Does Occult Hepatitis C Virus Infection Exist?

Occult hepatitis C virus (HCV) infection has been found in anti-HCV and serum HCV RNA-negative patients with abnormal results of liver function tests of unknown origin and in patients with spontaneous or treatment-induced recovery from hepatitis C (2–6, 9–11).

In a recent issue of the Journal of Clinical Microbiology, Halfon and coworkers questioned the existence of occult HCV infection as they have not detected HCV RNA in the peripheral blood mononuclear cells (PBMCs) of patients with cryptogenic liver diseases (7). However, negativity for HCV RNA in PBMCs does not exclude the existence of occult HCV infection because the “gold standard” method to identify this occult infection is by detection of viral RNA in liver cells. Thus, the authors should have tested liver samples in order to refute the original report (3). If no liver samples are available (as seems to be the case in Halfon’s report), already published alternative approaches, such as detection of HCV RNA in ultracentrifugated serum samples (1), should be performed in addition to HCV RNA detection in PBMCs to confirm negative results.

There are also several concerns about the method used by Halfon and coworkers. First, the authors have not provided any information on how PBMC samples were preserved for RNA isolation. This is an important issue when testing for HCV RNA because improper storage of samples hinders viral RNA detection (8). Positivity of HCV RNA in PBMCs of patients with chronic hepatitis C does not ensure good preservation of samples because the amount of HCV RNA in PBMCs is higher in these patients than in occult HCV-infected patients. Consequently, viral RNA detection would be less affected by the quality of the isolated RNA. In addition, even though the authors used 1 million PBMCs for RNA isolation, the efficiencies of RNA extraction may differ among samples, which would affect HCV RNA detection. Thus, it is important to quantify total RNA to perform each PCR assay with the same amount of RNA. Second, when negative data are reported, the sensitivity of the assay is critical to ensure that the lack of HCV RNA detection is not due to the low sensitivity of the technique used. In this regard, the method employed to determine the sensitivity of HCV RNA detection in PBMCs was inadequate. Adding an HCV RNA-positive serum to uninfected PBMC lysates only ensures that the lysate by itself does not interfere with viral RNA detection. However, it does not indicate whether intracellular HCV RNA from infected PBMCs would be detected. A more appropriate method would be to use PBMCs from a patient with chronic HCV infection for RNA isolation and HCV RNA detection and then to test serial dilutions of the isolated RNA. Finally, the patient population studied was too small and heterogeneous to dispute previous results on occult HCV infection.

In conclusion, the data reported by Halfon and coworkers (7) do not provide any evidence against the existence of occult HCV infection.

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Authors’ Reply

We thank Dr. Carreño and colleagues for their letter giving us an opportunity to debate on the so-called “hepatitis C virus (HCV) occult infection”. Since the publication of our study in the Journal of Clinical Microbiology (4), two other papers recently published have confirmed our findings (1, 8).

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The first issue addressed is related to the concept of peripheral blood mononuclear cell (PBMC-based) HCV replication without presence of HCV plasma viremia. Numerous publications have analyzed extrahepatic compartments of viral replication that could potentially contribute to plasma viremia, most frequently PBMCs (2, 3–6). The contribution of PBMC-based HCV replication to total viremia remains unclear, as the level of negative-strand HCV RNA in PBMCs is very low compared to the level in the liver (6, 12). The continued presence of viral RNA in the PBMCs of subjects who had either spontaneously cleared their plasma viremia or cleared viremia following antiviral therapy has recently been reported, raising concerns that PBMCs may serve as a long-lived HCV reservoir capable of rekindling systemic infection (4, 5). To address this question, Bernardin et al. determined whether HCV RNA could be detected that was associated with PBMCs of seropositive blood donors who had spontaneously or therapeutically cleared their plasma viremia (1). Blood donor plasma viremia status was first determined with a highly sensitive transcription-mediated amplification (TMA) test performed in duplicate assays. PBMCs from 69 aviremic and 56 viremic blood donors were then analyzed for the presence of HCV RNA with TMA adapted to detect viral RNA in PBMCs and with a reverse transcription–nested-PCR assay. PBMC-associated HCV RNA was detected in none of the 69 aviremic donors, including all 6 subjects with a sustained viral response following antiviral therapy, whereas PBMC-associated HCV RNA was detected in 43 of the 56 viremic donors. The 13 viremic donors with no detectable PBMC-associated HCV RNA all had very low viral loads. They concluded that the PBMC HCV RNA detected in all 69 aviremic donors reported was possibly a result of the higher sensitivity of the TMA assay used to test for plasma viremia and that PBMC-associated HCV is unlikely to be maintained as a viral reservoir with the potential to rekindle plasma viremia in aviremic subjects, as determined by plasma TMA assays.

Unfortunately, our paper was accepted only as a short report, thus not permitting us to detail the complete methodology. A systematic detection of an RNA cellular internal control was done in all PBMCs assessed in our study. Regarding the storage and handling of sera and PBMCs, all of these materials (from centrifuged plasma and PBMC isolation) were stored at −80°C immediately after blood sampling and tested without cycles of freezing and thawing as previously recommended (5). The method proposed by Carreño and coworkers using PBMCs from a chronic HCV-infected patient for RNA isolation and HCV RNA detection and then testing serial dilutions of the isolated RNA cannot be a “gold standard” due to the limit of detection of HCV RNA by an in-house reverse transcription-PCR. Moreover, PBMCs of our patients were subjected to HCV RNA TMA assays without any HCV RNA detectable using this high-sensitivity assay (7).

It has been demonstrated that the slow decrease in anti-HCV antibody titers found in HCV-infected patients with spontaneously cleared viremia as well as the complete seroreversion detected in 7% of transfusion-transmitted infections may also reflect an absence of ongoing antigenic stimulation, indirectly supporting clearance of infection in persons who test HCV RNA negative in plasma (11). These findings are supported by the lack of HCV transmission following 11 of 12 fresh whole-blood transfusions (containing approximately 10^9 PBMCs) from aviremic donors (using duplicate TMA) (9).

The second issue addressed is related to the concept of the presence of HCV RNA in the liver in the absence of HCV RNA detected in PBMCs (10). Carreño et al. suggested that negativity for HCV RNA in PBMCs does not exclude the existence of occult HCV infection because the gold standard method to identify this occult infection is detection of viral RNA in liver cells. To address this question, Maylin et al. recently assessed the presence of residual HCV RNA in serum, liver, and PBMCs using TMA (sensitivity, <9.6 IU/ml) in a long-term follow-up study of chronic hepatitis C patients who achieved sustained virological response after interferon-based treatment. A total of 114 patients had a posttreatment liver tissue specimen, and 156 had PBMC specimens. Serum HCV RNA remained undetectable (1,300 samples), indicating that none of the patients had a relapse. HCV RNA was detectable in 2 of 114 (1.7%) liver specimens and in none of 156 PBMC specimens. These authors strongly suggest that sustained virological response may be considered to show eradication of HCV infection (10).

In conclusion, our publication and the two more recent studies using sensitive assays and liver/PBMC assessment re-inforce the absence of evidence of occult HCV infection.

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