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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 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 damage3 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 bilirubin 9.56 mg/dl, direct bilirubin 9.08 mg/dl, international normalized ratio 0.9 and albumin 4.23 mg/dl. Anti-nuclear 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 hepatitis with minimal inflammation, irregular lobulation, steatosis and bridging fibrosis. Serology was negative for blood-borne viruses, autoimmune markers, Wilson disease and mitochondrial disorders. Serum autoantibodies were positive for antinuclear antibody with HEp-2 substrate (1:80) and anti-liver kidney microsomal antibody (1:40). No SARS-CoV-2 mRNA transcripts were detected in AIH 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
certain circumstances and deliver mRNA in high quantities that could be used by the translational machinery of the cells to produce spike. These peptides could then be presented through the MHC class I antigen presentation machinery and promote their recognition by previously sensitized CD8 T-cell clones. In our case, like in others described recently, \(^1,4\) hepatitis occurred after the second dose of the vaccine, suggesting that previous exposure could enhance the severity of hepatocyte targeting by cytotoxic T lymphocytes. To the best of our knowledge this is the first report on \(\textit{in situ}\) hybridization of vaccine mRNA in hepatocyte cytoplasm using commercially-available \(\textit{in situ}\) RNA hybridization probes.

Another important teaching point is that these very rare cases of acute hepatitis after mRNA vaccines may resolve spontaneously and, may not always require the use of steroids (Fig. 1E). In the more severe cases, a rapid steroid tapering and avoiding long-acting immunosuppressants would likely be safe and effective, in contrast with the usual approach to AIH. Whether the duration of the expression of the spike protein by mRNA vaccine-transduced hepatocytes could be related to the duration or the intensity of the liver damage or the relapse during or after steroid tapering are unanswered questions that deserve further investigation.

Finally, these findings should be taken into account in clinical trials of cancer vaccines using lipid nanoparticle-packed mRNA. The expression of tumor neoantigens by hepatocytes could modify the response to vaccines and perhaps trigger similar cases of liver injury.

Loreto Martin-Navarro\(^1\)
Carlos de Andrea\(^2,5\)
Bruno Sangro\(^1,3,4,5\)
Josepmaria Argemi\(^1,3,4,5,7\)

\(^1\)Liver Unit, Clinica Universidad de Navarra, Pamplona, Spain
\(^2\)Pathology Department, Clinica Universidad de Navarra, Pamplona, Spain
\(^3\)Hepatology Program, Center for Applied Medical Research (CIMA), Pamplona, Spain
\(^4\)Centro de Investigacion Biomedica en Red (CIBER-EHD), Madrid, Spain
\(^5\)Instituto de Investigacion de Navarra (IdisNA), Pamplona, Spain

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**Fig. 1.** \(\textit{In situ}\) SARS-CoV-2 mRNA measurement using quantitative fluorescence and patient’s biochemical tests. SARS-CoV-2 mRNA transcripts (yellow channel) were detected using \(\textit{in situ}\) hybridization in (A) the liver of an individual with hepatitis after the second dose of the Pfizer-BioNTech (BNT162b2) vaccine, (B) a post-mortem liver tissue from an individual diagnosed with severe COVID-19 (as a positive control), (C) and (D) No SARS-CoV-2 mRNA transcripts were detected in the liver tissues from individuals with autoimmune hepatitis unrelated to COVID-19. Nuclei are highlighted with blue. Scale bars represent 200 \(\mu\)m (A-D). (E) Patient’s course of AST, ALT, total bilirubin, ALP and GGT activity. ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT, gamma glutamyltransferase.
Letters to the Editor

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Conflict of interest
None of the authors have any conflict of interest regarding this manuscript. Please refer to the accompanying ICMJE disclosure forms for further details.

Authors’ contributions
L. M.-N. followed the case patient, captured clinical and lab data and helped selecting the control patients for histological analyses, drafting the manuscript. C. A. performed the RNA- in situ hybridization and the quantification, revision of the manuscript. B.S. diagnosed and followed the patient during her disease, revision of the manuscript. J.A. conception, design of the histological test, interpretation of the data, design of the figure and writing of the manuscript.

Supplementary data
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Ammonia - an old friend with a new area of application

To the Editor:
We read with great interest the article by Tranah et al. recently published in the Journal of Hepatology. Ammonia is known to play a key role in the pathophysiology of hepatic encephalopathy. Recent evidence has also indicated the pathophysiologic role and the value of measuring ammonia even in hospitalized patients with acute decompensation. In their current multicenter study, Tranah et al. evaluated the predictive value of ammonia in outpatient with cirrhosis regarding liver-related hospitalizations and mortality. We would like to take the opportunity to congratulate the authors on this important study. However, extensive validation of biomarkers in the current analysis, we included 147 outpatients with available ammonia levels and follow-up data. Venous ammonia levels were measured according to a standard operating procedure that involved rapid sample transport on ice to the central laboratory within 5 min and the upper limit of normal (ULN) was 72 μmol/L. Patients were followed for liver-related hospitalizations (defined as the work of Tranah et al.) and mortality (composite of death or need for liver transplantation). Kaplan-Meier curves were used to illustrate incidences of the respective endpoints, attached p values were calculated with a log-rank test. Additionally, receiver-operating characteristic curve analyses were performed. Statistics were performed with IBM SPSS Statistics Version 27 and GraphPad Prism Version 9.4.0. The study was approved by the ethics committee of the Landesärztekammer Rheinland-Pfalz and written informed consent was obtained from all participants.

Overall, 25 of 147 participants (17%) were hospitalized during a median follow-up of 569 (IQR 371; 745) days and 17 died or needed liver transplantation (12%; n = 13 died; n = 4 liver transplantation). At baseline, most participants were in a compensated cirrhosis stage (Child-Pugh A/B/C: 73%/24%/3%) and the median MELD score was 9 (IQR 7; 12). Only a minority of participants had ammonia levels above the ULN (13%) and the median ammonia level was 46 μmol/L (IQR 36; 57 μmol/L). Baseline characteristics of the cohort are displayed in Table S1. Frequency of liver-related hospitalization was higher in participants with ammonia levels above the ULN (9 of 19, 47.4%) than in those with ammonia levels below the ULN (16 of 128, 12.5%) (Fig. 1). The AUC of ammonia for predicting 6-month and 1-year liver-related hospitalization was 0.74 (95% CI 0.57–0.92) and 0.68 (95% CI 0.52–0.84), respectively. During the total follow-up time, the mortality-rate did not differ between participants with ammonia levels above or below the ULN (13/147, 9%) and 17/128, 13%).

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Author names in bold designate shared co-first authorship

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