univariate and multivariate analyses of OS (Table S3 in this study[1])?

Financial support
Supported by Adjunct Talent Fund of Zhejiang Provincial People's Hospital (No: 2021-YT).

Conflict of interest
All authors have declared no conflict of interest.

Please refer to the accompanying ICMJE disclosure forms for further details.

Authors' contributions
K-C. W and L-Y. S contributed equally to this letter. K-C. W designed the letter; K-C. W and L-Y. S wrote the letter; M-D. W revised the letter. All the authors reviewed the paper and approved the final version.

Supplementary data
Supplementary data to this article can be found online at https://doi.org/10.1016/j.jhep.2022.04.027.

References
Author names in bold designate shared co-first authorship

[1] Beaufrère A, Caruso S, Calderaro J, Poté N, Bijot JC, Couchy G, et al. Gene expression signature as a surrogate marker of microvascular invasion on routine hepatocellular carcinoma biopsies. J Hepatol 2022;76(2):343–352.
[2] **Duvoux C**, **Roudot-Thoraval F**, **Decaens T**, Pessione F, Badran H, Piardi T, et al. Liver transplantation for hepatocellular carcinoma: a model including α-fetoprotein improves the performance of Milan criteria. Gastroenterology 2012;143(4):986–994.e3; quiz e14-5.
[3] Reig M, Forner A, Rimola J, Ferrer-Fàbrega J, Burrel M, Garcia-Criado Á, et al. BCLC strategy for prognosis prediction and treatment recommendation: the 2022 update. J Hepatol 2022;76(3):681–693.
[4] Erstad DJ, Tanabe KK. Prognostic and therapeutic implications of microvascular invasion in hepatocellular carcinoma. Ann Surg Oncol 2019;26(5):1474–1493.
[5] Zhang X, Li J, Shen F, Lau WY. Significance of presence of microvascular invasion in specimens obtained after surgical treatment of hepatocellular carcinoma. J Gastroenterol Hepatol 2018;33(2):347–354.
[6] Johnson PJ, Berhane S, Kagebayashi C, Satomura S, Teng M, Reeves HL, et al. Assessment of liver function in patients with hepatocellular carcinoma: a new evidence-based approach-the ALBI grade. J Clin Oncol 2015;33(6):550–558.
[7] Hanazaki K, Kajikawa S, Shimozawa N, Mihara M, Shimada K, Hiraguri M, et al. Survival and recurrence after hepatic resection of 386 consecutive patients with hepatocellular carcinoma. J Am Coll Surg 2000;191(4):381–388.
[8] Berardi G, Morise Z, Sposito C, Igarashi K, Panetta V, Simonelli I, et al. Development of a nomogram to predict outcome after liver resection for hepatocellular carcinoma in Child-Pugh B cirrhosis. J Hepatol 2020;72(1):75–84.

Ke-Chun Wang[1,2,3]
Li-Yang Sun[1,4]
Ming-Da Wang[1,3,*]
[1]*Department of HBP Surgery, Zhejiang Provincial People's Hospital, Affiliated People's Hospital, Hangzhou Medical College, Hangzhou, China*
[2]*College of Basic Medicine, Naval Medical University, Shanghai, China*
[3]*Department of Hepatic Surgery, The Third Affiliated Hospital of Naval Medical University, Shanghai, China*
[4]*Graduate School, Bengbu Medical College, Bengbu, China*
[*]Corresponding author. Address: Department of Hepatic Surgery, the Third Affiliated Hospital of Naval Medical University, Shanghai, China; Department of HBP Surgery, Zhejiang Provincial People's Hospital, Affiliated People's Hospital, Hangzhou Medical College, Hangzhou, China.
E-mail address: wangmdehbh@smmu.edu.cn (M.-D. Wang)

Reply to: Correspondence regarding "Gene expression signature as a surrogate marker of microvascular invasion on routine hepatocellular carcinoma biopsies"

To the Editor:

We strongly appreciate the interest motivated by our recently published article entitled "Gene expression signature as a surrogate marker of microvascular invasion on routine hepatocellular carcinoma biopsies",[1] and are pleased to consider the different concerns raised by Chao Li *et al*,[2] Zi-Xiang Chen *et al.*[3] and Ke-Chun Wang *et al.*[4] Altogether, they demonstrate that microvascular invasion (MVI), a very major prognostic risk factor, is a challenging issue, and its prediction at the time of hepatocellular carcinoma (HCC) diagnosis remains a significant unmet need.[5,6] This led us to propose an original surrogate 6-gene signature of MVI directly from routine HCC biopsy.

First, as highlighted by Zi-Xiang Chen *et al.*, we fully acknowledge the well-known limits of HCC biopsy (mostly invasiveness and tumor seeding), which are not usually discussed in oncology practice since, as far as we know, tumor biopsy is a prerequisite to treat all patients with cancer, except for HCC. Indeed, the high diagnostic performance of imaging in patients with cirrhosis has pushed the international guidelines to restrict the use of biopsy to atypical cases on imaging.[7] However, a multicentre UK audit showed that the imaging-based diagnostic criteria in advanced HCC had a positive predictive value of 91.4% with differentials that require different patient management.[8] Beyond diagnosis, comprehensive molecular studies have deeply refined the pathological understanding of HCC, with the description of different subgroups associated with different prognostic and theranostic factors.[9] Whereas such information is not yet

Received 25 May 2022; accepted 7 June 2022; available online 19 June 2022
https://doi.org/10.1016/j.jhep.2022.06.011

included in the routine patient pathway, the ability to fine-tune or personalize therapies is garnering increasing interest among clinicians and national healthcare systems. Accordingly, we fully believe that future guidelines may consider that point and propose a biopsy strategy according to the potential therapeutic options.[10,11] If so, among molecular approaches to screen for surrogate tissue biomarkers, NanoString technology, already routinely applied in breast cancers, is more rapid and cost-efficient than next-generation sequencing.[12,13] Zi-Xiang Chen *et al.* propose the use of liquid biopsy techniques, which are a promising new minimally invasive method for multiple molecular analyses. We agree on the future potential of liquid biopsy, even though numerous challenges remain, such as type of circulating biomarkers, time to perform genetic testing, and cost, not to mention the unknown frequencies of HCC shedding. As far as we know, while there is great interest surrounding liquid biopsy, it has not yet been routinely applied in the field of oncology.[14]

Then, Chao Li *et al.* questioned the statistical analysis and the pathological variables used. The authors claimed that similar to MVI, satellite nodules are only detectable by microscopy in the peritumoral liver of surgical specimens. We strongly disagree with Chao Li *et al.*, as satellite nodules are more and more often detected pre-operatively by imaging and they have been shown to predict MVI in patients with HCC as a stand-alone or more often combined with other factors in several studies.[15,16] Therefore, we think that satellite nodules recognized on pretreatment imaging should be integrated in the uni- and multivariate analysis. In cases with tumor multiplicity (13% of cases), we have to clarify that NanoString analysis was only performed on one nodule, usually the largest. As far as tumor encapsulation is concerned, we followed the WHO classification, which does not consider this feature as prognostic.[17] We must also stress that data on such a feature (presence or absence, and even complete or incomplete) is not always available when using pathological reports retrospectively. Lastly, resection margin was not integrated into the analysis since 9 patients (5%) underwent liver transplantation and margin distance was not available in 23 surgically resected patients (13%).

Finally, Ming-Da Wang *et al.* discussed the potential overlaps of clinicobiological variables (AFP score, tumor size and number, AFP and BCLC score) used in the statistical analysis. We agree with this comment and found a strong association between the 6-gene signature and overall survival (hazard ratio 2.15; 95% CI 1.12-4.14; p = 0.021) when performing the multivariate analysis without including the overlapping data (BCLC stage and AFP score). We also agree that recurrence free survival (RFS) is an appropriate endpoint in such a study. However, given the heterogeneity in the follow-up of patients (inclusion period 1995-2017), we were unable to evaluate RFS and liver function scores (ALBI and Child-Pugh) as too many data points were missing.

In conclusion, we strongly believe that adequate management of patients with HCC will be improved by a comprehensive tumor characterization. So far, HCC biopsy appears to be the best current routine diagnostic and prognostic approach. In that view, our 6-gene signature predicts very well MVI in routine FFPE biopsies. We acknowledge that further validation in prospective multicentric cohorts is required before translation into clinical practice.

Financial support

The authors received no financial support to produce this manuscript.

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

Manuscript preparation: A.B; Critical revision: V.P and V.V. All the authors reviewed the paper and approved the final version.

Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jhep.2022.06.011.

References

[1] Beaufrère A, Caruso S, Calderaro J, Poté N, Bijot J-C, Couchy G, et al. Gene expression signature as a surrogate marker of microvascular invasion on routine hepatocellular carcinoma biopsies. J Hepatol 2022;76:343–352. http://dx.doi.org/10.1016/j.jhep.2021.09.034.

[2] Li Chao, Ouyang Wei, Yang Tian. The association of microvascular invasion with satellite nodule, tumor multiplicity, tumor encapsulation and resection margin of hepatocellular carcinoma. J Hepatol 2022;77:890–891. http://dx.doi.org/10.1016/j.jhep.2022.03.036.

[3] Chen Zi-Xiang, Liu Si-Yu, Tong Xiang-Min. Preoperative prediction of microvascular invasion: Is invasive biopsy of HCC necessary? J Hepatol 2022;77:892–893. http://dx.doi.org/10.1016/j.jhep.2022.04.016.

[4] Wang Ke-Chun, Sun Li-Yang, Wang Ming-Da. Methodological considerations regarding a gene signature to predict microvascular invasion in hepatocellular carcinoma. J Hepatol 2022;77:893–894. http://dx.doi.org/10.1016/j.jhep.2022.04.027.

[5] Lim K-C, Chow PK-H, Allen JC, Chia G-S, Lim M, Cheow P-C, et al. Microvascular invasion is a better predictor of tumor recurrence and overall survival following surgical resection for hepatocellular carcinoma compared to the Milan criteria. Ann Surg 2011;254:108–113. http://dx.doi.org/10.1097/SLA.0b013e31821ad884.

[6] Rodríguez-Perálvarez M, Luong TV, Andreana L, Meyer T, Dhillon AP, Burroughs AK. A systematic review of microvascular invasion in hepatocellular carcinoma: diagnostic and prognostic variability. Ann Surg Oncol 2013;20:325–339. http://dx.doi.org/10.1245/s10434-012-2513-1.

[7] Di Martino M, De Filippis G, De Santis A, Geiger D, Del Monte M, Lombardo CV, et al. Hepatocellular carcinoma in cirrhotic patients: prospective comparison of US, CT and MR imaging. Eur Radiol 2013;23:887–896. http://dx.doi.org/10.1007/s00330-012-2691-z.

[8] Childs A, Zakeri N, Ma YT, O'Rourke J, Ross P, Hashem E, et al. Biopsy for advanced hepatocellular carcinoma: results of a multicentre UK audit. Br J Cancer 2021;125:1350–1355. http://dx.doi.org/10.1038/s41416-021-01535-2.

[9] Calderaro J, Couchy G, Imbeaud S, Amaddeo G, Letouzé E, Blanc J-F, et al. Histological subtypes of hepatocellular carcinoma are related to gene mutations and molecular tumour classification. J Hepatol 2017;67:727–738. http://dx.doi.org/10.1016/j.jhep.2017.05.014.

[10] Russo FP, Imondi A, Lynch EN, Farinati F. When and how should we perform a biopsy for HCC in patients with liver cirrhosis in 2018? A review. Dig Liver Dis Off J Ital Soc Gastroenterol Ital Assoc Study Liver 2018;50:640–646. http://dx.doi.org/10.1016/j.dld.2018.03.014.

[11] Di Tommaso L, Spadaccini M, Donadon M, Personeni N, Elamin A, Aghemo A, et al. Role of liver biopsy in hepatocellular carcinoma. World J Gastroenterol 2019;25:6041–6052. http://dx.doi.org/10.3748/wjg.v25.i40.6041.

[12] Geiss GK, Bumgarner RE, Birditt B, Dahl T, Dowidar N, Dunaway DL, et al. Direct multiplexed measurement of gene expression with color-coded probe pairs. Nat Biotechnol 2008;26:317–325. http://dx.doi.org/10.1038/nbt1385.

[13] Baskota SU, Dabbs DJ, Clark BZ, Bhargava R. Prosigna® breast cancer assay: histopathologic correlation, development, and assessment of size, nodal status, Ki-67 (SiNKTM) index for breast cancer prognosis. Mod Pathol Off J

U S Can Acad Pathol Inc 2020. http://dx.doi.org/10.1038/s41379-020-0643-8.
[14] Labgaa I, Villanueva A, Dormond O, Demartines N, Melloul E. The role of liquid biopsy in hepatocellular carcinoma prognostication. Cancers 2021;13:659. http://dx.doi.org/10.3390/cancers13040659.
[15] Xu X, Sun S, Liu Q, Liu X, Wu F, Shen C. Preoperative application of systemic inflammatory biomarkers combined with MR imaging features in predicting microvascular invasion of hepatocellular carcinoma. Abdom Radiol N Y 2022;47:1806–1816. http://dx.doi.org/10.1007/s00261-022-03473-w.
[16] Liu W, Zhang L, Xin Z, Zhang H, You L, Bai L, et al. A promising preoperative prediction model for microvascular invasion in hepatocellular carcinoma based on an extreme gradient boosting algorithm. Front Oncol 2022;12:852736. http://dx.doi.org/10.3389/fonc.2022.852736.
[17] WHO classification of Tumours Editorial Board. Digestive system tumours. 5th ed, vol. 1. Lyon: International Agency for Research on Cancer; 2019.

Aurélie Beaufrère[1,2,3]
Valérie Vilgrain[1,3,4]
Valérie Paradis[1,2,3,*]
[1]*Université Paris Cité, Faculté de Médecine, 16 rue Henri Huchard, Paris, 75018, France*
[2]*Department of Pathology, Hôpital Beaujon, FHU MOSAIC, AP-HP.Nord, 100 boulevard du Général Leclerc, Clichy, 92110, France*
[3]*INSERM UMR 1149, Centre de Recherche sur l'Inflammation, 16 rue Henri Huchard, Paris, 75018, France*
[4]*Department of Radiology, Hôpital Beaujon, FHU MOSAIC, AP-HP.Nord, 100 boulevard du Général Leclerc, Clichy, 92110, France*
[*]Corresponding author. Address: Université Paris Cité, Faculté de Médecine, 16 rue Henri Huchard, Paris, 75018, France.
E-mail address: valerie.paradis@aphp.fr (V. Paradis)

Decrease in viral hepatitis diagnoses during the COVID-19 pandemic in the Netherlands

To the Editor:

The COVID-19 pandemic has put a major strain on healthcare systems around the globe. Early reports have shown sharp reductions in most non-COVID diagnoses, from aortic dissections to cancer.[1,2] Efforts to control the pandemic have also had an important impact on the availability and capacity of viral hepatitis testing services.[3] In addition, the perceived risk of COVID-19 may affect a patient's willingness to visit healthcare professionals. Together, this may result in delayed or missed opportunities to diagnose patients with chronic viral hepatitis. A recent modelling study by Blach *et al.* published in *Journal of Hepatology*, underscores the potential detrimental effect on patient outcomes associated with disruptions in interventions aimed at viral hepatitis elimination.[4] However, the magnitude of the impact of the COVID-19 pandemic on viral hepatitis care in the EU region is currently unclear.

HBV/HCV infections are notifiable conditions under the Dutch Public Health Act, and testing facilities automatically report novel positive test results to the local Public Health Service. After assessment by specialised staff the cases are classified as novel acute or chronic infections and reported electronically to the National Institute for Public Health and the Environment. In order to assess the impact of the COVID-19 pandemic on novel chronic HBV and HCV diagnoses, we compared the number of reported cases in 2020 to the number of cases reported in 2019. In 2019, there were 1,105 novel chronic HBV and 664 novel chronic HCV diagnoses, which declined to 674 and 379 in 2020. We therefore observed an overall reduction of novel chronic viral hepatitis diagnoses of 40% (39% for HBV and 43% for HCV, p <0.001 compared to 2019). Interestingly, the weekly relative reduction in new chronic HBV and HCV diagnoses mirrored the weekly number of COVID-19 admission in the Netherlands. The sharpest drops in novel reported cases coincided with the peaks of the first and second COVID-19 admission waves (COVID-19 data from the NICE foundation,[5] Fig. 1). Still, even during the summer months when the number of COVID-19 admissions

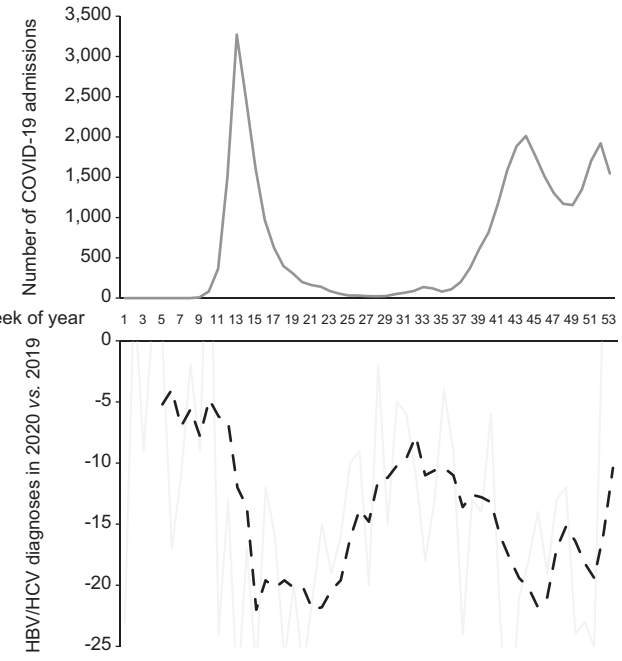

Fig. 1. COVID-19 admissions and HBV/HCV diagnoses in the Netherlands. The number of COVID-19 admissions in the Netherlands per week in 2020 (upper panel) and the reduction in the number of weekly reported new chronic HBV and HCV diagnoses in 2020 compared to the same week in 2019 (lower panel, with weekly reported cases in grey and the moving average in dashed black).

Keywords: HBV; HCV; COVID-19.
Received 19 March 2021; received in revised form 9 April 2021; accepted 12 April 2021; available online 20 April 2022
https://doi.org/10.1016/j.jhep.2021.04.015