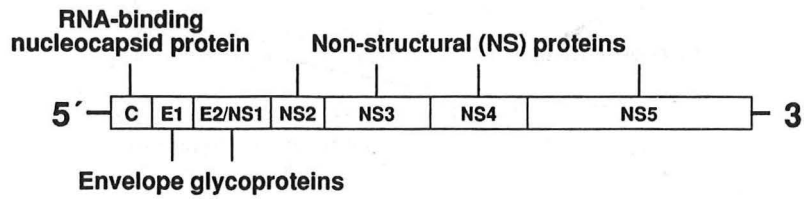
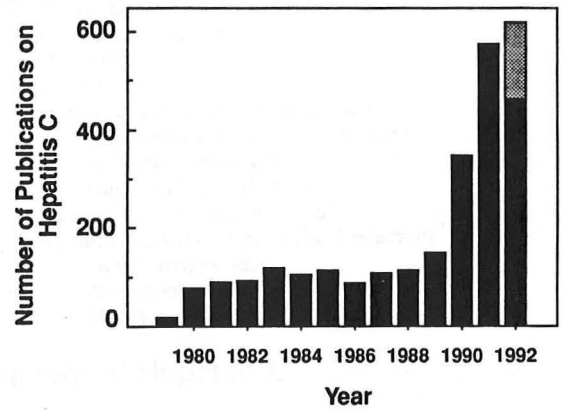
Hepatitis C Update: Progress and Problems



Hepatitis C virus genome organization



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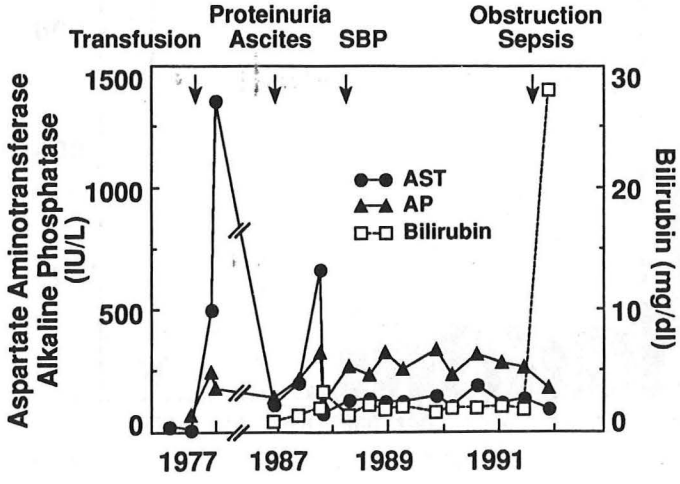
Case Report

In August 1975, R.C., then 44 years old, had an acute, infero-lateral myocardial infarct. Coronary angiogram in December demonstrated 99% stenosis of the distal right coronary artery and 90% stenosis of the distal circumflex artery and multiple stenotic lesions in the distal left anterior descending artery. He underwent successful 4 vessel coronary artery bypass graft surgery in November 1976, never developing significant ischemic heart disease again but during the operation he required transfusion with 4 units each of fresh frozen plasma and packed red blood cells. He developed acute hepatitis in December 1976, ~6 weeks after transfusion. He was then well until 1987 when he presented with proteinuria, peripheral edema and ascites. A history of 2-6 beers/day alcohol consumption for ~20 years, discontinued 3 years prior to admission, was elicited. He had microscopic hematuria, granular and hyaline casts, hypoalbuminemia (2 g/dl) and a low C3. Both aspartate aminotransferase (AST) and alkaline phosphatase were elevated, gamma-glutamyl transferase was normal. Paracentesis demonstrated low protein ascites (total protein 0.6 g/dl, gradient ≥1.4). Liver-spleen scan showed patchy distribution in the liver and shunting to bone marrow. A diagnosis of glomerulonephritis of unknown etiology was made and his renal function improved over the ensuing months. His liver disease was considered to be alcohol-related.

During the next 12 months he continued to have intermittent hematuria, pyuria and proteinuria with low C3 and cryofibrinogen was detected. He developed increased aminotransferases in December 1987, AST 662 IU/L and then in January 1988 he noted increasing abdominal girth followed by the onset of abdominal pain and fever. Paracentesis revealed polymorphonuclear leukocytosis (WBC 3,700/mm^3, 94% polys), low protein ascitic fluid (total protein 0.7 g/dl, gradient ≥1.8) and *Streptococcus pneumoniae* was cultured from blood and ascitic fluid. Bilirubin and ammonia were elevated at 3.4 mg/dl and 61 µmols/L respectively. The spontaneous bacterial peritonitis was treated with parenteral antibiotics and his ascites was managed with dietary salt restriction and diuretic therapy. A diagnosis of cirrhosis secondary to chronic post-transfusion non-A, non-B hepatitis was made. During out-patient follow-up, anti-hepatitis C virus antibody was found to be positive.

In June 1992, he presented with hepatic encephalopathy, precipitated by sepsis, secondary to obstructive jaundice. On admission to hospital, laboratory investigations revealed ammonia 400 µmols/L, bilirubin 28.1 mg/dl, amylase 296 IU/L, lipase 444 IU/L, creatinine 2.6 mg/dl; WBC 14,900/mm^3, 82% polymorphonuclear leukocytes. An endoscopic retrograde cholangiogram demonstrated obstruction at the ampulla of Vater by choledocholithiasis. His hospital course was complicated by gastrointestinal hemorrhage, azotemia and continuing infection from which he succumbed. Permission for autopsy was not obtained. This patient's medical history illustrates much of the wide spectrum of hepatitis C.

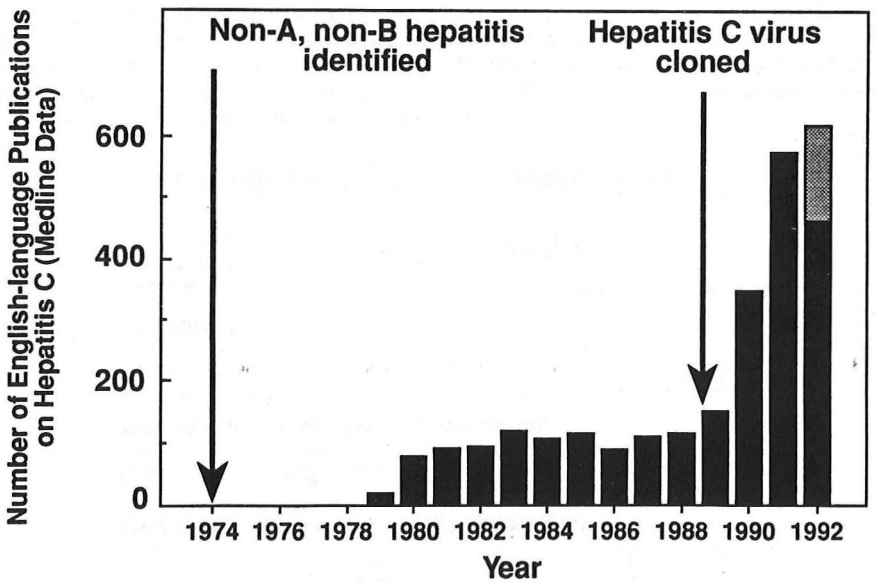


History of Hepatitis C

In 1974, Prince and co-workers at the New York Blood Center reported that of 204 cardiovascular surgery patients followed prospectively, 51 patients (25%) developed post-transfusion hepatitis.[1] In 36 patients (18% of all the patients and 71% of those with post-transfusion hepatitis), this was not caused by the hepatitis B virus.[1] They concluded "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". Similarly, neither hepatitis A nor hepatitis B accounted for the post-transfusion hepatitis in a group of cardiac surgery patients followed prospectively at the NIH.[2] A *Lancet* editorial in 1975 coined the term "non-A, non-B hepatitis" to describe the entity of hepatitis that was neither A nor B, emphasizing that the diagnosis was one of exclusion.[3]

In 1989, 15 years after the first suggestion that hepatitis C existed, a molecular biological approach was successful where many other techniques had failed and the hepatitis C virus was cloned.[4,5] The virus responsible for most post-transfusion hepatitis, and probably at least a large proportion of sporadic or community-acquired non-A, non-B hepatitis, has been isolated.[6,7] In blood banks, utilization of 1st generation screening tests measuring antibody to the hepatitis C virus (anti-HCV) has identified donor blood potentially capable of transmitting hepatitis C to recipients, and has substantially decreased the rate of post-transfusion hepatitis.[8,9] Presumptive transmission of hepatitis C by anti-HCV positive donor blood has also been documented.[10] However, the mode of transmission of hepatitis C to many of the 0.2-1% of normal blood donors that carry the virus is still unknown. Hepatitis C causes acute and chronic hepatitis,[6] cirrhosis[11,12] and hepatocellular carcinoma[13] in some people. In others, there may be no apparent effect, even though significant liver damage occurs silently.[7] Future studies should elucidate the many aspects of hepatitis C infection that remain unknown.

Impact of Cloning the Hepatitis C Virus: a Literature Explosion



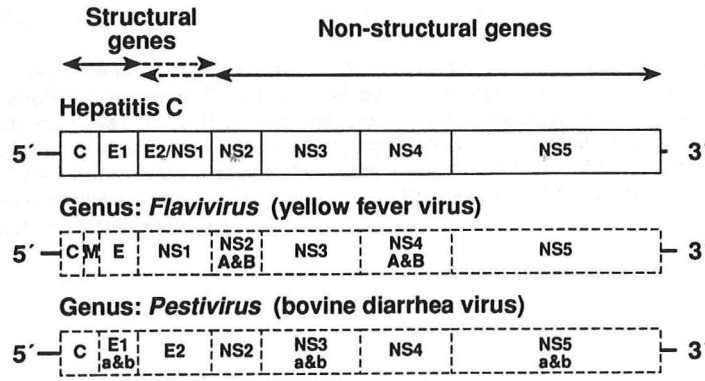
Virology

The hepatitis C virus (HCV) was isolated and cloned using a recombinant immunoscreening approach.[4] Human factor VIII concentrate that transmitted non-A, non-B hepatitis was inoculated serially into chimpanzees to obtain an infectious plasma pool of $\geq 10^6$ chimp infectious units/ml. Nucleic acids were extracted from a crude viral pellet and complementary DNAs synthesized by reverse transcriptase using random oligonucleotide primers. A cDNA library was generated in the vector λgt11 and immunoscreened using sera from a patient with post-transfusion hepatitis. A single positive plaque, 5-1-1, was isolated after screening ~10^6 recombinant clones. This positive plaque contained a 155 bp insert that was then used as a hybridization probe to isolate a 353 bp insert, clone 81. A series of experiments demonstrated that the cDNA hybridized to RNA from infected chimpanzee liver and serum, but neither uninfected liver RNA nor DNA. A larger cDNA was expressed in yeast as a fusion polypeptide of 363 HCV amino acids (C100-3) and human superoxide dismutase.[5] Immunoblot analyses of bacterial lysates demonstrated that sera from most patients with post-transfusion hepatitis were reactive. The complete nucleotide sequence of the RNA genome of the original HCV isolate was then determined from overlapping clones.[14] Thus, without specific knowledge of the type of virus involved, viral nucleic acid was cloned from infectious plasma samples.

Classification of HCV:

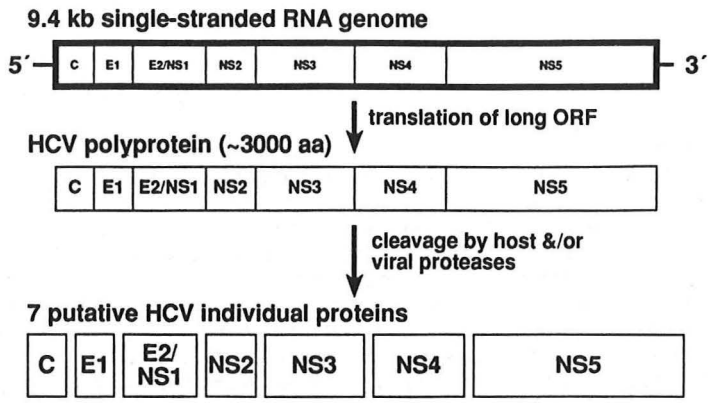
Properties of viruses that are used in taxonomic classification include: 1) virion size, shape and structure; 2) genome type (RNA or DNA), structure and sequence; 3) protein number, size and function; 4) replication, transcription and translation characteristics; 5) physical stability; and 6) biological properties.[15] The hepatitis C virion is small (30-34nm), enveloped and has a single-stranded, positive-sense RNA genome. Icosahedron-shaped particles of average diameter 33nm recovered from HCV-infected plasma[16] may represent intact virions. A lipid envelope is suggested by chloroform-sensitivity of infectious material[17] and changes in buoyant density with detergent treatment.[18] There is a single, large, open reading frame (ORF) that is predicted to encode a viral polyprotein precursor of ~3000 amino acids (3010 in the prototypic U.S. strain HCV-1; 3011 in the Japanese strain HCV-J1 and other Japanese isolates).[19,20] The nucleotide sequence upstream (5′) of the large ORF is similar to that of animal pestiviruses. In contrast, sections of the polyprotein have amino acid sequence similarities to animal pestiviruses, plant potyviruses and human flaviviruses.[19,20] The nucleotide and amino acid similarities to the genera *Pestivirus* and *Flavivirus* of the family *Flaviviridae* have resulted in the proposal that the hepatitis C virus belongs to a separate genus of the family.

Genetic Organization of *Flaviviridae*



Modified from: Choo *et al*, *Proc Natl Acad Sci USA* **88:** 2451-2455, 1991

Genetic Organization of the Hepatitis C Virus



Putative individual proteins

	kDa	
C	22	RNA-binding nucleocapsid protein
E1	gp33	envelope glycoprotein?
E2/ NS1	gp72	envelope glycoprotein?
NS2	23	? function - extremely hydrophobic
NS3	~60	helicase/protease
NS4	[52]	? function - extremely hydrophobic
NS5	[116]	RNA-dependent RNA polymerase

Modified from: Houghton *et al*, *Hepatology* **14:** 381-388, 1991
Bradley, Beach and Purdy, *Microb Pathogen* **12:** 391-398, 1992

Viral replication:

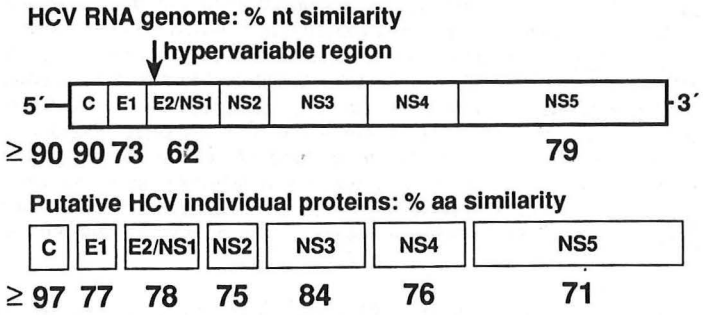
The HCV genome is a positive-sense, single-stranded RNA and minus-strand replicative forms are detected in infected liver tissue,[21,22] serum[22] and, by some investigators, in peripheral blood mononuclear cells.[23] In chimpanzees, HCV RNA was detected in the cytoplasm of most hepatocytes by *in situ* hybridization 2 days after inoculation with hepatitis C-infected serum[24] and was present in the serum by 3-7 days.[25-29] Hepatocyte HCV RNA remained detectable during the period of elevated transaminases.[24] There was no correlation between intrahepatic HCV RNA and hepatocyte necrosis, suggesting that the virus may not be directly cytotoxic.

Sequence diversity of HCV:

The ability to isolate and sequence HCV, using reverse transcriptase (RT) to synthesize cDNA and the polymerase chain reaction (PCR) with oligonucleotide primers from specific areas of the genome, has allowed comparison of isolates from different individuals.[19,20] There is an overall sequence similarity of ~70-80%. The nucleotide substitutions have a non-random distribution, with the amino terminal end of NS1/E2 constituting a hypervariable region. A segment of 28 amino acids demonstrates more than 50% variation in different isolates. This segment, which comprises <1% of the total amino acids in the polyprotein, contained 8 of the total 44 amino acid substitutions observed in an experimentally infected chimpanzee over 8 years.[30] Even more striking, 9 of 13 amino acids in the hypervariable region of NS1/E2 were changed in the strain HCV-H when compared over a 13 year period in patient H.[31] The 5′ untranslated region contains the highest degree of sequence conservation, with ≥90% similarity overall.[32,33] The region consists of highly conserved domains interspersed with variable domains.[32] In other viruses, similar conserved 5′ untranslated regions have regulatory importance in replication of the viral genome or expression of viral genes. Elements in the 5′ untranslated region of genomic-length HCV RNA that repress translation have been described.[34] Such elements may not be present in sub-genomic-length HCV RNA species,[35] thereby potentially allowing efficient translation and expression of viral genes. The presence of five distinct but related genotypes (I-V) throughout the world has been reported, based on sequence analysis.[36]

A nucleotide mutation rate of $\approx 1.9 \times 10^{-3}$ base substitutions per genome site per year has been calculated, based on HCV isolates from the single patient examined over a 13 year time period.[31] Similarly, the Japanese HC-J4 strain was isolated from an experimentally infected chimpanzee early during acute infection and also during chronic infection more than 8 years later.[30] Sequence comparison revealed a mutation rate of $\approx 1.4 \times 10^{-3}$ base substitutions per site per year (111/9412 nucleotides differed). The mutation rate of most RNA viruses ranges from 10^{-1} to 10^{-4} substitutions per site per year, considered to be a reflection of the lack of proof-reading ability of the RNA dependent RNA polymerases. In comparison, the proof-reading ability of DNA-dependent DNA polymerases that replicate chromosomal DNA results in a mutation rate of 10^{-9} nucleotide substitutions per site per year. The mutations detected in HCV RNA are unlikely to be introduced during PCR amplification, since the error rate of *Taq* polymerase is much lower than the observed mutation rate ($\sim 5 \times 10^{-6}$ errors per nucleotide per cycle).

Sequence Variability of Different Genotypes of the Hepatitis C Virus



Modified from: Cha *et al*, *Proc Natl Acad Sci USA* **89:** 7144-7148, 1992
Houghton *et al*, *Hepatology* **14:** 381-388, 1991

HCV variants, immune selection and therapeutic responses:

The extremely high rate of amino acid changes in the hypervariable region of the E2/NS1 segment suggests that functional and structural constraints on the encoded protein are low, as predicted by analysis of the region.[37] In the analogous segment of flaviviruses (NS1) and pestiviruses (E2), antigenically distinct variants are favored by selective immune pressures. A similar immune selection of HCV variants was observed.[37] Thus, antibodies were detected that bound to distinct linear peptides which were encoded by the specific virus isolated from the patient.[37] Differences in a 20 amino acid span of the N-terminal hypervariable region of E2/NS1 occurred in a single patient over time and were associated with distinct episodes of hepatitis. Furthermore, individuals could be co-infected with different variants.[37] The occurrence of such variants may contribute to chronicity if the mutations permit escape from neutralizing antibodies or cytotoxic T cell effector function.

Like other RNA viruses, HCV genomes in a single patient can be a mixed population,[38,39] with a dominant sequence accounting for the majority of isolates. Other variants, present in minor proportions, then have the potential to become dominant if selection pressures are applied. In a group of 6 patients with chronic hepatitis C, there was heterogeneity in the hypervariable region sequence from the three individuals that did not respond to interferon-β therapy.[40] In contrast, the three patients that demonstrated a sustained response and clearance of HCV RNA from the circulation had little or no heterogeneity in the hypervariable region. Furthermore, pre-treatment nucleotide and amino acid sequences may also influence response rates to α-interferon therapy.[41] Thus, patients with strains of hepatitis C genotypically resembling the prototypic U.S. strain, HCV-1, had higher viral titers and lower response rates to treatment than those with other strains (5/39, 13% compared with 16/26, 62% complete response). Future evaluation of hepatitis C-infected patients may include genotyping of the viral strain and assessment of homogeneity before selection for particular treatment protocols.

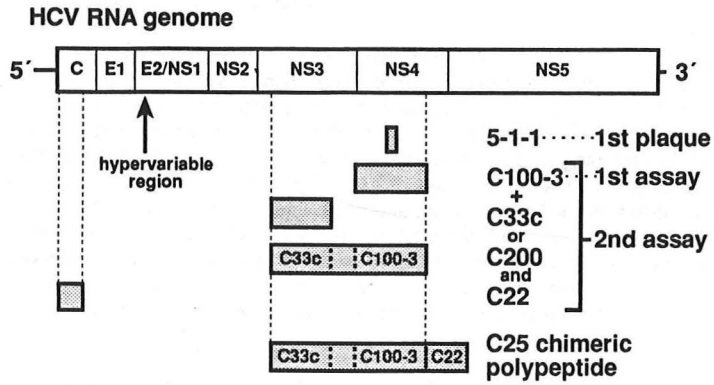
Detection of Hepatitis C Infection

Prevention of post-transfusion hepatitis C, by identifying blood donor specimens capable of transmitting the virus to recipients, was a major goal once the virus was isolated. Antibodies that recognize HCV protein epitopes have been detected in blood donor specimens and used to identify patients with hepatitis C infection. The original fusion protein, expressed in yeast, consisted of 363 virus-encoded amino acids from the NS4 region and was used in the 1st generation assays for the detection of antibodies to hepatitis C viral epitopes. This assay was able to identify hepatitis C in the majority of transfusion recipients developing post-transfusion non-A, non-B hepatitis.[42,43] In addition, donors implicated in the transmission of non-A, non-B hepatitis were also identified.[42,43] However, some donors and recipients were negative for hepatitis C infection by the 1st generation assay.[42,43] Furthermore, although many patients with chronic hepatitis C had antibodies that reacted with the C100-3 amino acid sequence, a proportion remained negative, perhaps because of sequence variation.[44] Alternatively, continued lack of antibody response, despite documented HCV infection without sequence mutation,[45] may reflect immunologic differences. Antibody positivity was often delayed when only the C100-3 sequence was used for antigen expression, with seroconversion not occurring until many weeks after the onset of hepatitis,[46] decreasing the usefulness of the 1st generation assay in the diagnosis of acute hepatitis C. Thus, the first generation assay lacked sensitivity, particularly for recognition of acute disease.

The initial assays were also lacking in specificity. Sera with antibodies that cross-reacted with superoxide dismutase, the fusion partner used for expression of viral sequences in yeast, accounted for some of the lack of specificity and were identified in a recombinant immunoblot assay (1st generation). False positive results with antibody assays for hepatitis C infection have also been associated with stored and/or "aged" sera,[47] sera from tropical communities,[48] and following influenza vaccination.[49]

Hyperglobulinemia and rheumatoid factor (anti-immunoglobulin) positivity also resulted in false positive anti-HCV tests with 1st generation assays.[50,51] Attempts to improve the specificity of blood donor screening and the sensitivity of detecting patients with hepatitis C infection have resulted in a number of different assays.

Sequences Expressed in Assays for Antibodies to the Hepatitis C Virus



Modified from: Chien *et al*, *Proc Natl Acad Sci USA* **89:** 10011-10015, 1992

Second generation assays:

Other putative HCV proteins were examined for potential capacity to diagnose acute infection earlier and chronic infection with greater reliability. Linear peptides encoded in the putative nucleocapsid or core (C) region and the E2/NS1 region are differentially recognized by human antibodies, suggesting that there are immunodominant regions.[37,52-54] Serologically distinct responses to the putative core protein were demonstrated in patients with genotypically different viral isolates.[55] Antibodies recognized peptides encoded in a variable segment of the otherwise highly conserved region. In comparison with 1st generation assays, synthetic peptides from the core (C) region allowed earlier detection of hepatitis C infection.[56-59] HCV RNA is also more likely to be detected in samples containing antibodies to core sequences.[60]

Sequences from the NS3 region (C33c) appear to be immunogenic in the majority of those with hepatitis C infection.[61] Furthermore, the C200 polypeptide that includes C33c, C100-3 and the intervening region (NS3 and NS4 sequences), was the most effective at blocking labeled antibody binding to hepatitis C virus antigen(s) in hepatocytes.[62] These peptide sequences have been included in 2nd generation assays to provide multiple separate antigenic epitopes, C100-3 + C33c + C22 or C200 + C22.[61] A single fusion polypeptide that includes the relatively conserved NS3 and NS4 regions and core sequences (C25 = C33c/intervening region/C100-3 + C22) that is not only simpler but as reliable as the multiple polypeptide 2nd generation assay may be the next improvement.[61]

Second generation assays have increased sensitivity in identifying blood donors with hepatitis C infection[10] and in detecting both acute and chronic hepatitis C infection.[63-70] In addition, increased specificity (decreased false positivity) has also been demonstrated by some, but not by all, investiga-

tors.[63,71,72] Cross-reactivity to the superoxide dismutase fusion partner of C100-3 expressed in yeast and to proteins in lysates of yeast and *E. coli* may be minimized by including these proteins in the diluting solutions, thereby preventing attachment of the non-specific antibodies to the immobilized, specific proteins.[63] Anti-HCV antibodies persist in the serum in most patients.[6,73] Thus, in a follow-up study of 81 patients who developed non-A, non-B hepatitis after receiving hepatitis C-infected immunoglobulin, 52/56 (93%) were anti-HCV positive 6-12 months after infection and 45/65 (69%) were positive 9-10 years after.[73] Loss of antibody is more common in those who have resolving disease clinically.[6,73]

HCV RNA and detection of infection:

Although 1st generation assays were able to identify blood donations with antibodies to hepatitis C, the positive specimens were not all associated with transmission of hepatitis.[10,74] Confirmatory testing, with a second generation immunoblot assay, was more specific.[10] The presence of HCV RNA, detected by PCR amplification, was also associated with infectivity.[10,74-76] In a prospective study of post-transfusion hepatitis, anti-HCV antibodies and HCV RNA were measured in serum samples obtained from 1100 donors and 300 recipients.[74] Of 6 donor samples (0.6%) repeatedly reactive in a 1st generation assay, only 1/6 (17%) transmitted non-A, non-B hepatitis to a recipient. HCV RNA was detected by PCR in the serum of the transmitting donor, but not in the sera of the other 5 donors, demonstrating that the presence of detectable viral sequences was a better predictor of infectivity. Detection of HCV RNA in specimens implicated in transmission of hepatitis C that were unreactive by 1st generation assays[76] additionally indicated that there were false negative results as well as false positive results in the early assays.

The new 2nd generation assays cannot identify blood donors with hepatitis C viremia with complete accuracy either.[72,77-80] HCV RNA was positive in only 52% of 23 samples selected randomly from a total of 400 with anti-HCV positivity confirmed by supplemental testing.[77] HCV RNA was not detected in any of 23 samples with a false positive 1st generation assay randomly selected from a total of 718 such samples. Similar results were reported by other investigators in that HCV RNA was not amplified from any of 203 sera with false positive results from a 1st generation assay but was present in 89/182 (49%) of samples with anti-HCV positivity confirmed by a 2nd generation immunoblot assay.[72] In earlier reports examining fewer samples, most donors with positive 1st or 2nd generation immunoblot assays were PCR positive (7/8[78] and 5/5[80]). Together, these data suggest that the new assay will be an effective screen for detecting blood donations capable of transmitting hepatitis C. However, when 108 donors with alanine aminotransferase concentrations >100IU/L were studied, 19 were PCR positive for hepatitis C but only 13 had antibodies detected by second generation anti-HCV assay,[79] implying that large-scale screening by PCR might be needed to eliminate post-transfusion hepatitis C.

Hepatitis C virus sequences can be detected, after reverse transcription to generate cDNA, either by standard PCR amplification techniques using conserved oligonucleotide primers followed by hybridization of a labeled probe to internal sequences or by "nested" or 2-stage PCR amplification with external and internal primers and visualization after gel electrophoresis. As with all PCR techniques, detection of HCV RNA requires strict adherence to procedures designed to reduce contamination. Using PCR amplification, HCV RNA has been detected in liver tissue[22,75,81,82] and in peripheral blood mononuclear cells[23,81-85] as well as serum. Special problems have been encountered in PCR amplification of HCV RNA because the sequence is variable. Oligonucleotide primers from highly conserved regions such as the 5′ non-coding sequence are more reliable.[86,87] Correct storage of samples used for PCR amplification is important and heparinized plasma is not suitable.[88,89] Faster and simpler methods of detecting and quantifying HCV RNA are currently being explored.[90-93] In addition, specific application of "nested" PCR for genotyping of HCV strains has also been reported.[94]

Transmission of HCV Infection

Transfusion of infected blood and blood products transmits hepatitis C and hepatitis C infection accounts for 80-90% of post-transfusion hepatitis.[6,68,69,95-97] Other "parenteral" risk factors for hepatitis C infection include transplantation with organs from hepatitis C-infected donors,[98-100] intravenous drug use,[101] tattoos[101,102] and needle-stick accidents.[103,104] Parenteral transmission studies that measured neither antibodies to peptides other than C100-3 nor HCV RNA probably failed to detect all positive recipients and underestimate the true transmission rate.[100] Transmission of hepatitis C from anti-HCV positive organs to transplant recipients was documented in 13/14 (93%) patients (see Table below).[100] These recipients were initially negative for anti-HCV antibodies and HCV RNA. The only patient who failed to demonstrate HCV infection was transplanted with a kidney from a donor positive for anti-HCV antibody but negative for HCV RNA. Of particular note, anti-HCV antibody tests were insensitive in transplant recipients both before and after transplantation (see Table below). This lack of sensitivity of routine tests may explain the apparently low rate of transmission of hepatitis C in other reports.[99]

Transmission of Hepatitis C by anti-HCV Positive Organ Donors

HCV RNA in Recipient Pre-Transplant	Donor HCV RNA	POSITIVE HCV RNA in Recipient Post-transplant
Negative n = 14	Yes	13/13
	No	0/1
Positive n = 6	Yes	5/5
	No	1/1

Differences Between Anti-HCV Tests and HCV RNA in Transplant Recipients

HCV RNA in Recipient	Positive Anti-HCV Test		
	1st generation	2nd generation	2nd immunoblot
Pre-Transplant:			
Positive (n=7)	3/7 (43%)	4/7 (57%)	4/7 (57%)
Negative (n=19)	4/19 (21%)	2/18 (11%)	1/19 (5%)
Post-transplant:			
Positive (n=23)	8/23 (35%)	16/23 (70%)	12/23 (52%)

From: Pereira *et al, N Engl J Med* **327:** 910-915, 1992

World-wide, intravenous drug use is probably the most common risk factor for HCV infection with ~80% seropositivity in this population in the United States.[64,105] In Australian blood donors, a history of intravenous drug use was the most common risk factor in those found seropositive for hepatitis C infection (105/220, 48% compared with 3/210, 1% in seronegative controls).[101] In pregnant women in Dallas, anti-HCV antibodies were positive in 23/1005 (2.3%) when screened by 1st generation assays.[106] Intravenous drug use was reported by 10/23 (43%) anti-HCV seropositive women but only 13/982 (1.3%) of seronegative women.

Multiple episodes of hepatitis in intravenous drug users were previously considered evidence of more than one non-A/non-B, parenterally-transmitted, infectious agent. The recent demonstration that chimpanzees may develop recurrent hepatitic responses and viremia when sequentially inoculated with different strains of HCV[107] suggests an alternative explanation. Re-infection of the chimpanzees, not re-activation of previously occult virus, was demonstrated by PCR sequencing of the virus strain after inoculation.[107] If the responses of humans are similar, then clearance of HCV from the blood and liver is not associated with protective immunity. Failure of the immune system to eradicate the virus may lead to chronic infection in many, if not all, those infected.

Hepatitis C infection in patients with parenteral exposure:

Patients with bleeding disorders or chronic renal failure requiring dialysis are commonly and frequently exposed to blood and blood products. A high percentage of hemophiliacs are positive for anti-HCV antibodies. In Italy, 82% (161/193) were positive by 1st generation assays.[108] Slightly lower rates were obtained from countries with lower overall prevalence of hepatitis C infection, 76% (100/131) in the U.S. and 59% (76/129) in the U.K.[109,110] Hepatitis C reactivity was associated with both hepatitis B virus (HBV) and human immunodeficiency virus (HIV) infection.[109,110] Hemophiliacs treated only with heated concentrates had lower rates of anti-HCV reactivity (22%, 5/23 compared with 67%, 71/106 treated with unmodified products).[110] Those receiving only vapor-heated factor VIII or IX concentrates (0/9), cryoprecipitate or single donor products (0/9) in the U.S. had no anti-HCV antibodies by 1st generation assays.[111] However, later studies demonstrated a false negative rate of up to 20% with 1st generation assays[112-114] and markers of infection were present in virtually all hemophiliacs.[112,114,115] Patterns of viremia in hemophiliacs[116] closely resembled those in chimpanzees with experimental hepatitis C.[26,29] HCV RNA was detected transiently in patients with acute resolving hepatitis and either intermittently or continuously in chronic hepatitis. With the current ability to detect HCV RNA and anti-HCV antibodies more reliably, future studies evaluating vapor-treatment, pasteurization and other methods of virus inactivation should determine the most effective means of preventing hepatitis C transmission during treatment of hemophiliacs.

Patients with renal disease on hemodialysis also have the potential to be continually exposed to infectious materials. The prevalence of markers for hepatitis C infection varies widely, depending on geographic origin of the patients and the presence of other parenteral risk factors. In Belgium, only 6/79 (8%) patients were positive for anti-HCV antibodies (2nd generation enzyme-linked immunosorbent assay, EIA) and HCV RNA was detected in only 4/79 using "nested" primers from the 5′ non-coding region.[117] In Japan, 2nd generation EIA assays detected anti-HCV antibodies in 74/167 (44%) patients compared with 34/167 (20%) using 1st generation assays.[118] Similarly, in the U.S. 31/87 (36%) patients were positive for anti-HCV assays (2nd immunoblot).[119] Reactivity correlated with length of time on dialysis but not to blood transfusion history nor to aminotransferase levels.

Occupational hazards:

Health-care workers represent 2% of acute hepatitis C patients[120] and may have higher rates of chronic hepatitis C infection than the general population. Thus, ~2% (8/456) of dentists in New York, particularly oral surgeons (9%, 4/43) were anti-HCV positive by 1st generation immunoblot, significantly

more than the control population (0.14%, 1/723).[121] The magnitude of the potential risk to health-care providers was underscored in a study of the prevalence of seropositivity for hepatitis C in emergency room patients of a large inner-city hospital, Johns Hopkins.[105] Antibody to hepatitis C (2nd immunoblot) was present in 18% (458/2523) of patients with rates as high as 51% in some groups. Similar results have been reported from the Washington D.C. Veterans Administration Hospital, with 40% of consecutive in-patients testing positive for anti-HCV by 2nd generation EIA assays (L. B. Seeff, personal communication). Since needle-stick exposure may lead to transmission in 10% of instances[104] the risk of contracting hepatitis C is considerable.

Risk of Hepatitis C Infection after Needlestick Accident

Source	HCV RNA in Source	HCV RNA in Recipient		Hepatitis in Recipient	
Anti-HCV positive n = 91	Yes	7/68	10%	5/68	7%
	No	0/8	0%	0/8	0%
Anti-HCV negative n = 68	n.d.	0/54	0%	1/54	2%

From: Mitsui *et al, Hepatology* **16:** 1109-1114, 1992

Non-parenteral transmission:

Non-parenteral transmission of hepatitis C may account for infection in patients with sporadic or community-acquired disease who lack classical parenteral risk factors. In patients with either hepatitis B or non-A, non-B hepatitis, multiple heterosexual partners were reported more commonly (hepatitis B patients 26%; non-A, non-B 12%) than in matched controls (7% hepatitis B controls; 1% non-A, non-B controls).[122] Anti-HCV antibodies were more common in heterosexually promiscuous groups (female prostitutes 9%; clients of prostitutes 16%)[123] and homosexual men (2-5%)[123-125] when tested by 1st generation assays. Heterosexual spouses of anti-HCV positive hemophiliacs also had modestly increased risk of anti-HCV positivity in some[126,127] but not all[128] studies, co-infection with human immunodeficiency virus raised the likelihood.[126] Intra-familial transmission of hepatitis C was reported in 26/530 (5%) household contacts of 225 anti-HCV positive subjects (1st EIA)[129] and 10/186 (5%) family members of 48 index patients (2nd EIA).[130] Spouses had the highest prevalence (21%), with increasing age and longer duration of marriage being risk factors.

HCV RNA has generally not been detected in body secretions[131-133] and, when present, blood contamination was potentially the cause of the positive result.[134] The route of intra-familial and sexual transmission is therefore obscure as yet. Indirect data suggest that non-parenteral transmission of HCV is different or less prevalent than non-parenteral transmission of HBV infection. Thus, anti-HCV antibodies were not detected in any of 113 outpatients with developmental disabilities, whereas 24 (21%) showed serologic evidence of past HBV infection and 3 (3%) were positive for hepatitis B surface antigen (HBsAg).[135] Even more striking, 0/128 institutionalized Down's syndrome residents and 0/136 residents with other developmental handicaps were anti-HCV positive (2nd EIA).[136] Again exposure to the hepatitis B virus was extremely common, 116/128 (91%, 35 HBsAg positive) with Down's syndrome and 103/136 (76%, 8 HBsAg positive) with other disorders. Furthermore, in 3 separate studies none of 200 Japanese

children,[137] 1,000 Chinese children,[138] and 2,111 Peruvian natives[139] had markers of HCV infection despite "normal" levels of HBV exposure. Additional epidemiological studies are necessary to solve the persistent problem of transmission of HCV in the absence of parenteral exposure.

Vertical transmission of hepatitis C, although uncommon, has been well-documented.[140-142] Chronic non-A, non-B hepatitis was observed in 2/13 live-born infants of 11 mothers with chronic non-A, non-B hepatitis,[143] suggesting the possibility of mother-to-child transmission. In 8 infants born to mothers positive for both anti-HCV and HCV RNA, HCV RNA was either intermittently positive or positive in all samples of blood up to 12 months of age.[140] HIV infection was present in 3 of the mothers but only one child. Anti-HCV antibody was initially detected in the infants but was absent from all samples of infants more than 5 months old, suggesting that the initial positive results reflected passive transfer of maternal antibody. Aminotransferase elevation was intermittent or absent in the infants. Thus, neither antibody status nor biochemical studies indicated infection with HCV. However, in additional studies vertical transmission was not detected[144] or was infrequent.[145] Thus, HCV RNA was not detected in 24 infants followed for at least 3 months after birth and only 1/24 was positive at birth whereas 16/23 mothers were positive for HCV RNA (all had confirmed anti-HCV positivity).[144] Since only one sample was assayed for HCV RNA, intermittent positivity may have been missed. In another study, HCV RNA was present in only one of 21 children born to 14 women with chronic hepatitis C, despite multiple samplings.[145] In summary, vertical transmission of hepatitis C infection is probably infrequent, particularly in the absence of HIV co-infection. The lack of anti-HCV antibodies in the infants with HCV RNA in one study[140] emphasize the inability of seropositivity to detect all infected persons.

Hepatitis C and Liver Disease

The spectrum of liver disease observed in patients demonstrating seropositivity for the hepatitis C virus covers the gamut from asymptomatic blood donors with normal liver function tests and no apparent sequelae through acute and chronic hepatitis to hepatocellular carcinoma and hepatic failure requiring liver transplantation. Unanswered questions include the long-term natural history of hepatitis C infection not acquired by blood transfusion and the characteristics, in the virus and the patients, that determine outcome.

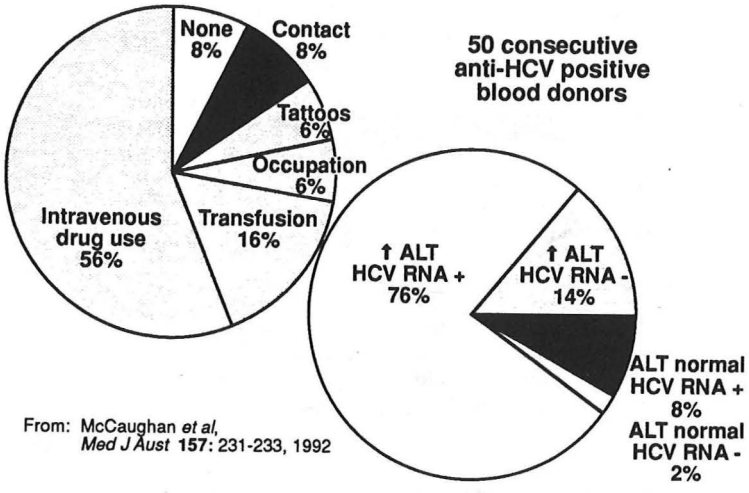
Blood donors and hepatitis C:

The largest hepatitis C-infected group is that of blood donors, who have been identified since the introduction of routine screening, which was in mid-1990 in the United States. In this group, with a low prevalence of true hepatitis C infection, the false positive rate with 1st generation and 2nd generation EIA screening assays may be 50%. However, those with antibodies directed against specific hepatitis C peptide epitopes (i.e. confirmed by immunoblot or neutralization assays) have a high rate of HCV RNA positivity and have been shown to transmit post-transfusion hepatitis, both demonstrations of bona fide infection.

The presence of liver disease has been evaluated in anti-HCV positive blood donors in a number of studies.[146-148] Only 13/50 (26%) anti-HCV positive Italian blood donors (1st generation enzyme-linked immunosorbent assay, EIA) were confirmed positive by recombinant immunoblot, despite repeatedly reactive screening EIA in all 50 donors.[146] An additional 17 patients (34%) reacted with only the 5-1-1 peptide or the C100-3 peptide but not both and did not react to superoxide dismutase alone (indeterminate). All donors with positive confirmatory tests had elevated alanine aminotransferase (ALT) and gamma-glutamyl transferase (GGT) levels, as did 6 of the donors with indeterminate reactivity. Other biochemical tests of liver function were normal in all 50 blood donors. In Spain, 104/150 (69%) anti-HCV positive blood donors (1st EIA) were confirmed positive (2nd immunoblot) and 76/104 had elevated transaminases.[147] Liver biopsies showed a range of abnormalities, with chronic hepatitis the most

common finding (see below). These studies demonstrate that asymptomatic volunteer blood donors can harbor hepatitis C virus and have active and histologically progressive liver disease.

Risk Factors and Aminotransferases in anti-HCV Positive Blood Donors



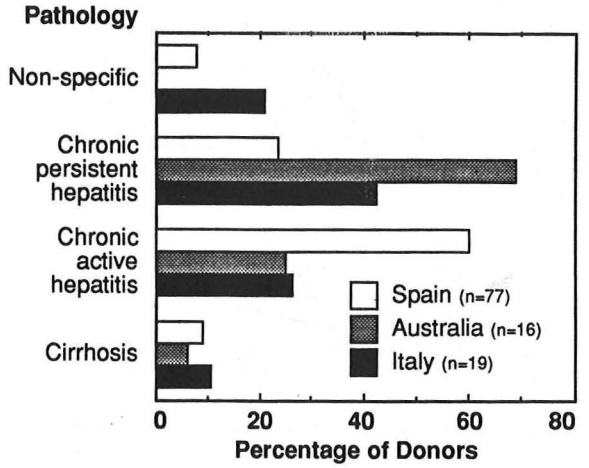
From: McCaughan *et al*, *Med J Aust* **157**: 231-233, 1992

In a similar study in Australia, 50 blood donors with anti-HCV positivity, all confirmed by recombinant immunoblot (1st generation) were evaluated.[148] Parenteral risk factors for exposure to hepatitis C were present in 42 (28 intravenous drug use, 8 blood transfusion, 3 health-care workers, 3 tattoos) and 4 had a history of close contact with a patient with hepatitis of unknown cause. Almost all (92%) were asymptomatic, the remaining 4 having fatigue with right upper quadrant abdominal discomfort additionally reported by 1. Spider naevi were present on physical examination in 14 and there was hepatomegaly in 2 of the latter patients. Alanine aminotransferase levels were followed for 6 months and were repeatedly normal in 5 (10%), elevated but <100 IU/L in 34 (68%) and elevated with some results >100 IU/L in 11 cases. In 22 patients, ALT levels were intermittently normal, the classic pattern for chronic, parenterally-acquired, non-A, non-B hepatitis. Of 16 patients undergoing liver biopsy, 1 had cirrhosis, 4 CAH and the remaining 11 CPH. HCV RNA was detected by PCR in 42 cases (84%) using two different sets of primers (5′ non-coding and NS3 regions). Of donors with persistently normal alanine aminotransferase levels, 4/5 were positive and 38/45 (84%) donors with aminotransferase elevation were HCV RNA positive. Thus, volunteer blood donors with hepatitis C infection generally were asymptomatic with mild disease but active and progressive disease was also observed.

Although many blood donors with hepatitis C may have elevated alanine aminotransferase levels, the converse is not true.[149] Before the introduction of anti-HCV testing, elevated alanine aminotransferase levels and anti-hepatitis B core (anti-HBc) antibody positivity were used as surrogate markers for blood donations likely to transmit hepatitis. Of 100 blood donors in the United States with elevated alanine aminotransferase levels, suggesting the possibility of hepatitis C infection, only 17 had anti-HCV antibodies by initial screening, all were confirmed positive by 2nd generation recombinant immunoblot.[149] The only risk factor present more often in the anti-HCV positive group was a history of intravenous drug use (5/17, 29% anti-HCV positive; 4/83, 5% anti-HCV negative). Alcohol-related liver disease (n=48) and obesity (n=22) were more common causes of elevated alanine aminotransferase levels. Similarly, in 101

U.S. blood donors with elevated aminotransferases, markers of hepatitis C infection were found in only 11 (anti-HCV positive) and 8/11, but no seronegative donors, had HCV RNA detected.[150]

Liver Biopsy Findings in anti-HCV Positive Blood Donors

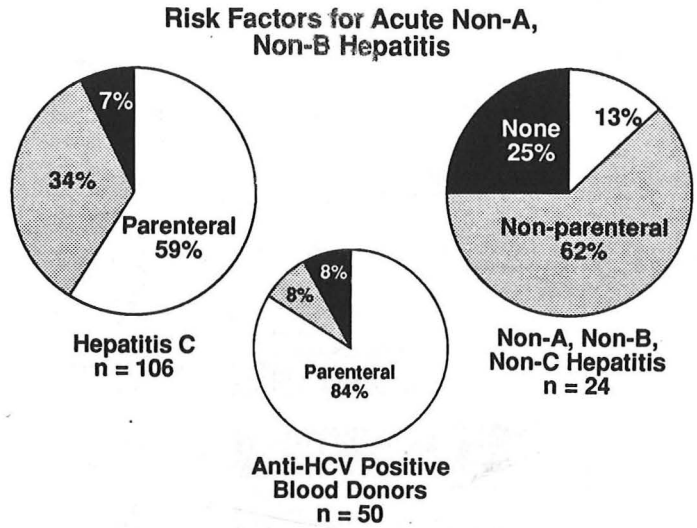


Acute hepatitis C:

At any one time, the number of patients with acute hepatitis C is likely to be the smallest clinical group. However, since everyone of these patients could become chronically infected, they may all represent new additions to the reservoir of infected persons. The incidence of acute non-A, non-B hepatitis in the U.S., derived by the Centers for Disease Control from the Sentinel Counties Non-A, Non-B Hepatitis Studies, is 7.1 per 100,000 and ~80% have hepatitis C markers.[6,122,151]

In the Sentinel Counties study, specific questioning elicited the presence of parenteral risk factors in 59% and sexual or household contact with hepatitis in 6%.[6] An additional 28% had completed less than 12 years of schooling and were therefore classified as "low socio-economic level", a finding previously shown to correlate with non-A, non-B hepatitis cases when compared with controls.[122] In 7% there were no identifiable risk factors. For comparison, in 24 patients with acute non-A, non-B hepatitis that was **not** hepatitis C (non-A, non-B/D, non-C), no risk factors were identified in 25% and only low socio-economic level was identified in an additional 54%. Parenteral risk factors were less frequent (13%) although close contact was similar to those with hepatitis C markers (8%). An investigation of acute non-A, non-B hepatitis in 50 consecutive patients in Dallas,[152] identified parenteral risk factors in 25/34 patients with hepatitis C infection but none in patients without hepatitis C.

Acute hepatitis C, presenting *de novo* for diagnosis and management, is clinically indistinguishable from other causes of viral hepatitis. Following a viral prodrome, non-specific gastrointestinal symptoms of anorexia and nausea usually develop, abdominal pain may occur and jaundice is noted. Laboratory investigations reveal elevated aminotransferases and alkaline phosphatase and elevated bilirubin in most. Results of serologic assays exclude acute hepatitis A and B (IgM anti-hepatitis A virus antibody negative, HBsAg negative, IgM anti-HBc antibody negative) and a careful history and physical examination can aid in excluding drug hepatotoxicity, ischemic hepatitis, biliary tract disease and chronic liver disease.



From: Alter *et al*, *N Engl J Med* **327:** 1899-1905, 1992
McCaughan *et al*, *Med J Aust* **157:** 231-233, 1992

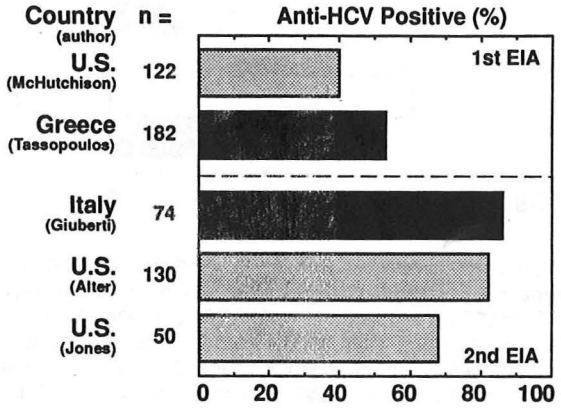
A positive diagnosis of acute hepatitis C can be made by documentation of seroconversion to anti-HCV positivity. When anti-HCV antibodies are positive at the time of presentation, one problem is that of differentiating newly-acquired, acute infection from an exacerbation of underlying chronic infection. Thus, patients with chronic hepatitis C may develop an acute hepatitic illness with re-infection. Such a response is suggested by chimpanzee studies.[107] Viremia occurred after inoculation of different strains of hepatitis C or inoculation with the same strain of hepatitis C after clearance of detectable virus from the circulation, suggesting that protective immunity did not develop.

Some patients with hepatitis C infection and clinically apparent acute hepatitis may not develop seropositivity as measured by current 2nd generation EIA assays.[6] Of 106 patients with acute hepatitis and evidence of hepatitis C infection, 93 (88%) were anti-HCV positive by commercial assay. Every anti-HCV positive patient who was tested (n=21) had HCV RNA in serum by PCR amplification. An additional 9 patients (8%) were anti-HCV negative when tested but had HCV RNA present, as a marker of hepatitis C.[6] In 4 patients who were anti-HCV negative on commercial testing (one of these patients was HCV RNA negative, 3 were not tested), antibodies that recognized hepatitis C viral antigen in liver were present.[6] These antibodies presumably recognize structural or other determinants not present on the linear peptide antigens used in EIA assays. They blocked the binding of fluorescent-labeled human IgG that is reactive with HCV antigens in frozen liver biopsy specimens from experimentally infected chimpanzees.[62] Similar blocking antibodies were also present in 19/19 anti-HCV EIA positive patients and 5/9 patients with HCV RNA but without anti-HCV EIA reactivity. Thus, readily available tests were able to identify 88% of hepatitis C-infected patients whereas research assays were needed for the remaining 12%.

Although hepatitis C infection can now be detected, there are still some unresolved questions concerning the etiology of acute non-A, non-B hepatitis. Hepatitis E virus, the causative agent of enterically-transmitted or epidemic acute non-A, non-B hepatitis, has been identified in Mexico. Furthermore, evidence of hepatitis E infection has been reported in patients with fulminant non-A, non-B hepatitis in the United States.[153] The patients had not travelled outside the U.S. and thus had not been in areas where hepatitis E is endemic. Thus, hepatitis E may account for some cases of non-fulminant, acute non-A, non-B hepatitis. In addition, there may be another parenterally-transmitted agent that causes acute hepatitis.[17] Both chloroform-sensitive (hepatitis C) and chloroform-resistant (non-C

hepatitis) infectious agents transmitted hepatitis to chimpanzees.[17] Intravenous drug users, in particular, and others with continuing parenteral risk factors may be chronically infected with hepatitis C without overt disease, as the blood donors described above. Exposure to another parenterally-transmitted agent, not yet identified, may then explain the acute hepatitic illness.

Hepatitis C in Community-Acquired Acute Non-A, Non-B Hepatitis



The proportion of acute non-A, non-B hepatitis patients exhibiting hepatitis C markers varies with the assay used, geographic origin of the patients and the risk factor profile of the selected patients (see above).[6,68,95,96,154,155] The highest rates are observed with 2nd generation EIA and immunoblot assays and HCV RNA detection in Italy.[68,155] In Los Angeles, anti-HCV positivity was higher in intravenous drug users (31/51, 61%) than in patients with "sporadic" infection (11/50, 22%).[95] Of note, 11 patients with "sporadic" infection in that study had risk factors for hepatitis C (multiple sexual partners, sexual contact with drug users, close contact with hepatitis patients). Hepatitis C infection is usually demonstrated in ~80% of patients with post-transfusion hepatitis, but not 100%, even with the latest assays (see Table).[6,68,69,95-97]

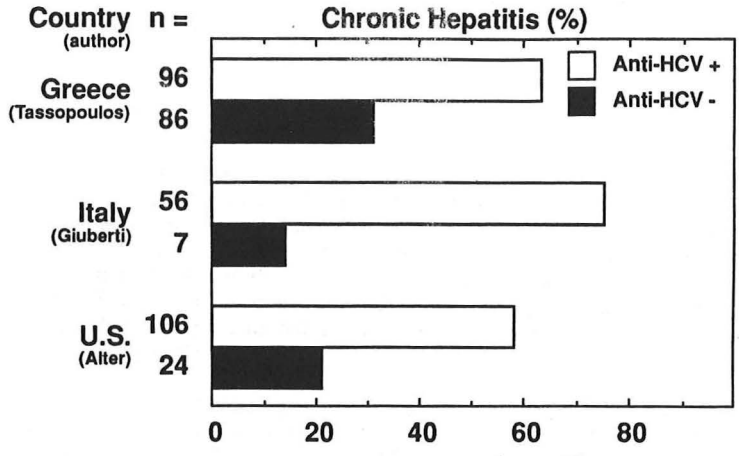
Contribution of Hepatitis C to Post-transfusion Acute Non-A, Non-B Hepatitis

Author	Country of Origin	Assay	Anti-HCV Positive	
			Number	Percent
Aach	U.S.	1st EIA	51/111	46%
		2nd EIA	67/111	60%
			67/74*	91%
Mattsson	Sweden	1st EIA	15/28	54%
		2nd EIA	23/28	82%
Alter	U.S.	2nd EIA	19/25	76%
		Total**	21/25	84%
Wang	Taiwan	1st EIA	20/30	67%
		2nd EIA	23/30	77%
		HCV RNA	24/30	80%

* excludes 37 cases (= 3% hepatitis rate in non-transfusion controls)

** includes HCV RNA and anti-HCVAg positive cases

Chronic Hepatitis Following Acute Non-A, Non-B Hepatitis



Symptoms gradually resolve in the majority of patients. Laboratory investigations, however, may be persistently abnormal for months or years. The percentage of patients with documented chronic hepatitis following acute hepatitis C is usually very high (see Figure above and Table below).[6,68,96,154] Chronic hepatitis following acute post-transfusion hepatitis is no more common than following "sporadic" or community-acquired disease when anti-HCV positive cases are compared (see Figure above and Table below).[6,69,96,97] When anti-HCV tests are non-reactive, chronic hepatitis is less common than when these tests are positive.

HCV RNA could be detected by PCR in most patients with chronic hepatitis (12/13, including 2 intermittent) in the Sentinel Counties Non-A, Non-B Hepatitis Study when tested 42-48 months after the onset of the acute illness.[6] Similarly, HCV RNA was detected in 19/21 Chinese patients who developed chronic disease following an episode of acute post-transfusion hepatitis C.[97] Of greater concern, however, HCV RNA was detected in all 15 patients (intermittent in 2) with normal aminotransferase levels who were tested in the Sentinel Counties study after 42-48 months.[6] These data suggest that hepatitis C infection may persist, even when there is no evidence of clinical or biochemical disease. Whether hepatitis C infection is ever eradicated remains unanswered, but the results from this extensive follow-up of patients with initial clinical acute hepatitis suggests that eradication/cure is rare at best.

Chronic Hepatitis Following Post-transfusion Acute Non-A, Non-B Hepatitis

Author	Country of Origin	Chronic Hepatitis* Anti-HCV Positive		Anti-HCV Negative	
Tassopoulos	Greece	18/32	(56%)	5/11	(45%)
Mattsson	Sweden	14/23	(61%)	0/5	(0%)
Alter	U.S.	14/21	(67%)	?/4	(?%)
Wang	Taiwan	21/24	(88%)	0/6	(0%)

* persistent or intermittent elevation of aminotransferases

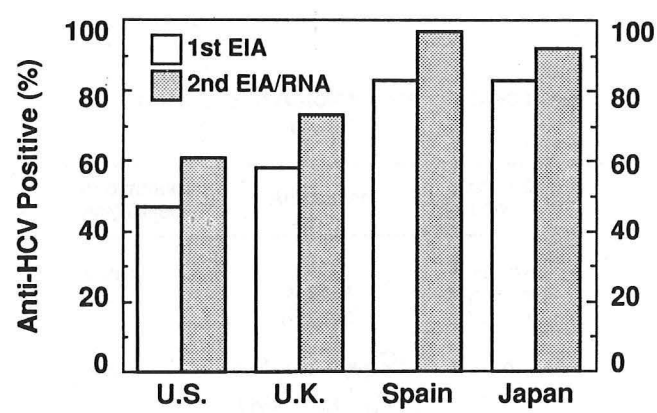
Chronic hepatitis C:

Evidence of hepatitis C infection is found in 60-100% of patients with chronic hepatitis when other causes are excluded and 2nd generation EIA screening tests are used (see Figure below).[67,156-159] Higher rates of hepatitis C infection are observed in Japan and Mediterranean countries and lower rates in the U.S. and the United Kingdom. Measurement of HCV RNA by PCR further increases the number of patients demonstrated to have hepatitis C infection, particularly if the initial screening is with 1st generation assays.[157] Thus, in 156 Japanese patients with chronic non-A. non-B liver disease, HCV RNA was detected in 121/129 (94%) patients with anti-HCV antibodies (1st generation EIA assay) and in an additional 15/27 (56%) patients negative for anti-C100-3 antibodies.

Detection of HCV RNA in patients with chronic hepatitis who are negative for anti-HCV antibodies is apparently uncommon, except in the immunosuppressed population (see below), although there are few data to evaluate.[159-161] In French alcoholics, HCV RNA was detected in 1/5 patients negative for anti-HCV antibodies[160] however no clinical data were provided regarding chronic hepatitis or possible immunosuppression from co-infection with HIV. Of 4 Japanese patients **negative** for both anti-C100-3 and anti-HCVcore (C22) who had chronic non-A, non-B liver disease (1 CPH, 1 cirrhosis, 2 cirrhosis with hepatocellular carcinoma), only one with CPH was positive for HCV RNA by PCR.[161] Similarly, 2 Japanese patients (1 chronic hepatitis and 1 cirrhosis) negative for anti-HCV antibodies (2nd EIA) were positive for HCV RNA by PCR.[159] Different primer sets were required for 1 patient, indicating sequence variability. Anti-HCV antibodies recognizing antigenic epitopes not represented in current assays may be present in such patients, thereby accounting for the negative result on 2nd generation testing.

In patients with anti-HCV antibodies by 2nd generation assay, HCV RNA is very frequently detected.[159,162] In Hong Kong, 67/81 (83%) of patients with anti-HCV antibodies (2nd EIA) were viremic when 2 different primer sets (5′ non-coding and NS4) were used for PCR amplification.[162] In Japan, 89/100 patients with chronic non-A, non-B liver disease were positive for anti-HCV antibodies by 1st EIA, 98/100 by 2nd EIA and 100/100 were positive for HCV RNA by PCR.[159] Chronic hepatitis C infection as an etiologic agent in chronic non-A, non-B liver disease can thus be readily identified in most patients with currently available 2nd generation assays.

Anti-HCV Seropositivity in Chronic Non-A, Non-B Liver Disease



Hepatitis C and other liver diseases:

The potential role of hepatitis C infection in the chronicity or progression of liver diseases considered secondary to other etiologic agents has also been examined. The proportion of patients with ostensibly other liver diseases, but having evidence of hepatitis C infection, is highly related to the country of origin of the patient group. Anti-HCV antibodies were not detected in 31 British patients with primary biliary cirrhosis[50] for example, but were positive in 15/88 Italian patients.[163] The finding of anti-HCV antibodies in patients with alcoholic liver disease is also variable with geographic origin (see below).

Hepatitis C and autoimmune chronic liver disease

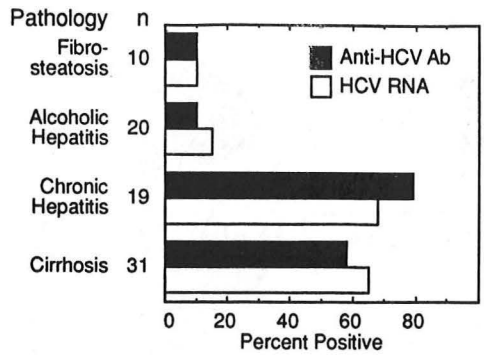
There is an especially complicated relationship between hepatitis C infection and autoimmune chronic liver disease. In Italy, anti-HCV antibodies found in patients with autoimmune chronic hepatitis on 1st generation testing were generally confirmed reactive on 2nd generation assays[164-166] regardless of the type of autoimmunity. Additionally, antibody reactivity was often associated with HCV RNA positivity in Italian patients with autoimmune features (10/15, 67% using single primer set)[165] indicating true infection. Japanese patients with autoimmune chronic hepatitis were also likely to be reactive on both 1st and 2nd generation assays for anti-HCV antibodies, but HCV RNA was less often detected (5/26, 19% using nested primers from the 5′ non-coding region).[167] In contrast, Swedish and British patients with autoimmune chronic hepatitis had a high rate of false positive anti-HCV reactivity that correlated with disease activity and hyperglobulinemia.[50,51,164]

The association between hepatitis C and type-2 autoimmune chronic liver disease suggests a different relationship than false positive anti-HCV or co-existence of both diseases. In type-2 autoimmunity, antibodies to liver and kidney microsomes (anti-LKM) are present. Anti-LKM1 recognizes cytochrome P450IID6[168] and patients with anti-LKM1 antibodies are distinguishable from those with antibodies against liver and kidney microsomes in association with ticrynafen-induced liver disease (anti-LKM2) or infection with hepatitis B and D viruses (anti-LKM3). Patients with anti-LKM1 antibodies frequently have antibodies to hepatitis C virus and HCV RNA detected.[169-172] This is particularly so in older patients, especially men, with low titers of anti-LKM1 antibodies.[171] Younger patients are more often negative for anti-HCV antibodies.[164,171] Additionally, patients with hepatitis C infection and anti-LKM1 antibodies are also frequently positive for antibodies directed against a determinant expressed in infected chimpanzee liver (GOR). The development of autoantibodies in association with hepatitis C infection appears to be confined to a minority of patients in a limited geographic distribution. This may reflect differences in the major histocompatibility complex and the selection of specific peptides for presentation to autoreactive lymphocytes.

Hepatitis C and Autoimmune Chronic Liver Disease

Autoimmune Liver Disease	Antibodies	Anti-HCV Seropositivity
Type 1	ANA A-SMA (F-actin)	Italy - yes Japan - yes U.K. - no
Type 2	LKM1 (younger, ♀)	No
	LKM1 (older, ♂)	Yes
	LKM2, LKM3	?No

Frequency of Hepatitis C Infection in Japanese Patients with ALD



From: Nishiguchi *et al, Hepatology* **14:** 581-589, 1991

Hepatitis C and alcoholic liver disease

Early studies of U.S. patients with alcoholism demonstrated an increased frequency of anti-HCV positivity (27% by 1st EIA) in those with clinical liver disease.[173] There was no such relationship for antibodies to hepatitis B. However, many of the reactive sera were negative by confirmatory immunoblot. The reported decreased survival in those patients with unconfirmed reactivity[173] was unexplained. Antibodies to the hepatitis C virus found in up to 68% of selected Japanese patients with alcoholic liver disease were associated with HCV RNA positivity and increased histopathologic damage (see Figure above)[174] in this study and in a Spanish investigation.[175] In France, 22/62 (35%) patients with chronic alcoholism had anti-HCV antibodies (2nd immunoblot) and HCV RNA was also detected in 18 of the 22 patients.[176] Liver biopsies demonstrated only alcoholic liver disease in 7 patients with viremia but a mixed picture of viral- and alcohol-related pathologic lesions was observed in 5 patients. Infection with hepatitis C thus appears to increase the likelihood of significant liver disease in alcoholic patients. Whether there is an interaction between the pathogenetic processes, however, is less clear.

Hepatitis C and hepatitis B co-infection

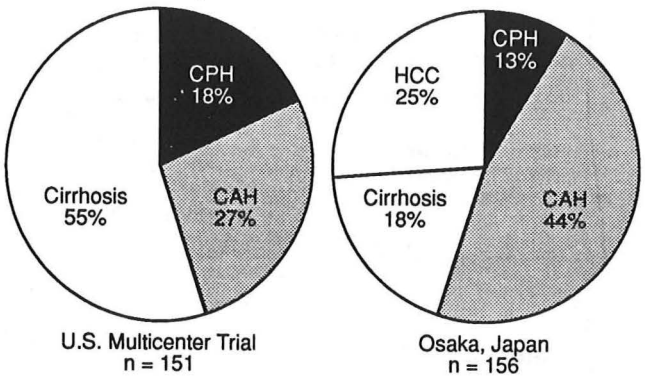
Evidence of co-infection with both B and C hepatitis viruses was observed in 16/148 (11%) patients followed at the NIH.[177] These patients were more likely to have cirrhosis and decompensated liver disease and have HIV infection. In Denmark, 22/69 (32%) patients with acute hepatitis B (IgM anti-HBc antibody positive) had serologic evidence of preceding or past infection with hepatitis C (1st EIA) and 4 were considered to have simultaneous co-infection with both viruses (seroconversion for anti-HCV).[178] All patients cleared HBsAg, however, 2 patients had chronic hepatitis despite being HBsAg-negative, perhaps representing chronic hepatitis C. In Chinese patients with chronic hepatitis B who underwent spontaneous clearance of HBsAg, markers of hepatitis C infection (2nd EIA) were found much more frequently than in matched controls who remained HBsAg positive (see Table below).[179] Other investigators following small numbers of patients have not observed any significant interaction between hepatitis B and hepatitis C.[97]

Association of Hepatitis C with Spontaneous Clearance of HBV Infection

Hepatitis B Disease	HBsAg clearance	Anti-HCV Positive	
Asymptomatic carrier	Yes	8/32	25%
	No	1/64	2%
Chronic hepatitis	Yes	9/22	41%
	No	8/88	9%

From: Sheen *et al, J Infect Dis* **165:** 831-834, 1992

Chronic Progressive Liver Disease in Hepatitis C



From: Davis *et al, N Engl J Med* **321:** 1501-1506, 1989
Hagiwara *et al, Gastroenterology* **102:** 692-694, 1992

Progression of chronic hepatitis C:

At the time of diagnosis, patients with chronic hepatitis C (abnormal aminotransferases during a 6-12 month period of observation and anti-HCV antibody positive) may have any of a range of abnormalities on liver biopsy. Chronic hepatitis was diagnosed in 50/100 consecutive patients with chronic non-A, non-B liver disease admitted to a Japanese hospital, 30/100 had cirrhosis and 20/100 hepatocellular carcinoma.[159] In contrast, of patients with post-transfusion chronic liver disease followed long-term at the NIH (mean 10 yrs), chronic persistent hepatitis was diagnosed in 3/39, chronic active hepatitis in 32/39 and cirrhosis in 4/39 initially and in 8 patients altogether.[180] Patients entered into the U.S. multicenter trial of recombinant interferon-α had chronic persistent hepatitis in 27 (18%), chronic active hepatitis in 41 (27%) and active cirrhosis in 83/151 (55%).[11] Longitudinal studies of patients with post-transfusion chronic liver disease indicate that chronic hepatitis is present on biopsy an average of 10 years after transfusion, cirrhosis by 20 years after and hepatocellular carcinoma at 30 years.[181] Cirrhosis can be present in <2 years in individual patients with particularly aggressive disease.[180]

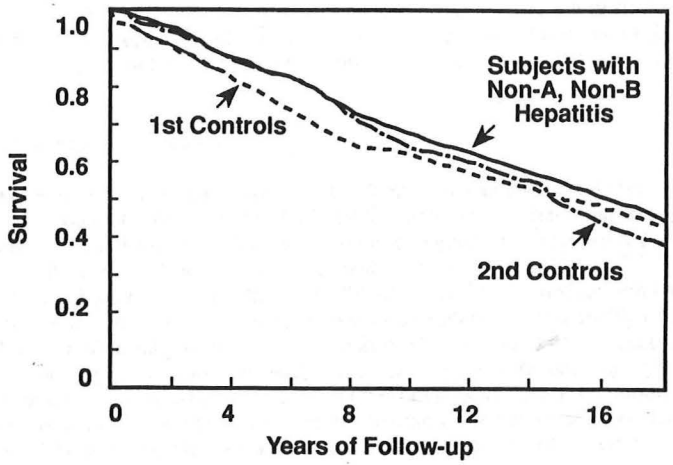
Pathologic comparison of hepatitis C and autoimmune chronic hepatitis has revealed histological features that are more often seen in one or the other disease, but there are no pathognomonic findings (see Table below).[182] The more indolent nature of hepatitis C in many patients, with relatively less aggressive disease and a lower incidence of cirrhosis, was apparent in the comparison. A histological pattern of mild chronic hepatitis with portal lymphoid follicles, mild fatty infiltration and loss of bile ducts is the most common finding.[183,182] Hepatitis C virus-infected cells in human liver have been identified immunohistochemically using monoclonal antibodies to the core, envelope and NS3 regions of the HCV genome.[184] Hepatocyte cytoplasmic staining was detected in liver tissue from 19/48 patients with 1-3 antibodies. Inflammation and fibrosis scores were higher in positive tissue.

Comparison of Histological Features of Chronic Hepatitis

Feature	Hepatitis C	Autoimmune Liver Disease
	n=50	n=21
Bile duct loss	91%	19%
Steatosis	72%	19%
Lymphoid follicles	49%	10%
Piecemeal necrosis	10%	81%
Parenchymal collapse	6%	76%
Cirrhosis	58%	90%

From: Bach *et al, Hepatology* **15:** 572-577, 1992

Long-term Follow-up of Subjects with Non-A, Non-B Hepatitis and Controls: Mortality from All Causes



From: Seeff *et al, N Engl J Med* **327:** 1906-1911, 1992

Long-term outcome of hepatitis C infection:

In many patients, hepatitis C infection may not be symptomatic, even when cirrhosis has developed. Furthermore, overall survival may not be altered by hepatitis C infection acquired from transfusion.[7] Thus, in a follow-up study of patients prospectively diagnosed as having post-transfusion non-A, non-B hepatitis, survival was the same as in controls. There was a modest increase in liver disease-related mortality (total 3.3%, compared to 1.1% and 2% in controls). Alcohol was potentially a co-factor in this increase. These data suggest that, in the majority of patients, hepatitis C infection follows an indolent course. Similarly, when patients receiving organ transplants from anti-HCV positive donors were followed clinically, few developed significant liver disease.[99] In contrast, a subgroup of patients clearly has progressive disease and develops complications of chronic liver disease and hepatocellular carcinoma. This group of patients is most easily recognized by examining patients undergoing liver transplantation.

Hepatitis C and liver transplantation

Patients with hepatitis C infection have been frequently identified when considered for liver transplant programs. In addition, the problem of recurrence of hepatitis C post-transplant has been examined. Of 128 transplant recipients at U.C. San Francisco, 15/30 with chronic non-A, non-B liver disease, 7/19 with a diagnosis of alcoholic cirrhosis and 3/11 with hepatitis B-related liver disease were positive for anti-HCV antibodies (1st EIA and neutralization confirmation).[185] No patients with other causes of chronic liver disease were reactive. In contrast, 105/372 patients transplanted at the University of Pittsburgh were seropositive and there was little difference in the disease categories,[186] suggesting either a high rate of false positivity or the possibility of hepatitis C contributing to the progression of other diseases.

Hepatitis C infection post-transplant can be newly acquired or recurrent infection. These two groups have been separated in studies using PCR to identify patients that have hepatitis C infection pre-transplant.[187-189] In 89 patients with alcoholic or cryptogenic cirrhosis, HCV RNA was detected in 30/35 anti-HCV positive patients and in 6/54 patients negative for anti-HCV.[187] In the post-transplant period, all the patients with anti-HCV reactivity were viremic. In addition, 4/6 patients with detectable HCV RNA pre-transplant but no anti-HCV antibodies were also viremic post-transplant. Another 17 patients had HCV RNA first detected post-transplant. Post-transplant hepatitis in the allograft was diagnosed in 15/34

patients with HCV RNA (44%) but only 1/21 non-viremic patients. Of note, however, a hepatitic pattern was **not** seen on liver biopsy in the majority (19/34, 56%) of these immunosuppressed patients with hepatitis C viremia. In another study, hepatitis C infection was also detected by PCR in 60% (15/25) patients with chronic hepatitis in an allograft, whereas only 7/15 (47%) patients were positive for anti-HCV antibodies.[188] Thus, hepatitis C after liver transplantation, either recurrence or new infection, may be a frequent cause of chronic hepatitis and difficult to diagnose by conventional methods. Long-term follow-up studies are needed to determine the importance of hepatitis C in the survival of liver transplant patients.

Hepatitis C and immunosuppression

When initially evaluated, the rate of hepatitis C infection in liver transplant recipients was underestimated because few patient seroconverted for anti-HCV antibodies post-transplant and some lost pre-existing antibody.[185,187] Similarly, when the transmission of hepatitis C by anti-HCV positive organ donors was examined, anti-HCV antibodies were insensitive markers of infection.[98,100] Loss of anti-HCV antibodies has been observed concomitant with HIV infection[125,190] as well as with immunosuppression for organ transplantation. Whether immunosuppression also alters the relationship between the virus and host is less clear. In individual patients, exacerbation of chronic liver disease occurs with cessation of immunosuppression but in HCV-positive, HIV-positive persons liver disease can be aggressive.[191] The mechanism of liver cell damage in hepatitis C infection is not known. Clearly immunoglobulin is produced by reactive B lymphocytes[192] and specific T lymphocyte responses have also been detected.[193] However, the importance of immunologically-mediated hepatocyte necrosis is not yet determined.

Hepatitis C and hepatocellular carcinoma

Hepatitis C virus infection is associated with the development of hepatocellular carcinoma, as reported by a number of investigators and reviewed recently.[194-200] The highest association with hepatitis C infection has been observed in countries where hepatitis C is common, such as Japan. As noted above, in patients followed long-term with post-transfusion hepatitis, hepatocellular carcinoma is a late occurrence, taking ~30 years to develop.[201]

Treatment of Hepatitis C

Recombinant α-interferon was approved for treatment of chronic hepatitis C following trials demonstrating sustained normalization of aminotransferases in ~25% of patients.[11,12] Selection of patients for α-interferon requires care and consideration. In occasional patients with features of autoimmunity, liver disease is exacerbated by α-interferon[202,203] and occult, autoimmune-related thyroid disease[204] and thrombocytopenia can become overt. Patients with advanced disease may also be less likely to respond,[205,206] as well as being poorly able to tolerate side-effects. Contra-indications to therapy may also include active substance abuse, alcoholic liver disease, hemodialysis, hemophilia, HIV infection, organ transplantation, psychiatric disease and significant cardiac disease.[207] Treating patients that are most likely to respond and benefit from therapy is the goal. Those with persistently or intermittently abnormal serum transaminases and progressive disease (development of increased fibrosis) on liver biopsy are candidates for therapy. Decisions regarding patients with inflammation but no fibrosis (chronic persistent hepatitis) are more difficult. Such patients can be followed and observed without treatment or be offered a course of therapy.[207]

A number of investigators have demonstrated that HCV RNA becomes undetectable or titers diminish in the patients (~50%) responding to α-interferon therapy.[208-213] During relapse, HCV RNA once again emerges. Long-term follow-up has confirmed these findings in patients initially entered in pilot studies.[211] The possibility that HCV genomic type or viral titer may influence the likelihood of achieving remission

has been suggested.[41] Patients with HCV genomes similar to the prototype U.S. strain had higher virus concentrations and lower complete response rates (5/39, 13%) than patients with strains more commonly isolated in Japan than in the U.S. (10/16, 63% and 6/10, 60% complete responses).

Natural β-interferon has also been tested in short-course therapy (intravenous t.i.w. for 4 weeks) for hepatitis C and non-A, non-B hepatitis in Japan.[214,215] In an early pilot study, 5/5 patients with chronic post-transfusion non-A, non-B hepatitis responded initially, with decreases in aminotransferase levels but there were no sustained responses.[214] In 6 patients diagnosed as having acute post-transfusion non-A, non-B hepatitis, 5/6 responded with decreases in aminotransferase levels and at 1 year follow-up all 6 patients had normal transaminases.[214] A second study randomized patients with acute non-A, non-B hepatitis to treatment with β-interferon or no therapy.[215] HCV RNA was detectable in 10/11 treated patients and 12/14 controls initially. After a single 4 week course of therapy, 7 patients responded with sustained normalization of aminotransferases and 3/4 patients, who initially were non-responders and were re-treated after 1 year, also responded. In the control group, only 3 patients spontaneously normalized transaminases, between 10 months and 2½ years after onset. Another randomized study of interferon therapy in acute post-transfusion hepatitis used α-interferon in 15 patients.[216] After 3 months, transaminases were normal in 11/15 treated patients but only 5/13 controls. By 12 months follow-up, there were no significant differences between the groups although more treated patients had normal aminotransferases (8/15, 53% compared with 4/13, 31%). Additional studies of therapeutic regimens for acute disease are clearly needed.

Newer anti-viral agents are undergoing testing. Ribavirin therapy decreased HCV RNA titers and normalized serum transaminases in 4/13 (31%) treated patients, however the response was not sustained.[217] Until better agents are available, α-interferon is the mainstay of therapy.

Hepatitis C and Other Diseases

Hepatitis C has **not** been implicated in a number of diseases that are associated with non-A, non-B hepatitis. Fulminant hepatitis that is the result of neither hepatitis A infection nor hepatitis B infection does not appear to be caused by hepatitis C either, in most instances.[218] HCV RNA could not be amplified from hepatic tissue of patients referred for liver transplantation on the basis of fulminant acute non-A, non-B hepatitis.[218] Occasional cases of fulminant hepatitis or fatal submassive hepatic necrosis in patients positive for anti-HCV antibodies have been reported but they are rare.[154,219] HBV DNA sequences were isolated from some patients with clinical fulminant non-A, non-B hepatitis.[220] These patients subsequently developed hepatitis B in the transplanted liver, suggesting that these patients may have had hepatitis B-related fulminant hepatic failure despite the lack of HBsAg and anti-HBc antibody in the serum.[220] Aplastic anemia following non-A, non-B hepatitis is also **not** associated with hepatitis C infection.[221,222]

Porphyria cutanea tarda, a disease characterized by cutaneous lesions and commonly associated with liver disease, appears to become clinically manifest when extrinsic factors that cause liver disease are present. In Italy, ~80% of 74 patients had markers of hepatitis C infection by 2nd generation EIA and immunoblot assay.[223] Among patients with cirrhosis and either homozygous or heterozygous α1-antitrypsin deficiency, anti-HCV antibodies (2nd EIA) were detected in 62% (33/53).[224] Additionally, cirrhotic patients were HBsAg positive (3/53), consumed excess alcohol (22/53) or had features of autoimmune liver disease (2/53). Markers of hepatitis C infection were positive in only 5% (4/78) patients with fatty liver and either heterozygous or homozygous α1-antitrypsin deficiency. The data suggest that most patients with significant liver disease and α1-antitrypsin deficiency have another etiopathologic factor, in addition to the abnormality in α1-antitrypsin.

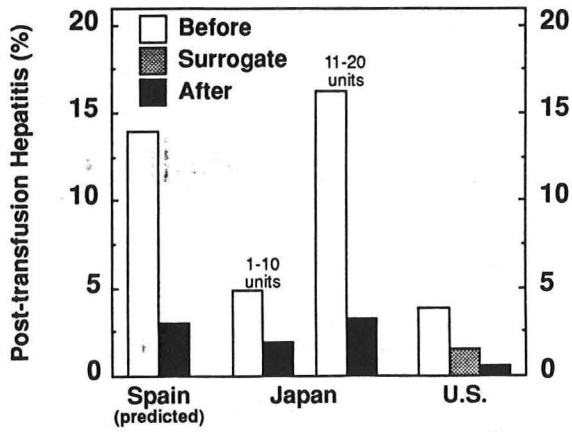
Essential mixed cryoglobulinemia is strongly associated with hepatitis C infection.[225-228] Recent case series have detected HCV RNA and anti-HCV antibodies in the majority of patients with type II and type III mixed cryoglobulinemia, suggesting that circulating hepatitis C viral antigen and antibody may induce the disease in susceptible individuals. Hepatitis C infection has **not** been associated with the chronic fatigue syndrome.

Prevention of Hepatitis C Infection

By screening the blood supply[8,9,229] and by inactivating undetected virus,[230] it is hoped that future transmission of hepatitis C by blood and blood products may be eliminated. Even products with currently low or negligible rates of hepatitis transmission, such as intravenous immunoglobulin, need to be monitored.[231] Introduction of screening tests for anti-HCV antibody detection has resulted in a decrease in the incidence of post-transfusion hepatitis.[8,9,229] Furthermore, the low rate of post-transfusion hepatitis in countries like the U.K. and Sweden can be further decreased by the use of anti-HCV screening, without discarding too high a percentage of donations.[232,233] In the U.S., of 118,396 consecutive donors, 607 were anti-HCV positive (0.5%), alanine aminotransferase was elevated in 2,038 (1.7%) and anti-HBc was positive in 1,507 (1.3%).[234] Only 60 persons were HBsAg positive (0.05%). Selected populations in the U.S. have considerably higher rates of positivity. Of 475 persons in Dallas participating in a voluntary screening program for hepatitis, 23 (5%) were anti-HCV positive, 23 (not coinciding) had elevated alanine aminotransferase levels, 57 (12%) were anti-HBc positive and 4 HBsAg positive.

Post-exposure prophylaxis recommendations for needle-stick transmission are not uniform. In general, intramuscular immune serum globulin is prescribed[235,236] since some studies, including recent investigations,[237] have shown statistical benefit. The prospects for true prevention, vaccine development. are gloomy at the present time. The lack of evidence for sustained clearance of the virus in humans or development of immunity to cross-challenge makes the task of vaccine formulation daunting.

Decrease in Post-transfusion Hepatitis with Screening for Anti-HCV Positivity



Summary

The virus that is the major cause of post-transfusion hepatitis, the hepatitis C virus (HCV), is a single-stranded RNA virus with sequence similarities to the *Flavivirus* and *Pestivirus* genera of the family *Flaviviridae*. Nucleotide changes between different strains of the virus are concentrated in hypervariable regions and may be related to immune selection. Genotypic variants may also be associated with different therapeutic responses. Antibodies to the hepatitis C virus can be detected by assays using recombinant antigens from conserved core, NS3 and NS4 regions in most infected persons or by amplification of RNA from the serum by polymerase chain reaction using oligonucleotide primers from highly conserved areas of the 5′ non-coding reion. Transmission of hepatitis C by parenteral route is frequent, non-parenteral spread by sexual contact or vertical transmission is much less common. Acute infection with hepatitis C can lead to classical acute hepatitis, but most anti-HCV antibody positive subjects have no history of acute disease. Almost all infected people apparently remain carriers of the virus, with varying degrees of hepatocyte damage and fibrosis ensuing. Chronic hepatitis is present after an average of 10 years, cirrhosis by 20 years on average and hepatocellular carcinoma after 30 years, however the range for each stage is very wide. Whether host or viral determinants are more important in deciding outcome is unknown. Following liver transplantation for complications of cirrhosis, hepatitis C infection recurs in the tranplanted liver but is generally not aggressive in nature. Currently, α-interferon, which results in sustained remission of aminotransferase elevation in ~25% of selected patients, is the only available therapy.

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