BLOOD REVIEWS
Haemostasis and Thrombosis

Chronic Hepatitis in Haemophilia

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SUMMARY. **Chronic hepatitis affects almost all haemophiliacs treated with non-virally inactivated clotting factor concentrates. The virus responsible is hepatitis C (HCV) and most patients have non-neutralising antibodies with circulating virus. Although the majority also have evidence of past infection with hepatitis B, less than 5% are chronic carriers of HBsAg. Chronic hepatitis C can be associated with severe and progressive liver disease but the development of complications is slow. Treatment with recombinant interferon alpha given subcutaneously normalises the liver function in 50% of patients, but 50% of responders relapse on stopping treatment. Liver transplantation is successful in patients with advanced liver disease and it offers the added advantage of phenotypic cure of the haemophilic state.**

It has been recognised for more than half a century that a proportion of recipients of blood and blood products developed hepatitis.[1] Even earlier it was known that vaccination with human derived vaccines transmitted hepatitis.[2,3] The infectious nature of the agent involved was demonstrated by serial passage in the chimpanzee model.[4,5] We now know that most of these episodes of hepatitis were due to two viruses, hepatitis B (HBV) and hepatitis C (HCV).

Although the introduction of clotting factor concentrates transformed the lives of severe haemophiliacs to the point that in the late 1970s their life expectancy was close to that of the normal population,[6] this was not without its problems. The impact of the human immunodeficiency virus is well known but the importance of hepatitis C infection is less well recognised.

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Treatment of the Bleeding Disorder

The treatment of bleeding in haemophiliacs has changed enormously over the last 40 years with the successive use of whole blood, plasma, cryoprecipitate and clotting factor concentrates. Although these processes increased the efficiency of providing a greater concentration of clotting factor in a progressively smaller volume they were associated with increased risk of transmitting viral infections as more, potentially infectious, donors were involved. The risk of viral transmission did not become a major problem until the introduction of concentrates prepared from many thousands of donors. Although transmission of hepatitis by concentrates was recognised during the early 1970s,[7] it was more than a decade later that measures to eliminate it from concentrates were introduced. Currently there are a number of viral inactivation processes used in the preparation of concentrates (Table 1). Although highly effective, reports of new viral transmissions associated with their use continue to occur.[8–11] The introduction of recombinant products will hopefully eliminate the risk of transmission of donor infection.[12]

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Table 1 Methods of viral inactivation of products currently available in the UK.

Dry heat 80°C 72 h
Solvent detergent treatment
Heat 20 h 60°C in n-heptane
Pasteurisation
Immunoaffinity Chromatography
Na thiocyanate treatment

The Hepatitis Viruses

Hepatitis A. Hepatitis A (HAV) is a non enveloped single stranded RNA virus normally transmitted by the faecal-oral route; a few rare cases of transmission by blood have been documented[13] when the blood was donated by a person in the acute (viraemic) phase of the disease. It does not lead to chronic hepatitis. Recently interest has been aroused following the reports of three outbreaks of hepatitis A associated with the use of a clotting factor concentrate which had undergone viral inactivation by a solvent detergent method;[14–16] a process that does not inactivate viruses that lack a lipid envelope such as HAV. Normann and colleagues using the nested polymerase chain reaction detected hepatitis A virus RNA in one of the implicated clotting factor concentrate batches.[17] If these cases are proven to be due to the clotting factor concentrate then vaccination using the newly available HAV vaccine[18] should be offered to all haemophiliacs likely to receive solvent detergent treated products. These reports of possible transmission of HAV by solvent detergent processed clotting factor concentrates have focussed attention on the limitations of this method of viral incativation for non-lipid enveloped viruses. Other non-lipid enveloped viruses include polio, parvo and coxsackie.

Hepatitis B and D. Hepatitis B is an enveloped double stranded DNA virus and was the first to be identified as a cause of post transfusion hepatitis. It can cause both acute and chronic hepatitis. 5–10% of acute cases develop into chronic hepatitis and of these, 25% develop chronic active hepatitis and ultimately cirrhosis and liver failure.[19] Although 44 to 90% of haemophiliacs who received non-virally inactivated clotting factor concentrates have serological evidence of previous hepatitis B infection,[20–22] very few are chronic carriers.[20–22] It is estimated that in the UK only 2 to 5% of haemophiliacs are HBsAg positive. The risk of new infection in haemophiliacs is very small as donors are tested for the presence of HBsAg and the concentrates undergo viral inactivation. Potentially infectious donors may still enter the donor pool however, due to very low levels of circulating HBsAg which fall below the sensitivity of the assays,[19] due to HBV pre-core mutants[23] or due to the recently recognised HBV DNA positive group of HBsAg negative patients with high titre anti-HBc.[23] This together with a possible failure of the viral elimination process could explain recent cases of hepatitis B in haemophiliacs.[8,9] In an attempt to eliminate any possibility of new HBV cases, all haemophiliacs with absent markers of previous infection should be vaccinated with either the plasma derived or recombinant HBV vaccine.[24] The effectiveness of HBV vaccination has been demonstrated in controlled studies of at risk populations such as homosexuals[25] and haemodialysis patients.[26]

Hepatitis D is a defective single stranded RNA virus that requires the surface antigen of the hepatitis B virus to replicate and infect the host. Originally recognised in Italy,[27] its occurrence has been demonstrated worldwide.[28] Rizzetto and colleagues have reported that in Italian HBsAg positive haemophiliacs 49% of adults and 25% of children are co-infected with hepatitis D.[28] Because of the low incidence of chronic HBV carriage in the UK, chronic liver disease due to the hepatitis D is rare. Patients with hepatitis D have more severe liver disease than patients with HBV alone.[29]

Hepatitis C. Ever since the availability of assays for the detection of hepatitis A and B it was recognised that at least one other virus existed which was responsible for the majority of post transfusion hepatitis, hence the name non-A non-B hepatitis (NANBH).[30] This virus has now been fully cloned and sequenced.[31,32] It is a single positively stranded RNA virus of around 10 000 nucleotides and 33 nm in size.[33] It shares aminoacid and nucleotide homology with the flaviviruses such as yellow fever virus and with pestiviruses such as hog cholera virus.[34] A number of groups round the world have sequenced different HCV viral strains and these are now divided into three different subgroups.[34] As one would expect from a single stranded RNA virus it undergoes frequent mutations. Some areas of the virus appear to mutate much more readily than others and one such hypervariable region between amino acid positions 384–414 has been identified (Fig. 1). This rapid mutation rate could explain how the virus escapes host immunological surveillance and the development of chronic infection.[35] The development of chronic infection is the remarkable feature of HCV infection and occur in 50 to 85% of infected subjects.

The first assays for the detection of this virus became commercially available in 1989 and were

Table 2 Hepatitic viruses transmitted by blood and blood products

Virus	Acute hepatitis	Chronic hepatitis
Hepatitis A	+	−
Hepatitis B	+	+
Hepatitis C	+	+
Hepatitis D	+	+
Hepatitis E*	+	−
Cytomegalovirus	+	−
Epstein Barr virus	+	−

*Theoretical. No reports of transmission by blood.
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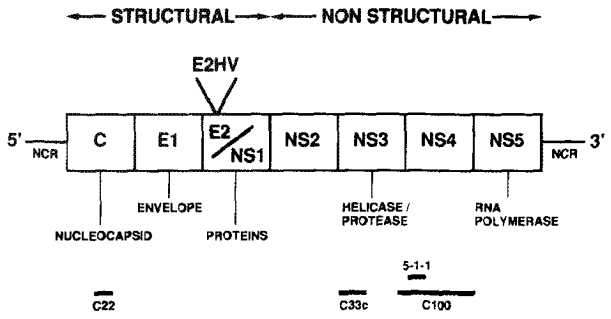

Fig. 1 The hepatitis C genome showing the structural (C, E1, E2/NS1), non-structural (NS2–5) and hypervariable region (E2HV) which is located within the E2/NS1. The two ends of the genome are flanked by non-coding regions (NCR). The lower part of the diagram shows the proteins used for HCV antibody testing and their relationship to the genome. (Reproduced with kind permission of the publisher, Churchill Livingstone, Edinburgh, from Bloom and Thomas, Haemostasis and Thrombosis, 3rd Edition).

based on the C100 antigen (first generation assays) (Fig. 1). The C100 antigen was obtained from the expression of part of the NS4 region of the HCV genome in a yeast vector.[32] Antibodies to this in haemophiliacs ranged from 59 to 85%.[36,37] Reasons for these differences included different patient selection criteria as well as different assay systems. False positive results were common with the first generation assays.[38,39] The next generation of tests incorporated structural antigens as well as the C100 antigen and these assays increased the sensitivity and specificity of the test. With second generation tests, haemophiliacs treated with non virally inactivated concentrates had an almost 100% incidence of antibodies[40,41] to this virus as would have been predicted from studies in Oxford and London where an incidence of 100% of NANBH was identified in the patients treated for the first time with these products.[42,43] A further advantage of the second generation assays is that they become positive earlier in acute HCV infection. These second generation assays are now in routine use world-wide, screening every donation that goes into the pool to produce the concentrates. At the same time as the second generation ELISA assays were introduced a recombinant immunoblot assay system (RIBA-2) became available for confirmation of positive results. In this system four different recombinant HCV antigens (C22, C33c, 5–1-1 and C100) are used thus increasing the specificity of the assay.[44] In haemophiliacs all Elisa positive results confirm on RIBA-2 testing suggesting that in this setting false positives are uncommon.[40,41]

The antibody tests indicate past infection and do not distinguish between ongoing infection and immunity. There are currently no antigen detection assays available but the polymerase chain reaction (PCR) has been used to detect circulating viral RNA.[45] The use of nested primers for PCR amplification increases the sensitivity and specificity of the technique.[46] In view of the heterogeneity of the different strains of HCV the choice of primers is critical.[47,48] Fortunately the 5′ end of the HCV genome is very highly conserved with >99% homology between all viral strains so far reported[49] so this offers a reliable system for the detection of HCV RNA. Pozzato and co-workers have recently shown a relationship between HCV genome structure and clinical outcome.[50] The different HCV genotypes may also be important in the rate of response of patients to interferon.[51]

In acute NANBH, HCV viraemia can be demonstrated using PCR within 2 weeks of infection which is several weeks before the appearance of HCV antibodies[52,53] (Fig. 2). Using PCR it has been demonstrated that most haemophiliacs with antibodies to HCV are in fact viraemic indicating ongoing infection, a situation reminiscent of HIV infection.[54,40]

PCR for HCV has also been used for the detection of HCV virus in FVIII concentrate, the presence of which strongly correlates with infectivity.[55] The development of quantitative PCR offers the exciting possibility of monitoring and modifying treatment with interferon based on the concentration of circulating virus.[56]

Other Hepatitis Viruses

Hepatitis E has recently been identified and cloned.[57] It causes epidemic non-A non-B hepatitis in 'developing world' countries.[58] It is usually transmitted via the enteral route and we are aware of no data suggesting transmission by blood or blood products.

Although on theoretical grounds other viruses which cause hepatitis such as Epstein-Barr, cytomegalovirus and herpes simplex viruses, can be transmitted by clotting factor concentrates, their importance is likely to be small as transmission is usually associated with cellular products. No clearly documented cases of transmission of these viruses have been reported and they do not cause chronic hepatitis.

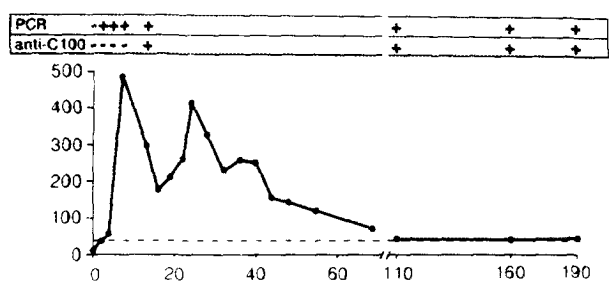

Fig. 2 Acute hepatitis C in a haemophiliac after first exposure to FVIII concentrate. HCV RNA was detected in serum at 2 weeks and seroconversion for anti-C100 antibody occurred at 13 weeks. This patient continues to be viraemic despite normal ALT. (Reproduced with kind permission of the publisher, The Lancet from Garson J A, Tuke P W, Makris M et al. Demonstration of viraemia patterns in haemophiliacs treated with hepatitis C virus contaminated factor VIII concentrates. Lancet 1990; 336: 1022–1025.

Assessment of Incidence and Severity of Liver Disease

It was recognised soon after the introduction of clotting factor concentrates in the early 1970s that a number of patients developed jaundice.[7] In carefully conducted studies the incidence of subclinical hepatitis was found to be much greater than those developing jaundice. Fletcher in Oxford in 1983 found that all 9 patients receiving clotting factor concentrate for the first time developed non-A, non-B hepatitis[42] and Kernoff in London in 1985 confirmed this, reporting the development of hepatitis in 19 of 21 patients treated for the first time.[43] In both the Fletcher and Kernoff studies, all patients with hepatitis developed NANBH and not hepatitis B. Overall, although only 10% of the patients developed jaundice following first exposure, almost 100% developed hepatitis.

The remarkable feature of NANBH/HCV infection is its tendency to develop into chronic hepatitis. After an episode of acute hepatitis a substantial proportion of patients develop chronic liver disease and a number of groups have attempted to quantify this. The differing reported incidence of 45 to 81% relates at least in part, to differences in methods of analysis.[59–62] Fluctuating liver enzymes are a characteristic feature of chronic HCV related liver disease; thus studies based on single determinations[59] are likely to indicate a lower incidence than those in which serial results have been determined.[60–62] The largest study is that reported by Cederbaum and colleagues who studied 1332 US haemophiliacs. On the basis of their last 3 ALT estimations 26% of these subjects had persistently raised ALT, 47.5% intermittently raised and 28.9% normal ALT.[60]

Measurement of liver enzymes released in the circulation is an indirect method of assessing liver damage and does not correlate with severity.[63] The most accurate way of assessing severity is liver biopsy which, in haemophiliacs, were first performed in the late 1970s. Most biopsies showed chronic persistent hepatitis (CPH) which by analogy with autoimmune chronic active hepatitis was considered to be mild and non-progressive. A significant proportion of patients however, were found to have more serious liver disease such as chronic active hepatitis (CAH) and cirrhosis[61,64,65] (Fig. 3). Some of the observed differences were undoubtedly due to different selection criteria for biopsy. Some groups biopsied patients with persistently abnormal transaminases,[61,64] whilst others biopsied those with intermittent abnormalities[66–68] or a combination of the two.[69]

There is considerable debate on the natural history of the hepatitis. Mannucci and colleagues found no evidence of progression of chronic liver disease in rebiopsied Italian haemophiliacs.[66,70] Similar conclusions on the lack of progression were drawn by White and co-workers in the US and by Stephens et al in the UK. In the US study, CPH or mild inflammation was observed in 14 of the 15 biopsied

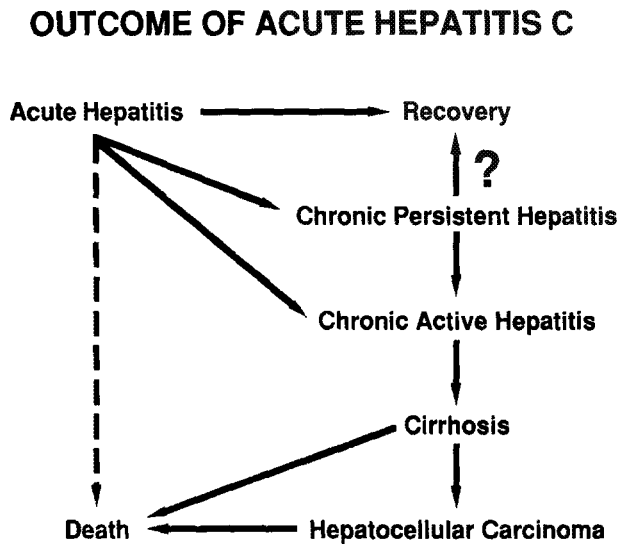

Fig. 3 Diagram showing the possible outcomes of acute hepatitis C. (Reproduced with kind permission of the publisher Churchill Livingstone, Edinburgh, from Bloom and Thomas, *Haemostasis and Thrombosis*, 3rd Edition).

patients.[67] Similarly Stevens et al[68] in the UK also found no serious liver disease. These studies suggested that chronic hepatitis in haemophilia is a mild disease and is not associated with any serious morbidity.

These observations were at variance with those reported by Hay et al[71] and Schimpf[69,72] from the UK and Germany respectively. In the report by Hay et al[71] from Sheffield, progression to serious chronic liver disease (CAH/Cirrhosis) was observed in 6 of 9 patients with persistently abnormal liver enzymes. In 2 patients, progression from CPH to cirrhosis was observed after 48 and 67 months.

A further recently recognised complication of chronic HCV in haemophiliacs is hepatocellular carcinoma. Colombo and colleagues[73] from Europe and the US found 10 patients with hepatocellular carcinoma in a population of 11 801 haemophiliacs. The rate of hepatocellular carcinoma was 3.2/100 000 patients per year which is at least 30 times the background incidence of this tumour in the patients countries of origin.[73]

Evidence is now available on the rate of disease progression of NANBH/HCV in patients who acquired the infection following blood transfusion. In Italy, Tremolada and colleagues found that 32% of patients biopsied on average 51 months after an episode of post transfusion hepatitis had cirrhosis and when patients with an initial histological finding of chronic hepatitis were re-biopsied 35 months later 50% of these had progressed to cirrhosis.[74] In Japan, Kiyosawa and co-workers reported that the rate of progression of chronic hepatitis was relatively slow with a mean time to cirrhosis of 17 years and to hepatocellular carcinoma of 23.4 years after transfusion.[75] Thus, in the setting of post-transfusion hepatitis, chronic NANBH does progress to severe liver disease but the interval from exposure to development of cirrhosis is long. This slow pro-

gression of chronic HCV related hepatitis could explain the differences in conclusions arising out of liver biopsy studies in haemophiliacs; most of the patients would have been infected in the early 1970s coinciding with the introduction of clotting factor concentrates. Thus, the first biopsy studies were undertaken at a relatively early stage in the development of chronic hepatitis.

There is an understandable reluctance to perform liver biopsies in haemophiliacs and consequently, a number of non invasive methods of assessing chronic liver disease have been attempted. Using CT scanning, Miller and colleagues found splenomegaly in 28/47 haemophiliacs with raised transaminases and collateral veins (implying portal hypertension due to cirrhosis) in a 25% of these.[76] Dynamic tests of liver function such as galactose elimination and bromsulphthalein clearance have been considered but in haemophiliacs early promising results[68] have not been confirmed. Serum procollagen III peptide is a marker of hepatic fibrosis, but although levels in haemophiliacs were higher than in controls they did not correlate with histological severity.[77] A stepwise increase in serum IgG has been reported in UK haemophiliacs correlating with disease severity.[63] This however is often complicated by co-infection with HIV which is associated with hypergammaglobulinaemia in the absence of hepatitis.[63]

Morbidity and Mortality Due to HCV in Haemophilia

We are aware of no publications directly addressing the problem of morbidity from liver disease in haemophilia, but limited mortality data and reports of liver transplantation in haemophilia lend support to the view that chronic liver disease in haemophilia is an emerging problem.[78–80]

There are varying reports of the incidence of mortality from liver disease in haemophilia. In a report from the US, Aronson reported that of 949 haemophilic deaths during the period 1968 to 1979, 8 were due to hepatitis and that in 8% of all cases cirrhosis was the primary or associated cause of death.[78] Also in the USA, Eyster and colleagues followed 79 patients with lymphocytopenia and thrombocytopenia and found that the cause of death was cirrhosis in 5 of the 10 who died in the 5 year observation period.[79] More recently Eyster and colleagues found that 18 of 164 (11%) of all deaths in Pensylvania during the period 1976 to 1991 were directly due to liver disease.[80] In Germany, cirrhosis accounted for 17% of all haemophilic deaths during 1978 to 1987.[81] Lower liver associated mortality has been reported from the UK (2% of deaths during 1976–1980)[6] and Sweden (4% of deaths in 1957–1968 and 9.1% of deaths during 1969–1980).[82] Most of the deaths in these reports occurred within 10 to 15 years of infection with the hepatitis (assuming patients were infected with the introduction of concentrates in the early 1970s). Since significant mortality increases after 15 to 25 years[75,83] we can expect that in the future liver associated mortality will be higher.

Treatment of Chronic Hepatitis C

In an attempt to alter the natural history of chronic HCV-related liver disease, a number of therapeutic agents have been tried. Of these, only interferon has shown real promise; steroids,[84] acyclovir[85] and inosine prabonex[86] have all been shown in controlled studies to be ineffective.

The early success of interferon in the treatment of patients with chronic hepatitis B, prompted Hoofnagle and colleagues to study this agent in chronic transfusion related NANBH. In an uncontrolled study they found that 8 of 10 patients treated with recombinant interferon alpha at 3 million units (mu) thrice weekly normalised their previously persistently abnormal transaminases.[87] A large number of publications of controlled and uncontrolled trials of interferon for chronic NANBH have confirmed the initial observations of Hoofnagle. The largest trial to date is a multicentre US study of 166 patients with chronic hepatitis C.[88] Patients were randomised to receive either thrice weekly subcutaneous interferon alpha at 3 mu or 1 mu or no treatment for 6 months. Complete response was seen in 38% of patients treated with 3 mu compared with 16% treated with 1 mu and 4% in the no treatment group. However, on discontinuing interferon 50% of the 3 mu and 44% of the 1 mu groups relapsed.[88] A recent meta-analysis of all the published studies supports the value of interferon in chronic hepatitis C;[89] overall the commonest regime used was 3 mu thrice weekly which is usually associated with a 50% response rate. Unfortunately there is approximately 50% relapse on stopping the treatment. Thus 25% of patients treated can expect to achieve long term remissions off interferon.

Experience in haemophilia is much more limited. Lee et al treated 2 haemophiliacs both of whom responded but relapsed on stopping treatment.[90] We conducted a randomised controlled liver biopsy trial of recombinant interferon alpha 2b given subcutaneously 3 times weekly in haemophiliacs. Our findings were almost identical to those in non haemophiliacs with chronic HCV, with 56% normalisation of ALT and 56% relapse on stopping treatment (Fig. 4). Although the number of patients was small, HIV positive patients responded as well as HIV negative ones and histological improvement was seen in some patients who did not normalise their liver enzymes with interferon.[91]

The aim of treatment of chronic hepatitis C with interferon is to alter the natural history of the disease. We will not know if this ambitious aim has been met for many decades. In the meantime the facts that interferon reduces hepatic inflammation as shown by the transaminase normalisation, improves liver his-

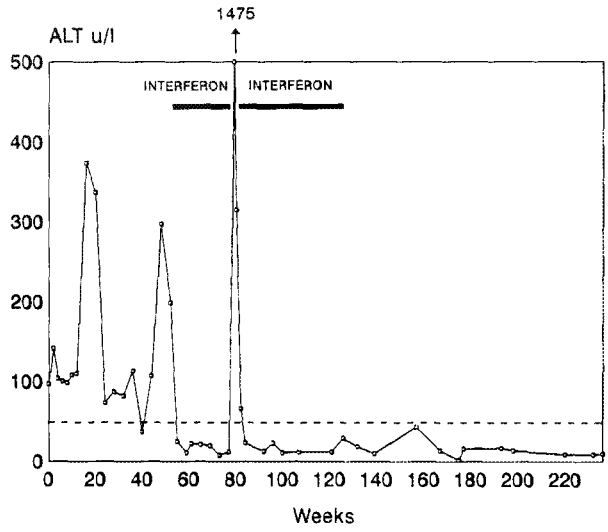

Fig. 4 Normalisation of alanine aminotransferase in a haemophiliac with chronic hepatitis C treated with interferon. Early relapse after a first course on interferon was treated with a second longer course of treatment. ALT has remained normal after the second course of interferon.

tology and eliminates circulating HCV viraemia as detected by the polymerase chain reaction[56] suggest that the ultimate aim may be achieved.

Recently a new agent Ribavarin has been undergoing trials in patients with chronic hepatitis C.[92] It is licensed for use in patients with severe bronchiolitis caused by respiratory syncytial virus. Although it has the advantage of oral administration it causes a mild haemolytic anaemia and responses so far appear to be inferior to those on interferon.

Liver Transplantation

For patients with advanced liver disease, liver transplantation offers the chance of increased life expectancy. In view of transplant related morbidity and mortality, careful selection of eligible patients is necessary. Although liver transplant for post-transfusion and community acquired non-A non-B hepatitis are common with a 5 year survival rate of over 70%,[93] to date there have only been 11 liver transplants reported in haemophiliacs. Liver transplants have been performed in patients with haemophilia A,[94,95] and B[96] as well as in 1 patient with type III von Willebrand's disease.[97] Survival in haemophiliacs appears to be similar to non haemophiliacs undergoing transplantation. An added advantage in haemophiliacs is that the transplant procedure results in a phenotypic cure as the new liver produces sufficient factor VIII/IX to raise the patients level into the normal range. HIV positive patients undergoing liver transplantation do not fare as well, as the medication given to prevent graft rejection results in further immunosuppression.[98] Another problem of transplantation for hepatitis C is that circulating HCV virus at the time of the transplant reinfects the new liver in a proportion of the patients.[95,99]

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