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Reply to: "Evidence supporting a beneficial role of vitamin D in chronic hepatitis C"

To the Editor:

We thank Pang *et al.* for their interest in our recently published systematic review and meta-analysis involving 2605 patients which found no association between baseline 25-hydroxyvitamin D level and sustained virologic response (SVR) to interferon-based antiviral therapy in chronic hepatitis C infection [1]. They are correct to highlight the influence of ethnicity on both vitamin D status and genetic polymorphisms in key proteins involved in vitamin D synthesis. The studies included in our meta-analysis contained only a small number of participants of Asian [2] or African-American [3] ethnicity, leading us to highlight the study's inability to adjust for ethnicity as one of its limitations.

With regards to the recently published meta-analysis by García-Álvarez *et al.* [4] evaluating vitamin D status and response to hepatitis C therapy, this study differs from ours in that it includes those with HCV-HIV co-infection. Furthermore, we believe this study has significant methodological issues such as the inclusion of three studies involving the same Italian cohort of approximately 200 patients, and the exclusion of five large studies [2,5–8] from Europe and Australia involving 1569 patients that were readily identifiable using the stated search strategy. Our concerns about this study have recently been published [9] and the validity of the study's findings should be viewed with caution.

Our meta-analysis only evaluates the relationship between baseline vitamin D status and SVR. The impact of vitamin D supplementation on outcomes to interferon-based antiviral therapy, although interesting, is a different clinical question that has not been definitively assessed in prospective, randomized controlled trials. We agree that vitamin D has potential anti-viral, anti-inflammatory, anti-fibrotic and immunomodulatory actions relevant to liver disease, which have been highlighted in a number of pre-clinical studies [10]. However high quality prospective clinical research studies are needed to support the hypothesis that vitamin D deficiency may be responsible for the worse outcomes in HCV related liver disease.

Conflict of interest

The authors declared that they do not have anything to disclose regarding funding or conflict of interest with respect to this manuscript.

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Hepatocyte senescence explains conjugated bilirubinaemia in chronic liver failure

To the Editor:

Conjugated bilirubinaemia in patients with chronic liver disease (CLD) reflects hepatic decompensation and a poor prognosis [1]. The pathophysiology that underlies conjugated bilirubinaemia in hepatic decompensation is poorly understood. There is no demonstrable flaw in processing unconjugated bilirubin and a more likely explanation is altered hepatocyte handling of conjugated bilirubin.

Hepatocyte senescence is present across diverse aetiologies and as many as 80% of hepatocytes show the senescent phenotype in advanced liver disease [2]. Metabolic activity is altered when a cell becomes senescent and one potential consequence is an alteration of conjugated bilirubin transport in senescent hepatocytes, which accumulate in advanced CLD.

Serum bilirubin and hepatocyte telomere length were measured in 70 patients within the spectrum of NAFLD. Mean hepatocyte telomere intensity, a surrogate marker of telomere length, was measured using quantitative fluorescent *in-situ* hybridization, as described [3]. There was an inverse relationship between serum bilirubin and hepatocyte telomere length ($p = 0.04$, Fig. 1). Thus, accelerated hepatocyte ageing is associated with jaundice.

Liver sections from five of those patients were double-stained using unconjugated mouse monoclonal anti-p21 (Dako; concentration 1:100, heat-induced EDTA-based antigen retrieval, 20 min) and unconjugated mouse monoclonal anti-MRP2 (Merck Millipore; concentration 1:20, heat-induced citrate-based antigen retrieval, 20 min). MRP2 was negative in p21-positive (senescent) hepatocytes and was only detected in p21-negative hepatocytes (Fig. 1). Reliable immunohistochemical staining could not be achieved with available MRP3 antibodies.

An *in vitro* model was used to examine gene expression of MRP2 and MRP3 in senescent hepatocytes by real-time PCR. Cellular senescence was induced in HepG2 cells by incubation with 0.5 mM H_2O_2 in culture medium for 60 minutes, as described [4]. Expression of MRP2 was downregulated in senescent HepG2 cells; in contrast, expression of MRP3 was upregulated (Fig. 1).

Hepatocytes are polarised cells; MRP2 is restricted to the canalicular (apical) membrane, whereas MRP3 is found only in the sinusoidal (basolateral) membrane [5]. Both MRP2 and MRP3 are unidirectional efflux pumps, which transport conjugated bilirubin into the canalicular space (bile) or the sinusoid (blood), respectively [5]. Reduced MRP2 expression in senescent hepatocytes *in vitro* and an absence of MRP2 protein in p21-positive (senescent) hepatocytes suggest reduced conjugated bilirubin transport into the biliary canaliculi. Increased MRP3 expression in senescent hepatocytes may be compensatory, increasing transport of conjugated bilirubin into the hepatic sinusoid (Fig. 1). It is, however, not clear why changes in MRP2 and MRP3 accompany hepatocyte senescence.