

## *Elsewhere Reviews*

### HEPATITIS C: IMPROVING THE DIAGNOSTIC ARMAMENTARIUM

*Van Der Poel CL, Cuypers HTM, Recink HW, Weiner AJ, Quan S, Di Nello R, Van Boven JJP, et al.* Confirmation of hepatitis C virus infection by new four-antigen recombinant immunoblot assay. *Lancet* 1991;337:317-319.

#### ABSTRACT

A new four-antigen recombinant immunoblot assay (4-RIBA) for confirmation of hepatitis C virus (HCV) C-100 enzyme-linked immunosorbent assay (ELISA) reactivity was tested in stored serum samples (1984-86) of blood donors and recipients and compared with results from polymerase chain reaction (PCR) analysis of fresh (1990) plasma samples in donors and recipients from the original study. Of 37 HCV C-100 ELISA-positive blood products, 8 were 4-RIBA positive, of which 7 were implicated in post-transfusion non-A, non-B hepatitis (PT-NANBH) and/or PCR confirmed recipient HCV infection. Of 9 recipients with PT-NANBH, 8 were reactive in 4-RIBA (6 positive and 2 indeterminate). With fresh plasma samples, 3 donors and 6 recipients who were 4-RIBA positive were also PCR positive. 4 4-RIBA indeterminate and 78 4-RIBA negative samples of donors and recipients were PCR negative. Of 6 4-RIBA positive recipients, 5 were PCR positive four to six years later. 1.6% of the 383 recipients became chronically infected with HCV. The new 4-RIBA represents a candidate confirmation test to discriminate between infective and non-infective HCV C-100 ELISA-positive blood donors.

#### COMMENTS

Serological testing for hepatitis C virus (HCV) has rapidly emerged from the research laboratory into general clinical use (1). The ELISA antibody test using an HCV-related antigen, C-100, is limited by a lack of sensitivity, particularly early in the infection (1). In one study of 15 patients with well-documented posttransfusion non-A, non-B (NANB) hepatitis, antibody production was delayed for a mean of 21.9 wk. In addition, the antibody was not invariably present even in about 20% of patients who had a firm clinical diagnosis of posttransfusion NANB hepatitis (2). Application of the polymerase chain reaction (PCR) to sera and liver tissue of patients with NANB hepatitis has confirmed the presence of viral RNA antedating development of an antibody or even in the complete absence of antibody development (3). Therefore a negative ELISA anti-HCV test cannot be taken as definitive proof that HCV infection is absent, particularly early in the illness.

A potentially more difficult problem to deal with is a patient who is found to be anti-HCV ELISA<sup>+</sup> when clinical liver disease has not been suspected, such as an asymptomatic blood donor. Important questions that the patient will want answered include whether he or she has liver disease and whether he or she is potentially infectious to other individuals. The first step in approaching such a patient is to consider the possibility of a false-positive anti-HCV result, which is always a concern in an individual from a population with a low prevalence of a condition. A report from Britain has drawn attention to the presence of apparently false-positive anti-HCV ELISA tests in patients with autoimmune CAH (4). It has been felt that the false-positive ELISA result relates to hypergammaglobulinemia and that anti-HCV seropositivity is lost with successful immunosuppressive therapy (5). In such cases of suspected autoimmune CAH, the clinician should evaluate the patient in the routine manner, including performing a liver biopsy. A much more difficult challenge is presented by an apparently well patient with normal liver biochemical tests and a positive anti-HCV serological result in the absence of confounding factors such as hypergammaglobulinemia. Many patients likely will present in this manner.

In such a patient who is positive for anti-HCV by ELISA, it will be important to confirm that the result is truly positive. A number of additional tests have been used in the research setting to improve the accuracy of anti-HCV testing, notably the recombinant immunoblot assay (RIBA) and PCR. An early RIBA test detected antibodies to three recombinant antigens: the C-100 also present in the ELISA assay; the 5-1-1-polypeptide, a product of an overlapping segment of the hepatitis C genomes; and superoxide dismutase, which is used to enhance efficient expression of proteins in plasmids. Experience with this first generation RIBA test indicated that it tended to be positive in patients whose anti-HCV ELISA tests were unequivocally positive with a high optical density/cut-off ratio. As with any infectious inoculum, the gold standard of diagnosis is the demonstration of infectivity. In this regard, this first generation RIBA test appeared to be a good indicator of infectivity. Thus researchers have reported (Ebeling F, et al. *Lancet* 1990;335:982-983, Correspondence) that all six blood donor samples that were RIBA<sup>+</sup> were implicated in the transmission of posttransfusion hepatitis. Similar information was reported by Garson et al. (6) using PCR to evaluate infectivity of donor blood units. Thus only one out of six ELISA<sup>+</sup> units was implicated in the transmission of posttransfusion NANB hepatitis, and this donor's blood, but not the others, was positive for HCV RNA by PCR.

Overall, application of the first generation RIBA test and PCR can improve the specificity and sensitivity of the anti-HCV ELISA test in diagnosing HCV infection. It is unlikely, however, that most clinicians will have routine access to the technique of PCR through hospital laboratories any time in the near future. As described in the paper by Van der Poel and colleagues, a newer RIBA test, 4-RIBA, has now been developed. Two additional HCV recombinant antigens have been added to the original panel of HCV antigens. One antigen is from the nonstructural NS3 region (C33c), and the other is an HCV core-associated antigen (C22). A positive 4-RIBA test is defined as reactivity with two or more of the HCV antigens, and an indeterminant result is defined as reactivity of one antigen only. In the study by Van der Poel and colleagues, this second generation RIBA test was validated in a series of anti-HCV ELISA<sup>+</sup> donors and recipients with the additional confirmatory use of PCR.

Four different groups of transfusion recipients were studied. Group A included anti-HCV ELISA<sup>+</sup> recipients with a clinical diagnosis of posttransfusion NANB hepatitis; group B included ELISA<sup>+</sup> recipients with no clinical evidence of posttransfusion NANB hepatitis; group C included recipients of ELISA<sup>+</sup> blood who had not become ELISA<sup>+</sup> or developed clinical posttransfusion NANB hepatitis; and group D included recipient controls matched for a number of factors, including the number of blood products received. Fresh blood samples drawn in 1990 were tested by 4-RIBA and PCR, and banked sera from the transfusion study period 1984 to 1986 were tested by ELISA and 4-RIBA.

Open heart surgery patients (383 transfusion recipients) received a total of 5,150 blood products prepared from 4,906 blood donations of 4,123 blood donors. Of the recipients, 86 were identified in groups A, B, C and D, and 76 of these were available for follow-up in 1990. Thirty-seven blood donations from 30 units were identified to be anti-HCV ELISA<sup>+</sup> on retrospective testing during this time, and all were retested with 4-RIBA. Of these 30 donors, 15 were still donating blood in 1989, and all 15 were enrolled in a follow-up study.

In group A, of the nine recipients found retrospectively to be anti-HCV ELISA<sup>+</sup> after transfusion, six were 4-RIBA<sup>+</sup>, two were 4-RIBA-indeterminant and one remained negative during the 26 wk after transfusion. All had been 4-RIBA<sup>-</sup> before transfusion. On follow-up in 1990, five of the eight recipients available for study were both 4-RIBA<sup>+</sup> and PCR<sup>+</sup>, and three were PCR<sup>-</sup>, of whom two were 4-RIBA<sup>-</sup> and one was indeterminant by 4-RIBA.

In group B (i.e., individuals who developed anti-HCV ELISA seropositivity but no clinical evidence of hepatitis), one individual was 4-RIBA<sup>+</sup> after transfusion on retrospective testing, and the individual on follow-up in 1990 was both 4-RIBA<sup>+</sup> and PCR<sup>+</sup>. All recipients in group C (i.e., recipients of ELISA<sup>+</sup> blood without seroconversion or clinical hepatitis), and all group D control recipients were negative by 4-RIBA or PCR testing.

With regard to the ELISA<sup>+</sup> donor units, 8 of 37 were 4-RIBA<sup>+</sup>, 3 were indeterminant and 26 were negative. Seven of the eight 4-RIBA<sup>+</sup> blood products were implicated in recipient posttransfusion NANB hepatitis, HCV infection or both. None of the 4-RIBA<sup>-</sup> but ELISA<sup>+</sup> specimens were implicated in the transmission of hepatitis. Six donors had contributed the eight 4-RIBA<sup>+</sup> units, and four of these six donors were available for study in 1990. Three of the four were both 4-RIBA<sup>+</sup> and PCR<sup>+</sup>; one donor had become 4-RIBA indeterminant after having been positive between 1984 and 1986 and was PCR<sup>-</sup> in 1990.

The 4-RIBA seropositivity tended to persist. Three of four 4-RIBA<sup>+</sup> donors and five of six 4-RIBA<sup>+</sup> recipients in the period 1984 to 1986 were 4-RIBA<sup>+</sup> and PCR<sup>+</sup> in 1990. One recipient who had been 4-RIBA-indeterminant in the period 1984 to 1986 but who had developed posttransfusion NANB hepatitis had become 4-RIBA<sup>+</sup> and PCR<sup>+</sup> in 1990.

It would appear from this study that the 4-RIBA test is strongly predictive of infectivity of blood for HCV because all eight 4-RIBA<sup>+</sup> blood donor units had been implicated in the transmission of NANB hepatitis and/or presence of HCV RNA by PCR on follow-up in 1990. The remaining 29 4-RIBA<sup>-</sup> or indeterminant blood products were not infective. More widespread use of the 4-RIBA test should help determine which ELISA positive patients are viremic. Notably, the study draws attention to the occurrence of continued viral infection in the absence of biochemical evidence of liver dysfunction. Thus one recipient who had been classified as having had an acute resolved case of posttransfusion NANB hepatitis as assessed by the pattern of serum aminotransferase elevations in the period 1984 to 1986 was PCR<sup>+</sup> and 4-RIBA<sup>+</sup> in 1990. In addition, three of six PCR<sup>+</sup> recipients had normal serum aminotransferase levels at the time of testing in 1990. The authors also comment, although they do not provide data to support this, that up to 40% of anti-HCV ELISA<sup>+</sup> blood donors with normal ALT levels have chronic hepatitis, based on liver histological studies.

Marcellin and colleagues (7) also reported the use of the 4-RIBA test in 100 patients with a clinical diagnosis of NANB hepatitis, 76 of whom were anti-HCV ELISA<sup>+</sup>. Ninety-six were 4-RIBA<sup>+</sup>, and two were indeterminant. All 76 ELISA<sup>+</sup> positive patients were 4-RIBA<sup>+</sup>. This study suggests that the 4-RIBA test is not only more specific but also more sensitive than the ELISA test. Of note is that 18 of the 20 patients in Marcellin's study were ELISA<sup>-</sup> but 4-RIBA<sup>+</sup> with a positive signal on the C33C and C22 bands only. This latter pattern of reactivity would also have been sufficient to classify the test as positive in Van de Poel's study, with presumably the same implication in potential transmission of HCV.

Future reports about the application of 4-RIBA testing will be awaited with interest. It would appear, however, that the 4-RIBA test is a useful adjunct in determining the significance of anti-HCV ELISA<sup>+</sup> test in blood donors, including those with no clinical and biochemical evidence of liver disease. Marcellin's data

also suggest that the 4-RIBA test will increase the sensitivity of HCV testing generally. Support for this view is provided by a report from Ebeling et al (8), commenting on Van der Poel's study. Three of nine blood donors' sera implicated in posttransfusion hepatitis transmission, nonreactive both to the ELISA C-100 test and to the HCV-related antigens combined in the first generation RIBA test, were reactive to the additional antigens C33c and C22 in the second generation RIBA test. A brief report has also appeared from Italy (9) describing a newer ELISA test, HCV C-200 C-22 EIA, which uses three HCV antigens instead of one HCV antigen, as in the original C-100 ELISA. In that study, 61 sera from a series of 420 were anti-HCV<sup>+</sup>, 43 by both C-100 ELISA and C-200 C-22 EIA and 18 by one or other test only. All four of the tests positive by C-100 ELISA alone were 4-RIBA<sup>-</sup>. Of the 14 sera positive by C-200 C-22 EIA but not C-100 ELISA, eight were positive, four indeterminate and two were negative on testing by confirmatory 4-RIBA. This newer test, as suggested by 4-RIBA confirmation, is more sensitive and specific than relying on C-100 ELISA alone. Addition of further viral antigens to RIBA testing may allow even more accurate and earlier diagnosis of HCV infection. The significance of anti-HCV in an otherwise healthy and biochemically normal individual remains uncertain and awaits studies of liver biopsy results and observation of the natural history of HCV infection in such patients.

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#### HEPATOCTE GROWTH FACTOR RECEPTOR AND THE c-MET ONCOGENE

Bottaro DP, Rubin JS, Faletto DL, Chan AM-L, Kmiecik TE, Vande Woude GF, Aaronson SA. Identification of the hepatocyte growth factor receptor as the c-met proto-oncogene product. *Science* 1991;251:802-804.

#### ABSTRACT

Hepatocyte growth factor (HGF) is a plasminogen-like protein thought to be a humoral mediator of liver regeneration. A 145-kilodalton tyrosyl phosphoprotein observed in rapid response to HGF treatment of intact target cells was identified by immunoblot analysis as the  $\beta$  subunit of the c-met proto-oncogene product, a membrane-spanning tyrosine kinase. Covalent cross-linking of <sup>125</sup>I-labeled ligand to cellular proteins of appropriate size that were recognized by antibodies to c-met directly established the c-met product as the cell-surface receptor for HGF.

#### COMMENTS

Hepatocyte growth factor (HGF) is a peptide growth factor that stimulates DNA replication of hepatocytes in primary culture. The factor was originally purified from human rabbit plasma and rat platelets and subsequently cloned from various sources including human plasma, leukocytes and fibroblast culture medium (1-3). Rat and human HGF are synthesized as a precursor that is cleaved to a 728 amino acid peptide (1) consisting of two disulfide-linked chains of 440 ( $\alpha$  subunit) and 233 amino acids ( $\beta$  subunit). HGF messenger RNA (mRNA) is expressed in many organs (including human liver, placenta and leukocytes and rat liver, kidney and spleen), and the protein has been detected in various tissues including brain, thyroid, intestine and pancreas (1, 3, 4). In the liver HGF mRNA is present in nonparenchymal cells but apparently not in hepatocytes (5). The HGF precursor protein is a plasminogen-like molecule (38% homology), but it lacks proteolytic activity. The  $\alpha$  chain of HGF contains four kringle structures, whereas plasminogen contains five; the  $\beta$  chain has extensive homology with a serine protease domain of plasminogen but no proteolytic activity (1). Although HGF was originally described as hepatocyte specific, it is now clear that HGF stimulates growth of many different cell types including melanocytes, keratinocytes, kidney tubular cells and mammary gland cells (6).

The *met* gene was originally discovered in 1984 as an oncogene present in a human osteogenic sarcoma cell line (7, 8). In these cells, the oncogene originated from a rearrangement of the *c-met* protooncogene locus in chromosome 7 and the *tpr* locus in chromosome 1. The *c-met* protooncogene product was characterized and found to be a receptor protein with tyrosine kinase activity, but no ligand for the receptor was identified. The *c-met* mRNA has been detected in mouse lung, brain, kidney, skin and epididymis but apparently not in normal liver. Constitutive overexpression of *c-met* can cause transformation and tumorigenesis as shown by transfection of the *c-met* protooncogene under the control of the SV-40 promoter into NIH 3T3 cells (7, 8).