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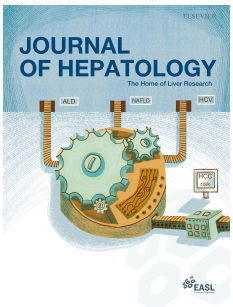

In situ detection of vaccine mRNA in the cytoplasm of hepatocytes during COVID19 vaccine-related hepatitis

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Title Page

Title

In situ detection of vaccine mRNA in the cytoplasm of hepatocytes during COVID19 vaccine-related hepatitis

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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 hibridization 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.

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Dear Editor,

We have read with high interest the article published in Journal of Hepatology by Boettler T. et al [1] where they report a case of acute hepatitis after the first dose of 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 describe the predominance of CD8 T cells infiltrate with a panlobar distribution with the presence of spike-specific CD8 T clones, which points 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-CoV2 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-CoV2 spike protein [4]. Interestingly, most described cases of SARS-CoV2 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.

Here we present a case of post-SARS-Cov2 vaccination AIH-like hepatitis in which we could detect the RNA encoding the spike protein within the hepatocytes using a highly sensitive and specific In situ hibridization (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 AST 1201 UI/L, ALT 1618 UI/L, alkaline phosphatase 211 UI/L, GGT 71 UI/L, total bilirrubin 9,56 mg/dL, direct bilirrubin 9,08 mg/dL, INR 0,9 and albumin 4,23 mg/dL. Antinuclear antibody with HEp-2 Substrate (1:80) and anti-LKM (1:40) were only mildly positive. Laboratory tests were negative for hepatitis A, B, C and E viruses, cytomegalovirus and Epstein-Barr virus. Polymerase Chain Reaction (PCR) for the detection of N and E genes of SARS-CoV2 was negative. A liver ultrasound was normal. Liver biopsy showed chronic portal and interface hepatitis with lymphocytes 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 AIH unrelated to COVID-19 and one post-mortem liver tissue from a patient 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 a severe COVID-19 patient. No SARS-CoV-2 mRNA transcripts were detected in AIH unrelated to COVID-19.

In line with the case reported by Boettler el at [1], our results suggest that lipid nanoparticles bearing mRNA molecules encoding SARS-CoV2 proteins can reach the 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 be then 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 ocurred 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 communication of vaccine mRNA in situ hibridization in hepatocyte cytoplasm using commercially-available In situ RNA hibridization 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. In the more severe cases, a rapid steroid tappering and avoiding long-acting immunosuppressants would likely be safe and effective, in contrast with the usual approach to autoimmune hepatitis. Whether the duration of the expression of the spike protein by mRNA vaccine-trasduced hepatocytes could be related to the duration or the intensity of the liver damage or the relapse during or after steroid tappering are unanswered questions that deserves further investigation.

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

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Figure Legend and Figure

Figure 1. 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 in situ hybridization in (A) the liver of a patient with hepatitis after the second dose of the Pfizer-BioNTech (BNT162b2) vaccine, (B) a post-mortem liver tissue from a patient 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 patients with autoimmune hepatitis unrelated to COVID-19. Nuclei are highlighted with blue. Scale bars represent 200µm (A-D). E. Patient's course of serum transaminases, total bilirubin, alkaline phosphatase (ALP) and Gamma Glutamyl Transferase (GGT) activity.