

HEPATITIS C - THE VIRUS, CLINICAL FEATURES AND TREATMENT

The General Field

There are five main hepatitis viruses (HAV, HBV, HCV, HDV, and HEV) which replicate predominantly in the liver. Additional viruses, such as the cytomegalo and Epstein Barr viruses replicate to a minor degree in the liver. HAV and HEV are enterically transmitted – by ingestion of infected food or water - and cause an acute infection followed by recovery. HBV and HCV are parenterally transmitted – by introduction of infected material through the skin or mucosal surfaces - and may cause acute or chronic infection. It is the chronic infections that put patients at risk of hepatocellular carcinoma.

Hepatitis C virus (HCV).

Discovery of HCV by Chiron (an American Biotech Company)

Mike Houghton and colleagues, working at Chiron, reported the cloning of HCV in 1989, by antibody probing of an expression library made from a reverse transcribed RNA extract of the serum of an infected chimpanzee initially inoculated with a serum sample from a patient with post-transfusion hepatitis (PTH). The antibody source was a serum specimen from a subject who had recovered from post-transfusion NANB hepatitis and assumed to have produced antibodies to NANB agent.

The Virus and its replication

HCV is a flavivirus sharing some properties with other members of this family, including dengue, yellow fever, and West Nile viruses. It is a single positive stranded RNA virus which exhibits considerable genetic heterogeneity. This diversity exists at several levels. The greatest differences are between the 6 major genotypes (1-6) with additional differences between the strains found within a genotype. Even within an individual infected with a single inoculum the genetic sequence of each virus particle is different and changes over time as the virus comes under various selection pressures including the host's immune response and more recently exposure to potentially

therapeutic drugs such as the protease and polymerase inhibitors. HCV exhibits greater genetic diversity than most other viruses and this is a major contributor to the high rate of chronicity (60-80% of those infected), the difficulty in producing a vaccine, and the rapidity of emergence of virus strains that are resistant to the new protease, polymerase and NS5a inhibitors.

Figure 1: structure of the HCV particle.

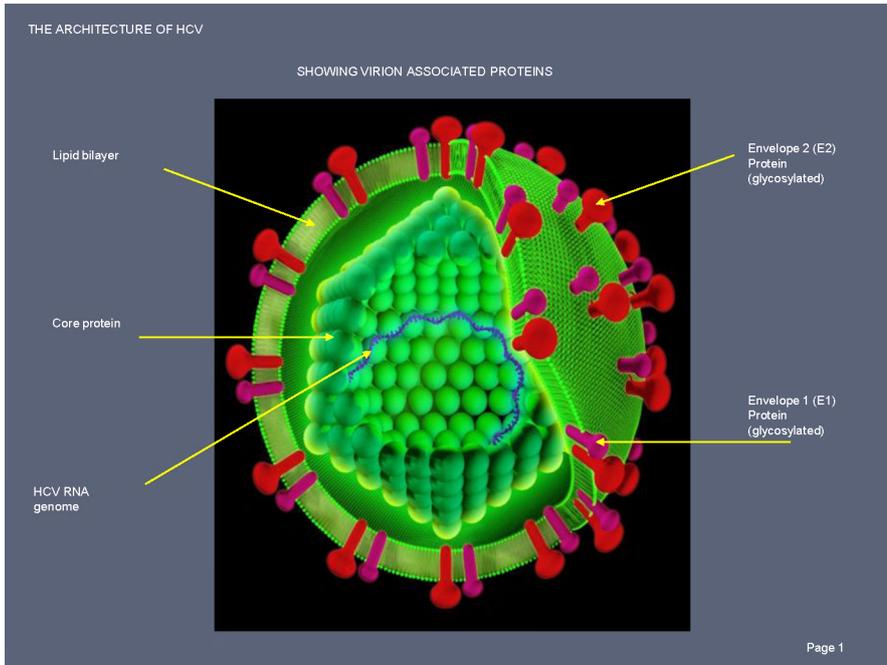


Figure 2: prevalence of HCV infection

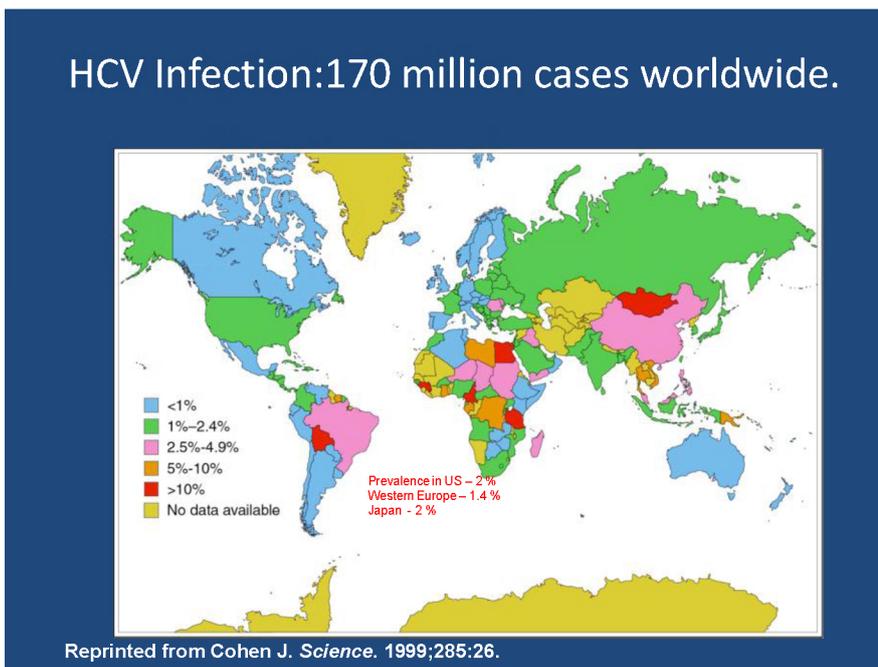
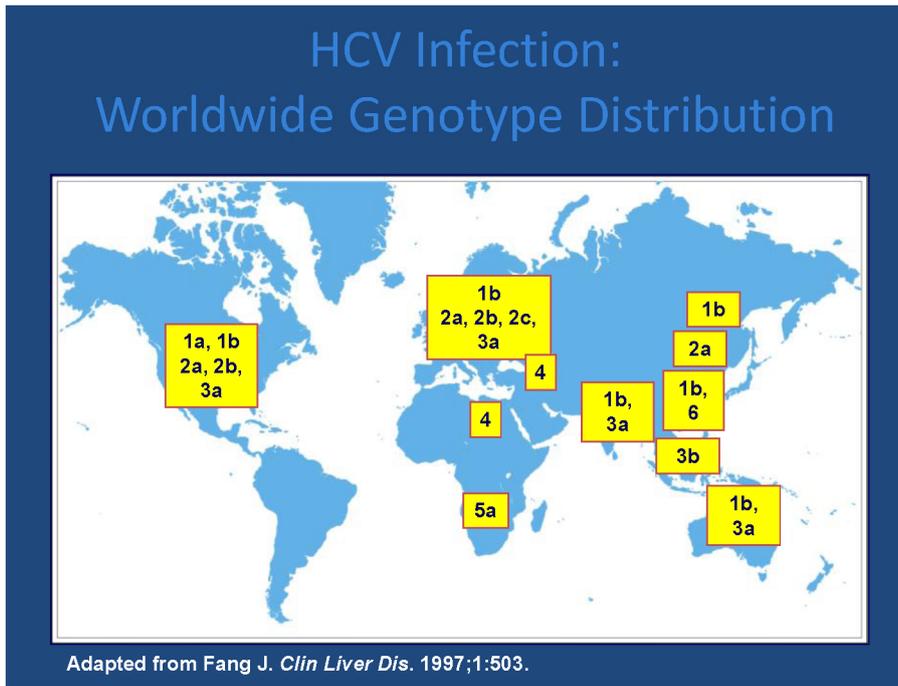


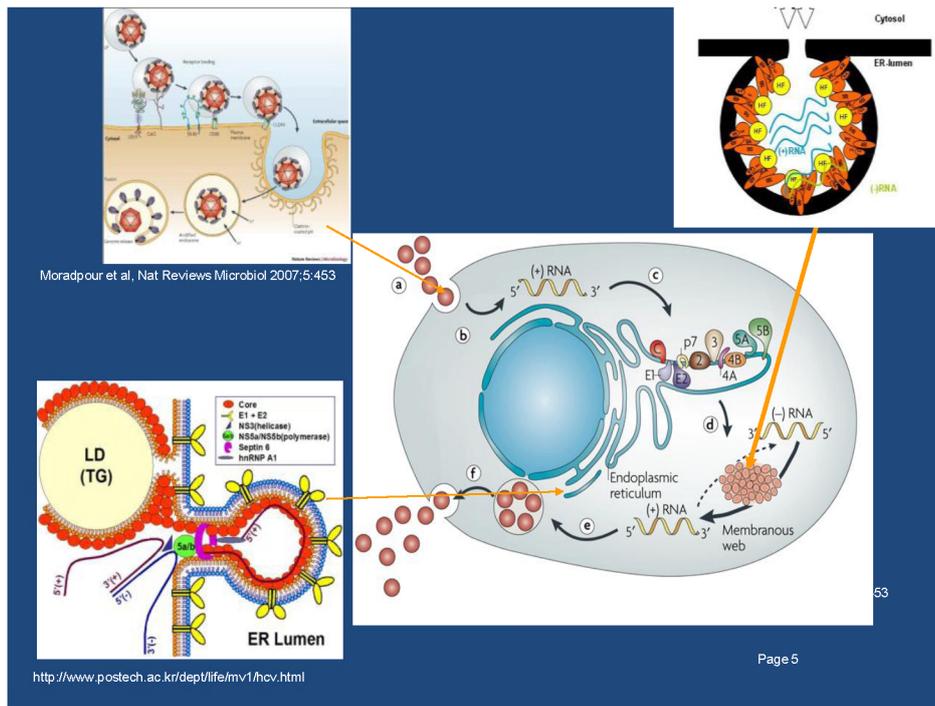
Figure 3: geographic distribution of HCV genotypes:



The virus bears two highly variable envelope proteins in its lipid coat. These hypervariable regions continually change under the selection pressure of the antibody response ie as virus neutralising antibodies are made by the host's immune response, new variants are selected which the antibodies initially do not neutralise. As the variant then becomes dominant a further antibody population is produced and the strain is again neutralised. Thus there is a 'race' between the capacity of the virus to undergo genetic and antigenic variation and the capacity of the host immune response to generate the appropriate immune response. This is why there has been difficulty in producing an effective prophylactic vaccine.

HCV Replication (see figure 4)

HCV gains entry to the liver by binding to lipid receptors on the liver cell surface. The virus is then taken up into the liver cell where it sheds its envelope and nucleocapsid proteins so that the RNA of the virus can be translated into the polyprotein from which the proteins of the virus are derived. New virus particles are then assembled and released from the liver to circulate in the blood stream.

Figure 4: HCV replication

The Liver and other Diseases caused or associated with HCV Infection.

The virus does not directly damage or kill liver cells (hepatitis). It is the host cellular immune response that recognises the infected cells triggering a response which inhibits the replication of the virus or destroys the infected cells.

The RNA of the virus is contained in the core (nucleocapsid) of the virus again coated in protective envelope protein.

HCV RNA can usually be detected in the blood 1-2 weeks after infection with an antibody response occurring at about 2 months (see figure 5).

Only 30-40% of people spontaneously clear HCV and the vast majority do so in the first 6 months of the infection. Thereafter very few (c1% pa) clear the virus spontaneously.

It is probable that the level of viraemia does influence the severity of the liver disease in that patients with HCV/HIV co-infection and patients on immune-suppressants after liver transplantation, have the highest levels of viraemia, and also the most rapidly progressive liver fibrosis. Outside of these settings, the level of viraemia is not positively correlated with severity

of liver injury or fibrosis and in any one patient may diminish as the disease progresses, possible related to progressive reduction of the liver volume. Severity of disease is not, as far as I know, related to the genotype or number of genotypes infecting the patient.

The virus may also cause non-Hodgkins B cell lymphoma, particularly in southern Europe.

Genotype 3 infection is associated with accumulation of fat in the liver (steatosis) and type 2 diabetes mellitus.

Cognitive function (brain fog) and mood disorders are probably causatively related to HCV infection, supported by the observation that the virus can infect the brain.

Mechanisms of HCV persistence

HCV persists in 60-70% of cases: these patients remain HCV RNA positive.

During an acute hepatitis in which the virus is eliminated, there is a cellular and humoral (antibody) immune response to epitopes derived from various virus encoded proteins. In general the stronger the CD4 and CD8 cellular response the more likely recovery is to occur. There is an antibody response to the envelope proteins of HCV but rapid antigenic shift occurs in the dominant quasi species presumably due to selection against variants recognised by the prevalent virus neutralising antibody. Thus, although the majority of patients will have antibody that binds to the variable and hypervariable regions of the E1 and E2 envelope proteins, the hypervariable region, as the name implies, undergoes rapid mutation and variants emerge.

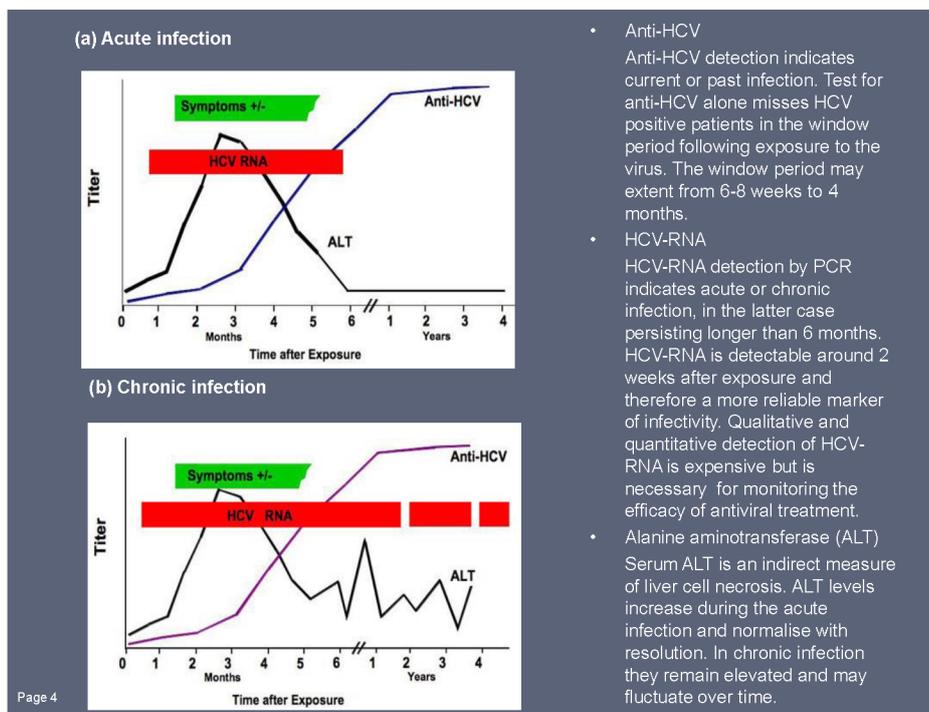
In patients developing persistent infection, the CD4 helper T cell and CD8 cytotoxic T cell responses are at a lower level although they are still detectable. The anti-envelope response is strong in this group of patients and again undergoes rapid change in specificity as new strains of the virus emerge and evoke a new anti-envelope response.

Several studies have now indicated that the immune response may contribute to the outcome of interferon therapy. 50% of patients show no response to interferon and this presumably relates to

interferon resistance. This may relate to inhibition of hepatocyte response to cytokines by HCV proteins, particularly the HCV protease NS3.

The diminished CD4 and CD8 lymphocyte responses may result from infection of monocyte/macrophage derived cells including professional antigen presenting cells (dendritic cells).

Figure 5: the serological profile of acute and chronic HCV infection. Chronic viral hepatitis is defined as infection persisting more than 6 months. Of the hepatotropic viruses, hepatitis B and C (HBV and HCV) cause chronic infection, resulting in chronic liver disease and, in 2-3% per year, hepatocellular carcinoma.



Transmission of HCV

HCV is present in body fluids and was spread via the administration of unscreened blood or blood products from infected individuals prior to 1991. Hepatitis C can also be spread vertically (mother to baby) and sexually but the rate of transmission is very low, less than 5% in each case.

Currently HCV infection usually occurs after use of shared (blood contaminated) needles during intravenous drug use.

In the past, widespread infection with hepatitis C in Egypt was associated with parenteral treatment of Schistosomiasis.

In general, the higher the concentration of virus in the blood – measured by quantitative assay of the plasma HCV RNA - the greater the risk of transmission particularly via the sexual and neonatal routes.

Issues around transmission by blood and blood products

In 1970-90, there was debate as to whether blood products derived from volunteer blood donations prior to screening tests being introduced in 1991, were safer in terms of transmission of HIV and HCV, than those derived from paid donors particularly those imported from the USA and Central and South America. In the case of HCV where the prevalence of infection in the UK blood donating general community was around 0.5% and several thousand donations were used to make each batch of factor 8 and 9 concentrate, the majority of batches made from volunteer blood donations were infected and the frequency of transmission was similar following use of both English NHS (Kernoff Lu Karayiannis and Thomas 1984, Brit J of Haematology), Scottish NHS (Ludlam Chapman, Cohen and Litton 1989, Lancet) and commercial material. Prior to 1983 (discovery of HIV), the prevalence of HIV in the donating volunteer community was very much lower and many volunteer batches prior to screening, were not therefore infected with this virus while the commercial/ paid donor derived material was more frequently infected because of the higher HIV prevalence in this group which often included IV drug users who were frequently HIV infected. Thus the frequency of serological evidence of HIV infection in treated patients with haemophilia A or B was influenced by the severity of the haemophilia determining the

frequency of administration of factor 8 or 9 concentrates (Ludlam et al 1989). In summary the risk of blood born virus (BBV) contamination of coagulation concentrates was related to:

- number of donors used for each batch;
- prevalence of each BBV in the donor population;
- severity of haemophilia determining frequency of coagulation factor therapy and therefore number of batches to which each patient was exposed .

The risk was reduced by using repeated donations from a single donor thereby reducing the number of donors needed for each batch, by screening out the population at high risk of having BBV by pre-donation questionnaires asking about IV drug use, receipt of blood transfusions prior to 1991, and frequency and type of sexual partners and limiting the number of batches to which each patient was exposed.

The paper by Minor et al (July 1990 Lancet) in which pools of plasma were tested for anti-HCV, did establish that the commercial concentrates had higher titres of anti-HCV, presumably because more donors used in these batches were infected, but NHS batches were only weakly anti-HCV positive, consistent with inclusion of a smaller number of HCV infected individuals donations in each batch, reflecting a lower prevalence of HCV infection in the UK donor population (2 out of 538, 0.4%) than in the USA commercial donors (probably around 60% HCV infection in donors who were using IV drugs).

Before the availability of serological tests for NANB (now HCV) in 1989/1991 the transmission of NANB hepatitis agent was established by screening for ALT abnormalities after receipt of concentrates. Virtually 100% of patients serially screened after Scottish NHS, English NHS or American and Austrian commercial concentrates developed abnormal ALT levels indicating infection with the NANB agent. Until 1983 no serological or surrogate tests were available to detect transmission of HIV. Thus when heat inactivation procedures were to be evaluated the surrogate for NANB infection, namely ALT screening, could be used before and after transfusion

of concentrates whereas there was no way to establish whether HIV transmission had occurred. This led to the suggestion at the Haemophilia directors meeting (1982-3) that in future studies testing to see whether heat inactivation was reducing the transmission of NANB, NHS concentrates (heat inactivated) should be used because they were demonstrably less likely to transmit HIV while invariably transmitting NANB (HCV).

The changing Perception of Severity of NANB hepatitis

Liver biopsies were done to try to establish the prognosis of patients with NANB hepatitis. This was believed necessary because of the fluctuating levels of the serum transaminases in NANB hepatitis, a measure of liver cell damage and the knowledge that in chronic hepatitis B histological features such as the presence of piecemeal necrosis and bridging fibrosis were predictive of prognosis. Initially (1970 -1985) the liver biopsies taken from NANB haemophilia cases (Manchester, Oxford and London Centres) showed mainly lobular hepatitis and chronic persistent hepatitis – usually indicative of a good prognosis – and only after several years of infection did they show chronic active hepatitis (with peri-portal piecemeal necrosis and bridging fibrosis) – indicative of a poor prognosis with a risk of development of significant fibrosis. Thus in the early days the liver disease was thought to be relatively mild compared to that seen with HBV for instance. This view started to change on the basis of the accumulating data from liver biopsies. Well illustrative of this changing view was the fact that Sheila Sherlock stated in the 6th edition of her textbook in 1981 that NANB hepatitis had a good prognosis but on the basis of a study by Bamber, Sherlock, Scheuer and Thomas ((London), “Clinical and histological features of a group of patients with sporadic non-A, non-B hepatitis”, J Clin Pathol, 1981;34:1175-1180) showing that biopsies from patients with chronic NANB hepatitis ‘ covered the whole spectrum of acute and chronic hepatitis and 1 patient had cirrhosis’, views changed. Studies from the USA (Koretz et al (Los Angeles), “Non-A, non-B post-transfusion hepatitis: disaster after decades?”),

(Abstract), *Hepatology*, 1982;2(5):687) also reflected this changing view and pointed out that NANB was a silent and slowly progressive disease which ultimately did result in cirrhosis in a proportion of cases. As follow-up has continued, it has become apparent that it is indeed the case that many NANB cases will develop cirrhosis over 30-50 years. By 1989 Sherlock in the 8th edition of her textbook on liver disease stated that 68% of patients with NANB hepatitis developed chronic disease and 20% developed cirrhosis. Recent studies from Asian patients, who have often been infected in early life, suggest that in middle and older age the majority may develop cirrhosis because of the longer duration of infection ie > 30 years.

Clinical Features of Liver Diseases caused by HCV

Clinical features common in acute hepatitis include influenzal type symptoms with malaise, myalgia, arthralgia, anorexia and nausea. There may be a mild pyrexia and an ache in the upper abdomen. This is followed by the biochemical features of hepatocyte necrosis as the immune system attempts to clear the virus infected liver cells. Transaminases (ALT and AST) increase in the blood as they are released from dying infected liver cells, typically reaching levels of 300-500 iu/l. An increase in the serum bilirubin may occur. As a result the urine becomes dark, the stools pale and the patient may become jaundiced and develop itching. Jaundice is rare in HCV infection. The jaundice usually lasts 1-4 weeks and heralds improvement in most cases. The feeling of malaise and of being generally unwell may last many months. As a general rule, if the acute infection is only mild or asymptomatic, then there is an increased risk of viral persistence. Rarely patients with HCV infection develop fulminant hepatitis (liver failure).

Chronic infection may present in a number of ways. First (and unusually) it may be documented during the follow up of an acute hepatitis episode and be diagnosed by definition when the patient has failed to clear virus from the blood after 6 months. More usually an asymptomatic course is run. The acute infection may pass unnoticed with its timing often unknown or inferred

from recall of at risk activity such as intravenous drug use, blood transfusion etc. In the asymptomatic patient the infection may be picked up incidentally during health screening or following detection of abnormal liver biochemistry in a blood test taken for an unrelated reason. The infection may not come to medical attention until late stage disease is reached and the patient presents with signs of chronic liver disease or a complication of cirrhosis such as variceal haemorrhage, ascites or the development of hepatocellular carcinoma (HCC). An unknown number of individuals may be infected, asymptomatic and destined never to develop complications. There is growing recognition that in some cases, HCV causes chronic ill health with decreased quality of life, depression and general malaise regardless of the degree of liver damage. This effect is independent of mode of acquisition. Early work suggests that the brains of HCV infected individuals may also be infected.

The most reliable way of monitoring the severity of the liver disease is to obtain histology by liver biopsy. Degree of necro-inflammation (grade) and fibrosis (stage) are the key to interpretation and consequent clinical decision making regarding treatment.

Treatment of HCV infection

Acute infection

If interferons are given within 6 months after infection, the vast majority (>90%) can be cured.

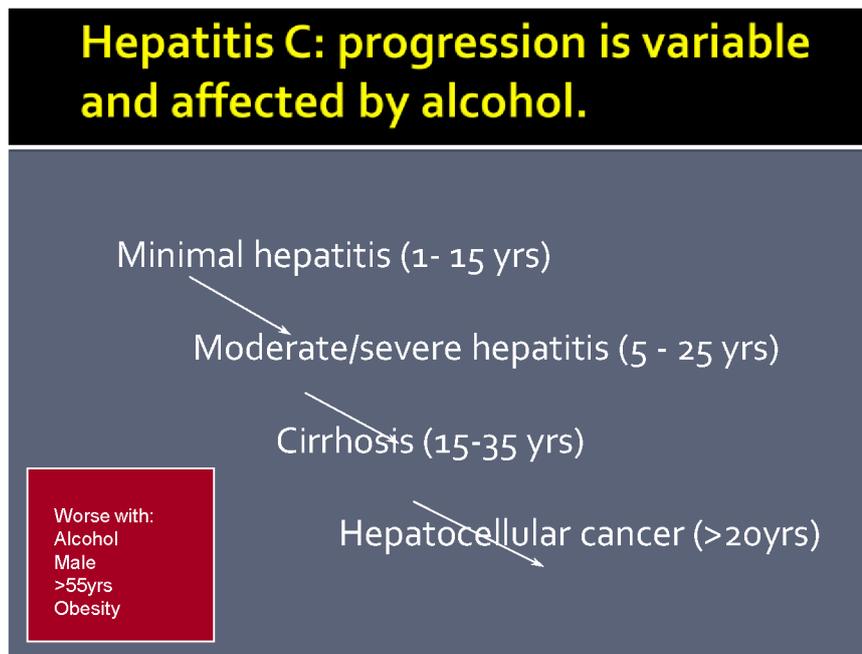
Chronic infection

The main aim is to prevent the development of cirrhosis and death from liver failure and liver cancer (hepatocellular carcinoma (HCC)). It is worth noting that it is only those that have progressed to cirrhosis who are at risk of HCC. Even those who have cleared the virus either spontaneously or on treatment but already have cirrhosis, are at risk of developing HCC. The incidence of HCC in those with established cirrhosis is around 2-3% per year.

Clearance of the viruses from infected individuals reduces transmission rates.

In chronic hepatitis C the presence and quantity of virus in the blood stream is determined by reverse transcriptase polymerase chain reaction (rt-PCR). Levels fluctuate from month to month and tend to be higher in immunosuppressed people such as those infected with HIV. The level of viraemia in HCV does not appear to determine the rate of progression to liver cirrhosis in the non-immunosuppressed. Levels of transaminases also fluctuate over time. The presence of normal transaminases does not exclude significant liver fibrosis. The rate of progression to cirrhosis varies among individuals chronically infected with HCV. Different sub-groups of patients progress at different rates. Major risk factors have been identified: male gender, excess alcohol and age >50 at acquisition.

Figure 5: factors influencing rate of progression of liver fibrosis



1/4 are predicted to progress from infection to cirrhosis in <20 years, 1/3 won't progress to cirrhosis for at least 50 years with an intermediate group in between.

The decision regarding which patients to treat depends predominantly on histology. There is general agreement that patients with continuing necro-inflammatory activity and fibrosis and people with compensated cirrhosis should be treated.

Current gold standard treatment involves pegylated interferon and ribavirin given for 12 months to patients with genotypes 1 and 4 and for 6 months to those with genotypes 2 and 3. Outcome from therapy with current agents is also affected by viral genotype and at the time of writing those with genotype-1 and 4 are cured in 40% and those with genotypes 2 and 3 are cured in 80% of cases.

Studies of the kinetics of HCV infection have indicated that approximately 10^{12} virus particles are produced by the liver per day. These virus particles have a relatively short half life in the circulation. In contrast, infected liver cells have a relatively long half life. The initial rate of disappearance of HCV RNA during interferon therapy is rapid and is followed by a second clearance curve which may reflect the presence of an interferon resistant second site of HCV replication either within the liver or an extra-hepatic site.

If the HCV RNA is undetectable by the 4th week of therapy, treatment duration can be shortened to 6 months in those with G1 and G4 and to 3 months in G2 and G3. If the HCV RNA is still positive at the 12th week of therapy, treatment is usually stopped because the chance of a sustained viral response is insignificant.

Several protease inhibitors (telaprovir and boceprevir) are now licenced and will increase the response rate in G1 patients to around 80%. In addition variations in the IL28 gene appear to influence the chance of response and this genetic predictive test is now finding its place in the selection of patients for treatment.

Future therapies for HCV

The protease, polymerase and NS5a enzymes of the virus are future targets. Combination therapy will be the most likely to succeed and although the above drugs are currently used with pegylated interferon and ribavirin, in future they may be tried without these drugs. When used without

interferon the appearance of drug resistance is of concern. In order to minimise this all three classes of new drugs are being used in combination and preliminary results are encouraging.

Bassendine Review of Natural History of HCV infection

The recent review of the natural history of HCV infection by Bassendine et al which summarised the basis for the Skipton Fund - ex gratia payments for those infected by NHS products - is an accurate summary of the views prevalent over the relevant period. The recent adjustments made to provide annual, non-taxed, indexed linked non-discretionary payments to those receiving stage 2 Skipton payments or their widowed spouses, similar to those paid to patients with HIV infection transmitted by NHS products, and discretionary charitable payments determined by the Caxton Fund, address my concerns over the adequacy of the original Skipton payments, for the future.