Whole Blood as an Alternative to Plasma for Detection of Hepatitis C Virus RNA

Hubert Darius J. Daniel, Joel David, Paul R. Grant, Jeremy A. Garson, and Priya Abraham

Departments of Clinical Virology and Clinical Gastroenterology, Christian Medical College, Vellore, India, and Department of Virology, UCL Hospitals NHS Foundation Trust, and Centre for Virology, Department of Infection, University College London, London, United Kingdom

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Peripheral blood mononuclear cells are reported to be one of the extrahepatic replication sites contributing to the persistence of hepatitis C virus (HCV) infection. Whole-blood and plasma samples from 61 individuals were compared as sources for the detection of HCV RNA. Forty-four of the individuals were receiving antiviral therapy, while 17 were treatment naive. The quantitation of HCV RNA was done by a sensitive in-house real-time reverse transcription-PCR. When the viral loads in the two types of samples were compared, a correlation coefficient of 0.858 (P < 0.001) was found, indicating that plasma and whole blood are equally acceptable sources for testing for HCV RNA.

Approximately 18 million people in India are estimated to be infected with hepatitis C virus (HCV) (13). HCV infection is chronic in 75% to 85% of infected individuals (3). Chronic HCV infection can lead to hepatocellular carcinoma after many years. Even though hepatocytes are the major replication site for HCV, a broad range of extrahepatic complications and diseases are associated with chronic HCV infection. These include mixed cryoglobulinemia, non-Hodgkin’s lymphoma, cutaneous vasculitis, membranoproliferative glomerulonephritis, neuropathy, lymphoproliferative disorders, porphyria cutanea tarda, lichen planus, and Sjogren’s syndrome (1, 7). HCV is reported to replicate in extrahepatic sites like interstitial cells of the kidney, acinar cells of the pancreas, peripheral blood mononuclear cells, and mononuclear cells of lymph nodes (5, 14). Currently, the diagnosis of HCV infection is achieved by the detection of HCV RNA in plasma or serum. Testing for HCV RNA in plasma or serum might give a false-negative result if HCV RNA is present inside peripheral blood mononuclear cells (PBMCs) or as precipitates of immune complexes and cryoglobulin aggregates. Hence, the recovery of RNA from whole blood is likely to help with the diagnosis of HCV infection in individuals with low HCV loads, since both intracellular RNA and plasma RNA are detected. This can also have implications in the monitoring of individuals receiving antiviral therapy (12). Screening for HCV by the use of whole-blood samples will be useful, since all blood components are screened and the time required to separate plasma and serum is saved (leaving the remaining sample usable for serology). This study was done to investigate whether whole blood is more sensitive than plasma for the detection of HCV RNA and to assess the impact of testing of whole blood on the viral load result.

Blood samples were collected from 61 patients who came to the Department of Clinical Virology, Christian Medical College, as referrals from the Departments of Hematology, Gastroenterology, and Nephrology. All patients were recruited after they provided verbal consent, in addition to a general consent that is obtained in our hospital for all investigations as part of our routine patient management. These 61 patients consisted of 44 men and 17 women with a mean age of 42 years (age range, 16 to 66 years). Of these 61 patients, 17 (27.9%) were not receiving treatment and 44 (72.1%) had been receiving pegylated interferon and ribavirin, interferon and ribavirin, pegylated interferon alone, or interferon alone for durations that ranged from 12 to 24 weeks. Whole-blood samples were collected in EDTA tubes, and the contents were mixed by gentle shaking and stored in two aliquots (400 μl each) at −60°C. Plasma was separated from the remaining sample after centrifugation at 1,500 rpm for 10 min and was stored in multiple aliquots at −60°C for testing. Five whole-blood samples were tested in duplicate to assess the reproducibility of the results.

HCV RNA was extracted from 200 μl of whole blood or plasma with a High Pure viral nucleic acid kit (Roche Diagnostics GmbH, Mannheim, Germany), according to the manufacturer’s protocol. This extraction protocol was easy to perform and is reproducible. This method can be used for the extraction of viral RNA and DNA from whole-blood and plasma samples. Five microliters of 5 × 10^−6 μg brome mosaic virus (BMV) RNA was added to 1 ml of binding buffer, which served as the internal control (IC) to rule out false-negative results. Elution was done with 50 μl of prewarmed (70°C) elution buffer. The eluate was always processed immediately.

The primers used for the in-house real-time reverse transcription (RT)-PCR were HCV TAQ1 (GTC TAG CCA TGG CGT TAG TA), HCV TAQ 2 (GTA CTC ACC GGT GCT GCC), BMV TAQ 1 (GTT CAC CGA TAG ACC GCT G), and BMV TAQ 2 (AAG AGC CCG GAA TGT CAA GA); and the probes were HCV TAQPR (6-carboxyfluorescein–CCC TCC CGG GAG AGC CAT AGT G–6-carboxytetramethylrhodamine)
Some reports have suggested that HCV replicates in extrahepatic sites, like PBMCs, in HCV-infected individuals. Further studies are needed to confirm this hypothesis.
other reports suggest that the HCV RNA load detected in plasma might not represent the exact viral load of the patient. The low viral load of HCV RNA in plasma samples could be attributed to viruses that adhere to blood cells or HCV RNA found within cryoprecipitates, which might sediment during the centrifugation process. Two earlier studies that used whole-blood and plasma samples have shown that the viral loads in whole blood are higher than those in plasma and that the excess viral load in the whole-blood samples was attributed to the combined viral load of plasma and RNA from the cellular pellet and cell washes following separation of the plasma. In those studies, extraction was done with the cationic surfactant tetradecyltrimethylammonium oxalate (Catrimox-14) and guanidinium isothiocyanate (10, 11). Contrary to these findings reported earlier, our study did not find a significant increase in the HCV viral load in whole blood compared to that in plasma. This finding is in accordance with that of another study in which no significant increase in the viral load in whole-blood samples compared with that in plasma samples was observed. In the earlier study, extraction was done by use of the tetradecyltrimethylammonium oxalate–Trizol method (4).

Watkins-Riedel et al. (12) compared the HCV RNA titers in whole-blood, serum, and plasma specimens obtained from 56 patients who did not respond to initial interferon alpha 2b monotherapy in order to determine whether the specimen type can predict the rate of virologic response to high-dose treatment with interferon and ribavirin (12). Of the 56 patients in that study, serum and plasma obtained from 18 patients tested negative for HCV RNA at the end of treatment, indicating a complete virological response. In contrast, analysis of whole-blood specimens obtained at the same time revealed the pres-
ence of viral RNA in 12 of these 18 patients. The testing of whole blood for the detection of HCV RNA was highly predictive of viral relapse (positive predictive value, 100%) and may thus be a useful tool for the monitoring of patient responses to interferon and ribavirin therapy (12). Testing of only serum or plasma specimens could underestimate the true circulating HCV load and could lead to an overestimation of the antiviral response rates (9, 12). Contrary to the findings reported earlier, among the 44 patients receiving antiviral therapy in our study, the plasma sample from only 1 additional patient whose whole blood was positive for HCV RNA (22 IU/ml) was negative for RNA. This patient was lost to follow-up, and hence, the virological response to therapy could not be ascertained.

Amplification of HCV RNA from whole-blood samples is done by different methods. Most of the studies have used RT-PCR for the amplification of HCV RNA from whole blood. Watkins-Riedel et al. (12) compared the LightCycler assay, the Cobas Amplicor assay, and two different in-house RT-PCRs and found that one whole-blood sample gave a positive result by the LightCycler real-time assay but was negative by the other assays evaluated. In the present study, despite the use of a sensitive in-house TaqMan real-time assay, there was no significant difference in the rates of detection of HCV RNA between whole blood and plasma. This may be attributed to the efficacy of the High Pure viral nucleic acid kit for the extraction of RNA from both types of clinical samples.

The detection of HCV RNA in whole blood was previously considered to be difficult due to the RNases present in blood samples and the inhibitory effect of hemoglobin. However, the detection of HCV RNA in whole blood will remove the need to separate plasma. Earlier studies have used tetradecyltrimethylammonium oxalate, a surfactant used for the extraction of HCV RNA from whole-blood samples (2, 9). Newer nucleic acid extraction protocols which can remove these factors from whole blood are now available. In this study, we have used the High Pure viral nucleic acid protocol (Roche Diagnostics GmbH) to extract HCV RNA from whole blood. Our study shows a good correlation between the whole-blood HCV viral load and the plasma HCV viral load in individuals chronically infected with HCV (r = 0.858), as reported earlier (8).

In summary, the rates of detection of HCV RNA in whole blood and plasma compared favorably under the conditions used in this study. The direct detection of viral nucleic acid in whole blood minimizes the need for sample processing and has the potential for widespread use in blood banks and diagnostic laboratories.

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