HEPATITIS C

Introduction

With the recent identification and sequencing of the viral genome of the hepatitis C virus (HCV) and the subsequent development of an ELISA assay, a wealth of recent literature has emerged.

The purpose of this brief review is to discuss the recent literature, to evaluate the current available assay systems, and to preview future developments.

Epidemiology

In the United States, non-A non-B hepatitis (NANBH) accounts for approximately 25% of the cases of clinically apparent infectious hepatitis.(1,8,27) The remaining 75% are composed mostly of hepatitis A and B cases.(2) In studies looking at seropositivity to HCV, approximately 80% of patients presenting with clinical NANBH were seropositive.(3,4,5,52) Only 5-10% of these patients report a history of transfusion.(7) A higher percentage (40-50%) of HCV seropositive NANBH patients provide a history of intravenous drug abuse (IVDA).(7,8) As many as 50% of patients with hepatitis C will present with no identifiable risk factors.(14) Numerous epidemiologic studies have attempted to identify additional risk factors for the transmission of hepatitis C, however a strong association has only been found with parenteral exposure either by IVDA or transfusion.(8)

The blood donor population has a prevalence of seropositivity to HCV of between 0.5 and 1.0% and it has been estimated that the non-blood donating general population has a prevalence rate of 2 to 3%.(8-10) Hemophiliacs have been shown to have an HCV seroprevalence of 75%, intravenous drug abusers between 40 and 80% and hemodialysis patients between 1 and 30%.(6,7,11,12,13) The general homosexual male population has only a slightly increased prevalence, approximately 4.0%, certainly lower than would be expected if the virus was transmitted efficiently by sexual contact.(15) However, studies have also demonstrated that HIV positive homosexual men have a much higher rate of HCV positivity than HIV negative homosexual men.(12,13) One can speculate that HIV positive homosexual men may have a higher incidence of parenteral exposures than HIV negative homosexual men or that HIV is more efficiently transmitted to immunosuppressed patients.

To investigate the importance of heterosexual transmission of HCV, studies have looked at partners of HCV positive subjects and have found either no increase or a slight increase in HCV seropositivity.(8,9,13) A recent study has implicated multiple sexual partners as a risk factor. However, this study defines multiple partners as greater than 2. does not define lifetime vs. recent contacts and looks at a small patient population in which only 6 NANBH patients with multiple partners (>2) were identified and compared to a single control subject.(14) Furthermore, it seems unlikely that sexual transmission would be
more important in heterosexuals than in homosexuals.

Employment as a health care worker has been found to be associated with a very slightly increased risk of HCV seropositivity. Other risk factors such as household contact, shellfish ingestion, acupuncture or tattooing, have not been found to significantly increase the risk of HCV seropositivity.

Scattered reports have documented both lack of and a low rate of maternal fetal transmission. Larger studies need to be done to definitively show the importance of vertical transmission. Thus, how HCV is maintained in the general population is not known.

The incidence of NANBH and prevalence varies by geographic location. In third world countries as many as 50% of all cases of hepatitis are NANB. However, the majority of these may be due to epidemic enterically transmitted hepatitis E. In studies looking at acute and chronic NANBH in developed countries (US, Japan, Germany, Australia, Spain, Italy, Netherlands), the seroprevalence of hepatitis C is between 20 and 40% in acute and between 70 and 90% in chronic NANBH. This apparent discrepancy between seroprevalence in acute and chronic disease reflects the delay in seroconversion to HCV antibody positivity.

Clinical Features

Hepatitis C has no clinical features that definitively distinguish it from hepatitis B. It has been reported to have an incubation time (infection to clinical hepatitis) of 7 weeks which is intermediate between hepatitis A and B. However, the time of seroconversion has been found to be highly variable, with a range from several weeks to up to a year. One feature that has been described in hepatitis C is a fluctuation of transaminase levels from abnormal to normal. This poses problems in the diagnosis of both acute and chronic hepatitis C and in the interpretation of a positive HCV antibody in the face of normal transaminase levels.

In cases of acute hepatitis C infection, the clinical symptomatology seems to be milder and more often anicteric than hepatitis B. Indeed, many of the patients exposed to hepatitis C virus are completely asymptomatic. However, up to 50% of transfusion recipients with NANBH (clinical or subclinical) develop biochemical evidence of chronic hepatitis and up to 20% of these develop chronic active hepatitis or cirrhosis. Of blood product recipients who develop chronic NANBH, approximately 70-90% are seropositive for HCV. In sporadic NANBH cases, approximately 30-80% are seropositive.

Other studies have investigated HCV seropositivity in patients with hepatocellular carcinoma (HCC). Colombo et al, found HCV antibodies, using the recombinant C100-3 protein and radiolabelled sheep anti-human immunoglobulin, in 65% of stored sera from patients with HCC with no relation to the presence or absence of hepatitis B surface antigen. The results have suggested an oncogenic role of HCV which may be potentiated by co-infection with HBV and/or alcohol consumption.
Pathology

The histopathologic features of HCV associated NANB hepatitis have been described to border between those of chronic active and chronic persistent hepatitis.(39-40) The changes seen in liver biopsies are: prominent lymphoid follicles in the portal tracts, fatty change, focal lobular necrosis and acidophil body formation.(39) These changes are not specific to HCV, although the finding of lymphoid follicles in cases of clinical acute hepatitis and the presence of lobular necrosis in cases of clinical chronic hepatitis is suggestive of HCV infection. Lymphoid follicles are usually associated with chronic and lobular necrosis with acute hepatitis.(54)

Therapy

Currently, there is no approved therapy for HCV infection. There has been an interest in the anti-viral effects of alpha 2b-interferon (IFN-a). Recent clinical trials have reported that IFN-a, given to patients with chronic NANBH thrice weekly in doses ranging from 1 to 3 million units for 6 months was associated with normalization of liver transaminases and histologic improvement of sequential liver biopsies.(41,42) However, the majority of responders relapse following cessation of therapy. The medical community awaits the long term follow-up of the sub-set of apparent long term responders, the results of studies looking at different dosing regimens and maintenance treatment of patients that relapse.

The prophylactic use of immune globulins for the prevention of post-transfusion NANBH has been shown to be of questionable benefit in several studies.(55) However, the Morbidity and Mortality Weekly Report (Feb. 1990) states "For persons with percutaneous exposure to blood from a patient with post-transfusion NANBH, it may be reasonable to administer IG (0.06 ml/kg) as soon as possible after exposure."(55)

Identification of HCV

The agent of NANBH has been long sought after and elusive to traditional techniques of viral isolation. Indeed, conventional immunological techniques have consistently failed to demonstrate the presence of viral antigens or antibodies, presumably due to the relative paucity of viral particles in the serum. Filtration studies demonstrated that the agent was smaller than 80nm in diameter and sensitivity to organic solvents demonstrated that it likely possessed an envelope containing an essential lipid.(4.43.44) Electron microscopy (EM) studies suggested the presence of a 50-60nm viral particle in livers of patients with NANBH.(39) The above findings (subsequent sequence analysis) have suggested that the virus belongs to the family of flaviridae or its subtype of pestiviridae.(45)

Choo et al(4), provided a major scientific breakthrough by using molecular biologic techniques to isolate a nucleic acid sequence of what is now referred to as hepatitis C virus
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(HCV). Highly infectious serum from a chimpanzee experimentally infected with NANBH was ultracentrifuged, the nucleic acids were extracted and complementary DNA (cDNA) was made to RNA. A lambda phage expression library was constructed and then screened with a patient's serum known to be infected with NANBH. A single positive clone (5-1-1) was identified, then amplified and used to probe the cDNA library and 3 overlapping clones were identified.

One of these clones (81) was tested in hybridization assays and found to anneal only to RNA from infected chimpanzee liver and not to DNA extracted from human placental control or infected chimpanzee liver. Thus, it appeared that the nucleic acid was RNA, not derived from the host, and therefore, likely to be viral in origin. Further evidence supporting the RNA origin was that pretreatment of the liver nucleic acid extracts with RNAse abolished hybridization. To determine if the RNA was single stranded the + and - cDNA strands were cloned into a ssDNA viral expression vector (M13) and it was determined that only one strand hybridized to both infectious RNA and to cDNA from clone 81. Thus, the single stranded (ss) nature of the virus was confirmed.

To determine the size of the putative viral genome, the cDNA of clone 81 was used to screen RNA extracted from infected chimpanzee liver. Hybridization revealed a heterogeneous RNA population, which probably represented partially degraded RNA, ranging in size from 5000 to 10,000 nucleotides in length. Thus, the genome was thought to be 10,000 nucleotides in length. This RNA appeared to possess an adenosine rich sequence which would be consistent with a poly A tail and would suggest that it represented messenger RNA. Hybridization was demonstrated with single stranded cDNA to both the RNA derived form infected chimpanzee liver and to the 5-1-1 clone producing the fusion protein, thus confirming that the RNA was sense (positive stranded) with respect to protein translation.

Sequencing of clones 5-1-1 and 81 revealed that they consisted of 155 and 353bp respectively. The nucleotide sequences of these and other overlapping clones suggested a continuous translational open reading frame encoding for a single polypeptide. To demonstrate the relationship of these clones to NANBH, a fusion polypeptide (using human superoxide dismutase as an expression enhancer, and the 5-1-1 clone as an insert) was synthesized using a bacterial expression vector. Reactivity to this antigen was demonstrated with both NANBH infected human and chimpanzee serum. In addition, seroconversion was demonstrated in experimentally infected animals.

Development of an ELISA assay

Kuo, et al, used the sequences derived from 3 clones overlapping the 5-1-1 clone and constructed a yeast expression vector was, which encoded for a fusion polypeptide which contained 363 viral amino acids.(24) This polypeptide was used to coat microtiter well plates and was found to react with well defined test panels of sera from NANBH patients and not to negative control sera. This fusion polypeptide (C100-3), has become the antigen
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used in the currently employed ELISA assay systems. It represents a region of the viral genome (NS3) encoding for a non-structural protein, most likely important in viral replication.

Weiner, et al (46), have employed the polymerase chain reaction (PCR) to investigate the presence of a portion of their published sequence, specifically a portion of the NS3 region, in livers of patients with post-transfusion chronic NANBH and found that 70% (7/10) of C100-3 positive patients were positive by PCR and 40% (2/5) of C100-3 negative patients were positive by PCR. They suggest by these results that antibody positive patients are likely to be viremic and that the rate of HCV infectivity is probably higher than predicted by antibody testing alone.

A similar approach to develop an ELISA test was used by Arima, et al. (47) They used serum from discarded units from 1047 Japanese blood donors with elevated ALT levels and negative HBV markers. (47) In these studies a high correlation was found again with NANBH test panels and the cloned cDNA. The Japanese researchers have also employed PCR to look for HCV sequences in patients with acute and chronic NANBH and have found their PCR reaction product, a 400bp sequence corresponding with the NS5 region of the genome, in both acute and chronic disease. (48) This suggests that the NS5 region codes for a protein which may be a potential marker for acute infection.

Vyas, et al (49) have also employed PCR to evaluate 101 non-transfusable units eliminated because of elevated transaminase levels (ALT) from asymptomatic volunteer blood donors. Their reaction product (a 210bp sequence corresponding to a portion of the NS3 region of the genome) was derived by ultracentrifugation from 0.6 mls of specimen, in 8 of 11 anti-HCV positive samples and none of the 90 HCV negative samples. Interestingly, the 3 PCR-, anti-HCV+ samples were PCR positive when 2.4mls of each patient sample was evaluated. Unfortunately, the 90 PCR- samples were not re-tested with higher sample volumes. This study suggests that there is excellent correlation between anti-HCV positivity and the presence of viremia. In addition, 2 of the 11 anti-HCV positive specimens were indeterminate on the recombinant immunoblot confirmatory assay (RIBA, Ortho Diagnostics). These specimens were confirmed positive by PCR which implies that the manufacturers criteria may be too stringent on the RIBA.

Vyas, et al (49) also attempted PCR with other primer pairs corresponding to the NS2 region of the genome. Apparently, this region displays high rates of sequence divergence and sequence differences in the primer binding sites were likely responsible for failure of primer binding and thus failure of PCR to detect small quantities of RNA. This is in contrast to the NS3 region which appears to be a highly conserved region where there is little sequence variation and therefore efficient primer binding. Finally, Vyas, et al (49), addressed viral quantitiation using quantitative PCR techniques. They found that estimated viral loads varied from 100 to 50,000,000/ml which is higher than estimated in animal studies looking at chimpanzee infectious doses (CID). (47,49,50,51)

Current research is now underway investigating the structural region of the genome with the hopes of finding antigens which may serve as markers of acute infection.
Sensitivity and specificity of the ELISA assay

The manufacturers of the ELISA assay claim sensitivity approaching 100% in the diagnosis of chronic HCV infection. These figures are based on seroconversion of chimpanzees who were experimentally infected with NANBH. In humans with chronic post-transfusion NANBH, sensitivity has been estimated to be approximately 86%.

The specificity of the ELISA assay, according to the manufacturer, is 99.53%. They based their calculation on a study of 13,153 blood donors at low risk for HCV.

One of the major problems in test interpretation lies in evaluating patients with acute NANBH. After 6 weeks following the onset of symptoms, only 50% of patients with acute NANBH will be seropositive. Because of this, the sensitivity of the assay has been reported to be lower in cases of acute NANBH with only 60% (3/5) of acute resolving post-transfusion NANBH cases seroconverting 6 months following exposure.

A problem in evaluating patients with chronic disease is that a subset of patients will lose anti-C100-3 antibody positivity during the course of infection. This has been reported in 5% (1/15) of patients with chronic NANBH after a 7 year follow-up and in 100% (3/3) of patients with acute resolving NANBH after a mean follow-up of 4.1 years.

Confirmatory Assays

The manufacturers recommend a recombinant immunoblot assay (RIBA, Ortho Diagnostics) or an antibody neutralization assay (Abbott Laboratories) as supplemental tests. They use as criteria for HCV positivity that a patient be reactive on two replicate ELISA assays and be confirmed by a supplemental assay. The Ortho Diagnostics RIBA uses recombinant fusion proteins from both the complete C-100-3 protein and the 5-1-1 protein. Since both contain human superoxide dismutase (SOD), which had been employed as an expression enhancer, the RIBA tests for reactivity to SOD as a control. The final control is immunoglobulin (IgG). The criteria for positivity is that both C-100-3 and 5-1-1 are positive and the controls are all negative. With these stringent criteria only 35 to 50% of repeat reactive patients from a low risk group (i.e. blood donors) will confirm on the confirmatory assays. From a higher risk group (i.e. paid blood donors) approximately 80% will confirm positive. As it stands now, the confirmatory assays are not FDA approved and are costly and time consuming to perform.

If PCR is used to detect viral sequences, not all anti-HCV antibody positive NANBH patients will have detectable HCV sequences by PCR. Conversely, some anti-HCV antibody negative NANBH patients will have detectable levels of HCV sequences when tested by PCR. Thus, if PCR positivity is taken as evidence of viremia then it is clear that the ELISA test may be misleading. PCR has the advantage over the ELISA assay in that primers may be chosen so that any region of the HCV genome may be amplified instead of looking for antibodies directed at limited number of protein products from a single region.
of the genome. Thus, by amplifying a number of different sequences, the chances of detecting HCV in the acute phase of infection would theoretically be much improved. In the various cited references, PCR looks promising due to its ability to detect early infection. Of course, PCR has its limitations and though touted to be highly specific and sensitive it may only be slightly better than the ELISA assay. In addition, it is a labor intensive and expensive test, in its present format, and therefore unlikely to find a place in routine clinical practice in the near future.

Summary

At present, we are left with a single screening test for a disease which, outside of parenteral exposure, is poorly understood. Perhaps the most important problem with the current assay is the lack of a gold standard for hepatitis C virus infection. The virus has failed to be definitively identified by EM and has never been cultured. The presence of antibody suggests exposure to HCV but does not prove that it is the causative agent of disease. The presence of antibody correlates fairly well with infectivity, at least in the blood donor setting. However, antibody positivity does not insure that a patient has hepatitis, will develop hepatitis or will be infectious to others. Clearly, further epidemiologic studies need to be done to understand the non-parenteral modes of transmission and also to determine the significance of HCV in the low-risk population.

Once given the information of antibody positivity, the clinician is left with the problem of interpreting the test, the problem of counselling a patient about questionable risk to their loved ones and finally, offering them entry into trials of an unproven experimental therapy. In addition, antibody positivity does not prove that HCV is the causative agent, it only documents previous exposure. Thus, if a patient is in a population with a high prevalence of HCV exposure, he/she may have to be worked up for other infectious (i.e. CMV and EBV) and non-infectious etiologies in order to interpret a positive antibody test. If the patient has NANBH and is in the window of the first year of infection or their history is not well documented, a negative HCV antibody test can be misleading. In that setting, the patient would have to be counselled, informed about being a possible risk to household contacts or to loved ones and would need to be retested at regular intervals and/or worked up for other causes of infectious and non-infectious hepatitis. Documenting seroconversion concordant with clinical and laboratory evidence of hepatitis, in the absence of seroconversion or evidence of other etiologies for hepatitis, is perhaps the only way that the current assay may be used to provide convincing evidence that HCV was responsible for an episode of hepatitis.

One may raise the point that in NANBH a positive HCV antibody test may prevent a more costly laboratory and medical work-up. However, it is quite possible that the exact opposite is true. For instance in acute HCV infection, a negative test may guide a clinician into ordering numerous other tests depending on the clinical situation (i.e. CMV, EBV, alpha-1-antitrypsin, ceruloplasmin, liver ultrasound, etc.).
Thus, until more is known about the epidemiology and/or therapy for HCV infection, and until a better test for acute HCV is developed, the diagnostic utility of the current assay in the setting of NANBH is academic at best.

CONCLUSIONS:

1) Hepatitis C exposure has been seen to correlate with the majority of NANBH cases in developed countries.

2) Hepatitis C seroprevalence is extremely high in transfused patients and IV drug abusers.

3) Hepatitis C exposure is highly correlated with biochemical evidence of chronic hepatitis.

4) Hepatitis C exposure in transfusion recipients is associated with an increased risk of chronic active hepatitis and cirrhosis.

5) Hepatitis C is transmitted most efficiently by parenteral exposure either by IVDA or transfusion. No studies have conclusively demonstrated the importance of any other type of exposure.

6) Hepatitis C has no definitive distinguishing clinical characteristics to separate it from hepatitis B.

7) Hepatitis C remains a diagnosis of exclusion and seropositivity for HCV only documents previous exposure and not active infection.

8) There are currently no available assay systems which can reliably detect acute hepatitis C.

9) There is currently no licensed confirmatory assay for HCV and PCR is experimental.

10) There is currently no approved therapy for HCV infection. Preliminary results with IFN-alpha show a transient benefit for a small subset of patients.
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