Hep

HEPATITIS C:
A WORK IN PROGRESS

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Clinical and Research Interests:

- Viral Hepatitis
- Autoimmune Hepatitis
- Alcohol-Induced Liver Disease
- Primary Biliary Cirrhosis
- Drug-Induced Liver Disease

The recognition that hepatitis C is the most important cause of chronic liver disease in the United States and many other parts of the world has become abundantly apparent over the past decade. (4, 32, 76) The latest estimates from the CDC indicate that almost 4,000,000 people in the United States have chronic hepatitis C with 8,000 - 10,000 deaths occurring each year from complications of the disease. (4) Appreciation of the importance of hepatitis C burst on the scene as the 1990s began following development of a reliable test (anti-HCV) to detect the presence of the hepatitis C virus. In a remarkable display of the power of clinical investigation, in less than a decade it has been shown:

1. That hepatitis C was the most important virus causing post-transfusion hepatitis and that testing blood donors for hepatitis C and removing these individuals from the donor pool made the blood supply remarkably safer.

2. That most patients diagnosed with hepatitis C are young and middle-aged adults between the ages of 30 and 49 years.

3. That users of illicit drugs who share needles almost all become infected with HCV, often acquiring the virus within the first several months of drug use.

4. That chronic hepatitis C is more prevalent in African-Americans and Hispanics than in whites.

5. That many patients who had increases in serum aminotransferase levels hitherto considered insignificant actually had chronic hepatitis C and that hepatitis C is among the leading causes of elevated aminotransferase levels in asymptomatic individuals.

6. That chronic hepatitis is the leading cause in most centers of the cirrhosis which leads to liver transplantation.

7. That the natural history of chronic hepatitis C is usually one of slow progression with much of the liver damage occurring in an insidious fashion below the level of clinical recognition.

8. That chronic hepatitis C induced cirrhosis is an important - likely the most important- precursor to the development of hepatocellular carcinoma in the United States.

9. That effective therapy based on the use of interferon leads to favorable sustained biochemical and virologic responses in some patients. Use of combination therapy of interferon and ribavirin has been associated with a 30%-40% sustained virologic response defined as no detectable HCV-RNA six months after the completion of therapy.

10. That further therapeutic approaches under investigation include induction (daily) versus thrice weekly interferon and the use of high dose interferon as well as additional approaches focused on identifying protease inhibitors, helicase inhibitors, and the potential use of ribozymes and antisense oligonucleotides.

11. That prospects for an effective vaccine in the near future are not bright.

FEATURES OF HEPATITIS C VIRUS

HCV is a lipid-enveloped single stranded RNA virus of the Flaviviridae family. (32) The genome for HCV has been completely cloned, and consists of approximately 10,000 nucleotides in a single open reading frame encoding a large polyprotein that subsequently processes into at least 10 proteins.

There are several (at least 6) rather distinctive genotypes of hepatitis C, and there is considerable variation in genotype distributions around the world. Specific genotypes appear to differ somewhat as to the natural history of the liver disease each causes, and influences the likelihood of response to interferon or interferon-ribavirin therapy. (112)

In addition, HCV rapidly changes under immune pressure with the development of multiple quasispecies thereby creating in an individual patient a heterogeneous population of viruses. (48) These quasispecies result from minor variations which are sufficient to change the virus in some ways but do not interfere with the ability to replicate. The RNA polymerase introduces random errors at a high rate with some sites highly conserved and others especially prone to variability (hypervariable regions). A patient may have numerous quasispecies at any time and the profile may shift dramatically during attempts at therapy. Quasispecies vary in resistance to treatment and in determining the course of the disease. The rapid emergence of quasispecies have added to the major problems (thus far insurmountable) in the development of an effective vaccine.

EPIDEMIOLOGY AND TRANSMISSION

Hepatitis C is most readily transmitted as a blood borne disease and is spread primarily through percutaneous and mucosal exposures. (3,72) Many patients have been identified with advanced liver disease from hepatitis C in whom the only risk factor likely to have been responsible for acquisition of hepatitis C was a blood transfusion before 1990 when widespread and subsequently mandatory testing of blood for anti-HCV was introduced. Patients who have used illicit drugs and have shared needles, even casually and on few occasions many years before, are at extremely high risk of acquiring hepatitis C with 50% - 60% of new drug abusers infected within 6 months of beginning drug use, and 90% infected by a year. (43)

It is apparent that there are routes of transmission of hepatitis C other than use of illicit drugs or transfusion which have not been as well defined. For example, utensils used for body-piercing, tattoos, and acupuncture may transmit HCV if proper precautions regarding sterilization are not taken. There are similar concerns of transmission for those who share razors and toothbrushes with infected persons. Of more recent vintage is the observation that the "straws" used for intranasal cocaine administration may have blood on the tip and thereby when shared become a vehicle for transmitting hepatitis C. (26)

There is some evidence that hepatitis C can be transmitted (albeit rather inefficiently) by close personal contact such as is found in sexual relationships. (3) Individuals who have had multiple sexual partners appear to be at increased risk, although it is uncertain which of several often associated activities leads to transmission. Fortunately the risk of sexual transmission is apparently quite low (much less than 5%) in monogamous relationships in the United States, and it is quite unusual to find the sole sexual partner of a person infected with hepatitis C to have evidence of the disorder.

Individuals who have hepatitis C and who have multiple partners are well advised to pay special attention to safe sexual practices. In situations in which sexual partners are found to be hepatitis C positive, it is often rewarding to take a detailed history looking for evidence of other risk factors such as previous experience with the use of illicit drugs.

In a few instances (less than 5 percent), an infected mother may transmit hepatitis C to her neonate. (66,101) This low rate of maternal to fetal transmission is in contrast to the >90% transmission to a neonate from a mother who has chronic hepatitis B and is HbeAg positive. It appears that mothers who are infected with HIV and HCV are more likely to transmit HCV. (66) It is recommended that a baby born to a mother who has chronic hepatitis C should be tested for the presence of the virus at one year of age by which time maternally acquired antibodies are no longer present. There is no evidence that any form of gamma globulin is of use in preventing transmission of hepatitis C. The mode of birth (vaginal versus Caesarean section) does not appear to affect the likelihood of transmission. There have been no recorded instances of transmission of hepatitis C by breast feeding. (66)

Fortunately there have been few well-documented instances of transmission of hepatitis C in medical settings, either from patient to physician or vice versa. However, even a few is cause for concern. There are increased risks of transmission in certain situations, such as for surgeons who may cut themselves while performing procedures in an infected patient. (39) Exposure to wire sutures or the sharp edges found on resected bone present special risks.

RECOMMENDATIONS TO AVOID HCV TRANSMISSION

- Avoiding household transmission
  - -- No sharing of toothbrushes or razors
  - -- Cover open wounds
  - -- No need to avoid close contact with family members
  - -- No need to avoid sharing meals or utensils
- Pregnancy not contraindicated
  - -- Risk of transmission to baby <5%
  - -- Breast-feeding considered safe
- Needle-exchange programs
- Avoiding tattooing and body-piercing under unsterile conditions

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CDC studies have suggested a higher incidence of hepatitis C in patients from lower socioeconomic groups although the interpretations of these observations are uncertain. (3,4) There are many patients in whom it is not possible to establish with any certainty how hepatitis C virus was acquired.

PREVALENCE OF HEPATITIS C

The number of cases of acute hepatitis C appears to be dropping likely through mandatory testing of blood and blood products, and possibly through increased awareness of hepatitis C in users of illicit drugs and participation in needle exchange programs. The latest CDC estimates indicate that there are at present 25,000 to 35,000 new cases a year in the United States as compared to estimates of

150,000 to 175,000 a year at the beginning of the 1990s. (4) The CDC estimates that there are 3.9 million persons infected with the hepatitis C virus in the United States with an overall prevalence of 1.8%. Chronic infection is estimated to be present in 74% (2.7 million) of the 3.9 million infected individuals, 73.7% of patients with hepatitis C in the United States have genotype 1. The majority of infected individuals are in the 30 to 49 year age group.

It is estimated that the number of patients who present with decompensated liver disease from infections acquired 20 or more years ago, will increase dramatically over the next several years as the cohort of already infected patients move through life. Suggested explanations for the cohort effect of hepatitis C include entry into the pool of many of patients who used illicit drugs, even if only occasionally, beginning in the 1960s and 1970s as well as the addition of patients who were transfused prior to 1990.

DETECTION OF HEPATITIS C

GROUP 1: ROUTINELY TESTED

Risk History (Lifetime)

- Persons who injected illegal drugs, even once
- Clotting factor recipients (pre-1987)
- Transfusion or solid organ recipients (pre-July 1992)
- Persons notified that they received blood from donor who later tested HCV-positive
- Long-term dialysis patients
- Persons with evidence of liver disease
- Health care workers exposed to anti-HCV antibody – positive blood
- Infants >12 months old born to HCV-positive women*

GROUP 2: UNCERTAIN NEED

- Intranasal cocaine and other noninjection illegal drug users
- Persons with history of tattooing and body piercing
- Recipients of transplanted tissue (eg, corneal, musculoskeletal, skin, ova, sperm)
- Long-term sexual partners of HCV-positive persons

GROUP 3: ROUTINE TESTING NOT NECESSARY

Risk History (Lifetime)

- Men who have sex with men
- Health care workers
- Pregnant women
- Household contacts of infected persons

CDC = Centers for Diseases Control and Prevention; HCV = hepatitis C virus;
NA = not available.
* Tests are unreliable in children under 1 year.

IMPACT OF IDENTIFICATION AND REMOVAL OF INDIVIDUALS WHO ARE ANTI-HCV POSITIVE FROM THE BLOOD DONOR POOL

Following the introduction of mandatory testing of blood donors, there has been a remarkable decrease in the number of patients who acquire hepatitis C through transfusion of blood products. (4,45) Post-transfusion hepatitis C is now a rare event. Through educational programs emphasizing the risks of sharing needles in the transmission of HIV, hepatitis B and hepatitis C, there appears to be some impact on hepatitis C transmission among users of illicit drugs.

PROGRESSION FROM ACUTE TO CHRONIC HEPATITIS

A major problem for patients who acquire hepatitis C virus is the remarkable frequency with which the acute infection does not resolve and leads to chronic infection. Whereas immunocompetent adults who acquire hepatitis B have only a 1% to 7% chance of becoming a chronic carrier of hepatitis B, patients who develop acute hepatitis C have a 75% or more likelihood of retaining the hepatitis C virus. (32) Once chronic hepatitis C develops, there is scant evidence that spontaneous remission ever occurs. Cirrhosis develops in 20% to 30% of patients with chronic hepatitis C within 20-30 years. Hepatitis C virus causes liver injury both through direct viral effects and in eliciting a host immune response. (7,20,21,23)

Several preliminary studies suggest that recognition of hepatitis C during the acute phases of the illness might have therapeutic implications. It is possible (even likely) that institution of interferon therapy immediately upon recognition of acute hepatitis C may prevent viral retention. However, it is often difficult to recognize acute hepatitis C. (3,32) Only a few of these patients have evidence of jaundice, abdominal pain or other signs pointing toward an acute liver disease. A vast majority of patients who are discovered to have hepatitis C are already well into a chronic phase at the time of diagnosis. Interestingly there have been few, if any, cases of acute liver failure (fulminant hepatitis) which can be reliably attributed to hepatitis C virus. However, there are reports of a fulminant course if a patient who has chronic hepatitis C acquires a superinfection with hepatitis A. (106)

A STEALTHY AND OFTEN RATHER SILENT CLINICAL COURSE

It appears that HCV generally has a relatively unannounced onset, then quietly sets up residence within the liver cells and leads to ongoing chronic hepatitis. (59,96,97,102,103) The chronic hepatitis is often present with few if any symptoms. Many individuals are surprised (and often devastated) when told of the presence of hepatitis C following evaluation of an elevated aminotransferase level found at the time of attempting to donate blood or obtain life insurance. The symptoms of chronic hepatitis C, when present, are usually quite non-specific. Many are asymptomatic whereas others experience at most slight to moderate fatigue. However in some, the fatigue associated with hepatitis C is sufficient to limit activities. The chronic hepatitis may remain clinically silent and stealthily progress to cirrhosis over many years.

THE IMPORTANCE OF CIRRHOSIS

There is general agreement that 20%-40% of patients who have chronic hepatitis C will progress to cirrhosis often detected after 20 or more years of infection. (32,59,97,102,103) There are considerable variations in individual patients. However once cirrhosis is present the patient is in a precarious state. In a follow-up study of 384 European cirrhotic patients who had chronic hepatitis C, the survival rate probably was 91% at 5 years and 79% at 10 years. (40) The 5-year risk of developing hepatocellular carcinoma was 7% and the risk of hepatic decompensation was 18%.

There is no question that chronic hepatitis C is a disease associated with considerable morbidity and mortality once cirrhosis develops. (80)

EXTRAHEPATIC MANIFESTATIONS OF HEPATITIS C VIRUS INFECTION

While hepatitis C virus is an infectious disease with a propensity to cause liver injury, the virus does cause a variety of diseases with clinical manifestations apart from liver disease. Hepatitis C is a leading cause of cryoglobulinemia. (1,32) In this syndrome, patients often present with fatigue, arthralgias, and an erythematous raised rash over the lower legs. In some patients with hepatitis C induced cryoglobulinemia, there is scant or no evidence of liver injury. Furthermore, hepatitis C is an important cause of membranoglomerulonephritis accounting for 10% to 20% of patients with this condition. (42) In membranoglomerulonephritis, as in cryoglobulinemia, it appears that deposition of hepatitis C containing immune complexes causes the disorder. (56) Therefore all patients who present with significant proteinuria should be tested for the presence of anti-HCV. Conversely all patients who have hepatitis C should have a test for urine protein.

There is an increased incidence of hepatitis C virus in patients who have porphyria cutanea tarda (PCT) suggesting the virus may cause or unmask the disease. (78) These patients often have evidence of chronic hepatitis C infection in addition to characteristic bullae on sun exposed parts of the body. How the hepatitis C virus might lead to the abnormalities in porphyrin metabolism and subsequently to the clinical manifestation of PCT is unknown. It is of note that many patients who have PCT are chronic alcoholics. One of the first lines of treatment for PCT is the removal of iron through venesection. These observations are of further interest when it is recalled that alcohol is an important factor in promoting the progression of hepatitis C and that hepatitis C is the only viral induced liver disease which is regularly associated with increased deposition of iron in the liver. There are interactions between HCV, alcohol, iron, and porphyrins which need to be further elucidated.

More recently an association between hepatitis C and diabetes mellitus has been suggested. (49,65) A further association is the appearance of Mooren's corneal ulcers in patients who have chronic hepatitis C. (109)

EXTRAHEPATIC MANIFESTATIONS OF HCV (1,19,42,56,65,78,109)

- Extrahepatic manifestations or syndromes (immunologic origin)
  - -- Essential mixed cryoglobulinemia
  - -- Membranes
  - -- Glomerulonephritis
  - -- Arthritis
  - -- Keratoconjunctivitis sicca
  - -- Lichen planus
  - -- Porphyria cutanea tarda
  - -- Mooren's corneal ulcers
  - -- Diabetes mellitus

HEPATOCELLULAR CARCINOMA

Chronic hepatitis C induced cirrhosis is an important risk factor in the development of hepatocellular carcinoma. (25) The constant stimulation of cell damage over many years, followed by regeneration and repair, ultimately leads to cirrhosis in approximately 20% of patients within 20 years. Hepatocellular carcinoma rarely if ever occurs in a patient who has chronic hepatitis C who does not have cirrhosis. In patients who have HCV-induced cirrhosis, the risk of developing hepatocellular carcinoma is estimated to be 1% to 4% a year. (40) Men appear to be at increased risk. Patients who have chronic hepatitis C and cirrhosis should be regularly monitored by ultrasonography and determination of alpha-fetoprotein to facilitate detection of early hepatocellular carcinoma.

HEPATITIS C AND HEPATOCELLULAR CARCINOMA (HCC)

- Chronic HCV infection increases risk of HCC
  (1%-5% per year after 20 years, especially in patients who develop cirrhosis)
- Most HCV-related HCC occurs in presence of cirrhosis
  - -- Rate of development of HCC after cirrhosis increases to 1%-4% year
- HCV-related HCC more common in men and in older Patients
- Up to 70% of patients with HCC are HCV-infected

DIAGNOSIS

Hepatitis C is most often diagnosed following evaluation of a patient found to have elevated aminotransferase levels. Prior to the availability of serologic tests to detect hepatitis C, many clinicians did not regard slight elevations in aminotransferase levels in asymptomatic patients to be of much consequence. There are surely many causes for slight aminotransferase elevations including obesity and reactions to a variety of therapeutic drugs (including alcohol). However, the prevalence of hepatitis C is high enough to warrant screening all patients who have elevated aminotransferases for the presence of anti-HCV.

It must be noted however that not all patients with hepatitis C have elevated aminotransferase levels. (32) Normal aminotransferase levels are found at least intermittently in 30% to 50%. It is acknowledged that patients who have hepatitis C and normal aminotransferase levels generally (although not invariably) have less liver disease than those who do not have elevations. (67) However, significant on-going liver injury may occur even in those in whom ALT values remain within the normal range or are only slightly elevated and progressive fibrosis may occur.

The second generation EIA test provides a highly accurate inexpensive, automated way to screen patients for hepatitis C. The test is 92% to 95% sensitive using determination of HCV-RNA presence by PCR as the standard. In patients who have elevated aminotransferase levels and a history of behavior that suggests a posible (or likely) exposure to hepatitis C, a positive anti-HCV test almost always indicates the presence of hepatitis C. (98) However in patients in whom a positive test is found in asymptomatic blood donors in whom there are no risk factors, only 25% to 60% of individuals who test positive on an initial screening test are subsequently shown to have hepatitis C. It is in this latter group of low-risk individuals who have a positive EIA that supplemental tests including radioimmunoblot assay (RIBA) are useful. Within the RIBA test, there are immunoassays against individual epitopes of the hepatitis C virus.

DIAGNOSTIC TESTS FOR HCV INFECTION

TEST	USE
Anti-HCV antibody (EIA)	Initial diagnosis
Supplementary RIBA	Resolve a doubtful EIA result, particularly false-positive in low-risk populations (eg: volunteer blood donors who have no risk factors)
Qualitative PCR for HCV RNA	Resolve indeterminant RIBA and confirm ongoing infection with virema
Quantitative HCV RNA tests (PCR,bDNA)*	Determine level of serum HCV RNA

EIA = enzyme immunoassay; RIBA = recombinant immunoblot assay;
PCR = polymerase chain reaction; bDNA = branched DNA
* Is less accurate at lower levels of HCV-RNA.

RT-PCR and bDNA techniques are available to measure the presence and, in certain circumstances, to quantitate the amount of hepatitis C virus. There are a several RT-PCR based tests on the market and these vary in the primers used. The advantage of the RT-PCR test relates to its sensitivity. Disadvantage of the RT-PCR based tests have included the variability in the performance and lack of reproducible results, especially from laboratory to laboratory. The bDNA technology, which employs a signal amplification technology, has proven quite reliable. (32) However, the disadvantage of this approach is that it is not as accurate at detecting low levels of virus as are RT-PCR methods.

HCV RNA TESTS

- Polymerase chain reaction (PCR)
  - -- Qualitative and quantitative types
  - -- Qualitative most sensitive and specific to detect HCV viremia
  - -- Quantitative especially useful to measure viral load before, during, and after treatment
- Branched DNA amplification (bDNA)
  - -- Quantitative, but less sensitive than PCR method
  - -- Diminished sensitivity at low levels of HCV-RNA limits usefulness (concentrations $<2 \times 10^5$ Eq/mL undetectable)
  - -- Easier to perform, less variability in results than PCR tests

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ROLE OF LIVER BIOPSY

The liver biopsy is important in the evaluation of chronic hepatitis C in providing an accurate determination of the stage of the underlying liver injury. (5,32,76) Once a patient with chronic hepatitis C develops cirrhosis, there are considerable short and long-term risks of hepatic decompensation. Clinicians are often surprised to find evidence of more extensive liver disease on liver biopsy than was clinically suspected in a patient who has chronic hepatitis C. The insidious nature of the disorder and the clinically silent development of fibrosis can lead to a clinically unsuspected cirrhosis. It must be noted that there is no such thing as "benign cirrhosis". Any patient who has the structural rearrangements of the liver found in cirrhosis with the resultant changes in blood flow is at considerable risk of decompensation. The development of cirrhosis is the most important negative milestone in the natural history of hepatitis C. A further occasional dividend of liver biopsy in patients who have hepatitis C is in the identification of disorders other than hepatitis C that may be present.

HISTOLOGIC FINDINGS

There is a continuum of liver disease in patients with chronic hepatitis C, which ranges from mild inflammation to advanced cirrhosis. In mild disease the inflammation is quite localized to the portal triad and the adjacent periportal regions. In moderate disease, in addition to the portal and periportal necrosis, areas of interlobular focal necrosis are often evident. As the disease progresses, increasing fibrosis develops with the end result of cirrhosis in some. (9,67,89) In addition, patients with chronic hepatitis C often have increased fat in the liver and may have evidence of excessive iron deposition. (58)

FACTORS THAT INFLUENCE PROGNOSIS

Proposed explanations for the variability in the course of hepatitis C in individual patients are many: (74,80)

- The genotype of the virus may influence the rate of progression of the liver disease and the response to therapy. There are 6 major genotypes. 70% of patients in the United States are genotype 1 (1a and 1b) and these patients are more likely to have progressive disease. However it must be noted that decisions regarding prognosis and/or the need for treatment in an individual patient should not at present be influenced by genotype.

- Age at the time of acquisition of hepatitis C appears important. There is some evidence that the disease is more progressive in patients who acquire chronic HCV after age 40.

- The histologic stage of disease is of great importance in determining prognosis. Once cirrhosis develops, the patient is at considerable risk of developing decompensated liver disease and for developing hepatocellular carcinoma.

- The immune status is important in determining the course of chronic hepatitis C in that immunosuppressed patients do not respond as well to treatment with interferon as do immunocompetent individuals. Patients who have had combined infections of chronic hepatitis C and hepatitis B or HIV generally have responded less well to treatment than those who have HCV alone. (34) Formerly patients who had co-infection with HIV and HCV were seldom treated for HCV because of the higher likelihood that HIV presented the far greater risk to health. With the recent development of protease inhibitors and other drugs for the treatment of HIV infections, it has been necessary to reconsider whether patients who are on an effective multi-drug treatment program and have achieved effective suppression of HIV should be treated for chronic HCV.

- Women appear to respond more favorably to treatment than do men.

- Race may be important as regards the course of chronic hepatitis C and the likelihood of response to therapy. In thus far somewhat limited strides, it appears that African-Americans respond less readily to treatment. (70,91)

- Viral load is important in that patients who have large viral loads generally respond less well than those who have lower viral loads. In part, the variation in viral load may relate to the underlying immune status of an individual or to the viral genotype . In immunocompromised patients, those most bereft of defenses would be expected to have higher viral loads and also to respond less well to therapy.

The issue of hepatic iron as a prognostic indicator is one in which there has been considerable interest and great uncertainty. It has been established that hepatic iron stores are increased to a greater extent in chronic hepatitis C than in other types of chronic viral hepatitis. (14,58) It remains to be established if therapeutic phlebotomy will be effective in making patients more responsive to interferon. Preliminary results do not suggest a major advantage.

Factors that Influence Prognosis in Patients who have Chronic Hepatitis C

- Age
- Gender
- Race
- Extent of Liver Injury Found on Biopsy
- Concomitant Use of Alcohol
- Immune Status
- Viral Genotype
- Viral Load
- Hepatic Iron

THE INTERACTIONS OF ALCOHOL AND CHRONIC HEPATITIS C

One of the prognostic factors of greatest significance, and one that can be potentially controlled, is the use of alcohol. The range of anti-HCV positivity in chronic alcoholics with liver disease has been reported to be from 20% to 85%. (8,17,24,27,28,35,41,73,75,77,83,85,95) There seems no question that patients who have chronic hepatitis C and are chronic users of alcohol do less well and progress more rapidly to cirrhosis. The additive effects of the injuries produced by alcohol and hepatitis C co-promote the development of cirrhosis. Furthermore, patients who have cirrhosis, and are chronic alcoholics appear to be much more likely to later develop hepatocellular carcinoma. (25,35)

Chronic Hepatitis C - Effects of Concomitant Use of Alcohol

- Additive Injuries
- More Rapidly Progressive Disease
- Higher HCV Viral Load

The additive and likely synergistic relationships between the liver injuries caused by alcohol and hepatitis C virus has been a major theme of the 1990s. Soon after the identification of anti-HCV in 1989 and development of effective tests, it was recognized that the prevalence of anti-HCV was high in chronic alcoholics. There are reports that 15% to 50% of chronic alcoholics who had evidence of liver disease were positive for HCV-RNA measured by a reverse transcription polymerase chain reaction assay. (55)

The degree of alcohol consumption appears to correlates with the HCV-RNA level. (Pessione) Conversely reduction in alcohol intake leads to a decrease in serum HCV-RNA. In one study a relationship was shown between the extent of alcohol consumption in the week before testing and the level of HCV-RNA. (28)

The combination of long-term alcohol consumption and age promotes the progression of fibrosis. (87,88,108) Alcohol promoted increases in fibrosis in patients who have chronic hepatitis C as well as the heightened viral titers related to chronic alcohol use accelerates progression to

cirrhosis and increases the likelihood that hepatocellular carcinoma will develop. (35,61,99) Hepatocellular carcinoma in patients who have chronic hepatitis C is largely confined to those patients who have progressed to cirrhosis. (25) The increasing incidence of hepatocellular carcinoma in many parts of the world appears to be in large measure related to chronic HCV infection, and is especially likely to be found in patients who are also chronic users of alcohol.

The synergistic effects of alcohol and HCV may relate in part to effects of alcohol (or HCV) on the immune system, on viral replication, or on hepatocellular responses to injury. In a controlled study, mice fed on ethanol or isocaloric control liquid diet were immunized with HCV core DNA expressing constructs. (44) The mice who then received ethanol showed evidence of decreased Th cells and CTL activity and reduced cytokine expression. In addition there was a switch from Th1 to Th0 subtype in proliferating CD4+T cells in the ethanol fed mice. Crossover experiments to an isocaloric control diet restored immunity in these mice supporting the concept that the observed immunosuppressive efforts were due to ethanol.

In several studies of patients receiving interferon therapy for chronic hepatitis C, it has been shown that alcohol consumption (> 60 g/d) decreases the likelihood of a sustained response to therapy. (75,82,95) Since sustained treatment responses to interferon in patients with chronic hepatitis C are more likely to occur in patients who have low viral loads, and since alcohol promotes higher viral loads, there is every reason to insist on abstinence from alcohol during treatment for chronic hepatitis C.

TREATMENT:

Approved Drugs

Interferon alfa 2b, interferon alfa 2a, and alfacon-1 are approved by the Food and Drug Administration for the treatment of chronic hepatitis C. (2,76,104) In addition, the combination of interferon alfa-2b and ribavirin is FDA approved. (2) Ribavirin alone has not been demonstrated to be an effective drug in hepatitis C. (13,33,38,93)

Indications for Therapy

Therapy in chronic hepatitis C is indicated in patients who are anti-HCV positive, have elevated aminotransferase levels, and have evidence of chronic hepatitis on liver biopsy. (2,5,76) Patients who have compensated cirrhosis are candidates for therapy, although it appears the outcome may be less favorable. (105) It is uncertain whether patients who are anti-HCV positive and have normal aminotransferase levels benefit from interferon therapy. (67)

Contraindications to Therapy

Patients who have evidence of decompensated cirrhosis with a history of bleeding from esophagogastric varices, ascites, hepatic encephalopathy, spontaneous sepsis, low platelets or severe leucopenia are not candidates for presently available therapy. These patients should be considered as candidates for liver transplantation. Furthermore patients who have a history of a definite autoimmune disease or major depression are not candidates.

Goals of Therapy

The principal goals of therapy in hepatitis C are long lasting virologic elimination of hepatitis C virus, return to normal of serum alanine aminotransferase levels, and reduction of liver inflammation assessed on liver biopsy.

Determinations of Responses to Therapy

Response to therapy is defined biochemically as a return to normal of the serum aminotransferase levels at the end of treatment (ETR) or at the end of six months post-treatment follow-up (sustained response; SR). An additional and far more precise marker of treatment effectiveness is to determine the virologic response based on whether the patient has lost serum HCV-RNA at the end of treatment (ETR), or after 6 months post-treatment (SR), suggesting a prolonged hopefully long-lasting and even permanent response.

Interferon Monotherapy

Interferons are cytokines and have been the most widely used treatment in hepatitis C. (46,51) The specific mechanism – or more likely mechanisms – by which interferon favorably affects the course of chronic hepatitis C remain to be defined. There are antiviral and antiproliferative actions as well as immune regulatory effects and likely anti-angiogenesis effects, which appear to be of importance. Interferon further interferes with the spread of infection to uninfected cells.

In the initial studies of interferon therapy in patients with chronic hepatitis C, it was established that approximately 40% of patients who received interferon-alfa 2b (3 mu subcutaneously tiw) for 6 months had a return of aminotransferase levels to normal (biochemical response). (29) Unfortunately relapses following cessation of treatment were frequent and only 7-20% of patients had a long-term sustained response. Response-predicting factors were the same as the aforementioned prognostic factors. Patients who were genotype 1a or 1b responded less readily; however, some type 1a and type 1b patients did have sustained responses.

The biochemical ETR with interferon monotherapy has been in the 30% to 50% range with a biochemical SR of 15% to 20%. (32,71) The virological responses with interferon monotherapy are in the range of an ETR of 30% to 40% and SR of 6% to 20%. Responses were improved where patients received 12 months of therapy (or more) compared to those who received 6 months of therapy.

It is of note that virtually every patient who achieves a biochemical or virological response has concomitant evidence of histologic improvement. (36,71) Of great interest are those observations which suggest that even in patients who have not had complete biochemical or virological responses, there is often histologic improvement.

From analysis of many clinical trials in patients with chronic hepatitis C who have received interferon monotherapy, a few general conclusions can be reached. First, the ultimate outcome of interferon therapy in an individual can usually be predicted by early events. In the vast majority of patients who respond, a response (biochemical and/or virological) will occur by 12 weeks.

Conversely, if a patient has not experienced a biochemical or virological response of considerable magnitude by 12 weeks, there is no reason to continue therapy.

Studies carried out over the past several years have suggested that the duration of treatment with interferon monotherapy is more important than the dose in determining a sustained response and that the earlier after institution of interferon therapy that a response occurs, the more likely it is that the response will be sustained. (71,89,99) For patients in whom an early response has been detected as defined by return to normal of aminotransferase levels and disappearance, or at least marked reduction in titer of the virological markers, it is recommended that the patient continue on therapy until 12 months therapy has been given. Patients who respond to therapy have a significantly greater decrease in hepatic inflammation than partial non-responders.

In a trial comparing the response to interferon in several racial groups, African-Americans had a lower sustained response rate (2%) then did white patients (12%). (70,91) Responses in Hispanics and Asian-American patients were not different from non-Hispanic white patients.

There are several studies that indicate interferon therapy prevents the development of hepatocellular carcinoma in patients who have chronic hepatitis C. (12,53,54,81,111) However there are other follow-up studies in which interferon therapy did not appear to affect the role of progression to hepatocellular carcinoma. (52) The presence and extent of fibrosis is a most important marker of the progression of liver disease in a patient who has chronic hepatitis C. Fibrosis increases with duration of disease is markedly accentuated by alcohol. There are suggestions that the extent of fibrosis correlates with body mass index and steatosis. (57,68) The extent of fibrosis has been categorized in an F0 (no fibrosis), to F4 (cirrhosis) scale by a European group. (88) These investigations calculate the role of fibrosis progression (or regression) a year based on interpretation of several liver biopsies. Use of the scale (designated the METAVIR scale) allows assessment of the effects of therapy on progression. Through these studies, it has been concluded that interferon therapy reduces the natural progression of fibrosis in patients with chronic hepatitis C independently of genotype. (88) Other investigators have confirmed an interferon related decrease in the rate of progression of fibrosis. (11,39,37,100)

LONG-TERM FOLLOW-UP

There is increasing evidence from clinical trials using extended (12-18 months) interferon monotherapy, that patients who maintain a complete biochemical and virologic response for 6 months following cessation of therapy, more than 90% will maintain the response and demonstrate increasingly significant improvement in histology. (62,64)

SIDE EFFECTS OF INTERFERON

Several of the side effects from interferon are tabulated. (32,63) The flu-like symptoms are generally more pronounced following the first few injections and tolerance to interferon often develops within 2-3 weeks. In order to minimize side effects, many patients take interferon at bedtime along with acetaminophen. The gastrointestinal side effects of nausea, anorexia and occasional diarrhea are generally mild. The decrease in white blood cell and platelet counts relate to the antiproliferative effects of interferon and usually are transient with rapid recovery following dose reduction or temporary cessation. Modest alopecia is frequent, mild, and usually transient. Rash and pruritus may occur. Side effects lead to a reduction in interferon dose in 10% to 40% of patients and discontinuation of therapy in 5% to 10%.

Side Effects of Interferon Therapy

- Systemic, Flu-like
  - Fever
  - Chills
  - Headache
  - Myalgias
  - Fatigue
- Gastrointestinal
  - Anorexia
  - Nausea
  - Vomiting
  - Diarrhea
- Endocrine
  - Hypothyroidism
  - Hyperthyroidism (rare)
- Hematologic
  - Leucopenia
  - Thrombocytopenia
- Alopecia
- Neuropsychological
  - Depression
  - Irritability
  - Cognitive Change
- Hypertriglyceridemia
- Rash
- Pruritus

The major adverse effects of interferon therapy are a fall in platelets and white blood cells, and depression. Almost all patients have "flu-like" symptoms of malaise, fatigue, myalgia, and insomnia that are especially prominent early in the course of therapy. In many patients these side effects improve as treatment continues. Interferon should be used with caution and careful follow-up in patients who have a history of clinical depression. There are reported instances of interferon causing or markedly exacerbating depression and promoting suicidal ideation. Fluoxetine or other antidepression agents may be helpful in these patients.

Thyroid problems, usually hypothyroidism, develop in a few patients receiving interferon. The thyroid problems may become clinically significant and thyroid replacement therapy may be needed. It is advisable to obtain a TSH level before beginning therapy in order to provide a baseline for further assessment.

Interferon therapy has been reported to exacerbate a wide variety of autoimmune disorders, and if used in patients who have suspected autoimmune disorders the patient must be carefully monitored. Interferon has been associated with exacerbation of psoriasis and exacerbation of sarcoidosis. Pericarditis likely autoimmune in nature has been rarely observed. (16)

RIBAVIRIN THERAPY

Ribavirin is a synthetic nucleoside analog that structurally resembles guanosine. (47) The drug was initially evaluated in patients with hepatitis C following demonstration of its favorable antiviral activity on other RNA viruses including respiratory syncytial virus. Ribavirin monotherapy had a favorable effect on serum aminotransferase levels but has little if any effect on HCV-RNA levels. (13,33,38,93) However when given in combination with interferon, marked synergistic effects were found. (92)

The mechanism of inhibition of hepatitis C virus by ribavirin is unknown. (47,62,84) There is increasing evidence that ribavirin has direct anti-hepatitis C actively by promoting misincorporation into the viral RNA. (50) It is likely that there are antiviral and immunomodulary antivities. It has been suggested that ribavirin depletes intracellular phosphate pools and may inhibit hepatitis C viral polymerase.

Ribavirin is contraindicated in a patient who has anemia (hemoglobin<10g/dl), active or significant cardiovascular disease, or renal failure. (63) Furthermore the drug must not be given to a patient who is unwilling to meticulously follow contraception procedures during and for 6 months following therapy.

INTERFERON-RIBAVIRIN COMBINATION THERAPY

The results of treatment with interferon-ribavirin combination therapy has been more favorable than any achieved with interferon as a single agent. (6,22,69,71,94) In trials evaluating combination therapy, the results in patients who had initially responded to interferon and subsequently relapsed has shown a response of greater than 40% sustained response, and in naive patients a virologic sustained response rate of 30% - 40%. (30,69,71,88) It was noted that patients with genotype 1, the major genotype in the U.S., have a decreased sustained virologic response rate (approximately 28%) compared to the much more favorable responses in patients who had genotype 2 or 3.

The use of combination therapy of interferon with ribavirin has greatly improved the likelihood of achieving a sustained therapeutic response when compared to interferon monotherapy.

Dose, Duration, and Side Effects of Interferon-Ribavirin Therapy

The interferon component of therapy is administered subcutaneously at 3mu three times a week. The dose of interferon may have to be reduced if the patient develops a drug-related marked decrease in platelet or white blood cells, or if severe depression occurs, especially if there is any indication of suicidal ideation . Ribavirin is given orally at a recommended dose of 1200 mg a day (divided dose) to patients who weigh >75 kg, and 1000 mg a day for those who weigh ≤75kg. Dose reductions are indicated if significant decreases in hemoglobin resulting from drug-induced hemolysis occurs. If the hemoglobin levels fall to <10g/dl, the dose should be reduced to 600mg, and if the hemoglobin falls to <8.5g/dl the drug should be discontinued. There is increasing evidence that a ribavirin dose of 600-800mg/day may suffice. Ribavirin therapy must be administered with great caution to any patient who has evidence of unstable or significant cardiac disease which might be adversely affected by a fall in hemoglobin. Most drug-induced disease in hemoglobin occurs within the first month of therapy and the mean maximum drop is 2.6-2.8g/dl. (63)

The duration of combination therapy is determined by early response and by genotype. In patients receiving interferon monotherapy, a determination of HCV-RNA at 12 weeks of therapy gives a reliable indication as to whether treatment would be effective in that situation. If HCV-RNA was detectable, there is no reason to continue treatment. However, with combination therapy, the 12 week measurement proved to be less predictive and an additional 15% achieved a loss of HCV-RNA during a subsequent 12 weeks of therapy. (71) Furthermore from multicenter trials, it was determined that patients who had genotype 1 should be treated for 48 weeks, whereas patients who have genotypes 2 or 3 achieved as much benefit from 24 weeks of therapy as from 48 weeks. (71)

A further consideration when using combination therapy is that ribavirin has established teratogenic and embryocidal potential. (47,63) It is mandatory that patients (male and female) practice effective contraception during and for 6 months following treatment.

During therapy patients should be regularly monitored using standard hematologic tests (weeks 1, 2, 4, and then monthly), and pregnancy tests. An occasional liver biochemical panel helps monitor progress.

Interferon-Ribavirin Combination Therapy in Naive Patients with Chronic Hepatitis C

Following the favorable results from several small trials of interferon-ribavirin (IFN/RB) combination therapy, two large multicenter trials were carried out to determine the safety and efficacy of the approach. (69,90) In the trial in the United States, 912 patients were randomized into 4 groups and received either IFN/placebo for 24 weeks, IFN/placebo for 48 weeks, IFN/RB for 24 weeks, or IFN/RB for 48 weeks. (69) In an international trial of 832 patients there were 3 treatment groups: (90) IFN/placebo for 48 weeks; IFN/RB for 24 weeks and IFN/RB for 48 weeks. IFN was administered 3mu tiw and RB given in divided doses of 1000 or 1200 mg/day depending on weight. Patients were stratified based on genotype (1 or non-1); viral load (above or below $2x10^6$ copies/ml), and on the presence or absence of cirrhosis. Liver histology was assessed within 6 months of study entry and

again at the conclusion of a 6 month follow-up period. Histologic improvement was defined based on a necroinflammatory index. (71) The measure of success was a sustained virologic response with no detectable HCV-RNA 6 months after completion of therapy.

In the United States trial, patients who received interferon monotherapy had a SR of 6% (24 weeks therapy) and 13% (48 weeks therapy) compared to 31% for those who received IF/RB for 24 weeks and 38% response at 48 weeks. (69) Responses in the International trial were slightly higher, likely the result of inclusion of more patients who were genotype non-1. The degree of histologic improvement was greater in patients who received combination therapy and the improvement correlated with a sustained virologic response.

Combination therapy was more effective than monotherapy in all categories regardless of HCV genotype, viral load, or extent of fibrosis. Response rates were lower for genotype 1 compared to non-1, and for patients who had lower viral load. There was a suggestion in the International trial that patients who had severe fibrosis responded better with 48 weeks of therapy than within 24 weeks regardless of genotype.

In an International study of IFN/RB therapy in patients who had cirrhosis at the time of entry, it was concluded that combination therapy significantly enhanced response rates compared to interferon monotherapy. (94) Therefore the presence of compensated cirrhosis is not a contraindication to therapy.

Interferon-Ribavirin Combination Therapy in Patients Who Have Relapsed Following Response to Interferon Monotherapy

Combination therapy is certainly indicated in patients who relapse following interferon monotherapy. (18,30,31) Treatment of IFN relapses with combination therapy led to a sustained loss of detectable HCV-RNA in 49%. Furthermore there was histologic improvement in nearly two-thirds of these patients. (31)

Interferon-Ribavirin Combination Therapy in Non-Responders

It is uncertain whether interferon-ribavirin therapy is of value in patients who have had scant response to interferon monotherapy. Undoubtedly some of these patients respond although the numbers of sustained responses is quite low. (86)

Side Effects in Patients Receiving Interferon-Ribavirin Combination Therapy

The incidence and range of side effects in patients receiving combination therapy of interferon alfa-2b and ribavirin appear to be similar to that found with interferon monotherapy with two important exceptions. (63) Ribavirin caused a near universal dose dependent hemolytic anemia which usually becomes manifest within the first month of therapy. Dose reduction of the ribavirin may be necessary in up to 10% of patients. Furthermore, ribavirin is fully established to be teratogenic and patients of both sexes must be strongly advised to practice effective contraception during and for six months

following treatment. It is necessary to follow patients on combination therapy carefully throughout treatment in order to detect decreases in hemoglobin which may be dangerous. Patients who have active cardiac disease should not receive combination therapy.

QUALITY OF LIFE

Several prospective studies have evaluated health-related quality of life (HQL) in patients who have chronic hepatitis C before and after therapy. (15,60,79,107) Patients who have a sustained response to treatment for chronic hepatitis C have demonstrably improved quality of life. Patients receiving interferon-ribavirin combination therapy had significant improvement in general health, vitality, social functioning, health distress, chronic hepatitis C specific health distress, and chronic hepatitis C specific limitation domains of the SF-36 health survey. (79) Nonresponders to treatment showed no improvement when evaluated over a similar interval.

COST EFFECTIVENESS OF TREATMENT FOR CHRONIC HEPATITIS C

Cost effectiveness analyses based on modeling of the expected natural history, morbidity and mortality resulting from chronic hepatitis C indicate that a treatment induced sustained virologic response prolongs life. (10,110) In one study of the effects of interferon monotherapy there was increased life expectancy of 3.1 years if treatment was given at 20 years of age, 1.5 years if given at 35 years of age, and 22 days at 70 years of age. (10) The discounted marginal cost effectiveness was $500, $1,900, and $62,000 per year of life gained respectively.

FUTURE THERAPIES

Pegylated interferons have been developed and will likely soon be in the market place. The pegylation of the interferon markedly increases the half-life and prevents renal excretion so that a once a week injection is possible. Advantages of the approach related to patient acceptance and more constant levels of interferon availability. A potential disadvantage would be the appearance of a side effect soon after injection of pegylated interferon in a setting in which elimination of the drug will be prolonged. There is great hope that hepatitis C specific protease inhibitors, or helicase inhibitors will soon be available and prove to be useful. The helicase protein is coded in the NS3 region and is critical for viral unwinding of the RNA genome after polymerase replication. The structure of the helicase is established, and hopefully drugs which affect the unwinding will be forthcoming.

There is also interest in therapeutic approaches using specially designed ribozymes and antisense oligonucleotides. Ribozymes (ribonucleic acid enzymes) are RNA molecules that lead to sequence-specific cleavage. Ribozymes are designed so that a catalytic site is flanked by antisense sequences, which mediate a highly specific binding to target RNA. Because of a "touch and go" mechanism, a single ribozyme can cleave many target RNAs in sequence.

REFERENCES:

1. Agnello A, Chung RT, Lee MK. A role for hepatitis C infection in type II cryglobulinemia. *N Engl J Med* 1992;327:1490-1495.

2. Ahmed A, Keeffe EB. Treatment strategies for chronic hepatitis C: Update since the 1997 National Institute of Health Consensus Development Conference. *J Gastroenterol Hepatol.* 1999;14 (Suppl):12-18.

3. Alter MJ. The detection, transmission, and outcome of hepatitis C virus infection. *Infect Agents Dis* 1993;2:155-166.

4. Alter MJ, Kruszon-Moran D, Nainan OV, et al: The prevalence of hepatitis C virus infection in the United States, 1988 through 1994. *N Engl J Med* 1999;8:556-562.

5. Anonymous. EASL International Consensus Conference on Hepatitis C. *J Hepatol* 1999;30(5):956-961.

6. Barbo G, Lorenzo G, Belloni G, et.al: Interferon alpha-2b and ribavirin in combination for patients with chronic hepatitis C who failed to respond to, or relapsed after, interferon alpha therapy: A randomized trial. *Am J Med* 1999;107:112-118.

7. Battegay M. Immunity to hepatitis C virus: A further piece of the puzzle. *Hepatology* 1996;24:961-963.

8. Befrits R, Hedman M, Blomquist T, et al: Chronic hepatitis C in alcoholic patients: Prevalence, genotypes, and correlation to liver disease. Scan J Gastroenterol 1995;30:1113.

9. Bedossa P, Poynard T, for the French METAVIR cooperative study group. An algorithm for the grading of activity in chronic hepatitis C. *Hepatology* 1996;24:289-293.

10. Bennett WG, Inoue Y, Beck JR, et al: Estimates of the cost-effectiveness of a single course of interferon -α2b in patients with histologically mild chronic hepatitis C. *Ann Intern Med* 1997;127:855-865.

11. Benvegnú L, Chemello L, Noventa F, et al: Retrospective analysis of the effect of interferon therapy on the clinical outcome of patients with viral cirrhosis. *Cancer* 1998;83:901-909

12. Benvegnú L, Chemello L, Noventa F, et al: Does interferon therapy prevent hepatocellular carcinoma in patients with chronic hepatitis C. *Gastroent* 1999;117:738-740.

13. Bodenheimer HC Jr, Lindsay KL, Davis GL, et al: Tolerance and efficacy of oral ribavirin treatment of chronic hepatitis C: A multicenter trial. *Hepatology* 1997;26:473-477.

14. Bonkowsky HL, Banner BF, Rothman AL. Iron and chronic viral hepatitis. *Hepatology* 1997;25:759-768.

15. Bonkovsky HL, Woolley JM, and the Consensus Interferon Study Group. Reduction of health-related quality of life in chronic hepatitis C and improvement with interferon therapy. *Hepatology* 1999;29:264-269.

16. Boonen A, Stockbrügger RW, van der Linden Sj. Pericarditis after therapy with interferon-a for chronic hepatitis C. *Clin Rheumatol* 1999;18:177-179.

17. Brillanti S, Masci C, Siringo S, et al: Serological and histological aspects of hepatitis C virus infection in alcoholic patients. *J Hepatol* 1991;13:347.

18. Camma C, Giunta M, Chemello L, et al: Chronic hepatitis C: Interferon retreatment of relapsers. A meta-analysis of individual patient data. *Hepatology* 1999;30:801-807.

19. Caronia S, Taylor K, Pagliaro L, et al: Further evidence for an association between non-insulin-dependent diabetes mellitus and chronic hepatitis C virus infection. *Hepatology* 1999;4:1059-1063.

20. Cerny A, Chisari FV. Pathogenesis of chronic hepatitis C: Immunological features of hepatic injury and viral persistence. *Hepatology* 1999;3:595-601.

21. Chang KM. The mechanisms of chronicity in hepatitis C virus infection. *Gastroent* 1998;115:1015-1018.

22. Chemello L, Cavalletto L, Bernardinello E, et al: The effect of interferon alfa and ribavirin combination therapy in naïve patients with chronic hepatitis C. *J Hepatol* 1995;23:8-12.

23. Chen M, Sallberg M, Sonnerborg A, et.al: Limited humoral immunity in hepatitis C virus infection. *Gastroent* 1999;116:135-143

24. Coelho-Little ME, Jeffers LJ, Bernstein DE, et al: Hepatitis C virus in alcoholic patients with and without clinically apparent liver disease. *Alcohol Clin Exp Res* 1995;19:1173.

25. Colombo M. Hepatitis C virus and hepatocellular carcinoma. *Semin Liver Dis* 1999;19:263-269.

26. Conry-Cantilena C, Van Raden M, Gibble J, et.al: Routes of infection, viremia, and liver disease in blood donors found to have hepatitis C virus infection. *N Engl J Med* 1996;334:1691-1696.

27. Corrao G, Arico S. Independent and combined action of hepatitis C virus infection and alcohol consumption on the risk of symptomatic liver cirrhosis. *Hepatology* 1998;27:914.

28. Cromie SL, Jenkins PJ, Bowden DS, et al: Chronic hepatitis C: Effect of alcohol on hepatic activity and viral titre. *J Hepatol* 1996;25:821.

29. Davis GL, Balart LA, Schiff ER, et al: Treatment of chronic hepatitis C with recombinant interferon alfa: A multicenter randomized, controlled trial. *N Engl J Med* 1989;321:1501-1506.

30. Davis GL, Esteban-Mur R, Rustgi V, et al: Interferon alfa-2b alone or in combination with ribavirin for the treatment of relapse of chronic hepatitis C. *N Engl J Med* 1998;339:1493-1499.

31. Davis GL. Combination therapy with interferon alfa and ribavirin as retreatment of interferon relapse in chronic hepatitis C. *Semin Liver Dis* 1999;19 (suppl 1):49-55.

32. Davis GL. Hepatitis C. In: *Schiff's Dis of the Liver, 8th edition*, Schiff ER, Sorrell MF, Maddrey WC (eds.), Lippincott-Raven, Philadelphia, 1999;1:793-836.

33. Di Bisceglie AM, Conjeevaram HS, Fried MW, et al: Ribavirin as therapy for chronic hepatitis C. A randomized, double-blind, placebo-controlled trial. *Ann Intern Med* 1995;123:897-903.

34. Dieterich DT, Purow JM, Rajapaksa R. Activity of combination therapy with interferon alfa-2b plus ribavirin in chronic hepatitis C patients co-infected with HIV. *Semin Liver Dis* 1999;19 (Suppl 1):87-94.

35. Donato F, Tagger A, Chiesa R, et al: Hepatitis B and C virus infection, alcohol drinking, and hepatocellular carcinoma: A case control study in Italy. *Hepatology* 1997;26:579.

36. Duchatelle V, Marcellin P, Giostra E, et.al: Changes in liver fibrosis at the end of alpha interferon therapy and 6 to 18 months later in patients with chronic hepatitis C: Quantitative assessment by a morphometric method. *J Hepatol* 1998;29:20-28.

37. Dufour JF, CeEllis R, Kaplan MM. Regression of hepatic fibrosis in hepatitis C with long-term interferon treatment. *Dig Dis and Sciences* 1998;43:2573-2576.

38. Dusheiko G, Main J, Thomas H, et al: Ribavirin treatment for patients with chronic hepatitis C: Results of a placebo-controlled study. *J Hepatol* 1996;25:591-598.

39. Esteban JI, Gomez J, Martell M, et al: Transmission of hepatitis C virus by a cardiac surgeon. *N Engl J Med* 1995;334:555-560

40. Fattovich G, Giustina G, Egos F, et.al: Morbidity and mortality in compensated cirrhosis type C: A retrospective follow-up study of 384 patients. *Gastroent* 1997;112:463-472.

41. Fong T-L, Kanel GC, Conrad A, et al: Clinical significance of concomitant hepatitis C infection in patients with alcoholic liver disease. *Hepatology* 1994;19:554.

42. Garcoa-Valdecasas J, Bernal C, Garcia F, et al: Epidemiology of hepatitis C virus infection in patients with renal disease. *J Am Soc Nephrol* 1994;5:186-192.

43. Garfein RS, Vlahov D, Galai N, et al: Viral infections in short-term injection drug users: The prevalence of the hepatitis C, hepatitis B, human immunodeficiency, and human T-lymphotropic viruses. *Am J Pub Health* 1996;86:655-661.

44. Geissler M, Gesien A, Wands JR. Inhibitory effects of chronic ethanol consumption on cellular immune responses to hepatitis C virus core protein are reversed by genetic immunizations augmented with cytokine-expressing plasmids. *J Immunol* 1997;159:5107.

45. Gerlich WH, Caspari G. Hepatitis viruses and the safety of blood donations. *J Viral Hepatitis* 1999;6 (Suppl):6-15.

46. Gish RG. Standards of treatment in chronic hepatitis C. *Semin Liver Dis.* 1999;19 (Suppl 1):35-47.

47. Glue P. The clinical pharmacology of ribavirin. *Semin Liver Dis.* 1999:19 (Suppl 1): 17-24.

48. González-Peralta RP, Qian K, She YS, et al: Clinical implications of viral quasispecies in chronic hepatitis C. *J Med Virol* 1996;49:242-247.

49. Hadziyannis S. Diabetes mellitus and chronic hepatitis C virus infection. *Hepatology* 1999;29:604-605.

50. Hong Z, Ferrari E, Wright-Minogue J, et al: Direct antiviral activity of ribavirin: Hepatitis C virus nNS5B polymerase incorporates ribavirin triphosphate into nascent RNA products. *Hepatology* 1999;30:354A.

51. Hoofnagle JH, DiBisceglie AM. The treatment of chronic viral hepatitis. *N Engl J Med* 1997;336:347-356.

52. Hu Ke-Quin, Tong MJ. The long-term outcomes of patients with compensated hepatitis C virus-related cirrhosis and history of parenteral exposure in the United States. *Hepatology* 1999;29:1311-1316.

53. Ikeda K, Saitoh S, Arase Y, et al: Effect of interferon therapy on hepatocellular carcinogenesis in patients with chronic hepatitis type C: A long-term observation study of 1,643 patients using statistical bias correction with proportional hazard analysis.

54. Imai Y, Kawata S, Tamura S, et al: Relation of interferon therapy and hepatocellular carcinoma in patients with chronic hepatitis C. *Ann Intern Med* 1998;129:94-99.

55. Ince N, Wands JR. The increasing incidence of hepatocellular carcinoma. *N Engl J Med* 1999;340:798-799.

56. Johnson RJ, Gretch DR, Couser WG, et al: Hepatitis C virus associated glomerulonephritis. Effect of alpha interferon therapy. *Kidney Int* 1994;46:1700-1704/.

57. Kage M, Shimamatu K, Nakashima E, et al: Long-term evolution of fibrosis from chronic hepatitis to cirrhosis in patients with hepatitis C: Morphometric analysis of repeated biopsies. *Hepatology* 1997;25:1028-1031.

58. Kazemi-Shirazi L, Datz C, Maier-Dobersberger T, et.al: The relation of iron status and hemochromatosis gene mutations in patients with chronic hepatitis C. *Gastroent* 1999;116:127-134.

59. Kenny-Walsh E for the Irish Hepatology Research Group. Clinical outcomes after hepatitis C infection from contaminated anti-D immune globulin. *N Engl J Med* 1999;340:1228-1233.

60. Koff RS. Impaired health-related quality of life in chronic hepatitis C: The how, but not the why. *Hepatology* 1999;29:277-279.

61. Kubo S, Kinoshita H, Hirohashi K, et al: High malignancy of hepatocellular carcinoma in alcoholic patients with hepatitis C virus. *Surgery* 1997;121:425-529.

62. Lau DTY, Kleiner DE, Ghany MG, et al: 10-year follow-up after interferon-alpha therapy for chronic hepatitis C. *Hepatology* 1998;23 (Suppl 4 pt2):1121-1127.

63. Maddrey WC. Safety of combination interferon alfa-2b/ribavirin therapy in chronic hepatitis C-relapsed and treatment-naïve patients. *Semin Liver Dis.* 1999:19 (Suppl 1):67-75.

64. Marcellin P, Boyer N, Gervais A, et al: Long-term histologic improvement and loss of detectable intrahepatic HCV RNA in patients with chronic hepatitis C and sustained response to interferon-alpha therapy. *Ann Intern Med* 1997;127:875-881.

65. Mason AL, Lau YN, Hoang N, et.al: Association of diabetes mellitus and chronic hepatitis C virus infection. *Hepatology* 1999;29:328-333.

66. Mast EE, Alter MJ. Hepatitis C. *Semin Pediatr Infect Dis* 1997;8:17-22.

67. Mathurin P, Moussalli J, Cadranel JF, et.al: Slow progression rate of fibrosis in hepatitis C virus patients with persistently normal alanine transaminase activity. *Hepatology* 1998;27:868-872.

68. McCullough AJ, Falck-Ytter Y. Body composition and hepatic statosis as precursors for fibrotic liver disease. *Hepatology* 1999;29:1328-1239.

69. McHutchison JG, Gordon SC, Schiff ER, et.al: Interferon alfa-2b alone or in combination with ribavirin as initial treatment for chronic hepatitis C. *N Engl J Med* 1998;339:1485-1492.

70. McHutchison JG, Poynard T, Gordon SC, et al: The impact of race on response to anti-viral therapy in patients with chronic hepatitis C. *Hepatology* 1999;30(4):302A.

71. McHutchison JG, Poynard T. Combination therapy with interferon plus ribavirin for the initial treatment of chronic hepatitis C. *Semin Liver Dis* 1999;19 (Suppl 1):57-65.

72. McQuillan GM, Coleman PJ, Kruszon-Moran D, et.al: Prevalence of hepatitis C virus infection in the United States: The national health and nutrition examination surveys, 1976 through 1994. *Am J Public Health* 1999;89:14-18.

73. Mendenhall CL, Seeff L, Diehl AM, et al: Antibodies to hepatitis B virus and hepatitis C virus in alcohol hepatitis and cirrhosis: Their prevalence and clinical relevance. *Hepatology* 1991;14:581.

74. Minuk GY. The influence of host factors on the natural history of chronic hepatitis C viral infections. *J Viral Hepatol* 1999;4:271-276.

75. Mochida S, Ohnishi K, Matsuo S, et al: Effect of alcohol intake on the efficacy of interferon therapy in patients with chronic hepatitis C as evaluated by multivariate logistic regression analysis. *Alcohol Clin Exp Res* 1996;20:371A.

76. National Institutes of Health consensus development conference panel statement: Management of hepatitis C. *Hepatology* 1997;26 (3 Suppl 1):2S-10S.

77. Nalpas B, Thiers V, Pol S, et al: Hepatitis C viremia and anti-HCV antibodies in alcoholics. J *Hepatology* 1992;14:381.

78. Navas S, Bosch O, Castillo I, et al: Porphyria cutanea tarda and hepatitis C and B viruses infection: A retrospective study. *Hepatology* 1995;21:279-284.

79. Neary MP, Cort S, Bayliss MS, et al. Sustained virologic response is associated with improved health-related quality of life in relapsed chronic hepatitis C patients. *Semin Liver Dis.* 1999;19 (Suppl 1):77-85.

80. Niederau C, Lang S, Heintges T, et.al: Prognosis of chronic hepatitis C: Results of a large, prospective cohort study. *Hepatology* 1998;28:1687-1695.

81. Okanoue T, Itoh Y, Minami M, et al: Interferon therapy lowers the rate of progression to hepatocellular carcinoma in chronic hepatitis C but not significantly in an advanced stage: A retrospective study in 1148 patients. Viral Hepatitis Therapy Study Group. *J Hepatol* 1999;30(4):653-659.

82. Okazaki T, Yoshihara H, Suzuki K, et al: Efficacy of interferon therapy in patients with chronic hepatitis C. *Scan J Gastroent* 1994;29:1039.

83. Pares A, Barrera JM, Ercilla G, et al: Hepatitis C virus antibodies in chronic alcoholic patients: Association with severity of liver injury. *Hepatology* 1990;12:1295.

84. Patterson JL, Fernandez-Larsson R. Molecular mechanisms of action of ribavirin. *Rev Infect Dis.* 1990;12:1139-1146.

85. Pessione F, Degos F, Marcellin P, et al: Effect of alcohol consumption on serum hepatitis C virus RNA and histological lesions in chronic hepatitis C. *Hepatology* 1998;27:1717.

86. Pol S, Couzigou P, Bourliere M, et al: A randomized trial of ribavirin and interferon-alpha vs. interferon-alpha alone in patients with chronic hepatitis C who were non-responders to a previous treatment. Multicenter Study Group under the coordination of the Necker Hospital, Paris, France. *J Hepatol.* 1999;31:1-7.

87. Poynard T, Aubert A, Lazizi Y, et al: Independent risk factors for hepatocellular carcinoma in French drinkers. *Hepatology* 1991;13:896-901.

88. Poynard T, Bedossa P, Opolon P. Natural history of liver fibrosis progression in patients with chronic hepatitis C. *Lancet* 1997;349:825-832.

89. Poynard T, Leroy V, Cohard M, et.al: Meta-analysis of interferon randomized trials in the treatment of viral hepatitis C: Effects of dose and duration. *Hepatology* 1996;24:778-789.

90. Poynard T, Marcellin P, Lee SS, et.al: Randomised trial of interferon α2b plus ribavirin for 48 weeks or for 24 weeks versus interferon α2b plus placebo for 48 weeks for treatment of chronic infection with hepatitis C virus. *Lancet* 1998;352:1426-1432.

91. Reddy KR, Hoofnagle JH, Tong MJ, et al: Racial differences in responses to therapy with interferon in chronic hepatitis C. *Hepatology* 1999;30:787-793.

92. Reichard O, Norkrans G, Fryden A, et al: Randomized, double-blind, placebo-controlled trial of interferon alpha-2b with and without ribavirin for chronic hepatitis C. *Lancet* 1998;351:83-86

93. Schalm SW, Hansen BE, Chemello L, et al: Ribavirin enhances the efficacy but not the adverse effects of interferon in chronic hepatitis C. Meta-analysis of individual patient data from European centers. *J Hepatol* 1997;26:961-966.

94. Schalm SW, Weiland O, Hansen BE, et.al: Interferon-ribavirin for chronic hepatitis C with and without cirrhosis: Analysis of individual patient data of six controlled trials. *Gastroent* 1999;117:408-413.

95. Schiff ER. Hepatitis C and alcohol. *Hepatology* 1997;26:395.

96. Seeff LB, Buskell-Bales Z, Wright EC, et.al: Long-term mortality after transfusion-associated non-A, non-B hepatitis. *N Engl J Med* 1992;327:1906-1911.

97. Seeff LB. The natural history of hepatitis C – A quandary. *Hepatology* 1998;26:1710-1711.

98. Shakil AO, Conry-Cantilena C, Alter HJ, et.al: Volunteer blood donors with antibody to hepatitis C virus: Clinical, biochemical, virologic, and histologic features. *Ann Intern Med* 1995;123:330-337.

99. Simonetti RG, Camma C, Fiorello F, et al: Hepatitis C virus infection as a risk factor for hepatocellular carcinoma in patients with cirrhosis. *Ann Intern Med* 1992;116:97.

100. Sobesky R, Mathurin P, Charlotte F, et al: Modeling the impact of interferon alfa treatment on liver fibrosis progression in chronic hepatitis C: A dynamic view. *Gastroent* 1999;116:378-386.

101. Thaler MM, Park C-K, Lander DV, et al: Vertical transmission of hepatitis C. *Lancet* 1991;338:17-18.

102. Tong MJ, El-Farra NS, Reikes AR, et.al: Clinical outcomes after transfusion-associated hepatitis C. *N Engl J Med* 1995;332:1463-1466.

103. Tong MJ, El-Farra NS. Clinical sequelae of hepatitis C acquired from injection drug use. *West J Med* 1996;164:399-404.

104. Tong MJ, Reddy KR, Lee WM, et al: Treatment of chronic hepatitis C with consensus interferon: A multicenter, randomized, controlled trial. *Hepatology* 1997;26:747-754.

105. Valla DC, Chevallier M, Marcellin P, et al: Treatment of hepatitis C virus-related cirrhosis: A randomized, controlled trial of interferon alfa-2b versus no treatment. *Hepatology* 1999;29:1870-1875.

106. Vento S, Garofano T, Renzini C, et.al: Fulminant hepatitis associated with hepatitis A virus superinfection in patients with chronic hepatitis C. *N Engl J Med* 1998;338:286-290.

107. Ware JE Jr, Bayliss MS, Mannocchia M, et al: Health-related quality of life in chronic hepatitis C: Impact of disease and treatment response. *Hepatology* 1999;2:550-555.

108. Wiley TE, McCarthy M, Breidi L, et al: Impact of alcohol on the histological and clinical progression of hepatitis C infection. *Hepatology* 1998;28:805-809.

109. Wilson SE, Lee WM, Murakami C, et al: Mooren-type hepatitis C virus-associated corneal ulceration. *Ophthalmology* 1994;101:736-745.

110. Wong JB, Bennett WG, Koff RS, et al: Pretreatment evaluation of chronic hepatitis C. *JAMA* 1998;280:2088-2093.

111. Yoshida H, Shiratori Y, Moriyama M, et al: Interferon therapy reduces the risk for hepatocellular carcinoma: National surveillance program of cirrhotic and noncirrhotic patients with chronic hepatitis C in Japan. IHIT Study Group. Inhibition of hepatocarcinogenesis by interferon therapy. *Ann Intern Med* 1999;131(3):174-181.

112. Zein NN, Rakela J, Krawitt EL, et.al: Hepatitis C virus genotypes in the United States: Epidemiology, pathogenicity, and response to interferon therapy. *Ann Intern Med* 1996;125:634-639.