

Hepatitis C virus: An overview

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In May 1988, Kuo and Houghton and their colleagues in Chiron Research Laboratory, in cooperation with the physicians from NIH and CDC, announced their discovery of the agent which is responsible for the majority of Non-A Non-B Hepatitis (NANB) in post-transfusion hepatitis (PTH)^{1,2}, concluding a 15-year effort to search for this elusive agent. Although it is believed that there is another blood-borne agent responsible for a smaller percentage of the post-transfusion hepatitis, the new agent (an RNA virus closely related to Flavivirus) was named Hepatitis C (HCV).

Subsequently, a serological test, an enzyme-linked immunoassay (ELISA), was developed and become commercially available. Blood banks throughout the whole country have incorporated this assay into one of their screening test batteries. In less than 3 years, a surge of research effort has produced much data in the epidemiology, virology, genetic mapping and treatment of HCV. With the availability of routine diagnostic testing for HCV and the FDA approval of Interferon as an efficacious therapeutic modality, the practicing clinicians will now need to know how to interpret accurately and understand the limitations of this diagnostic test, as well as the indications for Interferon therapy and what benefits it might hold.

The recognition of NANB hepatitis as a nosological entity is derived from the study of transfusion-associated Hepatitis (TAH). In 1975, Feinstone, Alter et al from NIH described a form of TAH which has no viral A or B markers or any other serologic markers³. With the use of more sensitive assays for hepatitis B virus (HBV) surface antigen, 2/3 of the TAH case were found to be negative. From 1975 until 1988, numerous attempts failed to identify the agent or agents responsible for NANB hepatitis.

We knew that there are probably 2 viral agents involved in blood-borne NANB hepatitis. Although NANB hepatitis is responsible for the majority of post-transfusion hepatitis, at least a third of NANB hepatitis cases are not infected via the

percutaneous route (sporadic cases)⁴.

Epidemiologic data of NANB hepatitis are extensive and have been reviewed recently¹⁶.

Clinically, the disease has a shorter incubation period — somewhere between that of hepatitis A and B — a greater propensity to become chronic and it frequently progresses on to cirrhosis. It has been suggested that approximately 50% of NANB hepatitis patients develop biochemical evidence of chronic hepatitis; among those cases with chronic ALT (SGOT) elevations that are biopsied, approximately 60% have chronic active hepatitis (CAH) and/or cirrhosis. Five to 10% of those who develop acute NANB-TAH will eventually end up with cirrhosis. Despite the relatively mild symptomatology in chronic NANB hepatitis and the usual absence of physical abnormalities, mortality directly as a result of liver illness has been reported to be as high as 25%⁵. Furthermore, reports from Spain, Italy and Taiwan have implicated HCV positivity in the majority of cases of non-HBV hepatocellular carcinoma^{6,7,8}.

The path to the identification of HCV was tortuous. Attempts to isolate the virus, ie the antigen, were unsuccessful due to the minute amount of virus in the serum. Bradley et al at CDC in Atlanta have studied a group of chimpanzees with highly infectious chronic NANB hepatitis⁹. The authors stored sera which were believed to contain very high titers of infectious virus in the samples.

Adopting a different approach to the problem, a group of geneticists in the Chiron Lab in California, with expertise in genetic engineering, used the chimps' sera to achieve the final identification of HCV. By means of ultracentrifugation, all the viral particles were concentrated and all the nucleic acid was extracted, because whether the virus is RNA or DNA was unknown at the time.

Reverse transcriptase was then used with random primers (a synthetic oligonucleic acid produced by recombinant genetic engineering) to construct a cloned DNA library (cDNA) complementary to all the nucleic acids present in the pellet. It was then assumed that this pellet contained genetic material from both the chimps' genes and the viral genes. This cDNA was then digested by restriction enzymes to provide fragments which were next inserted into a bacteriophage GT-11 as a vector, and then to express their corresponding peptides through *E.Coli*.

Serum from a patient with confirmed NANB hepatitis was used as a source of viral antibody to screen through more than a million clones expressed in the bacteriophage GT-11. Final-

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ly, clone 5-1-1 was found to react with the patient's serum. This 155-base pair clone was then extracted from the vector and used to hybridize to, and identify with, a larger clone in the original cDNA library, clone 81, which reacted solely with the original, highly infectious, chimpanzee's serum but not with the normal chimps' host genome¹². At that point, the virus was determined to be an RNA virus because of its sensitivity to ribonuclease; it was single-stranded because it bound only to 1 of the 2 strands in the clone 81 cDNA. It was also determined to have about 5,000 to 10,000 nucleotides and had a lipid envelope.

This virus is distantly related to the Flavivirus and the Pestivirus Family, in terms of the general structure and organization of the positive-stranded RNA genome and its encoded viral polyproteins. Furthermore, with the understanding of the RNA sequences, most of the structural and non-structural proteins encoded by HCV have now been identified from in-vitro RNA translations and mammalian-cell expression studies. It is now known that on the 5' end, the HCV genome has one, single, open-reading frame (ORF), followed by structural protein codons, nucleocapsid and envelope, then 5 non-structural protein codons (NS1-NS5).

The clone 5-1-1 genome was found in the NS3-NS4 region, which through mammalian cell expression produced a 363 viral aminoacid fusion polypeptide (c100-3), which in turn was used in a radio-immunoassay to capture circulating anti-HCV antibodies in blood. This assay was then tested to validate the specificity of this clone in detecting viral antibodies associated with NANB hepatitis. The specificity and sensitivity of this radio-immunoassay was most convincingly confirmed by its successful decoding of a panel of coded serum samples prepared by Alter in NIH in the past; this has been used to test the validity of the previously described immunoassay for NANB hepatitis¹⁰.

Currently, there are 2 ELISA tests available commercially to detect HCV: one from Abbott Lab and the other from Ortho Diagnostic System. This first generation assay, based on the use of the c100 polypeptides, can detect HCV antibody only after a long "window" period of 1 to 3 months, or even longer, after the onset of acute hepatitis. In fact, some cases have been reported to take more than a year to become seroconverted. Nevertheless, based on the studies of the stored sera, at least 80% of patients with a firm clinical diagnosis of NANB-PTH are anti-HCV positive¹¹. A much lower frequency of seropositivity is noted in acute resolving NANB hepatitis, ie as low as 15% in the original study². Several groups are at risk for acquiring HCV infection, such as iv drug-abusers, hemodialysis patients and hemophiliacs receiving multiple transfusions¹².

So far, homosexuality has not been shown to be a risk factor.

Blood banks in the USA have incorporated HCV testing into their routine of screening donors. In the 1970s, due to the high incidence of PTH (up to 20% of recipients), commercial blood was excluded and the incidence dropped to 10%. As blood was screened for HBsAG, HIV, and the so-called surrogate markers, ie HBcAB and ALT, the incidence dropped further to between 3% and 5%. It is estimated that it will drop into the 1% range as the result of HCV antibody screening.

However, there are pitfalls with this first generation assay.

In addition to the prolonged interval between disease and serum conversion before the antibody can be detected, there are up to 20% of well-characterized NANB hepatitis cases that remain negative.

False positives were quite common in diseases involving hypergammaglobinemia, such as chronic autoimmune hepatitis. This ranged from 44% reported by Esteban-Mur and colleagues¹², and 78% of patients with type II chronic autoimmune hepatitis as reported by Lenzi et al¹³. Ikeda et al, in a letter to the editor in *Lancet* in 1990, suggested that this false positivity may be due to the reactivity to the superoxide dismutase moiety of the recombinant c100-3 fusion polypeptide used in the ELISA assay¹⁴.

Because of these problems, a more sensitive assay was developed called RIBA, Recombinant Immunoblot Assay. This refined test detects an antibody directed against 3 recombinant antigens: The original c100-3, the 5-1-1 polypeptide in a fusion protein with superoxide dismutase and superoxide dismutase protein itself. This is now a so-called confirmatory test in some of the reports we have received.

There is a more accurate but technically more cumbersome test called PCR, Polymerase Chain Reaction assay. It is essentially an immunoamplification test. Advancement in genetic engineering technology has resulted in a novel way to detect minute amounts of genetic material and to sequence it by using commercially developed primers (oligonucleotides) to clone specific DNA rapidly, or in this case RNA, without the need for a living cell. It allows the RNA from a selected region of a genome to be amplified by more than a million-fold, provided that at least part of its nucleotide sequence is already known, through polymerase action.

Recently, in the International Symposium on HCV in Los Angeles, the Chiron group announced their successful use of a bacterial recombinant immunoscreening method to identify many other immunogenic regions of the HCV-encoded polyprotein. Subsequently, recombinant proteins derived from both the non-structural as well as the structural regions of the HCV polyprotein have been purified from recombinant yeast; specific individual radioimmunoassays for the circulating antibody have been developed.

Additionally, in the same meeting, Dr. Krawczynski from CDC, using fluorescein isothiocyanate (FITC)-labeled polyclonal IgG obtained from a patient with HCV hepatitis, and from chimps experimentally infected with HCV, has identified morphologically the hepatitis C antigen in hepatocytes. As early as 10 days after symptoms appear, HCV antigen can be detected in the hepatocyte. This is a very important tool used to study the pathogenesis and natural history of HCV¹⁵.

Summary

An immunoassay for the detection of the majority of NANB-PTH cases is available now, although at least 20% of well-characterized NANB-PTH remains negative. False positive cases in hypergammaglobinemic states in certain chronic active hepatitis are also common. The virus has been named hepatitis C (HCV). It is believed that there is at least another blood-borne viral agent that may be responsible for a smaller cohort of patients with NANB-PTH. It usually takes 1 to 3 months after onset of acute illness for HCV antibody to be

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formed on every donated unit. Thus, almost half of the units with anti-HCV would have been discarded and not transfused anyway (Table 2).

By contrast, of those units with repeatedly reactive ELISA but negative or indeterminate RIBA tests (40 + 23 = 63), abnormal results in the surrogate tests were found in only 8 (12.7%).

During this period of time, 28,807 units of blood were collected, with a prevalence of repeatedly reactive anti-HCV test by ELISA of 0.03% (or 3:1,000 donations). A third of those were confirmed to have antibody to HCV by RIBA for a frequency of 0.01% (or 1:1,000).

Lookback

In addition to informing all donors of abnormal test results, the Blood Bank has undertaken a "lookback" procedure if the donor gave blood prior to the availability of the HCV test. In the lookback, the intent is to notify former transfusion recipients that there may have been a risk of contracting HCV from the earlier transfusion. Although we are unable to determine when the donor was infected with HCV or whether a reactive HCV test truly means infectivity, follow-up of these patients is recommended for the following reasons:

(1) HCV is usually subclinical, so the patient would not be coming in to see the doctor, even if infected.

(2) HCV is often associated with chronic liver disease (30-50%)—a much higher incidence than with HBV. HCV is also associated with primary liver-cell carcinoma.

(3) There are recent studies reported in the *New England Journal of Medicine* that offer hope of treatment of chronic NANB hepatitis with alpha interferon^{4,5}.

Our standard lookback procedure is as follows: The Blood Bank contacts the hospitals, provides them with unit numbers, transfusion dates and blood products transfused from donors in the past whose blood today is ELISA and RIBA reactive for anti-HCV. The hospitals, in turn, are to notify physicians to advise their patients of the possible exposure to HCV. This 2-step procedure is necessary, since the Blood Bank of Hawaii does not have a file of the names of recipients of blood products. We have suggested to hospitals, in their message to physicians, that information about HCV be given, and to include especially the recommendation that their patients be notified of the possible exposure and the importance of follow-up.

We realize that not all who have given blood will return to donate again. Thus, HCV may have been transmitted to patients whom we would not be able to identify through our usual lookback procedures. We therefore recommend that physicians include, as part of their general medical history, questions that relate to blood transfusion. This information may have clinical significance that relates to HCV as well as providing a basis for future expansion of scientific knowledge about possible transmission of infectious agents through blood transfusion. There are steps that can be taken today, and should be taken, to treat these patients, as well as for the protection of their family members (eg vaccination against HBV, alpha interferon treatment for HCV, etc).

As the sole provider of blood products to all civilian hospitals in Hawaii, the Blood Bank wants physicians to feel confi-

dent that blood for transfusion to their patients is the safest possible. As new and refined tests and procedures are developed and approved for blood bank use by the Food and Drug Administration, physicians can be assured that we will continue to adopt such for the well-being of Hawaii's patients. When a lookback becomes necessary, we hope you will offer continued support and assistance.

The Blood Bank of Hawaii is licensed and regulated by the Food and Drug Administration and is an accredited member of the American Association of Blood Banks and the Council of Community Blood Centers.

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