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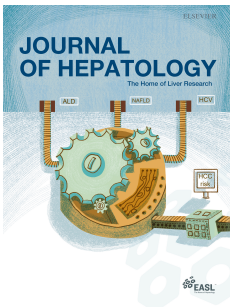


End-of-treatment HBcrAg and HBsAb levels identify durable functional cure after Peg-IFN-based therapy in patients with CHB, by Huang et al. J Hepatology 77, 42-54 (2022)

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**End-of-treatment HBcrAg and HBsAb levels identify durable functional cure after Peg-IFN-based therapy in patients with CHB, by Huang *et al.*** *J Hepatology 77, 42-54 (2022)*

Dear J. Hepatology Editors,

There is a growing demand to find biomarkers that can help the clinical management of CHB. This exigence has initiated an active discussion in the HBV research field about integrating virological and clinical parameters with immunological ones to better select patients for novel immunotherapies and manage their treatments.

For example, this journal has recently published a collaborative "state of the art" manuscript that discussed several methods of analysis of HBV-related immunity in CHB patients[1] (Gehring A et al, doi: 10.1016/j.jhep.2022.02.020) linked with a specific commentary[2] (Bertoletti A,doi:. 10.1016/j.jhep.2022.03.028) where the importance of defining robust and reliable assays to measure immunological parameters was highlighted. One point of discussion was that the complexity of the assay to measure HBV-specific T cell response poses challenges to accumulate reliable results derived from large clinical trials. This limitation can explain why the measurement of HBV-specific T cell response as a biomarker for the clinical management of CHB patients has only been evaluated in few studies, despite the consensus on the importance of HBV-specific T cells in the control of HBV[3,4,5].

Indeed, the problem of obtaining a reliable quantification of HBV-specific T cells in CHB patients remains diffused, and we think it is present in the manuscript recently published in J Hepatology entitled *"*End-of-treatment HBcrAg and HBsAb levels identify durable functional cure after Peg-IFN-based therapy in patients with CHB*"* [6]*.x*

In this large study of CHB patients treated with Peg-IFN, Huang et al. proposed that the quantity of HBcrAg and HBsAb levels can predict the persistent functional cure of HBV in CHB patients treated with Peg-IFN. The manuscript, however, also indicates that the level of "HBV-specific CD8 T cell response" does not provide any meaningful indication since, as the results displayed in Figure 2 of the manuscript indicate, the frequency of CD8 T cells specific for core, envelope and polymerase is identical at the end of treatment in CHB patients responding or non-responding to Peg-IFN therapy.

The issue with such a conclusion is that we have severe doubts that the data shown in Figure 2 of this manuscript constitute a "reliable" measurement of HBV-specific CD8 T cells.

The problems with these data are the following:

a) The HBV-specific CD8 T cell response in the CHB patients was measured using 3 HLA-tetramers consisting of "*phycoerythrin (PE)-labeled tetrameric peptide-HLA class I complexes representing the HLA-A2-restricted epitopes on the core peptide spanning amino acids 18 to 27 (FLPSDFFPSV), envelope peptides spanning amino acids 335 to 343 (WLSLLVPFV), and polymerase peptides spanning amino acids 575 to 583 (FLLSLGIHL*)". In addition to the fact that a measurement of the frequency of three single CD8 T cell specificities cannot be used as representative of the global HBV-specific CD8 T cell response (HBV-specific CD8 T cell response is often multi-specific, particularly in patients who control the HBV infection), these "*Tetramers based on HLA-A2-restricted epitopes*" detect HBV-specific CD8 T cells only in HLA-A2 (actually HLA-A*02:01+) positive patients. There is no mention in all the paper and in the inclusion and exclusion criteria of the ANCHOR study whether the "HBV-specific CD8 T cells" were measured only in HLA-A*02:01+ patients or not. HBV-specific CD8 T cells cannot be

measured in the total treated patient population unless the authors have selected only patients carrying HLA-A2. The authors should therefore clarify this point.

b) Furthermore, one of the three HLA-tetramers used to monitor HBV-specific CD8 T cell response in these patients is an HLA-A2 core tetramer based on the sequence FLPSDFFPSV that is the sequence of core in HBV genotype A and D but not in HBV genotype B and C. Since the study was done in China, the CHB patients were likely infected by HBV genotype B or C. The sequence of core 18-27 in HBV genotypes B and C is characterized by the sequence FLPSDFFPS**I** and the AA Isoleucin at position 27, which differentiated the core sequence of the different genotypes (Gen A, D= FLPSDFFPS**V,** Gen B, C= FLPSDFFPS**I)** and alters the binding of the FLPSDFFPSI region to HLA-A2, making this epitope less immunogenic[7,8]. This might be why core 18-27-specific CD8 T cells are rarely found in HLA-A2 patients infected by HBV genotype B and C[9]. However, in this paper the authors detected high frequencies of core-specific CD8 T cells with an HLA-tetramer designed to visualize HBV-core CD8 T cells specific for a sequence of core that is unlikely to be present in the population studied. Therefore, the authors should clarify whether their CHB population is infected preferentially by HBV genotypes B or C and explain why, contrary to previous data, they are apparently able to detect FLPSDFFPS**V-**specific CD8 T cells**.**

c) Finally, the results obtained using the method of HLA-A2-tetramer staining are apparently performed on PBMC directly ex vivo (thus without previous in vitro expansion in vitro). The results show a frequency of CD8 T cells specific for the three different epitopes, with numeric values of about 3-4% out of total CD8 T cells for each HLA-tetramer. There are a couple of problems with such findings: the first is that there is a consensus derived from data of different laboratories that envelope-specific T cells are particularly compromised in patients with CHB infection[10,1112]. Thus, the detection of CD8 T cells specific for envelope at a frequency that appears even higher than core and polymerase does not fit with evidence showing that persistent HBsAg production progressively alters the HBV-specific T cell repertoire[12]. The second point is that the results indicated by the authors suggest that about 1 in 10 CD8 T cells (or even more) are HBV-specific in these patients (3 epitopes studied only), a frequency of HBV-specific T cells approximately 2 logs higher than the one detected by all the other laboratories in the world that have been measuring HBV-specific T cells ex vivo[13–15]. A question for the authors is whether their results are representative of ex vivo measurement or if they analyzed samples after in vitro expansion. In addition, the authors' only representative original flow cytometry data (Fig. 5A) raises concerns regarding the experimental validity of the T cell analyses. Indeed, the HLA class I tetramers (expected to bind specifically to CD8+ T cells only) stain CD8+ and CD8-negative cells at similar proportions, and no distinct cell populations (‘clouds’) are visible.

Without clarifying the protocols of patient selection and PBMC staining, the HBV-specific T cell data of this manuscript are incompatible with our current knowledge of the magnitude and hierarchy of HBV-specific CD8 T cell response. Furthermore, the authors should explain why an analysis of only three specificities was deemed sufficient to evaluate the global HBV-specific T-cell response.

We believe the HBV-specific T cell data should be retracted from this manuscript since they are sending the message that analysis of HBV-specific T cell response in CHB patients does not offer any predictive value. While such a conclusion might even be correct, it should be based on adequate measurements of the global HBV-specific T-cell response and not by an analysis of only three single specificities in CHB patients without proper patient selection and negative controls.

Sincerely,

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