Hepatitis C Virus Infection: Detection and Treatment

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Hepatitis C virus (HCV) is a blood-borne virus that infects the liver. HCV affects millions of Americans, and poses a serious public health threat with sequelae such as cirrhosis, hepatocellular carcinoma, and liver failure. This paper reviews means of transmission, characteristics of the various risk groups, and clinical presentations of both the acute and chronic stages of HCV infection. Diagnostic methods, including screening and confirmatory tests, along with relevant clinical and physiologic findings are also described. Additionally, treatment strategies, in particular combination therapy with interferon α-2b and ribavirin, are discussed. Contraindications, side effects, and monitoring of this therapeutic modality are considered. Finally, prospective treatments are presented.

ABBREVIATIONS: ALT = alanine aminotransferase; AST = aspartate aminotransferase; EIA = enzyme immunoassay; FDA = Food and Drug Administration; GGT = gamma glutamyltransferase; HCV = hepatitis C virus; NAT = nucleic acid amplification testing; PKR = RNA-dependent protein kinase; PT = prothrombin time; RIBA = recombinant immunoblot assay; RT-PCR = reverse transcription-polymerase chain reaction; URL = upper reference level.

INDEX TERMS: hepatitis C virus; hepatocellular carcinoma; interferon; peginterferon; ribavirin.

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Hepatitis C is the most common blood-borne infection and is the leading cause of chronic liver disease.[1] In the United States, approximately four million people are infected with Hepatitis C virus. Nearly three million of those are in the chronic stage and about forty thousand new cases occur each year.[2-4] HCV infection has a 1% to 2% mortality rate with approximately 10,000 deaths occurring annually in the United States.[2] One of several hepatitis viruses (A through G), hepatitis C accounts for 20% of viral hepatitis cases and is potentially the most harmful to the host because of its propensity to cause chronic infection and severe liver damage often requiring liver transplants.[3,5]

The hepatitis C virus (HCV) is a small, enveloped, single-stranded RNA virus of the family Flaviviridae, and has a genome of 9.6 kilobases coding for structural and non-structural proteins.[6,7] HCV has a high rate of mutation and genomic heterogeneity (the greatest variability is seen in the envelope glycoproteins).

Genomic sequencing by reverse transcription-polymerase chain reaction (RT-PCR) has lead to the identification of several HCV genotypes.[7,8] The genotypes vary in geographic distribution, viral RNA levels, clinical severity, and response to treatment.[2,7,9,10] Simmonds nomenclature describes six genotypes, some of which have closely related subtypes, e.g., 1a-c, 2a-c, 3a-b, 4a, 5a, and 6a.[9] Other sources report that 11 genotypes and greater than 90 subtypes exist.[10,11] Genotypes 1a (58%), 1b (22%), 2b, and 3a are the most common genotypes in the United States and are most commonly associated with chronic hepatitis.[9] Genotype 1b produces the most severe presentations such as hepatocellular carcinoma and cirrhosis and is least responsive to therapy, i.e., 40% response rate. Genotypes 2b and 3 are most responsive to therapy, i.e., 80% response rate, and successful treatment can occur within 24 weeks.[7,9] The highest viral RNA levels are associated with 1b, while the lowest involve 2b and 3a.[7,12]

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Because of the ability of the virus to alter its viral envelope proteins, the rapid, spontaneous rate of mutation, and the inability of the host's immune system to target the virus effectively, i.e., protective antibodies able to neutralize the virus are not produced by the host, the virus efficiently can infect and re-infect the host multiple times.[3,9] Moreover, the mutability of HCV proteins make production of a vaccine extremely problematic; hence, no vaccine is currently available.[3-5]

TRANSMISSION

HCV is transmitted by parenteral exposure to infected blood.[2] Routes of infection include organ transplantation, IV drug use, sexual contact, exposure to blood, perinatal transmission, or occupational exposure.[5] Blood transfusion is another source, and in the U.S. 3% of donors are positive for HCV antibodies.[6] However because of improved screening methods initiated in 1992, the incidence of infection due to blood or blood product transfusion or organ transplantation has greatly decreased.[5]

The highest risk group for HCV are intravenous drug users (60% of cases).[2,5] Other high risk groups are recipients of clotting factors made before 1987, and those who received blood transfusions prior to 1992.[5] Healthcare workers and sexual contacts of infected persons are considered to be at a low, but not insignificant, risk for contracting the virus.[5] In 10% of cases, the source of infection is unidentified, but these cases are thought to occur by exposure to the virus through cuts or wounds, and the fact that the virus can remain viable for weeks outside of a host may be relevant in these cases.[8]

CLINICAL PRESENTATIONS

The incubation period for HCV ranges from 40 to 120 days.[13] Two types of HCV infection occur, acute and chronic. The clinical presentations of both types of HCV infections vary. The acute stage may be asymptomatic, or may involve jaundice, anorexia, malaise, abdominal pain, fatigue, migraines, nausea, or dark urine.[5] Liver enzyme levels in the serum may be elevated, and damage to liver cells may be detectable at fifty days. In 15% of acute cases, the infection resolves within five months and liver enzymes return to normal levels suggesting full recovery. Full recovery is defined by absence of HCV RNA in serum and normal levels of the liver enzyme, alanine aminotransferase (ALT). Unfortunately, 85% of acute infections result in chronic disease and are signaled by persistent elevation or fluctuating levels of ALT for greater than six months.[2] A very small percentage of acute cases fulminate with fatal liver destruction.[6]

Chronic HCV infection is typically insidious, progressing at a slow rate without symptoms in most patients during the first two or more decades after initial infection. Symptoms ultimately result from the cytopathic effect of the virus on the liver over time by a constant, low rate of hepatocyte destruction.[5,14] An inflammatory reaction to the infection within the liver is also evident.[9] Symptoms can vary. Patients may be asymptomatic for years, frequently 10 to 20 years, and then enter a chronic stage. Those persons with chronic infection are at risk for one of the following: fibrosis, cirrhosis, hepatocellular carcinoma, or liver failure. In addition, patients may present with hepatosplenomegaly, muscle wasting, portal hypertension, or ascites.[2,5] Subsequent systemic abnormalities may develop that include cutaneous, endocrine, hematological, renal, and circulatory abnormalities.[8]

Serum liver enzyme levels are typically increased during this chronic stage. An elevation of these enzymes for more than six months, coupled with detection of antibodies to HCV, i.e., anti-HCV, is characteristic of chronic hepatitis.[5] Factors associated with more severe liver injury due to HCV include advanced age, alcoholism, and immunosuppression.[4,7]

DIAGNOSIS

Diagnosis of HCV infection relies on detection of antibodies to HCV via screening and confirmatory tests, i.e., detection of HCV RNA in serum by RT-PCR or use of a recombinant immunoblot assay. Clinical presentation and risk factors, e.g., exposure, are also taken into consideration when making a diagnosis. Indirect evidence of liver damage can be assessed by chemistry profiles that measure liver enzymes; while direct evidence of liver damage can be ascertained by liver biopsy.[8,15]

Enzyme immunoassay (EIA) serves as the screening test and detects antibodies to HCV. An EIA acute panel for viral hepatitis includes tests for hepatitis A, hepatitis B, and hepatitis C.[14] Individuals with HCV will test positive for anti-HCV, which is detectable in 80% of patients by six weeks, and in 90% of patients by twelve weeks.[13] Presence of anti-HCV does not confer long-term immunity to HCV, and persistence of this antibody indicates that actively replicating HCV remains in the patient's body.[13]

The FDA approved an EIA test for antibodies for HCV in May 1990, followed by a more sensitive and reliable test introduced in July 1992. In 1996 a modified second-generation test (anti-HCV 3.0) was introduced to screen all blood supplies for antibodies to HCV.[3,5,6] However, these very sensitive tests suffer from false-positive results and require supplemental confirmatory tests to eliminate them. In 1999, FDA approved an improved test for confirming positive results from

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screening tests. In the same year, blood transfusion centers began nucleic acid amplification testing (NAT) of pooled donor samples for human immunodeficiency virus (HIV) and HCV under FDA's Investigational New Drug application. In 2002, the FDA approved the NAT test, Procleix HIV-1/HCV assay, which directly detects the genetic material of all HCV and HIV genotypes in pooled donor samples.[16]

If viral nucleic acid is detected using NAT, then the blood donor units making up the pool are retested individually to determine which unit contains the virus. Once determined that an individual unit is contaminated by HIV or HCV, it is taken out of the blood supply; thus stopping transmission of the virus to blood recipients. NAT is more effective than antibody testing since it decreases the window period for detection of HCV infection, i.e., from 70 to 80 days time between exposure to the virus and when antibodies to the virus become detectable, to 30 days.[17] The Procleix HIV/HCV assay, developed by Gen-Probe Inc. is now being manufactured and marketed by Chiron Corporation worldwide.[18] Presently 75% of the nation's blood supply is being screened by the Procleix™ system.[18] This system is restricted to screening blood donors and is not being used to diagnose individuals with HCV.[16]

Results from individuals who test positive for HCV antibodies using EIA screening techniques are verified using methodologies with increased specificity. For confirmatory testing, a recombinant immunoblot assay (RIBA) is most often used. RIBA is a type of Western blotting, and like the EIA, it tests for the presence of antibodies against HCV. In patients not producing significant amounts of antibodies, RT-PCR may be used as the confirmatory test because of its increased sensitivity.[9] RT-PCR directly measures HCV virions in serum by detection of HCV RNA genome. The test measures viral load which is helpful in predicting response to treatment.[5] RT-PCR for HCV may be positive two weeks following the initial infection.[13] A qualitative RT-PCR assay for HCV, the COBAS Amplicor® HCV test manufactured by Roche Molecular Systems, Inc, has recently been approved by the FDA. A positive test indicates active infection, i.e., replicating virions in the serum.[5]

Hepatitis C infection may produce characteristic serum chemistry findings. These findings can aid in diagnosis and provide, along with liver biopsy, an estimation of the extent of liver damage.[8] Table 1 shows serum chemistry findings that occur with HCV infection. Serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) levels are markedly increased, i.e., 0 to 20 times the upper reference level (URL). In acute hepatitis, ALT is frequently increased greater than 10-fold URL, while AST is increased more than 3-fold URL. In chronic hepatitis increases are generally more modest. Alkaline phosphatase (ALP) and gamma glutamyltransferase (GGT) levels may also be increased but usually less than 2-fold URL.[5,8,15] Increases in ALP and GGT may indicate that liver damage has occurred, e.g., cirrhosis. Also reflecting liver damage, total serum bilirubin levels are typically increased, while direct (conjugated) and indirect (unconjugated) bilirubin levels are either increased or normal.[15] Additionally, serum ammonia levels are increased, serum urea levels are decreased, and the blood urea nitrogen/creatinine ratio will be low.[15] Albumin and total protein levels may be normal in acute hepatitis, but are often decreased in chronic hepatitis.[8]

Other changes are seen in urine and hematological parameters. Urinalysis is typically positive for bilirubin and normal or increased for urobilinogen.[15,19] The amount of biliru-

Table 1. Laboratory findings associated with liver damage

Chemistry	Result
ALT	
Albumin	
Ammonia	
AST	
ALP	or N
Bilirubin – total	
Direct	
Indirect	or N
Blood urea nitrogen	
BUN/creatinine ratio	
GGT	or N
Total protein	
Coagulation	
Prothrombin time	
Hematology	
Platelet count	
White blood cell count	
Urinalysis	
Bilirubin	
Urobilinogen	or N

= increased levels
= decreased levels
N = normal reference range

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bin or urobilinogen found in urine varies with the extent of hepatocellular damage. Finally, a complete blood count may reveal low platelet and white blood counts, and the prothrombin time (PT) may be increased, which occurs more frequently in advanced disease.

A percutaneous liver biopsy can be performed to aid in diagnosis, determine the degree of liver damage and the severity of the disease, and provide baseline data to gauge disease progression and help determine the best treatment. The degree of necrosis, inflammation, and fibrosis is determined through immunohistochemical staining of biopsy sections. Liver biopsy is contraindicated, however, in patients with advanced disease and/or clotting problems, as indicated by an increased PT, marked ascites, and thrombocytopenia.[8]

TREATMENT

Patients are eligible for treatment when there is evidence of HCV infection.[5] Evidence of HCV infection includes the presence of antibodies to HCV and/or viral RNA as well as elevated levels of liver enzymes in the serum.[14] Additionally, a physician would look for evidence of liver disease (fibrosis, inflammation, and necrosis) by liver biopsy, if appropriate.[5] The goal of all HCV treatment protocols, whether monotherapy or combination therapy, is to eliminate detectable HCV RNA in the blood. Treatment is considered successful if HCV RNA is undetectable six months after the treatment regimen is completed.[20] All treatments involve the use of interferon-α, a glycoprotein naturally released by virally-infected cells, either as monotherapy or in combination with other agents.[20] The interferon-α produced by infected cells diffuses to nearby cells, and stimulates the synthesis of an RNA-dependent protein kinase (PKR).[21] PKR helps to inhibit viral replication in these cells, and stimulates macrophages and natural killer cells.[21] This indirect mechanism described appears to play a role in the efficacy of synthetic interferon treatment.[20] There is evidence that interferon also has a direct antiviral effect.[22]

Monotherapy involves the use of genetically engineered interferon α-2a, interferon α-2b, interferon alfacon-1, or a branched-chain, polyethylene glycol modified form of interferon, peginterferon α. Pegylation changes the uptake, distribution, and excretion of the interferon, which prolongs its half-life. Peginterferon can be given once weekly and provides a constant level of interferon in blood. Two forms of peginterferon have been developed: peginterferon alfa-2a (Pegasys®: Hoffman LaRoche) and peginterferon alfa-2b (Pegintron®: Schering-Plough Corp.). At this time only peginterferon alfa-2b has been approved for use in chronic hepatitis in the United States. Formerly monotherapy typically lasted six months to two years, with a low success rate of less than 15%.[5] However, an improved success rate is seen with a branched-chain, polyethylene glycol modified form of interferon, peginterferon α administered for 48 weeks.[5,20,23] Because of relatively low success rates of monotherapy with different interferon formulations, combination therapy with additional therapeutic agents is recommended, unless contraindications to those supplemental agents exist (contraindications will be discussed later).[5]

Combination therapy consists of interferon α injections and oral ribavirin.[20] Prior to June 1998, the FDA approved the combination treatment as a secondary treatment regimen only when interferon alone failed. However, in June 1998 the FDA approved combination therapy as a primary course of treatment and as of September 2002 the optimal regimen is a 24- to 48-week course of combination peginterferon alfa-2b (1.5 mcg/kg/week) and ribavirin (400 mg twice/day).[3,5] Safe dosage levels of ribavirin have not yet been determined for pediatric patients; in these cases monotherapy is indicated.[5] Ribavirin is a synthetically produced nucleoside that inhibits a broad spectrum of viruses by inhibiting viral DNA and RNA synthesis and replication.[20] Overall, combination therapy, as compared with monotherapy, has both a higher rate of elimination of HCV RNA (up to 70% of patients), and a lower rate of relapse following completion of therapy.[3,5]

Among patients who become HCV RNA negative during treatment, a proportion will relapse when therapy is stopped. Undetectable levels of HCV RNA for at least six months following completion of therapy indicate sustained response to therapy.[5] Recommended duration of therapy is six months up to a year, depending on HCV genotype. This regimen has been shown to be effective in approximately 40% of patients, resulting in the elimination of the virus and improvement in liver inflammation (indicated by a decrease in liver enzyme levels in the serum).[5] HCV genotypes 2 and 3 are most responsive to therapy, and a 24-week course of combination therapy (70% to 80% response rate) yields similar results to those of a 48-week course. On the other hand, HCV genotype 1 is less responsive (40% to 45% response rate) to combination therapy and a 48-week course is necessary for a more sustained response rate.[5]

Contraindications for use of interferon α-2b and peginterferon include conditions such as cirrhosis, ascites, persistent jaundice, and wasting; age greater than sixty years; or concurrent HIV infection with low CD4 levels. Additional contraindications include evidence of neuropsychological problems, recent alco-

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hol abuse (must abstain for at least six months prior to treatment), autoimmune disease, and bone marrow hypoplasia. Contraindications for use of ribavirin include anemia, renal dysfunction, coronary artery disease, or cerebrovascular disease.[5]

Side effects of interferon use include fatigue, flu-like symptoms, depression and other psychiatric disorders, rashes, birth defects, exacerbation of autoimmune disease or existing heart conditions, and suppression of bone marrow hematopoiesis. Bone marrow suppressive effects appear to be more common with peginterferon than standard interferon; thus, frequent monitoring of cell counts is recommended for patients receiving this treatment. These adverse effects occur in more than 10% of patients and vary from mild to severe, but can be mitigated by adjusting the dosage of interferon administered. Most side effects are worse during the first few weeks of treatment and may diminish as treatment is continued.

Undesirable reactions related to ribavirin include anemia (causes RBC hemolysis), ischemia, fatigue, itching, i.e., histamine-like effect, and rashes. These effects, too, can sometimes be relieved by dosage adjustment. Ribavirin is excreted mostly by the kidneys and patients with serum creatinine levels above 2.0 mg/dL should not given this drug. Ribavirin is contraindicated in pregnancy. Less common side effects of ribavirin include severe bacterial infections, marked thrombocytopenia or neutropenia, seizures, and retinopathy. Adverse effects of ribavirin are most severe early in treatment, and may be more severe when combined with interferon. Fatal myocardial infarctions and strokes have been reported with combination therapy.[5]

During treatment, the patient should be closely monitored at regular intervals to assess side effects and response to treatment. Blood counts and aminotransferase (AST and ALT) levels should be measured at weeks 1, 2, 4, and at 4- to 8-week intervals subsequently. If side effects are manifest, drug dosages should be adjusted to alleviate them.[5] A serious and rare side effect known as autoimmune hepatitis can be induced by treatment. This can be avoided by monitoring ALT levels and if they rise to greater than twice the baseline value, therapy should be stopped and the patient monitored. Corticosteroid therapy may be needed to control the hepatitis.[5]

Because disease progression and response to therapy is affected by HCV genotype, initial evaluation for genotype is recommended.[5,7] HCV RNA should be measured at 24 weeks. If at this time HCV RNA is still detected, therapy should be stopped and considered unsuccessful. If HCV RNA is not detected at 24 weeks, and genotypes 2 and 3 are implicated, then treatment is successful. If genotype 1 is implicated and

Figure 1. Treatment strategy and follow-up for patients infected with Hepatitis C

Algorithm for HCV treatment

Make diagnosis based on AST and ALT elevations, anti-HCV, HCV RNA in serum, and chronic hepatitis shown by liver biopsy.

Assess patient for suitability of therapy and contraindications.

Test for HCV genotype.

Discuss side effects and possible outcomes of treatment.

Start therapy with peginterferon α-2b dose of 1.5 mcg/Kg weekly and oral ribavirin 800 mg daily.

At weeks 1, 2, and 4 and then at intervals of every 4 to 8 weeks thereafter, assess side effects, symptoms, blood counts, AST, and ALT levels.

At 24 weeks, assess AST and ALT levels and HCV RNA. In patients with genotypes 2 and 3, stop therapy. In patients with genotype 1, stop therapy if HCV RNA is still positive, but continue therapy for a total of 48 weeks if HCV RNA is negative, retest for HCV RNA at the end of treatment.

After therapy, assess AST and ALT levels every two months for six months. In responders, repeat HCV RNA testing six months after stopping therapy.

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HCV RNA is not detected, therapy should be continued for an additional 24 weeks to prevent recurrence.

Long term monitoring is recommended. Following successful treatment, AST and ALT levels should be monitored every two months up to six months. Six months after stopping therapy, testing for HCV RNA by RT-PCR should be performed. If HCV RNA is still negative, relapse is unlikely.[5] Figure 1 summarizes the treatment protocol.[5]

Few approved options exist for those patients who fail treatment or relapse.[3] As mentioned, peginterferon α may be more successful, and has fewer side effects than interferon α-2b.[20,22] Currently, the FDA is considering approval of peginterferon combined with ribavirin.[20] Treatments on the horizon include recombinant interleukin 10 (and other cytokines), and antiviral agents that inhibit HCV-derived enzymes, such as RNA polymerases, helicases, and proteases.[2] Also being investigated are drugs including ribozymes (enzymes designed to breakdown viral RNA) and antisense oligonucleotides.[5]

Specific mechanisms being researched and targeted for antiviral action are blockage of HCV antigen production, inhibition of viral glycosylation, and blockage of viral entry into cells.[5] As a last resort, liver transplantation may be the only effective treatment for HCV.[8,20] However, the new liver frequently becomes infected. Liver transplantation is still considered beneficial because the patient may live the remainder of his/her life without complications due to the insidious course of the disease.[8]

SUMMARY

Although information continues to accumulate about HCV and treatment strategies continue to improve, hepatitis C remains a serious health threat. Current combination therapy has a reasonably good response rate and represents an improvement over prior strategies; however, treatment failures and serious side effects do occur, highlighting the need to research and design newer treatments. As a result of the successful cloning of the hepatitis C virus in 1991, researchers have been striving to develop a vaccine; but their efforts are complicated by the need to include all HCV genotypes to make an effective vaccine. Because HCV mutates rapidly within infected patients, an effective vaccine may become useless if new strains of mutant viruses arise. Thus, alternative strategies are needed to meet the challenge of HCV infection.[9]

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