Research Article

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Using non-invasive biomarkers to identify hepatic fibrosis in people with type 2 diabetes mellitus: The Edinburgh type 2 diabetes study

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Background & Aims: It is difficult to determine the different stages of non-alcoholic fatty liver disease without the use of invasive liver biopsy. In this study we investigated five non-invasive biomarkers used previously to detect hepatic fibrosis and determined the level of agreement between them in order to inform future research.

Methods: In the Edinburgh Type 2 Diabetes Study, a population-based cohort aged 60–74 years with type 2 diabetes, 831 participants underwent ultrasound assessment for fatty liver and had serum aspartate aminotransferase to alanine aminotransferase ratio (AST/ALT), aspartate to platelet ratio index (APRI), European Liver Fibrosis panel (ELF), Fibrosis-4 Score (FIB4) and liver stiffness measurement (LSM) measured.

Results: Literature based cut-offs yielded marked differences in the proportions of the cohort with probable liver fibrosis in the full cohort. Agreement between the top 5% of the distribution for each biomarker pair was poor. APRI and FIB4 had the best positive agreement at 76.4%, but agreement for all of the other serum biomarker pairs was between 18% and 34%. Agreement with LSM was poor (9–16%).

Conclusions: We found poor correlation between the five biomarkers of liver fibrosis studied. Using the top 5% of each biomarker resulted in good agreement on the absence of advanced liver disease but poor agreement on the presence of advanced disease. Further work is required to validate these markers against liver biopsy and to determine their predictive value for clinical liver-related endpoints, in a range of different low and high risk population groups.

Keywords: Hepatic fibrosis; Non-invasive biomarker tests; Non-alcoholic fatty liver disease; Type 2 diabetes mellitus.

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Abbreviations: NAFLD, non-alcoholic fatty liver disease; NAFL, non-alcoholic fatty liver; NASH, non-alcoholic steatohepatitis; CLD, chronic liver disease; NFS, NAFLD Fibrosis Score; BMI, body mass index; ET2DS, Edinburgh Type 2 Diabetes Study; LDR, Lothian Diabetes Register; USS, ultrasound scanning; TE, transient elastography; ALT, alanine aminotransferase; AST, aspartate aminotransferase; HA, hyaluronic acid; P3NP, aminoterminal peptide of pro-collagen III; TIMP1, tissue inhibitor of matrix metalloproteinase 1; SCD, skin capsule distance; LSM, liver stiffness measure; CVH, chronic viral hepatitis; PBC, primary biliary cirrhosis; AST/ALT, ratio alanine aminotransferase to aspartate aminotransferase ratio; APRI, aspartate to platelet ratio index; ELF, European Liver Fibrosis panel; FIB4, Fibrosis-4 Score; kPa, kilopascals; NPV, negative predictive value; spec., specificity.

Introduction

Liver dysfunction in people with type 2 diabetes mellitus is thought to be mainly caused by non-alcoholic fatty liver disease (NAFLD). The earliest stage of NAFLD is simple steatosis but this can progress to non-alcoholic steatohepatitis (NASH) and ultimately to hepatic fibrosis, cirrhosis and the long term complications of chronic liver disease (CLD) such as hepatocellular carcinoma. The prevalence of NAFLD is thought to be higher in type 2 diabetes than in the general population [1–4]. Research focusing on the identification of fatty liver using ultrasound suggests a prevalence of around 34% in the general population [1]; in type 2 diabetes our own group found the prevalence to be 42.6% [2] and this figure may rise to 70% in more selected sub-populations of diabetes [3,4]. The prevalence of NASH and NASH-related fibrosis is much harder to determine as currently the only widely accepted diagnostic method is liver biopsy. However, it is difficult to justify performing liver biopsy to determine the severity of liver disease in community based subjects, including volunteers in research settings for two key reasons (i) there is considerable variability in sampling and histopathological interpretation due

to the small volume of tissue sampled (typically 0.002% of the liver) [5] and subjective semi-quantitative scoring systems [6–8], and (ii) biopsy is associated with an adverse outcome profile including pain, bleeding and rarely death [9–12]. Thus, there is considerable interest in the adoption of validated non-invasive markers of fibrosis into clinical practice. In the few biopsy studies of populations with type 2 diabetes, the prevalence of advanced fibrosis in those with NAFLD was 7–12% [13–15].

Non-invasive markers have been extensively validated in secondary care for the diagnosis of hepatic fibrosis either for specific underlying pathologies (e.g., the NAFLD Fibrosis Score, NFS) or with varying disease specific cut-offs. There are three broad groups of biomarkers: single markers, combination marker panels and imaging. Increasing numbers of scales and scores are being developed, with most studies reporting acceptable diagnostic accuracy (AUC >0.7) for individual methods in diagnosing the presence of hepatic fibrosis in NAFLD. However, their reliability and utility in identifying undiagnosed liver fibrosis in wider clinical practice and in research settings is yet to be determined given the limited studies in primary care [16,17]. Our group has previously shown [18] that the utility of many simple marker panels (BAAT score, BARD score, NFS) is limited in a population with type 2 diabetes by the inclusion of age, body mass index (BMI) and diabetes and led to over-estimation of the prevalence of fibrosis and high levels of indeterminate results.

In this study we investigated five biomarkers used previously to detect hepatic fibrosis in clinical populations with NAFLD. We aimed to determine the level of agreement between these biomarkers, in a large, representative, well-phenotyped population of people with type 2 diabetes mellitus (the Edinburgh Type 2 Diabetes Study, ET2DS).

Patients and methods

Study population

Full methods of the ET2DS have been published previously [19]. In brief, patients aged 60–74 years were selected at random from the Lothian Diabetes Register (LDR), a comprehensive register of patients with diabetes living in Lothian, Scotland. 1066 patients were recruited and attended a baseline clinic for physical examination. Study recruits have been shown previously to be largely representative of all those randomly selected to participate (n = 5454) and therefore of the target population of older men and women with type 2 diabetes living in the general population [20]. Participants who were able and willing (n = 939) attended a liver assessment 1 year after baseline, including liver ultrasound scanning (USS) [21]. Subjects who were still living were invited to a further detailed assessment approximately four years after recruitment; these subjects (n = 831) form the study population for the current analysis.

Ethical approval was obtained from the Lothian Research Ethics Committee and all subjects gave written informed consent.

Clinical examination and liver assessment

Clinical examination at the year 1 liver assessment and year 4 follow-up was similar to that performed in earlier phases of the study, described in detail previously [19]. In brief, patients underwent physical examination (including height and weight measurements); venepuncture; self-administered questionnaire (including alcohol consumption) and liver imaging (including USS and transient elastography (TE)). Plasma glucose, HbA1c, platelets and liver enzymes (including alanine aminotransferase (ALT), aspartate aminotransferase (AST)) and albumin were measured on a fasting blood sample using a Vitros Fusion chemistry system (Ortho Clinical Diagnostics, Bucks, UK). European Liver Fibrosis (ELF) panel – comprising hyaluronic acid (HA), aminoterminal peptide of pro-collagen III (P3NP) and tissue inhibitor of matrix metalloproteinase 1 (TIMP1) – was measured using the ADVIA Centaur immunoassay system (Siemens Healthcare Diagnostics Inc, New York, USA) on serum stored at −80 °C. USS was performed using a Sonoline Elegra Ultrasound Imaging System (Sieman's Medical Systems Inc, Washington, USA), software version 6, using a 3.5 MHz transducer. A phantom (411 LE 0.5, GAMMEX rmi Ltd, Nottingham, UK) as described and validated previously using magnetic resonance spectroscopy [21], hepatic steatosis was graded as present or absent based on standard criteria.

One dimensional TE was performed using a FibroScan (Echosens, Paris, France) machine at the year 4 follow-up visit only. A single operator was formally trained by Echosens personnel prior to commencement of the study. Initial ultrasound assessment allowed measurement of the skin-capsule distance (SCD). For SCDs <2.5 cm the M probe was used, for SCDs $\geqslant$2.5 cm the XL probe was used in accordance with recommended standard Fibroscan operating procedures. The TE probe was placed in an intercostal space overlying the liver with the patient in the supine position. Using ultrasound to guide positioning, an area of the liver that was at least 6 cm deep and free from large vessels was selected for investigation. The area measured was between 25 mm-65 mm below the surface of the skin for the M probe and 35 mm-75 mm for the XL probe. The operator aimed to obtain ten valid liver stiffness measurements (LSM) with a success rate of at least 60% and IQR <30% of the final (median) result. All scans were undertaken in the fasting state (minimum 4 h). Every six months the probes were serviced and calibrated.

Any patient with plasma liver enzymes above the upper reference limit, any abnormality on liver USS (including steatosis) or LSM >8 kPa underwent a liver screen including viral serology, alpha-feto protein, ferritin, autoantibodies, immunoglobulins, caeruloplasmin and α1-antitrypsin. In addition, pre-diagnosed liver disease was identified from NHS National Services Scotland, Information Services Division data linkage to SMR01 general and acute inpatient discharge records and from patient self-report questionnaires on prior health conditions. Any liver disease identified from linkage and the patient questionnaire was confirmed using individual patient medical records and patients with confirmed pre-diagnosed liver disease (chronic viral hepatitis, CVH, haemochromatosis and primary biliary cirrhosis, PBC) were excluded from the final analyses.

Data analysis

The five biomarkers/panels evaluated in this investigation were derived as follows:

- Aspartate aminotransferase to alanine aminotransferase ratio (AST/ALT) calculated as AST(IU/L)/ALT(IU/L).
- Aspartate to platelet ratio index (APRI) calculated as [AST(IU/L)/upper limit normal]/platelets($\times 10^9$/L)] $\times$ 100 [22].
- European Liver Fibrosis panel (ELF) calculated as 2.588 + (ln(HA)$*$(ln(P3NP)$*$0.775) + (ln(TIMP1)$*$0.494) [23].
- Fibrosis-4 Score (FIB4) calculated as [age(years) $\times$ AST(IU/L)]/[platelets($\times 10^9$/L) $\times$ $\sqrt{}$AST(IU/L)] [24].
- Liver stiffness measurement in kilopascals (kPa) expressed as the median TE value from at least ten valid measurements.

Absolute change in serum biomarker was defined as the change between the year 1 liver assessment and year 4 follow-up. Serum biomarker change was also defined categorically as: increased (increase of >5% of liver assessment value); decreased (decrease of >5% of liver assessment value); and stayed the same (absolute change within 5% of liver assessment value).

NAFLD was defined as the presence of hepatic steatosis on USS without alcohol excess or use of hepatotoxic medication and a negative liver screen. Alcohol excess was defined according to established criteria as alcohol intake >14 units/week (female) or >21 units/week (male) [25], or participant self-report of current/previous alcohol excess [26]. Use of hepatotoxic medication included the use of (non-topical) glucocorticoids for >2 weeks, isoniazid, methotrexate, amiodarone, or tamoxifen within the 6 months prior to USS [3,26]. Clinically significant positive immunology titres were defined as ASmA titre >1:160 or AMA titre >1:40 [26,27].

All patients with data available for APRI, AST/ALT ratio, ELF, and FIB4 were included in the analysis. All continuous variables were assessed for approximation to the normal distribution with APRI and FIB4 showing a skewed distribution. Correlation between biomarkers was analysed after standardisation to Z-scores, and adjusted for age and sex. Cronbach's alpha was used to examine the inter biomarker agreement (using standardised Z-scores). Student's *t* test or the Mann-Whitney U test were used to compare means and Chi-squared test to compare proportions.