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Reply to: "Comment on "Autoimmune hepatitis developing after coronavirus disease 2019 (COVID-19) vaccine: Causality or casualty?""

To the Editor:

We read with interest the comment by Capecchi *et al.*[1] regarding our Letter to the Editor describing a case of autoimmune hepatitis (AIH) developing after COVID-19 vaccination.[2] We thank the authors for their interest in our manuscript and for their thoughtful insight, which complemented our report.

As we carefully expressed in our original letter, and Capecchi *et al.* further reinforced, a causal relationship between the vaccine and the development of AIH cannot be proven. Other factors, such as the postpartum status, could have acted as confounding factors. Another possibility is that the vaccine unmasked a process that was already developing. Yet, while we recognized that the temporal association could have been coincidental, we felt compelled to report this case for several reasons.

There is already significant evidence supporting a strong link between severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection and the development of autoimmunity.[3] Among 45 consecutive patients admitted with SARS-CoV-2 pneumonia, 35.6% were found to have positive antinuclear antibodies.[4] As the spike protein believed to be responsible for autoimmunity is the same used in vaccines, there is physiological feasibility for an association between COVID-19 vaccines and autoimmune conditions. In support of this, cases of immune thrombocytopenia,[5] acquired hemophilia A[6] and other autoimmune processes have been reported days after COVID-19 vaccination. Unfortunately, the proportion of vaccine-related adverse events reported to the Vaccine Adverse Event Reporting System (VAERS) is likely low. Therefore, there is an urgent need to share potential adverse events to increase awareness among patients and physicians, and to encourage them to report them to the appropriate authorities. Only a large and comprehensive pharmacovigilance effort will help us answer the question as to whether these vaccines can induce autoimmunity or not.

Since the publication of our letter, we have been approached by at least 4 patients throughout United States via email claiming that they have been diagnosed with autoimmune hepatitis after COVID-19 vaccination. Moreover, a quick glimpse at the 2021 VAERS database (available online at https://vaers.hhs.gov/data/datasets.html) describes several cases consistent with acute liver injury and even an AIH flare. While we do not have any further information and we understand that these cases are only anecdotal at this time, it further strengthens the importance of sharing information and experiences, in order to advance our understanding of SARS-CoV-2 infection and develop better strategies to contain the virus.

Finally, and most importantly, while we ratify that it is important to further explore the potential link between COVID-19 vaccines and autoimmunity, this should not discourage patients and physicians from prescribing and/or receiving these vaccines. Even if confirmed in the future, cases of vaccine-induced autoimmunity appear to be uncommon as evidenced by only a handful of reports despite massive vaccination worldwide. Furthermore, it is likely that SARS-CoV-2 infection can trigger the same autoimmune processes as its vaccines do, leaving unvaccinated people not only at risk of developing the autoimmune process, but also the other complications associated with COVID-19.

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Conflict 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

Both authors contributed equally.

Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jhep.2021.06.008.

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Fernando Bril[1,*]
David M. Fettig[2,*]
[1]*Internal Medicine, Department of Medicine, University of Alabama at Birmingham, Birmingham, AL, USA*
[2]*Division of Gastroenterology, Hepatology and Nutrition, Department of Medicine, University of Alabama at Birmingham, Birmingham, AL, USA*

[*]Corresponding authors. Addresses: Internal Medicine, Department of Medicine, University of Alabama at Birmingham, Birmingham, AL, USA; Tel.: 205-934-2490; or Gastroenterology, Hepatology and Nutrition, Department of Medicine, University of Alabama at Birmingham, Birmingham, AL, USA; Tel.: 205-934-2490.
E-mail addresses: fbril@uab.edu (F. Bril), dfettig@uabmc.edu (D.M. Fettig)

Understanding HBcrAg components improves the interpretation of clinical HBcrAg assay results

To the Editor:

We read with great interest the paper by Inoue *et al.* on the development of a novel high-sensitivity hepatitis B core-related antigen assay (iTACT-HBcrAg) and its application for monitoring hepatitis B e antigen (HBeAg)-negative patients and HBV reactivation.[1] Without a doubt, this high-sensitivity HBcrAg assay improves the sensitivity of the current HBcrAg assay and is timely for the ongoing pursuit of an HBV cure. However, a few important issues need to be clarified.

Firstly, the authors used the HBeAg chemiluminescence enzyme immunoassay (CLEIA) to select HBeAg-negative patients. However, the HBeAg CLEIA may have a lower sensitivity in detecting HBeAg than the HBcrAg assays. Thus, the "HBeAg-negative" patients in this study might not have been truly HBeAg-negative. The authors should clarify the detection limits of HBeAg by their HBeAg-specific CLEIA assay *vs.* their HBcrAg assays.

Secondly, the authors employed the OptiPrep gradient fractionation to define the HBcrAg components and concluded that HBcrAg detected before HBV reactivation represented empty virion particles containing the so-called 22-kDa precore protein (p22cr).[1] However, the characterization was incomplete and their conclusion may not be entirely correct. We have recently characterized HBcrAg components based on their biochemical and biophysical properties.[2,3] We found that HBcrAg includes hepatitis B core antigen or HBcAg (10%), p22cr (10%), but predominantly HBeAg (80%).[2] Indeed, both HBeAg and p22cr, which we renamed PreC owing to its closer relatedness to the precore than the core protein, contain multiple species resulting from genotype-specific C-terminal processing from their common precursor p25. We also found that the PreC/p22cr shared similar properties (*e.g.* buoyant density and size) to HBeAg, and neither PreC/p22cr nor HBeAg forms capsids or empty virions.[2] In contrast, HBcAg is the main or sole component of empty virions,[2,4] and empty virions (*i.e.* enveloped capsids devoid of DNA or RNA) are formed in the complete absence of the precore protein (*i.e.* no HBeAg or PreC/p22cr).[4,5] Although the mechanism of secretion of PreC/p22cr, which retains the N-terminal signal peptide, remains mysterious, we found that PreC/p22cr concentrations are always proportional (ca. 10 to 20%) to HBeAg in HBV-infected primary human hepatocytes and chimpanzees, and in HBeAg-positive patients.[2,3] We also detected glycosylated PreC/p22cr, as well as glycosylated eAg, in the woodchuck hepatitis virus-infected woodchucks.[3] Together, these results strongly indicate that secretion of PreC/p22cr, like HBeAg, involves the cellular secretory pathway where protein glycosylation occurs. Thus, it is extremely unlikely that PreC/p22cr can be produced in the absence of any HBeAg secretion, as PreC/p22cr should not be produced when HBeAg cannot be produced. We suggest that the HBcrAg that the authors detected in fractions 11 to 13 (Fig. 7A) and fraction 12 (Fig. 7B) was actually HBcAg in empty virion particles, instead of PreC/p22cr as the authors proposed,[1] assuming that this patient was truly HBeAg-negative. As we demonstrated,[2,3] HBcAg-specific antibodies, which do not cross-react with either HBeAg or PreC/p22cr, can be used to clarify this point. If the kU values can be converted to mass units (*e.g.* picograms), it would also facilitate the interpretation of results, such as those presented in Table S10, in terms of the contribution of HBcAg to the overall quantity of HBcrAg.

Thirdly, the authors repeated the OptiPrep gradient fractionation of the reference plasma 990776 used in a previous study[1,6] (Fig. S3). However, the results are difficult to interpret as presented. The authors referred to the "peaks" of empty virions and complete virions based on the previous analysis using sucrose gradient fractionation.[6] It is difficult to compare the profiles from 2 different gradient approaches (sucrose *vs.* OptiPrep) from 2 separate studies directly. The supposedly empty and complete virion peaks were not separated at all in this figure. How HBsAg (spheres and filaments), virions (empty or complete), HBeAg, and PreC/p22cr fractionate on the OptiPrep gradient will need to be more vigorously defined before any solid conclusions can be made regarding the association of the different HBcrAg components with these physical entities. In this regard, we have shown that CsCl gradient fractionation can reliably separate these HBV entities.[2,3]

Lastly, we believe that a better understanding of the fundamental biology of HBcrAg will guide its improved application as a marker for intrahepatic covalently closed circular DNA levels and activity, allowing us to better monitor the natural course of HBV infection and responses to antiviral treatments. First, we need to define the components detected by the HBcrAg assays. Our recent studies have helped to clarify a number of issues in this regard.[2,3] Second, detection of each HBcrAg component, separately, should allow more precise understanding of

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