Reply to: The use of acoustic radiation force-based shear stiffness in non-alcoholic fatty liver disease

To the Editor:

We appreciate the comments and clinical perspectives raised by Dr. Cross in response to our recent publication concerning the use of acoustic radiation force-based shear stiffness values in NAFLD patients to evaluate fibrosis scores [1], and we would like to take this opportunity to respond to those points. Dr. Cross mentions the significant number of failed stiffness reconstructions in our study (21.5%), which we agree would be too high for a screening test to evaluate liver fibrosis. It should be noted that the majority of those reconstruction failures (~84%) occurred in patients with BMI >30. As discussed in the manuscript [1], higher BMI is associated with deeper livers, reduced acoustic radiation force magnitudes, and therefore noisier data for performing the reconstructions. Many of these patients with failed shear wave reconstructions did not meet our quality control threshold of having an IQR/mean $\leqslant$0.3 over the replicate shear wave estimates in the three imaging locations. This reconstruction variability is an active research efforts focused on improving the next generation of shear wave reconstruction algorithms. Additionally, we would recommend restricting the imaging locations utilized in patients with high BMI to help improve the repeatability of the reconstructed stiffnesses. While the intercostal imaging windows tended to yield repeatable shear wave reconstructions, the addition of the subcostal window was associated with additional variability in this subset of patients, most likely due to the additional subcutaneous fat at this location.

Although an intention-to-treat analysis was not performed in the context of our study, one can argue that knowledge of the absence of advanced NAFLD (i.e. fibrosis stage 3–4) using a non-invasive test would decrease resource utilization and cost associated with performing liver biopsies in all patients with suspected NAFLD. As highlighted in Fig. 2 of the referenced manuscript [1], the shear modulus across the early stages (fibrosis stage 0–2) of disease is very narrow, and those patients do not have increased morbidity or mortality from liver and cardiovascular related outcomes. With the exception of optimizing risk factors for disease progression, patients with early stage disease do not require surveillance and monitoring for complications associated with advanced hepatic fibrosis. We agree with Dr. Cross that additional statistical analyses are necessary to better define the sensitivity, specificity, negative and positive predictive values as well as positive and negative likelihood ratios for such a non-invasive test to have widespread clinical applicability. However, such analyses would ideally include a much larger sample size. Therefore, larger studies powered to derive meaningful threshold values capable of guiding clinical decisions are necessary.

Conflict of interest

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Reference

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Transplanted hepatocytes: Wiped out or washed out?

To the Editor:

Hope for the long-term effectiveness of hepatocyte transplantation (HTx) for metabolic liver disease in pediatric patients is based on the premise that hepatocytes in the main constitute a static population, quite unlike for example, the small bowel where there is constant turnover. However, several recent studies on the dynamics of hepatocyte turnover raise important questions about the long-term success of HTx. For example, a recent lineage tracing study in the mouse has demonstrated that, within a year, almost the total parenchymal population is replaced by new hepatocytes differentiating from Sox9-expressing interlobular bile ducts [1,2], suggesting that transplanted hepatocytes will inevitably be 'washed out' of the liver within the turnover time – in about 1 year in the mouse. Other murine studies have pointed to a small sub-population of biliary duct cells, also being Sox9-positive, as being bipotential, driving progenitor cell-mediated liver regeneration [3], seemingly giving rise to Foxl1-expressing progenitor cells, also with bipotentiality, but with limited proliferation potential [4]. A further murine study has shown that 0.076% of all albumin-expressing hepatocytes are born from albumin-naïve cells every 4 days in normal healthy mice, again highlighting the dynamic cell state of the normal liver [5].

These murine studies have re-awakened the discussion as to whether hepatocytes 'stream' from portal tracts to hepatic veins, first described for the rat liver [6]; subsequently with the liver being formally proposed as a 'stem cell and lineage system', hierarchically organized into stem, amplifying and differentiating

compartments [7]. Very recently, these cell maturational stages have been characterized in great detail in terms of the likes of morphology, ploidy, proliferation, biochemistry and gene expression [8]. Moreover, our own recent studies in the human liver, demonstrating a portal tract to hepatic vein orientation of groups of clonally-derived hepatocytes, are in line with the concept of a dynamic lineage system [9]. On the other hand, not all studies have concurred with this concept, for example Bralet and colleagues [10] genetically labeled rat hepatocytes *in vivo* at 24 h after partial hepatectomy, but failed to observe any change in their location (periportal and mid-zonal) over the proceeding 15 months – observations not consistent with a 'streaming' liver.

There are recent examples of the fairly long-term beneficial effects of HTx and survival of engrafted cells, for example, a Crigler-Najjar patient survived well for 4 years after HTx before an OLT and he still had conjugated bilirubin in his blood at 3.5 years [11]. In 2006, Sokal's group performed a HTx for the correction of argininosuccinate lyase deficiency, and cell tracking confirmed their durable presence (12.5%) in the liver at 7 months after the last infusion [12]. More recent information on that same case confirmed that the patient was still doing well at up to 18 months when she received an OLT [13]. On the other hand, the majority of children undergoing HTx for urea cycle disorders have only been monitored for a relatively short time before OLT, though one 3-year-old patient with citrullinemia was still doing well 30 months after HTx [14]. Many other cases of HTx have also only provided short-term benefit including glycogen storage disease type I [15], and factor VII deficiency [16].

In most studies, the absence of a sustained benefit of HTx in the medium to long-term has been ascribed to rejection ('wipe-out') or other causes not directly related to HTx itself (e.g. infections), but we would like to suggest that hepatocyte egress ('wash-out') could be an alternative, but non-exclusive explanation. We believe it is beholden upon hepatologists to once and for all establish the cell replacement dynamics of the liver, preferably in a large animal model. If the 'streaming liver' hypothesis wins the day then attempts at the correction of metabolic liver disease should be directed towards targeting cholangiocytes and/or other hepatocyte progenitors or only transplanting hepatocytes into extrahepatic sites.

Conflict of interest

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Entecavir in the treatment of chronic hepatitis B in kidney transplantation

To the Editor:

We have read with interest the review by Vallet-Pichard *et al.* published in a recent issue of the *Journal* [1]. In the section "Recommendation for HBV therapy" they reviewed the scarce data published on this topic. They cited the article published by Kamar *et al.* as the only experience with NUC therapy in renal transplantation [2]. We have published our experience with entecavir treatment in a small population of chronic HBV patients with chronic kidney disease [3]. Eleven male patients – 1 with stage 4 chronic kidney disease, 7 undergoing hemodialysis in the waiting list for a transplant, and 3 kidney transplanted recipients – were included in the study evaluation. Six were treatment naïve, and 5 were