Reply to 'Are the 5-hydroxymethylcytosine-based wd-scores really superior over α-fetoprotein for the early diagnosis of hepatocellular carcinoma?'

We thank Chen and Lin for their interest in our article.[1] They raised concerns about the comparison between α-fetoprotein (AFP) and the 5-hydroxymethylcytosine (5hmC)-based weighted diagnostic (wd)-scores,[2] a debate worth further clarification.

First, it should be clarified that Chen and Lin's description did not represent the overall picture of the wd-scores.[1] The performance measures mentioned by Chen and Lin were specifically for distinguishing early hepatocellular carcinoma (HCC) and non-HCC (including not just chronic hepatitis B (CHB) virus infection but also benign liver lesions, liver cirrhosis (LC) and healthy controls) in the validation set. Notably, the 5hmC-based wd-scores consistently outperformed AFP in both training and validation sets for HCC versus non-HCC, early HCC versus CHB/LC, and early HCC versus controls (healthy individuals, benign liver lesions). Compared with AFP alone, the 5hmC-based wd-scores performed especially well when detecting early HCC from CHB/LC in the validation set (area under the curve (AUC)=84.6%), representing a 22% improvement in AUC when compared with AFP (AUC=69.2%) at the conventional cut-off of 20 ng/mL.

Second, Chen and Lin questioned the outperformance of wd-scores by citing Wong *et al*.[3] It should be clarified that Wong *et al* had a completely different study design, in which they conducted a retrospective–prospective study on 1516 patients with CHB who were on 'active entecavir treatment', and their findings were based on 'on-treatment AFP'. We do not challenge the findings from Wong *et al* because it was a completely different design and patient population (ie, patients with CHB on active antiviral treatment under close surveillance). The direct comparison between Wong *et al* and Cai *et al*, which was a case–control design including >1200 HCCs with CHB and other backgrounds and >1300 other individuals (CHB, LC, benign liver lesions and controls), however, is not scientifically valid. The low AFP cut-off as argued by Chen and Lin must be systematically investigated under a comparable study design, with similar risk factors and sample size. Importantly, even at the 6 ng/mL cut-off argued by Chen and Lin, our data still suggested a superior performance of the wd-scores for early HCC detection in terms of sensitivity and specificity (table 1).

Third, besides suggesting outperformance of the wd-scores over AFP and their independence from AFP, Cai *et al* further reported that combining AFP with wd-scores could slightly improve the detection of HCC.[1] We further investigated those early HCC samples that were misclassified by wd-scores in the training set of 333 early HCCs in Cai

Table 1 Comparison between the wd-scores and α-fetoprotein (6 ng/mL) in the validation set of Cai *et al*

Diagnosis	Marker	Cut-off	Sensitivity	Specificity
Early HCC versus CHB/LC	wd-score	27.9	0.827	0.674
	AFP	6 ng/mL	0.589	0.659
Early HCC versus CHB	wd-score	27.9	0.827	0.771
	AFP	6 ng/mL	0.589	0.600

CHB, chronic hepatitis B virus infection; HCC, hepatocellular carcinoma; LC, liver cirrhosis; wd-score, 5hmC-based weighted diagnostic score.

et al. Interestingly, 23 out of 38 misclassified samples (60.5%) would have been picked up by AFP alone at 20 ng/mL, while the wd-scores would have detected 90.6% of the 160 early HCCs that were misclassified by AFP.

Finally, we agree that cost-effectiveness analysis will be necessary to establish the superiority of the 5hmC-based wd-scores over AFP, as is true for all technologies. To date, however, as suggested by the European Association for the Study of the Liver, AFP is suboptimal in terms of cost-effectiveness for routine surveillance of early HCC.[4] Considering that it requires only low-coverage sequencing and its capacity for detecting multiple diseases in a single blood test,[5 6] the 5hmC-based technique as described in Cai *et al* suggested a promising alternative in HCC detection and surveillance for improving care of patients with high risks of HCCs. It also offers future potential for prognosis, which we will continue to follow and report.

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REFERENCES

1 Cai J, Chen L, Zhang Z, *et al*. Genome-wide mapping of 5-hydroxymethylcytosines in circulating cell-free DNA as a non-invasive approach for early detection of hepatocellular carcinoma. *Gut* 2019;68:2195–205.
2 Chen Y, Lin X-Y. Are the 5-hydroxymethylcytosine-based wd-scores really superior over α-fetoprotein for the early diagnosis of hepatocellular carcinoma? *Gut* 2020;69:1893–4.
3 Wong GLH, Chan HLY, Tse Y-K, *et al*. On-treatment alpha-fetoprotein is a specific tumor marker for hepatocellular carcinoma in patients with chronic hepatitis B receiving entecavir. *Hepatology* 2014;59:986–95.
4 European Association for the Study of the Liver. Electronic address: easloffice@easloffice.eu, European Association for the Study of the Liver. EASL clinical practice guidelines: management of hepatocellular carcinoma. *J Hepatol* 2018;69:182–236.
5 Li W, Zhang X, Lu X, *et al*. 5-Hydroxymethylcytosine signatures in circulating cell-free DNA as diagnostic biomarkers for human cancers. *Cell Res* 2017;27:1243–57.
6 Yang Y, Zeng C, Lu X, *et al*. 5-Hydroxymethylcytosines in circulating cell-free DNA reveal vascular complications of type 2 diabetes. *Clin Chem* 2019;65:1414–25.