(MELD) and the presence of sarcopenia and previous HE was performed. Co-presence of previous HE and sarcopenia were independently associated with mortality (HR 2.56, p = 0.0056, 95% CI 1.3-5), thus improving the prognostic value of MELD alone (HR 1.1, p <0.001, 95% CI 1-1.16). Moreover, the incidence of death was significantly higher in patients with sarcopenia and previous HE (log rank p = 0.009), as shown in Fig. 1.

While sarcopenia can be easily assessed with an appropriate imaging technique (*e.g.* CT), the objective evaluation both of presence and grading of HE remains more challenging. However, these results confirm the important role of muscle alterations and HE as concomitant and important co-factors for mortality in patients with cirrhosis.

It is therefore crucial to look at the emerging clinical determinants of cirrhosis, such as muscle alterations, from a different perspective, in which some various new factors could add prognostic value to the oldest and most well-established ones. Indeed, not only the presence of a severe liver disease, or a previous history of minimal/covert HE, or iatrogenic portosystemic shunts, but also sarcopenia, nutritional deficit or spontaneous portosystemic shunts could play a major role. Patients with cirrhosis should be considered under this complex and whole panorama, aiming to identify a well-defined subgroup of very high-risk patients, in which other factors with a deeper clinical and instrumental assessment and a consequent "non classical" management should be adopted.[5] The future could involve validation of new prognostic tools able to combine these robust clinical (HE or sarcopenia), radiological (spontaneous/iatrogenic shunts) and biochemical (MELD) variables with the aim of identifying and promptly treating extremely high-risk patients.

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Authors' contributions
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Silvia Nardelli: data analysis, critical revision for important intellectual content, final approval.

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Reply to: "Gut liver muscle brain axis: A comprehensive viewpoint on prognosis in cirrhosis"

To the Editor:
We would like to thank Ridola *et al.*[1] for their interest in our work[2] and for validating our conclusion.

The onset and progression of cirrhosis is accompanied by dysfunction of the gut-liver-brain axis. Gut dysbiosis is often observed in patients with cirrhosis compared to healthy individuals, which causes intestinal barrier dysfunction, increases bacterial translocation, activates circulating immune cells, and ultimately leads to cytokine production and systemic inflammation.[3]

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Systemic inflammation, as a cirrhosis-associated immune dysfunction, is associated with the development of liver decompensation, organ failure, bacterial infection and poor prognosis.[3] In addition, intestinal flora are closely related to several aspects of hepatic encephalopathy (HE) pathogenesis, including ammonia toxicity theory, bile acid cycle, gamma-aminobutyric acid hypothesis and neuroinflammation.[4] Sarcopenia, as a malnutrition-related complication of cirrhosis, has received increasing attention in recent years. Hyperammonemia and systemic inflammation are the core mechanisms responsible for the development of sarcopenia. Hyperammonemia upregulates myostatin levels, activates autophagy, impairs the mTOR pathway in a manner that increases mitochondrial dysfunction and production of reactive oxygen species.[5] Systemic inflammation also promotes sarcopenia by decreasing muscle protein synthesis and increasing protein degradation.[5] Thus, muscle can be added as a system to form the gut-liver-brain-muscle axis to better understand the progression and prognosis of cirrhosis.

The prevalence of sarcopenia in the study by Ridola *et al.* was 56%,[1] higher than that of 37.5% in our meta-analysis,[2] which may be attributed to a higher proportion of males, and patients with Child-Pugh class B/C cirrhosis, and previous HE. Since sarcopenia is associated with the severity of liver dysfunction, their study included patients with cirrhosis and relatively serious liver dysfunction, which can be reflected by higher mortality (39%) during a mean period of 12.7±10.1 months,[1] compared to the 1-year cumulative probabilities of survival of 76.6% (95% CI 66.4%–85.5%) in patients with sarcopenia in our study.[2]

Our meta-analysis showed that sarcopenia is independently associated with mortality in patients with cirrhosis, and this association was validated by Ridola *et al.*, who considered that the co-presence of previous HE and sarcopenia could improve the prognostic performance of the MELD score alone.[1] However, there is still controversy about whether sarcopenia can improve the predictive value of existing models. In a model of MELD-sarcopenia established by Montano-Loza *et al.*, they included 669 patients with cirrhosis evaluated for liver transplantation (LT), and found MELD-sarcopenia was not superior to MELD in predicting 3- or 6-month mortality, and MELD-sarcopenia had only a small advantage in predicting overall and 12-month mortality.[6] Subsequently, van Vugt *et al.* externally validated the MELD-sarcopenia model in 585 European patients, and showed that it was not superior to the MELD score alone in their cohort. Meanwhile, in the same cohort, van Vugt *et al.*, developed a novel score including MELD, sarcopenia, HE and age. However, the discriminative value of the model (c-index 0.851) was very similar to that of MELD alone (c-index 0.839).[7] In addition, the discriminative value of other combined models such as MELD-psoas score also failed to improve the accuracy of prediction.[8] Based on uniform and validated measurement methods and cut-off values, the predictive value of MLED-sarcopenia remains to be externally validated.

Considering the potential impact of sarcopenia on prognosis, some studies considered that indications for transjugular intrahepatic portosystemic shunt (TIPS) and LT could be expanded for patients with cirrhosis and sarcopenia, based on the fact that TIPS can improve sarcopenia and the prognosis of these patients.[7,9] However, there is still a lack of high-level evidence to recommend the expansion of these indications. High-quality studies need to be designed to directly demonstrate that the benefits of these operations outweigh the drawbacks, and the economic burden and resource availability should also be taken into account.

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Authors' contributions

XT, YHY: Drafting of the manuscript. JW, FJ: Study conception and study supervision, critical review of the manuscript. All authors: Approved the manuscript.

Supplementary data

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Active HBV replication in hypoxic pericentral zone 3 is upregulated by multiple host factors including HIF-1α

To the Editor:

With great interest, we read the manuscript "Hypoxia inducible factors regulate hepatitis B virus replication by activating the basal core promoter" by Wing *et al.* published in the *Journal of Hepatology*.[1] The authors identified 2 conserved hypoxia response elements (HREs) within the HBV genome, through which stabilized hypoxia inducible factor-1α (HIF-1α) bound to HBV DNA, activated the basal core promoter, and prompted HBV replication under hypoxic conditions. Physically, cross sections of liver lobules from the portal vein (PV) to the central vein (CV) could be arbitrarily divided into 3 concentric zones, including periportal zone 1, midlobular zone 2 and hypoxic pericentral zone 3,[2] and it would be interesting to explore whether the physically formed lower oxygen tension in zone 3 would be more adaptive for HBV replication. Herein, we aimed to provide more evidence for the promotion of HBV replication by hypoxia and HIF-1α, and search for other factors involved in the upregulation of HBV replication under hypoxic conditions.

To provide extra experimental evidence supporting the role of HIF-1α in HBV replication, precursor recombinant covalently closed circular HBV DNA (prcccDNA)/pCMV-Cre plasmids were co-transfected with HIF-1α expression plasmid (or vector control) into HepG2 cells for 72 h, then levels of HBcAg, supernatant HBV DNA and HBV RNA were detected. The increased levels of HBcAg protein, supernatant HBV DNA and supernatant HBV RNA further confirmed that HIF-1α could indeed prompt HBV replication (Fig. 1A). It has been reported that HBx could also stabilize HIF-1α by preventing its ubiquitin-dependent degradation, independently of hypoxia.[3] We next constructed HBx-depleted prcccDNA plasmid (prcccDNAΔHBx) by nonsense mutation at the second ATG within the HBx open reading frame, and co-transfected prcccDNAΔHBx/pCMV-Cre with HBx expression plasmid (or vector control) into HepG2 cells for 72 h, before measuring the levels of HIF-1α protein and indicators of active HBV replication. It came out that HBx could stabilize HIF-1α at the protein level and synergistically increase the levels of HBcAg protein, supernatant HBV DNA and HBeAg, indicating the involvement of HIF-1α in the upregulation of HBV replication induced by HBx (Fig. 1B). Taken together, HIF-1α could upregulate HBV replication, which further supported Wing *et al.*'s research.

The HBeAg-positive chronic HBV infection (EPI) phase is characterized by higher HBV replication due to a lack of host immune responses,[4] and is a suitable stage at which to measure the real influence of hypoxia on HBV replication. Therefore, liver samples from 38 chronic HBV-infected individuals within the EPI phase were collected from Beijing 302 Hospital and stained for HBcAg by immunohistochemistry (Table S1). In total, 158 zonated structures were recognized and it was found that the percentage of HBcAg-positive hepatocytes was significantly zonated, and was higher in hypoxic pericentral zone 3 (16.5%) than in midlobular zone 2 (7.0%) or periportal zone 1 (5.1%) (Fig. 1C), which further verified the upregulation of HBV replication induced by hypoxia.

Since the cellular gene expression profile was zonated within 3 zones,[5] we wonder whether the expression pattern of other host factors could also contribute to zonated HBV replication seen above. The single cell RNA-sequencing dataset (GSE115469) was downloaded for further analysis. According to the expression profile of established cell-specific marker genes, there were 639 cells clustered into periportal zone 1, 103 cells for midlobular zone 2 and 2,570 cells for pericentral zone 3 (Fig. S1A). Given that HIF-1α was stabilized at the protein level rather than the transcriptional level, we further compared the expression pattern of *SLC10A1*, *CTNNB1* and *HNF4A* within 3 zones (Fig. S1B-D). The level of sodium taurocholate cotransporting polypeptide (NTCP) encoded by *SLC10A1* was higher in zone 3 than in zone 2 or zone 1 (Fig. 1D, left panel). NTCP is a multiple transmembrane transporter and participated in the enterohepatic circulation of bile salts.[6] Physically, bile salts were mainly removed by periportal zone 1 hepatocytes, and pericentral zone 3 hepatocytes were recruited at higher bile salt loads,[7] thus higher expression of NTCP in zone 3 may guarantee the complete absorption of bile salts. Meanwhile, NCTP was reported as the functional receptor of HBV,[8] therefore its zonated expression pattern may support HBV infection in hypoxic pericentral zone 3. Apart from *SLC10A1*, the expression levels of *CTNNB1* and *HNF4A* were also higher in zone 3 than in zone 1, showing a similar pattern to each other (Fig. 1D, middle and right panel). Since the Wnt/β-catenin pathway and HNF-4α were both reported to promote HBV replication,[9,10] their zonated expression pattern may also contribute to the upregulation of HBV replication in pericentral zone 3.

Keywords: HBV replication; hypoxia; HIF-1α; NTCP; β-catenin; HNF-4α; liver zonation.
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