Non-invasive liver fibrosis scores are strongly associated with liver cancer mortality in general population without liver disease

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Word count:4,798

Number of figures and tables: 5

Number of supplementary figures and tables:4

Abbreviations: ALT, alanine aminotransaminase; APRI, Aspartate aminotransferase to platelet ratio index; AST, aspartate aminotransferase; BMI, body mass index; BP, blood pressure; CI, confidence interval; CVD, cardiovascular disease; EAC, excess alcohol consumption; HBsAg, hepatitis B surface antigen; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; HDL-C, high density lipoprotein cholesterol; HIV, human immunodeficiency virus; HR, hazard ratio; aHR, adjust hazard ratio; ICD-10, International Classification of Diseases and Related Health Problems, 10th Revision; IQR, interquartile range; LDL-C, low density lipoprotein cholesterol; NAFLD, non-alcoholic fatty liver disease; NFS, NAFLD Fibrosis Score; T2DM, type 2 diabetes mellitus.

Funding statement: None.

Conflict of interest disclosure: Nothing to report.

Acknowledgments

CDB is supported in part by the Southampton NIHR Biomedical Research Centre (IS-BRC-20004), UK. MPJ is supported by the National Institute for Health Research through the NIHR Southampton Biomedical Research Centre and Southampton Academy of Research.

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Abstract

Background & aims: In a general population without known liver disease, we tested whether: a) increased liver fibrosis scores (FIB-4 and APRI) are associated with liver cancer mortality, and b) the probability that a person with a higher score died of liver cancer.

Methods: In a retrospective occupational cohort who underwent annual/biennial health examinations (between 2002 and 2015), subjects were excluded with known chronic liver disease. Based on their baseline FIB-4 and APRI scores, subjects were categorised in low/intermediate/high risk groups for advanced liver fibrosis. Using Cox proportional hazards regression analyses adjusted hazard ratios (aHR) were estimated for liver cancer mortality, with the low risk FIB-4/APRI group as the reference. Harrell's C statistics were also calculated.

Results: In 200,479 participants, mean (SD) age was 36.4 (7.7) years. Median follow-up was 4.1 years (IQR 2.10-8.03) with 80 liver cancer deaths. High baseline FIB-4 or APRI scores occurred in 0.25% and 0.09% of subjects, respectively. A high FIB-4 or APRI score was associated with a markedly increased risk of liver cancer mortality (aHRs 629.10 [95% CI 228.74-1730.20]; and 80.42 [95% CI 34.37-188.18]), respectively. C statistics were FIB-4=0.841 (95% CI; 0.735-0.946); APRI=0.933 (95% CI; 0.864-0.999).

Conclusions: In a general population without known liver disease, high FIB-4 or high APRI (in keeping with a high probability of advanced fibrosis) occurred in 0.25% (FIB-4) and 0.09% (APRI) of subjects. Both scores were associated with a markedly increased risk of liver cancer mortality and FIB-4 and APRI models both strongly predicted liver cancer mortality.

Keywords: Liver Fibrosis Scores, Liver Cancer, Mortality

Lay Summary/Key Points

- **Primary liver cancer is a major cause of death but in the general population there are no good predictors to identify subjects at high risk of liver cancer mortality**
- **In 200,479 subjects not known to have existing liver disease, high FIB-4 or high APRI scores were both independently associated with a markedly increased risk of liver cancer mortality. Both high FIB-4 or high APRI scores were very good predictors of liver cancer mortality**
- **FIB-4 or APRI scores are easy and inexpensive to measure. In the general population high FIB-4 or high APRI scores could be used to target subjects at high risk of liver cancer mortality.**

1. INTRODUCTION

Hepatocellular carcinoma is far the most common form of primary liver cancer (comprising 75-85% of cases).[1] The incidence of liver cancer and specifically hepatocellular carcinoma (HCC) varies by country, depending on the relative prevalence of certain aetiological risk factors.[2] However, the global incidence of HCC has continued to increase in recent years, rising by 38% between 2006 and 2016.[3] This appears to be predominantly attributable to population aging and population growth[3] and cases of HCC due to NAFLD are predicted to increase between 2016 and 2030.[4] In the United States the number of prevalent cases of HCC due to NAFLD is expected to rise by as much as 130% between 2016 and 2030.[4] However, although NAFLD is estimated to affect up to a quarter of the global population, it remains the small minority of people with NAFLD who develop HCC.[5] Therefore, who to screen and how to screen for HCC remains a point of considerable debate.[6]

An unmet clinical need is how to identify individuals in the general population with undiagnosed liver disease who are at risk of liver complications such as HCC.[7] The early diagnosis of HCC is particularly important because of the poor prognosis and lack of curative options if HCC is diagnosed late in the course of the disease. Since liver fibrosis is an important risk factor for HCC, an attractive strategy to identify individuals in the general population who are at increased risk of HCC, is to use non-invasive scores that utilise simple, already available serum parameters that have been validated as useful proxy markers for liver fibrosis. One such liver fibrosis score is the FIB-4 score which is a non-invasive score that was initially developed to predict risk of significant fibrosis in populations with chronic hepatitis C (HCV) and human immunodeficiency virus (HIV) co-infection.[8] Aspartate aminotransferase (AST) to platelet ratio index (APRI) is another non-invasive score which was similarly developed to predict risk

of significant fibrosis in chronic HCV infection.[9] Both FIB-4 and APRI have been subsequently validated in populations with fatty liver disease.[10]

In patients with known NAFLD confirmed by liver biopsy, FIB-4 and APRI also appear to be predictive of liver-related outcomes including liver transplantation and liver-related mortality.[11,12] A large population-based study by Kim and colleagues, evaluated the ability of non-invasive scores to predict mortality in individuals with a diagnosis of NAFLD based on ultrasonography.[13] The scores that were evaluated included FIB-4, APRI and another non-invasive score, the NAFLD Fibrosis Score (NFS). The results in the study by Kim and colleagues demonstrated that APRI and NFS were predictive of all-cause mortality in individuals with NAFLD, and the data also suggested that FIB-4 may also predict all-cause mortality. There is some evidence in a general population (without chronic viral hepatitis) that the non-invasive score APRI shows an association with increased all-cause mortality, cancer-related mortality and liver-related mortality after adjustment for age.[14] The same study demonstrated that high scores of FIB-4 were associated with all-cause mortality, CVD-related mortality and liver-related mortality after adjustment for age. Furthermore, Hagstrom and colleagues have recently demonstrated the ability of APRI and FIB-4 (in addition to three other non-invasive scores of fibrosis risk) to predict the risk of the composite clinical outcome of 'severe liver disease' in a general population without known liver disease.[15]

However, to date these aforementioned studies have not been able to demonstrate whether there is an association between non-invasive markers FIB-4 and APRI, and liver cancer-related mortality. The performance of non-invasive scores for primary liver cancer in individuals without known liver disease would be of considerable importance for future consideration of a

screening programme for HCC, something which is not yet advocated by international guidelines.[16,17]

Consequently, our aim was to test the hypothesis that high FIB-4 and APRI scores are associated with liver cancer mortality in the general population. Specifically, we aimed to assess whether FIB-4 and APRI scores are associated with increased risk of liver cancer mortality in a general population cohort with negative viral serology, low levels of alcohol consumption, and no evidence of fatty liver disease on abdominal ultrasound, after adjusting for key potential confounders that included age, sex, body mass index (BMI) and other comorbidities.

2.0 METHODS

2.1 Study population

We utilised a retrospective study design of a cohort of adult Korean men and women who underwent well person health checks in a comprehensive health screening programme at Kangbuk Samsung Hospital, Seoul, Korea from 2002 to 2015 (N = 284,646). The Kangbuk Samsung Health Study is a cohort study of Korean men and women who underwent an annual or biennial health examination at one of the Kangbuk Samsung Hospital Total Healthcare Centres in Seoul and Suwon, South Korea.[18]

The health screening program at Kangbuk Samsung Hospital aims to promote health through early detection of chronic diseases and their associated risk factors. In Korea, employees are required by the Industrial Safety and Health Law to participate in annual or biennial health checks. About 80% of the participants in the cohort that we studied were either employees or spouses of employees of various companies and local governmental organisations. The remaining participants registered individually for the programme. Participants who were excluded from the final analysis were: participants with missing data at baseline; participants who were hepatitis B surface antigen (HBsAg) positive or HCV antibody positive; participants with evidence of fatty liver disease on ultrasound; participants with a history of excessive alcohol consumption, defined as alcohol intake ≥30 g/day for men and ≥20 g/day for women; participants with a history of malignancy; participants with missing data at follow-up; participants aged <20 years old. After exclusions, there was a total of 200,479 participants who were eligible for analysis.

This study was approved by the Institutional Review Board of Kangbuk Samsung Hospital. The review board exempted the requirement for informed consent because de-identified data was used for the analysis.

Mortality follow-up until the end of 2017 was based on Korean death certificate data from the Korea National Statistical Office. All Korean deaths are reported to the Korea National Statistical Office. We determined the cause of death using the underlying cause listed on each death certificate, as classified according to the International Classification of Diseases and Related Health Problems, 10th Revision (ICD-10). A previous study has evaluated the accuracy of Korean death certificate data.[19] This demonstrated that concordance between the cause of death on the death certificate and patient diagnosis from medical insurance data was 72.2% for all cause deaths and 94.9% for cancer deaths.[19]

2.2 Measurements

Data on medical history, medication use, and health-related behaviours were collected through a self-administered questionnaire. The self-administered questionnaire included an assessment of alcohol intake and the details of the questions used have been fully described previously.[20] In short, both the frequency of intake of alcohol per week and the average intake of alcohol per drinking day were recorded. Excessive alcohol consumption was defined as alcohol intake ≥30 g/day for men and ≥20 g/day for women and subjects consuming this level of alcohol were excluded from the analyses. Current smokers were identified and the weekly frequency of moderate- or vigorous-intensity physical activity was assessed. Physical measurements and serum biochemical parameters were measured by trained staff. Trained nurses measured sitting blood pressure with standard mercury sphygmomanometers. Blood specimens were sampled from the antecubital vein after more than 12 hours of fasting. Serum levels of glucose, total

cholesterol, triglyceride, low-density lipoprotein cholesterol (LDL-C), and high-density lipoprotein cholesterol (HDL-C) were measured using Bayer Reagent Packs (Bayer Diagnostics, Leverkusen, Germany) on an automated chemistry analyzer (Advia 1650™ Autoanalyzer; Bayer Diagnostics, Leverkusen, Germany). Regular calibration and quality control measurements were performed throughout the study period using a validated calibrator and quality control materials. The clinical laboratory has been accredited and participates annually in inspections and surveys by the Korean Association of Quality Assurance for Clinical Laboratories. Liver ultrasonography was performed at health checks. Ultrasonography is an adequate modality of imaging for detection of hepatic steatosis providing there is >30% hepatic steatosis.[21,22] Participants who had evidence of hepatic steatosis on ultrasonography were categorised as fatty liver disease and these subjects were excluded from the presented analyses.

The FIB-4 index was calculated with the following formula: FIB-4 = (age [years] × AST [U/L]) / (platelet count [$\times 10^9$/L] × √ALT [U/L]). Cut-off values from the curve were used to define low (FIB-4 ≤ 1.30), intermediate (1.30 < FIB-4 < 2.67), and high (FIB-4 ≥2.67) probabilities of advanced fibrosis.[23] The APRI was calculated with the following formula: APRI = 100 × (AST / AST upper limit of normal) / platelet count ($\times 10^9$/L). Cut-offs for low and high probabilities of advanced fibrosis were 1 and 2, respectively.[9]. Using these thresholds low risk (<1), intermediate risk (1-2) and high risk (≥2) categories were developed.

2.3 Statistical analysis

Subjects were categorised into three groups based on their FIB-4 and APRI categories (low, intermediate and high risk). The baseline characteristics of the study participants as well as their respective FIB-4 and APRI categories are presented as mean (standard deviation), median

(interquartile range), or number (percentage), as appropriate. Hazard ratios (HRs) and adjusted HRs (aHRs) and 95% confidence intervals (CIs) for all-cause and cancer-related mortality were estimated using Cox proportional hazards regression analyses with the low FIB-4 or low APRI score as the reference group. Two Cox regression models were used. Model 1 was adjusted for sex, year of screening examination, centre, BMI, smoking status, regular exercise, educational level, history of diabetes, and history of hypertension. Model 2 was Model 1 and adjustment for LDL-C, HDL-C, triglycerides, systolic blood pressure, glucose, alcohol intake. Age was used as the time scale, which was documented by the age at which subjects underwent their first health check-up examination (left truncation) and the age at which subjects exited the analysis at the date of death or on December 31, 2017. We conducted time-dependent analyses and covariates during follow-up were updated as time-varying covariates in the models. All reported P values were 2 tailed. Differences with a P value < 0.05 were considered statistically significant.

3. RESULTS

284,646 men and women participated in a comprehensive health screening program at Kangbuk Samsung Hospital, Seoul, Korea from 2002 to 2015. The following participants were excluded: 95 participants who were less than 20 years old, 3,418 participants with a history of cancer, 5,145 participants with missing baseline data, 829 participants with missing follow-up data, 4,453 participants with missing HBV and/or HCV serology, 73,581 participants with fatty liver disease on ultrasound at baseline. Data from 200,479 participants were analysed in the study as some subjects were excluded because of the presence of >1 exclusion criteria. The median follow-up of the participants was 4.1 years (IQR 2.10-8.03). The baseline characteristics of participants are summarised in **Table 1**. The mean age of the cohort was 36.4 years (± 7.7 years). There were differences between men and women. These differences

included: more male smokers (40.73% v 2.49%), more overweight males (23.78% v 8.36%), higher mean alcohol intake amongst males, proportionally more T2DM in males (1.75% v 0.73%), proportionally more hypertension in males (13.32% v 4.52%), more males with high FIB-4 score (0.37% v 0.14%), more males with high APRI score (0.16% v 0.03%).

The number of participants alive and dead at the end of follow-up is also summarised in **Table 1**. 510 (0.25%) participants had a high FIB-4 score. Amongst the 510 participants with a high FIB-4 there were 40 deaths, which is a crude death rate of 1,913 deaths per 100,000 persons per year. Amongst the 184 (0.09% participants with a high APRI score there were 10 deaths, which is a crude death rate of 1,326 deaths per 100,000 persons per year. For comparison, the crude death rate was substantially lower in subjects with T2DM or hypertension. For example, there were 717 deaths per 100,000 persons per year in subjects with T2DM and 371 deaths per 100,000 persons per year in subjects with hypertension.

Considering the high observed number of deaths per 100,000 persons per year amongst participants with high FIB-4 and APRI scores, we then sought to evaluate the association between: (1) FIB-4 and mortality; (2) APRI and mortality. We analysed the association between intermediate and high-risk FIB-4 or APRI scores and mortality for the entire cohort. For this analysis, we defined two mortality outcomes: (1) all-cause mortality, and (2) cancer-related mortality. We used either the low probability of advanced liver fibrosis FIB-4 or APRI scores respectively as the reference groups (see **Table 2** and **Table 3**). These results showed that for individuals with intermediate and high-probability of advanced liver fibrosis FIB-4 or APRI scores, there was an increased risk of all-cause mortality. **Table 2** summarises the aHR for mortality categorised by FIB-4 score, with the low-risk FIB-4 score as the reference category. After adjusting for sex and age, for a high-risk FIB-4 score, the magnitude of increase

in mortality risk was greater for both all-cause mortality (aHR 5.16 [95% CI 3.93-6.78]) and cancer-related mortality (aHR 8.55 [95% CI 6.04-12.10]). (Since age is a variable in the FIB-4 formula, it is important to stress that age was also adjusted for in the analyses).

The increase in all-cause mortality and cancer-related mortality for intermediate and high-risk FIB-4 scores was also observed for intermediate and high-risk APRI scores. **Table 3** summarises the aHR for all-cause mortality and cancer-related mortality when categorised by APRI score, with low APRI score as the reference category. After adjusting for age and sex, the magnitude of this mortality risk was again higher for a high-risk APRI score, both all-cause mortality (aHR 14.22 [95% CI 8.66-23.34]) and cancer-related mortality (aHR 18.06 [95% CI 9.63-33.84]).

In view of the markedly increased cancer-related mortality risk in the high probability of advanced liver fibrosis groups, we then investigated individual causes of death. These are summarised in **Table 4**. Cancer-related death was proportionally the single largest category for cause of death (49.7% of all deaths) in the cohort. Cardiovascular (CVD)-related deaths only made up 10.6% of all deaths. Malignant neoplasm of bronchus and lung (18.6% of cancer-related deaths) was the most common type of cancer-related death. Malignant neoplasm of liver and intrahepatic bile ducts ('liver cancer death') was the second most common type of cancer-related death (15.7% of cancer-related deaths). The characteristics of those individuals who died from liver cancer is described in full in **Supplementary Table S1**.

We then sought to evaluate whether there was an independent association between FIB-4 (or APRI) and liver cancer (HCC) mortality after adjustment for age, sex and covariates as shown in **Table 5**. There were 80 liver cancer deaths and of these 5, 41 and 34 deaths occurred in the

low, intermediate and high probability of advanced fibrosis FIB-4 groups, respectively. Only a small proportion of subjects had either a high FIB-4 score (0.25%) or a high APRI score (0.09%). Remarkably, in the high probability of advanced fibrosis FIB-4 group 34/510 (6.7%) of subjects died from liver cancer during follow up. Similarly, for APRI, 57, 15 and 8 deaths occurred in the low, intermediate and high probability of advanced fibrosis APRI groups, respectively. In support of the FIB-4 data, in the high probability of advanced fibrosis APRI group 8/184 (4.3%) of subjects in this group died from liver cancer. These data also showed that both FIB-4 and APRI scores were extraordinarily strongly associated with liver cancer mortality. Participants with either a high FIB-4 score or a high APRI score had a markedly increased risk of liver cancer mortality (aHR 629.1 [95% CI 228.7-1730.2]) and aHR 80.4 [95% CI 34.4-188.2], respectively). In view of the strong association between FIB-4 (and APRI) and liver cancer mortality, we then analysed whether there was any association between FIB-4 (and APRI) and mortality due to a different cancer type. As lung cancer was another common cancer cause of death, we tested for an association between FIB-4 (and APRI) and lung cancer mortality. These data (**Table 5**) showed there was no increased risk of lung cancer mortality with either high FIB-4 or APRI scores. Given the much smaller number of deaths for other causes of cancer-related mortality we did not test for an association between FIB-4 (and APRI) and other cancer types.

We tested the probability that a person with a higher FIB-4 or APRI score died of liver cancer. These data showed that the Harrell's c-index were for FIB-4=0.841 (95% CI; 0.735-0.946); and for APRI=0.933 (95% CI; 0.864-0.999). We calculated the sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) for liver cancer mortality at 3 years, 5 years and 10 years. Sensitivity was 6.3 (2.1-14), 25 (16-35.9) and 63.7 (52.2-74.2) respectively. Specificity was 99.6 (99.5-99.6), 89 (88.8-89.1) and 49.9 (49.7-50.1)

respectively. PPV was 0.55 (0.2-1.3), 0.09 (0.1-0.1) and 0.05 (0.00-0.10) respectively. NPV was 100 (100-100), 100 (100-100), 100 (100-100) respectively.

We performed a sensitivity analysis which censored incident HBV and HCV cases and this demonstrated the same marked association between high FIB-4 score and liver cancer mortality (aHR 880.46 [95% CI 241.8-3205.8]), and the same marked association between high APRI score and liver cancer mortality (aHR 121.87 [48.48-306.31]). (See Supplementary Table S2). A sensitivity analysis of individuals aged ≥ 35 years old and < 65 years old demonstrated the same marked association between high FIB-4 score (and APRI) and liver cancer mortality. (See Supplementary Table S3) This sensitivity analysis was performed because a previous study by McPherson and colleagues suggested FIB-4 may perform optimally in individuals aged ≥ 35 years old and < 65 years.[24] We performed a sensitivity analysis for individuals aged > 50 years but the relatively small number of individuals > 50 years in the entire population limited the analysis performed. (See Supplementary Table S4). Finally, we performed a sensitivity analysis which censored individuals with liver cancer-related mortality in the first year. However, there were no liver cancer deaths in the first year.

4. DISCUSSION

Our novel results show that in a large general population who are not known to have existing liver disease, and in whom we have excluded known common causes of chronic liver disease, increased FIB-4 or APRI scores are very strongly associated with increased risk of liver cancer mortality. Given that the predominant type of primary liver cancer is HCC, the most likely explanation for this finding is that there is a significant amount of undiagnosed liver fibrosis in the general population. We have also demonstrated that increased FIB-4 and APRI scores are

independently associated with all-cause mortality in a general population of working age. Given that age is one of the factors incorporated into the FIB-4 score, we also adjusted for this factor in our analyses. We also performed the same analyses using APRI, which does not incorporate age as a factor in the score. The finding that a high-risk APRI score was also associated with all-cause mortality verifies the high-risk FIB-4 score results.

The observation that FIB-4 and APRI are independently associated with liver cancer mortality has not been previously described in a general population. However, our findings are consistent with, and extend, previous findings as well. In 2017 Unalp-Arida & Ruhl demonstrated that both FIB-4 and APRI were associated with liver disease mortality (and all-cause mortality) in a much smaller unselected population of 14,841 participants in the third National Health and Nutrition Examination Study (NHANES III).[25] This study by Unalp-Arida & Ruhl also demonstrated an association between high APRI score and neoplasm mortality but unlike our study did not specifically evaluate any association between FIB-4 or APRI and liver cancer mortality. Kim and colleaguesa[13] previously carried out a population-based study which evaluated subjects with evidence of NAFLD in NHANES and demonstrated that APRI (and NFS) were associated with all-cause mortality in patients with NAFLD identified by ultrasonography. FIB-4 and APRI scores have also been shown to be associated with mortality in previous cohort studies of individuals with biopsy-proven NAFLD.[12,26,27] However, a 2018 systematic review and meta-analysis[28] of eight studies (four of which provided data for FIB-4 and APRI) investigated the association between non-invasive scores and mortality, and this analysis included the quoted studies by Kim et al[13] and Angulo et al[12]. In contrast to our results obtained in a much larger single cohort, this meta-analysis of four studies with data for FIB-4 and APRI scores failed to show that either FIB-4 or APRI scores were associated with all-cause mortality.[28]

Furthermore, as mentioned in the introduction, Hagstrom and colleagues recently demonstrated that the non-invasive markers FIB-4 and APRI appear to predict composite liver-related outcomes in a general population.[15] The study by Hagstrom and colleauges[15] utilised the AMORIS (Apolipoprotein-related MOrtality RISk) cohort which was originally set up to test if levels of apolipoprotein B and apolipoprotein A-I were more closely related to fatal myocardial infarction and stroke than conventional lipids.[29] In the AMORIS cohort, 26% of laboratory analyses in the cohort represented routine health screenings and 24% represented occupational health screening, whereas the remaining 50% of laboratory analyses represented outpatient care. Information on alcohol intake was not available. Therefore, there was presumably a proportion of the cohort with known liver disease, including alcohol related liver disease. This is in contrast to the population enrolled in the Kangbuk Samsung Health Study which represents a general population who underwent occupational health screening through their employer or who individually registered for the program, and which included detailed information on alcohol intake.

It is notable that cancer-related death was the most common cause of death in this general population of working age. This finding is consistent with previous evidence which has suggested that, in high income countries, cancer-related death is more common than CVD-related death.[30] The relationship between FIB-4, APRI and cancer is of considerable interest and could have clinical relevance for identifying subjects at increased risk of HCC in the general population. On the numerator of the FIB-4 and APRI equations, an increased concentration of aspartate aminotransferase (AST) increases both FIB-4 and APRI scores. Although alanine aminotransferase (ALT) is more liver-specific[31], a proportionally greater rise in AST has long been established as a predictor of significant fibrosis.[32] However, despite the independent association between an isolated increase in AST and all-cause mortality, there is

not a demonstrable independent association between AST and HCC mortality.[33] Possibly more relevant to predicting HCC mortality is the role of platelet count as one of the denominators in both the FIB-4 and APRI equations. Platelets play a fundamental role in the coagulation cascade, clot formation, wound healing and tissue repair.[34] Platelets also aid clot formation in the context of inflammation and may contribute to pathological processes which include atherosclerosis and thrombosis.[35] However, despite the fact that thrombocytosis and thrombocytopenia are both independent predictors of all-cause mortality and cancer-related mortality[36–38], it is thrombocytopenia which causes a high FIB-4 or APRI score. In an unselected population one would expect that the commonest cause of unexplained thrombocytopenia would be undiagnosed liver disease with portal hypertension.[39] Platelet count was incorporated into the FIB-4 and APRI scores on this basis and both scores have been validated in multiple studies of patients with biopsy proven fibrosis due to viral hepatitis and NAFLD.[40–42] Screening for HCC is recommended for individuals at highest risk, and that high risk group predominantly constitutes patients who have liver cirrhosis.[43] However, portal hypertension (which causes sequestration of platelets with thrombocytopenia) is also associated with an increased risk of HCC independent of cirrhosis.[44] Therefore, there is a considerable body of evidence and biological plausibility to explain the association between FIB-4 (and APRI) and liver cancer mortality demonstrated in our findings.

There are some limitations in our study. It is possible that there were other types of primary liver cancer, other than HCC (ICD-10 C22.0), which were coded as 'malignant neoplasm of liver and intrahepatic bile ducts' (ICD-10 C22). Unfortunately, ICD-10 data on the C22 sub-sets were not available for these analyses to specifically identify HCC. However, the predominant form of other primary liver cancer, which is intrahepatic cholangiocarcinoma (ICD-10 C22.1), globally only constitutes 10-15% of liver cancers[1] and therefore at most we

would predict that 8-12 of 80 liver cancers in our cohort would be this form of liver cancer. Furthermore, FIB-4 and APRI are well validated predictors of fibrosis and in the absence of primary sclerosing cholangitis fibrosis is not typically recognised as a risk factor for intrahepatic cholangiocarcinoma.[40–42,45] We have considered the possibility of reverse causality in our study because some participants may have had liver cancer that was not detected on ultrasound at the outset of the study period. Although this is possible, liver fibrosis rather than liver cancer increases FIB-4 or APRI scores. Therefore, if a primary liver cancer was present (but undetected) at baseline, it is likely that a primary liver cancer (e.g. HCC) would have been present with its associated risk factor, i.e. liver fibrosis. Therefore, the presence of co-existing liver fibrosis, rather than HCC *per se*, would have increased FIB-4 or APRI scores. Also most HCCs have a rapid doubling time of under 3 months and so the presence of HCC quickly becomes evident on interval imaging.[46]

It is important to consider the accuracy of death certification data and the potential for misclassification bias in our study. There is previous evidence which demonstrates 94.9% concordance between medical insurance data and death certificate for causes of cancer death in South Korea.[19] Nonetheless, there is the possibility of misclassification between liver cancer types, for example the possibility of liver metastases (ICD-10 code C22.9). However, there is no previous evidence to suggest that there is an association between raised FIB-4 or APRI score and hepatic metastases. After adjusting for age it is unclear what biological plausibility there would be for an association between hepatic metastases and raised FIB-4 (or APRI), given that in a chronic inflammatory state such as metastatic malignancy, platelets would tend to be higher and it has been demonstrated previously that high platelets are a risk factor for undiagnosed cancer.[47] High platelets would result in a lower FIB-4 and APRI score.

Furthermore, although the high observed liver cancer-related mortality in our study is striking, this finding is congruent with that of a previous study by Bertuccio et al which demonstrated that HCC mortality is exceedingly high in Korea, higher than 26 other counties globally, even in the 20-44 age demographic.[48] Furthermore, this study by Bertuccio et al considered ICD-10 codes C22.0, C22.2, C22.3, C22.4, C22.7 and, excluded ICD-10 code C22.9 (Liver, unspecified), suggesting that the high liver cancer mortality in Korea is not due to misclassification of liver metastases.

There is also the possibility that the systematic questionnaire used to assess alcohol intake was not accurate in all study participants. However, ultimately this is reflective of real world clinical practice where patients may not admit to previous alcohol excess and misjudge their current intake. Also, ultrasonography has a sensitivity of 67% with regards to the detection of any degree of liver steatosis, and a sensitivity of 89% for detectging hepatic steatosis >30%.[22] On this basis, we assume that the observed strong association between high FIB-4/high APRI scores and liver cancer mortality risk, is due to undiagnosed liver disease that remains undetected by ultrasonography in the study. Thus, it plausible to conclude that clinicians may be falsely reassured by a 'normal' ultrasound result, when in fact, there is already advanced fibrotic liver disease with minimal liver steatosis that is below the limit of detection of ultrasound.

A strength of our study is the large sample size. However, there were relatively few cases of liver cancer death. In view of the number of covariates used in the Cox regression analysis, it is possible that the model was overfitted. Therefore, the findings need to be replicated in another study.

Importantly, only a small proportion of the general population have either a high FIB-4 score (0.25%) or a high APRI score (0.09%). That said, there was a striking increase in risk of liver cancer mortality in this group, with ~5% of subjects with high baseline FIB-4 or APRI scores dying from liver cancer during follow up. Thus, it is plausible to speculate that identifying high FIB-4 or high APRI scores, could have very important implications for targeting a group of patients who need HCC surveillance in the general population who are not known to have existing liver disease. Currently, there is no accepted strategy for screening for subjects at high risk of HCC in the general population, and we suggest it now needs to be tested whether high FIB-4 or APRI scores have clinical utility for predicting risk of HCC.

5. CONCLUSION

In a general population not known to have existing liver disease, high FIB-4 and APRI scores in keeping with a high probability of advanced liver fibrosis occur in 0.09% (APRI) and 0.25% (FIB-4) % of subjects. Both high FIB-4 and high APRI scores are associated with a very marked increase in risk of liver cancer mortality.

ABBREVIATIONS

APRI, Aspartate aminotransferase to platelet ratio index; AST, aspartate aminotransferase; BMI, body mass index; CI, confidence interval EAC, excess alcohol consumption; HBsAg, hepatitis B surface antigen; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; HDL-C,

high density lipoprotein cholesterol; HIV, human immunodeficiency virus; HR, hazard ratio; aHR, adjust hazard ratio; IQR, interquartile range; LDL-C, low density lipoprotein cholesterol; NAFLD, non-alcoholic fatty liver disease; NFS, NAFLD Fibrosis Score; T2DM, type 2 diabetes mellitus

ACKNOWLEDGEMENTS

CDB is supported in part by the Southampton NIHR Biomedical Research Centre (IS-BRC-20004), UK.

MPJ is supported by the National Institute for Health Research through the NIHR Southampton Biomedical Research Centre and Southampton Academy of Research.

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