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The association between liver fibrosis and cognitive impairment in type 2 diabetes

To the Editor:

Kronsten and colleagues discuss the gut-brain-liver axis linking depression and chronic liver disease.[1] In their proposed model, gut dysbiosis and increased gut permeability lead to systemic inflammation, which drives liver disease and depression. As a further manifestation of gut-brain-liver axis dysfunction, the authors note that cognitive impairment is prevalent in chronic liver disease and that novel interventions, such as antibiotics and faecal transplantation, may improve cognitive function.

Whilst studies have found an association between cognitive impairment and chronic liver disease such as non-alcoholic fatty liver disease (NAFLD),[2] none has specifically in type 2 diabetes (T2D). This is important because T2D is associated with gut dysbiosis, increased intestinal permeability and chronic immune activation,[3] and further is a strong risk factor for NAFLD, cognitive impairment and depression.[4] For the first time in a T2D population, we tested whether patients with comorbid liver fibrosis have worse depression and poorer cognitive performance than those without fibrosis.

Our study was a follow-up sub-study of the South London Diabetes (SOUL-D) cohort. Originally recruited between 2008-2011, SOUL-D was an inner-London cohort study in individuals with newly diagnosed (<6 months) T2D.[5] In the present study, we consecutively invited patients (according to order recruited to the original study) for 10-year follow-up between April 2018 and October 2019. Ethical approval was granted (UK reference 17/EE/0272) and all participants gave informed consent.

Co-primary outcomes were current depressive symptoms using the Patient Health Questionnaire-9, previously validated in this cohort,[5] and cognitive function using the Rey-Osterrieth complex figure (ROCF) test (Fig. 1).[6] As secondary outcomes, we measured cognition using the telephone interview for cognitive status (TICS-M) (testing verbal memory); Stroop colour word test (testing attention and executive function); and verbal fluency (testing executive function by asking participants to name as many animals as they can in 1 min). These cognitive tests have been found to be discriminatory in T2D.[7,8]

The primary predictor was liver fibrosis, estimated using transient elastrography (Fibroscan) of the liver. All patients fasted ≥6 hours prior. We defined any liver fibrosis using the cut-off of liver stiffness measurement ≥7.0, validated for mild (F1) fibrosis in T2D.[9] As secondary predictors, we measured liver steatosis using controlled attenuation parameter ≥260 dB/m and serum liver biochemical enzymes (alanine

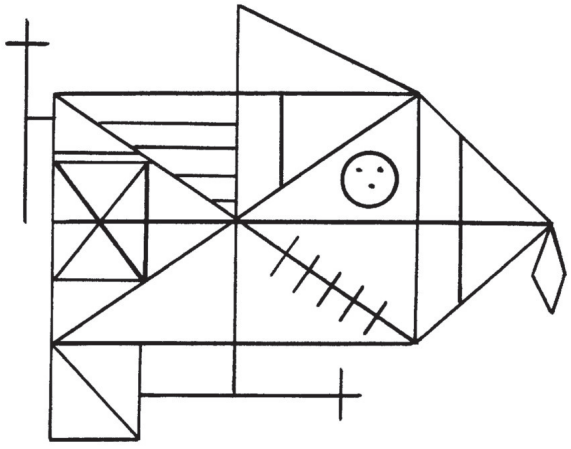

Fig. 1. The Rey-Osterrieth Complex Figure (ROCF) test. This is a neuropsychological assessment, in which participants reproduce a complicated line drawing under three conditions: 1) copying (testing attention and concentration, planning, organisation and visuospatial perception); 2) immediate recall without reference to the figure (testing immediate visuospatial memory); and 3) recall after a 30-minute delay (testing delayed visuospatial memory). Using the Osterrieth scoring system, the diagram is split into 18 units, each scored 2 if unit correct and placed properly; 1 if correct but placed poorly; 1 if distorted (but recognisable) and placed properly; ½ if distorted (but recognisable) and placed poorly; and 0 if absent or unrecognisable. The total on each condition is 36 and one assessor (CDM) scored all assessments to ensure consistency.

aminotransferase, aspartate aminotransferase and gamma glutamyltransferase). Findings were adjusted for age, sex, ethnicity, BMI, HbA1c, triglycerides and alcohol use using the AUDIT questionnaire. We used adjusted linear regression models to test associations between predictors and outcomes. Assuming a 25% prevalence of liver fibrosis, we estimated that depression and cognitive performance would demonstrate moderate-to-large (d = 0.65) differences between groups. At 80% power and 5% significance, we calculated at least 102 patients were needed.

Of the first 126 patients invited, 105 (83.3%) consented to all assessments including Fibroscan. Mean age was 63.6 (10.0) years, mean HbA1c was 60.6 (19.2) mmol/mol, mean BMI was 31.6 (7.4) kg/m^2, 49.0% were women and 49.0% were of non-white ethnicity. Twenty-nine patients (27.6%) had liver fibrosis (LSM ≥7 kPa). After adjustment for confounders, liver fibrosis was not associated with depressive symptoms (p = 0.28) but was associated with impaired performance on the ROCF copy condition (-3.40; 95% CI -5.56 to -1.23; p = 0.002) and impaired verbal fluency (-3.04; -6.06 to -0.01; p = 0.049). There were no associations between liver fibrosis and ROCF immediate recall (p = 0.21), delayed recall (p = 0.68), TICS-M (p = 0.98) and Stroop (p = 0.82) performance. Elevated controlled-attenuation parameter score showed a trend association (p = 0.058) with poorer ROCF copy condition performance but no other tests. Liver biochemical tests showed no associations with depression or cognition.

These preliminary findings demonstrate a novel association between liver fibrosis and cognitive impairment – but not depressive symptoms – in patients with T2D. The noted deficits in tests of executive function (ROCF copy condition, verbal fluency) suggest possible prefrontal dysfunction in patients with comorbid liver fibrosis.[10] As we found no evidence of impaired visual or verbal memory, subcortical structures are less implicated.

These findings have potentially important clinical implications. By increasing gut permeability and immune activation, T2D could be a key risk factor for dysfunction of the gut-brain-liver axis. Compared to depression, impaired cognitive performance could be an earlier clinical manifestation. Addressing T2D in early liver fibrosis provides a modifiable target to reduce stress across the liver and brain, for example through intensification of glycaemic control and weight loss interventions.

Our findings are limited by the limited sample size, cross-sectional design and lack of controls without diabetes. Although confounding by diabetes duration was minimised, the average age (63 years) was below that at which dementia is typically diagnosed. As such, these findings represent a potentially earlier onset of cognitive decline in T2D and suggest that Fibroscan – accepted by over 80% of our sample – could be a feasible risk tool for identifying such patients.

In sum, our study demonstrates a novel association between liver fibrosis and cognitive impairment in patients with T2D, which requires replication in prospective studies.

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Conflicts of interest

The authors declare no conflicts of interest that pertain to this work.

Please refer to the accompanying ICMJE disclosure forms for further details.

Authors' contributions

KI, KW and MAH designed the study. ES performed data collection. CDM wrote the first draft and performed the statistical analyses. All authors revised the manuscript for important intellectual content.

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Supplementary data

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In situ detection of vaccine mRNA in the cytoplasm of hepatocytes during COVID-19 vaccine-related hepatitis

To the Editor:

We have read with high interest the article published in *Journal of Hepatology* by Boettler *et al.*[1] where they report a case of acute hepatitis after the first dose of the BNT162b2 mRNA vaccine. The patient had an initial spontaneous recovery but relapsed after the second dose. Using imaging mass cytometry of the liver biopsy, the authors described an immune cell infiltrate predominated by CD8 T cells, which exhibited a panlobar distribution and contained spike-specific CD8 T-cell clones, pointing to the possibility of an autoimmune hepatitis (AIH)-like syndrome induced by the vaccine. There have been other reports of AIH-like hepatitis since the beginning of mRNA-based SARS-CoV-2 vaccination. Nevertheless, the incidence of AIH has not increased in 2021 during the COVID-19 vaccination period in Europe,[2] suggesting that triggering a bout of genuine AIH is unlikely the pathogenic mechanism of such vaccine-related events. Some authors have suggested molecular mimicry as a potential mechanism of liver damage[3] although no similarity was found between soluble liver antigen and SARS-CoV-2 spike protein.[4] Interestingly, most described cases of SARS-CoV-2 vaccine-related severe liver injury occurred after mRNA vaccines.[5] Boettler *et al.* could not detect the spike protein in the liver by immunohistochemistry, a fact they attribute to the biopsy being performed 4 weeks after the peak of hepatitis. Thus, whether the final mechanism of hepatocyte injury is by antigenic mimicry or by a direct expression of the spike protein by vaccine-transduced hepatocytes remains unexplored.

Herein, we present a case of AIH-like hepatitis following SARS-CoV-2 vaccination wherein we could detect RNA encoding the spike protein within hepatocytes using highly sensitive and specific *in situ* hybridization (RNA-ISH).

A 67-year-old female without past medical history was admitted to the emergency room 12 days after the second dose of Pfizer-BioNTech (BNT162b2), presenting abdominal pain, fatigue and jaundice. Liver tests showed aspartate aminotransferase 1,201 IU/L, alanine aminotransferase 1,618 IU/L, alkaline phosphatase 211 IU/L, gamma-glutamyltransferase 71 IU/L, total bilirrubin 9.56 mg/dl, direct bilirrubin 9.08 mg/dl, international normalized ratio 0.9 and albumin 4.23 mg/dl. Antinuclear antibody with HEp-2 substrate (1:80) and anti-liver kidney microsomal antibody (1:40) were only mildly positive. Laboratory tests were negative for hepatitis A, B, C and E viruses, cytomegalovirus and Epstein-Barr virus. PCR for the detection of N and E genes of SARS-CoV-2 was negative. A liver ultrasound was normal. Liver biopsy showed chronic portal and interface hepatitis with lymphocyte and plasma cell infiltration. Considering that the hepatic biosynthetic function was preserved, we decided to withold the initiation of corticosteroids. Liver tests progressively improved over the next three months until complete recovery with no treatment.

In situ detection of SARS-CoV-2 mRNA transcripts in FFPE tissue sections was carried out using the RNAscope assay (Cat No. 848561, Advanced Cell Diagnostics, Abingdon, UK) coupled to quantitative immunofluorescence. The probe spanned 20 nucleotides within the Spike region between nucleotides 21631 and 23303 of the SARS-CoV-2 isolate Wuhan-Hu-1 genome (NCBI reference sequence: NC_045512.2). Whole slide image analysis and SARS-CoV-2 mRNA quantification was performed using ImageJ software version 1.52c (NIH, Bethesda, MD, USA). Briefly, the fluorescence signal level of SARS-CoV-2 mRNA was measured in the cellular compartment given by an expansion of each detected nucleus which creates an approximation of the full cell area. Two cases of AIH unrelated to COVID-19 and one post-mortem liver tissue from an individual diagnosed with severe COVID-19 were included as control tissues. The liver tropism of SARS-CoV-2 has been extensively demonstrated in both biopsies and post-mortem tissue analyses.[6] The level of SARS-CoV-2 mRNA in the liver tissue of our patient with vaccine-related hepatitis was similar to the one found in the liver post-mortem biopsy obtained immediately after death from an individual with severe COVID-19. No SARS-CoV-2 mRNA transcripts were detected in AIH unrelated to COVID-19 (Fig. 1A-D).

In line with the case reported by Boettler *et al.*,[1] our results suggest that lipid nanoparticles bearing mRNA molecules encoding SARS-CoV-2 proteins can reach hepatocytes under