The management of chronic hepatitis C virus infection

Hepatitis C virus (HCV) was, prior to introduction of blood donor screening in 1991, the major cause of post-transfusion hepatitis and still is an important cause of sporadic hepatitsis throughout the world. The prevalence rates of infection in healthy blood donors range from 0.01–0.02% in the United Kingdom and northern Europe, 1–1.5% in southern Europe to rates of 6–5% in parts of equatorial Africa. Prevalence rates as high as 20% have been found in Egypt.

Although the virus was first cloned in 1989, only recently have there been claims of visualisation of the virus by immuneelectron microscopy.

The question of how best to treat patients with chronic HCV infection has been brought into focus with the recent licence of interferon for treatment, and the implementation of ‘HCV lookback’, where recipients of HCV infected blood products will be identified. In this article we discuss the management of patients chronically infected with HCV.

Natural history
Subclinical HCV infection is the rule with only 10% patients reporting an acute illness associated with jaundice. Recent studies have shown that HCV infection does not cause fulminant hepatitis. Severe acute HCV infections have been reported in liver transplant recipients, patients with underlying chronic liver disease, and in patients coinfected with HBV. Although the acute illness is usually mild, the sinister feature of HCV infection is the high proportion of patients progressing to chronic liver disease with the associated risks of developing cirrhosis and hepatocellular carcinoma. In a study of 135 patients with post-transfusion hepatitis, 77% developed chronic disease and of the 65 patients with sequential liver biopsies, 32% had developed cirrhosis after a mean follow up of 7-5 years.

In the same year, however, Seeff published a long-term follow up study of patients with post-transfusion non-A non-B (NANB) hepatitis. A total of 568 patients with post-transfusion hepatitis and two control groups of 526 and 458 patients who had received transfusions without developing hepatitis were studied. After an average follow up of 18 years the mortality related to liver disease was 3–3% in post-transfusion hepatitis cases compared with 1.5% in the control groups and most deaths occurred in patients with associated alcoholism. It seems therefore that most patients who develop progressive disease do so slowly. Longer follow up studies are needed to assess the contribution of HCV to mortality and morbidity.

Both viral and host factors influence the rate of disease progression. Viral factors associated with more rapidly progressive disease include high level viraemia, genotype 1b, and the degree of viral genetic diversity (quasispecies) found in an individual patient. Route of transmission may be important with one study suggesting that patients infected by blood transfusion tended to have more histologically active liver disease. The larger initial inoculum associated with blood transfusion and the finding of higher levels of viral replication in these patients adds support to these findings. Other host factors such as immune deficiency, associated alcoholism, and coinfection with HBV and HIV may also influence the rate of disease progression.

Investigation
Diagnostic serological assays
In 1989 Choo and colleagues isolated a clone (5-1-1) from a randomly generated cDNA library derived from the serum of a chimpanzee with NANB hepatitis using an antibody from a patient with post-transfusion hepatitis. By generating overlapping clones, the recombinant antigen C100-3 was prepared as a fusion peptide with human superoxide dismutase and expressed in yeast. This antigen was used in an enzyme linked immunosorbent assay (ELISA) for first generation anti-HCV antibody testing. The first generation assay lacked sensitivity and specificity prompting the development of second generation assays incorporating antigens from the nucleocapsid (C22) and NS3 (C33). Third generation assays (ELISA-3) now exist incorporating antigens from the putative nucleocapsid, NS3, NS4, and NS5 regions. ELISA-3 tests are now the most widely used screening test for HCV but despite the improved specificity of the test, positive results should be confirmed.

Confirmatory assays
The HCV antigens can be immobilised on nitrocellulose strips to create recombinant immunoblot assays (for example, Chiron RIBA, Chiron Diagnostics), which should be used to confirm all positive ELISA results.

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RIBA tests are deemed positive if there is reactivity to more than one antigen and indeterminate if reactivity occurs with only one antigen. Indeterminates are likely to be true positives with reactivity to core, NS3 or NS5 but false positivity with lone reactivity to the NS4 antigens 5-1-1 and C100-3 is common.25 27 Most RIBA positive patients will be viraemic (PCR positive). 28 Unfortunately the pattern of HCV antibodies does not correlate with disease outcome or predict future viral clearance.

Detection of viraemia

Direct detection of the virus using polymerase chain reaction (PCR) is needed in patients recently infected with the virus and in immunosuppressed subjects who may be antibody negative. In addition PCR is useful for determining the status of patients with indeterminate antibody profiles. All patients presenting for treatment must be tested for viraemia before starting treatment and PCR testing is necessary for monitoring the effect of treatment. The technique is laborious and therefore has a limited role in the routine diagnosis of HCV infection. Indeed a survey of European centres showed that only 16% of 31 centres were able to perform diagnostic PCR reliably.29 However, the development of enzymes capable of both reverse transcription and PCR and their incorporation into commercially available diagnostic kits, such as the Amplicor kit from Hoffman-La Roche, will make this test more widely available. The PCR can be adapted to assess the level of viraemia. 30 The branched chain DNA assay (Chiron Diagnostics) also provides quantitative data although it is less sensitive. 31

Liver function tests

The use of aminotransferase measurement to screen for chronic HCV infection is of limited value as about 50% of HCV infected (anti-HCV and PCR positive) patients will have normal values. Despite normal liver function tests these viraemic patients should not be considered ‘healthy carriers’ as most will have histological evidence of necro-inflammatory liver disease with or without cirrhosis. 32

Viral genotyping

Typing can be performed in several ways, either serologically with specific peptide ELISAs (serotyping), 33 or by analysis of PCR products by direct sequencing, 34 35 use of type specific primers, 36 restriction fragment length polymorphisms, 37 or with sequence specific DNA probes (genotyping). 38 Genotyping is available in certain specialist centres but it remains to be established how useful knowledge of genotype will be in the management of individual patients with HCV infection. The viral genotype in a patient may help predict the rate of disease progression 39 40 and response to antiviral treatment 41 42 but is also important in establishing sources of infection.

Histology

Examination of liver needle biopsy specimens often shows a mild hepatitis but all levels of inflammatory activity (grade) and fibrosis (stage) are recognised. In addition characteristic features are recognised in both the acini, with acidophili body formation and focal hepatocellular necrosis, and the portal tracts, with prominent lymphoid follicles and evidence of bile duct epithelial damage. 43-45 Despite the mild histological appearances a significant number of patients will progress to cirrhosis. 46 Unfortunately therefore the biopsy appearance at presentation does not always predict the rate of disease progression in an individual non-cirrhotic patient but biopsies taken every two years may be useful in predicting outcome if there is progressive accumulation of collagen. In patients with moderate to severe hepatitis, although improvement may occur, progression is more likely.

Although liver biopsy is imperfect in determining prognosis, it does provide some indication and is therefore recommended for those patients found to be viraemic with or without abnormal alanine transaminase activity. Most will have chronic hepatitis of varying severity with or without cirrhosis. 32 The severity of the necroinflammatory activity and degree of fibrosis found on liver biopsy will help to advise the patient on the need for antiviral therapy. The demonstration of cirrhosis is helpful prognostically. Some patients will test positive for antibody to HCV, have abnormal liver function tests but will be PCR negative: these patients should be screened for other liver diseases including autoimmune hepatitis and haemochromatosis. Finally anti-HCV positive patients found to be PCR negative with normal alanine transaminase activity should probably be followed up annually until the natural history (virological and biochemical relapse) is better understood. It may be recommended if there is a return of viraemia or a flare up of liver enzymes. The known morbidity and mortality risks associated with percutaneous liver biopsy 47 must be balanced against the potential benefits before a decision to biopsy is taken.

Screening and counselling

Estimates suggest that about 200 000 people are infected with HCV in the United Kingdom. The cost effectiveness of a mass screening programme for HCV is under debate at present. The British Liver Trust recommends that all patients infected with HCV should be referred by their general practitioner to a hepatitis care centre for regular monitoring. The natural history, treatment options, and likelihood of success should be discussed. Patients should be reassured that HCV infections are not usually associated with other infections such as HBV or HIV. Although the precise role of sexual transmission remains to be established 48-51 because up to 5% of spouses of infected patients are infected, 52-54 we advise couples in new relationships to use barrier contraception. In established relationships the small risk of transmission should be explained and the couple left to decide on barrier contraception or not. The risk of vertical transmission seems to be low (<6% of children becoming HCV positive) unless the mother is HIV positive or has a particularly high level viraemia. 55-57 Mothers should be advised that so far breast feeding has not been implicated in HCV transmission and that HCV RNA has not been found in breast milk. 58 59

Patients must also be screened for their suitability to receive interferon therapy. In particular patients should probably not be offered interferon if there is a history of depressive illness, autoimmune thyroid disease or evidence of ongoing alcohol or misuse of intravenous drugs. Patients should have access to reliable refrigeration and be able and willing to make regular clinic visits. Adequate warning should be given of the usual initial effects of interferon (fever and malaise) and in particular absence from work may be necessary during the early stages of treatment. Women should be advised not to conceive during a course of interferon.

Treatment (see Figure)

The beneficial effects of interferon α in NANB hepatitis were first reported by Hoofnagle in 1986. 60 Since then
several placebo controlled trials of interferon treatment in HCV have been reported with varying doses and duration of treatment.40 61-71 The early trials used alanine transaminase normalisation as the end point and overall 50% of patients showed an initial response to treatment but only half of these patients (25% of total) sustained normal alanine transaminase activity six months after the cessation of treatment. Later trials also measured the effect of treatment on HCV viraemia showing a 50% response rate (HCV RNA negative) on treatment, with only 15–25% of patients remaining PCR negative in the follow up period. Certain factors including infection with HCV 1b, high level viraemia, presence of cirrhosis, older age, longer duration of disease, and multiple quasispecies in the serum are associated with poorer sustained response rates to interferon.72 Most of the trials reported so far required the patients to have abnormal transaminase activity before treatment and as yet there are no accurate figures for the response rates in viraemic patients with normal liver function. It may be that treatment in the early stages of HCV infection, when patients are asymptomatic with normal transaminase activity and have minimal fibrosis on liver biopsy, offers the best opportunity for viral eradication.42 Despite evidence suggesting that interferon normalises liver enzymes, improves liver histology,73 and leads to loss of viraemia70 74 because of the long natural history of the disease there is as yet, no evidence that interferon reduces the risk of progression to cirrhosis and hepatocellular carcinoma.

\[ HCV \text{ algorithm}. \] ALT=alanine transaminase, PCR=polymerase chain reaction, US=ultrasonography, AFP=a fetoprotein.
Most treatment trials have used similar doses of between 1–3 million units of interferon three times per week for periods of three to six months. A dose of three million units is more efficacious than one million units resulting in a greater proportion of patients with normal transaminase activity while receiving treatment and less chance of relapse after treatment.75 Another study using 10 million units three times a week suggested that sustained response rates could be as high as 50%.76 Longer treatment regimens of 1273 77 or 1877 months also resulted in greater numbers of sustained responders. Further trials are required to clarify the optimum interferon regimen in terms of both dose and duration.78

All patients with moderate or severe inflammatory activity with or without fibrosis and any patient with fibrosis on liver biopsy should be offered treatment. Although patients with cirrhosis probably respond less frequently,75–77 79 the loss of viraemia and reduction in inflammatory activity may delay progression to liver failure and the development of hepatocellular carcinoma and therefore treatment should be considered. It is important to closely monitor cirrhotic patients with regular physical examination, ultrasound, and α-fetoprotein estimation to facilitate early detection of the complications of cirrhosis including hepatocellular carcinoma.

At present we do not recommend treatment for those patients found to have minimal/mild hepatitis on liver biopsy. As discussed earlier, however, some of these patients may progress to cirrhosis.46 We therefore recommend that patients with minimal/mild hepatitis should be reviewed every six months in the outpatient department with repeated liver biopsy every two to three years. If the biopsy shows worsening necroinflammatory disease or fibrosis, or both, then treatment should then be considered. In some cases of minimal hepatitis, after discussion with the patient, treatment may be requested at this early stage because of concern about infectivity. In this situation, after counselling on the relative minor nature of infective risks, if treatment is still requested, it should be given.

We treat patients with three to six million units of interferon three times per week for six to 18 months. Patients with risk factors for treatment failure should be treated with higher doses and treatment should be for at least one year. Doses of three to six million units of interferon three times per week are tolerated well and increased dosages result in more frequent treatment failures due to side effects including lethargy, leucopenia, thrombocytopenia, psychiatric disturbance, and autoimmune phenomena. Patients with a sustained response to interferon should be reviewed at six monthly intervals with transaminase estimation and PCR to monitor any return of HCV viraemia. Those patients relapsing after treatment should be considered for a second course of interferon at higher dose, or be considered for inclusion in a trial of interferon and ribavirin (see later). By monitoring the early period of treatment it may be possible to predict those patients likely to derive long term benefit from interferon treatment. Although patients rendered PCR negative during the first three months of treatment do not necessarily become sustained responders, a positive PCR result three months into treatment reliably predicts treatment failure.40 Thus patients failing to normalise liver function tests and becoming PCR negative during the first three months of treatment will not respond and interferon should therefore be withdrawn and other treatment strategies, such as higher interferon dose or alternative antiviral agents, should be considered.

Treatment failures

There are several treatment options to be considered for those patients not responding to interferon therapy. Ribavirin, an orally administered guanosine analogue, has been shown to reduce alanine transaminase activities in HCV infected patients but most will relapse following cessation of treatment.80–82 In addition there seems to be little effect on viraemia in these patients. There is hope that combination treatment with interferon and ribavirin will increase the numbers of sustained responders and results of these trials are eagerly awaited.

Studies93 have shown that non-responders tended to have higher values of hepatic iron compared with responders.83 84 and it was proposed that the higher iron concentrations result in a relative oxidative state in the hepatocyte rendering them more susceptible to cellular injury or necrosis.82 A further study showed that the combination of phlebotomy and interferon resulted in a greater reduction in transaminase activities, more patients rendered PCR negative on treatment, and reduced the likelihood of relapse after treatment.84 85 If the oxidative state of the hepatocyte is important then there is a theoretical argument to add an antioxidant, such as N-acetylcysteine, to the treatment regimen.

Finally ursodeoxycholic acid has also been used in the treatment of HCV showing reduction in transaminase activities in some patients95 and when combined with interferon results in a more prolonged period of transaminase normalisation after treatment cessation.86 However, ursodeoxycholic acid had no effect on viraemia or the histological appearances of the liver after treatment.8389 Further trials are necessary before any of these alternative treatment options can be recommended routinely.

Future developments

New antiviral agents

The development of new antiviral agents and vaccines has been hampered by the inability to develop a reliable cell culture system for the virus. However, there are a number of exciting prospects on the horizon. Knowledge of the molecular biology of HCV has led to the identification of specific functions associated with particular regions of the virus. These include a possible ribosomal entry site in the 5' non-coding region, protease and helicase activity for the NS3 region, and a RNA dependent RNA polymerase associated with the NS5 region. Once assays for these protein functions become available then compounds can be tested for specific inhibitory activity. In addition three-dimensional structural analysis will allow specific protein inhibitors to be designed.90

Preventive and therapeutic vaccines

Hope for the development of an effective vaccine has been dampened by two features of HCV. Firstly, the virus, like many RNA viruses, is known to mutate at a particularly high rate91–94 so that vaccination with one variant may well only confer homologous protection. Secondly, persistent infections are the rule despite reported humoral and cellular immune responses to the virus. There is some encouragement, however, as studies suggest that greater CD4 T cell proliferative responses are associated with a more benign course of HCV infection95 and a better response to interferon.96 Approaches for vaccine development include killed virus, attenuated virus, recombinant proteins, naked DNA injection, and the development of vector DNA vaccines. Use of killed or attenuated viral preparations is generally regarded as unsafe. Houghton and his colleagues at the Chiron Corporation have pursued the viral subunit approach using the chimpanzee model of HCV infection.
A preparation of core-E1-E2-NS2 in a vaccinia vector expressed in mammalian HELa cells was injected into seven uninfected chimpanzees. Strong antibody responses to these proteins developed and the five chimpanzees with the highest antibody titres were resistant to infection with a homologous viral strain. The two chimpanzees with poorer antibody responses could be infected but it was claimed that their disease course was milder than in non-vaccinated animals. It remains to be seen whether vaccination can prevent a viral strain. The potential of naked DNA injection in HCV is interesting as this type of vaccination seems to be particularly efficacious at stimulating the cellular arm of the immune system and may be useful as a therapeutic vaccine.

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