AIDS: A problem for the transfusion service?

John A J Barbara/Marcela Contreras/Patricia Hewitt, North London Blood Transfusion Centre, Middlesex

The first case of AIDS associated with blood tranfusion was described in 1982. Over the following two years great advances were made in the understanding and prevention of this new hazard associated with blood transfusion, culminating in 1985 with the introduction of a screening test for blood donation.

The realization that the acquired immune deficiency syndrome (AIDS) and HTLV-III/LAV infection can be transmitted to patients receiving transfusions of blood and its components, or products made from blood, has had a great impact on health services in general and on tranfusion services in particular.

One of the earliest signs of concern in the health services was a sensible drop in the number of non-essential tranfusions in some hospitals. On the other hand, from the recipient's point of view, there was a sharp increase in the number of requests from patients for blood from specified donors — relatives and friends — so-called "directed donations". In the National Blood Transfusion Service, these logistically impractical requests were vigorously resisted by pointing out their disadvantages, especially the constraint placed on the person "chosen" to donate the blood if he or she happened to be in a high-risk group for AIDS and did not want that fact to be known. Self-deferral from blood donation is practically impossible if you have been singled out as a "safe donor" by a worried patient!

Transmission by transfusion was clearly shown by Curran et al (1984), and more recently by Feorino et al (1985), and was one of the most potent arguments for a viral aetiology of AIDS prior to the actual isolation of the agent responsible, HTLV-III. In these studies, cases of carefully authenticated AIDS were identified where transfusion of blood or blood products was the only known risk factor. After careful interviews, homosexuality, intravenous drug-abuse, membership of ethnic groups at increased risk of AIDS and, in the case of children, infected parents or parents in high-risk groups were shown to be extremely unlikely. The blood donors involved in these transfusions were traced and studied; where investigations could be completed at least one donor in a "high-risk" group could be implicated in each case. Subsequently, the availability of specific serological tests confirmed the findings of transfusion-transmitted HTLV-III infection.

Undoubtedly, the human T cell lymphotropic virus type III (HTLV-III), which in a proportion of infected people causes symptoms of AIDS (12%) or AIDS-related complex (48%) (Weber et al, 1986), can be transmitted by either plasma or the cellular components of blood. Large pools of plasma, such as those from the raw material for products like the antihaemophilic preparation Factor VIII, increase the chance of transmitting the virus since infectivity may be retained even after the massive dilution incurred by pooling. Naturally, the need to combat the risks involved in transfusion became an urgent priority for the Blood Transfusion Service and hospitals.

How often is AIDS transmitted by blood transfusion?

By the end of 1985, 15 581* cases of AIDS had been reported to the Centers for Disease Control in the USA. Of these patients, 51% had died. A breakdown of the figures is shown in *Table 1.*

Table 1. AIDS in USA up to 16 December 1985, breakdown by risk group in adults and children

Risk group	Adults (% of adults)	Children (% of children)
Total	15 355	226
Homosexual/bisexual	11 237 (73%)	— (—)
Intravenous drug-abuser	2 643 (17%)	— (—)
Haemophiliac/coagulation disorder	117 (1%)	11 (5%)
Transfusion with blood or components	241 (2%)	33 (15%)
Heterosexual contacts	163 (1%)	— (—)
None of above	954 (6%)	12 (5%)
Parents have AIDS or are at high risk	— (—)	170 (75%)

Blood or blood components were implicated in 274 (1.8%) of the total cases in adults and children and products made from pooled plasma in 128 (0.8%). Of blood components, both cellular (red cells, platelets) and plasma components (FFP, cryoprecipitate) have been implicated. Overall, transfusion was associated with 402 (2.6%) of the cases. Naturally, the measures taken to reduce the risks of tranfusion-transmitted AIDS will now start taking effect, but because the incubation period of AIDS can be of several years, an unpredictable number of cases will not immediately become apparent and will influence the figures for some time to come.

In the UK, by the end of May 1986, 362 cases of AIDS had been reported to the Communicable Disease Centre (51% of whom had died). These cases included 17 haemophiliacs (4.7%) (one of whom had Factor IX deficiency), and six recipients of blood or blood components (1.7%). Two of the six blood transfusion recipients were transfused abroad. Overall, there-

**By April 1986, this figure stood at approximately 19 200.*

Dr John Barbara is Head of Microbiology, Dr Marcela Contreras is Director, and Dr Patricia Hewitt is Deputy Director at the North London Blood Transfusion Unit

fore, transfusion was associated with 23 cases (6.4%). Although transfusion cases in Britain constitute a greater proportion of the total than in the USA, much of the Factor VIII used here is of American origin and this biases the figures, as the incidence of AIDS in the UK is lagging 2–3 years behind that of the USA.

Up to the end of September 1985 in European countries including the USSR, 35 cases of transfusion-related AIDS had been reported from six countries. These cases constituted 2% of the total AIDS cases.

Approaches to prevent transfusion-transmitted AIDS

Obviously, prior to the employment of preventive measures AIDS posed a real risk for transfusion (though numerically small — estimated at 1 in a million) (Curran et al, 1985). In Britain, therefore, a two-pronged integrated and carefully planned approach was taken to prevent the transmission of AIDS by transfusion of blood or blood components. This was based on donor selection and testing of blood donations.

As an extra safety measure, the pooled plasma clotting factors are currently being heated to inactivate any HTLV-III that may be present. In Britain the Blood Products Laboratory at Elstree (BPL) has been heating the freeze-dried Factor VIII concentrate at 80°C for 72 h since April 1985. Factor IX is being similarly heat-treated. Although HTLV-III would be more susceptible to inactivation by heating Factor VIII concentrate in the wet state prior to freeze drying, 80°C for 72 h is such a rigorous treatment that BPL are confident that the virus will be totally inactivated. Certainly all the evidence to date indicates that heat-treated Factor VIII is safe and clinically effective, although doubt has been cast by recent reports of seroconversion following treatment with heat-treated commercial Factor VIII concentrates in the USA and the Netherlands. In assessing the data the potentially long incubation period of AIDS must be remembered before rushing to hasty conclusions that any heated products may have transmitted the virus.

Albumin undergoes pasteurization during its production and is therefore safe from the point of view of HTLV-III transmission. Furthermore, immunoglobulins prepared for intramuscular use have not been known to transmit any viral infection, including hepatitis B virus and HTLV-III, although the situation with regard to intravenous immunoglobulins is much less clear. Immunoglobulins cannot be heat-treated, but the method of preparation includes steps which are considered to inactivate the virus.

Donor selection and testing

The first, and probably most effective, line of defence for preventing transfusion-transmitted AIDS remains the careful education and selection of blood donors.

In Britain and elsewhere, as soon as AIDS was recognized as a potential problem for transfusion, great efforts were made to educate the general donor population and high-risk groups in particular. At the North London Blood Transfusion Centre (NLBTC) contact was quickly made with groups like the Terrence Higgins Trust for help and advice. Any blood donors in groups at risk were asked to exclude themselves from donating.

As will be seen below, people have responded extremely well to these requests. The Department of Health and Social Security provides explanatory leaflets which must be read by every prospective donor. These were first available in the Autumn of 1983 and were subsequently revised early in 1985, expanding the high-risk groups to include all male homosexuals and bisexuals. In mid-October 1985 a second revision was produced coincident with the initiation of screening to inform donors that they would be tested for anti-HTLV-III.

At blood donor clinics held by the NLBTC, additional "let-out" leaflets are provided setting out a range of varied conditions which render a person ineligible to donate (e.g. hypertension, homosexuality, pregnancy etc). In this way a person can defer him/herself without specifying "high risk of AIDS" as the reason for doing so. However, it is felt necessary to go further than this because in some situations it would be embarrassing for a regular donor suddenly to stop giving blood. This happens when donors attend sessions with their family (e.g. bisexual men with their wives) or workmates. A brief questionnaire is therefore provided for each donor to complete in privacy in a screened area; this allows the donor to ask that the donation is not used for transfusion purposes if he/she feels it is impossible to avoid giving his/her blood. Every donor also fills a declaration giving permission for his/her blood to be tested for anti-HTLV-III, hepatitis B and syphilis. Incidentally, the *Treponema pallidum* haemagglutination (TPHA) screen for syphilis used at many transfusion centres provides an extra safety measure by detecting donors in that group at risk of contracting sexually transmissible infections.

The confidential questionnaire was introduced at a static donor clinic in the West End in July 1984 and by October of that year revealed that 38 male homosexuals had continued to donate because the first DHSS leaflet implied that only "promiscuous" homosexuals should defer themselves from donating. Of nearly 4500 donors questioned at the West End Centre, 1.7% of established male donors and 1.3% of "first time" male donors admitted homosexuality. However, since the introduction of the first DHSS revision, it is rare to have donors who admit to belonging to risk groups. Nevertheless the use of the questionnaire (which has now been introduced to all NLBTC donor clinics) is continued since it provides an invaluable "escape route" for donors like the young "closet" homosexual who visited his mother for the weekend and was promptly brought along to donate at a Saturday session! Similarly, the northern factory boss who was dragged along to a donor session by his London workforce was able to confidentially inform us that he was a homosexual; this correlated with his positive TPHA result caused by a recent syphilis infection!

Anti-HTLV-III screening of blood donors

In mid-1985, as a response to immense public and political pressure due to the extensive AIDS problem in the USA, American blood banks initiated mass screening of donors for anti-HTLV-III. Perforce, this was implemented before all the potential problems inherent in such screening could be assessed and fully addressed. In Britain the Blood Transfusion Service resisted premature initiation of screening until three general problems had been dealt with:

- Assessment of the existing tests to find those best suited to the demands of the Blood Transfusion Service, especially the requirement for a low false-positive rate and adaptability to mass screening and rapid release of blood tested.
- Provision of reference laboratories and adequate donor follow-up and *completely* confidential counselling to cushion the impact on those donors found to be anti-HTLV-III positive.
- Availability of alternative test sites, in addition to sexually transmissible disease (STD) clinics, so that people in "at risk" groups would not come to the Blood Transfusion Service for the sole purpose of being tested anonymously.

Only when these requirements were met did the mass

screening officially start simultaneously in all British Transfusion Centres on 14 October 1985.

Selection of anti-HTLV-III assays

Prior to the commencement of routine screening, the Manchester and North London Blood Transfusion Centres ran extensive trials on 6000 donor sera collected randomly from around the country.

Two kits were "shortlisted" following trials with a range of commercially available kits on a smaller number of normal and "problem" sera (likely to cause false-positive results with some kits) at the Central Public Health Laboratory, Colindale (Mortimer et al, 1985). In the UK most transfusion centres favoured a "competitive" ELISA rather than an "antiglobulin" ELISA. The latter is the system licensed in the USA and all American commercial kits are based on the antiglobulin assay, as is the French Pasteur Institute test.

All the tests of American origin use the cell line derived by Dr Robert Gallo. These tests have the considerable disadvantage that the HLA antigens on the cells in which the virus grows are coated as impurities on the reaction wells of the croplates; HLA antibodies in donor sera can therefore react, and when enzyme-labelled anti-human IgG is added to complete the test, repeatable cross-reactivity (false positivity) can occur relatively frequently. These repeatable cross-reactions cause considerable doubt and delay in the laboratory because it is not possible to confirm the presence of an antibody as precisely as one can confirm detection of an antigen; for example, in the case of hepatitis B, the presence of the antigen HBsAg can be conveniently confirmed by neutralization with specific anti-HBs. In North America, these repeatable but "non anti-HTLV-III" positive results led to unnecessary alarm among donors who feared they would be falsely labelled as anti-HTLV-III positive. In the event, Red Cross Transfusion Centres in the USA do not inform donors of a repeatable positive result unless it can be confirmed by immunoblotting (the so-called Western blot test). However, since immunoblotting is generally less sensitive than the antiglobulin ELISA for anti-HTLV-III, there are several instances in the USA where likely "cross-reactive" donor blood is not used for transfusion.

Fortunately, at British transfusion centres this problem is not met because the first screen uses the very specific "competitive" format; any anti-HTLV-III present in donor sera competes with "conjugate" (the enzyme-labelled anti-HTLV-III) for HTLV-III bound on to the solid phase of the microplate. Furthermore, the competitive assay has the added advantage of allowing "immunopurification" of the viral antigen. The microplates are first coated with anti-HTLV-III; this can then "capture" HTLV-III which does not therefore need to be highly purified. The antigen coat is thus intrinsically "purified" and the competitive assay format is itself inherently less prone to cross-reactions and hence to false-positive reactions. Obviously, human antibody "immunopurification" cannot be used for antiglobulin assays because the enzyme-labelled anti-human globulin would bind the capture antibody and all the sera tested would appear positive!

Whatever test is used, stringent measures are taken to ensure that no-one is falsely labelled as "positive". All screen-positive tests are repeated in duplicate and a sample from the blood pack itself is checked to eliminate the possibility of labelling errors. A reference laboratory then checks the sample from the blood pack with a battery of tests. In England, these include an "antiglobulin" type assay, a "competitive" assay and, usually, an immunofluorescence assay or G-antibody capture assay. The immunoflorescence test employs HTLV-III infected *and* uninfected cells as a control for cross-reacting antibodies. Uninfected control cells are also available in some commercial ELISA assays.

Immunoblotting can serve as an adjunct to reference laboratory confirmatory testing; HTLV-III antigen is disrupted and run down a polyacrylamide gel; since such a gel is not permeable to antibody, a nitrocellulose "blot" imprint is taken off the gel and the protein bands can then be visualized with antibody, e.g. using ELISA. However, false-positive "sticky" sera could still cross-react in a blot test and if the proteins or polypeptides run in the same position as real viral proteins, interpretation could be difficult, requiring considerable experience for reliability.

A competitive ELISA, such as the kit produced by Wellcome Laboratories offers other advantages. The test takes less than 2 hours to perform, has only one incubation step and requires no predilution of the serum sample. Like most of the other commercial assays it is microplate based, lending itself to increased compatibility with the other tests and equipment in most of the transfusion centres in Britain. The test has proved extremely reliable, sensitive and convenient. ELISA plate readers are needed and computer analysis is of benefit to ensure reliable assessment of the results.

It has been estimated that the cost to the NHS of mass screening of all blood donations for anti-HTLV-III is in the order of £4 million per annum.

Prevalence of anti-HTLV-III positive donors

In the UK the rate of detection of confirmed anti-HTLV-III positive donors is so far very low (approximately 1 in 46 000) confirming preliminary indications (Cheingsong-Popov et al, 1984). This rate is lower than in the USA although prevalence in blood donors can vary dramatically there, depending on geographical location. Using a competitive ELISA the screen positive rate (i.e. samples that initially test positive, but on duplicate repeat test negative) is very low. Virtually no repeatable positive sera for which confirmation is doubtful have been recorded.

We consider that the low rate in British donors must largely

be due to the considerable efforts that have been made to persuade potential donors in high-risk groups to refrain from donating and to the responsible way in which would-be donors in high-risk groups have responded. An independent confirmation of this view comes from the fact that last year the number of NLBTC donors detected as HBsAg positive was half that of previous years (prior to the AIDS publicity) (Contreras and Barbara, 1985). It is well known that subjects at high risk of contracting AIDS are also at high risk of contracting hepatitis B. We now rarely find HBsAg-positive donors who are undergoing an acute hepatitis B infection, whereas previously they were mainly young males and constituted 15% of our HBsAg positives. Furthermore, the availability of alternative test sites seems to be an important factor in reducing the number of "high-risk" donors attending at transfusion centres.

Those working in blood transfusion centres consider it their own duty to inform donors personally who are confirmed to be anti-HTLV-III positive and to undertake primary counselling before referring them to counsellors who will look after them in the long term. All of this is carried out in strict confidentiality.

The future

Despite the very protracted incubation period for AIDS there is optimism that the already small risk of transmitting AIDS by transfusion in the UK has been reduced to minute proportions. Naturally, any reports of AIDS in patients who have received blood will be followed-up in great detail and with all the vigour with which post-transfusion hepatitis reports are pursued. We feel sure that hospitals will be much more diligent in reporting suspected transfusion-transmitted AIDS than they were in reporting transfusion-associated hepatitis! In addition, it will be necessary to follow-up the previous donations of ex-donors who are reported to hospitals or STD clinics to be HTLV-III positive or to have contracted AIDS or any HTLV-III related disease. We would urge clinicians to remember to ask patients in these categories if they had ever donated blood in the past.

Future assays for anti-HTLV-III are likely to incorporate genetically engineered antigen and monoclonal antibodies although direct tests for the antigen are likely to pose considerable problems. We would however add a note of caution. Despite the routine screening of all donations for anti-HLTV-III, people in high-risk groups should still *not* offer their blood for transfusion purposes. There have been reports of isolation of HTLV-III from the lymphocytes of anti-HTLV-III negative people in high-risk groups (Salahuddin et al, 1984). There is little detailed knowledge of the nature of the carrier state and of levels and longevity of infectivity, although it is known that some donors have transmitted HTLV-III infection on more than one occasion. In general, it can confidently be stated that the risk of not accepting blood transfusion when indicated far outweighs the minimal risk from transfusion-transmitted infection.

Cheingsong-Popov, R, Weiss, R A, Dalgleish, A et al (1984) Prevalence of antibody to human T-lymphotropic virus type III in AIDS and AIDS-risk patients in Britain. *Lancet*, ii, 477–480
Contreras, M, Barbara, J (1985) Acquired immune deficiency syndrome and blood transfusion. *Journal of Hospital Infection*, **6**, (Suppl. C), 27–34
Curran, J W, Lawrence, D N, Jaffe, H et al (1984) Acquired immunodeficiency syndrome (AIDS) associated with transfusion. *New England Journal of Medicine*, **310**, 69–75
Feorino, P M, Jaffe, H W, Palmer, E et al (1985) Transfusion-associated acquired immunodeficiency syndrome. Evidence for persistent infection in blood donors. *New England Journal of Medicine*, **312**, 1293–1296
Mortimer, P P, Parry, J V, Mortimer, J Y (1985) Which anti-HTLV-III/LAV assays for screening and confirmatory testing? *Lancet*, ii, 873–877
Salahuddin, S Z, Croopman, J E, Markham P D et al (1984) HTLV-III in symptom-free seronegative persons. *Lancet*, i, 1418–1420
Weber, J N, Rogers, L A, Scott, K et al (1986) Three-year prospective study of HTLV-III/LAV infection in homosexual men. *Lancet*, i, 1179–1182