Hepatitis C virus infection rate in volunteer blood donors from the Western Cape — comparison of screening tests and PCR

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Introduction. Hepatitis C virus (HCV) antibody seroprevalence studies overestimate the true infection rate. No data exist on the incidence of HCV or its clinical features in blood donors of sub-Saharan Africa.

Aims. To establish the true incidence of HCV infection in volunteer blood donors in the Western Cape, and compare risk factors and clinical and biochemical features of viraemic and non-viraemic subjects.

Methods. All donors attending the Western Province Blood Transfusion Service between December 1992 and August 1994 were screened prospectively for anti-HCV using the Abbott second-generation assay. Positive donors were evaluated clinically and biochemically. Their sera were examined for HCV-RNA by the polymerase chain reaction (PCR).

Results. Of 66 314 donors screened, 275 (0.41%) were anti-HCV-positive. Of these 13.6% were PCR-positive (0.056% of all donors). PCR-positive patients had more risk factors for HCV acquisition ($P < 0.01$), symptoms of hepatitis ($P = 0.02$) and clinical signs of liver disease ($P = 0.05$) and higher alanine ($P < 0.0001$) and aspartate aminotransferase levels ($P < 0.0001$) than PCR-negative donors. However, clinical and biochemical features did not discriminate adequately between PCR-positive and negative donors. Liver biopsies performed in 9 of 13 PCR-positive cases showed mild inflammation, but no cirrhosis.

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Conclusion. **Hepatitis C viraemia was present in 0.056% of blood donors. Only 13.6% of subjects with positive serological tests had evidence of viraemia. Risk factors were more common in viraemic donors. Viraemic subjects had clinical and biochemical features of liver disease more often than non-viraemic donors. Mild disease activity was present in all 9 subjects biopsied.**

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Hepatitis C virus (HCV) transmission by whole blood (and its components) has been significantly reduced since the introduction of routine blood donor screening with enzyme-linked immunosorbent assays (ELISAs) for anti-HCV antibodies.[1,2] The predictive value for viraemia of a positive ELISA result is affected by both the population seroprevalence and the method used to confirm viraemia (Bayes' theorem). Although second- and third-generation immunoassays have improved sensitivity and specificity compared with earlier ELISAs,[3,4] these continue to identify people without detectable viraemia.[1,2] This may in part be due to the retention of antibodies after clearance of HCV or may result from nonspecific antibody reactivity. The gold standard for confirming HCV viraemia is the reverse-transcription polymerase chain reaction (RT-PCR) using primers specific for the conserved 5' untranslated region of HCV. Although recombinant immunoblot assays have been used to confirm positive ELISAs, these lack both the sensitivity and specificity of PCR.[1,2,5]

Accurate estimates of HCV prevalence are essential, as public health planning depends on these results.[6] False-positive anti-HCV results reduce the size of the already small blood donor pool and, in addition, subjects informed of an initial positive ELISA result, their families and sexual partners experience unnecessary anxiety.

Although several seroprevalence studies have been performed in the general South African population,[7-9] no data currently exist on the true incidence of hepatitis C viraemia in the South African blood donor population. We prospectively studied all blood donors who presented at the Western Province Blood Transfusion Service (WPBTS) between December 1992 and August 1994 in order to determine the prevalence of hepatitis C viraemia in those with a positive ELISA. Where possible clinical, biochemical and additional serological studies were performed and, where indicated, histological evaluation of the liver was undertaken.

Subjects and methods

All donors attending WPBTS between December 1992 and August 1994 were tested for the presence of HCV antibodies using the Abbott second-generation ELISA (ELISA-2) (Abbott Laboratories, North Chicago, USA). Anti-HCV-positive donors were referred to the liver clinic, Groote Schuur Hospital for full clinical and serological assessment and, where clinically indicated, a liver biopsy. Clinical evaluation included a detailed history of possible risk factors for HCV transmission, such as intravenous drug abuse, past blood transfusions and tattoos, and a history of prior symptoms of hepatitis. The Ortho anti-HCV ELISA (Ortho Diagnostic Systems Inc., Raritan, NJ, USA) was used at Groote Schuur Hospital as a supplementary assay in the referred donors. Anti-HCV-positive donors who failed to present to the clinic also had supplementary serological tests and PCR performed on their stored sera (stored at −70°C), where sufficient serum was available. In addition, the Abbott ELISA was compared to the Ortho second- (until 04/94) or third-generation ELISA on all initially reactive serum samples.

HCV amplification

RNA was extracted from 200 µl serum using Total RNA Isolation Reagent (Advanced Biotechnologies Ltd, London, UK) and tRNA on ice; chloroform/iso-amyl alcohol was added in a ratio of 24:1 and the mixture vigorously blended and left on ice for 5 minutes; thereafter the homogenate was centrifuged at 12 000 rpm for 15 minutes. The supernatant containing the RNA was removed and precipitated in isopropanol overnight at −20°C, pelleted, washed twice with 75% ethanol, air-dried and resuspended in 20 µl diethyl pyrocarbonate (DepC)-treated ultra-pure water with 20 U RNasin (Promega Corp., Madison, WI, USA) per sample. A single tube RT-PCR was used to form and amplify HCV cDNA as described previously.[10] Both outer and nested primers used were those described by Chan *et al.*,[11] which gave a PCR product of 251 bp; this was visualised on a 2% agarose gel with ethidium bromide staining. The product was transferred to a nylon membrane and probed with a digoxigenin-labelled HCV probe (Boehringer Mannheim, GmbH Biochemica, Mannheim, Germany).

Statistics

Results are presented as the mean and standard deviation (SD), unless otherwise specified. The groups were compared using the unpaired *t*-test for continuous data and the chi-square test for categorical data; a P-value < 0.05 was considered significant.

Results

Serology and PCR

Of the 66 314 donors screened with Abbott ELISA-2, 275 (0.41%) were antibody-positive. Of these, 100 (36%) presented to the liver clinic at Groote Schuur Hospital where they underwent full clinical evaluation. Stored serum from an additional 84 anti-HCV-positive subjects who did not present for examination was analysed by PCR. Insufficient serum from the remaining 91 subjects was available for testing. Twenty-five of the 184 (13.6%) serum samples subjected to PCR analysis were found to be positive. The incidence of viraemia in the group as a whole was therefore 0.056%.

All PCR-positive donors were anti-HCV positive by both the Ortho and Abbott ELISA. One hundred and fifty-nine donors were found to be Abbott ELISA-positive but PCR-negative. Of these, 50 were positive, 107 negative and 2 indeterminate when the Ortho ELISA was used. It was not possible to comment on the comparative value of the Ortho ELISA, since it was only used in Abbott ELISA-2-positive donors.

Clinical analysis

One hundred donors were assessed at Groote Schuur Hospital, of whom 16 were PCR-positive and 84 PCR-negative. The proportion of PCR-positive subjects presenting for examination was similar to the PCR-positive proportion of the group that did not present for examination (16% v. 11%; $P = 0.30$).

Although the number of PCR-positive subjects was small, they had significantly more identifiable risk factors for HCV acquisition and more past symptoms compatible with hepatitis, while a significantly greater number had hepatomegaly and abnormal clinical findings on examination than those who were PCR-negative. Serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels showed significant differences between the PCR-negative and PCR-positive groups (Table I).

Table I. Donors with a positive anti-HCV ELISA with and without viraemia showed significant differences in respect of medical history, physical examination and biochemical analysis

	PCR-positive	PCR-negative	*P*-value
Risk factors	37.5%	11.9%	0.01
Symptoms	12.5%	1.2%	0.02
Hepatomegaly	25%	8.3%	0.05
ALT (U/l)	34.9 ± 18.8	16.8 ± 10.3	$P < 0.0001$
AST (U/l)	24.3 ± 10.25	14.4 ± 5.1	$P < 0.0001$

Combinations of ELISA with clinical and biochemical findings failed to enhance the ability to predict viraemic donors. Combining ELISA and transaminase results did not improve upon ELISA results alone, as only 62.5% of the PCR-positive donors had ALT levels above the upper limit of normal. In addition, 9 donors who were anti-HCV-positive but PCR-negative had a raised ALT level (4 positive for both Abbott and Ortho ELISAs and 5 with Abbott ELISA positivity only).

Nine PCR-positive donors were considered by their physicians to require a liver biopsy. All biopsies showed mild inflammatory activity and features compatible with HCV infection, but none showed evidence of cirrhosis. Portal tracts were enlarged in 77%, and bile duct damage was noted in 55%, periportal fibrosis in 45% and piecemeal necrosis in 45%. Thirty-three per cent had steatosis and 22% poorly formed lymphoid follicles.

Discussion

This study shows that only a small number of Western Cape blood donors with serology suggestive of HCV infection have detectable viraemia. The prevalence of antibodies in Cape Town donors (0.41%) is similar to that described in voluntary donors in northern Europe and the USA.[1,2,12] In our study virus was only detected in 13.6% of seropositive subjects or 0.056% of all donors. This is similar to a study of 287 332 British blood donors by Mutimer *et al.* where 0.35% of donors had HCV antibodies but only 5% of the latter had viraemia detected by PCR.[2] The poor predictive value of serology for viraemia may be at least partially due to the low HCV prevalence in the population we studied (Bayes' theorem). Other possible reasons for the discrepancy must include donors who retain antibodies to HCV after clearing the virus, as well as nonspecific binding of antibodies in the ELISA.

A small group of the PCR-negative donors (4.8%) had elevated transaminase levels. Although it is possible that a small number of these had true HCV infection, this is unlikely given that the sensitivity of our PCR assay is extremely high. Quality control studies at our laboratory, performed as part of the Eurohep2 study, showed that our PCR assay consistently detected as few as 20 genome equivalents/ml serum. While undetectable hepatitis C viraemia is possible in these donors, other common conditions including alcoholic liver disease, non-alcoholic steatohepatitis or hepatitis G infection[13] are more likely to be the cause of the elevated transaminase levels.

The combination of ELISA results and transaminase elevations and clinical variables did not assist in the distinction between patients with and without viraemia better than ELISA alone. The ELISA- and PCR-positive group had significantly higher AST and ALT levels than the ELISA-positive, PCR-negative group, but in both groups there was too wide a variation for this finding to be of any significant assistance in individual cases. Similarly, although clinical symptoms and signs and a history of risk factors were more common in the viraemic than the non-viraemic donors, these did not assist the prediction of viraemia.

In conclusion, screening for HCV infection by ELISA significantly over-estimates the incidence of HCV infection. All anti-HCV-positive subjects should be referred for confirmation of viraemia prior to appropriate management and counselling.

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