Viral markers in the treatment of hepatitis B and C

H Schmilovitz-Weiss, M Levy, N Thompson, G Dusheiko

Abstract

Acute hepatitis B virus (HBV) infection is typically distinguished from chronic disease by a positive IgM anti-hepatitis B core antigen (anti-HBc) test. Patients with chronic hepatitis B remain hepatitis B surface antigen (HBsAg) positive, often with raised serum alanine aminotransferase (ALT) activities, for more than six months. The presence of hepatitis B e antigen (HBeAg) and HBV-DNA correlates with infectivity (although patients infected with the pre-core mutated virus may be HBeAg negative). Immunity after HBV infection is characterised by the presence of anti-HBs and anti-HBc antibodies. Patients who respond to interferon alfa treatment lose HBV-DNA and HBeAg from serum and their ALT values return to normal; some also lose HBsAg and acquire anti-HBs. Diagnosis of acute hepatitis C virus (HCV) infection remains largely dependent on history and exclusion, as anti-HCV antibodies may appear late or never at all, although HCV-RNA may be detectable on polymerase chain reaction (PCR) within days of infection. Second generation ELISAs detect a range of anti-HCV antibodies in chronic infections, and confirmatory RIBAs have reduced the incidence of false-positive results. Direct tests for HCV antigens in serum are not yet available, although PCR testing for HCV-RNA can be used to confirm viraemia. Patients who respond to interferon alfa treatment show continuous normalisation of serum ALT values, and some lose HCV-RNA. Relapse occurs in about half of all those who respond.

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Chronic viral hepatitis B and C are important global causes of morbidity because of liver disease. Fortunately, diagnostic markers for both these diseases are now available and facilitate the selection of patients for treatment, and monitoring of this. Newer tools should improve our understanding of the mechanism of viral persistence in patients with chronic hepatitis and the pathogenesis of these diseases.

Hepatitis B

Hepatitis B is caused by the hepatitis B virus (HBV), a 42 nm, enveloped DNA virus. The

virus possesses an outer envelope, expressing hepatitis B surface antigen (HBsAg), and an inner nucleocapsid, expressing hepatitis B core antigen (HBcAg) and hepatitis B e antigen (HBeAg). The genome of HBV is a small, circular double-stranded DNA of about 3200 bases.¹ HBsAg consists of three envelope proteins (pre-S1, pre-S2, and S), which originate from one gene. The two nucleocapsid proteins, HBcAg and HBeAg, are the products of a single gene region on the viral genome,² which has two initiation codons. Initiation of translation at the first site (nucleotide 1814) produces a 312 amino acid polypeptide (P25). With subsequent processing, the resultant polypeptide is secreted as HBeAg (P15-18).³ Translation from the second initiation codon (nucleotide 1901) results in unprocessed core polypeptides which are assembled into HBcAg particles.

The pathogenesis of the disease is incompletely understood. Immunological mechanisms seem important, since most evidence suggests that HBV is not usually cytopathic, and that the expression of disease involves a poorly understood interplay between viral and host factors over time.

DIAGNOSIS OF HEPATITIS B: IMPLICATIONS FOR TREATMENT

Serological markers for clinical diagnosis of HBV infection have been well characterised, and are now complemented by newer molecular biological techniques. The most widely used test for diagnosis of HBV is an assay for HBsAg. Current immunoassays for HBsAg detect 100–200 pg HBsAg per ml of serum, corresponding to roughly 3×10^7 particles per ml. Most HBeAg positive carriers have more than 10^5 genomes per ml serum. Immunity after infection with HBV is characterised by the presence of anti-HBs and anti-HBc antibodies in serum. Immunity after vaccination is characterised by the presence of anti-HBs.

ASSAY OF HBV-DNA

The dot blot hybridisation test for serum HBV-DNA correlates with infectivity and is an important means of determining both the presence of viral genomes and the response to antiviral treatment. Amounts of HBV-DNA are expressed in pg per ml or, alternatively, genome equivalents per ml. One pg corresponds to 2.86×10^5 genome equivalents. The usual range of sensitivity is 0.1–1 pg or 10^5 genome equivalents per ml of serum. At least

University Department of Medicine,
Royal Free Hospital and School of Medicine,
London, United Kingdom
H Schmilovitz-Weiss
M Levy
N Thompson
G Dusheiko

Correspondence to:
Dr G M Dusheiko,
University Department of Medicine,
Royal Free Hospital and School of Medicine,
Pond Street,
London NW3 2QG, UK.

10^{3-5} virions have to be present in a specimen to be detected by dot blot assay. Liquid phase hybridisation assays for HBV-DNA have also been developed, in which extracted HBV-DNA is mixed with ^{125}I -labelled nucleic acid probe and allowed to hybridise, then free and hybridised probe is separated by column chromatography. Chemoluminescent assays are under investigation.

Southern blot assay is most useful in the study of liver DNA samples, allowing detection of integrated or free episomal HBV-DNA.⁴

POLYMERASE CHAIN REACTION FOR HBV-DNA

Polymerase chain reaction (PCR) permits the detection, cloning and sequencing of HBV genomes from patients with low level hepatitis B viraemia. It is at least 10^4 times more sensitive than dot blot hybridisation assays for HBV. In practice, 3×10^{-6} pg cloned HBV can be detected by ethidium bromide staining after 50 cycles of PCR.⁵ By using a nested primer technique, the sensitivity and specificity of the test is improved (1 molecule per 10^6 cells), and the amplified product can be visualised in a same day test. The sensitivity can also be improved to detection of less than 1 fg (100 ag) of original DNA if the amplified DNA is hybridised to labelled probe and detected by autoradiography. Contamination remains the major difficulty of the procedure.

ACUTE HEPATITIS B: DIAGNOSIS

HBsAg is the first marker to appear in serum, followed by HBV-DNA, HBeAg and DNA polymerase, and anti-HBc.⁶ HBV-DNA values usually reach 10^5 to 10^8 genome equivalents per ml with the onset of symptoms, after which they decrease. In contrast, in patients who develop chronic hepatitis B, HBV-DNA values remain high. Measurement of HBV-DNA by PCR in acute hepatitis has shown that in most patients HBV-DNA persists as long as HBsAg is present. A positive IgM anti-HBc test typically distinguishes acute from chronic hepatitis B. By the time the patient consults a physician, HBV-DNA and HBeAg are often no longer detectable in serum. The loss of HBeAg in the acute phase is a sign that the patient will clear HBsAg. Pre-S proteins show a good correlation with the detection of HBV-DNA. Anti-HBs is the last marker to appear in serum.

HBsAg may be present only transiently in serum. The only evidence of infection may therefore be the presence of IgM anti-HBc or subsequent development of IgG anti-HBc and anti-HBs. Some cases of fulminant hepatitis B may be caused by cryptic hepatitis B; usual markers of HBV, including HBsAg are absent, but HBV-DNA has been detected by PCR in liver tissue.⁷

CHRONIC HEPATITIS B: DIAGNOSIS

Many HBV carriers are detected through routine screening for HBsAg or the presence of abnormal liver function tests. Older patients

may present for the first time with complications of cirrhosis, or even hepatocellular carcinoma. Typically, serum aminotransferase activities are raised in patients with HBeAg/HBV-DNA positive chronic hepatitis, but some patients may have normal values. The aminotransferase activities fluctuate with time. A spontaneous remission in disease activity may occur in approximately 10–15% of HBeAg-positive carriers per year, characterised by disappearance of HBV-DNA from serum followed by loss of HBeAg. This may occur after a sudden exacerbation in serum aminotransferase values. Once HBeAg is cleared, the disease remits temporarily, and serum aminotransferase activities become normal. In many patients, there is a benign outcome. Progressive disease is believed to occur with persistence of viral replication, viral mutations, or transient exacerbations in viral replication. In particular, some patients lose HBeAg but continue to have active liver disease and HBV-DNA detectable in serum by direct hybridisation techniques and PCR. In these patients, HBcAg is apparent on histochemical staining of hepatocytes and they have histological evidence of chronic active hepatitis.⁸⁻¹⁰ They tend to have severe disease associated with intermittent serum HBV-DNA positivity. These carriers are frequently IgM anti-HBc positive. PCR amplification and subsequent sequencing of genomic DNA in patients with this form of chronic hepatitis B has indicated that one or more nucleotide substitutions in the pre-core region of the genome account for the absence of HBeAg expression. The commonest mutation is a point mutation from guanine to adenine, creating an in-frame stop codon – that is, converting codon 28 for tryptophan (TGG) to a termination or nonsense codon (TAG). This mutation prevents the translation of HBeAg (Fig 1). Several other mutations in the core and pre-core gene regions may also be present. Mutant HBeAg negative genomes may arise during infection, either as a result of a propensity to mutations in this region or because of immune selection pressure. Most evidence suggests that the nucleocapsid antigens (HBcAg and HBeAg) expressed on the cell membrane are the major target of the immune response and cytolytic T cells.¹¹ Liver cells that do not express HBeAg might, therefore, escape immune elimination.

Mixed HBeAg-positive and HBeAg negative infections can occur in both HBeAg positive and anti-HBe positive patients. Why some patients and not others develop severe disease has yet to be explained. The predominant viral population in anti-HBe positive patients with exacerbated disease consists of HBeAg negative, pre-core defective HBV, but core mutations may also be relevant. Pre-core mutant HBV genomes have also been detected in patients who seroconvert after interferon treatment.

Currently, PCR is the most sensitive technique for measurement of viraemia in HBV infection. Use of this assay indicates that loss of HBeAg is associated with a decrease

Figure 1 Markers of hepatitis B virus (HBV) infection. The arrows indicate points at which treatment is considered and the likely response.

rather than complete disappearance of HBV-DNA. With loss of HBeAg, HBV-DNA becomes undetectable by dot blot hybridisation, but can be detected by PCR.¹² This finding apparently represents low level continuation of DNA replication, but is frequently not associated with active liver disease. The continued persistence of HBV infection without disease is unexplained, and may be caused by infection with defective genomes or containment by the immune system.

Likewise, after successful antiviral treatment, HBV-DNA measured by dot blot hybridisation disappears, but in our experience and that of others, HBV-DNA remains detectable by PCR for more than a year after loss of HBeAg.^{13 14} HBV-DNA has been shown in liver tissue of patients in whom HBsAg reappeared, and in peripheral blood mononuclear cells in patients whose serum and liver were PCR negative for HBV-DNA, thus identifying a potential mechanism of reinfection from extrahepatic sites.¹⁵

TREATMENT OF CHRONIC HEPATITIS B: RELEVANCE OF DIAGNOSTIC MARKERS

Specific treatment has now become possible with the finding that interferon alfa inhibits replication of HBV and that prolonged treatment can lead to remission of the disease. In 25–45% of patients, interferon is clearly beneficial and may lead to clearance of HBsAg.

Interferon alfa treatment is indicated in patients with chronic hepatitis B who are HBsAg-positive, HBV-DNA negative and/or HBeAg-positive, and who have increased serum activities of alanine and aspartate aminotransferase (at least twice the upper limit of normal). Patients who are positive for HBsAg but negative for HBeAg and HBV-DNA should not be treated. Liver biopsies are advised in order to confirm the diagnosis and to stage the disease. The immediate goal of treatment is to eradicate viral replication and ameliorate the underlying disease. Responses to interferon alfa are characterised by a loss of HBV-DNA and HBeAg from serum, a fall in serum aminotransferase activities to within the normal range, and subsequent improvement in liver histology. In some individuals, HBsAg is also cleared, followed by the appearance of anti-HBs. A typical pattern of response is shown in Fig 1. Serum HBV-DNA values usually decrease when treatment is started but remain detectable until four to 12 weeks of treatment. Once HBV-DNA has become undetectable, HBeAg is cleared, usually within a few weeks although it may occur months later in some cases. The clearance of HBeAg and HBV-DNA during treatment is usually accompanied by a transient increase in serum aminotransferases. This pattern of response suggests that interferon acts by augmenting the immune response to HBV, perhaps triggered by the inhibition of viral replication. The loss of HBeAg and HBV-DNA is usually followed by a fall in serum aminotransferase values to within the normal range, and improved liver histology. Approximately 20% of patients who respond to treatment with clearance of HBeAg will also clear HBsAg within six months. Long-term follow-up studies have also shown that many more patients who lose HBeAg with interferon treatment will eventually lose HBsAg, although this may occur years after loss of HBeAg.

Several clinical features have been identified that predict response to interferon alfa.¹⁶ These features include:

- High initial serum aminotransferase activities;
- Low initial HBV-DNA values in serum (<100 pg/ml);
- A history of acute hepatitis (indicating more active disease);
- Short duration of disease;
- Female sex; and
- Absence of other major medical conditions, such as renal insufficiency, human immunodeficiency virus (HIV) infection, or immunosuppressive treatment.

The optimal dose and duration of interferon are still uncertain but an appropriate scheme would be 5 million units (MU) daily or 10 MU three times per week for three to four months given subcutaneously. Blood chemistry, haematology, and thyroid function tests should be measured at regular intervals. After treatment, patients should be tested at progressively spaced intervals (one to six months) for serum biochemistry and HBV markers, to determine whether the response is sustained.

It has been suggested that the presence of circulating pre-core mutants could predict which patients would respond favourably or otherwise to interferon alfa. To address this, we analysed data from 15 patients with chronic hepatitis B who were treated with interferon (nine responders and six non-responders). Serum samples were collected before and after treatment. Circulating HBeAg negative mutants were not found before treatment in either responders or non-responders. These results suggest that a predominance of HBeAg negative virions cannot be considered the overriding factor in patients who respond to treatment.¹⁴

Non responders represent a difficult problem in management. The use of a short course of prednisolone before interferon treatment has been advocated, but in prospective studies this has not led to higher response rates, except perhaps in those patients who have relatively inactive disease. Interferon may be used to treat anti-HBe positive, HBV-DNA positive patients, but many tend to relapse after stopping interferon. Anti-HBe and HBV-DNA positive patients with compensated cirrhosis but raised serum transaminase activities may be amenable to treatment with a lower dose (1 to 3 MU three times weekly) for a year. These patients may have lower serum concentrations of HBV-DNA (50 pg/ml) and may lose HBV-DNA with improvement in serum amino-transferases.¹⁷⁻¹⁹ Many of these patients have hypersplenism, however, and their thrombocytopenia and leukopenia require at least weekly monitoring. They are at greater risk of infections, and relapses also occur in these patients. Responses may be better in younger patients without cirrhosis compared with older patients and those with cirrhosis.

Other groups of patients who present difficult problems include Chinese patients, patients with advanced cirrhosis, and patients with other major illnesses who are immunosuppressed. Chinese patients and children with active disease and high ALT values probably respond to interferon, or pulsed corticosteroid treatment and interferon, in a similar manner to other adults.²⁰ Patients with advanced cirrhosis may decompensate with interferon and should not be treated.

MARKERS OF HBV AND LIVER TRANSPLANTATION

Reinfection of the grafted liver and development of chronic hepatitis is the major shortcoming of liver transplant for cirrhosis caused by HBV. Eighty five to 95% of patients positive for HBV-DNA at the time of transplant develop reinfection, despite immunoprophylaxis with HBV immune globulin.²¹ Twenty five to 40% of HBV-DNA negative patients also become reinfected as do 10% of hepatitis delta virus (HDV) positive patients. Strangely, some patients with delta hepatitis remain asymptomatic (despite detection of HDV antigen in the liver) until the reappearance of HBsAg in serum.

Hepatitis C

Acute infection with hepatitis C virus (HCV) may either be severe or asymptomatic and unnoticed. The acute disease may resolve completely, but has a disturbing propensity to lead to chronic hepatitis C, explaining the relatively high prevalence of the disease. Chronic HCV infection may lead to mild illness only, which may be asymptomatic for decades and not progressive. A carrier state is recognised in patients who are HCV-RNA positive but have normal ALT values. In others, the disease is inexorably progressive, and persistent life long infection leads progressively to chronic active hepatitis, cirrhosis, portal hypertension, and even hepatocellular carcinoma.

Sensitive and specific markers for identification of HCV infection are now available. However, the HCV markers used in diagnosis still rely on recombinantly cloned antigens, and in some circumstances the diagnosis remains difficult.

MOLECULAR VIROLOGY OF HCV: IMPLICATIONS FOR TREATMENT

HCV is an enveloped, positive-stranded RNA virus of 30–80 nm in size.²²⁻²⁴ The complete 9400 bp nucleotide sequence of the HCV genome has now been determined in a number of isolates.²⁵⁻²⁷ The nucleocapsid and envelope proteins are encoded at the 5' end of the genome, while the non-structural elements are located at the 3' end. The nucleotide sequences from the considerable number of isolates obtained to date indicate that HCV may be divided into at least five classes (HCV I-V) based upon nucleotide and deduced amino acid homology.²⁸⁻³⁰ The nomenclature of these genotypes remains confused. There are hypervariable regions in the E1 and E2/NS1 domains.^{31 32} These regions in the envelope glycoprotein(s) may be important antigenic sites, and variability in these regions has implications in diagnostic screening, immunoprophylaxis, and perhaps persistence of infection. The 5' untranslated region is usually highly conserved between isolates, although heterogeneous regions are found.^{33 34}

The cloning of the HCV genome has facilitated the production of a panel of recombinantly expressed antigens produced in yeast and *Escherichia coli*. Sequence data have also allowed the production of synthetic peptides for use in similar immunoassays.^{35 36}

ANTIBODIES TO HCV

HCV probably circulates in the serum at a concentration of between 10^2 – 10^7 particles per ml³⁷⁻³⁹ and, so far, it has proved impossible to detect viral antigens by conventional methods. Therefore, the detection of antibodies to HCV has become important as an indication of past or present infection. The first immuno-diagnostic ELISA detected antibodies to c100-3, a 363 amino acid fusion polypeptide representing part of the NS4 region of the HCV genome, and expressed in yeast with

superoxide dismutase.⁴⁰ The presence of antibody to c100-3 proved a good marker for infection with HCV in both blood transfusion associated and sporadic non-A, non-B (NANB) hepatitis.⁴¹⁻⁴⁵ Seropositivity was also shown to be associated with chronic infections and correlated with infectivity in blood donations.⁴⁵⁻⁴⁸

When screening low risk groups such as blood donors, however, false-positive results were common. False-positive results were also common in patients with hypergammaglobulinaemia, and in stored tropical samples.⁴⁹⁻⁵⁰ The assay also lacked sensitivity.

Second generation ELISAs which incorporate two extra HCV derived recombinant proteins have increased the sensitivity of the assay.⁵¹⁻⁵³ Antibodies to c22c are an earlier finding and occur more frequently than those to c100-3 during the course of HCV infection. A protein derived from the NS3 and NS4 regions (c200), or a smaller fragment of it (c33c) from NS3 alone, is also included in the second generation assays. The c200 protein is the product of a large cloned fragment, combining both the c33c and c100-3 regions. The detection of antibodies to the E1 and E2/NS1 glycoproteins has proven elusive, probably because of the sequence heterogeneity found in these regions. Antibodies to envelope proteins have been detected in viraemic patients, and it is therefore uncertain whether these antibodies are neutralising.⁵⁴⁻⁵⁷ IgM responses to HCV c100-3 antigen have been investigated in acute and chronic disease. Detectable levels have been found as early as four weeks after inoculation but can last for up to two years in individuals with chronic infection.⁵⁸⁻⁵⁹ Anti-HCV IgM is usually present in patients with raised ALT activities. The test is of uncertain importance at present, but may have some predictive value regarding progression to chronicity, and response to antiviral treatment.

SUPPLEMENTARY TESTS FOR ANTIBODY TO HCV

The most widely used method for supplementary testing is the recombinant immunoblot assay (RIBA) (Chiron Corporation) in which antibodies are sought to recombinant antigens of HCV coated on nitrocellulose strips. The second generation test (RIBA-4) includes bands 5-1-1, c100-3, c22-3, and c33c. Samples are regarded as confirmed positive if antibodies to two or more of the HCV proteins are present and indeterminate if antibody to only one antigen is found. The value of RIBA in excluding false-positive results has been extensively shown, as has a correlation between RIBA positivity and viraemia.⁶⁰⁻⁶¹

The utility of synthetic peptides as antigens in the immunodiagnosis of HIV infection has led to the production of similar peptides using the deduced amino acid sequence of HCV.

DETECTION OF VIRAL GENOME

Direct detection of HCV-RNA by conventional nucleic acid hybridisation

techniques is difficult, but HCV-RNA can be detected by an amplification technique (PCR).⁶²⁻⁶³ Using this method, HCV-RNA can be detected in most anti-HCV positive patients and also in a proportion of those who are antibody-negative, particularly neonates born to anti-HCV positive mothers.⁶⁴ The selection of PCR primers from the 5' region results in a great improvement in the sensitivity of the assay.⁶⁵⁻⁶⁶ Sensitivity is maximised by using double or nested PCR, which can detect as little as one molecule of cDNA, although sample handling can affect sensitivity.

HCV-RNA occurs in the blood long before other markers and often within days of infection.⁶⁵⁻⁶⁷⁻⁶⁸ Results of PCR tests should be assessed in combination with all available clinical, biochemical, and pathology information. The detection of negative stranded intermediates in the serum or other tissues may be of help in identifying active viral replication.⁶⁹⁻⁷⁰ Testing for such intermediates will also be of use in identifying sites of replication and also perhaps evaluation of response to antiviral treatment.⁶⁹

Methods for quantification have been based, until now, on dilution analysis. More accurate methods of HCV-RNA quantification are being developed for clinical use and for measuring response to antiviral treatment.⁷¹⁻⁷² The sequencing of PCR products enables information on genome diversity between isolates to be gathered. This will be important if, as has been suggested, the clinical outcome and response to treatment are determined by the infecting subtype.⁷³

DETECTION OF HCV IN THE TISSUES

There have been reports of visualisation of appropriate size viral particles in tissue from patients with NANB hepatitis.⁷⁴ Using immunohistochemical techniques with mono- and polyclonal antibodies to HCV proteins, several groups have detected evidence of HCV infection in liver tissue.⁷⁵⁻⁷⁷ The presence of virus specific antigen in the cytoplasm is a very early finding in the course of infection with HCV.⁷⁸ With the aid of in situ hybridisation, the HCV genome has also been detected in infected liver.⁷⁹ These results require verification, however, and may not be specific. HCV-RNA can be successfully amplified from fresh frozen sections or formalin fixed, paraffin embedded material.⁸⁰

DIAGNOSIS OF ACUTE HCV INFECTION

The mean incubation period of HCV is six to 12 weeks. With large inocula, however, such as after administration of factor VIII, the incubation period is reduced.⁸¹⁻⁸² The acute course of HCV infection is clinically mild, and the peak serum ALT increases are less than those encountered in acute hepatitis A or B. Only 25% of cases are icteric. During the early clinical phase, serum ALT activities may fluctuate, and may become normal or near normal, making the determination of true convalescence difficult.

The average time from transfusion to seroconversion is of the order of seven to eight weeks with the second generation tests; anti-c33 or anti-c22 antibodies often appear a week or two earlier than anti-c100-3. Seroconversion occurs much less frequently, and in lower titre, in acute self limiting infections compared with those that progress to become chronic.⁴¹⁻⁸³ Serological testing now indicates that seroconversion to anti-HCV occurs in 85–100% of patients with chronic blood transfusion associated NANB hepatitis.⁴⁶ A proportion of patients with transfusion associated and sporadically acquired NANB hepatitis remain anti-HCV seronegative, however. It will be important to define whether this represents HCV infection with poor serological response, or whether it is the result of other, as yet unclassified, NANB agents.

The acute disease may resolve completely with clearance of HCV-RNA from serum. A suitable immunodiagnostic test for resolved infection and immunity is not available, but antibodies to the envelope region are being sought.

In some patients, a very early appearance of anti-HCV may be the result of passively acquired antibody from the donor blood.

Serum HCV-RNA has been detected within one to three weeks of blood transfusion in patients with HCV; it usually lasts less than four months in patients with acute self limited HCV infection, but may persist for decades in patients with chronic disease.⁶⁸⁻⁸⁴

DIAGNOSIS OF CHRONIC HEPATITIS C

Most cases of chronic hepatitis C will not have been preceded by an episode of clinically apparent, icteric hepatitis. Fifty to 75% of patients with type C transfusion associated or sporadic hepatitis continue to have abnormal serum aminotransferase levels after 12 months, and chronic hepatitis histologically.⁸⁵ Serum aminotransferase activities decline from the peak values encountered in the acute phase of the disease, but typically remain two to eightfold higher than normal. Serum ALT concentrations may fluctuate over time, and may even be normal intermittently.

Many patients have a sustained increase in serum aminotransferases. Cirrhosis develops in approximately 20% of chronic hepatitis C patients within 10 years, although the cirrhosis may remain indolent and progress only slowly for a long period.⁸⁴⁻⁸⁶⁻⁸⁸ The disease is not necessarily benign, however, and rapidly progressive cirrhosis can occur. Older age at infection, concomitant alcohol abuse, and concurrent HBV or HIV infection or other illness may be important aggravating cofactors. Older patients may present with complications of cirrhosis, or even hepatocellular carcinoma. With progressive disease, the laboratory values become progressively more abnormal. Serum AST values greater than those of ALT, low serum albumin concentrations, and prolonged prothrombin time are all suggestive of cirrhosis. Low levels of autoantibodies may also become detectable.

Anti-HCV can persist for years, and even decades, in chronic hepatitis C but may decline in titre or disappear with resolution. A small percentage of patients seem to eradicate HCV-RNA permanently after chronic infection but this is usually seen in less than 5–10%.⁴¹⁻⁴⁷

HCV-RNA usually persists in patients with abnormal serum aminotransferase activities and anti-HCV antibodies. Although most patients with raised serum ALT are HCV-RNA positive, the converse is not always true. Isolates of HCV-RNA in individual patients may show nucleotide substitutions with time, suggesting that the HCV-RNA mutates at a rate similar to that of other RNA viruses.⁸⁹ The emergence of a mutant population does not always correlate with peaks in ALT.

It is not easy to project the prognosis for patients seen at one point in time. Episodes of hepatic necrosis may progress at variable rates to cirrhosis and, conversely, the lesion may revert in some patients to inactive hepatitis. Cirrhosis may develop in patients with an initially mild histological pattern; the mechanism for this transition is not known but may occur after repeated attacks of lobular necrosis associated with piecemeal necrosis. A relation between histological exacerbations and episodic clinical course is not proved, however.

The morphological features of cirrhosis caused by HCV are not specific to the disease; in the earlier stages, lymphoid aggregates may be seen.

AUTOIMMUNE HEPATITIS AND HEPATITIS C

There are conflicting reports regarding the occurrence of HCV antibodies in patients with autoimmune liver disease. Clearly, the ELISA for anti-HCV is prone to false-positive results in patients with high concentrations of immunoglobulins in serum.⁵⁰ These false-reactive anti-HCV antibodies in patients with anti-smooth muscle antibody (anti-SMA) may actually disappear with immunosuppressive treatment, as immunoglobulin levels decrease.⁹⁰ However, Italian patients with autoimmune chronic active hepatitis seem to have a high frequency of genuine exposure to HCV, whereas seropositivity in English patients usually represents a false-positive result. It is therefore not certain whether anti-HCV in patients with chronic active hepatitis represents persistent anti-HCV from earlier disease, whether the autoimmune disease is induced by HCV,⁹¹ or whether autoantibodies in autoimmune hepatitis patients cross react with HCV related antigens.⁹² In Japan, 80% of patients with chronic NANB hepatitis have circulating antibodies to a pentadecapeptide (Gor), an epitope of normal hepatocytes; this phenomenon may represent an autoimmune response peculiar to type C hepatitis.⁹³

Up to 50% of patients with type II autoimmune hepatitis (anti-liver kidney microsomal (LKM) antibody positive) are anti-HCV positive, and anti-HCV and anti-LKM in association may also represent another example of molecular mimicry. Anti-HCV

positive patients with anti-LKM autoimmune chronic active hepatitis are usually male, older, and have lower titres of anti-LKM than patients without anti-HCV. The target antigen of antibodies to LKM is a portion of the cytochrome P450 II D6 molecule; anti-LKM is not directed to a c100-3 epitope but some sequence homology between HCV and cytochrome P450 may exist.⁹⁴ This association has some therapeutic implications, as the autoimmune disease is responsive to corticosteroids, and may be aggravated by interferon alfa. It is preferable to confirm HCV viraemia in these patients by PCR.

TREATMENT OF ACUTE HEPATITIS C

The management of acute sporadic or transfusion related hepatitis C is along conventional lines, and is largely non-specific and supportive. The diagnosis remains one of exclusion in most patients, although if the patient is seen early enough, HCV-RNA in serum may be detectable at the time that serum aminotransferases are high. Anti-HCV may be detectable, particularly in severe, icteric cases, and in most of those patients destined to go on to chronic disease. Many patients will not be jaundiced. Approximately 50% of patients will still have raised serum aminotransferase activities six months after diagnosis. Therapeutic trials of interferon alfa have been undertaken. In general, these have been relatively small trials, and interferon has usually been administered for 12 weeks. A high proportion of patients (75%) have normal ALT at the end of treatment. This remission is not sustained when treatment is stopped, however, and the results do not differ significantly from untreated patients. Most have not reduced the rate of chronic disease, but might indicate an amelioration of the severity of the chronic hepatitis lesion. A Japanese trial of interferon beta, given intravenously for one to three months, did significantly reduce the risk of chronic hepatitis.⁹⁵ Until these findings can be reproduced, however, the routine administration of interferon for acute hepatitis C cannot be advised. Perhaps a longer course of treatment (six months to one year) may be beneficial.

TREATMENT OF CHRONIC HEPATITIS C

Asymptomatic patients detected through blood screening will require a supplemental test to verify their HCV status, as the rate of false-positive anti-HCV tests in low risk donors is high. Ideally, HCV-RNA should be measured in all patients to confirm viraemia, but the test is not generally available for routine diagnosis. If the test is reproducibly positive, then serum aminotransferases, bilirubin, alkaline phosphatase, and prothrombin time should be measured. In patients whose lifestyle or geographic origin suggest that they are at risk for other forms of viral hepatitis, HBV, HDV, and HIV infection must also be considered. Because autoimmune hepatitis is treated

differently, it is particularly advisable to exclude this diagnosis by measuring the titres of anti-SMA and anti-LKM antibodies, even in those with a positive anti-HCV test, and to measure HCV-RNA in anti-HCV-positive patients in whom interferon is contemplated.

Prospective studies have suggested that 10–20% of patients with chronic NANB hepatitis develop cirrhosis within 10 years. Therefore, individuals with chronic hepatitis C with raised ALT and chronic active hepatitis histologically should be considered for antiviral treatment. Trials are in progress to ascertain whether viraemic patients with normal ALT values respond to treatment. Preliminary treatment trials of interferon alfa for chronic hepatitis C indicated that a proportion of patients may respond to treatment with this agent. Larger, placebo-controlled studies have indicated that approximately 50% of patients will have normal serum aminotransferase activities after treatment courses of interferon alfa of approximately 3 MU three times weekly for six months.^{96 97}

Serum HCV-RNA may become undetectable after four to eight weeks of interferon alfa treatment in patients who respond, but an undetectable HCV-RNA at the end of treatment does not preclude relapse.

After stopping interferon at the end of six months' treatment, one half of the responsive patients will promptly relapse. Serum aminotransferase activities usually increase in patients who are HCV-RNA positive at the end of treatment.⁹⁸ Our studies at the Royal Free Hospital indicate that 20% of patients have a prolonged response to treatment and do not develop raised serum aminotransferase activities.⁹⁹ These patients also remain negative for HCV-RNA. Other regimens are being evaluated.¹⁰⁰ Starting treatment with a somewhat higher dose of 15 to 20 MU per week and prolonging therapy for a year may result in lower relapse rates. Relapses still occur, however, and patients have more side effects at higher doses. A second generation of trials is in progress to ascertain the effect of dose and duration.

The cost of six months of treatment is at least £1500. Treatment should not be continued beyond three months in patients who do not have reduced serum ALT activities. Responsive patients usually show histological improvement, and may have a decrease in collagen III propeptide concentrations.¹⁰¹ Unfortunately, responsiveness to interferon alfa remains somewhat unpredictable; patients with cirrhosis respond less well. Most patients will tolerate the treatment quite well. IgM anti-c100 or c22 declines in patients with a response to interferon alfa, and antibodies to E2/NS1 may also decrease.

When can treatment be considered successful? It is reasonable to infer that patients with normal serum ALT for more than a year after stopping interferon treatment, negative HCV-RNA for more than a year, histologically improved disease activity, and a normal serum

Hepatitis C: Natural history, markers and treatment

Figure 2 Markers of hepatitis C virus (HCV) infection and points at which treatment is considered.

procollagen III peptide have had a remission. Later relapse cannot be excluded, however. In some patients disease may actually worsen on treatment with interferon, and they may develop increased serum aminotransferase values. A positive anti-HCV antibody test in patients with autoimmune disease remains a pitfall in diagnosis, which has implications for treatment.¹⁰² These patients require confirmation by RIBA or PCR for HCV-RNA, as they may require corticosteroid rather than interferon alfa treatment.¹⁰³ It is possible that these patients have an underlying autoimmune status associated with hepatitis C and exacerbated by interferon treatment. For these patients, and for those who do not respond to treatment, ribavirin may be an alternative.¹⁰⁴ The most important predictive factors of response to interferon are absence of cirrhosis, younger age, HCV-RNA value, and possibly viral genotype.

RIBAVIRIN

Ribavirin is a synthetic nucleotide, a guanosine analogue, which possesses a broad spectrum of activity against both DNA and RNA viruses in vitro and in vivo.¹⁰⁵ Its efficacy has been shown in several viral diseases. The drug exerts its action after intracellular phosphorylation to mono-, di-, and triphosphate nucleotides. The precise mode of action probably includes perturbation of intracellular nucleoside triphosphate pools, interference with the formation of the 5' cap structure of viral mRNA by competitive inhibition of both guanylttransferase capping enzymes and methyltransferase capping enzymes, direct

inhibition of the viral mRNA polymerase complex, and possibly enhancement of macrophage inhibition of viral replication.

The pharmacokinetics of ribavirin have been studied. The bioavailability of oral formulations has been calculated at 19–65% (compared with intravenous administration). The distribution half life is one to three hours, but the terminal half-life is prolonged (27–52 hours), perhaps because of sequestration within red cells and other tissues. Ribavirin is concentrated 10–50 fold serum levels in red blood cells, and crosses the blood–brain barrier. Peak plasma concentrations range from 5–13 μ M after single oral doses of 600–2400 mg. The excretion of the drug is predominantly renal.

The major side effects of the drug that have been reported include anaemia, a metallic taste, dry mouth, flatulence, dyspepsia, nausea, headaches, irritability, emotional lability, fatigue, insomnia, skin rashes, and myalgia. Mild reversible anaemia is common. Modest increases in uric acid have been reported.

STUDIES OF RIBAVIRIN IN HCV INFECTION

In an unpublished study, Tong treated 22 patients with chronic hepatitis NANB with ribavirin 1200 mg daily for four weeks. Serum ALT and AST activities declined from a median of 145 to 78 and 86 to 52 IU/l, respectively. After four to eight weeks' follow up, median values of both ALT and AST had increased to those before treatment. An open-label study in Sweden, in which ribavirin was prescribed to 10 patients with chronic hepatitis C (1000–1200 mg/day) for 12 weeks, has been completed. Median serum AST values declined, but rose to values before treatment after the end of therapy.¹⁰⁴ A small study from the National Institutes of Health using escalating doses of ribavirin (600–1200 mg) showed a somewhat slower fall in serum aminotransferases, perhaps reflecting the lower starting dose of ribavirin.¹⁰⁶ There was a significant decrease in geometric mean titres of HCV-RNA. At the Royal Free Hospital, we have treated 15 patients with chronic hepatitis C with ribavirin. Seven patients (46%) had a 50% or greater decline in serum aminotransferase activity. Responsive patients may have negative serum HCV-RNA, but those with a lesser decline in ALT tend to remain positive. Ribavirin may have a role in patients with cirrhosis and leukopenia, or those with autoimmune disease and hepatitis C. Further controlled trials to assess response and relapse rates are warranted, and a multicentre, placebo controlled trial of ribavirin for hepatitis C is now in progress. Studies of ribavirin and interferon alfa used together are also indicated.

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