Target organ resistance to the action of insulin in non-insulin dependent diabetes mellitus may result in hyperinsulinaemia, which can promote atherogenesis, vascular and cardiac smooth muscle mitogenesis, and sodium retention. Essential hypertension and glucose intolerance occur together more often than is expected by chance (when occurring with a third feature, obesity, this comprises Reaven’s syndrome, also known as Syndrome X), but a causative link is not established.

Endothelin-1 is a potent vasoconstrictor peptide synthesised in the vascular endothelium and first described in 1988. Since then there has been rapid progress in developing potential anti-hypertensive drugs, such as endothelin receptor antagonists (e.g. RO47-0203) and endothelin converting enzyme antagonists (e.g. phosphoramidon). These tools have been employed to demonstrate the importance of endothelin-1 production in the physiological control of vascular tone. Patients with essential hypertension are more sensitive to endothelin-1.

In the rare syndrome of ‘Apparent Mineralocorticoid Excess’, renal mineralocorticoid receptor becomes unusually sensitive to cortisol because the enzyme 11β-hydroxysteroid dehydrogenase is defective and the conversion of cortisol to its inactive metabolite cortisolone is impaired. There is preliminary evidence that more subtle defects in this enzyme occur in essential hypertension. However, in essential hypertension, rather than there being renal hypersensitivity to cortisol, it is suggested that there is either vascular smooth muscle hypersensitivity to cortisol, or that adults with essential hypertension were exposed to greater levels of maternal cortisol in utero as a result of impaired placental 11β-hydroxysteroid dehydrogenase. This latter hypothesis may explain the recent observations that adverse cardiovascular outcome and adult hypertension occur in individuals who had a low birth weight and high placental weight.

The search for the cause of essential hypertension has been long and frustrating. In recognising that it is multi-factorial, and in establishing means to assess the contributions of different mechanisms in different individuals, we may be able to target at-risk individuals more accurately and then tailor their therapy. The two themes of this symposium, improved management of hypertension and better understanding of its aetiology, are likely to remain closely related.

WORKSHOP ON HEPATITIS C VIRUS

The six papers that follow are based on a workshop held in the College in 1993 but updated to 1995. They are a record of specialists talking to the specially interested, and general readers may find them difficult in places. But the effort of reading them is worthwhile as the viruses responsible for hepatitis are not only important clinically but present fascinating problems in biology. Older readers will remember that their textbooks in the 1930s contained a section on catarhal jaundice which was distinguished from obstructive and haemolytic jaundice. The cause of the catarh was unknown. The single diagnostic label was soon replaced by two, infectious hepatitis and serum jaundice, the latter being a common condition in patients being treated for syphilis with intravenous injections. When means were discovered for isolating and identifying viruses, these conditions were found to be due to separate viruses, hepatitis A and B (HAV and HBV). A third distinct virus with an affinity for the liver was yellow fever virus. Other identified viruses are hepatitis C virus (HCV), hepatitis D (HDV) and hepatitis E virus (HEV).

Yellow fever virus is spread by an arthropod vector from a pool of infection which still persists in some jungle primates. HAV and HEV infection is spread from case to case by the faecal-oral route. Infection by HBV is transmitted via intimate (usually sexual) contact or parenteral injection through a contaminated needle or transfusion fluid. HCV is rarely transmitted by sexual contact, occasionally by needle stick injury but usually by infusion fluid.

There is extreme variation in the clinical manifestations of infection with hepatic viruses. A self limiting attack of fever with jaundice is the common presentation with yellow fever and with HAV and HEV infections, but is often absent with HBV and HCV infection. A fulminating, usually fatal, hepatitis is common in yellow fever, very rare with HAV, HCV and HEV, a well known tragedy with HBV infection. A persistent inflammatory response, with or without the continuing presence of virus, leading to cirrhosis and carcinoma is the main clinical feature of HCV infection and common in HBV infection. It is rare, if it ever occurs, in yellow fever or in HAV infection. Hepatitis D virus is strongly related to intravenous drug use but has similar epidemiological and clinical features to HBV with which it is often associated in time. In the immunocompromised patient, as with AIDS, the liver may be affected by other viruses, in particular cytomegalovirus, herpes simplex virus, measles virus in adults and Coxsackie virus B, all of which may give rise to hepatitis in occasional individuals.

Do these marked variations in the clinical responses to infection arise from differences in the strains of infecting virus or in the nature of the immune response of individual patients? An understanding of these questions would help both in prevention of the infections and in the treatment of patients.

The Editors
Epidemiology of Hepatitis C

J. Gillon,* Edinburgh and SE Scotland Blood Transfusion Service, Royal Infirmary of Edinburgh

Historical perspective

In the 1960s, in Washington in the USA, a recipient of a blood transfusion had a 1 in 3 chance of developing post-transfusion hepatitis. In 1970 two things changed. Firstly, testing for hepatitis B became available, which reduced post-transfusion hepatitis by about 50%. Secondly, the blood donor service moved to an all-volunteer programme with a further significant reduction in hepatitis amongst recipients. In the 1980s, donors with raised ALT levels were excluded, but a rump of post-transfusion hepatitis cases remained. When testing for hepatitis C became available it became clear that 95% of non-A, non-B hepatitis was due to hepatitis C.

In 1982, the Centre for Disease Control, Atlanta, set up a ‘Sentinel Counties Study’, looking into the risk factors for non-A, non-B hepatitis in sporadically occurring cases in the community.1 It soon became apparent that transfusion was only accounted for 3% of such cases and in approximately 50% no risk factors could be identified. In the first year of this study, 13% of cases were associated with intravenous drug abuse, but by 1988 this figure had risen to over 40%. When second generation assays for HCV antibody became available, retrospective testing confirmed that HCV was responsible for 100% of cases except those occurring through intravenous drug abuse. In 1989, no source of infection could be identified for approximately one third of cases, and HCV was only identified in 52% of these. It should be noted that when testing for anti-HCV in patients with acute non-A, non-B Hepatitis, sufficient time must be allowed to elapse for seroconversion to take place. For example in one study2 only 10 of 20 (50%) patients who developed non-A, non-B hepatitis following blood transfusion and were tested within 6 weeks of the onset of illness, were found to be anti-HCV positive, compared to 19 of 25 (76%) who were tested 6 months after the onset of illness.

Geography

HCV is a worldwide problem. In the Far East there is a significant problem with hepatitis B virus infection, high rates of vertical transmission and a strong association with hepatocellular carcinoma. In most countries where there are significant rates of HBV infection, HCV is also prevalent, although usually less so. It is not known why the seroprevalence rates for HCV and HBV differ. In the majority of countries in the Far East, most hepatocellular carcinoma cases are associated with HBV infection. This is not the case in Japan where a greater proportion of cases of hepatocellular carcinoma are associated with HCV infection. Even within Japan, prevalence rates vary according to locality. In one town of 1,500 inhabitants, tested for HCV in 1990, 12.2% were found to be HCV seropositive, a much higher rate than that for blood donors (1.2% across the whole of Japan).3 However, when this town was subdivided into areas, there was even greater fluctuation in seroprevalence rates between areas, which ranged from 5.1% to 48.6% in the most densely infected area. The reasons for this are unclear, but might possibly relate to reuse of needles used for mass vaccination.

Risk factors in the United Kingdom

Having identified our seropositive cohort of blood donors, we looked for possible risk factors, and found that in 40% of cases seropositivity was related to previous

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In the Middle East high rates of HCV infection occur in Egypt, with 4% of Egyptian blood donors being anti-HCV positive.4 In rural communities, the figure is much higher. In Kuwait 1-2% of blood donors are HCV positive.5 In South American countries, blood donors tend to show seroprevalence rates of 0.6 to 0.9%, although the rate in Brazil is higher at 2.7%-6 and in rural Peru the seroprevalence rate is 0.7. Little is known about the epidemiology of HCV in Sub-Saharan Africa, but in the Republic of South Africa seroprevalence rates are similar to those found in South America with a 0.9% seroprevalence rate amongst blood donors. An identical rate has been obtained for blood donors in Zimbabwe, and it is of interest that the seroprevalence rates for HIV and HBV seem to be higher than this, the first indication that sexual transmission may be less important for HCV than it is for HIV or HBV.

United Kingdom

It should be appreciated that the data from the Blood Transfusion Service do not accurately reflect the overall population prevalence of HCV, as blood donors in the UK are a highly selected group of individuals. The Service tries very hard to exclude donors who have a risk of parenteral exposure to viruses. Initial screening for HCV was carried out on 10,633 blood donors from Glasgow, Newcastle and North London using a first generation test and 0.65% of donors were found to be HCV seropositive.8 However, when a confirmatory test was used, the vast majority of these tests were found to be false positives and the seropositive rate was readjusted to 0.047%. When all these specimens were tested by PCR, a few more positives were discovered, giving an overall prevalence rate of 0.06%. Since the adoption of routine screening of blood donations for HCV infection, the seroprevalence rate amongst blood donors has steadily fallen as seropositive donors are excluded from the pool. Any cases which turn up now should either represent seroconversion amongst regular blood donors or the introduction of a new donor to the transfusion service. Again because of the degree of selection, these data do not give a true reflection of the rate of infection amongst the general population. Nonetheless some information has been obtained. In the two years since testing began in our Service we have identified approximately 300 seropositive donors. We have also tried to extrapolate from this rate of donor seropositivity the number of transfusion recipients who have been infected in the past by these donors. In a small study in South East Scotland looking at the first 20 HCV seropositive donors to be identified, we found that over 70 potentially infectious units of blood had been donated by these individuals. However, many of the recipients of these units of blood died of their primary disease, some were untraceable and there remained fewer than 10 live patients who were available, could be tested, and were at risk of developing liver disease. We would therefore estimate that in Scotland there may be 150 infected recipients of blood transfusions. Translated to the UK population, a reasonable estimate is that there are 3,000 HCV seropositive recipients of blood transfusion alive and at risk of developing chronic liver disease.9 This has implications for identification and management of such individuals.
intravenous drug use. This was somewhat alarming to us, as we go to great lengths to exclude such individuals from the blood donor panel. We discovered that many cases were related to drug use up to 20 or 30 years previously, the inference being that the donors themselves thought this to be irrelevant. Other parental exposures e.g. blood transfusion, tattooing, ear piercing probably accounted for a number of cases. We also found 8 cases where the only discernible risk factor was sexual intercourse with an intravenous drug user, implying a possible role for sexual transmission of infection. As with other studies we were left with about a third of our cohort for whom we could identify no risk factors. It is possible that some of these may reflect undisclosed intravenous use of drugs in the past.

The risk of acquiring HCV through blood transfusion in relative terms appears to be small. In one study from North London, only one of 387 transfused patients was shown to seroconvert for HCV post-transfusion. However in haemophiliacs, who may be exposed to multiple donors in a lifetime, the situation is quite different, with seropositive rates approaching 100% in some groups exposed to non-heat-treated Factor VIII. Haemodialysis patients may also be at risk of HCV infection, with seroprevalence rates around the world that are usually up to 10%, but have reached 50% in some studies from the Far East.

**Sexual transmission**

Early work disclosed that sexual transmission of HCV from intravenous drug users to a sexual partner was much lower than might have been expected, and much lower than the transmission rate for HIV. A study from Glasgow has shown that the seroprevalence amongst male intravenous drug users is 85%, but amongst their female sexual partners the rate is only 8%. Amongst homosexual men tested in Glasgow, there have been no cases of HCV infection, except where there was a history of intravenous drug use, when 6/17 were seropositive, (E. A. C. Follett, personal communication). A study of prostitutes in the USA has shown that the seroprevalence for HCV amongst drug using women is 68%, but amongst non-drug using women is only 12%. It is now becoming clear that HCV seropositivity is one of the best markers available for identifying current or previous intravenous drug misuse, with much higher seroprevalence rates than for HIV or HBV. The reverse situation applied in a cohort of homosexual men from Barcelona, where the seroprevalence rates for anti-HIV, anti-HBc and anti-HCV were 84%, 81% and 16% respectively. Table 1 shows the changes over time in the seroprevalence of these three viruses in a cohort of Danish homosexual men, and it does show that the seroprevalence of HCV rises slightly during the follow-up period, although less so than for HBV and HIV. Turning to the female sexual partners of men with haemophilia, a study by Eyster published in 1991 showed only 5/194 female partners of HCV seropositive haemophiliac men to be infected. Moreover HCV transmission appeared to be affected by HIV infection in the index case. In a study from Glasgow on sexual partners of HCV seropositive blood donors, only 3/60 were found to be HCV infected, of whom 2 had previously undergone blood transfusion. One indeterminate result was also obtained in a sexual partner with a history of previous intravenous drug use. More recent studies have indicated higher rates of transmission with infection more likely the longer a sexual relationship continues. Vertical transmission, is a very important issue especially in parts of the world where HCV is more prevalent. There are difficulties in interpreting the results of serological tests of HCV in this setting, and PCR has proved essential in clarifying the transmission risk. The first important study was from Thaler in California, and rather worryingly all 8 neonates born to HCV seropositive mothers were found by PCR to be HCV infected, but further studies from New York and Sweden showed transmission rates of 0/24 and 3/21 respectively, suggesting a small risk of vertical transmission. In Edinburgh, of 58 babies born to HCV seropositive mothers, only 4 have been found to be infected with HCV. In addition, the rate of transmission is not increased by concomitant infection with the human immuno-deficiency virus.

### Table 1

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<th>Year of testing</th>
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<th>Anti-HBV cumulative % positivity</th>
<th>Anti-HIV cumulative % positivity</th>
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<tr>
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<td>4.1</td>
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</table>

*Melbye et al. 1990.*

**Household risks of transmission**

A study from Spain on 211 non-sexual household contacts of HCV seropositive patients found that 5.7% were infected with HCV. An identical seroprevalence rate was found in a study from Italy on the household contacts of seropositive blood donors. These results are contradicted somewhat by an identical study from Germany which produced a seroprevalence rate of only 0.5% for household contacts. The true picture is unclear and further studies are required. It is important to advise HCV infected patients not to share implements such as toothbrushes and razors, and it is possible that a failure to adhere to this advice may account for some cases of transmission.

**Risks to health care workers**

Healthcare workers are placed at risk of HCV infection through needlestick injuries. In Japan of 68 healthcare workers suffering needlestick injuries from patients who were HCV RNA positive, 10% seroconverted for HCV. This is a much higher rate of seroconversion than is seen for HIV. Further studies are required.

In summary, the data show that the main route of transmission for HCV is parenteral, with intravenous drug users representing the single biggest risk group. There should no longer be a problem with blood transfusion, and heat-treated blood products should have eliminated the risks to haemophiliacs. Modern screening methods should also significantly reduce the risk through organ donation. In addition to intravenous drug use, the other potential parenteral source of transmission that has not been fully investigated is tattooing. The risk of
sexual and vertical transmission of HCV appears to be small, and therefore gives some hope of being able to control the spread of this infection.

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Acute HCV infection

The incubation period of acute infection is about 6–8 weeks, although some patients have been reported to develop an acute hepatitis illness within a few days of transfusion, and it may be that the incubation period can be shortened by a high infecting dose. For most patients the acute illness appears to be completely asymptomatic, with jaundice only developing in about 10% of cases. Diagnosis of acute HCV infection is problematic. In one study of 17 patients with acute HCV infection, all patients had positive serum HCV RNA by PCR within three weeks of the onset of illness, but only 67% of the patients were positive for anti-HCV by RIBA. By 21 weeks this had risen to 86%. In chimpanzees who have been experimentally infected with HCV, RNA can be measured at 4–7 days post-infection, but levels fluctuate considerably and there may be days when HCV RNA is undetectable. In 20–40% of patients with acute HCV infection, transaminases normalise within 4–6 weeks, although in a proportion biphasic elevations of transaminases can make the determination of true convalescence difficult.

Sixty–80% of patients will develop chronic HCV infection. As with chronic HBV infection most patients are asymptomatic until late in the disease when they may present with liver failure. Most patients with chronic HCV are detected by screening and will not recollect an acute hepatitis illness. In contrast to HBV, the transaminase levels often fluctuate considerably over time, the reason for which is unclear. Screening for liver disease with transaminase levels may not be adequate, as it is now realised that patients with normal transaminase values who are anti-HCV positive and HCV RNA positive by PCR can nevertheless show chronic hepatitis in a biopsy. There would also seem to be a small group of patients who seem to fit the criteria of a healthy carrier. These patients have both normal transaminase values and normal liver histology.

Initial studies of post-transfusion NANBH had short follow-up periods and it is now realised that these underestimated the risks of liver disease. It is now believed that approximately 20% of patients with HCV infection will ultimately develop cirrhosis, although there is considerable individual variation in the rate of progression to this complication. A recent Italian study looking at 135 patients with post-transfusion NANBH showed that 77% of patients developed chronic hepatitis. Of 65 patients biopsied, 32% had developed cirrhosis by the end of the last year.
years follow-up. Almost 50% of the cirrhotic patients developed life threatening complications including portal hypertension. Of the remaining biopsied cases, 37% had chronic active hepatitis, 27% chronic persistent and 3% chronic lobular hepatitis. During follow-up half of those who were re-biopsied developed cirrhosis. Studies from Japan have shown a clear association between HCV infection and hepatocellular carcinoma (HCC) with approximately 50% of patients with HCC being anti-HCV antibody positive. It is unknown whether HCC develops as a result of an oncogenic effect of the virus or as a result of virus induced scarring and regeneration.

There are a number of viral and host factors that have been identified of possible relevance in explaining the differing outcomes of chronic HCV infection. It is now recognised that there are a number of HCV genotypes and a high mutation rate with HCV infection. The infecting strain may well be relevant to the disease outcome. Quantitation techniques for HCV RNA estimation are under development and early data suggest a correlation between levels of viral replication and severity of disease. Asymptomatic blood donors and patients with chronic persistent hepatitis tend to have lower levels than those with chronic active hepatitis or cirrhosis. The mode of acquisition of infection may also be an important factor in determining levels of viraemia, with highest levels seen in those infected by a blood transfusion. It has also been suggested that phasic elevations in transaminase levels may reflect immune driven mutation of HCV. A number of host factors are also relevant. As with chronic HBV infection, the outcome in male patients seems worse than in women in females. Alcohol seemed to be an important cofactor for disease progression and a more rapid deterioration is seen in high alcohol consumers. Immunosuppression either through the use of immunosuppressive drugs or as a result of coinfection with HIV infection seems to be associated with more rapidly progressive disease. The adverse effects of immunosuppressive drugs are particularly unfortunate, as chronic HCV infection is common amongst transplant patients.

Other clinical manifestations of HCV infection

The putative link between HCV and autoimmune disease of the liver is controversial. With the first generation serological tests for HCV, many patients with a previous diagnosis of autoimmune liver disease were found to be anti-HCV antibody positive. With the availability of more accurate confirmatory tests the majority of these results are now recognised to be false positives. There is no association between HCV and classic type 1 autoimmune hepatitis, which is usually seen in young women who have antibodies to nuclear proteins and smooth muscle. The treatment of choice in these patients is steroids. However, Type 2 autoimmune hepatitis can be associated with liver/kidney microsomal antibodies and in some adults appears to be associated with HCV seropositivity suggesting that perhaps an autoimmune process has been triggered by HCV infection. This complicates decisions regarding treatment as steroids can make viral infections worse and interferon can aggravate auto-immune processes.

Some patients with keratoconjunctivitis sicca (Sjoegren’s syndrome) have been found to be anti-HCV positive, suggesting a possible association. However, others have postulated that HCV may infect salivary glands, resulting in a focal sialadenitis.

Eight patients in one study were described with membranoproliferative glomerulonephritis in association with chronic HCV infection, all of whom were HCV antibody and RNA positive. All patients had proteinuria and hypocomplementemia and renal biopsies showed membranoproliferative glomerulonephritis with deposition of IgG, IgM and C3 on capillary walls. Electron microscopy was performed on 4 biopsies and the appearances in three suggested the presence of cryoglobulinaemia. All patients had elevated transaminase levels and liver biopsies demonstrated cirrhosis in 4 of the 5 biopsied cases. 4 patients were prescribed interferon alpha with a reduction in the protein loss, but a variable response in the serum creatinine.

There is a strong association between type 2 cryoglobulinaemia and HCV infection. An association also exists between HCV and porphyria cutanea tarda, although alcohol induced liver damage is probably a significant factor in many of these patients.

In summary, 60–80% of patients with HCV infection go on to develop chronic liver disease, and up to 20% may ultimately develop cirrhosis. Miscellaneous extrahepatic manifestations of HCV are seen rarely. Unfortunately, at this stage in our knowledge we are unable to predict the outcome for individual patients.

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PATHOLOGY OF HCV INFECTION

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Acute infection

There is little known about the changes seen in the liver during the acute phase of the infection, as biopsy is seldom undertaken at this time. Some reports however, describe features such as individual hepatocyte necrosis, Kupffer cell activation, lobular and portal triad inflammatory cell infiltration, which are no different from those seen in other forms of acute hepatitis. In most cases the acute infection follows a mild course with full clinical recovery. Nonetheless, in a few cases the infection has apparently caused a fulminant hepatitis.

Chronic infection

It appears that the majority of HCV infections result in chronic hepatitis. In a biopsy study of HCV infected haemophiliac patients from Sheffield, 24/35 who underwent liver biopsy showed features of chronic hepatitis, whereas only 11 had normal histology. Other studies have shown similar percentages. The histological findings in all patients with chronic liver disease associated with HCV infection are similar, and are not specific. The overall picture is often described as that of a mild chronic hepatitis with borderline features between chronic persistent and chronic active hepatitis but increasingly the presence of certain features is regarded as a helpful pointer towards the diagnosis. Amongst these, lymphoid aggregates in the portal triads are probably the most useful. At one end of the range these may be an ill-defined condensation of small lymphocytes, and at the other, definite lymphoid follicles with reactive general centres. (Fig 1) In addition to the lymphocytic infiltration seen in portal areas there is often involvement of the lobules—lobular hepatitis—with lymphocytes present within sinusoids. (Fig 2) This feature is seen in about a third of cases and is usually mild. A similar proportion of cases show damage to bile duct epithelium with infiltration by inflammatory cells of lymphoid type, and degenerative changes of the bile duct lining cells. Such abnormal bile ducts can be seen at the centre of a lymphoid aggregate. Within the lobules, hepatocyte necrosis is not usually prominent but rarely gives rise to bridging necrosis. More commonly steatosis is seen, which is mild, macro-vascular and of no consistent pattern of distribution.

A recent study of the phenotypic distribution of the lymphocyte infiltrate has shown a normal pattern of activated B cells in the centre of lymphoid nodules, and T cells, which when observed in areas of piecemeal necrosis are of the CD4/

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helper phenotype, and the CD8 suppressor/cytotoxic phenotype in the lobules. This does raise the possibility that some of the liver damage may be immune mediated, although this remains a controversial area.

In a few cases where there is extensive hepatocyte necrosis, the regenerative process leads to the formation of multinucleated giant cells, some of which may contain bile, suggesting an impaired ability to generate bile canaliculi. These changes are worrying as they tend to be associated with progression to more severe chronic liver disease. Approximately 20% of infected patients progress to liver cirrhosis. There is an association between HCV infection and hepatocellular carcinoma (HCC) although the prevalence of HCV among patients with this cancer appears to vary considerably in different parts of the world. It is high in Italy (65%) and Spain (75%), but much lower in Southern Africa (2%). In most cases the length of time for progression to HCC after infection appears to be 20 or more years. This interval may be shorter in patients who acquire the infection at an advanced age. There is some evidence to suggest that patients developing HCC after HCV infection have a poorer prognosis, as the lesions are often multifocal and present at a late stage of development. The finding of both Hepatitis B and C infection in patients with HCC is not uncommon in certain parts of the world, and it is postulated that the action of both viruses may be synergistic in the carcinogenic progress, although the mechanism of oncogenesis in HCV infection is not fully understood.

Patients with chronic alcohol liver disease

A higher prevalence of HCV antibodies has been shown in patients with severe forms of either alcoholic hepatitis or cirrhosis than in those with normal liver or steatosis only. Patients with HCV infection should therefore avoid alcohol consumption.

Liver transplantation

End stage chronic Hepatitis C infection is an increasingly common indication for liver transplantation. This procedure is usually followed by re-infection of the graft which may however respond to treatment. In biopsies taken from patients with deranged liver function following transplantation, it can be difficult to differentiate between the changes of rejection and those of re-infection with HCV.

Demonstration of HCV in liver tissue

HCV has not yet been directly visualised, nor has culture been possible. Specific sequences of viral RNA have been identified in hepatocytes using in situ hybridisation, indicating that the virus proliferates within these cells. More recently, immunohistochemical methods have been developed using antibodies to envelope non-structural proteins of the virus, with encouraging results. It is possible that these studies may shed some light on the problems relating to the mechanisms of action of the virus, and in particular whether the effects of the chronic infection are immune mediated—or directly related to cytotoxicity. It may also become possible to monitor the success of antiviral treatment using these methods.

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SCREENING TESTS FOR HEPATITIS C VIRUS


SCREENING TESTS FOR HEPATITIS C VIRUS

E. A. C. Follett,* Scottish National Blood Transfusion Service, Regional Virus Laboratory, Ruchill Hospital, Glasgow

When tests for hepatitis B became available in the early 1970s the blood transfusion service were able to identify carriers of the virus and thereby prevent the occurrence of post-transfusion hepatitis. However, despite the screen, cases of this condition occurred and the term non-A, non-B hepatitis was applied to these. Early hopes that the agent would be rapidly identified proved unfounded. Classical conventional techniques of virus isolation failed to identify it, even in well characterized cases of post-transfusion hepatitis and it was not until molecular biology techniques for cloning nucleic acids were applied to plasma derived from chimpanzees infected with the human non-A, non-B agent, that a virus was identified and characterized; subsequently its genome was sequenced and a series of diagnostic reagents derived. Hepatitis C virus (HCV) is an enveloped single stranded positive sense RNA virus classified in the family Flaviviridae. Comparison of genome sequence and organisation suggest a closer association with the pestiviruses rather than the flaviviruses within this family but at present HCV is classified as a separate genus within the flaviviridae.

Although no electron micrographs of HCV exist, much work has been done by molecular biologists to characterize this virus. The genome is composed of just over 9,400 nucleotides coding for 3,010 amino acids. There is a 5' noncoding region followed by core (c) and envelope (E1/E2) structural regions and finally 5 non-structural regions (NS1-NS5). (Fig)

Historical

The first commercial kits for detecting HCV antibody became available in late 1990. Recombinant antigen to only one region of the genome NS4, was included but as two components, 5-1 and c100. The type of assay used was known to produce nonspecific results and to identify these a supplementary assay was produced by Chiron, a recombinant immuno-blot assay, RIBA 1, containing the two recombinant antigens of the screening assay adsorbed to a nitrocellulose strip. Unfortunately, non-specific results remained a problem. This unsatisfactory situation was improved by the introduction of RIBA-2 which contained recombinant antigens to further regions of the genome, the core region with

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c22 antigen and the NS3 region with c33. Although not a true confirmatory test, because it did not contain antigens from an independent source, RIBA-2 has proved extremely useful and provided a degree of confidence in HCV data which was previously lacking.

Analysis of results has indicated that data from the original first generation screening assay not subsequently tested by RIBA-2 must be regarded as suspect especially in low risk populations such as blood donors. The RIBA-2 data also highlighted the importance of the core and NS3 regions in HCV infection. Consequently a second generation assay was introduced containing the c22 antigen and c33 antigen as well as 5-1-1 and c100. This combination of tests has provided most data on HCV infection in the past three years. Episodes of acute HCV infection could be identified in post transfusion hepatitis and in drug users, amongst others. These studies have shown a lengthy window period in most infections. The second generation tests have reduced but not eliminated this window and in many infections a second sample three to four weeks after the acute illness is necessary to confirm the diagnosis. Screening of all blood donations has been demonstrated to reduce the rate of post transfusion Hepatitis significantly.

Current status
The problem of confirmation of the antibody remains, especially now that the screening assay contains the same antigens as the supplementary assay. A further complication of the use of RIBA-2 is the unfortunately named ‘indeterminate’ result, when only antibody to one antigen can be detected. In blood donors, up to 40% of repeatedly reactive samples in the screening tests can be in this category. A partial solution to this problem is the use of polymerase chain reaction (PCR) methods to detect the presence of HCV genome in such samples. While not confirming the presence of antibody, the presence of viral RNA does make it much more likely that the indeterminate finding represents ‘real’ antibodies. Generally, <10% of indeterminates in blood donor populations are PCR positive. Detection of viral RNA by PCR remains the only method of determining potential infectivity in the patient. Commonly >70% of RIBA-2 positive samples are found to be PCR positive, indicating chronic infection to be a much more lasting consequence of HCV infection compared to HBV infection.

Alternative antigen products
The screening tests marketed by Ortho Diagnostic Systems and Abbott Diagnostics use material derived from the original Chiron Corporation isolate. Other manufacturers in Europe and North America have independently cloned HCV genomes and marketed screening and supplementary tests. While useful in their own right as screening tests, these alternative antigen products can also have value in interpreting the RIBA ‘indeterminate’ result. For example, in a study of blood donor repeat reactive samples from Scotland and Northern Ireland, all RIBA-2 positives detected originally by Abbott and Ortho tests were detected as positives by the Murex ELISA, the UBI ELISA and the Innolia supplementary assay. Of the RIBA-2 indeterminates all those which were PCR positive were detected by Murex and Innolia, but none one by UBI. Very few of the PCR negative indeterminates reacted in any of these alternative antigen assays. Great care is needed in the interpretation of results using these alternative antigen tests. Compared to the second generation Ortho and Abbott ELISAs, the Murex ELISA has an extra NS5 component, the UBI ELISA originally lacked an NS3 component, and the Innolia supplementary test lacked NS5 but had an NS5 component but the latest versions of both these contain NS3 components. Caution is required to ensure that false negatives do not arise through choice of inappropriate assays.

Future developments
The Chiron Corporation have developed an improved RIBA, RIBA-3 which contains 4 antigens, 2 as peptides (c100 and c22), and two as recombinant antigens (c33 and NS5). This test has improved specificity for c100 and c22 antibodies. In the study of Scottish and Northern Ireland blood donors mentioned above, RIBA-3 also detected all PCR positive, RIBA-2 indeterminates. In addition the small number of PCR negative RIBA-2 indeterminates detected with a c100 or c22 antibody was comparable to that found using the alternative antigen tests. The combined results of PCR, RIBA-3 and alternative antigen tests indicates that the great majority of RIBA-2 indeterminates are indeed false positives resulting from nonspecific reactions. Compared to RIBA-2, RIBA-3 has improved sensitivity as demonstrated in earlier detection of seroconversion samples and for particular subtypes (types 2, 3 and 6) especially in NS4 region. The introduction of synthetic peptides in place of the recombinant antigens for the c22 and NS4 regions has markedly improved this test and there is now a need for some form of alternative antigen to replace c33 and NS5 recombinant components.

A third generation of screening tests is currently in production in which the manufacturers seek to include antigens from as many regions of the genome as possible in an effort to exclude all potentially infectious donations from the blood bank. The Abbott and Ortho tests now have added NS5, the Murex improved
NS3 and NS5 components. These tests are more sensitive than their predecessors, the improvement mainly associated with the NS3 antigen. The role of NS5 remains enigmatic. A similar conclusion was reached after a study of 5 improved supplementary immunoblot assays. Improved detection was associated with improvements in the original antigens (core, NS3, NS4) not with the addition of new antigens, NS5, E2/NS1. The failure of these new antigens to detect a hitherto undiscovered population of new HCV positives suggests that further effort in this area may be unrewarding.

**Outstanding problem**

Diagnosis of the acute infection remains almost impossible. Even if the antibody can be detected, there is no IgM to differentiate current from past infections. Too many blood donations are being lost because of nonspecific reactions. The indeterminate result is meaningless and unhelpful. A significant number of RIBA 2/3 positive patients lack HCV RNA. Does this represent recovery from acute/chronic infection, remission of chronic infection, or lack of sensitivity of the PCR test? A cheaper and simpler alternative to PCR would be helpful.

**REFERENCES**


**SIGNIFICANCE OF SEQUENCE VARIABILITY OF HEPATITIS C VIRUS**

P. Simmonds, Department of Medical Microbiology, University of Edinburgh

Nucleotide sequence analysis is a powerful tool for functional, epidemiological and clinical investigations of hepatitis C virus (HCV). Analysis of the complete sequence of HCV has allowed inferences to be made concerning its method of replication that complement or extend in vitro experimental data, and help to reveal underlying relationships with other viruses. Comparison of the sequences of different isolates has allowed HCV to be classified provisionally into a number of different types, whose biological and clinical significance is currently being explored. Within an HCV type, sequence variability can be used to identify specific viral isolates and this may perhaps allow chains of transmission to be identified. Finally, micro-heterogeneity of viral sequences within an infected individual has been proposed to be the basis of mechanisms of HCV persistence along the lines of another chronic virus infection, human immunodeficiency virus (HIV).

The structure of HCV genome shows similarities to other positive-stranded (i.e., messenger sense) RNA viruses. The organisation of the coding sequences into a single open reading frame, and the cleavage of the translated polyprotein into functional sub-units is a strategy of replication shared by several virus groups infecting both animals and plants. These include the flaviviruses, a group containing the important human pathogens yellow fever virus and dengue fever virus, and the pestiviruses, which are respiratory pathogens of several domesticated animals (e.g. sheep, cow, pig). Another (more distantly) related group of viruses that cause human disease are the non-enveloped picornaviruses of which hepatitis A virus, poliovirus and Coxsackie viruses are the most familiar.

**Classification**

Nucleotide sequences of HCV variants obtained from different parts of the world show considerable diversity. However, HCV does not show a continuum of sequence variability, instead there appears to be a finite number of distinct virus clusters typically showing approximately 67–70% overall sequence similarity. Many groups have developed schemes for the classification of HCV variants by simple nucleotide sequence comparisons, or by more formal phylogenetic analysis. Classifications may be based upon comparisons of complete genomic sequences, or of subgenomic fragments of the genome amplified by polymerase chain reaction (PCR). Progress in the identification and mapping of the distribution of different HCV types has been rapid since nucleotide sequence data first became available, but has led to confusion regarding the nomenclature of HCV. Different research groups have published several alternative classification systems.

We have recently used phylogenetic analysis of nucleotide sequences derived from part of the gene encoding a non-structural protein (NS-5) to classify sequence variants of HCV (Table). We have obtained evidence for the existence of at least six major genotypes of HCV amongst a worldwide collection of 76 samples from HCV-infected blood donors and patients with chronic hepatitis. Many of these HCV types comprised a number of more closely related subtypes, leading to a current total of 11 genetically distinct viral populations. We have proposed a new nomenclature for HCV variants that classifies all known HCV variants to date, and describes criteria that would enable new variants to be assigned within the classification as they are discovered.

Classification of viruses purely on the basis of sequence comparisons rather than by biological properties is a relatively new approach to virus typing. It has advantages of objectivity and clarity and is not dependent on often complex biological assays, such as the cross-neutralisation and pathogenicity in experimental animals as has been used in the past for classification of the *picornaviridae*. Furthermore, without a satisfactory virus culture system, and with a host range restricted to man and higher primates, most of the biological assays that have been used would be laborious, expensive or impossible to carry out with HCV.

**Origins of HCV**

Little is known about the ultimate origin of HCV and the process of diversification that led to the appearance of the different HCV types and subtypes. However, it might be possible to extrapolate the time of divergence of the
HCV types and sub-types from estimates of the in vivo rate of sequence change for HCV over shorter time scales. This can be done by comparison of viral nucleotide sequence obtained over time within an infected individual,4-6 or from donor/recipient pairs in cases of transfusion transmitted infection.7 Comparison of the nucleotide sequence of the NS-3 region of variants from donor and recipient four years after transmission revealed 96.8%-98.9% sequence similarity, that translates to roughly 0-2% sequence drift/year. Remarkably similar figures of 0.192% (partial genome)4 and 0.144% (complete genome)5 of sequence divergence per year have been found in longitudinal studies of infected individuals.

<table>
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<tr>
<th>HCV variant</th>
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<th>Cha/ Unde.</th>
<th>Cha/ Simmonds</th>
<th>Enomoto</th>
<th>Mori/ Okamoto</th>
<th>Tsukiyama-Kohara</th>
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<td>I</td>
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<td>K-PT</td>
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<td>II</td>
<td>1b</td>
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<td>II</td>
<td>I</td>
</tr>
<tr>
<td>EG-28</td>
<td>[22]</td>
<td>1c</td>
<td>nc*</td>
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<td>nc</td>
<td>nc</td>
<td>nc</td>
</tr>
<tr>
<td>HC-J6</td>
<td>[23]</td>
<td>2a</td>
<td>III</td>
<td>2a</td>
<td>K-2a</td>
<td>III</td>
<td>II</td>
</tr>
<tr>
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<td>[1]</td>
<td>2b</td>
<td>III</td>
<td>2b</td>
<td>K-2b</td>
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<tr>
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<tr>
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</table>

Proposed nomenclature for published HCV sequences and comparison with existing schemes (CHA/URDEA: [25]; CHAN/SIMMONDS: [2, 22]; ENOMOTO: [10]; MORI/OKAMOTO: [1, 26]; TSUKIYAMA-KOHARA: [28].

*nc: sequences not classified by originating authors indicated as ‘nc’.

At a simplistic level, we could proceed to calculate that the major types originated 80-100 years ago (30-35% sequence divergence). However, this calculation makes a number of unwarranted assumptions concerning the rate of accumulation of substitutions that takes no account of selection pressure operating against variants with altered protein sequences. For these reasons, the origin of the different HCV types can be put back far beyond the calculated 80-100 years, possible to several thousand years or even longer. The difficulty is not unique to HCV; for example current attempts to date the time of divergence of the two human immunodeficiency viruses, HIV-1 and HIV-2, are also subject to widely different estimates, ranging from 20 years to several thousand years.

Identification of HCV genotypes
Identification of variants of HCV can be carried out by a variety of PCR-based and serological methods. Virus types may be identified by nucleotide sequencing of DNA amplified from HCV RNA sequences in plasma or liver biopsy samples to detect specific nucleotide differences associated with each virus type. Alternatively, amplified DNA may be cleaved by restriction endonucleases whose recognition sequences differ between genotypes of HCV. These polymorphic sites may also be identified by other techniques such as labelled primer extension, ligation methods, type-specific amplification with type-specific primers8,9 or hybridisation with type-specific probes.10

We have made extensive use of restriction endonucleases for virus typing. In a recent study, we have used this method to investigate the frequency of infection with the six classified major genotypes of HCV in 447 infected volunteer blood donors from nine countries, Scotland, Finland, the Netherlands, Hungary, Australia, Egypt, Japan, Hong Kong and Taiwan.11 Viral sequences in plasma from blood donors infected with HCV were amplified in the 5’non-coding region and typed by restriction fragment length polymorphism. Electrophoresis of DNA fragments produced by cleavage with HaeIII/RsaI and SphI/HinfI allowed HCV types 1, 2, 3, 4, 5, and 6 to be identified. Further analysis with MseI/HinfI allowed sequences of the type 5 genotype to be distinguished from type 1. Types 1, 2 and 3 accounted for almost all infection in donors from Scotland, Finland and the Netherlands. Types 2 and 3 were not found in the Eastern European country (Hungary), where all but one of the donors was infected with type 1. Donors in Japan and Taiwan were only infected with type 1 or 2, while types 1, 2 and 6 were found in those from Hong Kong. HCV infection amongst Egyptians was almost always by type 4. (Type 5 appears to be confined to South Africa.)

Antigenic variation of HCV
Donors infected with HCV type 1 showed broad serological reactivity with a wide range of antigens used in the 2nd Generation Ortho RIBA-2. These include 5-1-1 and c100-3 derived from sequences in the NS-4 region, c33c from NS-3 and finally c22-3 from the core region of the genome that encode the nucleocapsid (structural) protein. However, infection with divergent HCV genotypes elicited antibody reactive to mainly c22-3 and c33c. Reactivity with 5-1-1 and c100-3 was infrequent and generally weak, irrespective of the geographical origin of the donor. Reactivity with the NS-4 encoded antigens in the 1st generation anti-C100 assay was absent in 68% of donors infected with types 2, 3, 4, 5 and 6 compared with 10% for type 1.11 Even when combined with surrogate marker testing, 1st generation tests would have failed to detect 12% of HCV-infected blood donors. As the region of HCV encoding the external membrane glycoprotein of HCV (E1, E2) is even more variable than NS-4 between genotypes, it is likely that vaccines based on these proteins12 need to be multivalent and to be specifically adapted for different geographical regions.

A more detailed analysis of the effect of sequence variability upon the antigenicity of the NS-4 protein was investigated by epitope mapping and by enzyme-linked immunosorbent assay (ELISA) with branched oligopeptides.13 Epitope mapping of the region between amino acid residues 1679-1768 in the HCV polyprotein revealed two major antigenic regions (1691-1708 and 1710-1728) that were recognised by antibody elicited upon natural infection of HCV. The antigenic regions were highly variable between variants of HCV, with only 50-60% amino acid sequence similarity between types 1, 2 and 3. Although limited serological cross-reactivity between HCV types was detected between peptides, particularly in the first antigenic region of NS-4, type-specific reactivity formed the principal component of the natural humoral immune response to NS-4.
One of the limitations with PCR-based methods for virus typing is that it can only be carried out in individuals who are viraemic. Furthermore, RFLP or direct sequencing methods of virus identification cannot readily detect more than virus type co-circulating within an infected individual. We have recently developed an alternative typing method that is based on a serological assay for type-specific antibody to the NS-4 region that is elicited by infection with different HCV variants. Type-specific antibody to particular HCV types was detected in 89% of samples from anti-HCV positive blood donors, and correlated almost exactly with genotypic analysis of HCV sequences amplified from the samples by polymerase chain reaction. Whereas almost all blood donors appeared to be infected with a single virus type (97%), a higher proportion of samples (40%) from haemophiliacs infected from transfusion of non-hepatitis inactivated clotting factor contained antibody to two or even all three HCV types, providing evidence that long-term exposure may lead to multiple infection with different variants of HCV.

Variation in course of disease

It is impossible to predict with any certainty whether infections with different HCV types follow clinically distinct courses. Direct clinical studies of possible differences in the course of HCV infection associated with specific HCV types have so far been largely inconclusive. An immediate problem with this type of study is disentangling possible differences in pathogenicity of HCV variants from other incidental variables, such as duration of infection and origin of patient, both of which may show spurious epidemiological linkage to specific HCV types.

In our study of HCV-infected Scottish donors, the age distribution, incidence of past infection with hepatitis B virus and reported risk factors were found to be similar between those infected with type 1 and type 3 (mean age 31.9 and 29.9, 18% and 17.5% anti-HBc positive, and 47 and 48% past intravenous drug abuse). However, the distributions of alanine aminotransferase (ALT) levels were significantly different between those infected with type 3 (abnormally raised in 83% and those infected with type 1 (55% abnormal ALT; p<0.05), type 2 (60%; p<0.01) and those who were non-viraemic (8%; p<0.0001). These data suggest that infection with HCV type 3 may be associated with more severe liver disease, either because of more recent infection or because of a greater inherent pathogenicity of type 3 variants.

Whether different HCV variants show different responses to interferon therapy is currently an active and extremely important area of research. The response to interferon might reveal important biological differences between the virus types, and should differences be found, might influence patient and dose selection for treatment. Two studies in Japanese NANTBH patients have shown that HCV type 1 (specifically type 1b) was less responsive to treatment than type 2 variants.

In a recent collaborative study, we used the serological typing method described above to investigate possible type-specific differences in α-interferon response in a large group of Italian patients with NANTBH. The probability of achieving a long term response with IFN, and conversely, that of a non-response correlated closely with the pre-treatment serotype. The long-term response rate of the 65 patients infected with type 1 was only 29%, significantly different from a response rate of 52% found in patients infected with type 2 (n=23) and 74% with type 3 (n=19). These clear type-specific differences indicate that HCV serotyping by ELISA may be a valuable and practical method for pre-treatment assessment of candidates for interferon therapy.

In conclusion, I have tried to summarise a rapidly expanding area of HCV research. The distribution of existing HCV types shows considerable geographical variation, and it is likely that the full extent of sequence variability of HCV has yet to be discovered. Progress in the clinical investigations will rely in part on the development of agreed typing assays and nomenclature for HCV variants, and this should be regarded as priority for those actively involved in such studies.

REFERENCES

Interferons also modify the cellular and humoral immune response: expression and activation of natural killer cells and T lymphocytes are affected. Early and late events in the viral life cycle, such as uncoating of viruses, or integration of proviral DNA, are modified by interferons.

Viruses may decrease interferon production from infected cells, and certain viruses may have evolved mechanisms of inhibition of interferon production.1

**Therapeutic use of interferon alpha:**
Four preparations of interferon alpha are currently available, three of which are recombinant [‘Roferon A’, alfa-2a, Hoffmann La Roche; ‘Intron A’, alfa-2b, Schering, Con IFN (consensus interferon, Amgen)], and one of which is prepared from a lymphoblastoid cell line ['Wellferon' alfa n1, Burroughs Wellcome]. Alpha 2a and 2b are recombinant preparations produced in E coli. Alpha 2a has a lysine molecule at position 23 instead of an arginine present in alpha 2b. Alpha n1 is a natural mixture of IFN alpha produced by human lymphoblastoid cells after viral stimulation. Early pilot studies pointed out the inhibitory effect of recombinant interferon alpha interferon upon HCV replication and the therapeutic potential of this drug.2 Later, in controlled, dose-finding studies, predictors of response could be tentatively defined, dose ranges narrowed and therapeutic strategies outlined.3,4

The major early side effects of interferon include an influenza like syndrome, chills, fever, malaise, muscle aches and rigors. Later side effects include malaise, muscle ache, headache, poor appetite, weight loss, increased need for sleep, psychological effects, (irritability, anxiety, depression), hair loss, thrombocytopenia, and leukopenia. Unusual, or severe side effects include seizures, acute psychosis, bacterial infections, autoimmune reactions, thyroid disease (hypothyroidism) proteinuria, myocardopathy, skin rashes, exacerbation of diabetes, and formation of interferon antibodies. Patients should be monitored at intervals one to four weeks during treatment, and blood counts and serum aminotransferases should be measured on these occasions. Thyroid functions should be measured before, during and at the end of therapy.5-9 There are recent reports of interstitial lung disease and retinal changes in patients treated with higher doses of interferon alpha.

The role of interferon antibodies in reducing response is controversial, but neutralizing antibodies may be one variable which affects response to interferon. A higher proportion of patients with chronic hepatitis treated with alpha 2a develop neutralizing antibody, 20%, vs 1-2% among patients treated with alpha n1. Thus alpha 2a interferon may be more immunogenic.10,11

With respect to treatment of cases of autoimmune liver disease with hepatitis C antibodies, the ELISA assay for anti-HCV is prone to false positive results in patients with high concentrations of immunoglobulins in serum.12,13 However, in some countries, patients with type II autoimmune CAH appear to have a high frequency of genuine exposure to HCV, and antibodies to liver kidney microsomal antibodies are present in patients.14,15 These patients may also have circulating antibodies to a pentadecapeptide (Gor), an epitope of normal hepatocytes; this phenomenon may represent an auto-immune response peculiar to type C hepatitis.16 This association has some therapeutic implications, as in some of these patients the disease may be aggravated by interferon alpha and is responsive to corticosteroids.
TREATMENT OF CHRONIC HEPATITIS C

Indications
Anti-HCV positive HCV RNA-positive patients are considered for treatment. There is no clear logic in treating anti-HCV positive patients who have normal serum ALT and are not positive for HCV RNA, and the monitoring of antiviral efficacy in such patients is not possible without repeating a biopsy. The indications for treatment are usually clear when the patient has raised serum ALT (more than two fold elevated) and moderate or severe hepatitis on liver biopsy. Patients may also be considered for treatment if the ALT are raised and mild chronic hepatitis is observed on liver biopsy. It is unclear whether treatment of HCV RNA-positive patients with minor, non-specific changes on liver biopsy would benefit from treatment. Unfortunately, serum ALT correlates poorly with the stage of the disease, and biochemical testing may not be informative. This is obviously a dilemma in evaluating haemophiliacs, in whom liver biopsies are more hazardous.

Chronic hepatitis has been a common consequence of community acquired hepatitis C infection where patients may not have suspected exposure to the virus. Prospective studies have suggested that 10–20% of patients with chronic NANB hepatitis develop cirrhosis within a 10 year period. Individuals with chronic hepatitis C with elevated ALT and chronic active hepatitis histologically should therefore be considered for antiviral therapy. Ideally HCV RNA should be measured in all patients to confirm viremia. If the test is reproducibly positive, then serum aminotransferases, bilirubin, alkaline phosphatase, and pro-thrombin time should be measured. HBsAg (and hepatitis D) and HIV infection need also to be considered. Another form of chronic hepatitis, i.e. a non-A, non-B, non-C flavivirus (hepatitis G) has just been reported at the time of writing this review. Because autoimmune hepatitis is treated differently, it is particularly advisable to exclude this diagnosis by measuring the titres of anti-smooth muscle and anti-liver kidney microsomal antibodies, even those with a positive anti-HCV test, and to measure HCV RNA in anti-HCV positive patients in whom interferon is contemplated. Where possible, HCV type (genotype) and the level of viraemia should be measured, as these indicators have therapeutic implications.

Preliminary therapeutic trials of interferon alpha indicated that a proportion of patients may respond to treatment with this agent. Larger placebo controlled studies have shown that approximately 50% of patients will have normal serum aminotransferases after treatment courses of interferon alpha of approximately 3 million units (mu) three times a week (tiw) for six months. Serum HCV RNA may become undetectable in patients after 4–8 weeks of interferon alpha treatment in patients who respond, but an undetectable HCV RNA at the end of the treatment does not preclude relapse.

However, on stopping treatment after six months, one half of the responsive patients will promptly relapse. Serum aminotransferases usually increase in patients who are HCV RNA positive at the end of therapy, although in some cases the relapse may be delayed for several months. Our studies in patients at the Royal Free Hospital indicate that 20% of patients have a prolonged response to therapy and do not again develop elevated serum aminotransferases. These patients also remain negative for HCV RNA.

There is most information about 3 mu, tiw, given for six months. It is not yet clear whether this dose is optimal. Other regimens are being evaluated, and there is a suggestion that higher doses may be beneficial. Initiating therapy with a somewhat higher dose of 15–18 mu per week, and prolonging therapy for a year probably results in lower relapse rates. However, relapses still occur and the side effects are greater at higher doses. The cost of treatment for six months is at least £1,500. Treatment should not be continued beyond three months in patients who do not have reduced levels of serum ALT. Responsive patients usually exhibit histological improvement, and may have a decrease in collagen III propeptide concentrations.

Unfortunately responsiveness to interferon alpha remains somewhat unpredictable; Factors which predict a greater likelihood of response are now being studied. Multivariate analysis of several pre-treatment parameters indicate that patients without cirrhosis are more responsive to interferon, and are more likely to have a sustained response. The influence of genotypes of hepatitis C is the subject of considerable interest at present, as is the association between lower levels of viraemia and response. In worldwide studies on genotypes, it has become apparent that genotype 1b is associated with a poor response to interferon therapy. However, type 2 and 3 are sensitive to interferon in a high proportion of patients. Patients with diverse circulating quasispecies may be less responsive to therapy than those with a single major species. Improved responses have been observed in patients with lower levels of circulating HCV RNA. Unfortunately, the issue remains complex; there is not yet a standardized system for quantitating concentrations of HCV RNA in serum. Also, these factors may be interdependent, as particular viral strains may replicate with higher efficiency than others. Genotyping and HCV RNA quantitation are not generally available to clinicians. However, when serotyping becomes available together with quantitation of HCV RNA, clinicians may be able to rationalize the use of interferon and the dose required.

When can treatment be considered successful? The criteria vary, but it is reasonable to infer that those patients with normal serum ALT a year after stopping interferon treatment, and negative for HCV RNA year after stopping therapy, with histologically improved disease activity and a perhaps normal serum procollagen III peptide, have had a good response. HCV antigens may be cleared from the liver with successful treatment.

Some patients have a sustained biochemical response to treatment but remain HCV RNA positive. These patients appear to have a higher probability of later relapse off therapy. Others have a transient response, and have normal serum ALT on treatment but relapse soon after treatment is stopped. Other patients may have a partial response with improvement, but not normalization, of the serum aminotransferases. Unfortunately a proportion of patients may have a good initial response on treatment, but then the ALT rise again despite continuing treatment; it is possible that some patients develop neutralizing interferon antibodies; the timing of antibody development may be a factor in explaining the course of responsive patients who do so. Continuing interferon with a second course of treatment with a different interferon may be useful in some patients but only a small number of patients have been reported. The significance of response to a different interferon after failing treatment with a first interferon is less certain.
Other patients do not respond to treatment, and no improvement in serum ALT levels can be discerned. Some patients may actually worsen on treatment with interferon, and develop increased serum aminotransferases. A positive anti-HCV antibody in patients with autoimmune disease remains a pitfall in diagnosis, which has implications for treatment.26 Such patients require confirmation by immunoblot assay, or HCV RNA, as corticosteroid therapy may be more appropriate than interferon alpha;26 it is possible that they have an underlying autoimmune status associated with hepatitis C and exacerbated by interferon treatment. For these and others who do not respond to treatment, ribavirin may be an alternative.

Immunosuppressed patients and patients with HIV may respond, although long term responsiveness is uncertain. This is of particular importance in liver transplant patients.

OTHER THERAPIES
Ribavirin
Ribavirin is a synthetic guanosine nucleotide analogue, which possesses a broad spectrum of activity against both DNA and RNA viruses in vitro and in vivo.27 Efficacy has been demonstrated in several viral diseases. The drug exerts its action after intracellular phosphorylation to mono, di- and triphosphate nucleotides. The 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 guanylate and methyltransferase capping enzymes, direct inhibition of the viral mRNA polyadenylase 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 IV administration). The distribution half-life is 1-3 hours, but the terminal half life is prolonged (27-52 hours) perhaps due to sequestration within red cells and other tissues. Ribavirin is concentrated 10-50 fold in red blood cells, and crosses the blood brain barrier. Peak plasma levels range from 5-13 μM after single oral doses of 600-2,400 mg. The excretion of the drug is predominantly renal.

The major side effects of the drug that have been reported include anaemia, (mild reversible anaemia is common), a metallic taste, dry mouth, flatulence, dyspepsia, nausea, headaches, irritability, emotional lability, fatigue, insomnia, skin rashes and myalgia. Modest increases in uric acid have been reported. Iron accumulation may occur in treated patients.28

Studies in ribavirin in hepatitis C infection
In an open label study in Sweden, in which ribavirin was prescribed to 10 patients with chronic hepatitis C (1,000-1,200 mg/day) for 12 weeks, median serum AST levels declined, but rose to pre-treatment levels upon completion.29 A small study, using escalating doses of ribavirin (600-1,200 mg) showed a somewhat slower fall in serum aminotransferases, perhaps reflecting the lower starting dose of ribavirin.30 There was a significant decrease in geometric mean titres of HCV RNA. At the Royal Free Hospital, we have treated 40 patients with chronic hepatitis C.31 Thirty-eight percent had normal ALT during treatment, but a further third had only a 50% decline in ALT. The remaining third were not responsive. The time course of normalization of ribavirin is slower (mean time to normal, 5-5 months) in patients with biochemical improvement. Despite the significant improvement in serum ALT we have not observed a marked decline in serum HCV concentrations. A multicentre placebo-controlled trial of ribavirin for hepatitis C is now being analyzed; although ribavirin therapy was associated with a highly significant decline in serum ALT concentrations, the drug did not result in sustained responses after 6 months of treatment and had no effect on serum HCV RNA concentrations. More promising data using ribavirin together with interferon alpha have been reported: approximately 40% of patients have sustained virologic or biochemical responses.20 Combination therapy may be effective in some patients who are unresponsive to interferon alpha.

Other ancillary treatments have been attempted. These include ursodeoxycholic acid,32-34 non-steroidal anti-inflammatory agents, N-acetyl cysteine,34 and iron depletion.35-39 However, the long term benefit of these strategies is unknown. Patients who do not respond represent a difficult management problem. Better antiviral agents are required; protease inhibitors are being developed in the laboratory. The efficacy of new drugs will be greatly facilitated by growing hepatitis C virus in tissue culture.

REFERENCES


I am well aware of your study. An interesting question is at what point should a biopsy to detect the presence of liver pathology be done. My impression is that progress in liver disease in HCV infection is slow and it may be 15–20 years before the more severe forms such as chronic active hepatitis or cirrhosis develop.

Questions to Dr Main
If half of HCV infected individuals become chronically infected, this implies that the other half clear the infection. Is this a reflection of a more effective immune response and, if so, might this have implications for successful vaccination against HCV?

The nature of the immune response to HCV is poorly characterized. Some infected patients become anti-HCV antibody negative. It is not known if such patients would be protected against further infection. It is possible that the original infection might recur at some stage in the future, although this is purely speculative.

The use of PCR appears to be crucial in determining the presence of viraemia and hence continuing infection. How much daily variation is there in the level of viraemia and how accurate are the assay methods used in PCR laboratories?

There is evidence of interlaboratory variation, although it is difficult to pass judgement on individual laboratories. Levels of viraemia do fluctuate, but it is not possible to advise how frequently PCR should be repeated. If possible more than one result should be obtained.

Should healthy HCV positive carriers be regarded as infectious?

Yes.

Could you comment further on the interaction between HCV and HIV?

The interaction is completely the reverse of that for coinfection with HIV and HBV. As HIV positive patients become progressively more immunosuppressed, the immune mediated liver damage decreases although this is at the expense of increasing hepatitis B viraemia. With HCV infection there is still debate as to how much of the liver damage is induced by the direct viral cytopathic effect, and how much is immune mediated. There is probably a mixture of both, although there is general agreement that giving immunosuppressive drugs, e.g. steroids, to HCV infected patients increases hepatic inflammation. HIV induced immunosuppression appears to act in a similar fashion, and this might lead to a more aggressive liver disease. However in a substantial proportion of coinfected patients, other complications of HIV infection may intercede and result in death before liver failure develops.

There is much individual variation. In coinfected patients with similar CD4 lymphocyte counts, there may be marked individual variation in the severity of hepatic disease.

What is the outcome for neonates who have vertically acquired HCV infection?

Diagnosis of neonates is complicated because of passive transfer of maternal antibodies. There are anecdotal reports, especially from the Mediterranean, of cirrhosis developing in children who appear to have acquired vertical HCV infection. However, there have been no adequate prospective studies to allow the question to be answered fully.

Questions to Dr Dusheiko
What is the optimal dose of interferon?

I suggest 6 mega units for induction and, after 3 months, 3 mega units. Given that most people with HCV infection are well and that there is no evidence as yet that interferon reduces the rate of progress to cirrhosis, is it ethical to give interferon outwith a clinical trial?

It is ethical as the evidence to-date indicates that 20% of treated patients achieve long term remission. This is enough to justify treatment, although one hopes to achieve better results in the future.

What is the experience of treating children with interferon?

There are reports from Italy and Spain and we have treated a small number of children ourselves. In our experience children tolerate interferon well and the response rates are similar to that in adults. I believe that the principles of management of HCV infection in adults should apply equally to children.

What differences are there in the therapeutic response to the different interferons?

There does not appear to be much difference between the different types of interferon but this is still under study.

How do you think interferon works?

The drug appears to interfere with hepatitis virus translation and thus prevents replication. Other immune mediated mechanisms may also play a role.

Some patients who relapse are subsequently offered maintenance therapy? In what dose and for how long?

The dose is variable and should be titrated against the individual patient. In some patients transaminase levels are exquisitely sensitive to minor alterations in dose. After 2 or 3 years of therapy an increased dose may be required because of breakthrough in transaminase levels.

Is there a role for early treatment with interferon following needlestick injury?

It would be an advantage to know the level of viraemia in the infected patient. If this was low, the risk of transmission through the needlestick injury is small, almost certainly less than 10%. In such cases treatment would not be warranted. If however, this was a transplanted patient with significant viraemia, then a case could be made for early treatment with interferon. However, at present there are no data by which to judge if such an approach would be beneficial.

What is the drop out rate for patients on interferon therapy?

In most studies it is about 10%. It tends to happen early when patients decide after a few doses that they cannot tolerate the flu-like side effects.

DISCUSSION ON SPECIFIC MANAGEMENT POINTS

Chairman: Dr G. R. Scott
Lecturers: Dr E. A. C. Follett, Dr J. Gillon, Dr Janice Main, Dr P Simmonds, Dr J. Piris, Dr G. M. Dusheiko
Discussants: Dr C. A. Ludlam, Dr R. Robertson, Dr A. McMillan, Dr P. C. Hayes, Dr E. Ong

Chairman: This meeting arose from a 'phone call that I received from a general practitioner. He told me that he had a patient, a young woman in her twenties, who had become infected with hepatitis C virus (HCV) through blood transfusion. He asked a number of questions. Should this girl continue to have intercourse with her husband? Was it advisable or indeed safe to have intercourse with a condom? Should she kiss her children? Should she have her own cutlery and
crockery? If she wanted to become pregnant again, was this advisable? It was clear that these questions were difficult to answer. When this young woman came to see me, I asked her some questions about the investigations that she was undergoing and whether or not treatment had been contemplated. She had got the impression from the doctors looking after her that nobody was really sure what to do. So we had a long talk and after a while she went away. Afterwards I felt that it would be useful to organise a meeting like this to try to answer some of these questions in such a way that guidelines could be drawn up for doctors and patients faced with similar situations. So in this session we hope to answer the following five questions.

Who should be tested?
Why should we be testing?
What should be done if HCV infection is diagnosed?
How and to whom should we offer treatment?
When should treatment be started and for how long should it be given?

So let us begin with the first question. Who are we going to test for HCV? Can I begin by suggesting to the panel that in the work-up of any patient with hepatitis A test for HCV should be carried out in the same way as a test would be carried out for hepatitis A virus (HAV) and hepatitis B virus (HBV).

**Dusheiko:** I think that any patient with raised aminotransferases should be tested for HCV.

**Chairman:** Turning now to screening of asymptomatic individuals, which groups should we be screening? Given that the major risk is through percutaneous transmission, it is obvious that we should screen blood or tissue donors. The other groups where parenteral transmission is highly relevant is intravenous drug users (IDUs). Dr Robertson, you are a GP in Muirhouse, Edinburgh, with a large number of drug users in your practice. What is your view on this issue?

**Robertson:** I have great concerns about screening in this population. The number of IDUs in my practice is substantial, and the implications for testing, follow-up and possible treatment of this large group of potentially infected individuals are enormous. I also know of a large number of former drug users, many of whom gave up their habit 5 to 10 years ago. Are we going to chase them up with a view to testing for HCV, with all the implications that such a diagnosis brings?

**Chairman:** Dr Follett, you were telling us that there are 10,000 IDUs in Glasgow, approximately 80% of whom are HCV positive. Should an attempt be made to trace the 8,000 infected drug users and offer them treatment?

**Follett:** Clearly we do not have the resources to manage such a large pool of infected individuals. We do not routinely screen IDUs for HCV infection, unless requested by their doctor.

**Chairman:** Yet we know that 50% will develop chronic infection, and 20% cirrhosis. Does anybody in the audience think that we should be trying to do something to prevent what is a substantial morbidity?

**Member of audience:** In cases of acute hepatitis, some laboratories are reluctant to test for HCV because of the low rate of seropositivity at this stage of infection.

**Follett:** It is true that in acute hepatitis C infection, only 25–35% of patients can be diagnosed by serology.

**Chairman:** Yet there is evidence that early treatment may be beneficial in preventing chronic infection. What about other tests in this period, e.g. IgM antibody?

**Follett:** An IgM test has recently become available and our results so far suggest that in some patients this may give an earlier positive result. HCV RNA may also be detectable early in the course of infection.

**Chairman:** What about screening of IDUs?

**Main:** Counselling of individual patients is required. At present our treatment for HCV is largely experimental, and consists of a drug which has to be injected subcutaneously over a long period of time. If patients feel that they do not wish to undergo this treatment, then it is questionable if there is any point in diagnosing their HCV infection. There may be other factors to consider. For example in antenatal clinics, concerns about vertical transmission might lead one to suggest screening for HCV infection.

**Piris:** In my view the only reason for early detection of disease is to offer treatment that may affect the outcome. We screen for colorectal cancer because we know that early surgery is curative. We cannot have the same faith in the ability of interferon to improve the outcome for patients with HCV, so why diagnose the infection?

**Ludlam:** Can I offer a counterview? We have an accurate test and we know that a significant number of patients with HCV infection develop progressive and potentially life threatening liver disease. Interferon is not perfect, but in about 20% of patients viral infection can be suppressed and liver damage reversed. This drug has been approved by the FDA, and licensed in a number of European countries for this indication.

**Member of audience:** There is also a public health implication. One would hope that those identified as HCV positive would take steps to prevent transmission of infection to others.

**Chairman:** Perhaps it is worthwhile considering why we are testing? Are we testing with a view to offering treatment to all those identified as infected, or are we testing with a view to preventing transmission? Should all HCV infected patients be considered for interferon treatment?

**Dusheiko:** There is a logistic problem here, in that the number of HCV infected individuals in the community would overwhelm our clinic if they were all referred at the same time. Nonetheless it is reasonable to think of HCV as a treatable disease and to refer all infected patients for therapy. This has resource implications that must be addressed.

**Member of audience:** Our laboratory uses a first generation ELISA test for HCV. Given the evidence that these tests are less accurate, what should we do?

**Chairman:** These test should not be used in isolation. Any positive result should be confirmed by a more specific test.

**Chairman:** What about screening sexual partners?

**Gillon:** In the blood transfusion centre, when we identify a seropositive donor, we offer testing to their sexual partner. Most sexual partners accept a test in these circumstances. All patients require counselling.

**Member of audience:** As a paediatrician, can I ask if the panel feels we should be screening the children of patients with HCV infection?

**Main:** When I see patients with HCV infection, I offer screening to both their sexual partner and their children if they so desire. I would emphasise that the risk to both groups is low, but they may wish the reassurance of a negative result.

**McMillan:** What do you tell a patient with HCV infection about sexual behaviour and the risk of transmitting the infection to others? Should they be advised to use barrier methods of contraception?
Main: The difficulty here is that we cannot give hard and fast advice. There is evidence that a lifetime's sexual exposure to HIV carries an overall 10% chance of transmission. The risk is therefore low but clearly exists. If a patient wants to be certain, then barrier contraception is advisable. If barrier contraception is not used, periodic testing of the partner is probably advisable.

McMillan: Do we know what represents 'safe sex' with respect to HCV infection? Is oral sex safe?

Gillon: There have been no reports of studies on that question.

Ludlam: Is anything known about co-factors for sexual transmission? Does the presence of another sexually transmitted disease increase the risk of transmission?

Chairman: I know of no studies that attempt to answer that question. There are conflicting data over the issue of whether concomitant HIV infection increases risk of transmission. What is your advice to haemophiliacs, Dr Ludlam?

Ludlam: If there is a stable sexual relationship that has lasted for many years and one partner is HCV seronegative, I find it difficult to advise barrier contraception. The difficulty is in the younger age group. When a patient is about to embark on a new sexual relationship, I find it very difficult to know what to advise. We certainly offer to test sexual partners, and sometimes find one positive.

Chairman: Before we finish with reasons for testing, can I add two other scenarios where testing may be indicated. I think that it is sensible to test for HCV a patient with HIV infection particularly if a drug user. In some patients liver failure develops before clinical immunodeficiency and it is useful to know in which patients this might arise. It may also be advisable to consider testing patients for HIV if they are already known to be HCV infected. That would particularly apply to IDUs who have obviously been at risk of both infections.

Member of audience: Should all organ and tissue donations be screened for HCV?

Gillon: Yes

Chairman: One final question about screening. HCV infection is associated with membranoproliferative glomerulonephritis, porphyria cutanea tarda and cryoglobulinaemia. Should we screen for it in patients presenting with these conditions?

Main: Yes, as there is evidence that interferon may be of therapeutic benefit in some of these patients.

Chairman: May we now look at what we do once we have diagnosed HCV infection with particular reference to the investigations that should be undertaken. Having confirmed the diagnosis with a second or third generation RIBA, it seems important that PCR for HCV RNA is then carried out. Would you agree with that, Dr Follett?

Follett: Yes, particularly if treatment is contemplated.

Chairman: What about quantitation of HCV RNA?

Follett: At present there are reservations about the accuracy of this test and there is evidence of day-to-day fluctuation. I am sure the test will become more accurate in time. Multiple estimations may more accurately reflect the true picture, but are expensive.

Dusheiko: The whole area of investigating HCV infection is underfunded. Numerically this is a bigger problem than HIV infection. We need to collect accurate data on the prevalence of infection, the costs of testing and further investigation and we need to seek central funding for this.

Chairman: Dr Hayes, you are a physician and a specialist in gastrointestinal and liver diseases with a clinic full of HCV infected patients. How do you investigate them?

Hayes: A liver biopsy on all these patients can be justified.

Main: While there is a clear case for liver biopsy in patients with persistently raised aminotranferases the contentious issue is whether or not this applies to those with a normal ALT.

Hayes: There is clear evidence that patients with normal ALT can have significant liver disease. I think that any patient who is HCV RNA positive should undergo liver biopsy, especially if the duration of infection is unknown. The use of raised ALT to select patients for further investigation arose from clinical trials of interferon, where a fall in ALT was used as a marker of clinical response. But we know that many patients with normal ALT have chronic active hepatitis and/or cirrhosis.

Dusheiko: It is not my policy to biopsy asymptomatic patients with normal ALT and negative HCV RNA. I follow up such patients, measuring transaminase levels on several occasions, before considering biopsy.

Member of audience: What is the mortality associated with liver biopsy?

Hayes: It depends on who is carrying out the procedure. With expert hands the risk of bleeding after laparoscopic liver biopsy is 1 in 1,000 and of death, 1 in 10,000 patients.

Piris: Every liver biopsy from a patient with HCV infection that I have seen has shown some liver pathology. Obviously this is a selected group but nonetheless this is an infection associated with significant liver disease and in my view the possible benefits outweigh the risks.

Member of audience: Is knowing the pathology in the liver only of benefit if this alters the management?

Hayes: Yes. In most instances treatment is offered on the basis of the findings at liver biopsy.

Dusheiko: In patients with minor inflammatory change, a few lymphoid follicles and little else, the prognosis over the next decade is good. In such patients I do not recommend treatment with interferon. However, if there is bridging necrosis, and particularly early signs of fibrosis, then the prognosis is poor and then I strongly advocate intervention. There has to be a degree of selectivity, as this is an expensive drug and its efficacy rate is only 20%.

Robertson: May I ask a question about transaminases. How often would you repeat measurements during the follow up of a patient with HCV infection? Some patients appear to have significant swings in transaminase levels.

Dusheiko: If the first ALT level in normal, I repeat this at least twice before deciding whether or not to do a biopsy. If all three were normal, then I would repeat the test every three to four months. This is a manageable task for general practitioners. If the hepatitis C RNA test is positive a biopsy is justified.

Member of audience: The consensus appears to be that patients with cirrhosis should not be offered treatment. Is there a non-invasive method that would pick up the cirrhotic state, thus obviating the necessity for a biopsy?

Dusheiko: Unfortunately non-invasive tests lack sufficient sensitivity to replace liver biopsy. Cirrhosis can only be diagnosed reliably on liver biopsy.

Chairman: Do you undertake repeat liver biopsy as part of follow-up and, if so, how often?

Piris: It is possible that a biopsy sample might not be representative of the
pathology. However in HCV infected liver removed at transplantation, the changes are fairly uniform throughout the organ. If a biopsy shows minimal inflammatory change, there is no immediate need to repeat it.

Chairman: If the biopsy is normal, do you repeat it and if so, when?

Piris: If there is deterioration in liver function, as reflected by persistently abnormal aminotransferases, and possibly by evidence of increased viral load, then another biopsy is indicated. If the aminotransferases remain normal, then a further biopsy is not required.

Chairman: May I ask about typing? Should we be trying to identify the patients infected with genotypes that might carry a poorer prognosis?

Follett: At present viral typing is a research tool and not widely available. But it appears to be valuable in prognosis and I am sure it will be useful when more widely available.

Dusheiko: We are attempting to type the infections of all our patients. One confounding variable is that patients with type 3 infection tend to be younger than those with type 1. This age difference makes it difficult to assess the prognostic significance of viral type, because the severity of histological change is influenced by duration of infection as well as by viral type.

Chairman: Some patients may be infected with several virus types, for example haemophiliacs and drug addicts. Do such patients have a poorer prognosis than those infected with a single type?

Dusheiko: It is not possible to answer that question at present.

Ong: Do you give interferon to patients with combined HCV and HIV infection?

Dusheiko: Yes, although few such patients have been studied.

Ong: Can you give us an indication of the type of patient with co-infection to whom you would offer treatment?

Dusheiko: I treat patients with HIV infection and chronic active hepatitis secondary to HCV infection. I think it is important to consider HCV in this setting as worth treating, especially when there are no symptoms attributable to HIV infection.

Chairman: In such patients do you think that zidovudine is of additional benefit?

Dusheiko: There have been small trials. Some patients whom we have treated with interferon have also been on antiretroviral treatment. There are theoretical advantages in combining anti-HCV and anti-HIV treatments but no studies yet to give definitive answers.

Hay: May I ask about the goals in treatment of HCV infection? Obviously there is a goal of improving liver histology, but there is also the goal of reducing or eliminating the viral load. Those two aims may not always go hand in hand. Are we concentrating too much on the liver and not enough on the initial problem, the virus?

Dusheiko: I think that by and large the two go hand in hand. There is evidence that if the viral load is reduced, then the liver disease is improved.

Robertson: I have been aware recently of seroconversions with respect to HCV. Should I be offering treatment to these patients?

Dusheiko: In the light of recent trials, I think you have to consider seriously interferon in some of these patients. The evidence suggests that treating patients at this stage leads to clearance of virus in two-thirds of cases. We do not know what the relapse rate is but it seems reasonable to infer that in some cases chronic infection can be prevented.

Member of audience: Surely there is a problem of diagnosing these acute infections, in that it may take several months for serological tests to become positive.

Dusheiko: That is correct; HCV RNA can be measured and a diagnosis achieved in that way. If a patient is HCV RNA positive, then treatment with interferon should be considered.

Chairman: Would you do a liver biopsy first?

Dusheiko: I do not think that biopsy is mandatory in that setting.

Member of audience: PCR testing for HCV RNA is not widely available. What should one do if one does not have access to this test?

Dusheiko: We have to press for national availability of this test.

Ludlam: Are all patients with cirrhosis regarded as unsuitable for interferon treatment? Would you consider treating patients with well compensated, symptomless cirrhosis? Such patients are in the most urgent need of treatment as they are at greatest risk of liver failure in the short-term.

Dusheiko: You are right. There are patients with ‘early’ cirrhosis who would benefit from therapy. The difficulty is to select the individual patient who will respond to therapy. Perhaps genotyping would help.

Main: One of the difficulties has been that clinical trials tend to include only patients with raised aminotransferases. This tends to exclude patients with cirrhosis who usually have normal ALT levels. We ought to be flexible in prescribing interferon. One should not embark on a six month course of treatment and not review it until the six months are up. For example a cirrhotic might benefit from a prolonged course of low dose interferon. On the other hand patients with raised ALT who fail to show response after 4 weeks of therapy, possibly at an increased dose, should probably cease treatment as there is little likelihood of a subsequent response. One also has to remember that interferon is not without side affects.

Piris: Cirrhotics are not a homogenous group histologically. Some patients with established cirrhosis have no inflammatory reaction; others show extensive inflammatory reaction with piecemeal necrosis implying ongoing disease that might well benefit from interferon therapy.

Member of audience: Patients undergoing renal dialysis have a high rate of HCV infection. Should interferon therapy be offered to these patients either before or after transplantation?

Dusheiko: There is evidence to suggest that patients on dialysis who are HCV infected tolerate that infection well. The reasons for this are not clear. As regards therapy either before or after transplantation, there are no data to guide us. In transplanted patients, there is always a concern about interferon and possible graft rejection. The simple answer is that we do not know.

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