CHRONIC HEPATITIS C: 1996

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INTRODUCTION

The remarkable development of knowledge about the hepatitis C virus (HCV) since its identification in 1989 is surely a tribute to the power of present-day science.**(1-3)** It is now established that chronic hepatitis C infection is present in 3.5 to 4.0 million Americans and that hepatitis C is by far the most important cause of chronic hepatitis in the United States, Europe and Japan.**(4)** It has been estimated in studies from the CDC that there are 150,000 new cases of hepatitis C in the United States each year and that of these 75%-80% or more develop into chronic infection. Furthermore, it has been estimated that there are 8-10,000 deaths related to hepatitis C in the United States each year.

Estimates of Acute and Chronic Disease Burden for Hepatitis C
United States

Acute	150,000
Fulminant	Not Proven to Occur
Chronic Carriers	3.5 million
CLD Deaths	8-10,000

CDC

In many patients chronic hepatitis C causes progressive liver injury which develops over years.**(5-7)** 20-50% of patients who have chronic hepatitis C develop cirrhosis within 10-20 years of onset.**(8)** There are marked variations in the rate of progression of hepatitis C. Many patients live in peaceful co-existence with the virus showing little or no evidence of underlying liver disease whereas others insidiously develop cirrhosis and subsequently hepatocellular carcinoma. There is no apparent correlation between the mode by which hepatitis C was acquired or the severity of initial illness which is predictive of the subsequent course.

Natural history studies are available for patients who had post transfusion hepatitis C who have been followed for up to 20 years.**(9)** Many of these patients have scant evidence of symptomatic liver disease. There were no differences in survival for a matched group of post-transfused patients who did not have evidence of HCV. However, with time it has become apparent that many of the patients who have hepatitis C are showing late appearance of problems resulting from ongoing liver disease. It appears likely the survival curves will diverge over the next 10 years of

followup with the emergent of more symptomatic cirrhosis and hepatocellular carcinoma. Clearly the national history of hepatitis C for a population is one which spans decades and is characterized by stealth.

Almost immediately following the identification of HCV, it was firmly established that hepatitis C was the principal cause of post transfusion hepatitis.**(3,4)** Widespread testing for HCV and rejection of blood that tested positive markedly diminished the risk of transfusion.

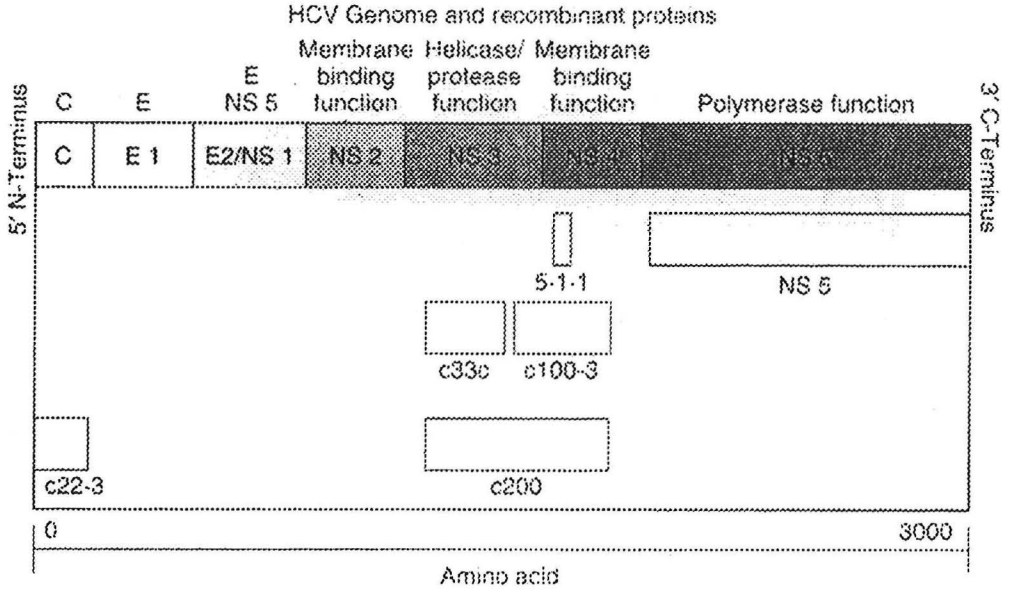
The availability of an accurate test for an antibody to HCV allowed recognition of the contribution of HCV not only to those patients who had post transfusion hepatitis but also to major problems of chronic hepatitis of uncertain etiology.**(10)** Furthermore, the role of co-existent HCV infection and acceleration in many patients who had underlying liver diseases of diverse etiologies was recognized.

THE HEPATITIS C VIRUS

Hepatitis C virus has been completely cloned by molecular biological techniques. Apace with the developments in molecular biology has been widespread application of increasingly accurate tests for antibodies to HCV in patients with a variety of liver diseases around the world.

HCV is a small single stranded RNA virus, 30-38 nm in diameter, with a lipoid envelope.**(11)** The genomic organization of HCV is similar to that of human flaviviruses, a family that includes Dengue virus, Japanese encephalitis virus and yellow fever virus. HCV shows some kinship with animal pestiviruses, a family which includes hog cholera virus and bovine diarrhea virus. HCV does not appear to replicate through a DNA template and integration of the viral genome into host DNA has not been found.

There is evidence that HCV replicates through a negative RNA strand template employing an RNA dependent RNA polymerase. There is one large open reading frame of 9379-9481 nucleotides.**(12)** There is little overall homology between the HCV genome or its encoded polypeptide sequences and other known viral sequences. At the 5' end, there is a terminal region of 329-341 nucleotides with a 92% homology among different HCV types. This region likely has a major function in directing translation of the viral genome, and its highly conserved character make it especially suitable for diagnostic detection of viral nucleic acids by polymerase chain amplification of HCV RNA.



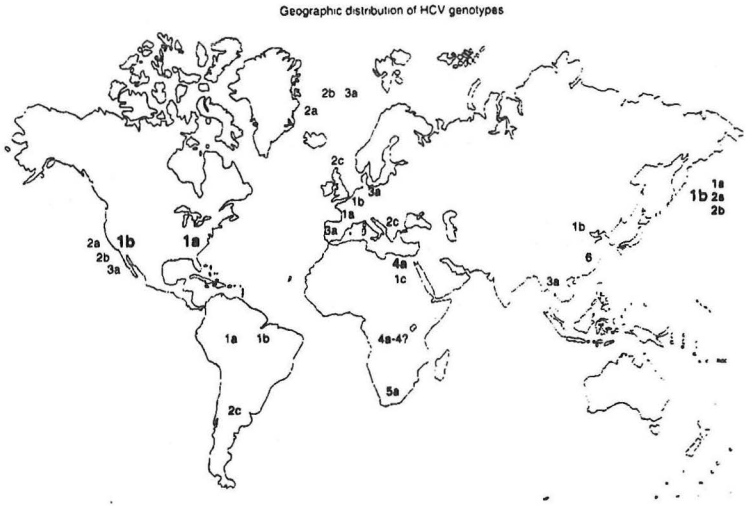
The HCV genome encodes a precursor from which individual proteins are processed through the actions of viral-encoded or host encoded proteases.**(13)** The virion structural proteins are encoded at the 5′ end and the non-structural proteins are encoded at the 3′ end. The nucleocapsid protein has two regions (E1 and E2) which encode for the envelope glycoproteins (gp 33 and gp 70). The E1-encoded glycoprotein definitely codes for an envelope glycoprotein and the E2/NS-1 encoded glycoprotein is probably also involved as an envelope protein. The E2/NS-1 protein corresponds to similar proteins in flaviviruses and pestiviruses. The NS-1 protein has not been shown to be secreted from the cell and is not established as an envelope protein.

In addition, there are four nonstructural regions in the HCV genome. These regions produce proteins with many functions including roles in direction of viral replication. Proteases are encoded by NS2 and NS3. There is a nucleoside triphosphate binding helicase encoded in NS3 that is important in the unwinding of the HCV-RNA genome for replication and a serine protease involved in the processing of non-structural proteins. There is RNA polymerase encoded in NS5 and evidence that within NS5 (region NS5a) that there is a region that influences interferon responsiveness.**(14)**

There is considerable variation at the 3′ terminal end of the genome with marked differences in length and sequence. As noted, the most conserved region is at the 5′ terminal with considerable conservation in the putative core regions. There is a hypervariable region of the virus at the N terminal part of the E2 envelope region. It is thought that mutations in this area are likely important in allowing viral escape from host immune responses.

There are a number of HCV genotypes similar to what has been found in other flaviviruses.**(15)** These genotypes are classified based on sequence analysis at the

5′ region and in the NS5 region. Recent studies report that 9 or 10 genotypes have been identified. The geographical distribution of genotypes around the world is quite remarkable. The specific genotype predominant in a population may relate to the age of the genotype (global dissemination over time) and be influenced by the efficiency of replication and transmission to hosts by many routes.

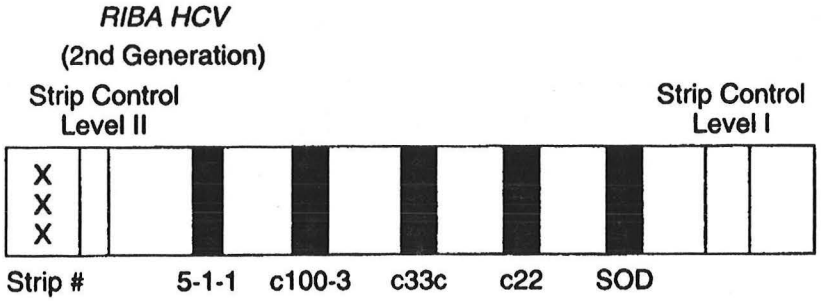


ASSAYS FOR DETECTION OF HCV

Over time, the assays for detection of HCV have become much more precise and specific.**(16-18)** The initial assay detected antibodies directed against sites in the NS (non specific) parts of the genome. The first HCV clone isolated (5-1-1) served as the basis for the production of a recombinant antigen (C 100) which served as the basis for the first generation enzyme-linked immunosorbent assay (ELISA). Subsequently, additional antigens from the putative core and from NS3, NS4, and NS5 regions were added to the test. With the further addition of detection sites, specificity has increased and the number of false positive tests has markedly decreased. At present, in the United States, ELISA II is the test uniformly employed. ELISA III assays are in use abroad and appear to add a small additional degree of specificity to the assay.

More recently, HCV-RNA levels have been quantitated by use of polymerase chain reactions and by a technique designated as the branched chain DNA signal amplification assay.**(16,18,19)** The signal amplification test is less accurate at low levels of virus than are several of the polymerase chain reactions, although constant improvements are being made to enhance detection of virus at lower levels. From studies of HCV-RNA levels and serum in liver tissue, it is established that there is generally a relationship between the serum levels of the virus and the concentration of the virus in the liver.

The diagnosis of HCV is based on the initial finding of a positive HCV antibody test (ELISA II). With the increasing specificity of the ELISA II assay, there is less need for previous supplemental assays such as those provided by recombinant immunoblot (RIBA). RIBA 2.0 detects 4 epitopes: c100-3, 5-1-1, c33c, and c22-3 with a positive result indicated by finding of a reaction to two or more of these epitopes. Many clinicians and investigators are proceeding directly from the ELISA II test to determination of HCV-RNA levels. However, RIBA testing remains useful in patients in whom the diagnosis is in question and in those in whom there are no identifiable risk factors.



HCV GENOTYPES

Once molecular cloning of HCV was achieved, it became apparent that there are several genotypes within the hepatitis C family.**(15,20)** Efforts are being increasingly directed towards determining the predictive value of the genotype in indicating the course of the disease, the likely outcome, and the likelihood that interferon or other therapies might prove successful in eradicating the virus.**(21,22)** Many quasispecies which result from relatively minor changes in the HCV genome have been recognized and are as yet of uncertain significance. These quasispecies apparently result from a failure of HCV to "reproductively proofread" and cull minor variations that develop during replication.**(23)** Immune pressure on viral replication may be important in the production of quasispecies.

PATHOGENESIS OF LIVER DISEASE FROM CHRONIC HCV INFECTION

Present evidence indicates that immune-mediated mechanisms are more important than viral cytotoxicity in the pathogenesis of liver disease from HCV infection.**(24)** One of the histologic characteristics in patients with chronic hepatitis C is the finding of aggregates of lymphocytes (lymphoid follicles) often in portal zones which consist of activated B lymphocytes encircled by a zone of activated T lymphocytes. The portal and periportal lymphocytes are CD8+ indicating the presence of cytotoxic cells.**(25)** Furthermore, lymphocytes which have been isolated from the livers of patients who have chronic hepatitis C have been shown to exhibit cytotoxic reactions

directed towards HCV proteins expressed on the surface of autologous target cells.**(25)**

Of note is that the presence of reactive T cell clones correlates inversely with the titer of HCV and directly with the extent of inflammation suggesting roles for immune mediation in control of viral replication and in the development of chronic liver injury.

It is further established that in many patients the virus lives in a symbiotic relationship within the liver cell in an uneasy truce that intermittently emerges causing episodes of active liver injury.

HCV, once acquired, is rarely eradicated spontaneously and often leads to cirrhosis which has a rather silent onset. HCV is localized in the cytoplasm of hepatocytes.**(26)** The explanations for how the HCV lives for years within hepatocytes and why the virus does not elicit effective antibodies leading to its eradication remain uncertain.

Range of Diseases Caused by HCV

The basic science of HCV is clearly ahead of the epidemiology. It is now established that HCV is associated with an array of hepatic and non-hepatic diseases. From extensive studies of HCV, it has been concluded that HCV is the most common cause of chronic liver disease, cirrhosis, and hepatocellular carcinoma in the United States and makes a major contribution to these disorders throughout the world.**(4)** Hepatitis B infection remains the most important cause of chronic liver disease, cirrhosis, and hepatocellular carcinoma in most of Africa and the Far East with the exception of Japan. Japan, surprisingly, is similar to the United States in that hepatitis C predominates.

As results from tests for the antibodies to hepatitis C were reported from around the world, it became apparent that HCV antibodies are found in patients with a variety of diseases affecting many organs. In addition to patients with chronic hepatitis, cirrhosis and hepatocellular carcinoma, it has been established that HCV is important in the pathogenesis of cryoglobulinemia and membranoglomeronephritis.**(27-30)** The membranoglomerulonephritis results from deposition of cryoglobulin-containing immune complexes containing HCV in the glomeruli. Membranoglomerulonephritis may occur when there is scant or no evidence of liver disease. Furthermore, an association of HCV infection with porphyria cutanea tarda has been established.**(31-33)** Of great interest, has been the observation that one type of autoimmune hepatitis (type II), characterized by the presence of liver-kidney-microsomal antibodies and the absence of antinuclear antibodies, is in many instances associated with, and likely caused by, HCV.**(34)**

Patients who have combined chronic hepatitis C and hepatitis B appear to have worse prognosis.**(35)**

MODES OF TRANSMISSION OF HEPATITIS C

Much more is known about the molecular biology of HCV than about the transmission of the virus.

RISK FACTORS ASSOCIATED WITH TRANSMISSION OF HCV

- **TRANSFUSION OR TRANSPLANT FROM INFECTIOUS DONOR**
- **INJECTING DRUG USE**
- **HEMODIALYSIS (YEARS ON TREATMENT)**
- **ACCIDENTAL INJURIES WITH NEEDLESTICKS/SHARPS**
- **SEXUAL OR HOUSEHOLD EXPOSURE TO ANTI-HCV POSITIVE CONTACT**
- **MULTIPLE SEXUAL PARTNERS**
- **INFANT BORN TO HCV-INFECTED WOMAN**

Retrospective studies have established that hepatitis C virus accounted for at least 90% of post-transfusion hepatitis before the initiation of screening of blood donors in 1989.**(3)** With the widespread use of the virus testing for HCV, the safety of the blood supply has been markedly improved and nowadays transfusion related infections are quite unusual.

The main route of transmission of HCV virus is parenteral.**(4)** In most HCV infected individuals in whom a route of transmission can be established, there is either a history of blood transfusion or the use of intravenous drugs.

Several small epidemics of HCV have been tracked to specific lots of immune globulin in which there was a breakdown in manufacturing techniques.**(36-38)** Nearly all

patients with hemophilia who required blood products before identification of HCV and screening became infected.(39) HCV transmission from patient to patient in hemodialysis units has been reported indicating the need for strict blood content control in these settings.(40) Furthermore, HCV has been transmitted by organ transplantation.(41)

Other parenteral risk factors that have been implicated in transmission include needle stick accidents among health care workers.(42,43) It has been estimated that needle stick accidents lead to transmission of HCV in 3% to 10% of individuals stuck with a needle from an HCV carrier. The risk is markedly lower than that previously found for needle sticks from patients who are carriers of hepatitis B. Reasons for the decreased risk of needle transmission of hepatitis C most likely relates to a much lower density of virus per unit of blood than is the case with hepatitis B. The risk of transmission of HCV from a needle stick is similar in incidence to that for HIV. Medical and dental personnel are a proven higher risk group for HCV infection.(44) In addition tattooing has been identified as a potential route of transmission.(45)

Transmission of hepatitis C within families has received a great deal of attention. Most evidence thus far suggests that transmission from an infected individual to a sexual partner is quite low but possible.(46,47) HCV-RNA has not been identified in semen or saliva from infected patients.(48) Observations from Japan have suggested a higher incidence of transmission within families than has been found in studies from Western countries.

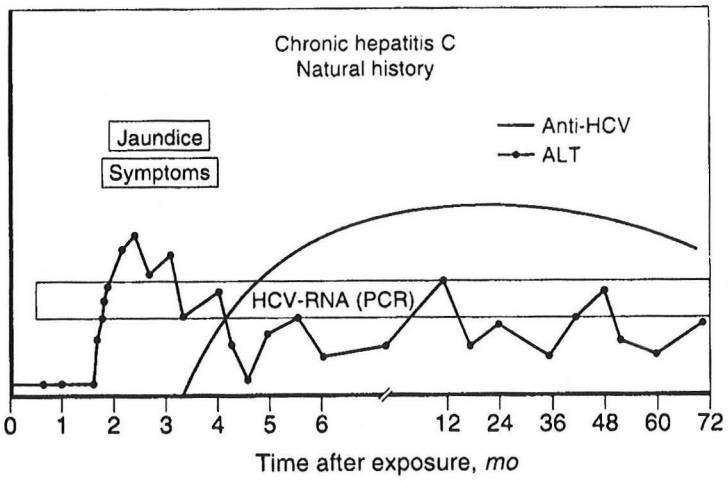
There are occasional reports from mother to infant transmission of hepatitis C.(49-52) Fortunately, as in the case of sexual transmission, only a very few babies have become infected in this setting. It has been suggested that mothers who have HCV and HIV are more likely to transmit hepatitis C, in part related to the higher HCV levels found in the mother in these situations.(53) There is no reason to believe that HCV is transmitted by breast-feeding.

In many patients who hare found to have HCV infection, no satisfactory explanation as to how the disease was acquired ever emerges. Many of these patients come from lower socioeconomic groups with no ready explanation as to how or why infection occurred.(4,54)

NATURAL HISTORY

There appear to be few (if any) effective ways in which a human can eradicate hepatitis C infection once acquired.(55) From CDC studies, it has been suggested that hepatitis C accounts for approximately 21% of all cases of acute hepatitis.(4) Overall, it seems that 80% of patients who acquire acute hepatitis C have persistence of the virus. There are no differences in the expected course or outcome for patients

who have chronic hepatitis C based on the way in which the disease was acquired. Many patients who have a positive anti-HCV test have little if any evidence of injury on liver biopsy, suggesting that there are some "healthy carriers."**(57-59)**



In many patients, the virus persists in an asymptomatic patient and causes little or no liver injury. However, chronic hepatitis is frequent and cirrhosis develops within two decades in 20% to 40% of patients who are chronically infected.**(55)** The time course to the development of cirrhosis and its complications is highly variable. Patients who have been infected for longer than 30 years and in whom cirrhosis has developed are at risk for the development for hepatocellular carcinoma. Hepatitis C has not been proven to be a cause of fulminant hepatocellular failure.**(60,61)**

Chronic hepatitis C infection is often clinically silent and many patients have developed advanced liver disease including cirrhosis with hepatocellular carcinoma without symptoms which suggest the presence of chronic hepatitis.

There are no tests which reliably predict which patients will progress to chronic hepatitis and cirrhosis. Studies are ongoing to determine if genotype analysis is of predictive value. There is general agreement that patients who have type 1b are more likely to develop cirrhosis and are resistant to interferon therapy.**(62-64)** However, it appears that chronic liver disease can develop in patients with any of the currently recognized HCV genotypes.

DIAGNOSIS

Most patients with chronic hepatitis C do not recall having had an episode of acute hepatitis. Many of these individuals are identified by finding elevated aminotransferases in a person who presents himself or herself as a blood donor or is

undergoing a routine medical examination for other reasons. Once an elevated aminotransferase level is found and antibody to HCV identified, the patient undergoes an evaluation to determine the extent of underlying liver disease and the suitability of candidacy for therapy. The most certain way to determine the type and stage of liver disease in a patient who has chronic hepatitis C is by liver biopsy.

Liver Diseases Associated with (or caused) by HCV

HCV is established as the most important cause of chronic hepatitis in the United States. As noted, insidious progression of chronic hepatitis towards cirrhosis is the usual pattern, although in some patients there is accelerated deterioration with rapidly evolving illness. It appears that contemporaneous excessive use of alcohol promotes additive injury.**(65,66)** In patients who have chronic hepatitis C that has progressed to cirrhosis and subsequently is complicated by the development of hepatocellular carcinoma, there are many in whom there has been excessive use of alcohol. There is considerable evidence that co-existent chronic hepatitis B in patients who have chronic hepatitis C leads to additional disease.**(35)**

HCV-Induced Hepatocellular Carcinoma (HCC)

There are studies from the United States, Europe and Japan, which suggest that HCV infection is a more important cause of hepatocellular carcinoma than is HBV.**(67,70)** Even in parts of the world including Southern Africa in which hepatitis B is the dominant viral infection affecting the liver, hepatitis C has been implicated as a cause of HCC.**(71)**

In southern Europe approximately three quarters of patients who have HCC are found to have evidence of HCV infection.**(69)** Similarly high rates of anti-HCV positivity are found in patients from Japan who have HCC while lower rates (<20%) have been reported in the United States.**(70)** Almost all patients who have HCC and are anti-HCV positive have evidence of serum HCV-RNA and most have HCV-RNA in liver tissue both around and within the HCC. The nucleotide sequence of the HCV-RNA within the HCC is identifical with that found in the non-tumor tissue.**(72)**

The mechanisms by which HCV causes HCC are not fully understood. There is general agreement that the most important factor is the duration of infection and the relatively low grade but constant cell turnover resulting from the inflammation.**(73)** Since HCV is an RNA virus with no associated reverse transcriptase. There is no viral integration into human DNA. HCV remains present in cytoplasm. Rarely HCC developed in a patient who has chronic hepatitis C and no evidence of cirrhosis.**(74)**

Patients with certain HCV genotypes (especially 1b) appear more likely to develop hepatocellular carcinoma.**(70)** Most observers believe that HCV-related hepatocellular carcinoma results from long term inflammatory activity with accelerated cell turnover and the constant pressure of hepatic regeneration and remodeling increases the likelihood of malignant transformation. It is not established if HCV effects P53, the tumor suppressor gene, although such studies are underway.

Hepatocellular carcinoma related to chronic HCV infection is usually found in patients in whom it is likely the infection has been present for longer than 20 years.**(70)** Sequential liver biopsies over many years (even decades) in patients who have HCC related to HCV have shown progression from mild chronic hepatitis to cirrhosis to hepatocellular carcinoma.**(73,75)** Occasionally a patient is encountered in whom the time from onset of liver disease to the development of HCC is only 5-10 years. Many of these rapid-onset patients have quite severe active liver disease with an accelerated course which compresses the usual more leisurely evolution.**(73)**

Additional factors which may accelerate or trigger the development of HCC is a patient who has chronic HCV infection include excessive use of alcohol, iron overload and associated porphyria cutanea tarda.**(70)**

There is no proven effective treatment for HCC associated with chronic HCV infection. There are reports from the Far East that suggests interferon therapy may favorably affect the course and prolong survival.**(76)**

CRYOGLOBULINEMIA:

HCV has been implicated as the cause type II cryoglobulinemia and vasculitis.**(27,29,77)** In these patients fatigue, arthralgias and purpura especially prominent over the lower extremity are usual findings. The vasculitis and purpura result from the deposition of the complexes in vascular capillaries. The immune complexes contain cryoglobulinemia consisting of polyclonal IgG and monoclonal IgM rheumatoid factors.**(29)** Hypocomplementemia is an almost constant feature.

Membranoglomerulonephritis

There is an established role for chronic HCV infection in the production of membranoproliferative glomerulonephritis.**(28,30)** The majority of these patients have evidence of cryoglobulinemia. It is established that the renal disease results from the deposition of immune complexes in the mesangium of the glomeruli. These immune complexes contain HCV, anti-HCV IgG and IgM rheumatoid factors. These patients generally have hypocomplementemia and deposition of immune complexes containing IgG, IgM, and C3 on capillary walls and in the subendothelial space. Patients with

HCV related membranoglomerulonephritis may have scant evidence of underlying liver disease. Interferon therapy have proved effective in treatment of membranoglomerulonephritis caused by HCV with a decrease in urinary protein associated with a fall in HCV-RNA. In one patient with HCV-related membranoglomerulonephritis cyclophosphamide therapy was effective.**(78)**

Porphyria Cutanea Tarda

Studies which report a marked association between chronic hepatitis C infection and porphyria cutanea tarda (PCT) have been published and widely discussed.**(31-33,79)** In studies from Spain, it was reported that the majority of patients who had sporadic (non-genetic) PCT have evidence of infection with HCV. How the virus might cause or accelerate the onset of PCT is unknown. One related observation which may prove important is that PCT often improves when iron is removed by venesection even when there is scant evidence that iron stores are markedly expanded. This observation takes on added significance when coupled with others that suggest that there is generally a moderate increase in iron storage in patients with chronic hepatitis C as compared to that found in patients with other types of chronic hepatitis.**(80)** Whether the excessive iron causes injury in addition to HCV, is uncertain. It has been suggested that patients who have excessive iron stores are particularly resistant to interferon therapy.

Polyarteritis Nodosa

Both chronic hepatitis B and chronic hepatitis C have been reported to cause at least some instances of polyarteritis nodosa.**(77)** In these patients evidenced liver disease may be overshadowed by manifestations of polyarteritis affecting skin and other organs. With both viruses, deposition of immune complex containing cryoglobulins and HCV, similar to those found in membranoglomerulonephritis is thought to be the mechanism by which polyarteritis occurs.

Mooren's Ulcers

An association between hepatitis C and Mooren's corneal ulcers has been established.**(81,82)** Interferon therapy has been effective in treating some of these patients.

HCV Related Autoimmune Hepatitis (Type II)

There are several types of autoimmune hepatitis all of which are characterized by hyperglobulinemia.**(34)** Type I, the most prevalent type, is characterized by hyperglobulinemia, female predominance, relentless progression, and presence of antinuclear antibodies. More recently, it has been recognized that there are other types of autoimmune hepatitis including one designated type II which is characterized by the absence of antinuclear antibodies and the presence of anti-liver/kidney microsomal antibodies. It has been shown especially from studies from the Mediterranean Basin that many patients with type II autoimmune disease have evidence of hepatitis C. How the hepatitis C virus might initiate an autoimmune process is unknown, although this observation serves as an opportunity to further study the interaction between viral infection and autoimmune disorders. Interferon therapy has proven more successful than has corticosteroid therapy in the treatment of these patients.

HISTOLOGIC STUDIES IN PATIENTS WITH HEPATITIS C

The present approach to the classification of chronic hepatitis is to independently assess and grade the extent and severity of necroinflammatory activity confined to the portal tract or that which extends into periportal areas.**(34,83)** As necroinflammatory injury becomes more advanced, periportal necrosis gives way to bridging necrosis which disrupts the scaffold of the liver and is a harbinger of cirrhosis. This classification replaces the previous emphasis upon the presence so called piecemeal necrosis, bridging necrosis, and cirrhosis. With more severe cases of chronic hepatitis, there is often confluent necrosis with bridging or multilobular injury.

The staging of chronic hepatitis requires an independent assessment of the extent of fibrosis which indicates the progression of the disease and the development of cirrhosis. If fibrosis is restricted to the portal tracts, it is assessed as mild. At the other end of the scale is cirrhosis.

Thus, the pathology of chronic hepatitis is graded based on necrosis and inflammation judged to be mild, moderate, or severe and also staged on the basis of the extent of fibrosis and extent of distortion of the hepatic architecture.

These distinctions become important in providing a rough assessment of prognosis and expectations of response to therapy. It is apparent that early disease as assessed by the extent of histologic damage responds more fully to interferon therapy. There is often considerable disassociation between clinical signs and symptoms of liver disease and the histologic findings. Since chronic hepatitis C is a smoldering disease that can lead to cirrhosis with few signs or symptoms suggestive of liver damage,

histologic evaluation becomes the most accurate way to stage the disease even when errors that might relate to sampling error of the biopsy are considered. Liver biopsy coupled with laparoscopy reduces the possibility of sampling error.

Additional histopathologic features found in chronic hepatitis C often include a mild to moderate deposition to fat and an increase in iron. Characteristic findings in a patient with hepatitis C include the histopathologic triad of lymphoid aggregates in portal tracts, bile duct changes, and mild to moderate steatosis.**(83-86)** Bile duct damage may be extensive and sometimes difficult to separate from other disorders characterized by biliary destruction.

The cause of significance of the fatty infiltration remains unknown. Fatty infiltration may be found even in patients who deny alcohol use. This issue becomes particularly important when considering the accelerating effect of alcohol-induced injury on the progression of the liver disease in patients with chronic hepatitis C.**(65,66)**

Iron deposition in patients with hepatitis C has attracted a great deal of attention.**(80,87)** There clearly is more iron deposited in these individuals than in patients with other types of chronic hepatitis. The iron content does not seem to correlate with the inflammatory activity of the disease but does with the degree of fibrosis. It has been suggested that there is a decreased response to interferon therapy in patients with chronic HCV who have iron overload.**(80-87)** Efforts have been directed towards discovering if reduction of iron through venesection will enhance the response to interferon.

THERAPY

Interferon therapy is the only proven treatment for chronic hepatitis C.**(34,88,89)** Interferons are glycoproteins produced by cells in response to infections by viruses.**(90)** Antiviral effects of interferon include inhibition of replication of many RNA and DNA viruses through a variety of mechanisms including prevention of virus attachment and uncoating, induction of ribonucleases and amplification of cytotoxic T lymphocytes and natural killer cells as immune responses to the exposure to viral proteins.

Overall, only 20%-40% of patients with chronic hepatitis C in the United States respond to interferon and many of these patients who respond initially subsequently relapse.**(88,89)** Questions as to why some patients respond and many do not has stimulated investigations of new therapies either in addition to or instead of interferon. Treatment responses based on the presence of specific genotypes and possibly quasispecies will likely prove important.

In pilot studies, there was evidence that serum ALT levels decreased promptly in some patients upon institution of therapy in some patients suggesting an anti-viral effect.**(88)** However, in many patients benefits were transient and there were relapses soon after treatment was stopped. In the past decade, there have been multiple studies of interferon therapy in chronic hepatitis C with the end result acceptance and licensing for treatment of interferon alfa 2b. The accepted dose has been 3 million units administered subcutaneously 3 times a week for 6 months.**(92)** The results have been encouraging. The ALT levels return to normal in approximately 40% of patients. Unfortunately, relapse following treatment occurs in 50-80% of successfully treated patients. Relapses are more common in patients who have fibrosis on liver biopsy and in those who have high levels of viremia pretreatment.

In the large multi-center study reported in 1989, it was established that interferon alfa 2b (3 million units 3 times a week) was effective when compared to patients receiving no treatment or those who received 1 million units 3 times a week.**(88)**

The major issue in the interferon treatment trials has been the lack of a durable response with persistently normal ALT levels found in only 15% to 20% of patients after treatment.**(88)** In addition, in many individuals there is detectible viremia despite the biochemical absence of apparent hepatic injury suggesting that interferon likely has an immunomodular effect beyond its anti-viral actions.**(91)**

A number of assumptions and issues under current investigation are as follows:

1. Interferon therapy appears to eradicate hepatitis C in only a small proportion of patients.

2. Interferon is suppressive of hepatitis C virus in some way in many patients. In patients who respond, there are decreases in HCV-RNA levels which can be detected within 4-8 weeks of initiating therapy.**(92-94)**

3. ALT levels decrease <u>after</u> there has been a demonstrable effect on the virus underscoring the concept that the level of HCV virus and the responses the virus engenders causes the liver injury.**(94,95)**

4. The response to interferon depends on many factors (discussed more fully below) with the major ones apparently being the age of the patient, duration of disease, presence or absence of cirrhosis, genotype of the HCV, and HCV-RNA levels.**(96)**

5. The ultimate goal of therapy is to eradicate the virus. It is now established that HCV may be present in cells other than hepatocytes and complete eradication may prove difficult. The completeness of the response of HCV-RNA to therapy has correlated well with the likelihood of long-term response.**(94)**

6. Reconsiderations are underway to determine the optimal dose and duration of therapy with interferon.**(91)** Clearly, for additional progress to be made new drugs must be developed which work instead of or in conjunction with interferon. Escalation of the dose of interferon beyond 3 million units three times a week in a patient who has not responded within 12 weeks of initiation of therapy rarely proves effective.**(97)**

Interferon therapy is associated with a number of side effects including an almost universal early appearance of fever, myalgias, and malaise. These manifestations usually abate over the first few weeks of treatment. Thyroid abnormalities occur with the occasional development of hypothyroidism in 1% to 3% of patients receiving alpha interferon.**(98,99)** It is recommended that TSH levels should be determined before and at the end of a course of interferon. In some patients interferon therapy exacerbates underlying autoimmune diseases. Whether an autoimmune disease might develop as the result of interferon therapy is unknown.**(100)**

Depression to some degree is seen in many patients receiving interferon. The drug is contraindicated in patients who have had major psychiatric problems. Suicidal ideation has apparently not been a problem. However, there is concern with giving interferon to a patient who has a major problem with depression.

Hair loss is rather frequent and fortunately, usually reversible following completion of therapy. In a few patients, the antiproliferative effects of interferon have been associated with a fall in the white blood cell count or platelet count to remarkably low levels. We recommend reducing the dose or discontinuing the drug if the neutrophil cell count falls below $1000/mm^3$ or the platelet count falls below $75,000/mm^3$.

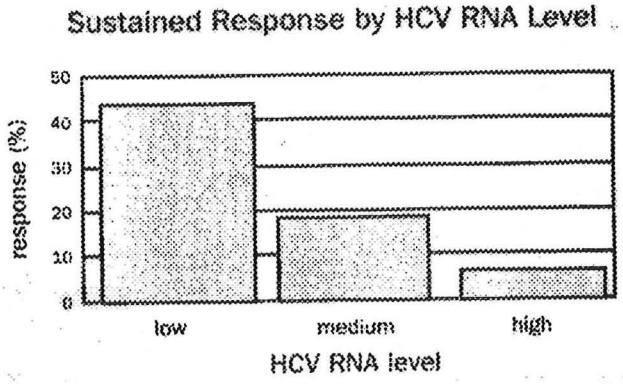
Early studies with interferon alfa 2b suggested that the optimal dose was 3 million units administered subcutaneously three times a week for six months.**(88)** The variables of dose and duration of therapy have been further evaluated. Evidence is conflicting as to whether a higher dose of interferon makes much difference in determining outcome.**(97)** It has been suggested that patients who are obese may respond less well than those who are young. This may in some way relate to a better response seen in females as compared to males.

The duration of interferon therapy appears to be an important determinant of outcomes. One set of observations from France bear special attention. In a multi-study by Poynard and colleagues, patients were treated for 6 months with 3 million units of interferon alfa 2b, 3 times a week.**(91)** These patients were then randomized into three groups with group 1 receiving an additional 12 months of interferon therapy at the same dose; group 2 received 1 million units of interferon alfa 2b for 12 months, and group 3 received no additional treatment unless there was a relapse defined as a rise in ALT for three consecutive months, in which case, 3 million units of interferon 3 times a week was given through the 18th month of the study. There were 100

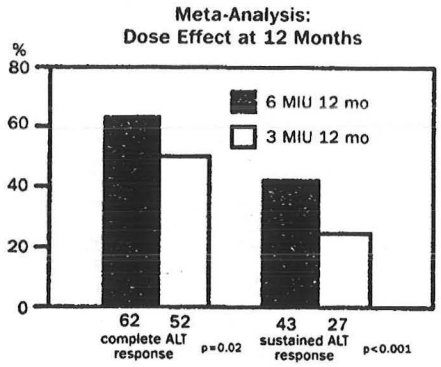
patients in each group. The differences in response as regards ALT levels was quite significant with a 45% return to normal of ALT at 18 months in group 1 compared to approximately 20% in the other two groups. During a two year follow-up after cessation of therapy, the differences in favor of group one remained.

It is of note that the main endpoint in this study was the improvement in histology. Group 1 patients had the greatest improvement. The efficacy was noted as regards pathologic findings of piecemeal necrosis, lobular necrosis, and portal inflammation. In patients evaluated at two years, the incidence of development of cirrhosis in those individuals who began the study without evidence of cirrhosis was 7% in group 1 versus 14% in group 2 and 16% in group 3. There were some patients in whom there was a dissociation between the ALT level and the histologic response. While in most, a fall in ALT was associated with improved histopathology, in a few there was significant histologic improvement despite continued elevations of the aminotransferases. Furthermore, there was a significant reduction in the HCV-RNA level in patients from group 1 when compared to groups 2 and 3.

Further information from this study has important implications. There was improvement of 31% of patients who had genotype 1b versus 60% for other genotypes. Patients who had type 1b were older and had twice the instance of cirrhosis of those with other types. However, it was noted that even in patients with 1b, there remained a significant difference in histology in favor of the longer term regimen.



In a compilation of three randomized controlled trials which employed identical protocols and were conducted in France and the United States, there was a return to normal serum ALT in 41% of the patients during treatment, and 70% of the responders had evidence of histologic improvement.(**101**) The histologic improvement was principally a decrease in inflammation within the liver. The response to treatment was greatest in patients who had mild to moderate hepatitis and did not have cirrhosis.



Poynard, 1996

The French group also reported a meta-analysis of 20 trials comparing interferon with control.**(101)** In these trials there was approximately 1,000 patients. The results supported earlier conclusions that there was approximately a 50% benefit with a complete ALT response and a 20% benefit for sustained ALT response. It was suggested that there was a clear difference in sustained ALT response in favor of 12 to 18 month therapy versus the standard 16 month therapy. Therefore, this group strongly supports a longer therapy of interferon alfa 2b in patients with chronic hepatitis C with a recommended duration of 12 to 18 months.

Interferon: Predictors of Success

There have been careful studies directed towards identifying factors which predict the likelihood of successful treatment of chronic hepatitis C with interferon therapy.**(21,22,62,80,94)** Based on current understanding of chronic HCV especially recognizing the insidious course of the disease towards cirrhosis, all patients who are found to have anti-HCV are candidates for therapy. Liver biopsy provides the most reliable information regarding the likelihood of success. Early disease characterized by inflammation with little or no fibrosis defines a group with the best chance to do well.**(102)** This shift to an emphasis on early therapy has been a gradual one spurred by recognition that patients who have considerable fibrosis and especially those who have cirrhosis are rather unlikely to respond.**(96)** A further observation likely to prove important is that patients who have chronic hepatitis C and persistently normal aminotransferase levels are unlikely to respond to therapy.**(103)**

INTERFERON THERAPY FOR CHRONIC HEPATITIS C: PREDICTORS OF SUCCESS

Host Factors	**Viral Factors**
Sex	Serum HCV RNA level
Age	HCV genotype
Body weight	Sequence diversity in HVR1 Sequence diversity in NS5a
Liver Disease	**Miscellaneous**
Cirrhosis	Hepatic iron
Chronic hepatitis	Immunosuppression
Disease duration	

Three host factors judged to be important are sex, age, and body weight. Females appear to respond more readily to interferon therapy than males. The increased response may relate to a generally smaller body size in the female, therefore, leading to a relatively larger dose of interferon. Furthermore, there is some evidence to which indicates that large patients respond less well which again may relate to a relative decrease in the dose on a per kilogram basis. Younger subjects respond more readily than older patients. However, whether this predictor can be separated from the effects of duration of disease is uncertain.

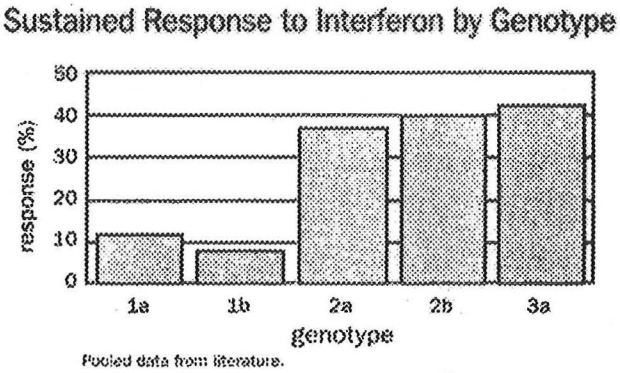
Interferon therapy has been less effective in patients with chronic hepatitis C who have persistently normal aminotransferase levels.**(103)** Interferon therapy has been effective in patients who have co-infection with HCV and HIV when administered before the CD4 count began to fall.**(104)**

There is general agreement that patients with cirrhosis respond much less readily interferon therapy than individuals who have minimal disease or those with chronic hepatitis in whom cirrhosis has not developed.**(96)**

Viral factors

Serum HCV-RNA level and viral genotype have been identified as significant factors in predicting the likelihood of success of interferon therapy. HCV genotype appears to be especially important when evaluating results of trials done around the

world.**(21,22,62)** In patients who have genotypes II or III, treatment response rates greater than 50% have been reported. However, in patients with genotype 1b, the most prevalent genotype in the United States, the results are much less favorable. The extent of diversity of quasispecies may also affect outcome.**(105)**



The pretreatment titer of HCV-RNA is also an important predictor of response.**(93)** Most patients with low pretreatment HCV-RNA levels responded regardless of genotype. Furthermore, it would go well beyond the data to suggest that the use of genotype or viral titer to determine which patients should be offered therapy (or not).

More recently, it has been reported that the extent of mutations in the nonstructural protein 5A gene affects response to interferon therapy in patients with chronic hepatitis C.**(14)** Japanese investigators have determined that a small region (NS5a 2209-2248) in patients with HCV-1b is important in predicting response to interferon. Nonstructural protein 5A is the amino-terminal half of nonstructural protein 5. In the carboxyl-terminal half (NS5b), there is coding for the RNA-dependent RNA polymerase that is important in the replication of the HCV-RNA genome. The function of NS5a is not known. Complete response to therapy did not occur in any of 30 patients whose NS5a 2209-2248 sequences were identical to that of the wild type of virus. However, 5 of 38 patients (13%) who had 1 to 3 changes in this region had complete treatment responses as did all 16 patients who had 4 to 11 aminoacid substitutions indicating that the mutant viruses were significantly associated with complete response. It must be noted that the baseline serum HCV-RNA levels as measured by bDNA signal amplification assay were lower in patients with the mutant type than in other types.

A multi-variant analysis revealed that the number of aminoacid substitutions in the region under study was the only variable associated with an independent effect on the outcome of interferon therapy.(Odds ratio, 5.3:95% confidence interval, 1.6 to 18; $P=0.007$) Whether mutations induced in this region during therapy might affect changes in susceptibility to interferon or even relapse remains to be determined.

ADDITIONAL THERAPEUTIC APPROACHES

Ribavirin

Based on results thus far observed in patients treated with interferon, it is apparent that additional therapies are needed. There is no evidence that patients with chronic hepatitis C who do not respond to the usual doses of alpha interferon will respond to higher doses even if these are given over markedly prolonged intervals. Therefore, there have been searches for alternative approaches. One such approach has been to use ribavirin which is a broad spectrum, oral, guanosine nucleoside analog that has been shown to have activity against DNA and RNA viruses including flaviviridae.

In preliminary studies, oral administration of ribavirin was found to lead to improvement in serum ALT levels in some patients with hepatitis C.**(106,107)** Therefore, additional trials were undertaken. In a randomized double-blind placebo controlled trial from the NIH, 29 patients with chronic hepatitis C received oral ribavirin (600 mg twice daily) for 12 months and 29 controlled patients received placebo for a similar interval.**(108)** There were striking differences observed in response between the two groups. Patients treated with ribavirin had prompt decreases in serum ALT levels (54%) compared with levels before treatment and with the levels noted in the controls who had only a 5% decrease. Ten of the 29 patients had a return of ALT to normal while receiving ribavirin. No control patient had a return to normal. Serum HCV-RNA levels did not change during or after therapy. Liver biopsies were done before and after the trial. There was a decrease in hepatic inflammation and necrosis amongst those patients who responded with a return of aminotransferases to normal in the ribavirin group.

Therefore, ribavirin therapy alone exhibits several beneficial effects in patients with chronic hepatitis C. Why the drug leads to a decrease in aminotransferases and improvement in the inflammatory component of inflammation found on liver biopsy while not affecting HCV-RNA levels is uncertain. One explanation is that ribavirin is immunomodulatory or immunosuppressive.**(108)** It is worth noting that patients on ribavirin often have a fall in the lymphocyte count, although such was not found to correlate with the decrease in aminotransferases in this study. The favorable results appear to last only as long as the drug is given. There was a rapid return to pretreatment levels once ribavirin therapy was stopped. The only side effect of ribavirin of note is the tendency to induce hemolytic anemia which may prompt an additional adverse effect in patients with chronic hepatitis C from the deposition of the iron within the liver.**(109)**

The encouraging aspects of these studies and small preliminary trials has led to the initiation of a randomized double-blind trial in which ribavirin and interferon will be given in combination and compared to interferon therapy alone.**(110,111)** It is established that in patients in whom interferon leads to an improvement in

aminotransferases that there is a concomitant decrease in the viral titer. Possibly, there will be favorable additive effects of these two agents.

Ursodeoxycholic Acid

Ursodeoxycholic acid has been shown to induce a reduction in serum aminotransferases in many patients with chronic hepatitis C.**(112-114)** The drug appears to have an anti-inflammatory effect as opposed to an anti-viral effect. There is no evidence that ursodeoxycholic therapy leads to a decrease in the HCV-RNA level in the serum or in the level of detectable virus in tissue. The benefits from ursodeoxycholic acid last only as long as the drug is given and therefore is similar to the situation when ursodeoxycholic acid is used in other liver diseases particularly cholestatic disorders.

Other Agents

Indomethacin, acetylcysteine and silymarin have all been suggested as adjunctive therapies in patients with chronic hepatitis C. None of these has been proven to be of benefit. Trials are underway to determine if removal of iron by phlebotomy will enhance the effectiveness of interferon.**(80,87)** While results from early small serves are encouraging, it is premature to conclude that this approach is ready for use in practice.

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