POSTGRADUATE DOCTOR MIDDLE EAST June 1995 vol 18 no 6 pp232-8

# Hepatitis C and what it means to be positive: diagnosis and epidemiology

**With the cloning of the hepatitis C virus (HCV) came the availability of laboratory tests to detect antibody to the virus by serological assays and to detect viraemia using the polymerase chain reaction. At present, HCV seropositive subjects must be assumed to be long-term carriers of the virus, capable of infecting others by blood to blood contact. This is most readily observed in intravenous drug users who share needles. The full extent to which sexual and materno-fetal transmission might occur is not yet clear. The clinical significance of HCV infection lies in the possible long-term sequelae of chronic infection.**

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## What does 'hepatitis C positive' mean serologically?

Until 1989 the term 'non-A, non-B hepatitis (NANBH) was somewhat clumsily, but quite accurately, used to describe hepatitis infections (often associated with blood transfusion but sometimes occurring "sporadically") where the serological tests for both hepatitis A and B were negative. With the original cloning[1] of what is now termed the hepatitis C virus (HCV) and the development of an assay to detect circulating antibody to the virus[2] the scene was set for a rapid widening of our understanding of NANBH. However, even now HCV has not been reliably isolated, nor visualised in the electron microscope, and only an artist's impression of the virus, with details based on indirect laboratory experiments, is available (fig 1).

Despite this, the complete sequencing of the RNA genome of the hepatitis C virus has

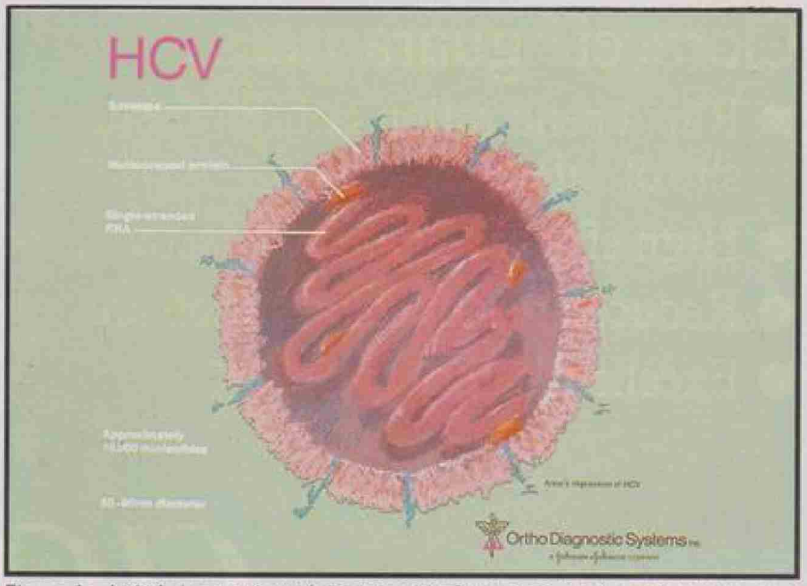


Figure 1. Artist's impression of the hepatitis C virus. Reproduced by courtesy of Ortho Diagnostic Systems Ltd.

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enabled molecular biologists to clone or synthesise a range of antigens that have been used to improve upon the prototype assay; this was based on a single viral antigen cloned (in yeast) from a region of the HCV genome that coded for a 'non-structural' antigen not incorporated into the virus particle. As such, detection of antibody directed against this antigen provided a sub-optimal assay in terms of sensitivity and range of detectable seropositivity. More recently the improved "second-generation" assays utilise structural and non-structural synthetic or recombinant protein antigens encoded by different regions of the HCV genome (fig 2).

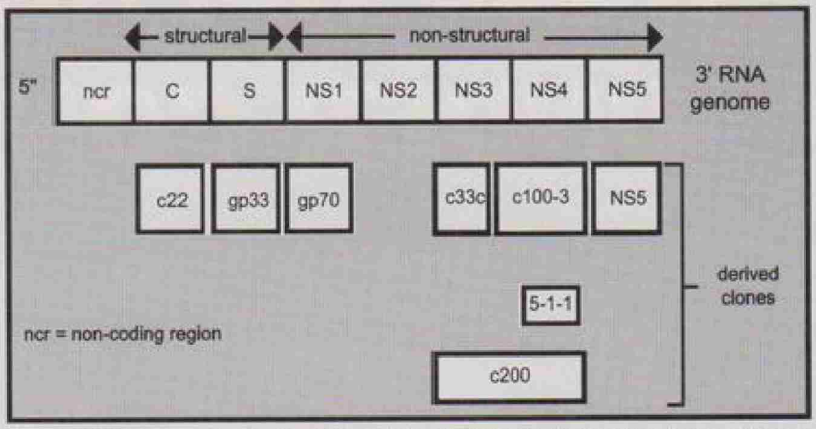


Figure 2. Genome of the hepatitis C virus, and the antigens that have been cloned or synthesised.

Even the improved commercial second-generation enzyme linked immunosorbent assays (ELISA-2 see fig 3), still give rise to positive results in approximately one in 350 UK blood donors, although this figure is improving as kits are further improved. Only a minority (approximately one in five) of these "anti-HCV reactive" donors are likely to be really infected with HCV, detected by the presence of antibody to HCV of the IgG type. Experimental assays have been devised which utilise an enzyme labelled anti-human IgM antibody, instead of the anti-human IgG depicted in figure 3. Such assays allow detection of *recently* acquired HCV infection and when developed for routine diagnostic laboratories, may well prove valuable for differentiating old from new HCV infections. This is of importance when trying to determine if the presence of anti-HCV during or after a clinical case of hepatitis is indeed due to an infection with HCV, or if the anti-HCV was coincidentally present following a previous infection. If pre-exposure samples are not available to check for existing seropositivity, the IgM assay would be the only way of making a definitive diagnosis of acute HCV infection.

Approximately one in 1,000 to one in 2,000 previously untested UK blood donors are confirmed as positive for anti-HCV by supplementary assays such as the Ortho Diagnostics' second-generation recombinant immunoblot assay (RIBA-2). This assay provides an analytical picture of the range of antibodies in a serum sample directed against the different HCV antigens. It is based on the same principle as the ELISA except that the different HCV antigens are layered in bands onto a 3 cm long nitrocellulose strip (see fig 4) together with IgG controls to show that serum sample has been added and to provide a baseline for scoring the intensity of any reactive antigen bands. If two or more antigen bands are positive, the test is considered reactive. RIBA-2 reactivity correlates very well with positivity in the polymerase chain reaction (PCR) assay. For this test, RNA is extracted from the serum sample and treated with the

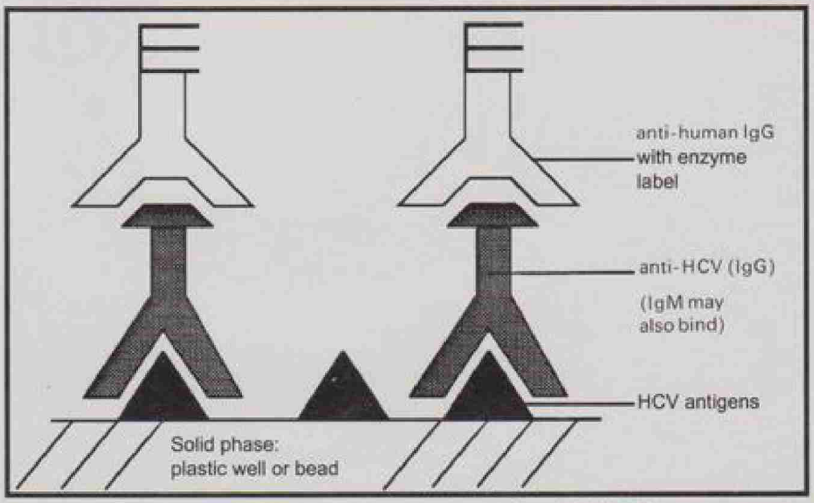


Figure 3. Basis of the ELISA system for detection of anti-HCV. The serum sample is added to a small plastic well or bead coated with HCV antigens. If anti-HCV is present it binds to the HCV antigen. The presence of bound human antibody, when probed with an antibody to human IgG (linked to an enzyme), can be detected by allowing the enzyme to catalyze a colour-producing reaction. The test takes approximately 2 hours to perform.

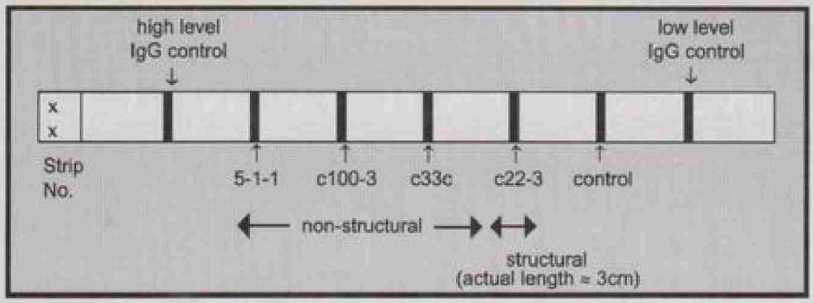


Figure 4. Antigens on the second generation nitrocellulose RIBA strip.

reverse transcriptase enzyme to make a double stranded DNA copy. Then, highly conserved sequences from the non-coding region (see fig 2) of the HCV genome, which will be constant in most HCV isolates, are amplified one million fold using appropriate oligonucleotide primers and a heat stable DNA polymerase enzyme by alternate heating and cooling. The latter steps enable separation of the double stranded DNA into separate strands and then replication to form new double-stranded fragments of DNA. This process is repeated until enough DNA is produced to be detected by biochemical techniques. For more information on PCR see Garson and Barbara, 1992[4].

An unconfirmed ELISA positive result for anti-HCV, especially in populations with a low prevalence of infection, is inadequate for provision of a definitive diagnosis of HCV infection. No donor or patient should be advised that they have been infected with HCV unless confirmatory testing has been carried out.

## Can one be immune to HCV?

Experience to date suggests that most subjects infected with HCV develop persistent infections with continuing viraemia as determined by PCR. Even if a patient, such as a haemophilia sufferer, becomes PCR negative for HCV while remaining seropositive, viraemia (for instance PCR reactivity) may recur after a period of time[5]. Manufacture of a safe and effective specific anti-HCV immunoglobulin will therefore not be as straightforward as with the preparation of hepatitis B immunoglobulin. In the latter case, a state of immunity to HBV, with development of anti-HBs, can be clearly demonstrated in the majority of persons infected with that virus and only a minority of infections lead to the carrier state.

## Clinical consequences of HCV infection

Acute hepatitis C infection is usually anicteric and the majority of anti-HCV positive blood donors, when interviewed, do not recall any episode of symptomatic hepatitis. The main clinical significance of HCV infection is therefore related to possible chronic sequelae. Most of the studies on the chronic effects of HCV infection are based on follow-up of patients who had developed NANBH after blood transfusion. In a recent histopathological study of chronic NANBH in such patients[6], liver biochemistry became normal within three years in 29 per cent, 55 per cent showed chronic persistent hepatitis and 16 per cent had chronic active hepatitis progressing to cirrhosis. In the long term, an association with the development of hepatocellular carcinoma (HCC) has also been identified. In Japan, a country with a high rate of post-transfusion hepatitis (PTH) prior to the introduction of anti-HCV screening of blood donors, in one study of HCV seropositive patients with cirrhosis or HCC, 48 of 119 (40·3 per cent) and 37 of 92 (40·2 per cent) patients respectively, had received blood transfusions in the previous nine to 53 years[7]. This contrasted markedly with findings in the UK where the incidence of PTH is very low, and no significant association of chronic liver disease with a history of prior blood transfusion was apparent[7].

## How is HCV transmitted?

By comparison with HBV infection, materno-fetal and sexual

### PRACTICAL POINTS

1. The presence of anti-HCV in patients and donors can be detected by serological assays. Confirmation of screening results is essential and some tests are available for this purpose. Current anti-HCV serological assays will probably miss a small proportion of HCV viraemic persons.
2. Definitive diagnosis of viraemia requires expensive polymerase chain reaction (PCR) testing which as yet is only reliably available in certain specialised laboratories. Although recombinant immunoblot (RIBA) reactivity correlates well with PCR results, a significant proportion of RIBA results are still indeterminate.
3. It is not yet known if a truly immune state can occur in a proportion of persons infected with HCV.
4. The clinical significance of HCV infection lies in its possible long-term sequelae.
5. Modes of transmission of HCV are not as well understood as for HBV or HIV. However, sharing of needles by intravenous drug users carries a very high risk of transmitting HCV infection.

transmission were predicted to be major routes of transmission. However, when assays for HCV became available it became obvious from several studies[3] that HCV may not be as readily transmitted by these routes as are HBV and HIV. This was most clearly demonstrated in a study of homosexual men in Denmark, but similar results have also been reported in studies of family and sexual contacts elsewhere. Unlike the situation with HBV infection, no marker of the level of infectivity in carriers is currently available and it is not known if the level of infectivity remains constant or varies over time. In a study in Italy there was a suggestion that HCV carriers with elevated liver function tests (for example alanine aminotransferase) might be more likely to transmit to contacts, but this finding requires confirmation.

One factor that has been clearly associated with HCV infection is intravenous drug use (IVDU) with shared needles[3]. When assessing the incidence of HCV transmission in sexual partners of HCV infected persons who admit to IVDU, care must be taken to exclude IVDU as a risk factor in the sexual partner.

The exact extent to which materno-fetal transmission of HCV occurs is currently unclear. Results vary in different studies but as further work is done, especially if PCR is used to distingush viraemia from passive anti-HCV in infants born to seropositive mothers, a clearer picture should emerge. In one such study[8], HCV RNA was found in eight of 10 infants born to anti-HCV positive mothers. In one of these eight, HCV RNA only became detectable 12 months after birth. This delay could be associated with passive transfer of maternal anti-HCV. Of the eight mothers who appeared to have transmitted HCV to their offspring, four were anti-HIV positive. In previous studies it had been suggested that immunosuppression in the mother rendered them more likely to transmit. However, the other four mothers in this study were anti-HIV negative.

As blood donors confirmed as anti-HIV positive are counselled, a better picture of the risk factors associated with HCV infection is emerging. Already, preliminary reports from several UK blood transfusion centres have shown that a history of IVDU (often many years in the past) can be elicited from approximately 50 per cent of the donors[9,10,11]. A significant number of histories of blood transfusion (prior to the introduction of routine anti-HCV donor screening) and of tattooing in uncontrolled conditions were also noted. However, in contrast to the patterns for HIV and HBV infection in UK blood donors, associations with male homosexuality or with origin in countries with high rates of HBV for HIV infection are not readily apparent. It is also noteworthy that for several seropositive donors, no obvious risk factor could be identified and the causes of sporadic HCV infections, which are known to occur, require clarification. Needlestick injuries leading to HCV infection have been documented but the full extent of risk is not yet clear. Measures to safeguard against HBV transmission by sharps' injuries should also be effective against HCV.

## Conclusion

Rapid advances in our understanding of HCV serology and molecular biology have provided us with a range of tests in a relatively short time. Nevertheless, several uncertainties relating to the specific diagnosis and epidemiology of HCV still remain. However, with advances in laboratory techniques and careful epidemiological studies in patient groups, large scale populations such as blood donors, and the family contacts of seropositive subjects, these questions should slowly be unravelled.

## Update

Currently 'third' generation ELISAs have become available incorporating the NS5 antigen in addition to those previously available. For confirmation, the RIBA likewise has the addition of a NS5 band and the formulation of the antigens (either cloned or synthetic) has been enhanced. Commercially available PCR (Ampiclor Roche Diagnostics) is helping to standardise and streamline necleic acid detection. Although European QC exercises reveal widespread shortcomings (false positivity and/or negativity) in many laboratories. A new amplification technique for detecting RNA directly (NASBA, Organon Laboratories) has also become available. With increased understanding has also come the identification of more than 6 distinct genotypes of HCV, work pioneered by Dr Peter Simmonds of Edinburgh.

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