Update on chronic viral hepatitis

K Walsh, G J M Alexander

Abstract
Many recent and significant advances in the field of chronic viral hepatitis, including therapy, suggest that an update on chronic hepatitis is timely.

Chronic hepatitis B virus infection remains a significant worldwide cause of liver cirrhosis and hepatocellular carcinoma, despite the wide availability of a long established and effective vaccine. Transmission occurs via perinatal, sexual, and parenteral routes (particularly intravenous drug abuse and although blood products still carry a risk, this is now extremely low in Western countries). Only a minority of infected adult cases develop chronic hepatitis but in children under 1 year, 90% develop chronic hepatitis. The clinical spectrum of chronic liver injury ranges from mild inflammation to end stage liver cirrhosis. Interferon alfa has been the mainstay of treatment for patients with active disease but nucleoside analogues (lamivudine and adefovir) are now available with similar efficacy. Patients with end stage liver disease and hepatocellular carcinoma can be offered transplantation but infection in the graft is commonplace. The combination of hepatitis B immunoglobulin and newer antiviral drugs reduce the incidence and severity of graft infection significantly.

The hepatitis C virus epidemic of the latter half of the 20th century now affects more than 1% of populations worldwide. This RNA virus is spread parenterally and is becoming the leading indication for liver transplantation. The majority of patients develop chronic hepatitis, which may be progressive, evolving to significant liver disease (cirrhosis or hepatocellular carcinoma) in about 20% cases after decades. Treatment with the combination of interferon alfa and ribavirin is successful in up to 40% cases. Liver transplantation is a therapeutic option for some but graft infection is universal and often complicated by progressive liver fibrosis. A vaccine remains a remote prospect so that prevention is crucial.

Hepatitis D virus infection occurs on a background of hepatitis B virus infection and can also cause liver damage. The response to antiviral therapy is poor.

The newer “hepatitis” viruses such as hepatitis G and TTV have been described, although their role in chronic hepatitis is in doubt. Hepatitis A virus does not cause chronic infection.

Hepatitis B virus

Epidemiology
Chronic hepatitis B virus (HBV) infection accounts for 5%–10% of cases of chronic liver disease and cirrhosis in the United States. It affects 350 million people worldwide. The major modes of transmission of HBV are perinatal, sexual (via semen and vaginal secretions), via blood products, and via contaminated needles in intravenous drug addicts. A recent US study found that the prevalence of HBV infection was 4.9% in serum tested in the period 1988–94. This was similar to the prevalence of 5.5% in serum tested in the period 1976–80, despite the widespread availability of vaccination. The prevalence did not increase until puberty suggesting that sexual transmission is the most important route of transmission in that social setting. Other factors associated with increased HBV infection were black race, lower income group, and foreign birth.

Virology
The genome of HBV is a small circular DNA of 3.2 kb, which is the smallest DNA virus known to be pathogenic for humans. HBV replicates in hepatocytes that then secrete hepatitis B surface antigen (HBsAg), hepatitis B e antigen (HBeAg), and release intact virions (containing HBV DNA) into the circulation. Chronic HBV infection is characterised by the persistence of HBV DNA and usually HBeAg in serum. Remissions are characterised by the disappearance of HBV DNA and HBeAg from serum despite the continued presence of HBsAg at lower titre. The pre-core mutation of HBV is found in a subgroup of patients who are HBeAg negative and HBV DNA positive in serum.

Clinical course
Chronicity is dependent mostly upon age at exposure. Thus, 90% of children infected before their first birthday become chronic carriers compared to 5%–10% of adults. The majority of patients infected with HBV in the Western world acquire the disease in adulthood and so do not develop chronic infection whereas in third world countries exposure occurs in the womb, early infancy, or childhood.

Immune compromise results in a less severe acute infection and is associated with a sharp increase in the probability of viral persistence.
Table 1 Features found in the various stages of chronic HBV

<table>
<thead>
<tr>
<th>Stage</th>
<th>ALT</th>
<th>HBV DNA</th>
<th>Interface hepatitis</th>
<th>Cytoplasmic HBeAg</th>
<th>Nuclear HBeAg</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Normal</td>
<td>High</td>
<td>No</td>
<td>Negative</td>
<td>Positive</td>
</tr>
<tr>
<td>2</td>
<td>High</td>
<td>Low</td>
<td>Yes</td>
<td>Positive</td>
<td>Positive</td>
</tr>
<tr>
<td>3</td>
<td>Normal/low</td>
<td>Negative</td>
<td>No</td>
<td>Negative</td>
<td>Negative</td>
</tr>
</tbody>
</table>

In homosexual men coinfected with HIV, the course of chronic HBV is more severe. A study of 132 homosexual men with chronic HBV infection with no history of intravenous drug abuse (65 with HIV) found that HBV replication and progression to cirrhosis were increased in those with HIV. This supports a cytopathic mode of liver injury.

Three clinical stages of chronic HBV infection can be distinguished (table 1).

1. Patients have a normal serum alanine aminotransferase and are asymptomatic. They have high serum levels of HBV DNA and HBeAg. Liver biopsy shows the presence of core antigen in nuclei only. These patients will not respond to interferon alfa therapy.

2. Patients have lower levels of HBeAg and HBV DNA in serum. Liver biopsy shows the presence of core antigen in nuclei and cytoplasm with marked hepatic inflammation. These patients have a high rate of seroconversion from HBeAg to anti-HBe, which can be accelerated by interferon alfa.

3. Patients have low levels of HBeAg and are HBV DNA negative in serum. There is no core antigen detectable on liver biopsy and no hepatic inflammation is seen. They may already have progressed to cirrhosis or may have insignificant fibrosis.

Seroconversion from HBeAg to anti-HBe, either spontaneously or secondary to antiviral therapy, is associated with an improved outcome with reduced liver injury despite the continued presence of HBV DNA in liver. The natural rate of clearance of HBeAg is 8%–12% per year.

HBeAg is lost much less often. In a 19 year follow up of 946 HBeAg carriers, the annual rate of clearance of HBeAg was 0.79%. Lower HBsAg levels and older age were associated with increased clearance. However, patients may still develop cirrhosis or hepatocellular carcinoma after HBeAg clearance. In adults there is a male predominance for development of cirrhosis and hepatocellular carcinoma.

Chronic HBV infection is generally benign in children. In 168 Italian and Spanish HBeAg positive children who were followed up for 20 years, 155 became asymptomatic carriers after HBe seroconversion and had biochemical remission. Only 6% cleared HBeAg in the same period.

Ikeda et al estimated the rate of progression to hepatocellular carcinoma in 645 patients with chronic HBV infection to be 2.1% at five years, 4.9% at 10 years, and 18.3% at 15 years.

**THERAPY**

**Interferon alfa**

This agent has direct antiviral effects but probably depends on its immunomodulatory effects for full effect. Interferon alfa is recommended for patients with detectable HBV DNA, raised serum transaminases, significant interface hepatitis on biopsy and compensated liver disease. Therapy is given at 10 million units thrice weekly for four months by subcutaneous injection. Response is defined in terms of clearance of HBV DNA and HBeAg, normalisation of transaminases and improvement in liver histology. After an episode of hepatitis in the six to 10 weeks after induction, a long term response with loss of HBeAg is seen in 25%–40% of patients. It may take considerable time for HBeAg to be cleared in responders who may develop anti-HBs after 2–7 years. A recent study with a follow up period of 11 years has shown that interferon alfa has beneficial long term effects in terms of viral clearance, prevention of hepatocellular carcinoma and prolonged survival. The features that best predict a response to interferon alfa are shown in box 1. Side effects seen with interferon alfa are shown in box 2. Interferon alfa should be used with caution in those with cirrhosis, because of the risk of hepatic decompensation during seroconversion.

**Lamivudine**

Lamivudine is a nucleoside analogue that inhibits viral DNA replication. A daily dose of lamivudine 100 mg daily for one year is associated with suppression of HBV DNA levels and substantial histological improvement in chronic hepatitis B. The major problem has been the development of drug resistant mutations with prolonged therapy. Long term therapy is associated with a higher response rate. Lamivudine is now licensed.

**Box 1: Factors indicative of a response to interferon alfa**

- High serum transaminases.
- Low serum HBV DNA.
- IgM antibodies to HBCAg.
- Active necroinflammation.
- Short duration of disease.
- Absence of complicating disorder.

**Box 2: Side effects of interferon alfa**

- Flu-like illness.
- Bone marrow suppression.
- Irritability.
- Inability to concentrate.
- Myalgia.
- Sleep disturbance.
- Depression.
- Weight loss.
- Alopecia.
- Skin rash.
- Fatigue.
- Headache.
- Arthralgia.
Adefovir
Adefovir dipivoxil is the oral prodrug of an acyclic nucleotide monophosphate analogue and inhibits viral polymerases and reverse transcriptases selectively with broad spectrum antiviral activity. Initial trials have shown that it is highly effective in reducing replication in chronic HBV infection. It is likely to have a major role in the treatment of lamivudine resistant HBV mutations, since cross resistance is not reported. It is almost certain that a combination of antiviral agents (for example, lamivudine and adefovir) will become standard practice in due course. Adefovir is not yet licensed.

Liver transplantation
The ultimate treatment of decompensated HBV related cirrhosis or hepatocellular carcinoma is liver transplantation. However, the major drawback has been that HBV infects the graft frequently resulting in a poor outcome. Fibrosing cholestatic hepatitis is a histological variant of HBV infection, which in the liver graft has a particularly poor outcome. Passive immunisation with HBV surface antibody (anti-HBs)—given intravenously as hepatitis B immunoglobulin (HB Ig)—can reduce HBV recurrence to 33% in those patients who are HBV DNA negative at the time of transplant but may drive resistant mutations. Thus, in the past, many centres have refused transplantation to HBV DNA positive patients. However, lamivudine has revolutionised our thinking as it has been shown to render patients HBV DNA negative pre-transplant and reduce HBV recurrence post-transplant. The major problem has been development of resistance to lamivudine. Promising results have been obtained combining HB Ig and lamivudine to reduce resistance, since cross resistance does not occur. Adefovir may also prove valuable in this context.

VACCINES
Hepatitis B vaccines were introduced in the early 1980s. The initial vaccines contained heat or chemical inactivated subviral particles derived from plasma collected from chronic HBsAg carriers but newer vaccines, for example, Engerix-B (SmithKline Beecham) contain HBsAg particles expressed from recombinant DNA in the yeast Saccharomyces cerevisiae. The vaccines are usually well tolerated with mild injection site reactions occurring in approximately one fifth of cases but fever and systemic reactions are rare. The only contraindication to vaccine administration is hypersensitivity to yeast or to a component of the vaccine. The vaccine is given in three stages, at 0, one to two months, and at six months. Booster injections are no longer recommended. Protective anti-HBs titres of >10 IU/l develop in 95%–99% of children and young adults who receive the series of three intramuscular doses. Factors reducing responses are shown in box 3. The vaccine has been shown to protect against HBV in all high risk groups including male homosexuals, intravenous drug abusers, infants born to HBsAg positive mothers, and health care personnel. Vaccination of the general population has been shown to be remarkably effective in the example of an Eskimo population who have now been followed up for 10 years with none developing chronic hepatitis or becoming HBsAg positive.

Despite successful vaccination in terms of satisfactory anti-HBs levels, some children have developed HBV infection due to an escape mutation in the “a” determinant of the HBsAg epitope driven by HB Ig given concurrently. This mutant may become more common in the next decade. Of some concern is that testing for the mutated HBsAg using modern monoclonal antibody based ELISA may be falsely negative and one has to resort to testing for HBV DNA or using older polyclonal assays to detect HBsAg.

Hepatitis C virus
Virology
By the early 1970s it was appreciated that most cases of post-transfusion hepatitis (also known as non-A, non-B hepatitis) were seronegative for markers of hepatitis A or B virus infections. Hepatitis C virus (HCV) was identified initially as the cause of non-A, non-B infectivity. HCV has a positive strand genome that is bound to the nucleocapsid and enveloped by a glycoprotein. There are 9400 nucleotide bases coding for 3000 amino acids. HCV has no DNA intermediate and hence cannot integrate into the host genome, but it does use a negative strand RNA in its replication cycle within the liver. The structure of the HCV genome is illustrated in fig 1. The three N-terminal HCV proteins (C, E2, E1/NS1) are structural while the four C-terminal proteins (NS2, NS3, NS4, NS5) are non-structural and critical to viral replication. The nucleocapsid and envelope glycoproteins are encoded at the 5' end of the genome while the non-structural elements are located at the 3' end. The HCV nucleocapsid is well preserved but the envelope and the NS5 regions are highly variable regarding both nucleotide and amino acid sequences. Thus, a number of genotypes of HCV have been described. At present, the genotypes described are 1a, 1b, 1c, 2a, 2b, 2c, 3a, 3b, 4a, 5a, and 6a. The worldwide distribution of the various genotypes is shown in box 4.

Epidemiology
Although there are significant regional and ethnic differences, the worldwide prevalence of
HCV is thought to be of the order of 1%, with an estimated 300 million carriers. In Western Europe, about five million people have chronic HCV infection, which accounts for 40% of end stage cirrhosis and 30% of liver transplant candidates. In the USA, approximately 3.5 million people have chronic HCV infection, with nearly 150,000 new infections annually. A multicentre study in the UK revealed that 0.61% of blood donors were anti-HCV positive by first generation antibody testing. A study of organ donors in the UK revealed that 0.72% were anti-HCV positive by recombinant immunoblot assay (RIBA)-2 testing.

Transmission of HCV is most efficient via the parenteral route with an infection rate greater than 90% in intravenous drug addicts (box 5). Historical methods of transmission have included transfusion of blood products and transplantation of tissues or organs from infected donors before the introduction of screening for HCV in the early 1990s. Current transmission methods apart from intravenous drug abuse may include tattoos placed with poor hygiene, intranasal cocaine use, and ear piercing. The risk of HCV transmission after needlestick injury varies from 0%–10%. Up to 90% of haemophiliac patients treated with commercially prepared and unheated clotting factors before 1985 were found to be anti-HCV positive. As a result of repeated blood transfusion and nosocomial transmission by machines, 10%–15% of haemodialysis patients were found to be infected.

Vertical transmission can occur and the chance of seroconversion in the infant is related to the maternal viral titre. Logic suggests that family members should avoid sharing potential sources of infection such as razors and toothbrushes, although no studies have ever been performed to demonstrate protection. Infection of sexual partners in long term relationships occurs very infrequently. HCV is not found in semen and patients in a stable sexual relationship are not advised to take extra precautions (EASL and AASLD guidelines).

**CLINICOPATHOLOGICAL FEATURES**

Acute HCV infection is usually subclinical with less than 1% of patients in the Cambridge series of over 900 patients reporting an acute illness associated with jaundice in relation to HCV infection. The sinister feature of HCV infection is the high proportion of patients progressing silently to chronic liver disease with the associated risks of developing cirrhosis and hepatocellular carcinoma. The histological features seen in chronic HCV are shown in box 6. Various cofactors augment or accelerate HCV mediated liver damage (box 7). Disease progression for chronic HCV should be measured in years or decades. In one long term follow up study, it took 18.4 years to develop significant chronic hepatitis, 20.6 years to develop cirrhosis, and 28.3 years to develop hepatocellular carcinoma.

Only 20%–35% of infected patients will develop significant liver complications. It is estimated that 20% of patients develop cirrhosis after 20 years’ duration of infection. Most patients with chronic HCV have relatively mild symptoms such as fatigue and only develop symptoms or signs with progression to advanced liver disease. Though not evidence based, we, like most liver centres, screen patients with HCV cirrhosis on a six monthly basis for hepatocellular carcinoma with ultrasound and measurement of alpha-fetoprotein.

While HCV is hepatotropic, it is also found in other tissues including peripheral blood mononuclear cells and lymph nodes and is associated with extrahepatic manifestations (box 8).

**DIAGNOSIS**

After exposure, seroconversion to detectable levels of anti-HCV antibodies takes an average
much earlier (at 30–60 days).65 The third generation assays have increased specificity compared with ELISA-1. In both assays antibodies were detectable after a mean of 15 weeks (range 0–52 weeks) after acute infection. Thus, there was a seronegative “window” during which, acutely infected patients may have tested negative.

Soon after, the first generation enzyme linked immunosorbent assay (ELISA-1) tested serum for the presence of IgG antibodies to the NS4 region of the HCV genome, the c100-3 antigen. This assay produced too many false positive results.64 Soon after, the first generation recombinant immunoblot assay (RIBA-1) was developed. This incorporated the 5-1-1 antigen, another antigen from the NS4 region, in addition to the c100-3 antigen. RIBA-1 had improved specificity compared with ELISA-1. In both assays antibodies were detectable after a mean of 15 weeks (range 0–52 weeks) after acute infection. Thus, there was a seronegative “window” during which, acutely infected patients may have tested negative.

The second generation ELISA-2 and RIBA-2 assays incorporated additional recombinant antigens from the viral genome. The RIBA-2 test is considered positive when two or more antigens react with the patient serum. Second generation assays have increased specificity and sensitivity and have the advantage that antibodies against c22–3 and c33c appear much earlier (at 30–60 days).65 The third generation assays, ELISA-3 and RIBA-3 incorporate additional HCV antigens.

Direct detection of viral RNA using polymerase chain reaction (PCR) is critical in patients infected recently with the virus or in immunosuppressed subjects who may remain antibody negative. In addition, PCR is useful for determining the status of patients with indeterminate antibody profiles. In clinical practice, PCR is useful for detecting viraemia in anti-HCV positive patients and in selection for antiviral therapy and monitoring responses. It is important to realise that though patients may be HCV RNA negative in serum, they nearly always are positive for HCV RNA in liver tissue.66 67

THERAPY
The success of antiviral therapy was once defined as normalisation of transaminases but is now based on loss of HCV RNA. Definition of “cure” is remaining serum HCV RNA negative at one year after completion of therapy,68 although relapse can still occur; loss of HCV RNA from liver tissue is demonstrated rarely. The ideal measures of response to therapy should be histological remission, prevention of progression to cirrhosis, and reduction in mortality but these long term data are not yet available.

The beneficial effects of interferon alfa alone in non-A, non-B hepatitis were first reported in 1986.69 Different regimens exist but the dosage is usually 3–6 million units of interferon alfa thrice weekly for 12–18 months. Higher doses improve responses a little, but lead to intolerable side effects. Response rates in terms of seroconversion to HCV RNA negative were unsatisfactory and varied from 13%–20%. Certain factors are associated with a poor response to interferon alfa (box 9).70

Most centres now use combination therapy with ribavirin and interferon alfa. Ribavirin is a guanosine analogue with no effect on HCV RNA when given alone. The combination provides a sustained response rate of 38%–43% when given for 12 months.71 72 Ribavirin has now been licensed for use in combination with interferon alfa for patients who have relapsed after interferon alfa monotherapy as well as for treatment naïve patients with chronic HCV infection. According to EASL guidelines, combination therapy is recommended for six months in those with genotype 1 and continued for a further six months if patients become HCV RNA negative. In all other genotypes, therapy should be given for six months. The principal side effect of ribavirin is haemolytic anaemia, which usually warrants dose reduction and sometimes withdrawal of the drug. In the UK a decision and guidelines concerning combination therapy are awaited presently from the National Institute for Clinical Excellence (NICE).

Development of new antiviral agents has been hampered by the inability to culture
HCV. However drugs are being developed that inhibit specific enzyme targets within the HCV genome, such as the protease and helicase. All patients with chronic HCV infection should be vaccinated against HBV (common risk factors) and hepatitis A virus, since the latter is reported to cause fulminant hepatic failure in HCV carriers.

Liver transplantation
The only treatment for HCV related end stage liver disease is liver transplantation. Graft infection occurs in almost all cases. At one year after transplant, about 50% of viraemic patients show evidence of hepatitis on biopsy.8 A longer term follow up showed that HCV infection is associated with accelerated rates of graft damage in some patients, especially those infected with genotype 1b.7 A recent worrying statistic is that 24% of patients have become cirrhotic at eight years after transplant.76

A recent Italian study has shown a 50% sustained response rate with combination therapy in transplant patients with HCV infected grafts, without precipitating rejection.75 Others have not achieved such success and combination therapy is considered unproven at present in liver graft recipients. Most centres concentrate on keeping immunosuppression to a minimum with the aim of decreasing HCV replication in the liver graft.

IMMUNOLOGY AND VACCINATION
Development of an effective vaccine is hampered by the extensive genetic and antigenic diversity among different HCV strains. The hypervariable region-1 (HVR-1) undergoes frequent nucleotide substitutions allowing viral persistence. Vaccination with a peptide vaccine of homologous HVR-1 has been effective in the chimpanzee, but high titres need to be maintained to prevent infection.6 HCV infection stimulates the production of neutralising antibodies and cytotoxic lymphocytes but new viral variants that escape these immune responses emerge frequently.7 In addition, HCV infection does not confer immunity against reinfection.8 Nevertheless, evidence suggests that CD4 T cell proliferative responses are associated with a more benign course and a better response rate to interferon alfa.8,9 It is of interest that ribavirin enhances this T cell response.

Hepatitis D virus
First described in 1977, the hepatitis delta virus (HDV) is a small defective virus that replicates effectively only in the presence of hepatitis B surface antigen (HBsAg). It has been shown in a transplant population that HDV infection alone and before HBV compared to those where HDV is a superadded infection in chronic HBV carriers. All patients with HDV have HBsAg in serum but most lack markers of active HBV replication such as HBV DNA and hepatitis B e antigen (HBeAg). The diagnosis is suspected by finding anti-HDV antibodies in an HBsAg positive patient and confirmed by finding HDV RNA in serum or HDV antigen in liver tissue.8

Therapy options are limited. Interferon alfa is effective in only a small proportion and has to be administered at high doses for a prolonged period.8 Relapses on cessation of therapy are common unless HBsAg is cleared, which occurs infrequently.6 Both ribavirin6 and lamivudine6 have proved ineffective in chronic delta hepatitis. The best approach is prevention using HBV vaccination.

Hepatitis G virus
Hepatitis G virus (HGV) is a recently discovered positive stranded RNA flavivirus,1 which was isolated from a surgeon with infective jaundice of unknown cause. Initial enthusiasm for the virus being responsible for non-A, non-B, non-C transfusion related hepatitis has waned. Although found commonly in chronic HCV carriers, it has been shown to have no impact on liver disease severity or response to antiviral therapy.8,9 Similarly, it has been shown to have no influence on the course of HBV.4 HGV positive blood donors are no more likely to have raised transaminases than HGV negative donors.3 HGV RNA could not be detected in the explant livers of 54 patients with cryptogenic cirrhosis.3

TT virus
First described in 1997, TT virus is an unenveloped single stranded DNA virus, which was described in the sera of 3/5 patients with biopsy proved non-A to G post-transfusion hepatitis.7 Further Japanese studies have indicated a possible causative role for TT virus in chronic hepatitis.7,8 However, studies from the UK and USA suggest that although the virus is detected commonly in blood donors, it is not clear if it has any pathogenic role in hepatitis.9,10


Chronic viral hepatitis
