Other vaccines include polypeptides containing specific HBV-antigenic determinants. Clinical trials of polypeptide vaccines are in progress. Hybrid virus vaccines for HBV utilising recombinant vaccinia viruses have been developed. These vaccines have certain theoretical advantages in that a single strain of vaccinia may be designed to present antigens characteristic of several viral diseases simultaneously. However, at present, the use of vaccinia virus remains experimental. Other recombinant viruses being investigated as vectors for hepatitis vaccines include adenoviruses and polioviruses, which may be effective when given by mouth.

The potential for the generation of vaccine-induced escape mutants in neonates born to HBV-infected mothers exists[27] but may be preventable by alterations in the recombinant vaccines.

Conclusion

A major concern in southern Africa is to control hepatitis B virus infection and thus prevent the appalling sequelae of chronic infection. Universal vaccination of infants in high-risk areas in South Africa will be an important step in the reduction of liver disease in our country.

The introduction of a national hepatitis B vaccination programme in South Africa is imminent. This policy must be supported by the political will to control HBV infection. This entails the provision of adequate resources for vaccination and surveillance, as well as general public health measures aimed at preventing transmission.

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Hepatitis C — a South African perspective

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The existence of non-A, non-B (NANB) hepatitis was established in the 1970s, when accurate serological tests allowed exclusion of hepatitis A and B viruses as the cause of most cases of post-transfusion hepatitis.[1] The term 'hepatitis C' was coined after molecular cloning of nucleic acid from highly infectious sera of chimpanzees[2] identified an RNA virus as the primary cause of post-transfusion hepatitis (PTH). Sequence analysis and expression of the RNA has shown it to be closely related to the flavi- and pestiviruses. It has marked genomic variability which may affect its biological and immunological characteristics, is transmitted parenterally and sporadically, by as yet unidentified routes, and causes chronic indolent liver disease in 50 - 75% of infected patients. It is associated with hepatocellular carcinoma, glomerulonephritis, cryoglobulinaemia, auto-immune liver disease, lymphocytic sialadenitis and porphyria cutanea tarda. Up to 500 million people worldwide may be infected with hepatitis C virus (HCV),[3] and many questions about the disease remain unanswered. Therapy is still largely ineffective and our current understanding of the long-term natural history, our methods of diagnosis, therapy, prevention and immunisation are suboptimal.

Virology of HCV

HCV was first obtained by screening the products of approximately 1 million c-DNA clones expressed in bacteriophage vectors, with serum from patients with NANB PTH.[2] The sequence of the first clone identified (5-1-1) encoded a 55 amino acid peptide containing an

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immunodominant epitope that reacted strongly with sera from NANB but not hepatitis A- or hepatitis B-infected patients. Hybridisation with and sequencing of overlapping clones allowed full identification of the original HCV isolate genome as a positive-sense RNA of 9 401 ribonucleotides. The genome contained a 5′ untranslated region of 341 bases, a large open reading frame of 9 033 bases encoding a polyprotein of 3 011 amino acids and a 27-base poly-A tail at the 3′-end.

It has now been confirmed that the hepatitis C agent is an enveloped virus of approximately 50 nm with a positive-sense RNA genome of roughly 9 500 nucleotides with a single, long, open reading frame. The HCV resembles the flaviviruses and pestiviruses[4] but is sufficiently different from them to be placed in its own genus.[5] Replicating virus uses a minus-strand RNA intermediate template. Detection of this minus strand may therefore indicate that active HCV replication is taking place in the tissue.[6] Genomic RNA is probably translated into a single polyprotein of approximately 3 011 amino acids, which is then processed into functional proteins.[4] The putative structure and function of these proteins has been ascertained by analysis of the amino acid sequence predicted from the ribonucleic acid sequence, by analysis of expressed recombinant proteins and analogies drawn from prior knowledge of the closely related flavi- and pestiviruses. The putative structural proteins, namely the single nucleocapsid and two envelope proteins, are located at the amino terminal end of the polyprotein and are derived from the 5′ third of the genome.[7] The two envelope proteins (gp33 and gp72) are thought to be glycosylated products derived from expression of the E1 and E2 regions of the genome respectively. Variable and hypervariable regions in the envelope glycoproteins have been described,[8,9] and may play a role in escape from host immunity. Downstream from the structural proteins are the non-structural (NS) proteins which are the products of the 3′ two-thirds of the genome (NS1-NS5 region). They have protease, helicase and replicase domains which may be involved in RNA replication and protein processing.[4] The 5′ untranslated end is highly conserved in different HCV strains from around the world (Fig. 1).[10,11]

Electron microscopic demonstration of putative HCV suggests that it has a 39 - 46 nm envelope with a 37 nm core.[12]

HCV variation

Genotyping

HCV may be classified into a number of distinct but related genotypes by sequence differences of both the coding and non-coding regions of the genome,[11,13-19] or into serotypes, by identification of antibodies which react with synthetic peptides from different genotypes.[20] Many different nomenclatures are currently in use to describe the genotypes, and this has resulted in difficulty in comparing results. Classifications described by Houghton *et al.*,[21] Cha *et al.*,[18] Simmonds *et al.*,[23] Okamoto *et al.*[24,25] and Enomoto *et al.*[19] are widely used. These classifications are compared in Table I.

Table I. Comparison of three HCV classifications

Chan *et al.*[22] Simmonds *et al.*[23]	Okamoto *et al.*[24,25]	Houghton *et al.*[21] Cha *et al.*[18]
1a	I	Group I
1b	II	Group II
2a	III	Group III
2b	IV	Group III
3a	V	Group IV
3b	VI	
		Group V

Genotypes may differ in their geographic distribution, virulence and in the levels of viraemia they produce, in their cell tropism, transmissibility and responsiveness to therapy with interferon. Genotypes 1 and 2 (Simmonds' nomenclature) are globally distributed, while others are more restricted (type 3 Europe; type 4 Middle East and Egypt; type 5 South Africa; type 6 Hong Kong).[16,18,22,23,25-27]

The sequence coding for amino acids 384-414 is hypervariable, with mutations occurring at a high rate from infection to infection and over time in individual patients. The variable regions may play a role in protection against immune surveillance and hence persistence of infection.[28-30] In contrast the 5′ untranslated region and N-terminal C (core) protein have highly conserved domains which are similar in all genotypes and, indeed, in the pestiviruses,[31] which suggests that these conserved domains perform crucial biological functions. Models of the 5′ untranslated region have shown it to have a complete stem-loop structure[23,31] with multiple functions.[31]

Different HCV genotypes may be associated with variations in the severity of clinical and histological disease, and its responsiveness to interferon therapy.[20,27,32] Patients infected with type 1 HCV appear to have more severe hepatitis, a greater risk of developing cirrhosis and a poorer response to interferon therapy.[27,32] Increased disease severity may be a specific feature of the type 1 genotype, as no correlation of disease severity with the other genotypes was found in two large series,[27,33] but results have been conflicting.[34] Several studies have shown a correlation between viral genotype and interferon responsiveness.[27,34-36] Type 1 infections appeared to respond relatively poorly[27] while type 2 infections responded better to therapy.[35] Levels of viraemia, which may be affected by the genotype, could influence the response to therapy.[27,33,35] It is unclear whether infection with several different strain of HCV affects responsiveness to therapy. Hino *et al.*[36] found that infection with multiple genotypes of HCV did not affect responsiveness to interferon therapy, although Okada *et al.*[37] have suggested that multiple infections may aid escape from immune responses. The number of mutations increases with time, and patients with long-standing infections could therefore have a poorer response to therapy as a result. Patients can be simultaneously infected with more than one genotype; flares of activity occur with each new infection, indicating the lack of cross-protective antibodies between different genotypes.[38]

Diagnosis of HVC

Antibody assays

Because hepatitis C viral antigens are present in very low concentrations, they are undetectable by conventional assays. The presence of HCV infection is ascertained either by detection of antibody to the virus or by detection of HCV RNA in serum or tissue.

First-generation C100-3 (NS4) assay

The first-generation tests used the C100-3 recombinant peptide derived from the non-structural NS4 region. It represented approximately 4% of the total genome and as a result there was a significant false negative rate, and numerous patients with HCV were missed with this assay.[39-41] This resulted in ongoing PTH from donors screened negative.

Second-generation assays

Second-generation assays incorporate recombinant antigens and/or synthetic peptides derived from NS3, NS4 and core regions (Fig. 1). Solid-phase enzyme-linked immunosorbent assays (ELISAs) based on the C33, C200 and C22 antigens, derived from these non-structural NS3, NS4 and core regions and expressed in *Escherichia coli* or yeast, form the basis of the Ortho (New Jersey) and Abbott (Chicago) second-generation assays. These assays have improved sensitivity and specificity.[42]

Antibody to HCV structural proteins (nucleocapsid and envelope)

A chimeric polyprotein called C25, comprising fused immunodominant regions of the nucleocapsid (C) and the non-structural NS4 and NS5 regions, has significantly improved the sensitivity and specificity for detection of both acute and chronic HCV infections.[43] Accuracy of HCV assays has been further improved by the identification of a small immunodominant epitope of the HCV capsid region comprising amino acids 31 - 45.[44-46] Assays based on this domain of the core protein, are highly specific (< 6% false-positive rate) and sensitive (68% correlation with PCR and 97% with antibody tests to the envelope plus NS3/4 regions) in identifying chronic HCV infection.[46] Antinucleocapsid antibodies appear early in the course of infection; these may therefore be more sensitive in early detection of acute HCV hepatitis.[47] Because anti-core titres fall with resolution of hepatitis but rise with active disease, they may be useful in assessing the intensity of HCV activity.[47] However, antibodies to core are probably not neutralising, as they have been detected in chronically infected subjects. In contrast, antibodies to (E) envelope glycoproteins may be neutralising in some, but not all, cases.[48]

IgM-based assays

Although not commercially available, IgM-based assays have been developed in an attempt to distinguish acute from chronic HCV infection. In acute infections, IgM antibodies to the core protein are detected early in the course of the disease and persist for approximately 8 weeks.[49] Unfortunately, IgM responses do not always follow a classic course, and may appear later than IgG antibodies[50] or remain positive in chronic HCV infections.[51] The persistence of IgM anti-core antibodies in chronic infection appears to indicate active infection as they correlate with the presence of HCV RNA.[50,52] IgM anti-core antibodies may be a good indicator for a positive response to interferon therapy.[51,53,54]

Supplemental (confirmatory) assays

False-positive results may occur because of detection of passively acquired HCV antibodies in transfusion recipients, cross-reactivity with vector or fusion proteins and in association with auto-immune diseases (*vide infra*).[55,56] In addition, positive antibody tests do not distinguish between resolved and chronic infections. These problems are exacerbated in blood donors, where a low prevalence of the

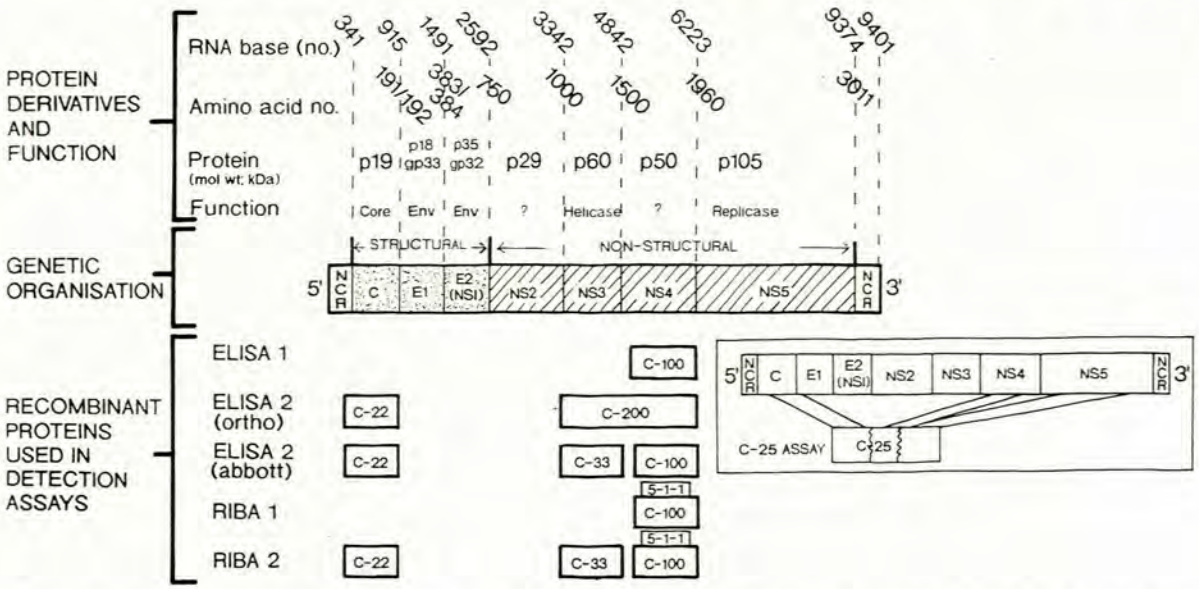

Fig. 1. Second-generation assays incorporate recombinant antigens and/or synthetic peptides derived from NS3, NS4 and core regions.

disease increases the number of false-positive cases, according to Bayes theorem. Supplemental and confirmatory assays, e.g. neutralisation, recombinant immunoblot assay (RIBA) and Western blot, have therefore been developed to improve diagnostic accuracy for HCV infection.[57] RIBA detects antibodies to antigens which are coated in separate bands on a nitrocellulose strip. The RIBA-2 tests for 2 antigens, 1 structural (C-22) and 1 non-structural (C33c), and positive results were found to correlated with the presence of HCV RNA.[58,59] The RIBA-4 detects 4 antigens (C100-3, 5-1-1, C33c, C22). Reactivity with 2 or more antigens indicates a high probability (> 90%) of true HCV infection. A strong correlation exists between positive RIBA-4 and viraemia, allowing discrimination between infective and non-infective sera.[53,60] However, sera with antibodies reactive only with C100-3 and 5-1-1 antigens from the NS4 region are often not associated with HCV RNA[61] as these may persist after the virus has been cleared. Sera reactive to a single C22 or C33 recombinant product have a low probability of containing HCV RNA.[62]

Cell-mediated immunity

Cell-mediated immunity appears to be important in host responses to the disease. Proliferative responses of CD4+ *T* cells to recombinant HCV synthetic core protein correlate with a benign course of the disease in subjects infected with the virus.[63]

HCV-RNA detection

Because HCV RNA circulates in low concentrations, sensitive detecting assays have been developed. Average plasma concentrations of virions vary from 10^2 to 5×10^7 virions/ml.[64] The reverse-transcription polymerase chain reaction (RT-PCR) for the detection of HCV RNA is the most sensitive marker of viraemia and infectivity. Specific oligonucleotide primers are used in single or nested PCR systems[65-67] and have a sensitivity which potentially allows for the detection of single molecules. Due to sequence variability of different HCV isolates, highly conserved regions of the genome especially the 5′ NCR are targeted. This allows for the detection of RNA from all strains of HCV.[68,69] Specificity and sensitivity of detection may be enhanced by means of a nested system,[70,71] and the need to probe may be obviated by use of labelled primers in nested systems.[70,72]

PCR remains largely a research technique as it is time-consuming, requires relatively sophisticated equipment, and standardisation and quality control are difficult. However, a commercial HCV RNA detection kit (HCV Amplicor; Roche Diagnostics) is available. Chiron have also introduced an HCV RNA assay that uses branched DNA amplification in which the detection system rather than the target sequence[34,73] is amplified (i.e. signal amplification). Although not as sensitive as PCR (detection limit 350 000 equivalents per ml), it may be used as a quantitative assay. Semi-quantitative competitive RT-PCR assays have also been described.[30,74] Measurement of HCV RNA levels is useful in correlating severity of disease and antiviral therapy. Lau *et al.*[73] have shown that patients with low HCV RNA serum levels respond better to interferon therapy than those with high RNA levels. The level of viraemia may also correlate with HIV co-infection, the mode of transmission, and with histological scores. High HCV RNA levels are associated with increased lobular inflammation, lymphoid aggregates and bile duct abnormalities.[73]

The PCR technique is highly sensitive, differentiates resolved from active infection in patients with antibodies,[75] allows identification of HCV infection early in the acute phase before the appearance of antibodies,[76,77] is useful in monitoring the effect of interferon or other antiviral therapies[78] and, by detecting minus strand RNA viral intermediates, is able to identify sites of active HCV replication.[6] Results of PCR should, however, be regarded with caution until techniques have improved.[79] When 31 laboratories tested a coded panel of serum samples, only 5 (16%) of these laboratories performed faultlessly on all samples. Both false positive and false negative results were common in this study, which included many leading research and academic laboratories. Apart from poor technique, other causes of incorrect results need to be considered:

1. False-positive results may occur as a result of amplicon contamination. Great care must be taken to separate pre- and post-PCR procedures; the guidelines of Kwok and Higushi[80] should be followed.
2. False-negative results can occur if inhibitors of the RT-PCR reactions are present in the RNA sample. An internal control for a single copy gene, e.g. actin or β-globin, should be included to identify this problem. Inadequately stored sera or those subjected to multiple freeze/thaw cycles may give false-negative results because of the degradation of the RNA.

Transmission

Hepatitis C circulates in low titres and is most efficiently transmitted by parenteral routes, especially by blood and blood products, shared intravenous (IV) drug needles, accidental needle-stick injuries[81] and via transplanted organs.[82-89] However, fewer than half of HCV-infected patients have had parenteral exposure to the virus. Community-acquired HCV is sporadic, transmitted mostly by unknown routes, but possibly also by sexual transmission, from mother to infant or via saliva. There is increasing evidence that transmissibility is increased when circulating HCV RNA titres are high.

Sexual transmission

Although sexual partners of HCV-positive haemophiliacs, and IV drug users, both homosexual[90] and heterosexual, have higher rates of HCV infection than the general population, especially when the latter are simultaneously HIV-positive,[91] this may reflect the effect of confounding risk factors and not sexual transmission *per se*. Osmond *et al.*[92] showed that HCV infection correlated with injection drug use, haemophilia and blood transfusion but not with sexual behaviour, number of sexual partners, history of sexually transmitted disease or HIV disease.[92] Similarly multivariate analysis has failed to show a significant sexual transmission of HCV in the sexual partners of high-risk individuals when other confounding risk factors were considered.[93] Multiple sexual partners in the previous 3 months and receptive anal

intercourse were thus associated with positivity to hepatitis B core antigen but not to anti-HCV.[93] In addition, HCV has not been found in semen samples.[94] However, up to 45% of commercial sex workers in some areas are HCV positive[95] although studies from other areas have identified antibodies in only 3,5% of this group. Higher prevalences may be due to simultaneous IV drug use or other risk factors. However, of 37 female partners of HCV-positive men, who had had no parenteral exposure or risk factors for HCV, 2 had HCV infection suggesting low-grade sexual transmission.[96] It is not clear whether HCV may be transmitted by kissing, but HCV is present in the saliva of affected subjects[97,98] and transmission by human bite has been reported.[99]

Intrafamilial transmission

Intrafamilial spread is uncommon,[100] but has been reported[101,102] and may be more common if the index patient has a high viral load.[101] Up to 8% of Japanese family members of index patients may be HCV positive; this suggests that transmission may be more common in certain areas.[103]

Vertical and perinatal transmission

Infants born to HCV-seropositive mothers with no HIV co-infection have a low but significant risk of between 0%[104] and 10%[105,106] of developing HCV infection. The risk in HCV RNA-positive mothers appears to be greater, if the mothers' HCV RNA titre is high. Ohto *et al.*[107] showed that if this titre exceeds 10^6 virions/ml the risk of transmission of HCV to infants is 36%, whereas if the titre was below 10^6 or when HCV RNA was negative, there was no transmission to the infant. Nucleotide sequencing of viruses from mothers and infants showed 97 - 99% homology, compared to homology of 66 - 92% between infants. This confirms that vertical transmission indeed took place in those affected.[107] In addition, mothers co-infected with HIV and HCV confer a risk of up to 50% for vertical transmission of HCV, and disease progression in these is often more rapid.[108,109]

The rate of vertical transmission may be greater in infants than is shown by seroprevelance studies. Thaler *et al.*[110] found that a high proportion of infants born to HCV-seropositive mothers were HCV RNA positive despite the absence of HCV antibodies, and that the disease may remain silent in many infants. (Seroprevalence studies of infants also lack specificity as anti-HCV antibodies may be passively transferred from mother to infant.[104]

Occupational exposure

Seroprevalence studies of hospital health care workers have failed to show a significant increase in HCV infection[111] although transmission by needle-stick injury is well documented in this group[81,111-114] and transmission may occur in up to 10% of needle-stick victims.[115] An increased seroprevelance has been found in dentists, especially those practising oral surgery and exposed to high-risk patients such as IV drug users.[116-118] The relative risk of being HCV seropositive was 12,9-fold in a study of New York dentists.[116] However, there was no increased risk documented in other studies.[119] Military personnel stationed in different geographical areas appear to have no increased risk of developing hepatitis C.[120]

Epidemiology

Prevalence: South Africa

The prevalence varies widely in different geographical regions and among ethnic groups within regions. Soni *et al.*[121] found a seroprevalence of 0,16 - 0,75% in the general blood donor population of Natal, with the highest prevalence in the black population (0,75% of blood donors). HCV antibodies were present in 40% of haemophiliacs and 5% of dialysis patients but in none of the nurses tested. Because up to 10% of haemodialysis patients may carry the virus without developing antibodies[122] the true incidence of HCV infection in renal dialysis patients in this study may have been as high as 15%. In a population-based study KwaZulu, 1,7% of urban blacks and 0,9% of rural blacks had HCV.[123] An earlier study from South Africa had shown a prevalence of at least 3,84% in rural and 1,2% in urban blacks,[124] indicative of a higher prevalence in rural communities. The prevalence in the latter study may be spuriously high due to the effect storage of serum has on the first-generation ELISAs, which have low specificity. Hepatitis C prevalence contrasted sharply with that of hepatitis B in all the above groups suggesting that there may be a different mode of transmission for the two viruses. The rates in the urban communities are similar to those in many Western countries.

Prevalence: General

Blood donor-based studies indicate a variable seroprevalence rate in different countries. Rates in most Western countries range from 0,3% to 0,7%, but are 0,9 - 1,2% in southern Europe and Japan, with higher prevalences in southern Italy and eastern Europe.[125] Japanese subjects younger than 20 years have a prevalence rate of 0,2% but this increases to 4% in subjects older than 50 years.[126,127] Egypt has one of the highest seroprevalence rates, up to 22% on ELISA and 13,6% on RIBA-2.[128] The very high rate has ben confirmed in numerous studies[129-132] and child recipients of blood products have PTH-C infection rates of up to 55%.[133] The geographical variation in prevalence may relate to blood transfusion practices before HCV tests became available in 1989, e.g. the use of paid donors who have HCV seroprevalence rates of approximately 10 - 15%[134] and the consequent use of contaminated blood. Other factors include the prevalence of IV drug users within a community, variations in HCV genotype, differences in socio-economic factors and possibly in previous medical practices. It has been postulated that the high prevalence in Egypt is due to the previous multiple re-use of glass syringes for treatment of schistosomiasis, while in parts of Italy self-injection of vitamins and tonics may have contributed to HCV spread (Colombo M. — personal communication).

Post-transfusion hepatitis

The incidence of PTH was 2 - 19% prior to surrogate or HCV testing of blood donors.[135] Although only approximately 15% of cases of HCV infection can be traced to transfusion of blood products,[136] HCV currently accounts for more than 82[47] — 100% of cases of PTH worldwide.[137-139] First-

generation (anti-C100-3) tests detected HCV antibodies in approximately 80% while the second-generation tests were positive in up to 98% of subjects with PTH.[140-141] PCR also detected HCV RNA in 4% of patients receiving transfusions but in whom neither antibodies nor clinical hepatitis developed; this implied that significant asymptomatic transmission may occur and that seroprevalence rates may lack sensitivity. NBNC cases may account for a small percentage (<10%) of PTH patients, but as accuracy of HCV testing increases, so the NBNC percentage decreases. These patients tend to have mild disease.

Donor screening has reduced the incidence of PTH significantly. Use of surrogate markers (ALT and HBcAg) for hepatitis reduced the incidence of NANB PTH by more than 60% and hepatitis B by 35%.[142] In Japan, screening with the Ortho anti-C 100-3 antibody test reduced the incidence of PTH in patients receiving 1 - 10 units of blood from 4,9% to 1,9% and in patients receiving 11 - 20 units of blood from 16,2% to 3,3%.[143] Similarly, Donohue *et al.*[144] showed that the PTH rate dropped from 3,84% in the pre-screening era to 1,54% with the use of surrogate markers and to 0,57% with HCV testing.[144] Currently, the risk of acquiring PTH increases with the volume of blood transfused and the use of pooled blood components.

A variety of strategies have been instituted in South Africa to prevent HCV transmission and are discussed by Bird and Gibbs on pp 570-572 of this issue. The reduced rate of PTH may be affected both by screening as well as low background rates of viral infection in the general population. Tests which indicate infectivity of blood products must be specific, in order to reduce discarding of non-infectious false-positive blood products. Positive surrogate markers (raised ALT levels) are 60% specific for infectivity of blood (i.e. 40% of blood positive by this test is non-infective), first-generation assays for antibodies are 70 - 80% specific[41] and RIBA-2 is 90% specific.[145] Antibodies do not always indicate HCV infection, but may represent convalescent antibodies after recovery form HCV. Therefore, PCR is the most accurate method to detect HCV RNA and hence infectivity,[146] but is not practical for routine screening. False-negative antibody tests also occur and, because of current imprecision of testing, significant numbers of PTH cases may still occur. It has been estimated that up to 2 000 new cases could occur annually in the UK.[147]

Community-acquired (sporadic hepatitis C

HCV alone does not account for all HAV- and HBV-negative cases of community-acquired hepatitis with negative markers. In acute sporadic NANB hepatitis, HCV infection accounts for only 50 - 82%[148-150] of cases. Of all HCV infections, approximately 50% are community acquired (sporadic) and the rest parenterally acquired.[150] In a prospective study of NANB hepatitis in 4 sentinal counties in the USA, 82% of NANB hepatitis cases were due to HCV infection (i.e. 18% NBNC hepatitis). Of the HCV cases, at least 40% were truly sporadic, in that no parenteral risk factor could be identified.[150] Chronic hepatitis developed in 62% of all HCV infections and this was similar for both post-transfusion and sporadic cases. Of the chronic cases, HCV RNA persisted in most patients, even those with normal liver biochemistry. Antibodies were lost at a rate of only 0,6 per 100 patient-years.[150-151]

High-risk populations for hepatitis C

Haemophilia

Prior to HCV screening and viral inactivation, 50 - 90% of haemophiliacs became HCV positive, depending on their degree of exposure to blood products and the source of the blood products.[91,100,152-158] The high degree of HCV contamination of factor VIII is the result of pooling of large numbers of donor sera in its production. HCV infection in haemophiliacs is easily missed by serological studies.[158] Seroconversion from an HCV antibody-positive to a negative state, especially in HIV-positive subjects, may also occur despite the persistence of HCV RNA in these subjects, many of whom have ongoing hepatitic inflammation that progresses to cirrhosis.[157] The situation is further complicated by the fact that many haemophiliacs do not develop a significant elevation in serum transaminase levels or liver disease, despite being HCV positive.[154]

The presence of HCV RNA or antibodies in blood products correlates closely with the probability of these causing infection in recipients. HCV RNA was detected by PCR in over 56% of stored clotting factor batches, before viral screening or inactivation became routine.[146] Viral inactivation should be by pasteurisation[156] or solvent detergent inactivation, as heat inactivation alone does not reduce the infectivity of blood products.[146]

Intravenous drug users

Intravenous drug users are an important reservoir for HCV; 45 - 92% are infected.[159-163] Co-infection with HIV and HBV is common, although transmission of the different viruses may occur independently.[161-164]

Haemodialysis

Approximately 10% of haemodialysis patients in populations with a low prevalence of HCV,[122,165,166] and up to 48% in high-prevalence areas[167-169] are HCV positive. Of these patients, 35 - 75% have abnormal liver tests or histology.[170] Parenteral transmission could only be identified in approximately half of the HCV-positive cases studied, suggesting that dialysis-associated transmission or increased sporadic transmission occurs in this group.[122,165] Recent work suggests that nosocomial transmission is probable, as HCV prevalence was low at initiation of dialysis (4,6%) and increased to 50% in those on dialysis for > 10 years. Up to 10% of dialysis patients, including those with active liver disease, may carry the virus without mounting an antibody response,[122] but if antibodies are positive, the majority of patients will also have HCV RNA.[171]

HCV may reduce antibody responses of HBV vaccination[172] and may cause liver disease in the renal transplant patient. Previously positive antibodies may become negative post transplantation because of immunosuppression, without the patient clearing HCV-RNA.

HCV in transplantation

Approximately half the recipients of HCV-positive organs may develop liver disease, which may be severe (subfulminant) in some cases.[173] It is controversial whether the virus is transmitted by corneal transplantation.[174-175]

Acute HCV infection

Clinical features

HCV accounts for the majority of cases (83[47] - 100%) of post transfusion and 20 - 40%[47] of the sporadic cases of NANB hepatitis.[176] The average incubation time is 6 - 12 weeks,[177] but may be as short as 14 days if the innoculum is large.[177] However, seroconversion may also be delayed up to 1 year or indeed may not occur. Antibody titres are lower in those with acute self-limiting HCV infection, compared with higher titres in those who have chronic disease.[136] Malaise, fever, jaundice and dark urine occur in a minority of acute infections and fewer than 10% of patients become seriously ill. Hepatitis C rarely causes fulminant liver failure[178-180] but may be a co-factor in causing more severe disease in cases of acute liver failure (ALF) caused by other agents.[180] A prospective study of 504 cases of PTH C has shown fulminant hepatitis to be rare and has suggested that detection of HCV in some studies of fulminant hepatitis was due to passive transfer of HCV RNA and antibodies to these patients who had received blood products or exchange transfusion.[181] Yoshiba *et al.*[182] identified 13 patients with fulminant liver failure and HCV infection and compared them with HBV fulminant hepatic failure and NBNC ALF. HCV was associated with less severe but more persistent disease, several peaks of enzyme rise and a higher mortality rate. The severity of the acute HCV infection may be affected by the genotype[183] and acute HCV infection may be caused by superinfection with a new genotype in a patient with pre-existing HCV.[184]

Diagnosis

Diagnosis of acute HCV infection depends on confirming the presence of HCV RNA (or antibodies) in patients with acute PTH or sporadic hepatitis, where other causes have been excluded. Reverse transcription PCR to detect HCV RNA is the current gold standard for diagnosing acute HCV infection,[148,176,185,186] but this is a research-based method which is expensive and requires scrupulous technique. Serological studies are generally less sensitive, as seroconversion may only occur weeks, months or years after the onset of clinical disease.[187] Anti-C100-3 antibodies, for example, became detectable only 9 ± 11 weeks after HCV RNA in 25 cases of acute HCV infections studied by Kato *et al.*,[176] and 1 - 80 weeks (mean 11,3) in patients studied by McHutchison *et al.*[188] Antibodies to the core protein are often the first markers to appear after HCV infection and therefore second- and third-generation assays are more sensitive in detecting early seroconversion than first-generation assays.[43,141,148] Core antigens currently used in detection of specific antibodies include a 62 Kda fusion protein of HCV core with Maltose-binding protein[189] and a fused chimeric polyprotein of the immunodominant epitopes of the C (core) and NS3-NS5 (non-structural) proteins.[43] Anticore antibodies were found to be predictive of the prognosis in acute HCV infection. If the disease became chronic, anticore antibodies increased, whereas they decreased and became undetectable if the disease resolved.[47] IgM antibody responses to a variety of recombinant proteins occur in acute HCV infections.[49] The most sensitive IgM antibodies were to the nucleocapsid antigens (core). These became detectable before IgG antibodies and remained positive for approximately 8 weeks.[49] Unfortunately, The presence of IgM antibodies do not always denote acute infection, as these may persist in chronic disease.

Course and management

As many as 90% of acute HCV infections become chronic[126] and many of these may progress to cirrhosis or hepatocellular carcinoma. It is unclear whether early treatment of acute HCV will improve the chances of long-term cure but several theoretical indications support this approach. The viral load (as quantified by b-DNA or semi-quantitative PCR)[190] as well as the number of mutations in the HCV genome appear to increase progressively with time.[191] As these factors may be indicators of poor response to treatment, early treatment may improve the ultimate long-term cure rate. Pilot studies of interferon α-2a treatment suggested possible improved cure rates in patients treated in the acute phase of the disease,[192] but a controlled trial of 3 months of INF α-2b, 3 million units three times weekly showed no difference in the prevalence of chronic hepatitis 15 months after cessation of therapy, although transaminases were significantly improved in the treatment group after 3 months of therapy.[193] A recent study has shown a 39% complete recovery rate in patients receiving 3 MU interferon α-2b for 12 weeks for acute HCV infection, v. none of the controls.[194]

Chronic hepatitis C infection

Many subjects with positive screening tests have never been exposed to HCV. The screening tests give false-positive results in the majority of cases (as expected from Bayes theorem). Positive tests should therefore be confirmed by PCR or another supplemental test.

Approximately 60%[152,195,196] to 90%[126] of patients infected with HCV develop chronic disease and 50 - 75% have abnormal transaminase levels at 12 months and chronic hepatitis on histology.[197] Although 40% of patients may experience symptoms at the onset of disease, the majority subsequently become asymptomatic.[198] Despite the lack of symptoms, 45 - 62% of HCV-positive blood donors may have active hepatitis and 7 - 15% cirrhosis.[199] Chronic HCV infection is usually mild, but is persistent and insidiously progressive; it rarely remits spontaneously[126] and significant sequelae occur after prolonged periods, including cirrhosis and hepatocellular carcinoma. Twenty to 50% of patients progress to cirrhosis after 10 - 16 years,[195,196,200] and approximately 10% develop end-stage liver failure[195] while the rest have features of chronic hepatitis. Patients developing cirrhosis are usually older and anti-HCV antibody titres are higher in active disease.[196]

The spectrum of disease in chronic HCV is variable, and rapidly progressive cirrhosis may occur, especially in older

patients and those with concomitant HBV[162,201-203] or HIV infection, alcoholic liver disease,[204] or associated haemochromatosis. The spectrum of disease also includes carriers with persistently normal serum ALT levels and minimal-to-mild inflammatory changes in the portal tracts, associated with low HCV RNA levels.[205] It is important to realise that chronic active hepatitis may be present on histology, despite the presence of normal ALT.[206]

The long-term mortality rate may be slightly increased in patients with chronic HCV infection. In a case-controlled study of 568 cases of PTH overall life table analysis of mortality at 18 years was 51% for PTH NANB hepatitis, v. 52% and 50% in the two case control groups. There was a small but significant increase in liver-related mortality (3,3% v. 1,1% and 2,0% in case controls $P = 0,033$) although alcoholic liver disease was diagnosed in approximately 70% of these cases.[207] All were cases of post-transfusion hepatitis and many may have been older subjects with heart disease. Koretz[208] similarly failed to show a significant increased mortality rate for chronic HCV. This may only be relevant to older patients and should not be generalised to younger subjects with HCV. This introduces a bias where competing causes for death could mask the relative effects of the HCV and liver disease on mortality. Thus prospective studies of sporadic and PTH cases need to be performed to allow estimation of the effects on mortality.

Pathology

Although histological changes are not specific for HCV, they may help to confirm the diagnosis of HCV hepatitis, to exclude other or associated causes of liver disease, e.g. alcoholism, and to document the extent of fibrosis and cirrhosis; this has therapeutic and prognostic implications. Histology may not be useful in assessing activity, as disease activity fluctuates.

The typical changes include piecemeal and spotty necrosis, acidophilic bodies (apoptotic cells), large droplet steatosis, Mallory-like bodies, lymphoid aggregates and follicles (predominantly in the portal regions), bile duct damage and loss.[209] Fibrosis and cirrhosis indicate chronic disease. While these lesions are characteristic of HCV hepatitis, they may also be found in other conditions including auto-immune chronic active hepatitis. The degree of piecemeal necrosis, fibrosis or cirrhosis, lobular hepatitis, cellular necrosis and portal inflammation forms the basis of the description of histology, and terms such as 'chronic active' of 'chronic persistent' hepatitis are currently not recommended (Scheuer PT — personal communication).

Relationship of HCV to other diseases

HCV and hepatocellular carcinoma (HCC)

Many studies have shown an increased prevalence of HCV markers (HCV RNA and HCV antibodies) in patients with HCC.[43,103,196,203,210-223] The association between HCV and HCC holds true even in areas of high HBV prevalence.[211] The prevalence of HCV infection and its contribution to the development of HCC varies geographically.[211,216] Thus approximately 20% of 128 cases of HCC in South Africa were positive for RNA or antibodies to HCV on 2nd generation tests, compared with 94,5% positivity to HBV markers.[211] A meta-analysis of 15 reports of 1 930 patients with HCC revealed that 47% of the cases were HCV positive (95% confidence interval 37- 57%), and HBV infection was present in 59% (95% CI 27 - 91%). The odds ratio for developing HCC in HCV-positive cases was 25 (95% CI 18 - 33).[218] There may be an additive effect in cases of HBV and HCV co-infection,[222] which may be indirect and mediated by cirrhosis.[219] Reverse transcription PCR assays for both positive and negative strand RNA, failed to detect evidence of HCV infection or replication in the cancerous tissue, although non-cancerous tissue was positive,[220] which supports an indirect effect of HCV in causing HCC.

HCV and alcoholic liver disease

Patients with alcoholic liver disease have an increased prevalence of HCV infection. The surprisingly high prevalence of HCV antibodies in patients with alcoholic liver disease is not due to false-positive cross reactions;[58] however, interaction between the virus and alcohol is poorly defined.[204,224-226] HCV was found in 18 - 30% of patients with alcoholic liver disease.[224,226] and infected patients developed liver disease at a significantly younger age than those without HCV.[224,226] Although clinical and pathological grading were similar in HCV-positive and negative alcoholic patients, positive patients had higher ALT levels and required significantly more hospital admissions.[226] Rosman *et al.*[204] found greater degrees of lobular and portal inflammation in HCV-positive alcoholic patients, but no difference in perivenular fibrosis or cirrhosis. HCV thus appears to have a synergistic effect with alcohol in aggravating and accelerating alcoholic liver disease. It is still unclear why HCV is so much more common in patients with alcoholic liver disease.

HCV and prophyria cutanea tarda (PCT)

HCV infection has consistently been found in 62 - 82% of subjects with sporadic PCT but only in 5% of those with familial PCT.[225,227-230] This strong association may imply that HCV can trigger or contribute to the development of PCT or that PCT in some way increases susceptibility to HCV infection.

HCV and cryoglobulinaemia

Essential mixed cryoglobulinaemia (type 2 cryobulinaemia: polyclonal IgG and monoclonal IgM rheumatoid factors; type 3: both IgG and rheumatoid factors are polyclonal) manifests with cutaneous vasculitis, palpable purpura, arthralgias, neuropathy, and renal and hepatic disease. No cause was identifiable in most cases, prior to identification of the hepatitis C virus. Several recent studies have identified HCV RNA in many of these cases (43 - 84%)[231-235] and quantitative assays indicated that it was concentrated in the cryoprecipitate with low levels in serum. Antibodies were only found in 42% of serum samples or cryoprecipitate suggesting a high false-negative rate for current antibody tests.[231] Clinically the subset of cases with positive HCV antibodies identified by ELISA-2 and RIBA-2 had increased cutaneous involvement, clinical, biochemical and histological

evidence of more severe liver involvement and lower CH50 complement levels.[232] Of 61 prospectively studied HCV cases, cryoglobulinaemia was found in 36%, although this was only clinically manifest in approximately 10%. Seventy percent of these patients had circulating rheumatoid factors.[236] Interferon-α may be the therapy of choice for essential mixed cryoglobulinaemia, in the light of previous therapeutic trials[237] and the presence of HCV in the majority of cases.[231]

HCV and renal disease

Glomerulonephritis with associated proteinuria and diminished renal and liver function abnormalities, cryoglobulinaemia, hypocomplementaemia, and membranoproliferative histology occurs in HCV infection.[238] Patients with HCV antibodies and glomerulonephritis all had HCV RNA in their serum, and circulating immune complexes and cryoglobulins containing HCV RNA, anti-HCV IgG and IgM rheumatoid factor to anti-HCV-IgG antibodies.[238] Renal biopsy of these patients showed IgG, IgM and C3 deposition in the glomeruli. Interferon therapy caused reduced viral replication and was associated with improved renal and liver functions. The pathogenesis of the glomerulonephritis is unknown, but it may be due to glomerular deposition of immune complexes containing HCV, anti-HCV-IgG, IgM rheumatoid factors and complement.[238]

HCV and auto-immune hepatitis

A complex and poorly understood relationship exists between HCV infection and auto-immune chronic liver disease (AICLD). The prevalence of HCV antibodies is increased in patients with AICLD compared with the general population,[216,239-246] but the association of the virus with auto-immune liver disease varies geographically.[216] Typically, type 2 auto-immune hepatitis,[241] which is characterised by positive liver-kidney microsomal (LKM-1) antibodies and nonsustained response to steroids, has been associated with HCV infection, but classic anti-nuclear antibody-positive type 1 auto-immune hepatitis has also been associated with this infection.[246] The matter is further complicated by the demonstration by Yamamoto *et al.*[247] that the LKM-1 antibodies in anti-HCV positive type 2 AICLD differed from the LKM-1 antibodies in HCV-negative cases of type 2 auto-immune hepatitis. In HCV-negative cases, LKM-1 antibodies recognised a variety of recombinant synthetic peptides spanning the amino acids between 72 and 456 of the P4502D6 isoform of the P450 microsomal enzyme. The HCV-positive cases (possibly representing primary HCV infection with a secondary auto-immune response) on the other hand reacted with the full length peptide but not with the smaller peptides.[247] However, in prospectively evaluated cases of HCV chronic active hepatitis, LKM-1 antibodies were rare, although anti-nuclear factor and anti-smooth antibodies were found in approximately 40% of cases.[236]

Anti-GOR antibodies appear to be uniquely associated with HCV infection. These antibodies develop to a host-derived pentadecapeptide in up to 80% of patients with chronic HCV infection, but less than 10% of patients with liver disease from other causes.[248] The majority of responders have HCV RNA, thus anti-GOR antibodies are a marker for active disease.[248,249] Antibodies to GOR target both a host peptide as well as the core antigen of the HCV; this suggests that these are auto-immune antibodies with host-viral cross-reactivity.[250]

In cases of hepatitis with both HCV and auto-immune markers, a subgroup behaves like classic auto-immune liver disease with good steroid responsiveness, whereas others respond to interferon therapy and behave more like a primary HCV infection. The presence of jaundice[251] found in 76% of cases of AICLD v. 0% of HCV), symptoms[251] (97% of AICLD v. 47% of HCV) and both anti-nuclear and anti-smooth muscle antibodies[239] at presentation are more suggestive of auto-immune hepatitis in these cases. The geometric mean titre (1:160 v. 1:500) of the anti-nuclear antibodies was lower in cases of HCV liver disease than AICLD.[251] It has also been shown that HCV-derived antibodies to a host epitope[243] may develop in the course of HCV infection; this suggests that the virus may precipitate auto-immune disease. Auto-antibodies in HCV infection occur in older patients,[251] but their presence does not appear to affect responsiveness to therapy or to be associated with an increase in side-effects of treatment.[252] In conclusion, until the relationship between auto-immune chronic active hepatitis and HCV infection is better understood, markers for both diseases should be sought. Therapy, be it interferon for HCV or steroids for AICLD, should be monitored carefully and changed if the desired effect is not achieved. Specifically, patients with AICLD who receive interferon may deteriorate markedly, while primary HCV infection may be aggravated by steroids.

Lymphocytic sialadenitis

Lymphocytic sialadenitis or lymphocytic capillaritis and sialadenitis, which cause xerophthalmia and xerostomia, were found in 50 - 57% of prospectively evaluated HCV patients, compared with 5% of control.[236,253] The mechanism of salivary gland involvement in HCV is unknown.[254]

HCV and lichen planus (LP)

There is an increased incidence of chronic liver disease in patients with chronic LP. A recent study found HCV infection associated with these cases. HCV was diagnosed prior to or at the same time as LP in the majority.[225] This association has been confirmed in a controlled study.[236]

Therapy

Patient selection

Patients with active chronic hepatitis C should be treated because of the persistent and often progressive nature of the disease which may lead to hepatocellular carcinoma, or cirrhosis in 10 - 20% of cases within a decade. Because the majority of patients with acute HCV remain persistently infected for many years and may develop cirrhosis, some authors recommend the treatment of acute HCV infection.[256] The diagnosis of hepatitis C must first be confirmed, preferably by demonstrating HCV RNA in blood by PCR

(currently only available at selected centres and research facilities), or by a supplemental test. Disease activity should also be confirmed by demonstrating raised serum transaminase values (ALT and AST), abnormal INR or albumin levels, and preferably also by showing active hepatitis on histology. The fluctuant nature of the HCV activity means that abnormal transaminase values may occur intermittently, so active disease cannot be excluded unless several tests performed at 1 - 2 monthly intervals have been normal. Other causes of hepatitis, e.g. alcoholic liver disease, auto-immune or HBV hepatitis, which may require different therapy, should be excluded where epidemiological or clinical features suggest the possibility of an alternative or compound diagnosis. Because auto-immune hepatitis is commonly associated with HCV infection and may require steroid treatment or be worsened by interferon therapy, anti-nuclear factor, anti-smooth muscle antibodies and anti-liver kidney microsomal antibody levels should be measured prior to initiating treatment. Specific contraindications to interferon therapy should be sought, including cirrhosis (as there is very poor responsiveness and increased risk), auto-immune collagen vascular disease, psychological depression, bone marrow insufficiency (especially low white cell count) and active sepsis. Patients with decompensated cirrhosis may benefit from low doses of interferon with normalisation of transaminase values, but therapy has to be given cautiously in these patients,[257] and closely monitored. Treatment should not be given until the risk-to-benefit ratio has been carefully weighed for each individual. Little may be gained in treating older asymptomatic individuals, as the disease is often more resistant to therapy in this group, the side-effects of interferon are greater and the indolent nature of the disease means that patients are unlikely to develop complications or symptoms in their normal lifespan.

Interferon therapy

Randomised controlled trials of approximately 3 million units of interferon α-2, three times per week for 6 months, have shown an approximately 50% apparent clearance of the virus (usually after 4 - 8 weeks of therapy) and histological resolution of disease,[258] which lasted until the end of therapy. Unfortunately there was a 50% relapse rate within 1 - 6 months after cessation of therapy, usually with rises in both HCV RNA and transaminase values.[259-261] Haemophiliacs had identical biochemical and histological response and relapse rates after receiving interferon 3 million units 3 times per week.[152] They also had a low relapse rate when treated for 1 year.[152] Longer therapy (≥ 1 year) has been associated with an improved cure rate, with an up to 42% sustained response at 24 weeks follow-up in some,[262,263] but not all, studies.[264] Recurrence of HCV during therapy may be the result of extensive mutational changes in the virus[265] or the development of antibodies to interferon.[264]

Acute PTH due to HCV responds to interferon therapy, 3 MU three times per week, but responses are not maintained and no difference in transaminase values was detectable between treated and untreated groups at 15 months.[193] Trials are needed for asymptomatic carriers of HCV, as the long-term natural history of these patients is not well documented.[266]

Predictors of good response to interferon

The absence of cirrhosis is probably the most important and consistent predictor of a good response to interferon.[267-270] Other markers of a good response include a short duration of disease,[270] low gamma glutamyl transferase activity,[267,268] lack of obesity, age less than 45 years[269] and the presence of IgM anti-core antibodies (which also signals a sustained response).[51,53,54] Others have not found any biochemical marker predictive of a good response.[264] A strong predictor of a good response is the presence of low levels of HCV RNA at the start of therapy, as measured by semi-quantitative PCR or b-DNA assays,[36,73,271] or HCV antigen levels in liver biopsy specimens.[272] Viral genotypes (which vary geographically)[273] and the number of strains and mutations (especially of the hypervariable region of the envelope E2 gene product) present in an individual may also influence the response to therapy. The mutations may assist in escape from immune surveillance.[274] Viral replication in peripheral blood mononuclear cells[275] may be associated with relapse following cessation of therapy. The response of HCV to, and host tolerance of, interferon is not significantly influenced by the presence of HIV infection[276,277] or the presence of haemophilia. The predictors of response mentioned above are too inaccurate to be of use in guiding patient management and it is inappropriate to withhold therapy on the basis of these predictors.

Alternative forms of therapy

Ribavirin

Ribavirin, a guanosine nucleotide analogue with good oral bio availability, may improve serum transaminase levels or reduce HCV RNA levels in approximately 30 - 60% of patients receiving 600 - 1200 mg/day, but relapses occur in nearly all patients on withdrawal of this drug.[278-283] Its use as a combination agent with interferon, to improve response and reduce relapse rates, is currently being studied.

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Hepatitis E

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Hepatitis E, enterically transmitted non-A, non-B hepatitis, is caused by an RNA virus transmitted via the faecal-oral route. It usually manifests as an outbreak of waterborne hepatitis in developing countries where sanitation is poor, but has also been found to be endemic in these regions.[1,2]

Properties of the hepatitis E virus

Hepatitis E is a spherical, 27 - 34 nm, non-enveloped single-stranded RNA virus.[3-5] On electron microscopy, hepatitis E virus (HEV) particles have spikes and indentations on their surfaces. The virus is sensitive to high salt concentrations, proteolytic digestion and freeze-thawing. The first two factors probably account for the relatively low numbers of intact viruses seen in the stools of patients with acute HEV.

HEV has a 7,5 kb, single-stranded, positive-sense RNA genome. It has three (overlapping) open reading frames (ORFs), with the non-structural genes located at the 5′ end of the genome and the structural genes at the 3′ end. Although most of the structural proteins are coded within ORF2, all three frames contribute to the morphology of the HEV.[3,6]

While early studies suggested that HEV was related to the picornavirus group, its physicochemical properties and morphology resemble those of the caliciviruses. Subsequent analysis of the non-structural genes have revealed similarity to the α-like virus supergroup which includes rubella and the beet necrotic yellow vein virus.[7] Final classification of HEV is pending.

Epidemiology and modes of spread

HEV has been responsible for several massive outbreaks of hepatitis, each producing over 10 000 cases. Many of these outbreaks have occurred as a result of sewage leaking into river water or a failure of the chlorination process for drinking water.[8,9] Indeed, hepatitis E is the principal agent for epidemic hepatitis in developing countries. Outbreaks have been reported from India, China, Nepal, Bangladesh, Pakistan and Indonesia.[10-14] In Africa major outbreaks have been documented in Algeria,[9] Ghana[15] and Ethiopia.[16]

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