REVIEW ARTICLE

Hepatitis C : An Update

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Summary

The identification of the Hepatitis C virus using molecular cloning techniques, besides making the term Non-A Non-B Hepatitis obsolete, enables the development of specific assays for the detection of antibodies in HCV-infected individuals, thus making it possible to obtain sero-epidemiological data of the disease. The carriage of Hepatitis C antibody varies worldwide. The disease is most prevalent in intravenous drug abusers or haemophiliacs. Parenteral transmission is the most important route of transmission. Sexual, intra-familial and perinatal transmissions are uncommon. About 40% could be community-acquired (sporadic). Diagnostic tests include enzyme-linked immunosorbant (ELISA) anti-HCV assay, recombinant immunoblot assay, HCV-RNA by polymerase chain reaction and HCV-Ag. More than 50% of acute cases becomes chronic and runs a benign and indolent course. About 20% progress to cirrhosis and some of these develop hepatocellular carcinoma. Several published trials have consistently shown that treatment with interferon in some patients is useful. There is however a relapse rate of 50%. Further trials with interferon and other anti-viral agents like ribavirin are awaited for more effective treatment.

Key words: Hepatitis C, epidemiology, diagnosis, treatment.

Introduction

The term Non-A Non-B (NANB) hepatitis was used in 1975 to explain the majority of cases of post-transfusion hepatitis (PTH) which were negative to tests for Hepatitis A, B, D and other hepatotropic viruses particularly the Cytomegalovirus (CMV), the Ebstein-Barr virus (EBV) and the Herpes Simplex virus (HSV).[1-3] The rather imprecise diagnosis of NANB hepatitis using such exclusion criteria was necessary up to the late eighties as attempts at isolating the virus proved frustrating. It was not until 1989 that a team from the Chiron Corporation, using molecular cloning techniques, reported cloning of the major causative agent of the parenteral form of NANB hepatitis, conveniently called Hepatitis C virus (HCV).[4] The virus causing the enteric (or epidemic) form of NANB, hepatitis E, has also been identified and cloned.[5] The discovery of both these viruses accounts for the majority of NANB hepatitis, leaving only a small minority that have yet to be identified (Fig. 1).

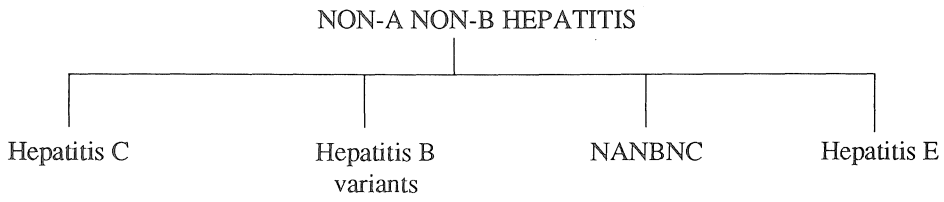


Fig. 1 : Non-A Non-B Hepatitis

Following the discovery of the virus, a specific assay for the measurement of antibody (anti-HCV) was developed[6] and it was found that hepatitis C accounts for 90–95% of PTH and 50% of sporadic or community-acquired hepatitis.[7,8] Recently, HCV-RNA in serum and tissue using the polymerase chain reaction (PCR) was found in almost 50% of patients with presumed chronic NANB with good correlation with the antibody test.[9]

Epidemiology

Blood donors

The disease is found worldwide and the carriage rate of Hepatitis C antibody in blood donors varies from 0.3 to 1.4% with a higher prevalence in New York and Southern Europe compared to Northern Europe and the United Kingdom. The frequency of the antibody in Japanese blood donors is 1.1%.[8,10] Preliminary epidemiological assessment suggests that there are about 100 million NANB carriers worldwide and an estimated 175,000 new cases annually in USA and Europe and double that figure in Japan alone.[11] Blood transfusion however accounts for only 5-10% of Hepatitis C cases.

High risk population

The highest prevalence worldwide is in intravenous drug abusers or haemophiliacs. The prevalence of anti-HCV in drug addicts is high, ranging from 70–92% in studies conducted in USA and Europe.[12,13] 78% of acute NANB hepatitis in drug addicts is seropositive to anti-HCV.[14] A follow-up study of IV drug abusers with acute hepatitis C suggested that they developed less severe chronic lesions compared to non-drug users.[15] The prevalence of anti-HCV worldwide in haemophiliacs is 50–90%,[16-21] the higher prevalence being found in those receiving multiple transfusions.[22]. Some haemophiliacs have HCV-RNA even without antibody response.

Other high risk groups in this category include multiply-transfused thalassaemics, patients on haemodialysis, renal transplant recipients, bone marrow and liver transplant patients.

Transmission of Hepatitis C

Parenteral

The major route of transmission is by parenteral and inapparent parenteral contact. A high prevalence of anti-HCV antibody is found in many risk groups exposed to blood or blood products. The estimated frequency of hepatitis in transfusion recipients is 3–4%, out of which more than 90% were due to NANB hepatitis.[23] IV drug abusers and haemophiliacs form the largest group being infected by the parenteral route.

Sexual and Household Contacts

Evidence for sexual transmission of HCV is lower and less efficient compared to hepatitis B.[24] It may however play a more significant role in patients with a higher viral load eg. during the early stage of infection and during co-infection with the human immunodeficiency virus (HIV). Similarly, the risk of intra-familial transmission to household contacts is very low.[25,26]

Perinatal

This is still controversial. Anti-HCV found in infants is usually due to passive transfer of maternal antibodies which disappear between three to twelve months after birth. Persistent HCV-RNA, however have been found in some children after the disappearance of the antibodies.[27,28] Vertical transmission is likely to be an important route of transmission if the mother has high titres of viramia, eg. if acute hepatitis C develops in the third trimester, or if there is co-infection with HIV. In an Italian

study,[28] almost 50% of newborns born to anti-HIV and anti-HCV positive mothers had anti-HCV sero-conversion between six – twelve months of age. Acute NANB infection was also present in about 50% of them.

Sporadic or Community-acquired

Twenty-five to fifty per cent of patients from most epidemiological studies of acute NANB hepatitis do not recall any percutaneous exposure. Alter[29] found up to 42% of patients with Hepatitis C without any obvious risk factor and considered them sporadic. Inapparent or covert percutaneous exposure in infected blood, sexual and/or vertical transmission could explain some of the sporadic cases. Further studies using HCV-RNA tests would be helpful.

The Virus

HCV has been cloned and has been visualised under electron microscopy.[30] It is a linear, single-stranded, enveloped virus which possesses an RNA genome of about 10,000 nucleotides.[4] It is 50–60nm in size and resembles either the pesti- or flavi-virus which is a member of the Group B arborviruses. Other members of this group include the yellow fever virus and the dengue virus. It has no genomic homology to retrovirus, HBV, HDV or other known viral agents.

Serology

Serological features of HCV differ from that of HBV because of its peculiar biological features. Being an RNA virus, it lacks the property of surviving in infected cells in the integrated DNA form although for reasons not yet well understood, it is still capable of inducing chronicity. The viral load in the blood is also very low in HCV infection contrary to that of HBV infection.

The cloning and sequencing of the HCV genome makes it possible for the development of a specific anti-HCV tests using radioimmunological and enzymatic assays based on recombinant proteins from various regions of the HCV genome (Fig. 2).

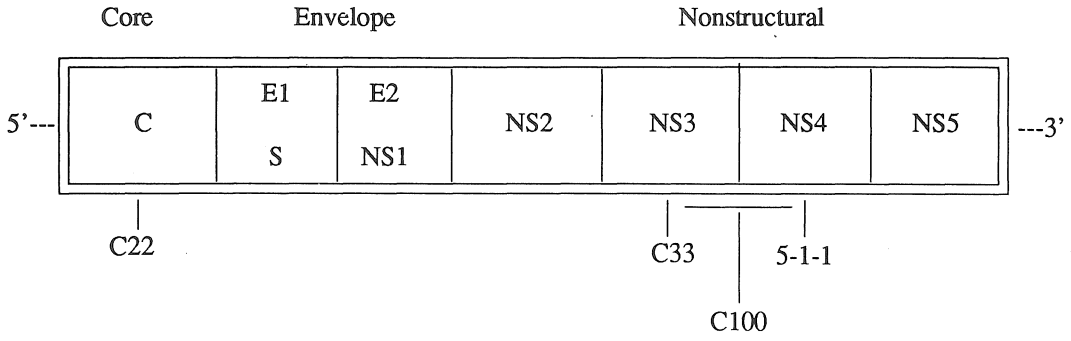


Fig. 2 : HCV genome and antigens

Antibodies to HCV appear in blood about three months after onset of acute illness but may appear a year later. Serum alanine aminotransferases (ALT) and HCV-RNA appear much earlier (Fig. 3). Anti-HCV persists in patients developing chronic hepatitis C but often is transient in those with acute resolving Hepatitis C.[31]

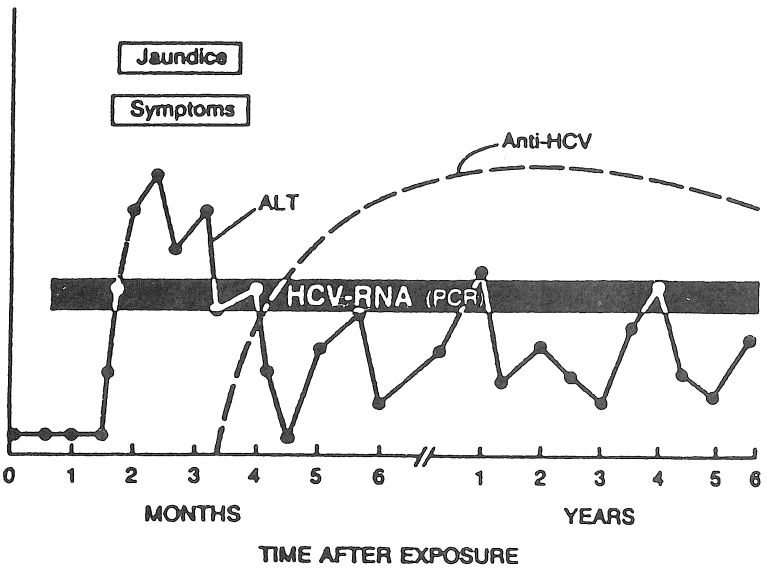


Fig. 3: Typical course of acute hepatitis progressing to chronic disease [after Hoofnagle and Di Bisceglie]

Enzyme-linked immunosorbant assay (ELISA) test for anti-HCV

The first generation test made use of a recombinant yeast polypeptide referred to as C-100-3 which is derived from the non-structural region NS4 of the HCV genome. Detection of anti-HCV by this method is not satisfactory nor sensitive and is detected rather late, usually between 4 to 24 weeks.[32]. The second generation test detects antibodies against multiple HCV proteins (C-33c, C-100-3 and C-22c) and therefore detect additional cases of HCV infection and yield earlier positive reactions in acute hepatitis.[33,34] It gives a diagnostic sensitivity of 95%,[35] the increased sensitivity being due to the presence of the HCV core protein (C-22p). It does not however distinguish between IgM and IgG antibodies and this together with the fairly long window period for seropositivity makes them unreliable for making the diagnosis of acute hepatitis C. It has been found that the second generation assay may not be suitable for monitoring the course of patients treated for chronic HCV infection because anti-C-22c persists for a long time (one – four years) whereas the anti-C100-3 and /or anti-C-33c decreases or disappears within two years in long-term responders, as indicated by persistent normalisation of aminotransferases.[36-37]

False-positives especially in autoimmune chronic active hepatitis and alcoholic liver disease have been reported. Two factors associated with false positives are: (i) storage of serum samples in hot weather and repeated freezing and thawing of the samples (Tibbs et al, personal communication) and (ii) the high globulin levels associated with these diseases[38]. False negatives also occur as some patients with undetectable anti-HCV have HCV-RNA in the liver though this is not very common.

Other tests available on a research basis include recombinant immunoblot assays (RIBA) for anti-HCV, immunostaining for HCV antigens in liver and polymerase chain reaction (PCR) for HCV-RNA in liver and serum.

Recombinant immunoblot assay (RIBA)

The recombinant immunoblot assay is developed as a supplemental test to invalidate the number of false positives with the C-100 ELISA test. The 4-RIBA has two additional HCV recombinant antigens

added to the C-100 RIBA, one from the nonstructural (NS3) region (C33c,) and the other, an HCV antigen derived from the core (C-22). In a study of 37 HCV C-100 ELISA positive samples, only eight were 4-RIBA positive and associated with PT NANB and/or PCR positive HCV infection.[39]

HCV-RNA

HCV-RNA can be detected in the serum and liver of infected individuals. It involves reverse transcription of viral RNA into cDNA, followed by amplification of the specific HCV cDNA by PCR.[9] Studies so far indicate that most patients with acute hepatitis C circulate HCV-RNA during the incubation period and the symptomatic phase of the disease and that 40-70% of patients with chronic hepatitis C have viral genome in their serum.[9,40,41] It correlates well with serum anti-HCV and can be considered a direct marker of HCV infectivity. It however does not distinguish between past and recent infection but will obviously be useful in anti-HCV negative infection and in early acute hepatitis as there is a period of several months of seronegativity during the early phase of acute HCV infection. HCV-RNA from the serum disappears when the disease resolves and persists when the disease becomes chronic.

HCV-Ag

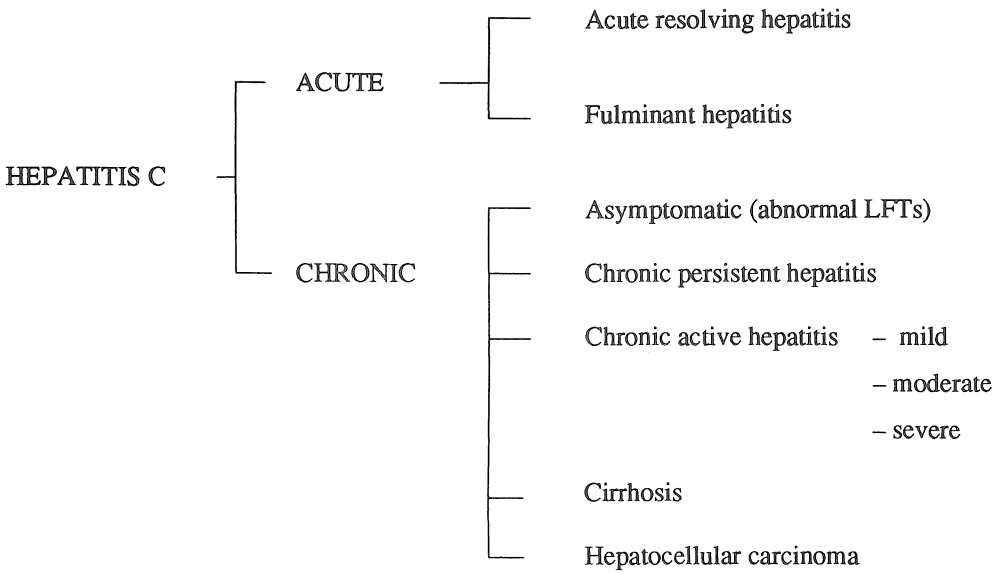
This is detected by immunofluorescence in hepatocytes of patients with acute and chronic hepatitis C even before the onset of the disease and the appearance of anti-HCV in the serum.[42] Its exact location on the HCV genome, its sensitivity and specificity, however, have not been fully evaluated. Since it can be found in both acute and chronic Hepatitis C, it can be used to confirm anti-HCV results and document the presence of active HCV infection. The differences in serological markers between Hepatitis B and C are shown in Table 1.

Table 1
Significance of serologic markers in chronic viral hepatitis [after Bonino et al (35)]

	HBV	HCV
Infection	HBsAg anti-HBc anti-HBe	anti-HCV
Replication	HBV DNA HBeAg	HCV RNA HC Ag (liver)
Virus-induced damage	IgM anti-HBC	?

Clinical Spectrum of Disease

Hepatitis C may present clinically as follows:



Acute Hepatis C

This accounts for 4–26% of hospital admissions for acute viral hepatitis.[43] Unlike acute hepatitis A and B, the disease is usually less severe and most patients present with fatigue and malaise, without any jaundice. The serum alanine aminotransferase (ALT) levels are usually elevated but not as high as observed in Hepatitis A and B. There is also a characteristic biphasic elevation of serum aminotransferases in acute PT-NANB, making it difficult therefore to determine true convalescence. Sporadic cases may present as fulminant hepatitis, an unusual feature of this disease. The case fatality rate for fulminant NANB hepatitis is greater than that Hepatitis of A and B. Extrahepatic manifestations such as rash, arthritis and aplastic anaemia have been reported.[44]

Chronic Hepatitis C

Persistent infection and chronic hepatitis are the hallmarks of HCV infection. Several studies have shown that > 50% of patients with acute PT and sporadic NANB hepatitis followed up prospectively develop biochemical and histological evidence of chronic hepatitis.[7,45] Those with persistent or intermittent ALT elevations for more than a year are considered to have chronic disease. The majority have few symptoms apart from fatigue. The diagnosis is usually suspected when abnormal LFTs are found on routine examination. Serum ALTs characteristically fluctuate throughout the chronic illness. Using HCV-RNA testing, some patients show persistent viral infection despite normalisation of ALT or disappearance of anti-HCV. About 50% of patients have various grades of chronic active hepatitis and about 20% has cirrhosis at first biopsy. Cirrhosis develops rather insiduously within 10 years and a number of these develop hepatocellular carcinoma. The rather slow and sequential progression from acute Hepatitis C to chronic Hepatitis C was exemplified in a Japanese study[46] which showed that the mean intervals between the date of transfusion and date of diagnosis of anti-HCV positive chronic hepatitis, cirrhosis and HCC was 10,21 and 29 years respectively.

Diagnosis

Acute Hepatitis C

Diagnosis is by exclusion in the high-risk patient as anti-HCV develops too late to be of any use. Tertiary centres may resort to HCV-RNA in equivocal cases.

Chronic Hepatitis C

Chronic Hepatitis C is suspected in patients with a history of parenteral exposure especially transfusion of blood or blood products or IV drug abuse and confirmed by obtaining a positive anti-HCV test after excluding other known causes of chronic liver disease particularly chronic Hepatitis B, autoimmune chronic hepatitis, Wilson's disease, alcoholic liver disease, drug-induced hepatitis, haemochromatosis and alpha-1 anti-trypsin deficiency.

Clinical Association with other diseases

1. Alcoholic liver disease

The reported prevalence of anti-HCV in patients with alcoholic liver disease is 25-50%.[47] The antibodies are found to be associated with clinical severity of the disease and a shorter survival, suggesting a role of HCV in alcoholic liver disease.

2. Autoimmune liver disease

In a study of 83 patients with Type 2 auto-immune chronic hepatitis (with seropositivity to anti-liver-kidney-microsomal antibodies or anti-LKM antibodies), Lunel F et al[49] reported that those with HCV were found in older males who had lower anti-LKM titres, moderately raised liver transminases and moderate liver pathology compared to those without HCV. The latter had typical features of the disease and were more frequent in young girls, with higher liver transminases, more severe liver pathology and higher anti-LKM titres. This distinction is important when treatment is being considered as, except for those who are anti-HCV positive, all patients with autoimmune hepatitis will respond very well to steroids.

3. Hepatocellular carcinoma

Sixty to seventy per cent of Japanese patients with HBsAg-negative hepatocellular carcinoma (HCC) were found to have anti-HCV antibodies.[47] In Europe, 65–75% of a similar group of patients were anti-HCV positive. There is also a suggestion that there is a synergistic effect of HBV and HCV because of the co-existence of anti-HCV and anti-HBc amongst patients with HCC compared to those without HCC. Apart from inducing cirrhosis, it is still not certain how HCV causes HCC.

Pathology

The histological features of acute Hepatitis C are similar to those of A and B except that parenchymal cells often show diffuse irregular eosinophilic granulomata associated with many acidophilic bodies and microvesicular steatosis. In chronic Hepatitis C, the presence of lymphoid follicles, large droplet fats, acidophilic bodies and bile duct damage are all good indicators of the disease but none of them is specific.

Treatment

Since chronic Hepatitis C is associated with progressive liver injury including cirrhosis and HCC, there is a need for effective treatment of this disease. The only effective treatment so far, at least in some patients, is alpha-interferon.

Interferon (IFN)

Several controlled trials[49,50] have shown that approximately 50% of patients with chronic hepatitis C respond to one–three million units of IFN given subcutaneouly thrice weekly for 24 weeks. Response was better with the higher dose in terms of improvements in serum aminotransferases and liver pathology. Liver enzymes decreased rapidly within two months of initiating treatment with IFN in responders. Discontinuation of treatment caused a relapse in at least 50% of initial responders who responded subsequently to a second course of IFN. It is not known how many of these will eventually achieve long-term sustained remission. Overall only 20–25% of treated patients did. Spontaneous remission occurred in 5–10% of patients.

Patients considered for treatment with interferon therapy must have a firm diagnosis which will encompass the following:

1. biochemical and histological evidence of chronic hepatitis. This would normally mean raised liver enzymes for a period of at least six months and typical findings of chronic necroinflammatory disease on liver biopsy.

2. serological or epidemiological evidence of HCV, meaning a positive anti-HCV, preferably using the second generation test. Anti-HCV negative patients who nevertheless have epidemiological features of NANB infection eg. history of exposure to blood, blood products or serum, can also be considered for treatment.

3. No other causes of chronic liver disease eg. alcoholic, drug-induced, metabolic and autoimmune liver diseases. The latter can sometimes be difficult as false positives to anti-HCV can occur. Absence of anti-mitochondrial antibodies and normal immunoglobulins will help exclude autoimmune disease. A trial of steroids would be helpful if doubt still exists.

Presently only patients with clinical, serum, biochemical or histological evidence of severe disease are considered for treatment. Hoofnagle and Di Bisceglie[51] define severity of disease as follows:

1. persistently raised liver enzymes: ALT>5–10X the upper limit of normal

2. presence of symptoms which interfere with the patient's normal life

3. histological changes of severe CAH and active cirrhosis.

It should not, at the present level of understanding, be offered to asymptomatic patients with mild to moderate disease activity.

Dose and regimen of IFN therapy:

Based on published controlled trials conducted so far, an accepted regimen will be: three million units (MU) of alpha-IFN subcutaneously thrice weekly for one year. If there is no response (i.e. failure of ALT to decrease by > 50%) by three months, the dose may be increased to 5 MU. IFN should be stopped if there is no response with this dose. If there is a response, treatment should be continued for a year with adjustment of the IFN dosage, according to side-effects and changes in liver enzymes.

If there is a relapse, as evidenced by raised liver enzymes, it is advisable to wait for about six months before considering a second course of IFN treatment, as transient, self-limited relapses have been observed.

Side effects.

Side effects to IFN are usually mild, well-tolerated and transient. They include flu-like symptoms, malaise, myalgia, anorexia, alopecia, arthralgia, concentration problems, headache and sleep disturbances. More serious side effects rarely occur, especially with prolonged treatment and these include depression, suicidal thoughts and even frank psychosis. Bone marrow supression especially involving white cell counts and platelets can occur. IFN may induce formation of auto-antibodies. These are usually transient, found in low titres and not associated with any morbidity. One to two per cent of patients have manifested some autoimmune condition eg. thyroiditis. An auto-antibody screening should therefore be done to avoid any confusion during treatment.

Monitoring of treatment

Rapid decline of ALT occurs with treatment. Serum levels of the virus, as measured by HCV-RNA, decrease in patients with long-term response to interferon treatment and remain undetectable despite discontinuation of treatment.

Since HCV-RNA is not readily available in all treatment centres, anti-HCV may be used instead as there is a good correlation between the two. It is usually done at the end of treatment. Response rate during treatment is assessed by measuring ALT levels. Full blood count needs to be measured at follow-up to detect any marrow suppression with IFN therapy.

There are presently no good clinical, biochemical or serological markers that can be used to predict response to IFN. It appears, however, that biopsy-proven cirrhotic patients have an unpredictable response compared to non-cirrhotic patients.

Others Treatment Options

Ribavirin

Ribavirin is a non-interferon-inducing nucleotide analogue with a broad spectrum of activity against RNA and DNA viruses, including those from the flavi-virus family. Hepatitis C resembles the flavi-virus. A pilot study on ten patients with biopsy-proven chronic hepatitis C, given oral ribavirin 1-2 gm a day for twelve weeks had been evaluated and found to be beneficial.[52]

Others

Treatment with acyclovir and prednisolone have both been tried but not found to be helpful.

Prevention

The main thrust for prevention is the exclusion of paid commercial blood donors and using only volunteer blood or autologous transfusion for elective surgery. Blood transfusion should be given only for massive blood loss causing severe haemodynamic decompensation. Blood donors should be routinely tested for the presence of anti-HCV (second generation) and for surrogate markers of HCV infection (anti-HBc and ALT). The use of these tests can help prevent PT-NANB hepatitis by 50-80%. In centres like Malaysia, where anti-HCV test is not freely available, serum ALTs can be used instead. Two independent studies in USA showed that donors with elevated ALTs were at increased risk of PT-NANB hepatitis. In one prospective PT-NANB hepatitis study, the calculated efficacy of anti-HCV C100 ELISA donor screening was comparable to ALT surrogate screening with a caveat that there may be HCV-infected individuals with normal ALTs. With ALT testing, 30-60% of PT-NANB hepatitis cases can be prevented, at the cost of discarding 2-4% of the donated blood.[53]

Use of recombinant-derived vaccines against HCV infection may present difficulty because of heterogeneity of the viral envelope.

Conclusion

Greater understanding of HCV replication and interferon treatment combined with recent advances in anti-viral therapy will help in the planning of safer and more efficacious treatment of this disease. Already trials are underway to assess higher doses and lower doses of IFN given for a longer period to improve the rate of sustained response.[54] Controlled trials using other agents like oral ribavirin either used singly or with IFN, are also eagerly awaited.

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