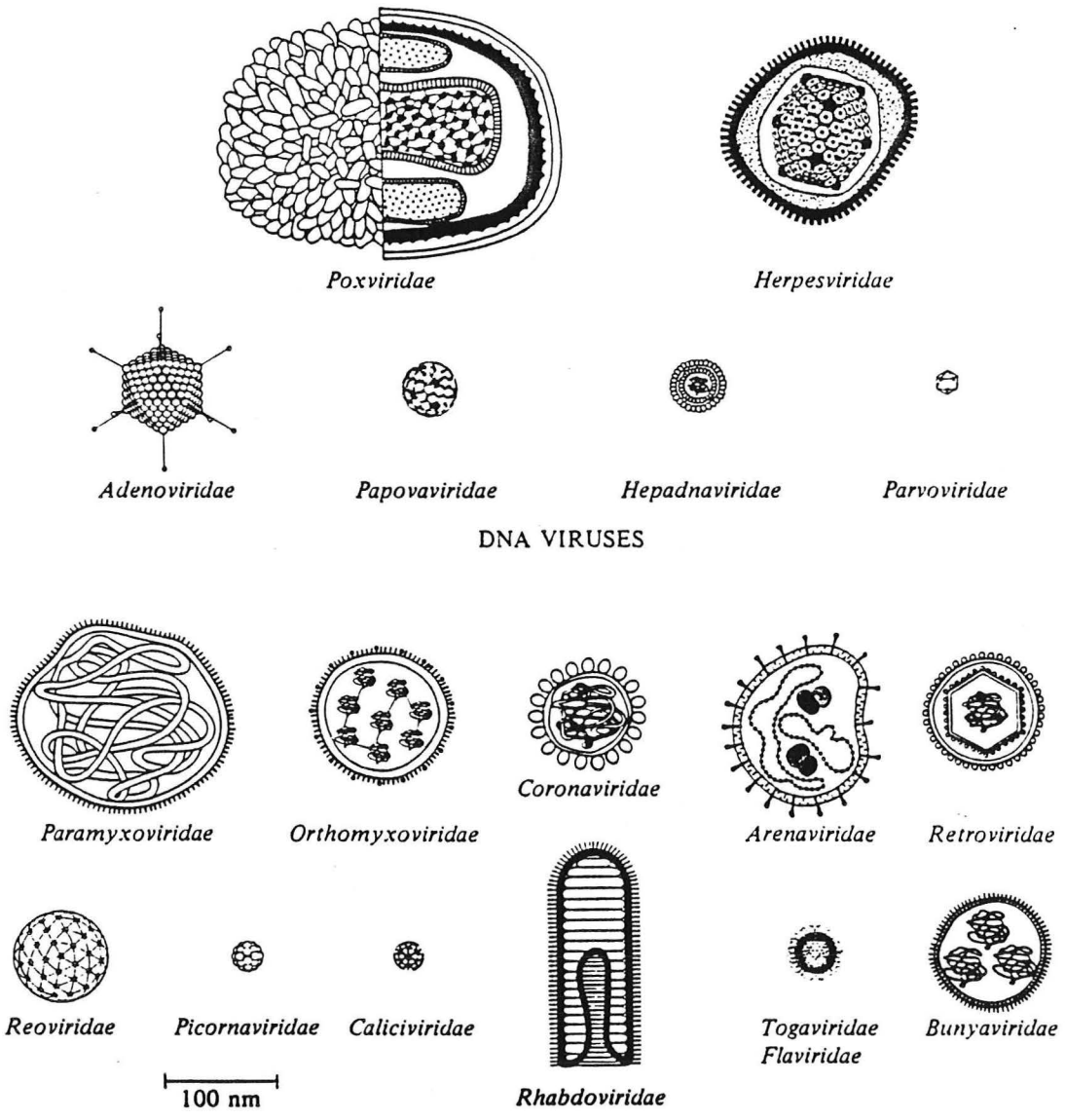
HEPATITIS C



From: **Medical Virology, D. O. White and F. J. Fenner eds; Academic Press 1986**

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CONTENTS

ABBREVIATIONS:

CAH	Chronic active hepatitis	CPH	Chronic persistent hepatitis
CMV	Cytomegalovirus	EBV	Epstein-Barr virus
HAV	Hepatitis A virus	HBsAg	Hepatitis B surface antigen
HBc	Hepatitis B core	HBV	Hepatitis B virus
HCC	Hepatocellular carcinoma	HCV	Hepatitis C virus
HDV	Hepatitis D virus	HEV	Hepatitis E virus
ISG	Immune serum globulin	NANB	Non-A, non-B
PTH	Post-transfusion hepatitis	SOD	Superoxide dismutase
TTV	Transfusion-Transmitted Viruses	ULN	Upper limit of normal

HISTORY:

In 1974, Prince and coworkers at the New York Blood Center reported that an agent other than the hepatitis B virus seemed to be the cause of a majority of cases of post-transfusion hepatitis (1). Of 204 cardiovascular surgery patients followed prospectively for 6 months, 51 (25%), developed post-transfusion hepatitis. In only 12 of these cases was there serological evidence of exposure to hepatitis B virus. Thus, in 36 of 51 (71%) there was no evidence of hepatitis B virus infection using sensitive assays. Of note, there were 15 cases of new hepatitis B virus exposure without hepatitis. CMV was not the cause of post-transfusion hepatitis in this cohort since there was equal evidence of cytomegalovirus exposure in all groups with and without hepatitis. Furthermore, hepatitis A virus infection did not appear to be the cause of non-B post-transfusion hepatitis, since the mean incubation period was similar to that of hepatitis B. The risk of non-B hepatitis in the recipients was 10 times higher with commercial blood sources than with all volunteer blood. In the summary of their report, the authors suggested "that a large proportion of long-incubation post-transfusion hepatitis is unrelated to hepatitis B, and that control of post-transfusion hepatitis will require identification of a hepatitis virus(es) type C" (1).

Shortly after the appearance of this original report, Feinstein and colleagues described 22 patients who had an episode of transfusion-associated hepatitis that was not due to viral hepatitis type A or B (2). The patients had undergone cardiac surgery at the NIH, and each had had at least one episode of transfusion-associated hepatitis. Hepatitis B surface antigen was not detected in acute phase serum samples obtained from these patients, and in none was there serologic evidence for hepatitis A infection (measured by immune electronmicroscopy). Nine of the 22 patients demonstrated anti-CMV responses, but these were unrelated to their hepatitis course. In all 22 patients, there was pre-existing antibody to Epstein-Barr virus. Of great importance, the authors noted that 50% of multiply transfused recipients of commercial blood developed hepatitis.

The occurrence of post-transfusion hepatitis that was neither type A or type B hepatitis was thus clearly demonstrated in 2 separate studies. A Lancet editorial published on July 12, 1975, first coined the term non-A, non-B to describe these findings (3). Although unwieldy, the terminology was accurate, since the diagnosis was one of exclusion, there being evidence for neither hepatitis A virus nor hepatitis B virus as the cause of the liver disease. Now, 15 years after the first suggestion that a hepatitis C virus existed, it has been identified and linked to most cases of post-transfusion hepatitis. The lag between the appreciation of its existence and the identification of this virus was the consequence of major technical difficulties, and it was not until the advent of new molecular biological approaches that the virus was successfully isolated. Before this occurrence, however, there was an increasing body of evidence that strongly indicated the transmissible nature of this disease.

Experimental Transmission of Non-A, Non-B hepatitis

In 1978, Alter and coworkers at the NIH demonstrated that non-A, non-B hepatitis was caused by a transmissible agent (4). They inoculated 5 chimpanzees with plasma or serum, obtained from four patients with post-transfusion hepatitis, and from one implicated donor. In all 5 chimpanzees there was biochemical and histological evidence of hepatitis. Of great importance, inocula obtained from both acute and chronic cases were infectious, suggesting that there existed a chronic carrier state of the agent responsible for non-A, non-B hepatitis existed. This suggestion was confirmed when four serum or plasma samples obtained from a single patient over a 6 year period were each inoculated into chimpanzees (5). One of these samples was obtained when the aminotransferases were transiently normal. All four transmitted hepatitis to chimpanzees, thereby demonstrating that a chronic carrier state existed, and that infectivity did not correlate with evidence of active liver disease. In similar experiments, a factor XI concentrate implicated as the cause of post-transfusion hepatitis also transmitted hepatitis to chimpanzees (6). Thus, experimental transmission of non-A, non-B hepatitis to chimpanzees demonstrated that a transmissible agent was responsible for the disease.

A re-evaluation of human studies, that had been carried out in the early 1950s, also confirmed that a transmissible agent was the cause of non-A, non-B hepatitis (7). In the initial studies, six sera implicated in post-transfusion hepatitis were each inoculated into 10-20 volunteers. Hepatitis occurred in the recipients of 5 out of the 6 implicated sera. A retrospective study demonstrated that only 2 of the 6 donor sera were hepatitis B surface antigen positive. All the recipients of a 1 ml inoculum of these HBsAg-positive sera had evidence of exposure to the hepatitis B virus, and in half of the recipients there was evidence of hepatitis. The remaining sera were negative for hepatitis B surface antigen, and yet three out of the six donor sera transmitted hepatitis to 10%-47% of recipients of a 1 ml inoculum. Of importance, 3/3 of the implicated non-A, non-B donors had evidence of chronic liver disease and chronic liver disease developed in 2 of the 6 non-A, non-B recipients who developed hepatitis.

THE "NON-A, NON-B" VIRUSES

The ability to identify both hepatitis A and hepatitis B quickly led to the suggestion that more than one virus may be responsible for non-A, non-B hepatitis. Thirty episodes of acute viral hepatitis were documented in 13 patients in California (8). Only two of the episodes (7%) were caused by hepatitis A, and an additional 12 (40%) were caused by hepatitis B. The remaining 16 episodes (53%) were therefore non-A, non-B episodes, and none of these could be ascribed to either Epstein-Barr virus or cytomegalovirus infection. In single patients, there was more than one episode of non-A, non-B hepatitis, suggesting that there may be more than one non-A, non-B agent.

Short-incubation Non-A, Non-B Hepatitis

A short-incubation non-A, non-B post-transfusion hepatitis was also described. A nosocomial outbreak of short-incubation (22-37 days) hepatitis occurred in patients and donors of a bone marrow transplant unit in 1972. Secondary spread was prevented by immune serum globulin, suggesting that hepatitis A virus may have been the cause. However, a re-evaluation of stored sera demonstrated that there was no evidence of acute hepatitis A virus infection, nor was there evidence of Epstein-Barr virus infection as the cause of this outbreak. This study therefore suggested that a parenterally transmitted non-A, non-B agent may also cause short-incubation hepatitis. A transfusion related short-incubation hepatitis has also been observed in hemophiliac patients (10). There were nine episodes of hepatitis in 6 children, occurring between 4 and 19 days after factor VIII concentrate was infused. The interval between previous infusions and the episodes of hepatitis ranged between 14 months and 14 years in 5 of the 6 children, making long- incubation post-transfusion hepatitis extremely unlikely. In these children, there was no evidence of seroconversion for cytomegalovirus, E-B virus or hepatitis A virus infection related to the episodes of hepatitis. The explanation for these findings appeared to be that a second, transmissible non-A, non-B agent may be responsible for short-incubation post-transfusion hepatitis.

Sporadic Non-A, Non-B Hepatitis

Non-A, non-B hepatitis agents also appeared to be responsible for viral hepatitis in patients with no history of parenteral exposure, either from transfusions or percutaneous injection. Of 103 patients with acute viral hepatitis in Costa Rica, 12 had neither hepatitis A nor hepatitis B by serologic testing (11). None of the 12 had a history of parenteral exposure. Of interest, four members of one family were infected. In California, evaluation of 45 episodes of sporadic hepatitis that were negative for hepatitis B surface antigen demonstrated that 2 of the 45 episodes were the result of hepatitis B virus infection since there was seroconversion for antibody to the hepatitis B core antigen (12). Hepatitis A virus infection accounted for 20 of the 45 episodes, and another 20 were concluded to result from a non-A, non-B agent. Therefore, as early as 1977, there was evidence that a non-A, non-B agent was responsible for both sporadic and transfusion- associated hepatitis in the United States.

The advent of testing for hepatitis A virus infection also made it clear that non-A, non-B hepatitis could occur in epidemics. A common-source of waterborne epidemic in India was evaluated to determine the (viral) etiology because some features were not typical of epidemic hepatitis A (13). For example, there was a high mortality (20%) in pregnant women. There was no evidence of either hepatitis A or hepatitis B virus as the cause of this epidemic. Clinically, epidemic non-A, non-B hepatitis resembled hepatitis A more than hepatitis B, with similarities in mode of spread, length of incubation, clinical features and biochemical tests. Furthermore, like hepatitis A, there was no evidence of chronic disease following epidemic non-A, non-B

hepatitis. Thus, there appeared to be another human hepatitis virus responsible for epidemic non-A, non-B hepatitis that was distinct from post-transfusion non-A, non-B type.

SEARCHING FOR HEPATITIS C

Representative Articles Published in the Lancet 1978-1984

Shirachi et al, 1978:	Hepatitis "C" antigen in non-A, non-B post-transfusion hepatitis
Kabiri et al, 1979:	Antigen-antibody system associated with non-A, non-B hepatitis detected by indirect immunofluorescence
Vitvitski et al, 1979:	Detection of virus-associated antigen in serum and liver of patients with non-A non-B hepatitis
Suh et al, 1981:	Specificity of an immunoprecipitin test for non-A, non-B hepatitis
Spertini and Frei, 1982:	Demonstration of a single antigen-antibody system in 26 patients with non-A, non-B viral hepatitis
Seto et al, 1984:	Detection of reverse transcriptase activity in association with the non-A, non-B hepatitis agent(s)

Although clearly demonstrated clinically, the non-A, non-B agents proved to be elusive to detect and identify. Between 1978 and 1984 there were at least 30 reports of serologic tests for non-A, non-B hepatitis antigens (14-19). However, none was sensitive, specific and reproducible. All were unable to diagnosed coded specimens and many appeared to detect a rheumatoid factor-like activity rather than the non-A, non-B agent. Indeed, this series of failures led to a number of investigators suggesting that the agent causing non-A, non-B hepatitis may be similar or related to the hepatitis B virus (reviewed in reference 20).

This hypothesis was based on a number of observations. First, the clinical and epidemiologic features of hepatitis B and post-transfusion non-A, non-B hepatitis were similar. Furthermore, analysis of donor blood in a number of studies had demonstrated that the transmission of post-transfusion hepatitis was increased in blood units that were positive for antibodies to hepatitis B core antigen and hepatitis B surface antigen. Finally, some investigators detected non-conventional markers of hepatitis B virus infection (using molecular hybridization and monoclonal antibody techniques) in non-A, non-B samples that were negative by conventional assays.

As pointed out by Feinstone and Hoofnagle in their editorial in 1984, however, there was lack of evidence supporting an HBV origin of non-A, non-B hepatitis. Thus, in all well documented studies, hepatitis B infection could be diagnosed by the appearance of conventional HBV markers and long-term follow-up demonstrated the persistence of these conventional markers in sera. In addition, there was no delayed development of conventional HBV markers in patients with non-A, non-B hepatitis. Furthermore, experimental transmission

studies demonstrated that non-A, non-B hepatitis developed in chimpanzees without HBV markers. Finally, there was no immunity to non-A, non-B hepatitis in chimpanzees who were immune to hepatitis B virus infection. The conclusion of Feinstone and Hoofnagle was that the agent responsible for non-A, non-B hepatitis was not likely to be a variant of hepatitis B.

Despite the difficulties in isolating single viruses, by ten years after coining of the term "non-A, non-B hepatitis", it became apparent that there were at least **three** viruses responsible for the diagnostic entity (reviewed in reference 21). One, now identified as the hepatitis C virus, caused post-transfusion hepatitis in 5-15% of recipients of 1-5 units of blood. This virus was inactivated by formalin, heating at 60° for 10 hours, and by chloroform. The second non-A, non-B hepatitis virus, now referred to as hepatitis E, was the causative agent of epidemic water-borne non-A, non-B hepatitis in India, Pakistan, Nepal, the Soviet Union and Mexico, but only demonstrated in the US in travellers from these areas. The third agent is still theoretic. It may be the causative agent of short- incubation post-transfusion hepatitis, transmitted in factor VIII and factor IV concentrates and in intravenous immunoglobulin. Clearly the distinction between this agent and hepatitis C has not yet been made, but it is implied by the close association between the hepatitis C virus and classic long-incubation post-transfusion hepatitis.

Hepatitis E

The existence of an epidemic form of non-A, non-B hepatitis was first demonstrated in 1980, as noted above (13). By 1987, Bradley and coworkers at the Centers for Disease Control had established an animal model for enteric non-A, non-B hepatitis by serial passage of the disease in cynomolgus macaques and tamarins and recovery of a disease-associated 27-34 nm virus-like particle (22). Their studies demonstrated an etiologic link to the virus-like particles initially, and in follow-up investigations, they identified a virus-associated antigen in experimentally infected cynomolgus monkeys (23). A fluorescent antibody blocking test was developed using this system, and human sera from enterically transmitted non-A, non-B hepatitis outbreaks were demonstrated to be positive. Epidemic non-A, non-B hepatitis or enterically transmitted non-A, non-B hepatitis as it has been previously called, is now identified as hepatitis E. The viral agent appears to be a calicivirus, a family with similar characteristics to the picornaviridae which include *enterovirus type 72*, the hepatitis A virus. The genome of hepatitis E virus is a single-stranded, polyadenylated RNA of 8.5 kb. The other physicochemical properties of the hepatitis E virus are similar to those of some caliciviruses, and significantly different from those of picornaviruses, although a final classification has not been attempted.

Hepatitis E virus is one of the leading causes of acute viral hepatitis in young to middle aged adults in developing countries. One important feature of the hepatitis is its high mortality rate in infected pregnant women, where it approaches 20%. An endemic reservoir of hepatitis E virus infected persons is likely to be found in the countries were epidemics occur, and it may be responsible for cases of sporadic non-A, non-B hepatitis in these countries. In contrast, sporadic cases of acute non-transfusion transmitted non-A, non-B hepatitis in the US were

found to be serologically unrelated to those from well-characterized hepatitis E virus outbreaks (24). The only sporadic cases of acute non-A, non-B hepatitis that were seropositive for anti-HEV antigen antibody were observed in a Pakistani and an Indian national permanently living in Western countries (USA and Italy) after returning from visits to endemic areas. In less than 10 years since its initial description, the viral agent responsible for epidemic non-A, non-B hepatitis has been identified.

Hepatitis C

The search for the elusive non-A, non-B agent responsible for long-incubation post-transfusion hepatitis has finally ended. Investigators at the Centers for Disease Control and the Chiron Corporation collaborated for these studies (25,26). Human factor VIII concentrate that was implicated in non-A, non-A hepatitis was inoculated into a chimpanzee and plasma, from the chronic phase of non-A, non-B hepatitis in this animal, was then serially inoculated into another chimpanzee. An infectious plasma pool was obtained from the second chimpanzee containing at least 10^6 chimp infectious units/ml. After a short, low speed, initial centrifugation, the supernatant was ultracentrifuged to pellet a crude viral preparation. Nucleic acids were extracted from this crude viral pellet, and complementary DNAs were synthesized from both RNA and DNA in the viral pellet by reverse transcriptase using random oligonucleotide primers. The resultant cDNAs were cloned into λgt11 vector to generate a cDNA library. The cDNA library was immunoscreened with a 10^{-2} dilution of patient serum. The patient had chronic non-A, non-B hepatitis, was HBV negative and IgM anti-HAV negative. A single positive plaque, 5-1-1, was isolated after screening approximately one million clones. This positive plaque contained a 155 base pair cDNA insert that was the used as a hybridization probe. It recognized clone 81, containing a 353 base pair cDNA insert.

Southern blot analysis of human and chimpanzee DNA was undertaken using clone 81 as a probe (25). There was no hybridization to human DNA or chimpanzee DNA obtained from a chronic non-A, non-B hepatitis animal. These findings indicated that clone 81 was not derived from the host genome. Furthermore, the lack of hybridization to chronic non-A, non-B hepatitis chimpanzee DNA suggests that no DNA replication intermediates related to the sequences of the cDNA were present. Clone 81 was also hybridized to RNA obtained from chimpanzee liver. There was specific hybridization to infectious chimpanzee liver RNA, but no hybridization to uninfected chimpanzee liver RNA. The abundance of the RNA was calculated to be 0.0001% of total RNA in the liver.

Specific hybridization of clone 81 was also observed with total nucleic acids prepared from high titer infectious chimpanzee plasma (25). This hybridization was resistant to deoxyribonuclease digestion but sensitive to ribonuclease treatment. Together with the lack of hybridization of the clone to chimpanzee liver RNA, these results suggested that clone 81 was derived from exogenous RNA, associated with non-A, non-B hepatitis. The RNA in infectious plasma was single stranded since it hybridized to only one strand of the cDNA probe.

INITIAL DETECTION OF HEPATITIS C VIRUS

Human Factor VIII Concentrate Implicated in Non-A, Non-B Hepatitis

⇓

Inoculation into Chimpanzee 771

⇓

Plasma from Chronic Phase of Non-A, Non-B Hepatitis

⇓

Inoculation into Chimpanzee 910

⇓

Infectious Plasma Pool ($\geq 10^6$ chimp infectious units per ml)

⇓

Centrifugation 10,000*g* 20 min

⇓

Ultracentrifugation of Supernatant 104,000*g* 5 hrs

⇓

Extraction of Nucleic Acid from Crude Viral Pellet

⇓

Synthesis of cDNA from Both RNA and DNA by Reverse Transcriptase with Random Primers

⇓

Clone into λgt11

⇓

cDNA Library

⇓

Immunoscreened with 10^{-2} Dilution of Patient Serum

Chronic Non-A, Non-B Hepatitis, IgM anti-HAV negative, HBV negative

⇓

1 Positive Plaque 5-1-1 Isolated from $\sim 10^6$

⇓

155-bp cDNA Insert Used as Hybridization Probe

⇓

Clone 81 - 353-bp cDNA

INITIAL DETECTION OF HEPATITIS C VIRUS

Southern Blot Analysis of Human and Chimpanzee DNA with Clone 81

⇓

No Hybridization to Human DNA or Chronic NANB Hepatitis Chimpanzee DNA

∴ Clone 81 NOT Derived from Host Genome

and NO DNA Replication Intermediates Related to Sequences

Hybridization of Clone 81 to RNA from Chimpanzee Liver

⇓

Specific Hybridization to Infectious Chimpanzee Liver RNA

Abundance 0.00001% of Total RNA

No Hybridization to Uninfected Chimpanzee Liver RNA

On analysis of RNA separated by gel electrophoresis, the approximate size of the RNA recognized by clone 81 was 5,000-10,000 nucleotides.

Sequencing of the cDNAs indicated a continuous translational open reading frame (25). This open reading frame was expressed in *E.coli* as a fusion polypeptide with human superoxide dismutase. Immunoblot analysis was then performed using bacterial lysates and patient serum, with bound antibody being detected with [^{125}I]-labeled sheep antibody to human immunoglobulin. A specific reaction was obtained with the original patient serum used to immunoscreen and identify the initial clone 5-1-1. In addition, serum from 7 of 11 non-A, non-B hepatitis patients but none of 10 blood donor controls was also reactive. In four experimentally infected chimpanzees, there was seroconversion with plasma initially negative and then becoming positive after inoculation with putative virus. Thus, without knowledge of the specifics of the virus involved, viral nucleic acid was cloned from infectious plasma samples.

A recombinant-based assay for hepatitis C virus antibodies was developed using three overlapping clones isolated by means of the cDNA in the hepatitis C virus clone 5-1-1 (26). These clones had one common open reading frame that encoded part of a viral antigen associated with non-A, non-B hepatitis. The continuous open reading frame was reconstructed from these clones and then expressed in yeast, as a fusion polypeptide with human superoxide dismutase, to facilitate the efficient expression of foreign protein. In this way, a superoxide dismutase/hepatitis C virus polypeptide (C100-3) containing 363 viral amino acids was synthesized at high levels in recombinant yeast.

After solubilization and purification, C100-3 was used as antigen in a radioimmunoassay. The specificity and sensitivity of the assay was examined in a blind fashion using a panel of well-pedigreed and well characterized samples that had been widely accepted as a crucial test of the validity of putative specific assays for non-A, non-B hepatitis. Six of 7 samples of proven infectivity in chimpanzees were detected with the assay.

DETECTION OF HCV ANTIBODIES IN PROVEN INFECTIOUS BLOOD SAMPLES

Development of an Assay for Antibodies to a Human Non-A, Non-B Hepatitis Virus

Serum	Positive Results
Proven infectious in chimp:	
Chronic non-A, non-B post-transfusion hepatitis patients	3/3
Acute non-A, non-B post-transfusion hepatitis patient	0/1
Implicated blood donors	3/3
Unproven infectivity in chimp:	
Acute non-A, non-B post-transfusion hepatitis patient	0/1
Implicated blood donor (3 cases)	0/1
Pedigreed normal controls:	
Blood donors - at least 10 donations without recipient hepatitis	0/5
Disease controls:	
Alcoholic hepatitis	0/1
Primary biliary cirrhosis	0/1

Kuo et al, Science 1989; 244:362-364

The only proven infectious sample that was negative by the assay was obtained during the acute phase of post-transfusion hepatitis. Normal control samples, disease control samples and two samples of unproven infectivity in chimpanzees were also negative. Thus, an assay had been developed that appeared to be extremely specific and highly sensitive in detecting an antibody associated with post-transfusion, long-incubation non-A, non-B hepatitis.

Longitudinal studies were also carried out in 10 cases of chronic non-A, non-B post-transfusion hepatitis from the United States (26). All but two of the blood donations received by these patients were assayed for hepatitis C virus antibodies. There were 138 blood donations of apparent negativity as measured by radioimmunoassay. Blood donations were regarded as positive when the result was more than three standard deviations beyond the mean of negative samples, and 12 blood donations were thus identified. In 9 of the 10 cases of chronic non-A, non-B post-transfusion hepatitis, there had been a transfusion with either one or two positive units of blood. There was also seroconversion for anti-hepatitis C virus antibodies in each of these 9 cases when assayed after 6 months. At three months after the episode of post-transfusion hepatitis, the recipients were negative or only had passively carried over antibody. In one of the 10 cases of chronic post-transfusion hepatitis, no positive units were demonstrated. However, there was seroconversion after 12 months in this patient. Thus, a specific assay has been developed that measures circulating viral antibodies associated with post-transfusion non-A, non-B hepatitis, the hepatitis C virus.

DETECTION OF HCV ANTIBODIES IN NON-A, NON-B POST-TRANSFUSION HEPATITIS

Development of an Assay for Antibodies to a Human Non-A, Non-B Hepatitis Virus

Case	Donors	Positive Donors	Anti-HCV Assay: Recipients (months after transfusion)			
			0	3	6	12
1	18	1	-	-	+	+
2	18	2	-	-	+	+
3	13	1	-	-	+	+
4	18	0	-	-	-	+
5	16	1	-	-	+	+
6	11	2	-	+*	+	+
7	15	1	-	-	+	+
8	20	1	-	-	+	+
9	8†	1	-	+*	+	+
10	15	2	-	+*	+	+

† 6/8 donors assayed

* Passive transfer of antibody

Kuo et al, Science 1989; 244:362-364

THE HEPATITIS VIRUSES

In summary, there are at least 5 viruses or virus-like agents. responsible for hepatitis. They are:

1. The **hepatitis A virus**, enterovirus type 72 of the family of *picornaviridae*;
2. The **hepatitis B virus**, the prototype virus of the family *hepadnaviridae*;
3. The **hepatitis D virus**, a defective virus that requires hepatitis B virus infection for processing.

The non-A, non-A viruses thus far identified are:

4. The **hepatitis C virus**, most closely linked to the family of *flaviviridae* which includes the arboviruses of yellow fever and Dengue fever;
5. The **hepatitis E virus**, the cause of epidemic or enterically transmitted non-A, non-B hepatitis that probably belongs to the family of *caliciviridae*.

THE HEPATITIS VIRUSES

Virus		Family	Structure of Nucleic Acid
A		*Picornaviridae*	ss linear RNA, + sense; serves as mRNA; 3′ end polyadenylated; 5′ end covalently linked to protein; *Enterovirus type 72*
B		*Hepadnaviridae*	ds circular DNA with ss tail
C		? *Flaviviridae*	ss linear RNA, + sense; serves as mRNA; 3′ end not polyadenylated; 5′ end capped
D		? Viroid	ss circular RNA
E		? *Caliciviridae*	ss linear RNA, + sense; serves as mRNA; 3′ end polyadenylated; 5′ end covalently linked to protein
	100nm		

CLINICAL FEATURES:

Transmission ofHepatitis C

Blood and single unit blood products have been implicated in the transmission of both short- and long-incubation non-A, non-B post-transfusion hepatitis (1,2,9). Similarly, factor VIII and factor IX concentrates have also been associated with short- and long- incubation post-transfusion hepatitis (6,10). Suspected sources identified in U.S. patients include IV drug users as well as blood transfusions. These observations suggest that groups at high risk for parenteral transmission of non-A, non-B hepatitis would be likely sources of hepatitis C virus transmission. Recent studies from Germany and Spain have demonstrated that the majority of patients with hemophilia have evidence of hepatitis C virus exposure, although it was much less common in hemodialysis patients (27,28). Intravenous drug users, another high risk group, also demonstrated a high rate of positivity. In contrast, homosexual men and female contacts of drug users were infrequently positive (28). The question of non-parenteral transmission of hepatitis C virus has not been completely answered as yet. Intrafamily spread of non-A, non-B hepatitis has been clearly documented (11). In addition, a recent study on the importance of heterosexual activity in the transmission of hepatitis suggested that non-A, non-B hepatitis as well as hepatitis B may be spread in such a manner (29). Preliminary studies from the NIH agree with those from Spain (28) that there is thus far little evidence of sexual spread of hepatitis C virus when measured by the current antibody test, with none of 62 familial and spousal contacts of HCV-positive patients having anti-HCV antibody (Hoofnagle, JH, personal communication). In addition, vertical or neonatal transmission of non-A, non-B hepatitis has not been well-documented in animal studies (30), or in clinical cases.

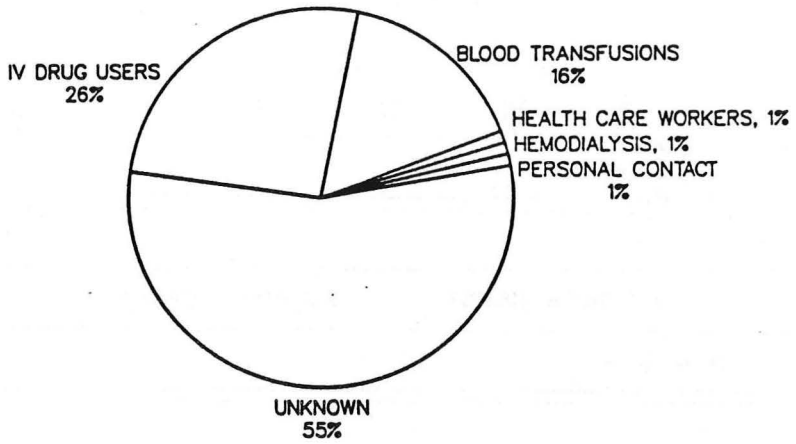
ANTIBODY TO HEPATITIS C VIRUS IN HIGH-RISK GROUPS

Country	Hemophilia		Hemodialysis		IV drug use	
	n	%	n	%	n	%
Germany	165/211	79%	8/146	6%	24/50	48%
Spain	62/97	64%	8/42	20%	59/83	70%

Roggendorf et al, Lancet 1989; ii:325-326
Esteban et al, Lancet 1989, ii:294-297

Another unanswered question is whether or not hepatitis C virus accounts for short-incubation post-transfusion hepatitis. The current data indicate that it is associated with long-incubation post-transfusion hepatitis, however, no definitive studies of short-incubation cases have been undertaken. Factor VIII, factor IX and intravenous immunoglobulin preparations have all been shown to transmit short-incubation non-A, non-B post-transfusion hepatitis (6,10,31). The current presumption is that if a viral agent is responsible for this disease, then it is an additional, as yet unidentified, virus.

Figure 1



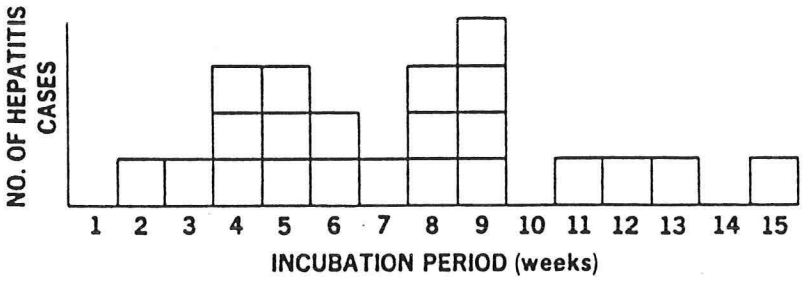
Reported Sources of Non-A, Non-B Hepatitis U.S.A. 1981-1985

From: Hadler SC and Margolis HS, Viral Hepatitis *in* Viral Infections of Humans, AE Evans ed 1989

Clinical Course

The clinical course of acute non-A, non-B post-transfusion hepatitis, one disease apparently associated with the hepatitis C virus, usually follows a fairly typical course for viral hepatitis (reviewed in reference 32). A prodrome of non-specific and gastrointestinal symptoms is followed by the onset of jaundice and eventual improvement of symptoms in most patients.

Figure 2

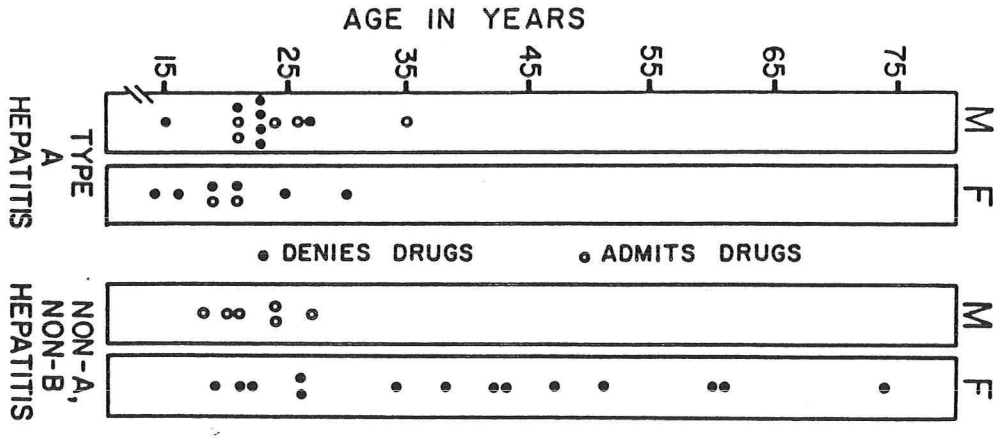


Incubation Periods of 22 cases of Transfusion-Associated HB_s Ag-Negative Hepatitis

From: Feinstone et al, New Engl J Med 1975; 292:767-770

The incubation period, from the time of transfusion to the development of symptoms or elevated aminotransferases, has a wide range, from 2-26 weeks with a mean of 7.8 weeks in the studies reviewed by Dienstag (32). In contrast, post-transfusion hepatitis B had a mean incubation period of 11.8 weeks in these studies.

Figure 3



Age and Sex Distribution of 20 Cases of Sporadic Urban Type A and 20 Cases of Non-A, Non-B Hepatitis

From: Dienstag et al, Ann Intern Med 1977; 87:1-6

When compared with post-transfusion hepatitis B, the clinical course of acute non-A, non-B hepatitis is generally less severe and there are more patients who are asymptomatic than symptomatic in many studies. Similarly, acute sporadic non-A, non-B hepatitis is generally less severe than either hepatitis A virus or hepatitis B virus infections. Peak levels of alanine aminotransferase tend to be higher in both hepatitis B virus and hepatitis A virus infections than in non-A, non-B hepatitis. In contrast, peak bilirubin levels tend to be higher in hepatitis B virus infections than both non-A, non-B and hepatitis A virus infections. Fulminant hepatitis is rare in post-transfusion hepatitis with no deaths in the 515 cases reviewed by Dienstag. The role of hepatitis C virus infection in non-A, non-B fulminant hepatitis remains to be investigated (see below).

OCCURRENCE OF NON-A, NON-B HEPATITIS

Comparison with Hepatitis A and Hepatitis B

Country	Hepatitis A		Hepatitis B + D		Non-A, Non-B	
	n	%	n	%	n	%
U.K. 1981 (n=368)*	220	60	103	28	48	13
U.K. 1983 (n=172)	88	51	58	34	22	13
Sweden 1981 (n=280)	84	30	129	46	63	24
Denmark 1979 (n=115)*	69	60	42	37	7	6
Greece 1988 (n=993)					134	14
Egypt 1986 (n=295)	8	3	115+19	45	146	50
Sudan 1987 (n=119)	1	1	15+15	26	88	74
India 1987 (n=684)	47	7	282	41	355†	52
Taiwan 1988 (n=101)	8	8	36	36	57	56
New Zealand 1982 (n=94)*	31	33	36	38	25	26
Australia 1984 (n=5477)	2174	40	2253	41	1050	19
U.S.A. 1984 (n=947)	328	41	261	33	211	26
U.S.A 1987 (n=80)	15	19	27	34	38	47
U.S.A. Dallas (n=469)	88	19	237	51	144	30

* includes patients with both hepatitis A and hepatitis B † 70/355 were chronic HBV carriers

Note: USA 1984 - passive reporting
USA 1987 - active reporting
(↑ (50%) HBV particularly homosexual men; ↑↑↑ (138%) NANB PTH 1987 cf 1984)

From references 32-44

The contribution of non-A, non-B hepatitis to cases of acute viral hepatitis varies depending on geographic location as well as source of patients. In Africa and Asia, non-A, non-B hepatitis accounts for more than half of the cases; however, many of these may be due to the epidemic form of non-A, non-B hepatitis, hepatitis E. In contrast, in Europe, North America and Australasia, non-A, non-B hepatitis contributes approximately 20-25% of cases.

OUTCOME OF VIRAL HEPATITIS

Fairfield Infectious Diseases Hospital 1971-1983

	Hepatitis A		Hepatitis B		Non-A, Non-B	
	n	%	n	%	n	%
Admissions (n=5477)	2174	40	2253	41	1050	19
Deaths (n=46)	3	7	19	41	24	52
Post-transfusion hepatitis (n=55)			48/2253	2	7/1050	0.7
Deaths (PTH n=10)			9/19	47	1/24	4.2

McNeil et al, Med J Aust 1984; 141:637-640

A retrospective analysis of over 5,000 patients hospitalized in Victoria identified 20% as being neither A nor B virus related (37). Only 7 of the non-A, non-B hepatitis cases were the result of a transfusion. There were many more cases of post-transfusion hepatitis B requiring hospitalization, further emphasizing the increased severity of hepatitis B compared with non-A, non-B in the post-transfusion setting. Indeed, hepatitis B virus infection accounted for 19 of 46 (41%) deaths, and 9 of these were transfusion-related. Thus, post-transfusion hepatitis B accounted for 2% of the admissions with hepatitis B, but 47% of the deaths with hepatitis B. Sporadic non-A, non-B hepatitis was disproportionately represented among those with fatal outcome, accounting for 19% of all admissions and 50% of all deaths.

Thus far, few studies have been published reporting the incidence of hepatitis C virus as a cause of acute hepatitis. One of the major problems is the delay in development of positivity with the currently available research tests. In both post-transfusion hepatitis and sporadic hepatitis, the antibody may not be detected until a mean of approximately 23 weeks after transfusion, although a preliminary report from California suggests that seroconversion in acute hepatitis C may occur earlier in many patients (46). In the future, direct measurement of the virus in plasma, probably using a polymerase chain reaction and/or measurement of an early-appearing antibody may allow differentiation of cases of acute non-A, non-B hepatitis due to the hepatitis C virus.

HEPATITIS C VIRUS AS A CAUSE OF ACUTE NON-A, NON-B HEPATITIS

Country	Source	Positive	
		n	%
Japan	Sporadic	2/13	15
Netherlands	Post-transfusion	4/9	44
Germany	Post-transfusion	4/20	20
U.S.A.*	Post-transfusion	3/15	20
	Drug users	20/43	47
	Sporadic	7/40	18

* Mean time to seroconversion 9 wks (range 3-80 wks)

Kuo et al, Science 1989; 244:362-364 *Roggendorf et al, Lancet 1989; ii:325-326* *McHutchison et al, Hepatology 1989; in press*

Fulminant Hepatitis

The contribution of HCV to fulminant hepatitis has not been clarified. In the U.S., non--A, non-B viral hepatitis may account for about 40% of cases but has a particularly dismal prognosis, contributing disproportionately to deaths. Current management of this complication includes early consideration for hepatic transplantation. Analysis of the role of HCV will be difficult until alternative methods, replacing the current late-appearing antibody tests, are available.

ETIOLOGY OF FULMINANT VIRAL HEPATITIS

Country	Hepatitis A		Hepatitis B		Non-A, Non-B	
	n	%	n	%	n	%
India 1987 (n=131)*	9	7	16	12	106	81
Deaths (n=79)	5/9	56	12/16	80	62/106	58
France 1987 (n=218)					61	28
Deaths					53/61	87
U.S.A. (n=52)†	1/52	2	32/52	60	20/52	38
Deaths (n=42)	0/1	0	23/32	72	19/20	95

* Note 1: 41/106 non-A,non-B hepatitis cases were in HBV chronic carriers

† Note 2: Apparent co-infection with HAV, HBV & HDV in 1 patient

A specific subset of patients with apparent fulminant, acute non-A, non-B viral hepatitis are those with underlying chronic HBV infection (47). Of 377 cases of fulminant HBsAg-positive hepatitis recently reviewed, 71 (19%) were considered to be the result of non-A, non-B infection in a chronic HBV carrier. The mortality in this group was nearly 80%.

Histology - Acute Hepatitis

The histologic features of acute non-A, non-B viral hepatitis are similar to those of the other viral hepatitides (32). Unusual features that have been reported include microvesicular steatosis, eosinophilic changes and sinusoidal cell activation.

Differential Diagnosis of Acute Hepatitis C Infection

The major causes of acute hepatitis are viruses and drugs. In addition, one must always consider an acute hepatitic response occurring in cholangitis or ischemia, an acute presentation of an underlying metabolic disorder such as Wilson's disease, or an acute presentation of autoimmune chronic hepatitis. In differentiating the cause of acute viral hepatitis, serologic results are of greatest importance. Hepatitis A virus infection is diagnosed by the presence of IgM antibody to HAV, acute hepatitis B virus infection is diagnosed by the presence of IgM antibody to hepatitis B core antigen, and in the future it is expected that tests will be available to allow the diagnosis of hepatitis E virus infection as well as hepatitis C virus infection in the acute phase. A fluorescence antibody blocking test is currently being used in research studies of hepatitis E virus infection (24). In 89 cases from outbreaks of non-A, non-B hepatitis in Asia, Africa and Mexico, 78 (88%) were positive for anti-HEV antigen antibody by this test. In contrast, none of 45 cases of sporadic non-A, non-B hepatitis in the US were positive.

Diagnostic difficulties may arise when acute hepatitis occurs in a previously unrecognized asymptomatic carrier of hepatitis B surface antigen. A recent study described 76 such patients, and 11 (14%) were considered to have non-A, non-B hepatitis (48). None had evidence of acute hepatitis A virus infection, 25 were diagnosed as having acute hepatitis D virus (delta) infection, and 40 patients were considered to have reactivation of HBV or an exacerbation associated with seroconversion to anti-HBe antibody. The occurrence of acute hepatitis complicating chronic HBV infection may also lead to fulminant hepatitis. In a recent analysis of 377 cases of hepatitis B surface antigen positive fulminant hepatitis, only 195 (52%) were IgM anti hepatitis B core positive, and therefore diagnosed as acute hepatitis B virus infection (47). An additional 111 (29%) had delta infection complicating either acute or chronic hepatitis B virus infection. Serologic markers suggested that 71 of the 377 patients (19%) had non-A, non-B hepatitis. There was a wide geographic variation in the incidence of non-A, non-B infection in chronic HBV carriers with particularly high rates in countries where epidemic non-A, non-B hepatitis is being described, such as India, Egypt and the Central African Republic.

Aplastic Anemia and Non-A, Non-B Hepatitis

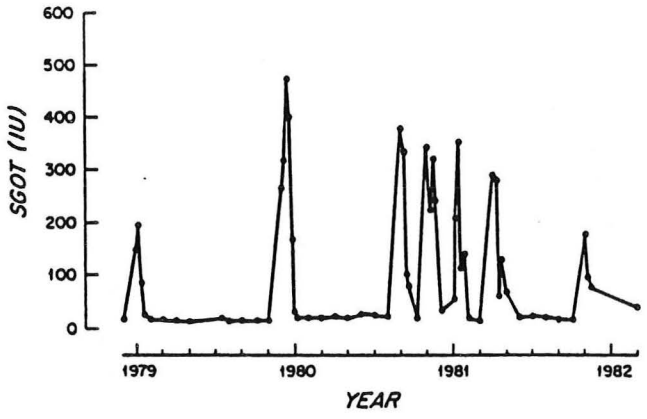
An association between viral hepatitis and aplastic anemia has been noted by a number of investigators. Retrospective analysis has revealed that in most of these cases the patients are negative for markers of hepatitis B virus infection or recent hepatitis A virus infection (49,50). Furthermore, aplastic anemia complicating orthotopic liver transplantation for non-A, non-B hepatitis has been reported recently (51). Of 32 patients undergoing liver transplantation for acute non-A, non-B hepatitis, 9 (28%) developed aplastic anemia 1-7 weeks postoperatively. In contrast, of 1,463 patients transplanted for other conditions, none developed aplastic anemia. In follow-up, 4 of the 9 patients died due to infections, and two had at least partially recovered 1-2 years after the onset. This association has not been confirmed by another center where 21 patients transplanted for fulminant or subacute non-A, non-B hepatitis were followed, and none developed aplastic anemia (52).

The possibility that non-A, non-B hepatitis viruses may inhibit human hemopoiesis has been investigated in vitro (53). Acute phase sera obtained from three chimpanzees inoculated with a putative non-A, non-B agent inhibited in vitro growth of human erythroid and granulocyte macrophage progenitor cells. However, similar inhibition was seen with hepatitis A virus and hepatitis B virus infected chimpanzees, and no clinical association of these viruses with aplastic anemia has been reported. It remains an intriguing possibility that one of the non-A, non-B hepatitis viruses may be responsible for aplastic anemia in a small subset of infected patients.

Chronic Hepatitis C Infection

The classic biochemical pattern in post-transfusion hepatitis is that of a waxing and waning course with intermittent normalization of aminotransferases (reviewed in reference 32). This pattern is rarely seen in other viral hepatitides and thus far appears to closely correlate with chronic hepatitis C virus infection.

Figure 4



Serial Determinations of SGOT in Chronic Non-A, Non-B Hepatitis

From: Dienstag, Gastroenterology 1983; 85:439-462

HEPATITIS C VIRUS AS A CAUSE OF CHRONIC NON-A, NON-B HEPATITIS

Country	Source	Positive	
		n	%
U.S.	Post-transfusion	17/24	71
	Sporadic	34/59	58
Italy	Post-transfusion	27/32	84
Japan	Sporadic	18/23	78
Germany	Post-transfusion	44/56	79
	Sporadic	47/65	72
Spain	Post-transfusion	46/54	85
U.S.	Post-transfusion	18/25	72
	Drug users	19/22	86
	Sporadic	5/15	33

Kuo et al, Science 1989; 244:362-364
Esteban et al, Lancet 1989, ii:294-297
Roggendorf et al, Lancet 1989; ii:325-326
McHutchison et al, Hepatology 1989; in press

Chronic infection appears to occur in 40-60% of patients with post-transfusion hepatitis, and since the majority of these may be accounted for by hepatitis C virus infection, the data indicate that chronic hepatitis C virus infection is likely to occur in 40% of patients infected by a transfusion.

OUTCOME OF ACUTE NON-A, NON-B HEPATITIS

Author	Source	Acute Hepatitis (Resolved)		Chronic Hepatitis	
		n	%	n	%
Koretz 1976*	Post-transfusion	18/47	38	29/47	62
Knodell 1977*	Post-transfusion	34/44	77	10/44	23
Gailbraith 1979	Hemodialysis Unit	21/29	72	8/29	28
Realdi 1982	Post-transfusion			13/21	62
Kryger 1983	Sporadic			15/94	16
Tassopoulos 1988	Sporadic	39/87	45	48/87	55
Feinman 1988*	Post-transfusion	34/53	64	19/53	36
Mean of 3 studies*	**Post-transfusion**	**86/144**	**60**	**58/144**	**40**

From references 44, 54-59

Of importance, 10-20% of patients with chronic non-A, non-B hepatitis progressed to cirrhosis. In contrast, many fewer patients with the sporadic form of non-A, non-B hepatitis and without percutaneous transmission progressed to chronic liver disease, with some estimates as low as 10%.

Histology - Chronic Hepatitis

Of 189 patients with chronic non-A, non-B viral hepatitis reviewed by Dienstag, 9 (5%) had non-specific or slowly resolving hepatitis, 72 (39%) had chronic persistent hepatitis or chronic lobular hepatitis, 74 (40%) had chronic active hepatitis, and 34 (18%) had cirrhosis. A number of studies have followed patients prospectively and documented evidence of progression to cirrhosis (54,55,57,60-63).

HISTOLOGIC FINDINGS IN CHRONIC NON-A, NON-B HEPATITIS

Author	Chronic Persistent Hepatitis		Chronic Active Hepatitis		Cirrhosis	
	n	%	n	%	n	%
Koretz 1976	2/15	13	9/15	60		
Knodell 1977	1/10	10	8/10	80	1/10	10
Realdi 1982	3/13	23	5/13	38	5/13	38
Wejstål 1987	24/49	49	12/49	24	13/49	27
Wejstål 1988	40/47	85	4/47	9	3/47	6
Mattsson 1988	14/37	38	10/37	27	12/37	33
Jové 1988	37/60	62	23/60	38		
TOTAL	**121/231**	**52**	**71/231**	**31**	**34/216**	**16**

From references 54, 55, 57, 60-63

However, this response has not been uniform, with a non-progressive course of non-A, non-B chronic hepatitis being observed in multi-transfused hemophiliacs (64). Thus, of 11 patients followed for 6 years with a liver biopsy every three years, 6 had either chronic persistent hepatitis which did not change, or merely changed to chronic lobular hepatitis, and 4 patients with chronic active hepatitis on initial biopsy demonstrated spontaneous improvement. This latter response, however, has not been seen in other studies.

HISTOLOGIC FINDINGS IN CHRONIC NON-A, NON-B HEPATITIS

Effect of Source of Infection and Age

Author	Source	Mean Age	Chronic Persistent Hepatitis		Chronic Active Hepatitis	
			n	%	n	%
Jové (Spain)	Post-transfusion	38	16/30	53	14/30	47
	Sporadic	31	18/25	72	7/25	28
Mattsson (Sweden)	Post-transfusion	54	15/37	41	22/37	59
	Drug use	26	20/33	61	13/33	39
	Sporadic	32	11/22	59	9/22	41

Jové et al, Liver 1988; 8:42-47 Mattsson et al, liver 1989; 9:120-127

Many fewer studies have reported on the prognosis of non-transfusion associated non-A, non-B hepatitis. In one such study, non-A, non-B hepatitis accounted for 157 (15%) of 1020 patients with biopsy verified acute hepatitis (58). Drug addiction was reported in 69 (40%) of the patients, but only 2 had hepatitis associated with blood transfusions. Follow-up liver biopsies were performed in 94 of the 157 patients, and 15 of the 94 had evidence of chronic liver disease, a much lower proportion than reported in studies of transfusion-associated non-A, non-B hepatitis. In the patients with chronic liver disease in the study, there was a predominance of elderly women with autoantibodies and no known exposure who developed chronic liver disease.

A similar investigation carried out in Greece followed 134 patients with acute non-A, non-B hepatitis (44). During the follow-up period, one patient died of aplastic anemia, and another of fulminant hepatitis. Of 87 patients assessed at 6 months, 48 (55%) had developed chronic hepatitis. A similar proportion (35 of 63, or 55%) had chronic hepatitis when assessed after 9-21 months. In this study, parenteral drug use was associated with chronicity.

A comparison study of 92 patients with biopsy proven chronic non-A, non-B hepatitis has suggested that age may be more important than route of infection in this group of patients (65). In keeping with other studies of patients with post-transfusion hepatitis, 22 of 37 (59%) had chronic active liver disease, and 46% cirrhosis. The mean age of this group was 54 years. In contrast, drug users and patients with sporadic chronic non-A, non-B hepatitis had significantly lower incidences of chronic active liver disease and cirrhosis, and the mean ages of these groups were 26 years and 32 years, respectively.

Autoimmune Liver Disease and Hepatitis C

The relationship between viral infection and autoantibody patterns in acute and chronic liver disease has been studied by a number of investigators (28,66,67). The finding of rheumatoid factor-like activity in a number of patients with post-transfusion hepatitis complicated development of antigen-antibody tests in earlier studies searching for hepatitis C (reviewed in reference 20).

When investigated retrospectively in 44 patients with non-A, non-B hepatitis, anti-nuclear antibodies were positive in 8 chronic sera and anti-smooth muscle antibodies positive in 10 chronic sera (66). Furthermore, when 34 Spanish patients with autoimmune chronic active hepatitis were examined, 15 of them (44%) had evidence of hepatitis C virus infection (28). In contrast, 19 US patients with autoimmune hepatitis, all positive for anti-nuclear and anti-smooth muscle antibodies, have been investigated for a possible association with hepatitis C virus infection, and only one of the patients, who had also received a blood transfusion, was positive for anti-hepatitis C virus antibody (67). Additional studies will be required to answer the question of the association between hepatitis C virus infection and autoimmune liver disease.

Hepatocellular Carcinoma

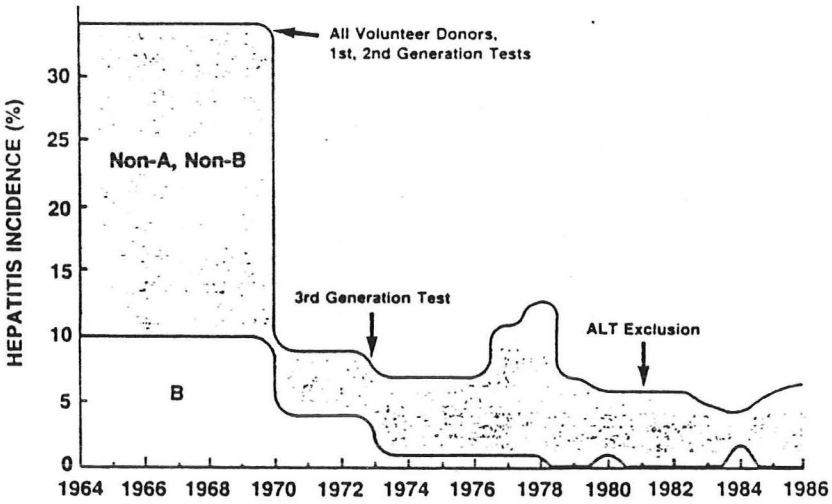
Hepatocellular carcinoma may complicate cirrhosis following non-A, non-B hepatitis. However, the association between hepatocellular carcinoma and hepatitis C virus infection has not yet been clarified. A number of studies suggest that there may be a close association. Thus, in 36 patients with non-B, non-B hepatitis, liver cell dysplasia was found in 17 of 40 specimens (43%), and three patients had hepatocellular carcinoma (68).

In Japan, where hepatocellular carcinoma is more common than in the US, many of the cases are associated not with hepatitis B virus infection but with non-A, non-B hepatitis (69). In a recent study of 319 patients with hepatocellular carcinoma, 243 of the patients were hepatitis B surface antigen negative, and of these 218 had evidence of chronic liver disease. Analysis of hepatic HBV-DNA was carried out on the subset of 79 patients in order to determine whether hepatitis B virus infection may have contributed to the development of hepatocellular carcinoma in the hepatitis B surface antigen negative cases. Hepatitis B virus DNA was detected in 21 of 26 hepatitis B surface antigen seropositive cases, but only in 4 of 53 hepatitis B surface antigen seronegative cases. These data suggest that hepatitis B virus infection does not significantly contribute to the development of hepatocellular carcinoma in non-A, non-B hepatitis in Japan. Preliminary reports, as yet unconfirmed, suggest that many of these patients have evidence of hepatitis C virus infection.

PREVENTION OF HEPATITIS C VIRUS INFECTION

The incidence of post-transfusion hepatitis has decreased remarkably since the introduction of testing for the hepatitis B surface antigen in the early 1970s (reviewed in reference 70).

Figure 5



Annualized Incidence of Transfusion-associated Hepatitis N.I.H.

From: Alter HA, *in* Transfusion-transmitted Viral Diseases, SB Moore ed 1987

CHANGING INCIDENCE OF POST-TRANSFUSION HEPATITIS

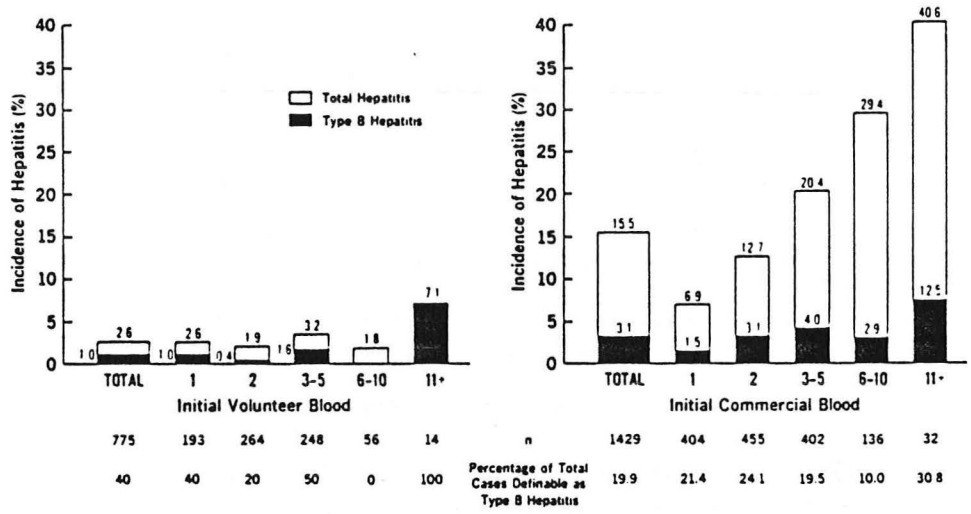
Comparison with Introduction of Screening Tests

Year	Screening Test	Incidence		
		Study	Rate	
			/1000 units	% patients
1970	HBsAg (counterelectrophoresis)	NIH	3.7	7.9
1975	HBsAg (radioimmunoassay)	NIH	6.4	11.1
1979		Sweden	12.0	19.0
		Australia	3.8	2.1
1981		NIH	10.7	12.7
1983	Voluntary AIDS	Italy	19.9	14.2
1985	HIV testing	Canada		9.2
1986	Anti-HBc testing	Spain		10.7
1986	ALT testing	Sweden	4.0	4.4

From references 59, 71-76

The development of improved sensitivity of detection of HBV infection with the advent of third generation tests such as radioimmunoassay further decreased the incidence of post-transfusion hepatitis B. At approximately the same time, the recognition that commercial blood was associated with a much higher risk of post-transfusion hepatitis, and subsequent changing to an all volunteer source, also considerably decreased the incidence. However, even the introduction of extremely sensitive assays for hepatitis B surface antigen did not completely prevent post-transfusion hepatitis B and had no effect on the majority of post-transfusion hepatitis, which was non-A, non-B.

Figure 6



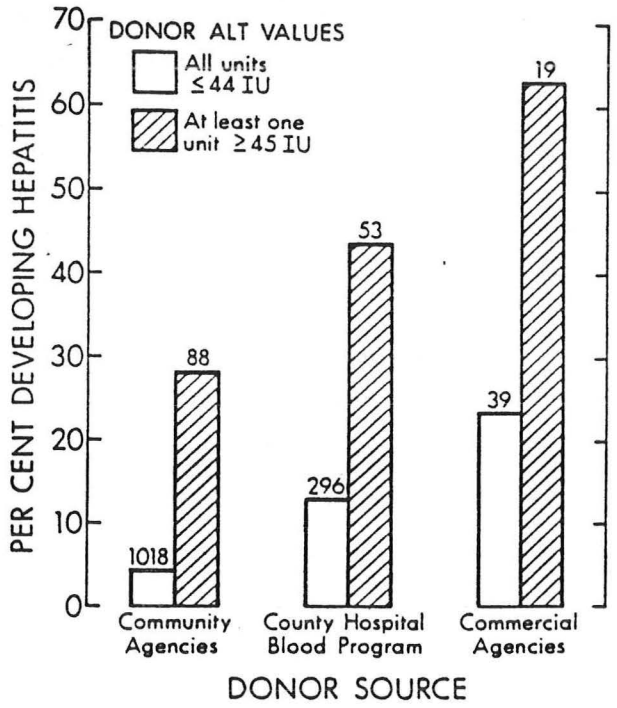
Incidence of Post-transfusion Hepatitis According to Source and Number of Administered Transfusions

From: Seeff et al, Gastroenterology 1977; 72:111-121

Prospective studies, the Transfusion-Transmitted Viruses (TTV) study and one conducted at the NIH clinical centers, reported that there was an increased incidence of post-transfusion hepatitis in recipients of donor blood having elevated levels of alanine aminotransferase (75,77). Furthermore, in the Transfusion-Transmitted Viruses study, there was an increased incidence of post-transfusion hepatitis in recipients of hepatitis B surface antigen negative donor blood having evidence of hepatitis B virus exposure (anti-HBc positivity), and this was also confirmed in the NIH study (78,79). It was proposed that these tests may be used as surrogate tests for non-A, non-B post-transfusion hepatitis until a definitive assay was available.

The problems with these observations were that the association with non-A, non-B post-transfusion hepatitis may have been artefactual, that two different carrier populations were detected by the test since the amount of overlap was relatively small, and unless two different viruses were being detected, the possibility of artefact was once again suggested. Furthermore, donor losses may have exceeded 7.5% if both tests were used to exclude donors. Additionally, 80% of anti-hepatitis B core positive donors were never implicated in the transmission of post-transfusion hepatitis, and donor units would thus be lost needlessly.

Figure 7



Relation between Highest Donor ALT Level and Incidence of Non-A, Non-B Hepatitis According to Donor Source

From: Aach et al, New Engl J Med 1981; 304:989-994

Theoretically, the introduction of screening for anti-HBV would reduce the incidence of post-transfusion hepatitis by 21-35%. The exact estimation of the effectiveness of such a screening procedure is complicated by the fact that in these two studies where patients were followed prospectively for the onset of post-transfusion hepatitis, a control group was similarly followed in parallel, and had a small but significant rate of hepatitis in the absence of transfusion.

ETIOLOGY OF HEPATITIS

Comparison of Transfused and Control Subjects in Clinical Trials

Study	Hepatitis	NANB Hepatitis	Other
Transfusion Recipients			
NIH	68/729	45/68	CMV 10 HBV 13
TTV	71/1022	65/71	HBV 6
Untransfused Controls			
NIH	1/203	1/1	
TTV	50/1588	46/50	HAV 3 HBV 1

Modified from Aach, 1987

RELATIVE RISK OF NON-A, NON-B HEPATITIS

Positive Screen	Non-A, Non-B Post-transfusion Hepatitis		Relative Risk
	n	%	
Nil	49/874	5.6%	1.0
Anti-HBc only	18/164	11.0%	2.1
ALT ≥45 only	20/79	25.3%	5.8
Both positive, different units	5/15	33.3%	8.7
Both positive, same units	14/19	73.7%	44.0

Modified from Hollinger, 1987

On the other hand, the relative risk of non-A, non-B post-transfusion hepatitis in the Transfusion-Transmitted Viruses study increased greatly when anti-hepatitis B core antigen, or an elevated ALT or both were present. The outcome of these observations was the introduction of screening for elevated ALT in 1986 and screening with antibody to hepatitis B core antigen in 1986-1987. In some countries it is being concluded that screening with such surrogate tests is not justified because of the very low incidence of donors with increased ALT (2.4% in the UK), and donors who are anti-HBc positive (2% in the UK) and the finding of no overlap in the groups (82).

In other countries, the problem of screening with antibody to hepatitis B core antigen is one of a high positive rate such as found in Spain, where 17.3% of the donor population was positive, coupled with the lack of clear association between receiving anti-HBc positive units and the development of post-transfusion hepatitis (76). Of 112 cardiac surgery patients in Spain, 12 (10.7%) developed non-A, non-B post-transfusion hepatitis. In 63 patients receiving at least one anti-HBc positive unit, the rate was 5 out of 36 (7.9%) whereas in 49 patients receiving no anti-HBc positive units, the rate was 7 out of 49 (14.3). Consequently, the introduction of surrogate testing for prevention of post-transfusion hepatitis is nowhere near universal.

An alternative test, that of guanase activity, has been evaluated in Japan. Initially the post-transfusion hepatitis rate in recipients receiving blood with low guanase activity was 3% (2 of 71), whereas in recipients receiving blood with high guanase activity, the rate was 44% (16 of 36) (83). However, when this test was prospectively evaluated, the rate of post-transfusion hepatitis in recipients of blood having low guanase activity was 8 of 112 (7%) and 2.4% of units were excluded from use because of guanase activity (84). These data indicate that although surrogate tests are useful in decreasing the incidence of post-transfusion hepatitis in some countries, they cannot be universally applied, and are not completely successful in preventing post-transfusion hepatitis.

ANTIBODY TO HEPATITIS C VIRUS IN BLOOD DONORS

Comparison with Other Screening Tests

Country	Anti-HCV positive		↑ ALT		Anti-HBc positive	
	n	%	n	%	n	%
Germany	13/3123	0.42%	2/150	1.3%		
Netherlands*	7/147		10/147		3/147	
U.S.A. (Dallas)	64/6526	0.98%	78/6526	1.2%	89/6526	1.4%
U.S.A. (Boston)			4861/ 300120	1.6%	7803/ 300120	2.6%

* Units given to recipients developing NANB PTH

Kühnl et al, Lancet 1989; ii:324 *van der Poel et al, Lancet 1989; ii:297-298* *Kruskall et al, Transfusion 1988; 28:286-288*

The introduction of testing for hepatitis C virus in blood donors is expected to decrease the incidence of post-transfusion hepatitis by 70-80%. However, the current test does not become positive until late in the course of illness, thus potentially allowing early asymptomatic cases that are hepatitis C virus antibody negative to be used for transfusion. Improvements in tests and the introduction of assays for early antigens and for the virus itself should improve the effectiveness of screening for hepatitis C virus and preventing post-transfusion hepatitis.

ANTI-HCV ANTIBODIES IN BLOOD DONORS

Correlation with Other Tests

	Anti-HCV positive		Anti-HBc negative Normal ALT		Anti-HBc positive AND ↑ ALT	
	n	%	n	%	n	%
New York	2/412	0.49%			16/36	44.4%
Dallas	64/6526	0.98%	44/6320	0.65%	3/5	60.0%
Netherlands*	7/147		7/10		0/3	

* Units transfused to recipients developing NANB PTH

In comparison with the surrogate tests, anti-hepatitis C virus antibody is not always associated with an elevated ALT, nor is it always associated with anti-hepatitis B core positivity (86). Indeed, in the Netherlands none of 7 anti-hepatitis C virus positive units were anti-hepatitis B core positive. Testing donor blood for elevated ALT levels and anti-hepatitis B core antibody is likely to be continued since hepatitis C virus infection may not account for all viruses transmitted by blood and blood products, in particular short-incubation hepatitis does not appear to be associated with a hepatitis C virus infection, and short-incubation hepatitis may

be decreased in incidence with ALT screening. Removing all units of blood having evidence of hepatitis B virus exposure, as is done with anti-hepatitis B core antigen testing, is also likely to be continued. Post-transfusion hepatitis B is a more severe disease than post-transfusion non-A, non-B hepatitis, as noted above, and is clearly not completely prevented by screening for hepatitis B surface antigen alone. Furthermore, antibody to the hepatitis B core antigen appears to identify a population at risk for HIV and other serious infections that may be transmitted by blood donation (Alter, HJ, personal communication). Therefore, it is likely that both surrogate tests will be continued where they have been introduced.

ANTI-HCV ANTIBODIES IN DALLAS BLOOD DONORS
Correlation with Other Tests

Screening Test Result	Anti-HCV positive	
	n	%
Normal ALT and anti-HBc negative	44/6320	0.70%
Elevated ALT	12/78	15.4%
Anti-HBc positive	11/89	12.4%
Elevated ALT and anti-HBC positive	3/5	60.0%
None (all donors)	64/6526	0.98%

Immunoprophylaxis

The question of immunoprophylaxis of post-transfusion hepatitis remains unclear (reviewed in reference 32). Between 1947 and 1972 there were 5 negative studies published. An additional negative study was the original report by Prince and coworkers which first described long-incubation post-transfusion non-B hepatitis (1). On the other hand, between 1945 and 1971 there were six studies which showed protection with immune serum globulin in preventing post-transfusion hepatitis. Of importance was the study by Knodell and coworkers, where 41 episodes of non-B post-transfusion hepatitis occurred in cardiac surgery patients (55,87). Progression to chronic liver disease occurred in 10 of the 41 cases, but in only one of these had ISG been given; the other 9 receiving no treatment.

The large VA Cooperative Study of 2,204 patients observed 241 cases of hepatitis, of which 189 were non-B (88). Immune serum globulin had a modest effect, decreasing icteric hepatitis in the recipients of greater than 3 units of commercial blood, but otherwise was ineffective. The most recent study examined 198 cardiac surgery patients of whom 14 developed hepatitis (89). In these cases, three patients had received immune serum globulin, whereas 11 were control patients. Thus, it appears that in some studies there is a benefit of immune serum globulin; however, screening for hepatitis C virus is likely to be much more effective than such a therapy.

IMMUNOPROPHYLAXIS OF POST-TRANSFUSION HEPATITIS

NEGATIVE STUDIES:	
1947-1972	- 5 studies
Prince et al, 1975	- 204 cardiac surgery patients
	- 51 cases hepatitis, 36 non-B
POSITIVE STUDIES:	
1945-1971	- 6 studies
Knodell et al, 1976	- 47/279 cases of hepatitis, 41 non-B
	- 10 progression to chronic liver disease (1 ISG)
Seeff et al, 1978	- 2204 VA patients, 241 cases hepatitis, 189 non-B
	- ↓ icteric hepatitis ≥3 units commercial blood
Sanchez-Quijano et al, 1988	
	- 14/198 cases of hepatitis in cardiac surgery patients
	- 3 ISG patients and 11 control patients

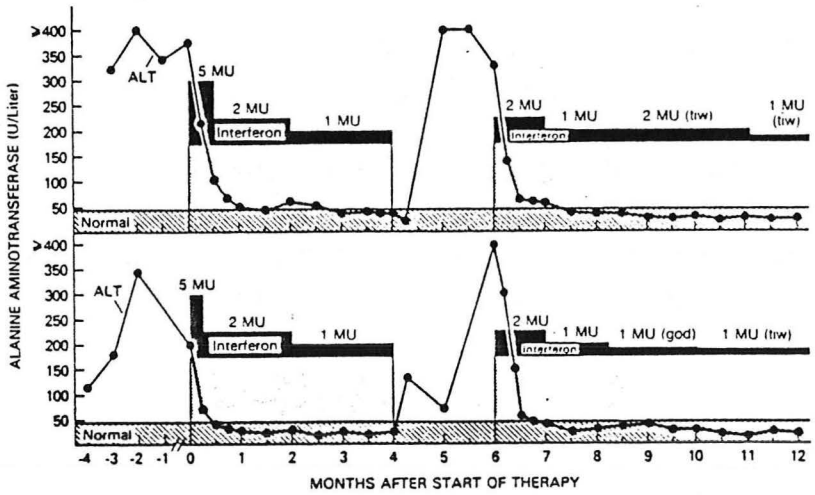
Prevention of short-incubation non-A, non-B post-transfusion hepatitis remains a problem. Recent studies demonstrating that dry heating of coagulation factors or treating them with psoralen compounds and exposure to ultraviolet light may prevent biochemical or histological evidence of hepatitis, and yet retain activity, are encouraging (90,91). Follow-up studies of these initial reports are awaited to determine whether a sustained effect is achieved.

TREATMENT OF CHRONIC NON-A, NON-B HEPATITIS

In 1986, Hoofnagle and coworkers from the National Institutes of Health published a preliminary report of treatment of chronic non-A, non-B hepatitis with recombinant human alpha-interferon (92). They treated 10 patients with chronic hepatitis, of whom 8 responded with a fall in aminotransferase levels to normal or near normal. Two patients relapsed after cessation of therapy, but responded once again after its reinstitution. Three of three liver biopsy specimens demonstrated improvement, and the overall response was regarded as very encouraging.

Recently the interim results of a randomized, controlled, open study have been published (93). Of 32 patients with chronic non-A, non-B post-transfusion hepatitis treated with interferon alpha-2b, all having had abnormal aminotransferase levels for at least one year, 14 of 21 treated patients normalized their ALT after 12 weeks of therapy, whereas none of 11 patients in the control group normalized aminotransferase levels. An additional study has evaluated liver biopsies in 12 patients before and after treatment with recombinant leukocyte interferon-alpha (94), there was a decrease in portal inflammation and hepatocellular degeneration after treatment. There was no improvement in liver biopsies in chronic hepatitis B patients treated with interferon-alpha.

Figure 8



Serial Determinations of ALT and AST in Chronic Non-A, Non-B Hepatitis Relapse with Cessation of α-Interferon Therapy

From: Hoofnagle et al, New Engl J Med 1986; 315:1575-1578

Two large trials are underway in the United States, evaluating treatment with alpha-interferon for chronic non-A, non-B hepatitis (95,96). A multicenter trial has enrolled 172 patients with chronic non-A, non-B hepatitis from transfusion or needle stick. Treatment is with one or three million units of recombinant interferon-alpha three times a week and an untreated control population was assessed in parallel. Patients were regarded as responding if their ALT levels fell to normal or declined to less than 1.5 times the upper limit of normal. The response rate was 46% in the high dose group, 28% in the low dose group, and 8% in the control group. Relapse rate after the discontinuation of therapy at 6 months was approximately 50%. Histological improvement was seen both in complete responders and in partial responders, that is, those whose aminotransferases fell but did not reach normal or less than 1.5 times the upper limit of normal. Unconfirmed reports indicate that 90% of this group are anti-hepatitis C virus antibody positive, and there were no apparent differences between HCV negative and positive groups.

The second large trial has enrolled 41 patients with chronic non-A, non-B post-transfusion hepatitis and treated them with 2 million units three times a week, or placebo. In the treated group 48% responded, whereas only 5% of the control group responded, and once again the relapse rate was approximately 50%. Final reports on these two trials are expected within the next few months, and it is likely that chronic non-A, non-B hepatitis will become an indicated diagnosis for treatment with alpha-interferon. At the present time, however, it is unclear what the optimal dosage is and how long treatment should be continued.

Currently, anti-viral therapy of chronic non-A, non-B hepatitis should be reserved for patients in controlled clinical trials. Under special circumstances it may be considered for specific patients with evidence of severe, progressive disease. The recommended dosage

regimen from the National Institutes of Health is for starting treatment at a dose of 5 million units three times weekly, however, lower doses of 2 and 3 million units three times weekly may suffice. Serum aminotransferases and complete blood counts should be monitored often until the response is clearly stable. If there is no improvement after 2 months of therapy at 5 million units three times weekly, therapy should be stopped. If there is a response to therapy, then interferon should be continued for at least 12 months. However, there are no data concerning longer therapy. Side effects may be troublesome, in particular psychological changes, cytopenias, infections and autoimmune conditions may occur. Additional studies determining the correct dose of alpha-interferon and the duration of therapy are still needed. Furthermore, alternative therapies for non-responders need to be evaluated.

SUMMARY

The hepatitis C virus, a single stranded RNA virus probably belonging to the family of flaviviruses has been identified as the probable cause of 90% of cases of transfusion-associated non-A, non-B hepatitis. The hepatitis C virus is present in approximately 1% of the blood donor population and is extremely common in high risk groups such as parenteral drug users and hemophiliacs. The contribution of hepatitis C virus infection to acute, sporadic and resolving hepatitis is as yet undetermined, but may in fact account for less than 50% of these cases. Infection with hepatitis C virus may be associated with aplastic anemia complicating non-A, non-B hepatitis, and may contribute to fulminant hepatitis, particularly in hepatitis B surface antigen chronic carriers. Further studies will define it's role in each of these categories. The contribution of hepatitis C virus infection to chronic liver disease has also to be established, but preliminary reports indicate that it may be a common cause and may be implicated in hepatocellular carcinoma occurring in the setting of non-A, non-B hepatitis. Therapy of chronic liver disease associated with hepatitis C virus infection is likely to be undertaken with recombinant interferon alpha in the future, however, prevention of hepatitis C virus infection will be the goal with screening of donor blood and exclusion of positive units likely to decrease the incidence of post-transfusion hepatitis to a large degree.

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