

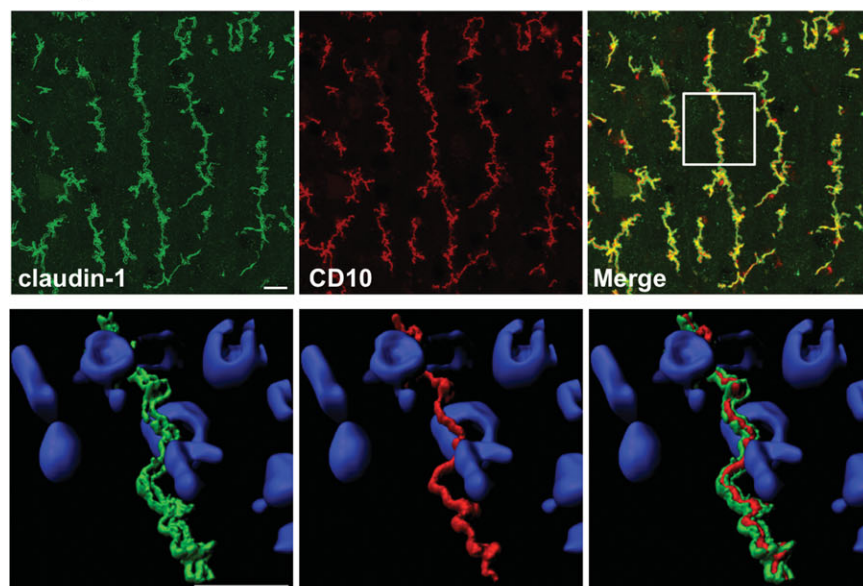
Heterogeneous Claudin-1 Expression in Human Liver

To the Editor:

We congratulate Mensa et al.¹ on their report studying the expression of hepatitis C virus (HCV) receptors claudin-1 and occludin after liver transplantation and their influence on early viral kinetics. The authors provide a unique insight into the potential role receptor expression levels play in modulating early phase viral kinetics. They observed an association between HCV recurrence and hepatocellular claudin-1 and occludin expression levels expression during the first week post liver transplant. The authors confirm the results of previous reports showing increased claudin-1 expression in HCV-infected liver.^{2,3} However, Mensa et al. conclude that claudin-1 is solely located at the apical pole of hepatocytes, in contrast to reports by Reynolds et al. and Zadori et al.^{2,3} We agree that claudin-1 is predominantly expressed at the apical membrane of hepatocytes in normal liver; however, a minor

pool of claudin-1 is observed at the basolateral membrane (Fig. 1). Basolateral expressed claudin-1 is more easily discerned when the liver section is co-stained with a marker for the hepatocellular membrane such as cytokeratin 8 (Fig. 1B), enabling one to observe heterogeneous patterns of localization across the liver parenchyma. The discrepancies between these studies are most likely explained by the imaging technique and analytical software employed. Spectral imaging of liver sections enables the accurate quantification of bound fluorescent antibody irrespective of signal intensity. However, volumetric imaging of claudin-1 at areas of high (apical) and low (basolateral) expression requires multiple threshold values (Fig. 1). In contrast, Mensa et al. quantified volumetric images of claudin-1 using a single threshold value, leading to a potential bias in their protein quantification and an underrepresentation of basolateral claudin-1. In conclusion, Mensa et al. have highlighted a role for viral receptor expression in defining HCV kinetics

A. Apical claudin-1



B. Apical and basolateral claudin-1

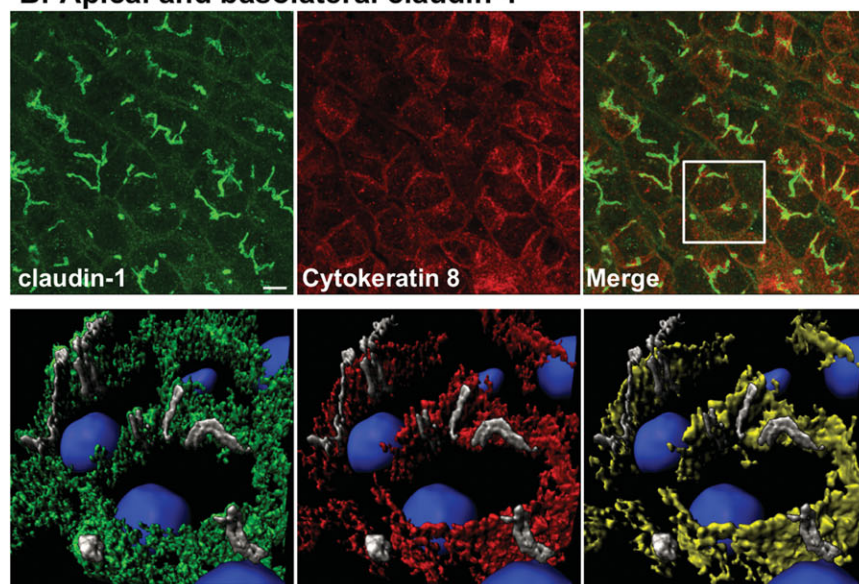


Fig. 1. Claudin-1 localization in normal human liver. Representative images of claudin-1 costained with markers specific for apical (CD10) or basolateral (cytokeratin 8) membranes. Heterogeneous patterns of claudin-1 at apical and basolateral membranes were observed. Magnified volumetric images (white box) prepared using Imaris software demonstrate claudin-1 localization around CD10, in keeping with a pericanalicular tight junction distribution. Application of low and high fluorescent intensity thresholds discriminates apical tight junction-associated (silver) and basolateral (yellow) pools of claudin-1. Nuclei were stained with DAPI (blue) and the scale bar represents 10 μm .

posttransplant, warranting further investigation to study the role of host pathways and inflammatory responses that regulate viral receptor hepatocellular expression.

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Potential conflict of interest: Nothing to report.

Reply:

We appreciate the observations made by Harris et al. regarding the differences in claudin-1 expression in human liver between the report by Reynolds et al.¹ and our work.² The main objective of our study was to assess the potential changes in tight junction pro-

teins claudin-1 and occludin following hepatitis C virus (HCV) graft infection. We observed an increased expression of claudin-1 and occludin over time in HCV-infected patients. The increase in claudin-1 was particularly significant in individuals with cholestatic hepatitis. It is important to notice that when we applied very low threshold values to create a surface for quantification, it was almost impossible to discriminate basolateral claudin-1 staining from either unspecific staining or tissue autofluorescence. More restrictive thresholding guarantees the quantification of specific signal, although it should be noted that this is at the expense of decreased sensitivity. Thus, it is a possibility that we may have underestimated claudin-1 expression in the basolateral membrane because we quantified fluorescence intensity using a single threshold value.

The detection of minor pools of basolateral claudin-1 is an interesting finding. Further studies are required to investigate the role of nonjunctional claudin-1 in HCV entry and the physiopathological consequences of increased levels of claudin-1 expression in HCV disease progression.

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Clinical Relevance of Detectable Hepatitis C Virus RNA in the Context of Direct-Acting Antivirals

To the Editor:

Harrington et al. discuss the clinical relevance of detectable, but not quantifiable, hepatitis C viral (HCV) RNA during treatment with the two recently approved direct-acting antivirals (DAAs), boceprevir and telaprevir.¹ The clinical trials used to assess the efficacy of these new DAAs were not designed to assess response-guided therapy using the less than lower limit of quantification [LLOQ] cutoff. However, a viremia below the LLOQ, but with detectable amounts of virus, clearly indicates that peripheral clearance has not occurred and, by implication, that replicating virus is still present in the liver. The endpoint for the LLOQ for most clinical trials is 25 IU/mL (1.39 log₁₀). The reduction in the sustained virological response (SVR) rate between those patients that have a viremia less than the LLOQ and those that have no detectable viremia clearly indicates that lack of peripheral suppression is still a good surrogate for persistence. No assay currently available detects HCV down to a level of 0.001 IU/mL, as outlined in Figure 1 of Harrington et al.¹

We have assessed the decreasing confidence interval (CI) associated with HCV reverse-transcriptase polymerase chain reaction (RT-PCR) on a panel of characterized HCV genotype 1b samples (100, 37, 10, 3.7, 1, 0.37, and 0.04 IU/mL; AcroMetrix; Invitrogen, Carlsbad, CA). The test platform was the Roche AmpliPrep and TaqMan 48 (Roche Molecular Diagnostics, Pleasanton, CA). Tests were replicated between 13 and 25 times. A 100% hit rate was achieved for the 100- and 37-IU/mL samples. A 95% CI was achieved at 9.914 (range, 5.737-26.578; n = 13). Probit analysis yielded a 60% hit rate at 2.624 IU/mL (95% CI: 1.782-4.241) and a 40% hit rate at 1.564 IU/mL (95% CI: 1.011-2.322). The assay did not yield detectable RNA for the 0.37 and 0.04 IU/mL samples (n = 25 and n = 18, respectively). We agree with Harrington et al.'s suggestion that validated cut-off LLOD points with appropriate CIs are applicable to the provision of optimal care and maximizing of SVR rates. An understanding of the decline in CIs surely makes the assessment of end-of-treatment detectable (but below the LLOQ) results as false positives too convenient an explanation.¹ These transient viremias