Edinburgh Haemophilia and Thrombosis Centre

Long term safety monitoring for transfusion transmitted infections

(with particular reference to HBV, non-A non-B hepatitis and HIV)

1974-1989

Draft

1. This document should be read in conjunction with 'Historical Summary of AIDS in haemophilia 1981 – 1985' (CAL15)

2. Historically the treatment of bleeding in haemophilia has been associated with adverse events. These include allergic reactions, haemolytic anaemia, antibodies to the deficient clotting factor, e.g., anti-factor VIII antibodies, and infections transmitted by blood, blood components and clotting factor concentrates. It is therefore important that patients are monitored for these complications of treatment.

3. The Edinburgh Haemophilia Centre has a long tradition, starting in the 1960s and 1970s, of systematically studying the bleeding pattern in those with haemophilia (1), describing arrangements at a Haemophilia Reference Centre (2) and was amongst the first to assess hepatitis and the risks of virus transmission with the initial studies on hepatitis B virus infection (3;4).

4. These studies in the 1970s were continued into the 1980s with a publication of liver function abnormalities in haemophilia (5) and a further follow up on the risk of hepatits B transmission from SNBTS clotting factor concentrates prepared from blood screened for hepatitis B infection. This demonstrated that despite screening of donors about 10% of susceptible patients became infected each year with hepatitis B virus (6). The implication of this was clear, that the then current screening tests for hepatitis B virus did not detect all infectious blood donations, and that patients should be encouraged to accept the hepatitis B vaccination when it became licensed in the mid 1980s.

5. As a component of our monitoring of NANBH, we collaborated in 1985/6 with Dr Robert Hopkins in SNBTS who was developing assays for hepatitis viral markers. He had developed a test which was initially considered might be specific for some forms of NANBH (7) and we undertook a pilot project to assess its utility in those with haemophila. Unfortunately on further investigation the marker was not found to be specific for NANBH.

6. To monitor treatment related adverse events (including infections), at clinic visits, blood would be taken for a full blood count (haemoglobin, white cell count including enumeration of the different types of white cells, and platelet count), assessment of blood chemistry, e.g. urea and electrolytes and liver function tests, assessment for the presence of an antifactor VIII antibody (inhibitor) and assessment for viral infection. These latter samples were stored long term in haematology and virology, as was customary for virology blood

samples from all patients. If there was evidence on any occasion of a possible new adverse event it was possible to retrieve the sample from the previous clinic visit and compare it to the latest sample. This was considered good clinical and laboratory practice (and made the interpretation of the laboratory results more accurate). This was not the arrangement in most laboratories in the UK because of the physical difficulty of storing many deep frozen samples.

7. AIDS was first reported in three patients with haemophilia in the US in 1982. Up to this time the cause of AIDS was not known but it had only been reported in homosexual men. In those with clinical AIDS immune tests revealed a severe deficiency of T helper (CD4) and an increase in T suppressor (CD8) lymphocytes. In addition many homosexual men, who had no symptoms suggestive of AIDS, were also found to have similar immune abnormalities although in a milder form. There were a number of differing explanations for the development of these immune abnormalities in this latter group of apparently well homosexual men, including the possibility of a virus which may have infected a large number of such men, but in only a small minority had it progressed to clinical AIDS.

8. In the light of the observation that may asymptomatic homosexual men had immune abnormalities, studies to assess the immune status of apparently well asymptomatic haemophiliacs were immediately undertaken in the US (summarized in Appendix 3). These demonstrated that there was a similar situation in those with haemophilia in that many asymptomatic individuals had similar immune abnormalities to homosexual men. Again the cause of these was unclear and furthermore it was uncertain whether they were progressive. Again, as in homosexual men, there were a number of possible explanations apart from an AIDS virus for the abnormal immune tests. (see 3).

9. The finding in 1982/3 of immune abnormalities in asymptomatic apparently well individuals with haemophilia in the US was perplexing and worrying. The cause of the immune changes was unknown but might have been related to the wide spread prevalence of an 'AIDS virus', or be due to some other side effect of factor VIII treatment, or it might even have been a previously undescribed feature of the condition of haemophlia.

10. With the possibility that people with haemophilia had apparent immune dysfunction, which might have been related to their treatment, might be progressive, and might lead to AIDS, I sought the help of a colleague Dr (now Professor) C M Steel at the Medical Research Council Unit at the Western General Hospital in Edinburgh. He generously established in his laboratory the facility to measure the proportion of CD4 and CD8 lymphocytes by microscopy in patients' blood. Similar studies were set up elsewhere in the UK, including haemophilia centres in Glasgow, Royal Free Hospital and Birmingham Children's Hospital.

11. When patients attended the Edinburgh haemophilia clinic for review, blood was taken for the investigations set out in para 6 above and was sent to the Haematology laboratory in the Royal Infirmary. The full blood count was

assessed in the usual way (except that instead of only counting 100 white cells under the microscope to quantify the number of the different types, 200 were enumerated to obtain a more accurate estimate of the number of lymphocytes, as they only form a relatively small proportion (approx 15-25%) of the total. The samples (labeled as 'AIDS study' – to identify them for different processing in the laboratory) were then couriered to Dr Steel's laboratory at the Western General Hospital where the proportion of CD4 and CD8 lymphocytes were measured. By this means it was possible to assess the proportion of each type of lymphocyte, as well as their absolute number, in the blood.

12. From our initial studies in 1983 what we observed, to our great surprise, was that the pattern of lymphocyte abnormalities in Edinburgh patients was similar to those in the US; yet none of the individuals had any symptoms or signs suggestive of AIDS. As the majority of patients had only received blood components, or products, prepared from Scottish blood donors, and there were at that time no AIDS cases in Scotland, it seemed rather unlikely that these lymphocyte changes were due to a possible ubiquitous AIDS virus. The cause of the immune changes in the Edinburgh patients was unknown but there were a number of possible explanations related the underlying condition of haemophilia and its treatment. It was imperative to monitor the patients because if the immune changes were becoming progressively more abnormal there might be a risk of their developing opportunistic infections, e.g. Pneumocystis carinii pneumonia, characteristic of AIDS.

13. Whilst contemplating these unexpected lymphocyte results, a letter appeared in the Lancet highlighting the immune abnormalities in the haemophiliacs in the US and stating that it would be very important to know if similar abnormalities were observed in those with haemophilia treated with blood products collected in a non-AIDS country (8). As I had such data it seemed important to submit it for publication because it would offer alternative explanations, other than wide spread infection by a putative AIDS virus, for the immune abnormalities observed in US haemophiliacs (9). This report concluded that the immune changes were likely to be due to 'infusion of foreign protein or a ubiquitous virus rather that a specific AIDS virus'.

    This preliminary report was followed by a more detailed description of our observations (10). The paper reported that the number of lymphocytes in the blood in haemophilia A was reduced with a reduction in Th/Ts ratio in half the patients' cells. In those with haemophilia B the immune changes were less marked with the number of Th cells being similar to controls, although the ratio of Th/Ts was reduced compared to controls due to higher level of Ts cells. The serum level of immunoglobulin IgG and IgA were raised and the IgG levels correlated to the ALT level (a measure of liver inflammation). The paper reviewed the possibility that these immune changes might have been due to some non-AIDS infection and/or chronic liver disease. The report concluded that the 'study had not identified the cause of the reduction of the Th but it is unlikely to be due to a specific AIDS virus in the blood products. It is more likely to result either from an as yet unidentified component of the therapeutic concentrates, or from a non-specific effect of foreign protein

infused intravenously. Because the reduction in Th is not dose related and because of it bimodal distribution in patients with haemophilia A, it seems that some patients are more susceptible to this immunological disturbance than others. What determines an individual's immunological response to transfusion remains elusive? Furthermore, the relation between lymphocyte subset abnormalities in symptomless haemophiliacs and the likelihood of eventual frank AIDS remains unclear although it may be connected with HLA status.'

14. At this time in 1983 it was unclear, in those with haemophilia, whether the lymphocyte changes we observed in Edinburgh were becoming progressively more abnormal and if so would serious clinical sequaelae follow. For this reason when patients attended the clinic, and blood was being taken for the routine tests, the lymphocyte subset numbers were also measured. The studies over the next two years demonstrated that although the lymphocyte numbers varied between individual patients the levels within each individual were reasonably constant and that there was not a consistent trend or change. This was reassuring (11;12).
15. By 1984 accumulating evidence indicated that AIDS was probably caused by a virus from blood donors, which was transmitted by clotting factor concentrates. By the autumn of this year Dr Richard Tedder, at the Department of Virology at the Middlesex Hospital, had established, as a research project, an anti-HTLVIII assay. This was an early antibody test, under development, for the detection of antibodies to the putative virus causing AIDS. He only had a limited supply of reagents and he was receiving many requests from other clinicians, but he agreed in October to test serum samples from 10 Edinburgh haemophilia patients. When the results were available he report to me that 3 of the 10 were anti-HTLVIII positive in his test. Our preliminary conclusion was that it was likely that these patients had been exposed to the putative AIDS virus. This was most unexpected because the patients had only been treated with factor concentrates prepared from plasma collected in Scotland where at this time there was only 1 known case of AIDS (reference). To investigate the situation, Dr Tedder agreed to test further samples from other patients. He found that a total of about 20 patients in Edinburgh were apparently anti-HTLVIII positive. Preliminary examination of the detailed transfusion records of the patents indicated that the simplest explanation to account for most of those who were anti-HTLVIII positive was to conclude that a single batch of concentrate given to patients in the Spring of 1984 was the source of exposure (McClelland 1984, (13). This was amongst the very first evidence that the UK blood supply had been contaminated by the AIDS virus.

16. These anti-HTLVIII data were immediately shared with senior SNBTS staff and haemophilia colleagues. This led to the urgent setting up of a meeting at SHHD with haemophilia directors and SNBTS on 29th November (para 11.208, ref 281).

17. Similarly a meeting was convened between UKHCDO Reference Centre Directors, UK blood transfusion colleagues and senior staff from Edinburgh and Elstree Protein Fractionation Centres at Elstree on 10th December 1984. At this meeting there was a major discussion about whether to introduce heat

treatment of concentrates in the UK. It was not an easy decision to make because of the possible adverse effects of heat treatment on the clotting factors and other proteins in the vials which could have had a range of adverse effects on patients. This was particularly difficult because it was very uncertain how effective the heat treatment would be at inactivating the HTLVIII virus to render it non-infectious. The decision was made, on very limited data, to heat treat NHS concentrates as soon as possible. If commercial concentrates were to be used only those that had been heat treated should be issued.

18. In Scotland SNBTS immediately made available 68 degree 2 hour dry heated factor VIII concentrate. Patients were invited to return the bottles of unheated concentrate they had at home and heat treated concentrate was given in exchange. This change over was complete by the end of December.

19. There was a need to inform patients of the latest information. The best way to do this was discussed and agreed between haemophilia directors in Scotland. It was decided to invite all patients to a meeting in Edinburgh on the evening of 16th December. It was anticipated that there might be very many patients and spouses and two lecture theatres were booked. At the meeting there were only about 30 people present. Dr Charles Forbes chaired the meeting, Dr Brian McClelland (Director Edinburgh Blood Transfusion Centre), Mrs Geraldine Brown (Haemophilia Social Worker) and I were present. We explained about the batch of factor VIII which appeared to have contained the HTLVIII virus and also that others who had received concentrate might have been exposed to the virus. We explained that there was some uncertainty in being able to interpret the anti-HTLVIII test result and what it might mean for individuals who were positive and negative. It was emphasized that everyone, irrespective of their anti-HTLVIII test result, should be careful with body fluids because they might be infectious. Any other person, e.g. spouse, cleaning up any spilled body fluids, especially blood, should do so using rubber gloves and the surface should be cleaned with diluted bleach. The possibility of sexual transmission was emphasized and all men were advised to use condoms during sexual intercourse, again irrespective of their individual anti-HTLVIII result. All patients present were encouraged, if they wished to know their anti-HTLVIII result, to make an appointment to see their haemophilia consultant to discuss their individual situation. At this time there was no specific anti-AIDS therapy which could be given to those who might be infected.

20. Following this open meeting for patients and spouses all patients were sent a letter and an information sheet about AIDS and the recommended safety precautions. All were encouraged to seek further information from their haemophilia consultant. General practitioners of all patients (irrespective of anti-HTLVIII result) were also sent a letter to inform that some patients with haemophilia had been exposed to the AIDS virus. As the individual anti-HTLVIII result for each patient was considered very sensitive and confidential information, especially because there was so much prejudice against people who were infected, general practitioners were not informed of the individual patient's result unless they gave consent. Furthermore neither the antibody result, nor any discussions or counseling in relation to a patient, was recorded in their medical case notes. This was also to protect patients from considerable

anxiety and prejudice amongst hospital staff who were concerned about their own personal safety.

21. Over the early months of 1985 most patients made appointments to be seen and most wished to know their anti-HTLVIII result. The uncertainties about its interpretation were explained. Irrespective of the antibody result the importance of the potential that body fluids were infectious was emphasized and therefore all men should protect their partner during sexual intercourse by using a condom.

22. To further investigate the possibility that there had been an HTLVIII contaminated SNBTS batch of factor VIII which had resulted in the development of the specific antibody, the following were undertaken;

    a. Firstly serial blood samples from patients who had become antiHTLVIII positive were retrieved from the deep freeze store in virology or haematology and their antibody status assessed. This way it was possible to demonstrate how long patients had been anti-HTLVIII positive and approximately when their test became positive. By this means it was possible to demonstrate that of the 31 patients who received the 'implicated' batch, 18 had been negative before receiving it and became positive some time later. This was very strong presumptive evidence of exposure to this batch.

    b. Secondly Dr McClelland reviewed in detail the transfusion records of all patients attending the haemophilia centre and all the batches of concentrates given during the first half of 1984. His report of this enquiry indicates that there was a very strong likelihood that batch 023110090 was the one which had resulted in the HTLVIII exposure (McClelland, 1984).

    c. Thirdly when the number of bottles of the concentrate received by each patient was reviewed against their HTLVIII antibody status, there was a clear indication of a dose effect, i.e. the more bottles received the greater the risk of being antibody positive. All patients who received more that 40 bottles were found to be anti-HTLVIII positive (13).

23. At this time, at the end of 1984 and beginning of 1985, as there was very considerable uncertainty about both the specificity and sensitivity of the anti-HTLVIII test result, interpretation had to be guarded. A positive result might be due to

    a. Active infection with HTLVIII
    b. Prior exposure to HTLVIII which had been cleared from the body, e.g., like following flu virus infection.
    c. Passive acquisition of antibody from the factor VIII concentrates, i.e., anti-HTLVIII from donor plasma – this was perhaps a possibility with NY because it was a low purity product containing a considerable quantity of immunoglobulin as a 'contaminant'.
    d. False positive, i.e., the patient had not been exposed to HTLVIII.

24. A negative anti-HTLVIII result could be interpreted as;

    a. The patient had not been exposed to the virus
    b. They had been exposed but the test was not of sufficient sensitivity to detect the antibody, i.e., a false negative
    c. The patient had been infected, produced an antibody, but with the passage of time its level had diminished to below the sensitivity of the assay.

25. With the anti-HTLVIII result being known on most patients and with the introduction of heat-treated factor concentrates in Scotland consideration was given as to how the patients should be followed up. It was decided for all patients to monitor their immune status as well as continue to assess the anti-HTLVIII and viral status. This approach would not only monitor the situation of each individual but it would provide evidence of the safety of the newly introduced heat-treated concentrates.

26. With the emergence in early 1985 of a relatively large number of HIV infected patients in Edinburgh, not only those with haemophilia, but also individuals belonging to other risk groups, needing to be cared for and assessed, arrangements and resources were required to provide clinical care, as well as monitor their immune and viral status. To meet this very considerable challenge a Lothian AIDS Advisory Committee was established, which I chaired, to recommend how clinical and laboratory services should be provided, and arrangements for staff safety promoted. Suddenly considerable resources were required and funds were sought from many sources, e.g. Lothian Health Board, Scottish Home and Health Department and Research funds especially to develop laboratory services. We were fortunate to obtain funding from the Scottish Home and Health Department and the Medical Research Council for the continued monitoring of the patients.

27. Although most of the investigations required for the patients were necessary for the continued clinical assessment, some of the virological investigations were especially important, particularly in the context of the 'implicated batch'. For example, were individuals who had received the 'implicated batch' and remained anti-HTLVIII negative infected or not with the virus? After a period of prolonged follow up we had substantial evidence that anti-HTLVIII negative individuals did not possess the virus. This provided very reassuring evidence for patients who knew they had been exposed to the 'implicated batch' but remained anti-HTLVIII negative (11;12). Additionally were anti-HTLVIII positive patients truly infected with the virus? Further studies confirmed that those who were anti-HTLVIII positive did possess the virus (see para29). Some of the investigations we were fortunately to be able to establish with research funds in 1985 were later to become routine for all patients infected with HIV. (see para 30 below).

28. With the continued monitoring over a period of time it became clear that patients negative for anti-HTLVIII at the beginning of 1985 remained negative despite it emerging later that some had been treated with batches of heat

treated SNBTS factor VIII concentrates which were later retrospectively found to have contained an anti-HTLVIII positive donation. Furthermore the lymphocyte subset numbers remained static in these individuals. This was very important information because it demonstrated that;

a. initial heat treatment introduced by SNBTS at 68C for 2 hours was probably effective in preventing infection possible because of the low HIV viral load in the plasma pool

b. heat inactivated HIV was not antigenic, i.e. it did not lead to the appearance of anti-HTLVIII, which was further evidence that anti-HTLVIII only appeared in response to active viral infection

c. the finding of static lymphocyte levels over a prolonged period of time was evidence that HTLVIII negative individuals were not infected with the AIDS virus. At this early stage there was concern that anti-HTLVIII negative patients might be either 'minimally infected' with the AIDS virus and had not yet produced a specific antibody response, or were truly infected and had not produced an antibody response. It is now known that relatively early in HIV infection individuals can have the virus in their circulation before the immune system produces a specific antibody response

d. it was reassuring that in anti-HTLVIII negative individuals with immune abnormalities that these were not 'deteriorating' and that the patients might be sliding into AIDS (due to non-HIV mechanisms).

e. a correlation could be demonstrated between depression of cell mediated immunity and the annual amount of factor VIII received. The study concluded that 'alloantigen or non-HIV viral exposure due to repeated administration of factor concentrates brings about alterations in the immune response, and that these changes are more marked following exposure to intermediate purity factor VIII compared with factor IX concentrate' (12). Edinburgh studies continued in this area (14). Other workers investigated possible non-viral mechanisms by which clotting factor concentrates might alter the recipient's immune system, which led to the suggestion that TGFbeta might be the 'immunologically active' 'contaminant' (15) although a subsequent Edinburgh study did not support this proposal (16). Further work elsewhere, however, did suggest that TGFbeta was a significantly immunomodulatory component of factor VIII concentrates (17) and that furthermore that immune modulation by plasma-derived clotting factor concentrates may reduce the risk of anti-factor VIII inhibitor formation in response to factor VIII infusions (18). This is still a actively researched topic as evidenced by the global Sippet study which is comparing the incidence of anti-factor VIII inhibitor appearance with plasma-derived, when compared to recombinant, factor VIII therapy (www.sippet.org).

29. The continued monitoring of the anti-HTLVIII positive patients demonstrated that in these individuals the CD4 lymphocyte numbers continued to decline. This was further evidence that being anti-HTLVIII positive was associated with active viral infection. The careful monitoring of these patients demonstrated that the virological and and specific immune responses varied markedly between individuals but several components of the immune system were identified as being associated with immune activation and faster clinical progression (19-23). The investigations also demonstrated that those individuals with a particular tissue type, A1B8DR3, progressed to clinical AIDS more rapidly and had higher levels of HIV virus in their blood (24).

30. There were 3 patients in Edinburgh who became anti-HTLVIII positive who did not receive the 'implicated' batch and who had only been treated with NHS products. It has not been possible to identify further their source of their infections.

31. In summary individuals with haemophilia attending the Edinburgh Centre were monitored long term clinically and by laboratory tests for hepatitis B and HIV viral infections, immune function and the clinical effects of non-A non-B hepatitis, during the period 1974-1989. The arrangements for the long term clinical care of both anti-HTLV positive and negative individuals are described elsewhere.

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