144 (53)**	303 (75)	235 (48)**	187 (51)**	251 (74)	129 (77)**
AST, U/L	20 (17–26)	19 (16–23)	25 (20–34)**	31 (23–50)**	19 (16–24)	21 (17–27)**	21 (17–28)**	20 (15–30)	70 (48–108)**
ALT, U/L	21 (15–30)	20 (15–29)	23 (15–36)**	23 (15–39)**	21 (15–29)	23 (17–34)**	18 (13–26)**	21 (15–30)	56 (35–93)**
Comorbidities, %									
Mental illness	10.4	9.8	11.6‡	14.8††	9.5	9.0	14.0††	10.4	11.2
Stroke/TIA	9.7	9.4	9.9	13.3†	10.1	7.6††	12.7††	9.7	13.3
PVD	5.3	5.3	5.2	5.5	5.5	4.1†	6.9†	5.3	2.0‡
PCI/CABG	6.8	6.3	8.8††	8.0	5.8	6.4	9.2††	6.9	4.6
Retinopathy	44.5	44.8	44.3	44.5	44.3	43.7	46.2	44.5	44.9
Liver cancer	0.06	0.01	0.05	0.7††	0.04	0.02	0.13	0.04	1.0†
Colon cancer	1.2	1.1	1.1	2.1	1.1	1.0	1.5	1.2	1.5
Medication, %									
Statin	87.6	87.8	87.7	83.6†	88.4	86.6	87.9	87.7	80.6†
Metformin	74.3	76.9	66.7††	62.2††	75.8	76.7	67.7††	74.3	70.4
Sulphonylureas	47.1	48.6	42.0††	42.3†	48.4	46.3	46.3	47.1	48.5
Glitazones	21.4	22.5	18.0††	17.5†	19.8	22.4†	22.5†	21.4	19.4
Insulin	14.0	14.0	13.4	15.0	12.8	13.7	16.5††	13.9	17.9
HbA1c, mmol/mol	58.5 (18.0)	59.3 (18.2)	56.5 (17.4)**	53.9 (16.8)**	58.6 (17.9)	59.9 (18.8)*	56.0 (16.9)**	58.5 (18.0)	57.5 (19.9)
Cholesterol, mmol/L	4.3 (1.1)	4.4 (1.1)	4.2 (1.0)**	4.0 (1.1)**	4.4 (1.1)	4.4 (1.1)	4.1 (1.0)**	4.3 (1.1)	4.3 (1.2)
Abnormal eGFR, %	40.8	38.7	44.5††	58.3††	39.6	31.3††	58.5††	40.9	35.2
NFS	−0.21 (−1.04 to 0.62)	−0.53 (−1.29 to 0.18)	0.67(0.13 to 1.24)**	1.76(1.19 to 2.45)**	−1.10 (−1.89 to −0.36)	−0.16 (−0.80 to 0.32)	1.27 (0.93 to 1.77)	−0.23 (−1.06 to 0.58)	1.73 (0.65 to 2.72)**
FIB4 score	1.22 (0.88 to 1.69)	1.05 (0.80 to 1.37)	2.04 (1.54 to 2.27)	3.38 (2.93 to 4.48)	1.01 (0.73 to 1.30)	1.14 (0.88 to 1.51)**	2.0 (1.56 to 2.61)**	1.21 (0.88 to 1.66)	5.09 (3.58 to 7.77)**
APRI score	0.22 (0.16 to 0.30)	0.19 (0.15 to 0.24)	0.34 (0.27 to 0.44)**	0.61 (0.41 to 0.92)**	0.17 (0.13 to 0.23)	0.23 (0.18 to 0.31)**	0.28 (0.21 to 0.43)**	0.21 (0.16 to 0.30)	1.42 (1.17 to 2.03)

Note: Low FIB4 score: <1.3 if aged <65 years or <2.0 if aged ≥65 years; Low NFS: < −1.455 if aged <65 years or <0.12 if aged ≥65 years; Low APRI score: <1 (independent of age); Intermediate FIB4 score: >1.3 if aged <65 years or >2.0 if aged ≥65 years and <2.67 (independent of age); Intermediate NFS: > −1.455 if aged <65 years or >0.12 if aged ≥ 65 years and <0.676; High FIB4 score: >2.67 (independent of age); High NFS: >0.676 (independent of age); High APRI score: ≥1.
Note: Values are proportions, and means (standard deviations) or medians (interquartile range).
Abbreviations: ALT, alanine aminotransferase; APRI, aspartate aminotransferase to platelet ratio index; AST, aspartate aminotransferase; BMI, body mass index; CABG, coronary artery bypass graft; eGFR, estimated glomerular filtration rate; FIB4, Fibrosis-4 score; HbA1c, glycated haemoglobin; NFS, nonalcoholic fatty liver disease fibrosis score; PCI, percutaneous coronary intervention; PVD, peripheral vascular disease;TIA, transient ischaemic attack.
*P < 0.01.**P < 0.001 for comparisons between subgroups after Bonferroni correction with reference low subgroup.‡P < 0.05.
†P < 0.01.
††P < 0.001 for chi-squared comparisons between subgroups after Bonferroni correction with reference low subgroup.

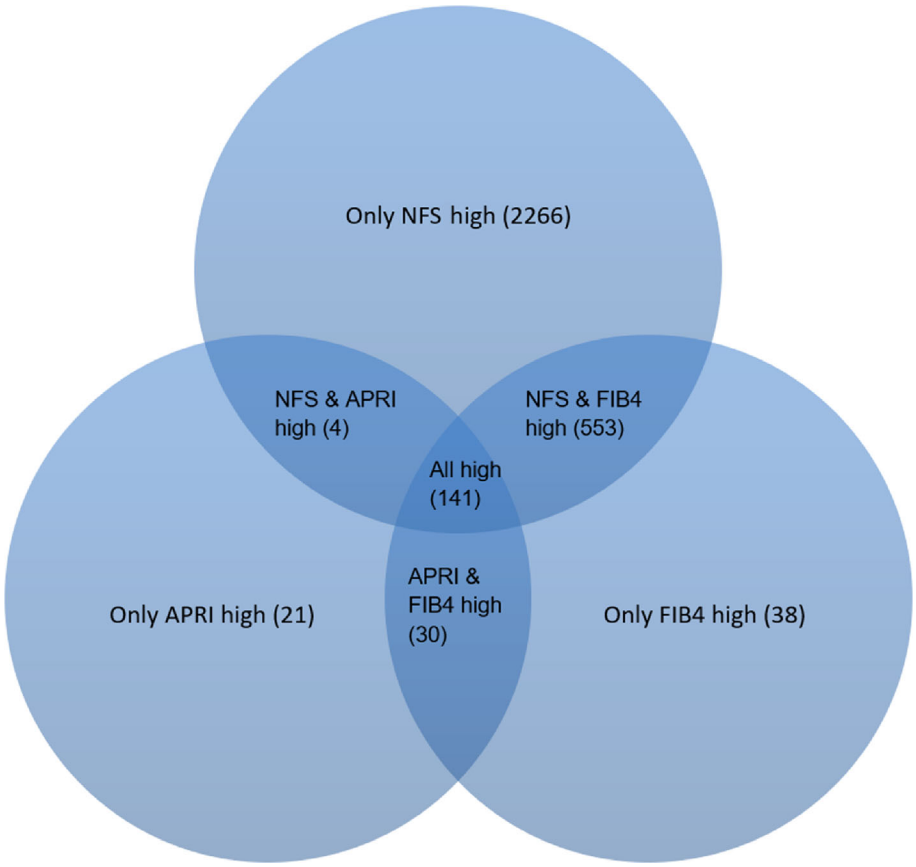

FIGURE 2 Venn diagram showing overlap between high risk scores and numbers of participants in each high-risk category for each fibrosis score. APRI, aspartate aminotransferase to platelet ratio index; FIB4, Fibrosis-4; NFS, nonalcoholic fatty liver disease fibrosis score

TABLE 2 Numbers of deaths, crude mortality rates and hazard ratios adjusted for age, sex, diabetes duration, smoking, presence of comorbidities at baseline, eGFR, HbA1c, cholesterol, prescription of statins and glucose-lowering drugs in patients with type 2 diabetes by Fibrosis-4 score, nonalcoholic fatty liver disease fibrosis score and aspartate aminotransferase to platelet ratio index categories

Fibrosis score	Deaths and mortality / 1000 PY (Total 3925)[a]	Hazard ratio (95% CI)[b]	*P* value
FIB4, low (n = 9706)	2778 (36.3)	1.0 (ref)	
FIB4, intermediate (n = 2121)	695 (43.5)	1.14 (0.98–1.32)	0.101
FIB4, high (n = 762)	452 (101.9)	3.69 (2.88–4.7)	<0.001
NFS, low (n = 4800)	1448 (38.5)	1.0 (ref)	
NFS, intermediate (n = 4825)	992 (24.9)	1.00 (0.88–1.13)	0.95
NFS, high (n = 2964)	1485 (75.7)	2.32 (1.95–2.75)	<0.001
APRI, low (n = 12 393)	3831 (40.0)	1.0 (ref)	
APRI, high (n = 196)	94 (74.0)	3.92 (2.88–5.34)	<0.001

Abbreviations: CI, confidence interval; APRI, aspartate aminotransferase to platelet ratio index; FIB4, Fibrosis-4 score; NFS, nonalcoholic fatty liver disease fibrosis score; PY, person-years.

[a]Number of deaths (crude incidence rates for 1000 person-years of follow-up).

[b]Hazard ratios (95% CI) estimated by Cox regressions adjusted for following covariates: age, interaction of age and fibrosis score, sex, diabetes duration, body mass index, smoking, estimated glomerular filtration rate at risk, presence of comorbidities at baseline, mean levels of glycated haemoglobin (58.5 mmol/mol), cholesterol and use of statins and antihyperglycaemic drugs.

1000 person-years. Numbers of deaths, crude all-cause mortality rate by fibrosis score category and the multivariable adjusted all-cause mortality ratio by fibrosis score category are given in Table 2. Further adjustment for ALT and AST levels had little effect on the hazard ratios for each of the fibrosis score categories (Table S1). Mortality was higher in the high-risk fibrosis groups than the low-risk fibrosis groups for each score, Kaplan-Meier cumulative mortality curves are shown in Figure 3.

There was a significant interaction between liver fibrosis score categories and age (<65 vs. ≥65 years) for all-cause mortality (Figure 4). There was no evidence of interactions with sex, duration of diabetes, glycaemic control or comorbidities (Figure 4). The hazard ratios for mortality for the high compared to low fibrosis score categories were significantly higher for people aged <65 years than for people aged ≥65 years for all three fibrosis scores (Table 3). As for the

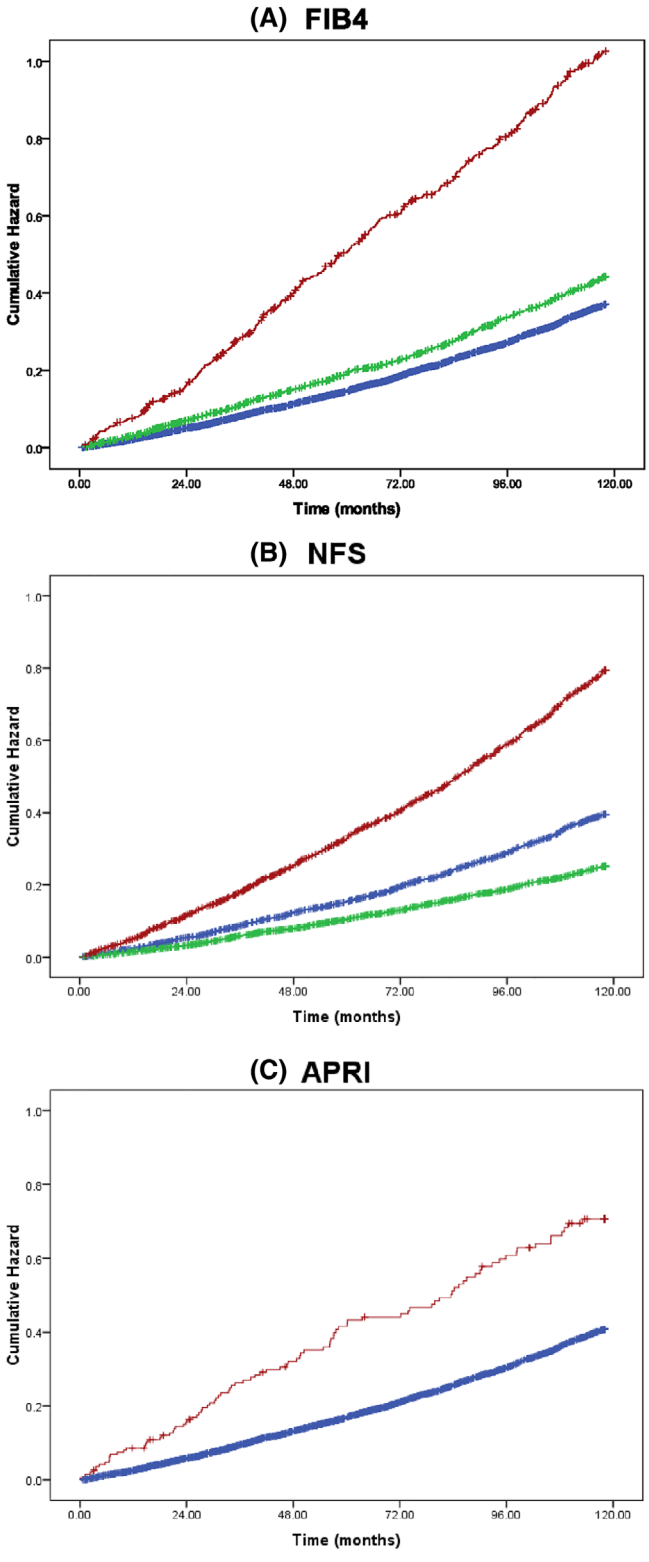

FIGURE 3 Kaplan-Meier estimation curves of cumulative all-cause mortality during follow-up in subjects classified by **A,** Fibrosis 4 (FIB4) score and **B,** nonalcoholic fatty liver disease fibrosis score (NFS) into high (red), intermediate (green) and low (blue) categories, and by **C,** aspartate aminotransferase to platelet ratio index (APRI) score into high (red) and low (blue) categories

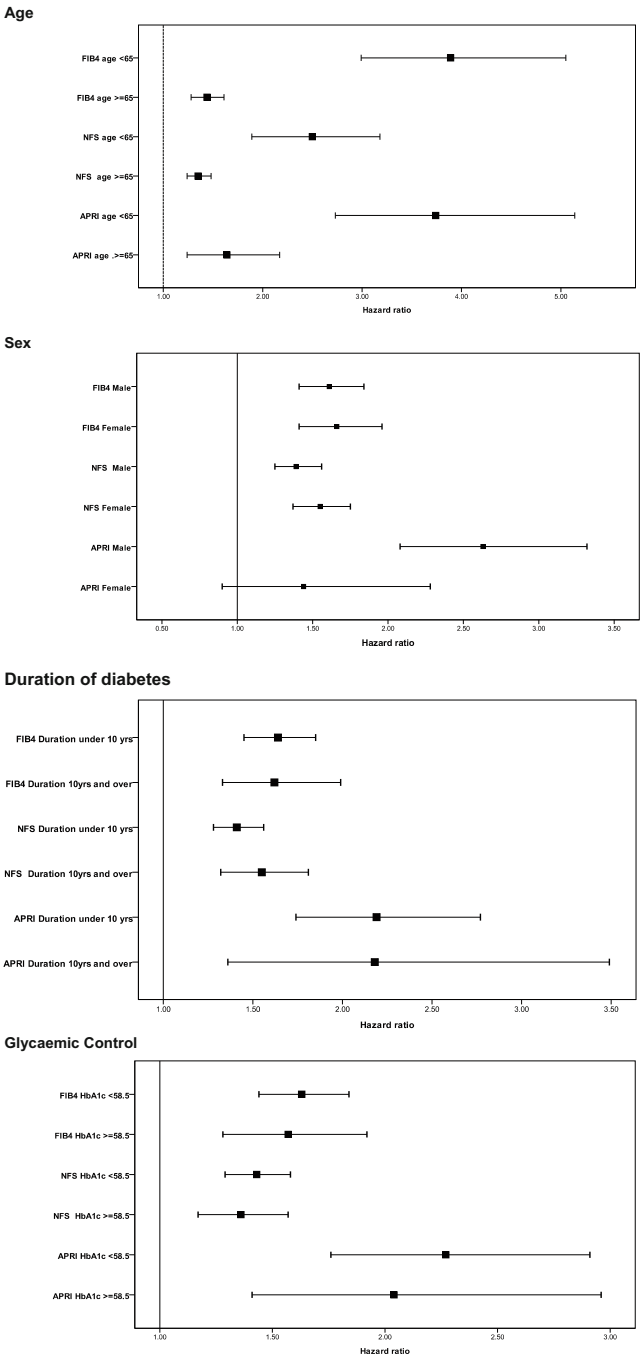

FIGURE 4 Forest plot of hazard ratios for all-cause mortality for high compared to low categories for Fibrosis 4 (FIB4) score, nonalcoholic fatty liver disease fibrosis score (NFS) and aspartate aminotransferase to platelet ratio index (APRI), stratified by age (<65 and ≥65 years), sex, duration of diabetes (<10 and ≥10 years) and glycated haemoglobin (HbA1c; <58.5 mmol/mol [<7.5%] vs. ≥58.5 mmol/mol [≥7.5%]), adjusted for age, sex, diabetes duration, smoking, presence of comorbidities at baseline, estimated glomerular filtration rate, HbA1c, cholesterol, prescription of statins and glucose-lowering drugs. Values are hazard ratios (95% confidence intervals) adjusted for the same covariates as in Table 2

TABLE 3 Stratified analysis for people aged <65 and ≥ 65 years and hazard ratios for all-cause mortality associated with liver fibrosis score categories adjusted for age, sex, diabetes duration, smoking, presence of comorbidities at baseline, estimated glomerular filtration rate, glycated haemoglobin, cholesterol, prescription of statins and glucose-lowering drugs

	Age < 65 years (n = 5729)		Age ≥ 65 years (n = 6860)		
Fibrosis score	Hazard ratio (95% CI)[a]	*P* value	Hazard ratio (95% CI)[a]	*P* value	*P* for interaction[b]
FIB4, intermediate	1.18 (0.99–1.41)	0.062	0.99 (0.90–1.09)	0.84	0.18
FIB4, high	3.89 (2.99–5.05)	<0.001	1.44 (1.28–1.61)	<0.001	<0.001
NFS, intermediate	1.03 (0.85–1.26)	0.75	1.04 (0.95–1.15)	0.40	0.44
NFS, high	2.50 (1.89–3.18)	<0.001	1.35 (1.24–1.48)	<0.001	<0.001
APRI, high	3.74 (2.73–5.14)	<0.001	1.64 (1.24–2.17)	<0.001	<0.001

Abbreviations: CI, confidence interval; APRI, aspartate aminotransferase to platelet ratio index; FIB4, Fibrosis-4 score; NFS, nonalcoholic fatty liver disease fibrosis score.

[a]Values are hazard ratios (95% confidence intervals) adjusted for the same covariates as in Table 2.

[b]*P* value of interaction term in the unstratified dataset.

overall analysis, further adjustment for AST and ALT levels did not make major changes to these results (Table S2). Additionally, Table S3, shows the hazard ratios for mortality after further adjustment for two thresholds of AST/ALT ratios (>0.8 and >1.0). An increased risk of all-cause mortality was observed for ratios above both these thresholds (>0.8 and >1.0) 1.57 (1.38–1.78) and 1.76 (1.56–2.00), respectively (both P <0.001, compared to people with ratios below the relevant threshold).

Table S4 shows the hazard ratios for mortality after adjustment for number of high-risk categorizations by the three scores NFS, FIB4 and APRI. There were 3053 cases. Seventy-six percent (2325) were categorized as high-risk by only one score, 19% (587) were categorized as high-risk by two scores, 5% (141) were categorized as high-risk by three scores.

Increased risk of mortality was observed in those categorized as high-risk by two or three scores compared to those categorized as high-risk by only one score (1.95 [1.32–2.90], $P < 0.01$ and 2.65 [1.83–3.83], $P < 0.001$, respectively).

In the comparison of discrimination between mortality and survival for the different scores, FIB4 score outperformed NFS (AUROC 0.667 vs. 0.650; $P < 0.05$). Both FIB4 score and NFS performed better than the APRI score (AUROC 0.486; $P < 0.05$).

3.3 | Sensitivity analysis

Sensitivity analysis among the subset of 7556 patients (60%) who had fibrosis scores calculated within a year of cohort entry showed slightly higher mortality rates compared to the total population but no substantive difference in crude and multivariable-adjusted hazard ratios for mortality for high- compared to lower-risk scores (Table S5).

4 | DISCUSSION

In this study we have described the distribution of three fibrosis scores, FIB4, NFS and APRI, and their associations with all-cause mortality in 12 589 individuals with type 2 diabetes in Ayrshire and Arran in Scotland, United Kingdom. We have shown that there is increased all-cause mortality for the highest compared to the lowest categories of all three fibrosis scores, with similar values for FIB4 and APRI and lower values for NFS after adjustment for age, sex, diabetes duration, smoking status, presence of comorbidities at baseline, eGFR, HbA1c, cholesterol, prescription of statins and glucose-lowering drugs. We also demonstrated significantly higher hazard ratios for all-cause mortality associated with higher fibrosis scores for individuals aged under 65 years compared to those aged 65 years and over. Patients categorized as high-risk by two or three of the scores had a significantly higher hazard ratio for all-cause mortality compared with those categorized as high-risk by only one score.

The strengths of this study include its large population of a well-defined group of patients with clinical and biochemical variables drawn directly from primary care electronic patient records. We believe it is the largest study to date in patients specifically with type 2 diabetes comparing the distribution of the different risk scores and describing their association with all-cause mortality. Prevalence of high-risk fibrosis scores in our population ranged between 1.6% and 23.5%, depending on the score used. In a US study of 501 people with type 2 diabetes aged ≥50 years who received noninvasive assessment of fibrosis using magnetic resonance elastography and vibration-controlled transient elastography, the prevalence of NAFLD, advanced fibrosis and cirrhosis was 65%, 14% and 6%, respectively.[28] The American Association of Clinical Endocrinologists guidelines recommend noninvasive screening for liver fibrosis in all patients with type 2 diabetes,[13] it is imperative that these risk scores are validated specifically in this cohort of patients, regardless of whether they are known to have NAFLD. The novel data presented in this study are particularly important considering the low prevalence of type 2 diabetes in other studies of liver fibrosis.[4,21]

There was a minimal amount of missing data and only a small proportion (5.7%) of the eligible population were excluded from the analysis as a consequence, so the potential for bias is limited. Our sensitivity analysis that was restricted to a subset of 7556 patients (60%) who had data to calculate fibrosis scores within a year of baseline demonstrated similar estimates of crude or multivariate-adjusted mortality to those reported in the overall analysis.

There are some limitations inherent to our study design. Whilst we excluded patients with a diagnosis of liver disease attributed to alcohol or viral hepatitis, it is possible that some people with other risk factors for liver disease were included. Accurate estimates of alcohol consumption are not available in the electronic patient record and NAFLD is not reliably coded in primary care.[29] It is therefore not possible to ensure accurate diagnoses of NAFLD, a diagnosis of exclusion of other liver diseases in our population, in which there is a relatively high prevalence of alcohol use and a nontrivial prevalence of hepatitis C.[30,31] We therefore took the pragmatic approach to compare fibrosis risk scores and their association with mortality regardless of presence or type of liver disease in this population of people with type 2 diabetes.

Several additional factors may limit our study. Due to the nature of the data, the cause of death was not available. Liver- and cardiovascular-related events have previously been identified as the major contributors to excess mortality in patients with NAFLD.[3] NAFLD is a risk factor for cardiovascular disease, and probably also chronic kidney disease,[7,8,32] independent of established cardiometabolic risk factors, such as obesity, hypertension and type 2 diabetes. Unfortunately, data on antihypertensive medication were not available and, as these medications are associated with a survival benefit, their use may represent an unmeasured confounding variable in our analyses if use differs by fibrosis score. Finally, the majority of the patients in this cohort are of white European ethnicity, reflecting the characteristics of the local population. This will limit extrapolation of our results to other regions that have a more ethnically diverse population.

Other noninvasive risk scores are used for fibrosis risk stratification, such as the BARD score and AST/ALT ratio.[26,33,34] We did not apply the BARD score as it consists of diabetes as well as AST, ALT and BMI, the same risk factors that are included in the NFS. The inclusion of diabetes status within the BARD and NFS scoring systems increases the chances of an individual with diabetes having a score that identifies them as high-risk. This is illustrated by our findings that the proportion of people in the high-risk category was considerably higher for the NFS compared to the FIB4 or APRI scores and only 24% of people with a high NFS had a high FIB4 or a high APRI score.

As noninvasive scoring systems can reliably exclude advanced fibrosis in patients with NAFLD, they can therefore provide an initial assessment of liver fibrosis.[18,35,36] Several studies have now validated their use in large populations of patients with NAFLD[36–38] and FIB4 has recently been recommended by the American Association of Clinical Endocrinologists as the first-line screening tool in patients with type 2 diabetes[13] given that it has been most extensively validated.[21,39,40] Our results confirm previous findings of associations between higher values of all three scores and all-cause mortality[4,21,36] specifically in people with type 2 diabetes and also showed that the FIB4 score offered better discrimination between mortality and survival than the NFS or APRI score.

Our finding of higher relative mortality for people aged under 65 years with high fibrosis risk scores compared to older people has not been described before and may be explained by several factors. It partly represents the lower absolute risk of mortality in younger patients. However, it may also reflect age-related changes in the deposition of fat in the liver, compared with visceral and intramuscular fat compartments.[41] It has been suggested that there is an age-related change in the kinetics of free fatty acids, leading to increased visceral adiposity relative to hepatic steatosis.[42] It is notable, however, that FIB-4 and NFS have demonstrated poor diagnostic performance in patients under 35 years of age and further research is needed to identity alternative forms of noninvasive fibrosis assessment in the increasing numbers of young people with type 2 diabetes.[25]

The ability to better predict histological stage of liver disease and mortality risk using noninvasive methods may be helpful in the management of patients. More intensive treatment strategies aimed at reducing cardiovascular, renal and liver-related morbidity and mortality may be appropriate in high-risk cohorts. Additionally, people categorized as low-risk may need fewer investigations such as vibration-controlled transient elastography and ultimately liver biopsy, even if they have abnormal liver function tests and no other obvious causes of liver disease.

The number of patients proceeding to further assessment if current guidelines were followed in our cohort, however, is high, with approximately 23% of patients having intermediate- or high-risk FIB4 scores. Presently, to refine this process of identifying risk of liver disease in patients with type 2 diabetes, the American Association of Clinical Endocrinologists suggest that a two-step process is used, combining FIB4 with a further noninvasive test such as vibration-controlled transient elastography or the enhanced liver fibrosis test (ELF™). ELF™ is a proprietary test consisting of a combination of biomarkers and is recommended by NICE in the United Kingdom for the assessment of patients with suspected NAFLD.[43] In combination with FIB4, ELF™ can help stratify indeterminate-risk patients, increasing the detection of advanced fibrosis.[20,44] ELF™ demonstrates good predictive values[43,45] but is not available routinely in many areas because of the cost and current laboratory infrastructure. Vibration-controlled transient elastography has a high negative predictive value for advanced fibrosis in patients with NAFLD,[46] but gives unreliable results in up to 20% of patients, particularly those with a high BMI.[47] Viewed in the context of our results in patients with type 2 diabetes these limitations highlight the need for further research to improve stratification of intermediate-risk groups, and the use of biomarker scores, combined with vibration-controlled transient elastography, to inform appropriate referrals to secondary care hepatology services.

In conclusion, this study shows that, in a large cohort of patients with type 2 diabetes, classification into higher liver fibrosis risk score strata is associated with higher all-cause mortality. We have also identified a significantly higher increased relative risk of mortality in individuals under 65 years classified as high-risk compared with those over 65 years of age. Given the large number of patients categorized as intermediate- or high-risk, further research is needed into the optimal implementation and application of these risk stratification tools, (particularly when combined with vibration-controlled transient elastography), as well as the identification and implementation of effective interventions for people at high risk of liver fibrosis.

AUTHOR CONTRIBUTIONS

Andrew Collier conceived the paper in discussions with Sarah Wild and Christopher Byrne. Lyall Cameron extracted the data. All the authors contributed to the drafting the article and revising it critically. All authors give final approval of this version to be published.

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CONFLICT OF INTEREST STATEMENT

There are no conflicts of interest.

PEER REVIEW

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DATA AVAILABILITY STATEMENT

The datasets generated during and/or analysed during the current study are not publicly available due to privacy & ethical restrictions but would be available from the corresponding author on reasonable request.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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