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Viral hepatitis and haemophilia

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Modern therapy with clotting factor concentrates has been dramatically successful in preventing and alleviating the worst effects of haemophilia. Before the mid to late 1980s, when effective methods of concentrate sterilization were introduced, such therapy was associated with a virtual certainty of transmission of viral hepatitis. Many patients who received intensive therapy before this time now have evidence of chronic and progressive liver disease, in which non-A, non-B agents are thought to be of dominant pathogenetic importance. Complex viral interactions involving both hepatotropic agents and HIV may occur in haemophiliacs, whose responses to infection may show atypical patterns. Interferon seems promising as a therapeutic agent. Vaccination against hepatitis B virus infection remains mandatory in patients without serological evidence of immunity.

HAEMOPHILIA AND BLOOD PRODUCT THERAPY

There are two types of haemophilia, A and B, which are identical in their clinical manifestations and modes of inheritance. In haemophlia A, the commoner variety, bleeding is caused by deficiency of factor VIII in the blood. In haemophilia B, factor IX is deficient. The clinical severity of haemophilia correlates with the level of circulating factor VIII/IX. Patients with severe disease (<2 u/dl) suffer from repeated, spontaneous and painful bleeding into muscles and joints which, if untreated, rapidly leads to crippling. Uncontrolled bleeding in other sites, such as the brain, causes premature death.

Although the first report of blood transfusion being used to treat

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haemophilia was as early as 1840,[1] effective therapy for the disease has only been widely available for the last 20 years. As recently as 1937, Birch described 113 patients with haemophilia of whom 82 died before the age of 15 years, often from trivial injury.[2] Fresh frozen plasma, the mainstay of treatment in the 1950s and 60s, improved this situation but it was the introduction of clotting factor concentrates from the late 1960s which revolutionized the treatment of haemophilia, realizing the prospect of a near normal life for many patients.

An early concentrate, cryoprecipitate,[3] currently accounts for less than 5% of factor VIII usage in the UK. It has the advantages of simplicity of manufacture and only low donor exposure for patients. The latter advantage may be lost in patients with severe haemophilia, who usually need very frequent transfusions. In most western countries, lyophilised clotting factor concentrates are now preferred for the routine treatment of haemophilia. These products are manufactured on an industrial scale, usually from plasma pools to which many thousands of donors have contributed. Their convenience and efficacy has led to a rapid escalation of usage (Fig. 1). Because demand usually outstrips the capacity of voluntary blood services, most countries remain dependent upon imported commercial preparations, largely derived from paid donors in the USA. Although not the case now, there is no doubt that in the past these commercial concentrates were associated

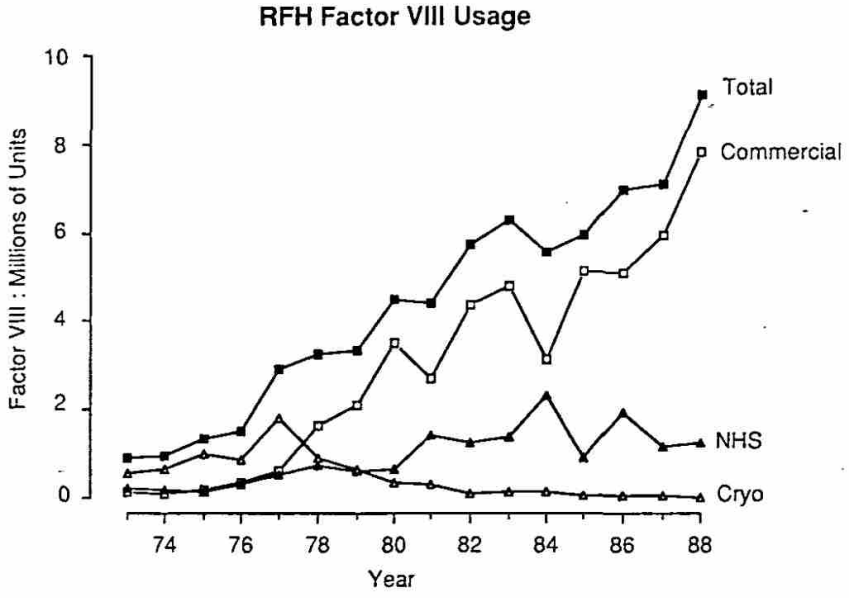

Fig. 1 Haemophilia-related blood product usage at the Royal Free Hospital.

with higher risks of viral transmission than products derived from voluntary donors.

EVIDENCE OF LIVER DISEASE IN HAEMOPHILIA

Transfusion associated jaundice was first described in the 1940s,[4,5] and became increasingly recognized as a complication of haemophilia treatment following the introduction of plasma product therapy in subsequent years.[6] By the 1970s, overall estimates of the rate of symptomatic acute hepatitis with jaundice in haemophiliacs ranged from 2–6% of treated patients per year, being particularly common in those patients who had previously received little or no treatment.[7–9]

Using the more sensitive but still nonspecific approach of assessment by biochemical liver function tests, many studies carried out since the mid-1970s showed a high prevalence of abnormalities (e.g. 50–90%), especially of serum transaminases, in populations of multitransfused haemophiliacs.[10–13] Although these abnormalities were not often severe, they were frequently persistent, suggesting (by definition) the development of chronic liver disease.

From the late 1970s, accumulating evidence from biopsy studies has confirmed that significant histopathological liver disease is not uncommon in haemophiliacs. In an international collaborative exercise described by Aledort and colleagues,[14] a panel of distinguished histopathologists examined samples obtained either by biopsy or at post-mortem from 155 patients from various parts of the world. By consensus classification, 15% of samples were judged to show cirrhosis and a further 7% chronic active hepatitis (CAH). Thus, 22% of patients had severe liver disease, and it was apparent that even patients without major abnormalities of liver function tests could have serious liver disease on biopsy. In smaller studies, including our own, the proportion of major histopathological abnormalities has been both higher and lower, probably reflecting sampling differences.[15–18]

Of perhaps even greater concern is the observation that supposedly relatively benign liver disease, such as chronic persistent hepatitis (CPH), may sometimes rapidly progress to serious disease. In one reported case, a single infusion of concentrate was followed by acute non-A, non-B hepatitis and then, 4 years later, by histologically proven cirrhosis.[19] Sequential biopsies in haemophiliacs have shown, in one series, progression of CPH to CAH

and cirrhosis in 4 of 6 patients within 4 to 6 years.[20] Although other groups have found a lesser rate of progression to serious disease,[21] it is clear that the course and progression of chronic liver disease in haemophiliacs may not necessarily follow patterns established in other patients. This is perhaps not surprising, in view of the unique nature of their therapy, and possibilities for multi-viral interactions.

There is little doubt that liver biopsy in haemophiliacs carries increased risks of bleeding. In one report cited above,[14] 12.5% of cases were complicated by bleeding necessitating prolonged hospitalization and/or increased concentrate usage, and there was a fatality rate of 1% compared to 0.01% in non-haemophiliacs. Efforts have therefore been made to find non-invasive methods of assessment of chronic liver disease. Using CT scanning with or without contrast, we found 28/47 patients (60%) with abnormal liver function tests to have splenomegaly, and 7/28 (25%) to have collateral oesophageal veins.[22] Serum procollagen III peptide levels may be helpful in the assessment of hepatic fibrosis.

NON-A, NON-B HEPATITIS

There can be little doubt that liver disease in haemophiliacs is most often a result of exposure to viral contaminants of blood and blood products. Hepatitis A virus (HAV) is only rarely transmitted by transfusion and never causes chronic disease. In contrast, non-A, non-B agent(s), hepatitis B virus (HBV) and hepatitis D virus (HDV; delta agent) may all contaminate blood products used to treat haemophiliacs and are all capable of causing serious problems.

In recent years it has become established that non-A, non-B hepatitis (NANB), a disease diagnosed by exclusion, accounts for a large majority of cases of post-transfusion hepatitis. Typically, acute NANB is clinically mild, but progresses to chronicity in a high proportion of cases. Undoubtedly, many cases of NANB, whether acute or chronic, may be asymptomatic. Therefore, reported frequencies based on clinical criteria alone lead to gross underestimates of prevalence, and it is of interest that Beeson commented in 1943 that 'the real frequency of this complication of transfusion (hepatitis) will be known only when there has been a concerted effort by physicians to recognize such cases'.[4]

Until the recent isolation of a blood borne NANB agent designated hepatitis C virus (HCV),[23] and the development of

serological tests for HCV, no reliable tests for NANB were available, and realistic estimates of incidence and chronicity rates could only be derived from prospective studies in which biochemical liver function tests, particularly transmináses, were monitored at frequent intervals. Chronic NANB is characterized by fluctuating abnormalities of these tests, and it can be difficult to distinguish recurrent acute attacks from chronic disease. Typically, frequently treated haemophiliacs show fluctuating low grade abnormalities of transaminases, not usually associated with clinical symptoms. The similarities between the clinical expressions and histological features of NANB and the liver disease found in haemophilia have led to the widely held view that NANB infection is responsible, to a sizeable degree, for the hepatic problems of haemophiliacs.[14,24]

In the USA, the incidence of acute post-transfusion NANB after treatment with non-pooled blood and blood products derived from commercial donors has been found in the past to average about 28%, compared with 7% after products derived from volunteers.[25] In the UK, the reported incidence after products obtained from voluntary donors is 2–4%.[26,27] Therefore, even on the basis of the lower reported attack rates in the UK, e.g. a 2.4% incidence after a mean exposure to 7.24 units of blood products,[27] it becomes virtually certain that a susceptible patient exposed to more than about 300 donor units of blood products will develop post-transfusion NANB. Since clotting factor concentrates are usually prepared from pools of several thousand donors, it would be expected that overall attack rates following transfusion of unsterilized products to previously untreated patients, i.e. those who would be unlikely to have acquired any resistance or immunity to infection, should approach 100%, whether the concentrates were of commercial or volunteer origin. Since this has been found to be the case in clinical studies,[28,29] it is probable that concentrates in use until the mid 1980s were invariably contaminated with NANB. In our studies, hepatitis was symptomatic in only 43% of the prospectively studied, and 12% of the retrospectively studied patients. Commercial products were generally found to cause more severe disease, with associated chronicity rates of 80%. It should be noted, however, that although relatively low exposure to volunteer donor cryoprecipitate may be accompanied by a lesser overall risk of NANB,[29,30] cryoprecipitate certainly has the capability to cause fulminant acute disease, and the resulting chronic disease may run a severe and protracted course.[31]

In the light of these observations, it is hardly surprising that a majority of haemophiliacs, particularly if they have been multi-transfused, should show evidence of chronic liver disease, and results of preliminary studies using the newly developed test for anti-HCV indicate, as might be expected, that a majority of heavily treated patients are seropositive.[32]

HEPATITIS B AND D VIRUS

The discovery in the mid 1960s of the 'Australian antigen', against which multitransfused haemophiliacs developed precipitating antibodies, led to the identification of the several specific serological markers which are now available for characterization of HBV infection, and the recognition that HBV was a cause of transfusion-transmitted hepatitis. Although a large majority of intensively treated haemophiliacs have serological evidence of previous HBV infection—over 90% in one of our own recent series[33]—most acute infections are probably subclinical, and followed by a state of acquired immunity. Only a small minority of infected patients have in the past become chronic carriers of HBsAg, with or without anti-HBe. In our own practice, the prevalence of HBsAg carriers amongst severely affected patients is 5%. Half these patients carry HBeAg. Coincident HIV infection has the potential to increase this proportion.

Because of their repeated and massive exposure to viruses other than HBV, it is difficult to know the extent to which chronic liver disease in haemophiliacs with HBV markers is attributable to this agent. In the majority of patients with detectable anti-HBs/HBc, this is perhaps unlikely, although it has been suggested that the persistence of HBV in hepatocytes, despite the presence of circulating antibodies, may indicate a pathogenetic role for HBV. Undoubtedly, however, coincident infection with other viruses, and massive exposure to other contaminants of concentrates, may modulate the effects of HBV and cause, for example, the delayed appearance of serological markers[34] (Fig. 2) which Brotman and colleagues previously observed in experimental animals,[35] and attributed to NANB interferon induction. In this respect, coincident infection with the human immunodeficiency virus (HIV) may assume increasing importance, and the course of HBV infection in multi-transfused haemophiliacs may clearly be expected to differ from that in 'normal' individuals.

Hepatitis D virus (HDV, delta agent) requires the presence of

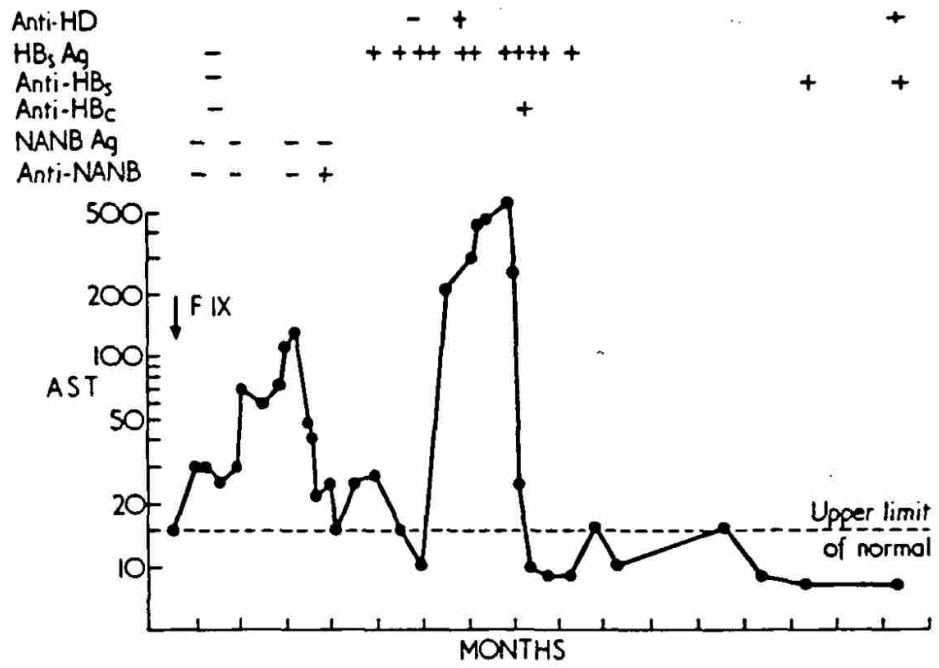

Fig. 2 Acute HBV and NANB infections in a patient with haemophilia B following a first exposure to NHS factor IX concentrate. NANB Ag and anti-NANB refer to results of an experimental system of serological markers[56] (Source: Lee et al, 1985[34]). Reproduced by permission of Elsevier Science Publishers.

HBV for its replication, and superinfection with HDV can occur in either acute or chronic HBV disease. Like HBV, HDV infection may be transmitted by blood and blood products, and is well documented in haemophiliacs.[36] The patient whose course is shown in Fig. 2 seroconverted for anti-HDV after receiving only a single infusion of NHS factor IX concentrate. Thus, the agent is clearly present in the UK volunteer donor population.

MULTI-VIRAL INFECTIONS

As noted above, haemophiliacs may become the sites of complex viral interactions, and it is sometimes difficult to disentangle the modulating influences which different hepatotropic viruses have on each other. This problem is illustrated by the patient whose course is depicted in Fig. 3.[34] He was first exposed to factor VIII concentrate in 1976, when he developed acute 'non-B' hepatitis. He continued to receive factor VIII, and in 1977/78 had a presumed sub-clinical attack of HBV infection which resulted in him becoming a HBsAg/HBeAg positive carrier. In early 1980, he seroconverted from HBeAg to anti-HBe and this was accompanied

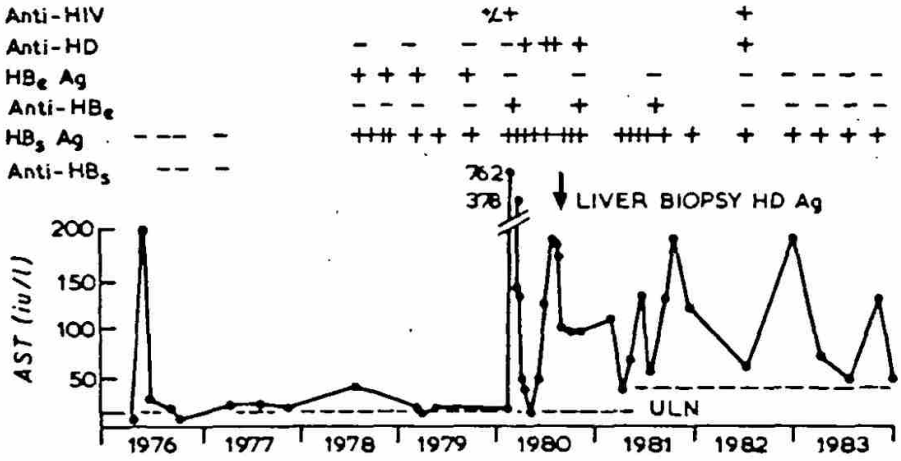

Fig. 3 Multi-viral infection in a patient with haemophlia A. For explanation, see text. (Source: Lee et al, 1985[34]). Reproduced by permission of Elsevier Science Publishers.

by a severe exacerbation of his hepatitis which was attributed to clearance of virus from infected hepatocytes. Retrospectively, however, anti-HDV became detectable shortly after this episode, and it seems more likely that the exacerbation was due to superinfection with HDV. This conclusion was supported by the biopsy finding of changes of acute hepatitis, despite the two year period which had elapsed since the first detection of HBsAg, and the observation that the biopsy sample was seropositive for HDV antigen. It may also be relevant that the patient was later to have seroconverted for anti-HIV at the onset of his exacerbation of hepatitis. It seems possible that he acquired HDV and HIV from the same batch of concentrate, and that a coincident HIV infection may have contributed to the severity of the exacerbation.

STERILIZATION OF CLOTTING FACTOR CONCENTRATES

Despite the application of progressively more stringent donor screening procedures, it must remain virtually inevitable, because of their origin from many thousands of donors, that source plasma pools used for fractionation contain contaminating viruses. Over the last decade, attention has therefore focused upon possibilities for viral removal/inactivation. Most commonly, the approach taken has been to apply a sterilization procedure, such as heating, to the final product. More recently, improved fractionation technology—for example, using monoclonal antibodies—has been

used to separate clotting factors from contaminants during the production process. The two general approaches may of course be complementary, and many variations on these themes are used by different manufacturers.

Whatever process of viral removal/inactivation is adopted, there remain two major difficulties. Firstly, all such processes have a detrimental effect on the final yield of clotting activity in the concentrate and, in general, the more effective the process of decontamination, the greater the yield loss. For example, 'wet heating' procedures (heating in solution, pasteurization), which are amongst the most efficacious as regards viral inactivation, can result in 30–40% loss of activity.[37] Such major losses of activity not only increase production costs, but also create a requirement for much larger quantities of source plasma, a source commodity. This causes problems in both commercial and voluntary sectors.

The second difficulty is that of proving the efficacy of sterilization procedures and the safety of final products. Where tests for transmissible agents are available, as they are for HBV and HIV, processes can at least partly be validated by in vitro experiments. Until recently, this has not been possible for NANB agent(s). Because the results of studies in experimental animals have also proved to be imperfect predictors, it has become widely accepted that reliable evidence of product safety can only be obtained by prospective and frequent biochemical and serological evaluation of 'first exposure' recipients, using the historical control of experience with unsterilized concentrates. There are many difficulties in mounting such studies, not least those of patient accrual. Certainly, however, valid conclusions can only be drawn if there has been adherence to a stringent protocol.

The development of clotting factor concentrates free from the risk of viral contamination is in a process of evolution, the process being a slow one because of the need to evaluate new methods and products by clinical trial. Formal 'seals of approval' from regulatory authorities have generally lagged far behind scientific advances, and treating physicians and patients have been faced with the difficult and often seemingly impossible task of juggling safety, cost and supply of these essential drugs in an increasingly litigious environment clouded by scientific uncertainty.

The impetus provided by the recognition of AIDS has, however, brought the issue of blood product transmission of other pathogens under closer scrutiny, and it has become clear that while some earlier processes, such as conventional 'dry heating' (heating

in the lyophilized state), were largely effective against HIV, they were only minimally or partly effective in reducing the risk of hepatitis transmission.[38–42] To a large extent, these processes have now been superceded and concentrates now in use, prepared by 'super dry heating', 'wet heating', solvent-detergent technology, vapour heating under pressure, and monoclonal immunoabsorption, all seem likely to have a very high margin of safety in both respects.[43–46] Clearly, however, complete absence of risk is an impossibility, and assurance of safety can only come from a continued high level of surveillance. It seems unlikely, at least in the short term, that recombinant technology will provide easy or cheap solutions to the many problems of replacement therapy in haemophilia, although it is of course anticipated that transmission of HIV and hepatitis viruses will be eliminated.

HEPATITIS B VACCINATION

Despite the advances which have been made in donor screening and concentrate sterilization, haemophiliacs continue to be at above average risk of acquiring HBV infection through transfusion therapy. This is not only because of the residual risks of concentrates, but also because other therapeutic products, such as cryoprecipitate and the cellular components of blood, cannot be effectively sterilized. As an additional method of risk reduction, vaccination against HBV is therefore regarded as mandatory in patients without serological evidence of immunity.

Hepatitis B vaccine is conventionally given intramuscularly, but because this route may cause serious bleeding in haemophiliacs subcutaneous administration has generally been preferred, and is well tolerated.[47–49] Both the magnitude of the response and the percentage of responders are higher in younger age groups, and we have found children to respond very well to intradermal vaccination using one tenth of the normal adult dose.[48] Although it was initially thought that a protective level of anti-HBs (>10 iu/l) would usually be retained for at least five years, evidence from haemophiliacs and other groups indicates that it may be lost much earlier. This is particularly so in anti-HIV positive patients (Fig. 4), who also show an impaired initial response to vaccination and accelerated loss of naturally acquired anti-HBs. It is important to note that anti-HIV seropositive patients known to have been previously anti-HBc positive remain at risk of acquiring HBV infection by transfusion,[50] since there is good reason to suppose

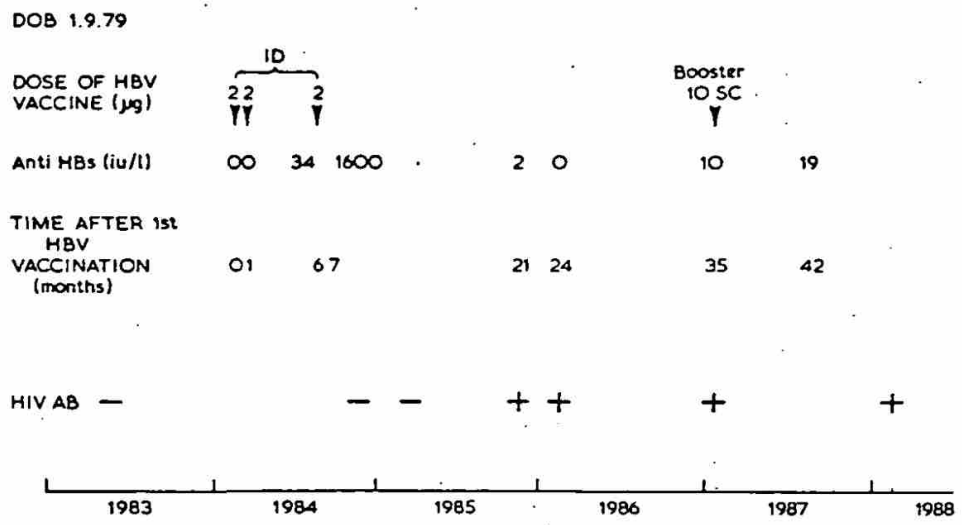

Fig. 4 Anti-HBs levels in a child with haemophilia A who became anti-HIV seropositive after hepatitis B vaccination (Source: Miller et al, 1989[48]). Reproduced by permission of Alan R Liss Inc.

that coincident HBV/HIV infection may carry a particularly poor prognosis.

For these reasons, our policy is to check anti-HBs levels in all our vaccinated patients at yearly intervals, with a view to maintaining levels above 10 iu/l. In poor responders to conventional doses of vaccine, and in some patients with low levels of naturally acquired anti-HBs, a booster injection or a repeated course using a double dose of vaccine is given, and it should be noted that it may be necessary to give booster doses at much more frequent intervals than in the average vaccinee.

POSSIBILITIES FOR TREATMENT OF ESTABLISHED DISEASE

Interferon and liver transplantation

Effective methods of prevention having now been implemented, the main problem for haemophiliacs has become the arrest and reversal of already established disease. Interest has centred on the use of antiviral agents, particularly interferon (IFN), which are given on the basis that termination of viral replication will be associated with a reduction in hepatocellular inflammation, and a therefore lessened risk of progression to irreversible cirrhosis and its complications. In chronic HBV infection, a relative immune competence is likely to be an important criterion for successful

antiviral therapy, and a favourable response seems less likely in anti-HIV seropositive patients. Using lymphoblastoid IFN to treat haemophilic carriers of HBsAg/HBeAg, we have confirmed this poor response in anti-HIV seropositive patients, which imposes a constraint on the application of this treatment in most haemophiliacs. In NANB, hepatocellular damage is more likely to be a direct result of viral replication rather than immunologically mediated injury, and a similar constraint may not apply. Low doses of alpha IFN have been shown to be capable of rapidly normalizing transaminase levels in patients with chronic NANB, including disease transmitted by plasma product therapy.[51,52] Initial results of studies in haemophiliacs seem promising.[53,54]

For those patients in whom irreversible hepatic damage has already occurred, antiviral therapy is clearly much less likely to be of benefit. Management of the complications of cirrhosis, particularly variceal bleeding, is usually much more difficult in haemophiliacs, and the prognosis in these circumstances is poor. Liver transplantation has been undertaken in several haemophilic patients, and has the apparent additional benefit of 'curing' their haemophilia.[55] However, the risks of the procedure are too great to consider it beyond the area of end-stage liver disease.

CONCLUSION

The seriousness of the viral hepatitis problem in haemophiliacs was underestimated in the past. Partly as a consequence of the emergence of AIDS, it is now recognized as a matter of dominant importance. Effective methods of prevention have now been implemented. The challenge for the future will be the development of successful strategies for treatment.

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