Detection of Serum Antibodies to Hepatitis C Virus in ‘False-Seronegative’ Blood Donors in Oman

Adel Alnaqy  Safia Al-Harthy  Geraldine Kaminski  Said Al-Dhahry

Department of Microbiology and Immunology, College of Medicine and Health Sciences, Sultan Qaboos University, Al-Khod, Oman

Key Words
Hepatitis C virus  •  Antibodies  •  Immune complexes  •  Blood donors, seronegative

Abstract
Objective: To detect hepatitis C virus (HCV) antibodies in seronegative donors by disruption of the immune complexes (ICs). Subjects and Methods: HCV antibody detection was carried out on 600 seronegative donors following an IC dissociation assay. Reverse transcription polymerase chain reaction (RT-PCR) was then performed on the positive results. Results: Nine of the 600 samples (1.5%) were positive for IC-dissociated HCV antibodies. Of the 9 only 3 antibody-positive samples had detectable HCV RNA. Conclusion: Screening for antibodies to HCV in combination with PCR appears to be the safest way to reduce the residual risk of HCV in blood transfusion.

Introduction
Hepatitis C virus (HCV), a small RNA virus of the Flaviviridae family, is the major etiological agent of non-A, non-B hepatitis [1]. Parenteral transmission and other modes of blood exchange are the most common routes of HCV infection [2].

Safe transfusions depend on the use of blood components that are not contaminated with infectious agents such as HCV. The first-line test for screening HCV infection is the HCV antibody test. This is an enzyme-linked immunosorbent assay (ELISA) that is easy to perform, cost-effective, and highly sensitive. The recombinant immunoblot assay (RIBA), on the other hand, is a more specific test that confirms the presence of anti-HCV antibodies thereby reducing the number of false-positive results, particularly in low-prevalence situations where there are no evident risk factors for HCV. Presence of the virus can be confirmed by the detection of HCV RNA using reverse transcription polymerase chain reaction (RT-PCR).

A small proportion of HCV-infected subjects can be detected as seronegative by ELISA. This may occur in what is known as the diagnostic window period, during which specific antibodies have not yet been produced, but the virus is present in the serum [3]. False-negative anti-HCV tests are also seen in infection by some HCV genotypes which are poorly immunogenic or where the antibody response to HCV infection is delayed, such as in case of immunosuppressed persons [4].

Research groups have suggested the presence of circulating immune complexes (ICs) formed between HCV...
and anti-HCV in the sera of patients with HCV infection [5, 6]. In patients in which a state of antigen excess exists, such antibodies may escape detection and may be revealed only after use of methods to dissociate such ICs. Confirmation of viremia by RT-PCR is thus necessary for making the diagnosis of infection.

This study was designed to investigate the possible presence of HCV-IC in the sera of blood donors who have tested anti-HCV antibody-negative on routine methods of disrupting the IC and retesting for antibodies. Samples giving a positive result following dissociation were subjected to RT-PCR for HCV RNA.

**Subjects and Methods**

**Study Subjects**

Serum samples from 600 blood donors, which had tested negative for anti-HCV by enzyme immunoassay, AxSYM, were studied.

**ELISA for Detecting Anti-HCV Antibodies**

Antibodies to HCV, for both serum and dissociation extracts, were measured by the AxSYM system (Abbott Diagnostic Division, USA), which uses antigen-coated beads with an antibody coupled to an enzyme to produce a fluorescent end product proportional to the amount of bound antibody. Control sera from patients who showed anti-HCV-positive ELISA results prior to and after IC dissociation assays were included in each run. Similarly, samples that were negative for both were also included as negative controls.

**IC Dissociation Assay**

The method of IC dissociation assay previously described by Troisi and Hollinger [7] was used. Briefly, 400 μl of serum was added to 3.6 ml of phosphate-buffered saline (PBS), pH 7.4, and was centrifuged at 1,700 g for 20 min at room temperature. The supernatant was mixed with an equal volume of ice-cold PBS containing 4% polyethylene glycol 6000 (PEG-6000) and 3% Tween-20 to precipitate ICs. Samples were incubated overnight at 4°C and then recentrifuged under the same conditions. The precipitate was washed twice with 3 ml of PBS containing 2.5% PEG-6000 and 3% Tween-20 and resuspended in 220 μl of 3 M potassium thiocyanate to dissolve ICs. The sample was incubated at 37°C for 30 min and then refrigerated at 4°C until the ELISA assay was repeated on both the original untreated serum sample and the resuspended pellet (usually within 1–2 days).

**In-House HCV RT-PCR Method**

Sera that were positive for anti-HCV following IC dissociation assay were further tested for the presence of HCV RNA. RNA was extracted from 0.1 ml of post-IC dissociation suspension from each of the anti-HCV-positive samples as described previously [8].

In-house HCV RT-PCR method (sensitivity, 1,000 genomes/ml) was performed as described by Ruster et al. [9]. RNA was reverse-transcribed and amplified using nested primers. Amplified sequences were then detected by agarose gel electrophoresis.

**Results**

Of the 600 samples, 9 (1.5%) showed positive results for anti-HCV. Original samples for all 9 samples were retested for anti-HCV antibodies and the results remained negative, but 3 of them tested positive for HCV-RNA.

**Discussion**

Post-transfusion HCV infection has been reduced dramatically as a result of the introduction of advanced assays for testing for antibodies to HCV in blood donors. However, the risk of transfusing blood-derived components from virus-carrying donors still remains [10–12] and HCV RNA has been found in some antibody-negative samples by PCR or nucleic acid amplification technology [13–16].

There are several possible explanations by which the HCV isolates in these individuals avoid detection by commercial antibody tests. Antibodies may be present in the subjects, but due to extensive sequence differences between the individual isolates and the commercial antigen, the antibodies may be different enough to prevent detection in the commercial assay. Alternatively, variation in host immune responses may lead to diminished or absent antibody production in some HCV-infected individuals [4].

This study focused on the possibility of the in vivo formation of IC bonds between the virus antigen and antivirus antibodies that hinders the detection of antibodies by the method we used. A higher incidence of anti-HCV detected among patients with chronic HCV using the same dissociation techniques has been reported in a similar study [7].

The fact that 6 out of 9 samples with postdissociation anti-HCV antibodies failed to have a detectable viral RNA by the PCR could be due to the presence of virus below the detection level of the viral load [17]. Various other problems could also be associated with this. Viral RNA present in the sample is fairly unstable, which means that the sera must be tested immediately or frozen as quickly as possible and stored at −20°C or −70°C. Other factors that may lead to false results are improper sample collection and handling, delayed serum separation and inadequate storage conditions [18]. On the other hand, it is possible that anti-HCV antibodies in those 6 samples are false-positive. Use of confirmatory antibody testing (RIBA) is recommended to confirm ELISA anti-HCV reactivity and exclude false-positive reaction after the IC dissociation.
The considerably low percentage of ‘false-seronegative’ donors identified by our study implies that current tests are highly sensitive and beneficial in reducing the risk of transfusion-associated HCV infection. Yet the question remains as to whether our observations warrant screening of blood donors for antibodies to HCV alone or in combination with HCV RNA. The incorporation of RNA detection in blood banks has been shown to reduce the residual risk of contracting post-transfusion HCV [19]. Furthermore, a new HCV core antigen assay (ELISA) based on dissociation of ICs [20] is recently available to reduce the residual risk of HCV transmission in the routine screening of blood donations.

Measures currently employed in some advanced centers, such as the correct traceability of blood components or follow-up of the recipients of infected blood-derived components, would help to improve the safety of blood bank products. The consequences of decisions to push toward a zero-risk blood supply are important to consider such as increased sensitivity weighed against increased cost.

**Conclusion**

A small proportion of HCV-infected subjects are seronegative when screened by ELISA. PCR appears to be the safest way to reduce the residual risk of HCV in blood transfusion.

---

**References**