Liver fibrosis markers and all cause mortality in people with type 2 diabetes

TITLE PAGE

Title: 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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Abbreviations list:

ALT – Alanine aminotransferase
APRI - AST to platelet ratio index
AST – Aspartate aminotransferase
BMI – Body mass index
CABG - Coronary artery bypass graft

CKD – Chronic kidney disease

eGFR – Estimated glomerular filtration rate

ELF - Enhanced liver fibrosis score

FIB4 - Fibrosis-4 score

HbA1c - Haemoglobin A1c

NAFLD – Non-alcoholic fatty liver disease

NFS - NAFLD fibrosis score

NICE - National Institute for Health and Care Excellence

PVD - Peripheral vascular disease

TIA - Transient ischaemic attack

ABSTRACT

Aims: International guidelines recommend non-invasive screening for non-alcoholic fatty liver disease (NAFLD) in people with type 2 diabetes mellitus. Several readily available biomarker scores have been developed to estimate the risk of liver fibrosis. These include the Fibrosis-4 score (FIB4), NAFLD fibrosis score (NFS), and AST to platelet ratio index (APRI). In a cohort of individuals with type 2 diabetes, we aimed to describe the distribution of these scores and the association 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-points used to identify low risk were: FIB4 <1.3 if age <65 years or <2.0 if age >=65 years; NFS <-1.455 if age <65 years or <0.12 if age >=65 years; APRI < 1 (independent of age). High risk cut points were FIB4 >2.67; NFS >0.676; 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: Mean±SD age was 65.2±12.1 years. 54.5% were men and median (IQR) diabetes duration was 5.8 (2.8-9.3) years. Prevalence of high risk categories was 6.1% for FIB4, 23.5% for NFS and 1.6% for APRI. During median follow-up of 9.8 years, 3925 patients (31.1%) died resulting in a crude mortality rate of 40.4 per 1000 patient-years. Overall adjusted all-cause mortality hazard ratios (95% 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 (2.99-5.05) and 1.44 (1.28-1.61) for FIB4, 2.50 (1.89-3.18) and 1.35 (1.24-1.48) for NFS and 3.74 (2.73-5.14) and 1.64 (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 minimise excess mortality in people at high risk of liver fibrosis.

Introduction

Non-alcoholic fatty liver disease (NAFLD) is characterised 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 the 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 non-invasive 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 non-invasive 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 score (FIB4) [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™) (that is not commonly used despite NICE Guidelines in the UK recommending its use) [17], the NAFLD fibrosis score (NFS), and the 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 the risk score categories and all-cause mortality and compare the strength of the association between the different scores and all-cause mortality.

Methods and materials.

We performed a retrospective cohort study of patients identified from electronic primary care records for adults >=18 years of age in 45 (out of 53) General Practices in the Scottish region of Ayrshire & Arran (covering around 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 1st January 2012. 214 people with a diagnosis of alcoholic liver disease or viral hepatitis at baseline or during follow-up were excluded (**Figure 1**).

Data were available on age, sex, date of diabetes diagnosis, smoking status and presence of co-morbidities (defined using read codes for mental illness, stroke/transient ischaemic attack (TIA), peripheral vascular disease (PVD), percutaneous coronary intervention (PCI)/coronary artery bypass graft (CABG), retinopathy, liver and colon cancer), factors in the FIB4, NFS and APRI fibrosis scores (body mass index (BMI), aspartate aminotransferase (AST), alanine aminotransferase (ALT), platelet count & albumin levels), HbA1c, estimated glomerular filtration rate (eGFR), lipid levels and prescribing of statins and drugs used in diabetes. Abnormal eGFR (estimated glomerular filtration rate) was

198 defined at an eGFR<60 ml/min/1.73m^2. eGFR was treated as a categorical variable due to
199 because a numerical value for eGFR is only provided by the laboratory if it is under
200 60mL/min/1.73m^2 otherwise it is reported as >=60 mL/min/1.73m^2. Measurements closest
201 to cohort entry date were used. In sensitivity analysis a limit of measurements within one
202 year of baseline was used.

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

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

208 **Missing data**

209 758 (5.7%) patients were excluded due to incomplete data. There were statistically
210 significant differences between people with incomplete and complete data for only five
211 baseline characteristics; duration of DM (median 6.7 yrs vs. 5.8 yrs), albumin (mean 4.3
212 vs. 4.2 g/l), prevalence of stroke/TIA (14.1% vs. 9.7%); retinopathy (38.8% vs. 44.5%) and
213 abnormal eGFR (30.5% vs. 40.8%). There was no significant difference in prevalence of
214 diabetes mellitus between practices that did and did not provide data (4.6% vs. 4.8% χ^2=
215 3.38, p = 0.07.

216 **Liver fibrosis score calculations.**

217 FIB4 (Fibrosis 4 score), NFS (NAFLD fibrosis score) and APRI (AST to platelet ratio
218 index), were calculated [16,18,19] using data measured as close to cohort entry as possible.
219 The three scores were categorized in low, intermediate and high groups at recommended
220 cut-off values [25,26]: The cut-points indicating low probability of advanced liver fibrosis were:
221 FIB4 <1.3 if age <65 years or <2.0 if age >=65 years; NFS <-1.455 if age <65 years or
222 <0.12 if age >=65 years; APRI < 1 (independent of age). The upper cut-points (indicating

high probability of advanced liver fibrosis) were all independent of age: FIB4 >2.67; NFS >0.676; APRI >=1 (21).

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 were compared by analysis of variance (ANOVA) 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-square (or Fisher's exact test when appropriate) again with post-hoc Bonferroni correction.

Kaplan-Meier curves and log rank tests were used to compare cumulative hazard of crude all-cause mortality during follow-up between individuals with low, intermediate and high FIB4/NFS scores 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, presence of co-morbidities at baseline, eGFR, HbA1c, cholesterol, prescription of statins and glucose-lowering drugs. AST and ALT were included as covariates in a sensitivity analysis. Age, diabetes duration, cholesterol and HbA1c were treated as continuous variables, with the others treated as categorical variables. Both analyses with continuous standardised 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 (HRs) 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 old), sex, diabetes duration (<10 vs. ≥10 years long), presence of co-morbidities 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 2-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 receiving operator characteristic curves (AUROCs).

Analyses were performed with SPSS version 17.0 (SPSS Inc., Chicago, Il., USA).

Results

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 to 20) months for FIB4, 10 (5 to 22) for NFS & 7 (3 to 18) for APRI. 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 .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%) are not in the high category for either FIB4 or APRI. Of the 762 cases in the FIB4 high category 694 (91%) are also in the NFS high category. Of the 196 cases in the APRI high category 141 (72%) are in both the FIB4 and NFS high categories.

Mortality during follow-up

During a median follow-up of 9.8 years (total 97055 patient-years), 3925 patients (31.1% of the cohort) died and crude mortality was 40.4 per 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 had little effect on the HRs for each of the fibrosis score categories (see **Supplementary Table 1**). 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 old) for all cause mortality (**Figure 4**). There was no evidence of interactions with sex, duration of diabetes, glycaemic control or co-morbidities (**Figure 4**). The hazard ratios for mortality for the high compared to low fibrosis score categories were significantly higher for people aged under 65 years of age than for people ≥65 years of age for all three fibrosis scores (**Table 3**). As for the overall analysis, further adjustment for AST and ALT did not make major changes to these results (**Supplementary Table 2**). Additionally, **Supplementary Table 3,** shows the hazard ratios for mortality after further adjustment for two thresholds of AST/ALT ratios (>0.8 and >1.0). 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.

Supplementary Table 4 shows the hazard ratios for mortality after adjustment for number of high risk categorisations by the three scores NFS, FIB4 & APRI. There were 3053 cases. 76% (2325) were categorised as high risk by only one score, 19% (587) were categorised as high risk by two scores, 5% (141) were categorised as high risk by three scores.

Increased risk of mortality was observed in those categorised as high risk by two or three scores compared to those categorised 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 the FIB4 fibrosis score outperformed NFS (AUROC 0.667 vs. 0.650; p<0.05). Both FIB4 and NFS performed better than the APRI score (AUROC 0.486; p<0.05).

Sensitivity analysis

Sensitivity analysis among the subset of 7556 (60%) of patients 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. (**Supplementary Table 5**).

Discussion

In this study we have described the distribution of three fibrosis scores, FIB4, NFS and APRI and their association with all-cause mortality in 12,589 individuals with type 2 diabetes in Ayrshire and Arran in Scotland, UK. 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, presence of co-morbidities 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 under 65 years of age compared to ≧65 year olds. Patients categorised as high risk by two or three of the scores had a significantly higher hazard radio for all cause mortality compared with those categorised 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 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. In a US study of 501 people with type 2 diabetes ≥ 50 years of age who received non-invasive 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 non-invasive screening for liver fibrosis in all patients with type 2 diabetes [13], it is imperative 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 is 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 and so the potential

for bias is limited. Our sensitivity analysis restricted to of a subset of 7556 (60%) of patients 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 within 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 [31]. 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 non-trivial prevalence of hepatitis C [29,30]. 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 CVD, and probably also CKD [7,8,32], independent of established cardio-metabolic 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 non-invasive 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 score. 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 risk score had a high FIB4 or a high APRI score.

As non-invasive 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 under 65 years of age with high risk 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 non-invasive 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 non-invasive 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 categorised 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 around 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 non-invasive 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 body mass index [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.

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 categorised as intermediate or high risk further research is needed on 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.

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Legends to figures

Figure 1 Flow diagram describing cohort selection.

Figure 2 Venn diagram showing overlap between high risk scores and numbers of subjects in each high risk category for each fibrosis score.

Figure 3 Kaplan-Meier estimation curves of cumulative all-cause mortality during follow-up in subjects classified by the FIB4 & NFS (A & B) scores into high (red), intermediate (green) and low (blue) categories; and APRI (C) into high (red) and low (blue) categories. Abbreviations: FIB4, Fibrosis 4 score; NFS, NAFLD Fibrosis Score; APRI, AST to platelet ratio index

Figure 4
Forest plot of hazard ratios for all-cause mortality for high compared to low categories for FIB4, NFS and APRI, stratified by age (<65 & ≥65 years), sex, duration of diabetes (<10 & ≥10 yrs) and HbA1c ((HbA1c <58.5 mmol/mol (<7.5%) vs. ≥58.5 mmol/mol (≥7.5%)) adjusted for age, sex, diabetes duration, smoking, presence of co-morbidities at baseline, eGFR, HbA1c, cholesterol, prescription of statins and glucose-lowering drugs. Abbreviations: FIB4, Fibrosis 4 score; NFS, NAFLD Fibrosis Score; APRI, AST to platelet ratio index. Values are hazard ratios (95% confidence intervals) adjusted for the same covariates as in Table 2.

Table 1: Baseline characteristics of study population stratified by FIB4, NFS & APRI categories.

	Total (n=12589)	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=12393)	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 (males %)	54.5	52.4	60.7††	63.0††	48.8	59.7††	55.1††	54.2	70.0**
Smoking (current/ex %)	56.4	56.7	55.4	55.8	58.4	55.6‡	54.5†	56.5	54.1
Diabetes duration (yrs)	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) **
Platelets count (x10^9)	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) **
Co-morbidities (%)									
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
Medications (%)									
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 score	-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)

Low FIB4 <1.3 if age <65 years or <2.0 if age >=65 years; Low NFS <-1.455 if age <65 years or <0.12 if age >=65 years; Low APRI < 1 (independent of age); Intermediate FIB4 >1.3 if age <65 years or >2.0 if age >=65 years and <2.67 (independent of age); Intermediate NFS >-1.455 if age <65 years or >0.12 if age >=65 years and <0.676; High FIB4 >2.67 (independent of age); High NFS >0.676 (independent of age); High APRI >=1

Values are proportions, and means (standard deviations) or medians (interquartile range).

*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-square comparisons between subgroups after Bonferroni correction with reference low subgroup.

Abbreviations: NFS, NAFLD fibrosis score; FIB4, fibrosis 4 score; APRI AST to platelet ratio index; HbA1c, glycated haemoglobin; AST Aspartate aminotransferase ALT Alanine aminotransferase, PVD Peripheral artery disease, PCI Percutaneous coronary intervention eGFR estimated glomerular filtration rate

Table 2: Numbers of deaths, crude mortality rates and hazard ratios adjusted for age, sex, diabetes duration, smoking, presence of co-morbidities at baseline, eGFR, HbA1c, cholesterol, prescription of statins and glucose-lowering drugs in patients with type 2 diabetes by FIB4, NFS & APRI categories

Fibrosis scores	Deaths and mortality / 1000 PY	Hazard ratio (95% CI)[b]	p-value
	(Total 3925)		
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=12393)	3831 (40.0)	1.0 (ref)	
APRI high (n=196)	94 (74.0)	3.92 (2.88-5.34)	<0.001

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

[b]Hazard ratios (95% confidence interval) estimated by Cox regressions adjusted for following covariates: age, interaction of age and fibrosis score, sex, diabetes duration, BMI, smoking, eGFR at risk, presence of co-morbidities at baseline, mean levels of HbA1c (58.5 mmol/mol), cholesterol and use of statins and anti-hyperglycaemic drug

Table 3: Stratified analysis for <65 and ≥ 65 year olds and hazard ratios for all-cause mortality associated with liver fibrosis score categories adjusted for age, sex, diabetes duration, smoking, presence of co-morbidities at baseline, eGFR, HbA1c, cholesterol, prescription of statins and glucose-lowering drugs.

Fibrosis scores	Age < 65 years (n=5729)		Age≥ 65 years (n=6860)		
	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; NFS, NAFLD Fibrosis Score; FIB4, Fibrosis 4 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 data set.

Figure 1

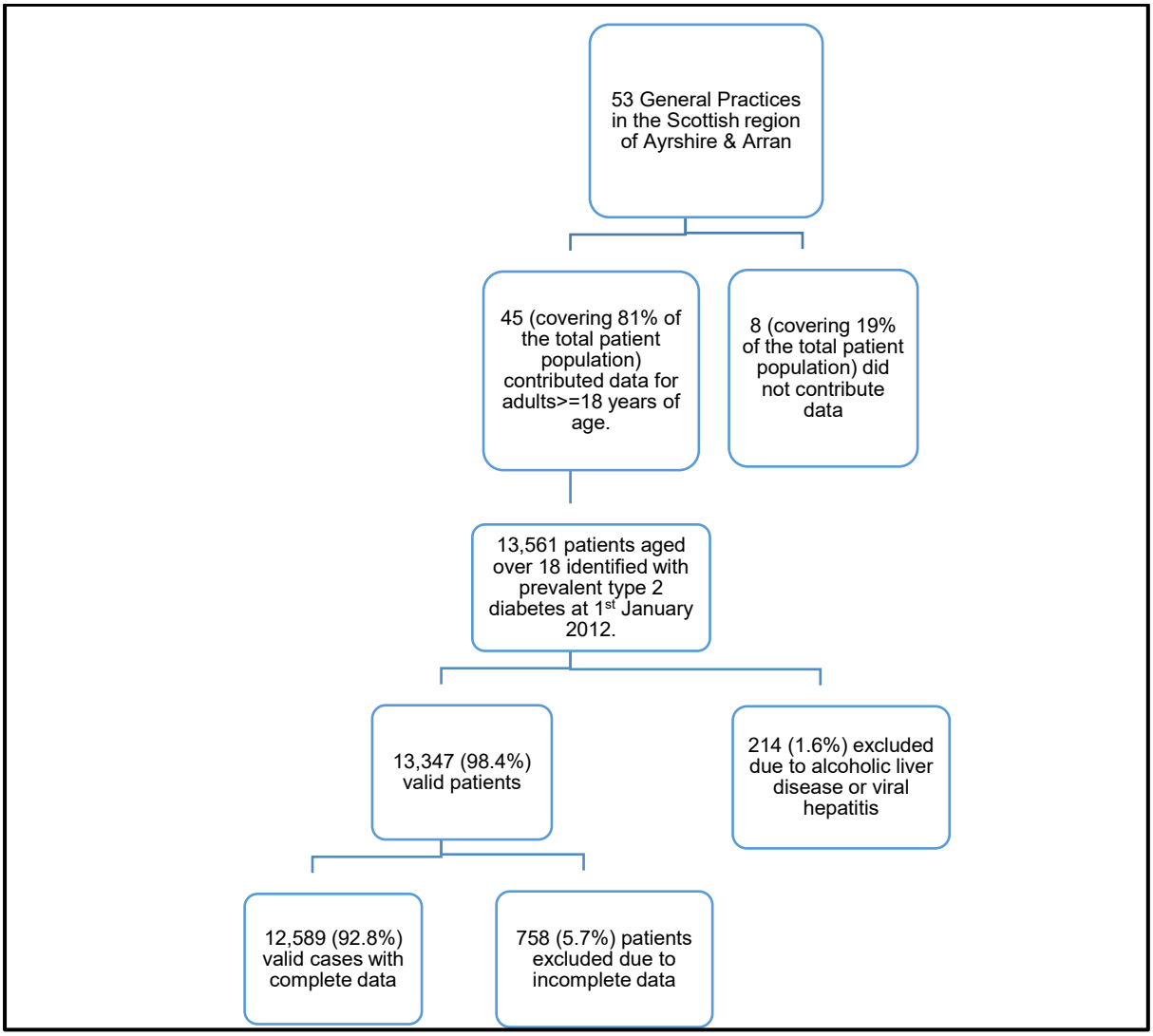

53 General Practices in the Scottish region of Ayrshire & Arran
45 (covering 81% of the total patient population) contributed data for adults>=18 years of age.
8 (covering 19% of the total patient population) did not contribute data
13,561 patients aged over 18 identified with prevalent type 2 diabetes at 1st January 2012.
13,347 (98.4%) valid patients
214 (1.6%) excluded due to alcoholic liver disease or viral hepatitis
12,589 (92.8%) valid cases with complete data
758 (5.7%) patients excluded due to incomplete data

Figure 2

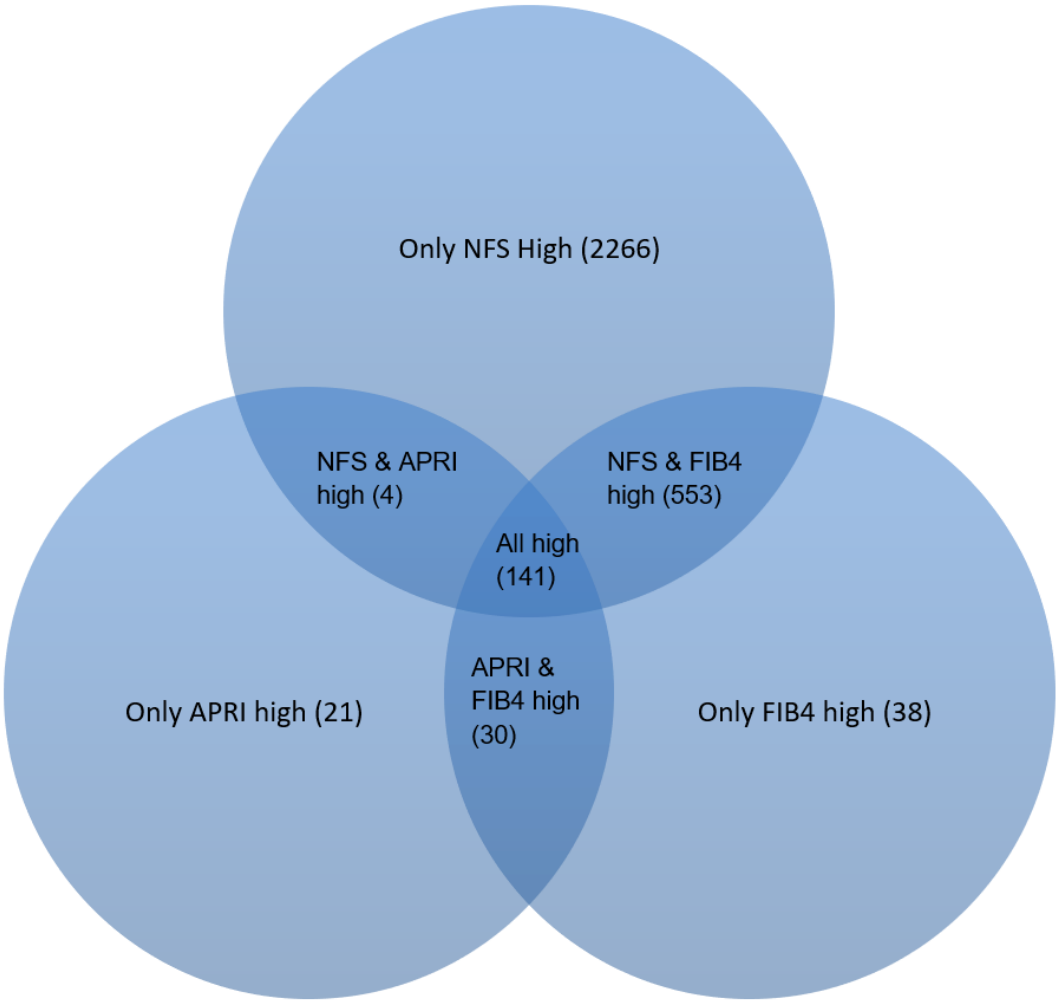

Only NFS High (2266)
NFS & APRI high (4)
NFS & FIB4 high (553)
All high (141)
APRI & FIB4 high (30)
Only APRI high (21)
Only FIB4 high (38)

Figure 3

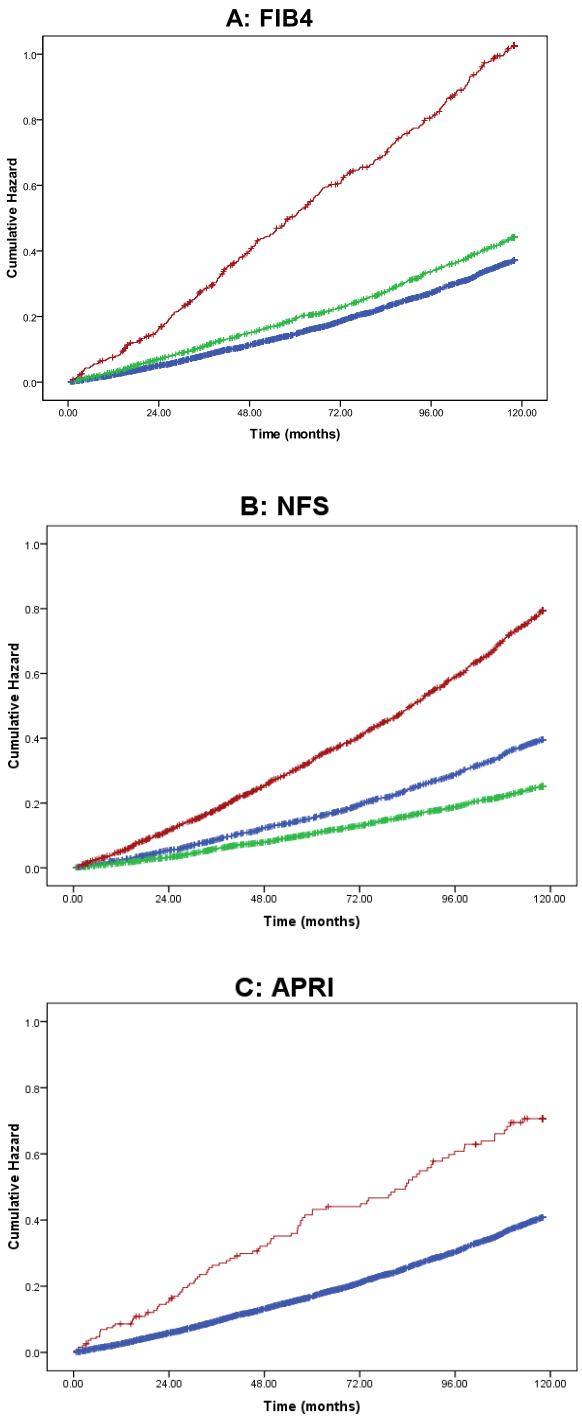
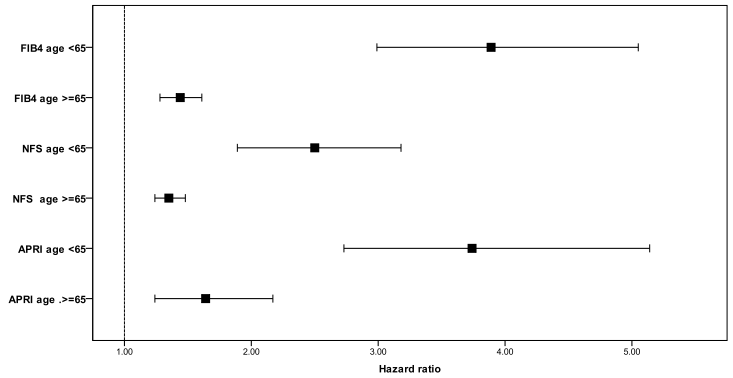
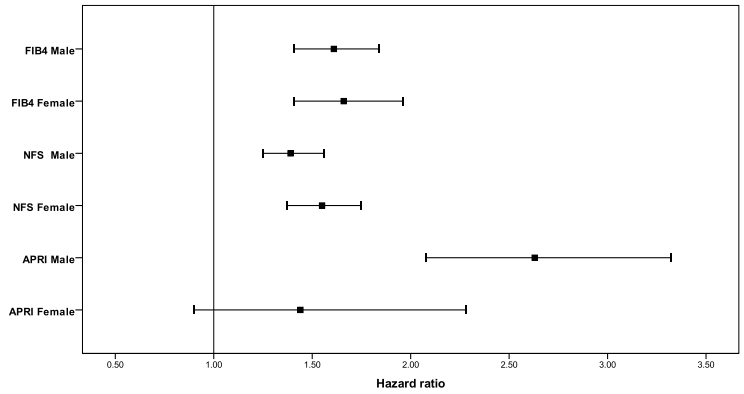

A: FIB4
Cumulative Hazard
1.0
0.8
0.6
0.4
0.2
0.0
0.00
24.00
48.00
72.00
96.00
120.00
Time (months)
B: NFS
Cumulative Hazard
1.0
0.8
0.6
0.4
0.2
0.0
0.00
24.00
48.00
72.00
96.00
120.00
Time (months)
C: APRI
Cumulative Hazard
1.0
0.8
0.6
0.4
0.2
0.0
0.00
24.00
48.00
72.00
96.00
120.00
Time (months)

Figure 4

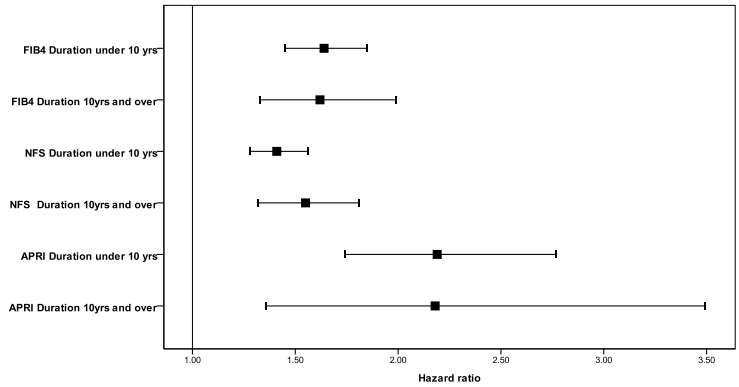
Age



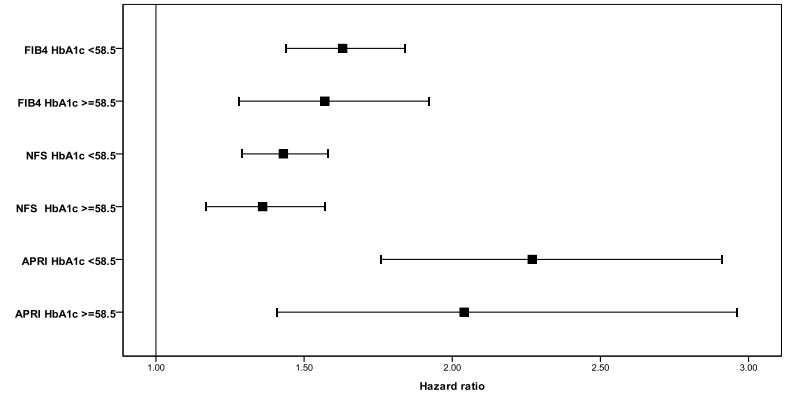
Sex



Duration of diabetes



Glycaemic Control



SUPPLEMENTARY TABLES

Supplementary Table 1: Numbers of deaths, crude mortality rates and hazard ratios adjusted for AST, ALT, age, sex, diabetes duration, smoking, presence of co-morbidities at baseline, eGFR, HbA1c, cholesterol, prescription of statins and glucose-lowering drugs in patients with type 2 diabetes by FIB4, NFS & APRI categories

Fibrosis scores	Deaths and mortality / 1000 PY	Hazard ratio (95% CI)[b]	p-value
	(Total 3925)		
FIB4 low (n=9706)	2778 (36.3)	1.0 (ref)	
FIB4 intermediate (n=2121)	695 (43.5)	1.15 (0.98-1.33)	0.083
FIB4 high (n=762)	452 (101.9)	3.73 (2.89-4.83)	<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)	0.96
NFS High(n=2964)	1485 (75.7)	2.27 (1.91-2.70)	<0.001
APRI low (n=12393)	3831 (40.0)	1.0 (ref)	
APRI high (n=196)	94 (74.0)	4.12 (2.98-5.70)	<0.001

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

[b]Hazard ratios (95% confidence interval) estimated by Cox regressions adjusted for following covariates: AST, ALT, age, interaction of age and fibrosis score, sex, diabetes duration, BMI, smoking, eGFR at risk, presence of co-morbidities at baseline, mean levels of HbA1c, cholesterol and use of statins and diabetes medications.

Supplementary Table 2: Stratified analysis for <65 and > 65 year olds and hazard ratios for all-cause mortality associated with liver fibrosis score categories adjusted for AST, ALT, age, sex, diabetes duration, smoking, presence of co-morbidities at baseline, eGFR, HbA1c, cholesterol, prescription of statins and glucose-lowering drugs.

Fibrosis scores	Age under 65 (n=5729)		Age 65 and over (6860)		
	Hazard ratio (95% CI)[a]	p-value	Hazard ratio (95% CI)[a]	p-value	p for interaction[b]
FIB4 intermediate	1.17 (0.98-1.39)	0.087	0.99 (0.90-1.10)	0.86	0.16
FIB4 high	3.65 (2.70-4.94)	<0.001	1.45 (1.29-1.62)	<0.001	<0.001
NFS intermediate	1.02 (0.83-1.23)	0.88	1.04 (0.94-1.15)	0.43	0.46
NFS high	2.28 (1.75-2.98)	<0.001	1.33 (1.22-1.46)	<0.001	<0.001
APRI high (n=197)	3.34 (2.29-4.88)	<0.001	1.87 (1.38-2.53)	<0.001	<0.001

Abbreviations:; CI, confidence interval; NFS, NAFLD Fibrosis Score; FIB4, Fibrosis 4 score

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

[b] p value of interaction term in the unstratified data set.

Supplementary Table 3: Numbers of deaths, crude mortality rates and hazard ratios adjusted for age, sex, diabetes duration, smoking, presence of co-morbidities at baseline, eGFR, HbA1c, cholesterol, prescription of statins and glucose-lowering drugs in patients with type 2 diabetes by FIB4, NFS, APRI & AST/ALT categories

Fibrosis scores	Deaths and mortality / 1000 PY	Hazard ratio (95% CI)[b]	p-value
	(Total 3925)		
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=12393)	3831 (40.0)	1.0 (ref)	
APRI high (n=196)	94 (74.0)	3.92 (2.88-5.34)	<0.001
AST/ALT ratio low (n= 3435)	575 (19.6)	1.0 (ref)	
AST/ALT ratio high 0.8 (n=9154)	3350 (49.5)	1.57 (1.38-1.78)	<0.001
AST/ALT ratio low (n=6303)	1288 (24.4)	1.0 (ref)	
AST/ALT ratio high 1.0 (n=6286)	2637 (59.7)	1.76 (1.56-2.00)	<0.001

[a] Number of deaths (Crude incidence rates for 1000 person-years of follow up)
[b]Hazard ratios (95% confidence interval) estimated by Cox regressions adjusted for following covariates: AST, ALT, age, interaction of age and fibrosis score, sex, diabetes duration, BMI, smoking, eGFR at risk, presence of co-morbidities at baseline, mean levels of HbA1c, cholesterol and use of statins and diabetes medications.

Supplementary Table 4: Numbers of deaths, crude mortality rates and hazard ratios adjusted for age, sex, diabetes duration, smoking, presence of co-morbidities at baseline, eGFR, HbA1c, cholesterol, prescription of statins and glucose-lowering drugs in patients with type 2 diabetes by number of high fibrosis scores on the three measures NFS, FIB4 & APRI.

Fibrosis scores	Deaths and mortality / 1000 PY	Hazard ratio (95% CI)[b]	p-value
	(Total 1520)		
High on only one measure (n=2325)	1085 (67.6)	1.0 (ref)	
High only on two measures (n=587)	359 (108.8)	1.95 (1.32-2.90)	<0.01
High on all 3 measures (n=141)	76 (85.7)	2.65 (1.83-3.83)	<0.001

[a] Number of deaths (Crude incidence rates for 1000 person-years of follow up)
[b]Hazard ratios (95% confidence interval) estimated by Cox regressions adjusted for following covariates: age, interaction of age and fibrosis score, sex, diabetes duration, BMI, smoking, eGFR at risk, presence of co-morbidities at baseline, mean levels of HbA1c, cholesterol and use of statins and diabetes medications.

Supplementary Table 5: Crude and multivariate-adjusted mortality rates in patients with type 2 diabetes by FIB4, NFS & APRI categories collected within one year of baseline

Fibrosis scores	mortality rate[a]	Hazard ratio (95% CI)[b]	p-value
	(n=2506)		
FIB4 low (n=5808)	1787 (40.2)	1.0 (ref)	
FIB4 intermediate (n=1278)	439 (45.6)	1.10 (0.90-1.34)	0.37
FIB4 high (n=470)	280 (106.0)	3.86 (2.79-5.35)	<0.001
FIB4 standardised (SD=1.46)[c]		1.11 (1.07-1.16)	<0.001
NFS Low (n=2946)	948 (42.2)	1.0 (ref)	
NFS intermediate (n=2804)	624 (27.7)	1.01 (0.86-1.19)	0.92
NFS High(n=1806)	934 (80.9)	2.41 (1.92-3.01)	<0.001
NFS standardised (SD=1.32)[c]		1.37 (1.22-1.52)	<0.001
APRI low (n=7445)	2448 (43.8)	1.0 (ref)	
APRI high (n=111)	58 (86.8)	4.53 (3.03-6.78)	<0.001
APRI standardised (SD=0.37)[c]		1.07 (1.04-1.10)	<0.001

[a] Number of deaths (Crude incidence rates for 1000 person-years of follow up)
[b]Hazard ratios (95% confidence interval) estimated by Cox regressions adjusted for same covariates as in table 2:
[c] Hazard ratios are estimated for linear increments of one standard deviation for continuous NFS,FIB4 & APRI fibrosis scores.