Prediction of Relapse following Treatment for Hepatitis C: Is Whole Blood More than the Sum of Its Parts?

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(See the article by Watkins-Riedel et al. on pages 1754–60)

Therapy for hepatitis C virus (HCV) has a long duration, is expensive, and is challenging for the patient and the health care professional. The current regimen consists of pegylated IFN (injected once weekly) and ribavirin (administered daily) together for 24–48 weeks, depending on the viral genotype. Combined toxicities of these medications include depression (which may be severe), fever, flulike syndrome, and multiple hematologic abnormalities, including anemia, neutropenia, and thrombocytopenia. These medications can cost thousands of dollars per year, and the requirement of weekly injections combined with the prolonged duration of flulike illness require clinicians to provide considerable support to patients who elect to be treated. Rates of sustained virologic response (conventionally defined as the absence of serum viremia 24 weeks after completion of treatment) vary widely, depending on the genotype and other host factors [1], and in clinical trials range from 80%–90% in patients with HCV genotype 2 and 3 infection to 40%–50% in otherwise healthy patients with genotype 1 infection. However, in community practice, the substantial barriers presented by this regimen result in far fewer patients receiving medication and achieving a sustained virologic response [2]. Obviously, both clinicians and patients have a substantial interest in early prediction of failure to achieve sustained virologic response, to minimize both unwarranted toxicities and costs.

In this issue of Clinical Infectious Diseases, Watkins-Riedel et al. [3] address the issue of laboratory prediction of relapse after initially successful treatment of hepatitis C. This study compared the kinetics of RNA positivity in serum, plasma, and whole-blood specimens both before and after the end of treatment. A group of patients who did not respond to initial standard-dose IFN monotherapy were treated with high-dose standard IFN alone or in combination with ribavirin. Fifty-six sequential patients were chosen to undergo testing for detection of HCV RNA in serum, plasma, and whole-blood specimens every 4 weeks, beginning at week 24 of the study (24 weeks before the end of treatment) and continuing through week 24 after the end of treatment. Of these patients, 18 had negative tests results for the presence of HCV RNA in serum at the end of treatment, and 14 ultimately relapsed. Watkins-Riedel et al. [3] found that detection of HCV RNA in whole blood at the end of treatment had a strong positive predictive value for viral relapse. The negative predictive value for sustained virologic response was lower but may have been limited by the small number of patients who achieved a sustained virologic response.

This is not the first study to compare HCV RNA detection in whole blood with that in plasma or serum. Several studies have previously shown, using “home-brew” methods for extraction and analysis, that there are more HCV RNA copies in whole blood than in plasma [4–6]. However, a limitation noted in one of these studies [4] was the initial inability to confirm the results of whole-blood analysis with a commercially available assay (Amplicor; Roche Diagnostics), which was probably due to higher concentrations of RNA used in the home-brew method. An additional source of controversy arose when another group was unable to validate these results using the same extraction techniques [7]. In the present study, Watkins-Riedel et al. [3] were careful to validate their results using commercially available assays, which is an important step toward wider use of whole-blood assays instead of serum assays.

Placed in the context of previous research, what does this study tell us about
the biology of HCV and its association with clinical practice? The hepatocyte is the primary site of HCV replication in vivo, but the role of other compartments as potential replication sites is less clear. Although it has been demonstrated that HCV RNA is present in association with PBMCs [8–10], the issue of whether HCV actually replicates in PBMCs or is adsorbed onto the cell surface remains unresolved. Some studies have detected markers of replication in PBMCs [11] and/or dendritic cells, even after clearance of the virus [12, 13]; however, not all studies have observed this phenomenon [14–16]. Other studies suggest that HCV is only lymphotropic in immunosuppressive situations, such as after transplantation or in persons with HIV-1 infection [17–19].

It has been shown recently that CD81 is a binding site for the envelope glycoprotein E2 region of the HCV genome [20]. CD81 is a multifunctional cell surface protein that is expressed on the vast majority of nucleated cells, with the exceptions of RBCs and platelets [21]. This protein is associated with CD4 and CD8 molecules on T cells and with MHC class II molecules and other molecular complexes, including CD21 and CD19, on B cells. However, it is not clear whether CD81 serves solely to initiate binding and whether there are other receptors involved in viral entry. Other putative cellular receptors include the cellular low density lipoprotein receptor [22], glycosaminoglycans [23, 24], and dendritic cell–specific ICAM-grabbing nonintegrin (DC-SIGN) [25, 26], all of which might mediate passive adherence to cells. Given the receptors’ wide range of expression and the long half-lives of some cells, this is a plausible explanation of why HCV RNA might be present in whole blood for a longer duration than it is in serum—even if virus in whole blood is nonreplicative. Although the detection of HCV RNA in whole blood in this study might suggest that whole blood is a potential viral reservoir, in the absence of studies to determine whether there were replicative intermediates in whole blood or occult virus in the liver [27], this is difficult to confirm.

In terms of clinical practice, it is important to remember that the study by Watkins-Riedel et al. [3] only addresses the issue of relapse in patients who have cleared virus by the end of treatment. Of more concern to most clinicians and patients is predicting as early as possible who will respond to treatment. Fried et al. [28] have shown that the predictive value of a PCR result positive for HCV RNA after 12 weeks of treatment is 97%–100%. If a patient did not have qualitative test results that were lower than the limit of detection (which, for the current Cobas assay, is 50 IU/mL) or a >2-log decrease in the initial viral load by week 12 of treatment, the likelihood of sustained virologic response is 0%–3% [28–30]. It is also important to note that achievement of early virologic end points does not necessarily guarantee that a patient will develop a sustained virologic response, so the predictive value of a negative test result at week 12 is not as high.

On the basis of these guidelines, given that all of the 18 end-of-treatment responders had virus in serum at week 24 after treatment began, it was predictable that few of the patients would have a sustained virologic response. However, even 12 weeks is a long time for some patients to experience the numerous toxicities of IFN and ribavirin. An important question for future studies is whether whole blood testing will predict failure to achieve sustained virologic response at an earlier time point than does conventional serum testing (e.g., 4–8 weeks after treatment begins). A recent study in which serum specimens were obtained for detection of HCV RNA suggested that use of 1- or 2-log decrease in HCV RNA load at 4 weeks after the start of treatment as a marker would miss 6% and 15%, respectively, of individuals who would develop a sustained virologic response, whereas use of failure to achieve a 2-log decrease or negativity at week 12 as a marker did not miss any patients with sustained virologic response [29]. Because this same study estimated that drug costs may be reduced 20% by adhering to a stopping rule at 12 weeks, there may be additional cost savings if therapy can be stopped earlier. Future studies will be needed to determine whether the use of whole-blood testing will offer enhanced sensitivity for predicting failure of therapy without a reduction in specificity for predicting the possibility of cure.

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