

TRANSFUSION SAFETY STUDY

PROTOCOL

STUDY OF RECIPIENTS OF BLOOD AND BLOOD COMPONENTS FROM DONORS WITH ANTIBODY TO HTLV-I

BACKGROUND AND RATIONALE

The first human retrovirus was isolated in the United States in 1980 (Poiesz, et al.) and was subsequently named *human T-cell leukemia/lymphoma virus type I* (HTLV-I). Since its discovery, HTLV-I infection has been associated with adult T-cell leukemia/lymphoma (Blattner, et al., Robert Guroff, et al., Hinima et al.) and with progressive neurological disease. Blood transfusion has been shown to be one of the means of transmission (Sato and Okochi).

Anti-HTLV-I screening of blood donors has been initiated in Japan to prevent transmission by this route (Okochi and Sato) and it has been suggested that screening is necessary in the United States. However, the magnitude of the problem of HTLV-I transmission by blood transfusion has not been systematically assessed in this country. The Transfusion Safety Study (TSS) is in a unique position to do so.

In 1984, under the emergency powers, the National Heart, Lung, and Blood Institute (NHLBI) issued a contract (NO1-HB-4-7002) for the collection of serum samples from routine blood donors prior to the availability of screening tests for antibodies to the human immunodeficiency virus (anti-HIV). The immediate use for the TSS/NHLBI Donor Repository was to identify potentially infectious donors and evaluate transmission of HIV to recipients by the components of the donation. However, the potential use of this Donor Repository for the study of transmission of other transfusion-associated viruses was also recognized and permission for tests for other infectious agents was included in the informed consent signed at the time of donation.

Samples were taken from donors in four geographic areas with the highest prevalence of AIDS: the Greater New York area, served by the New York Blood Center; the South Florida area, served by the South Florida Blood Service, now the American Red Cross Regional Blood Services; San Francisco and Bay area counties to the north of that city, served by the Irwin Memorial Blood Bank; and, Los Angeles County, served by the American Red Cross Regional Blood Services.

From early September, 1984, until early February, 1985, donors at the participating blood services were asked to permit the collection of an additional 10 ml of clotted blood for HIV antibody testing when a licensed test became available and for testing for other infectious agents which might be transmitted by blood. They were also asked for permission to contact them later if the results of such tests indicated that further observations would yield useful scientific information.

At the end of Repository collections in early 1985, a total of 200,000 donor serum specimens had been stored. Their distribution was as follows: New York Blood Center, 77,760; American Red Cross Blood Services, South Florida Region, 31,104; Irwin Memorial Blood Bank, 27,500; and American Red Cross Blood Services, Los Angeles-Orange Counties Region, 67,418.

Under NHLBI contract NO1-HB-4-7003, the TSS is following confirmed anti-HIV positive and negative control blood donors and recipients of components from their donated blood (IRB reference #04083).

The present protocol proposes to test the Repository for antibodies to HTLV-I and follow confirmed positive donors, a negative control group, recipients of components from these donors, and household members of donors and recipients.

SCIENTIFIC QUESTIONS TO BE ASKED

1. With what frequency do donors with HTLV-I antibody or other markers of HTLV-I infection transmit their infection to recipients of their blood and blood components. Can the impact of using an anti-HTLV-I EIA in blood banks be estimated?
2. What characteristics of *donors* influence whether transmission of infection takes place--sex; age; race/ethnicity; the presence of other infectious agents transmissible to the recipient? Study of donors may demonstrate parameters that will be helpful in advising all anti-HTLV-I-positive persons about their infectivity.
3. Are there any differences in relative infectivity of whole blood and components (packed cells, platelets, etc.)? The information may permit inferences about relative amounts of intracellular and extracellular virus in peripheral blood.
4. To what extent does the HTLV-I reservoir among donors extend beyond the populations presently believed to be at risk, and what are the probable sources or mechanisms of infection for persons outside these risk groups?
5. What characteristics of *recipients* influence whether transmission of infection takes place--sex, age; the underlying disease that resulted in a need for transfusion, the occurrence of immunologic stress by allogeneic exposures, the presence of immunodepression due to the underlying disease or therapeutic modalities used in its management, the acquisition of other transfusion-transmitted viral agents from the same or other donors?
6. When infection does occur, can host factors influencing its clinical expression be identified?
7. To what extent are household contacts of persons who receive a unit of anti-HTLV-I positive blood at risk of developing HTLV-I infection?
8. To what extent are household contacts of anti-HTLV-I positive donors at risk for infection with HTLV-I?

OBJECTIVES

1. To determine the frequency with which HTLV-I infection is transmitted from an infected donor to the recipient, especially in relation to the type of component administered.
2. To identify and observe as controls, recipients of blood donated by persons whose sera are anti-HTLV-I-negative by all applicable tests. It is not practical to apply case-control matching techniques to select this group. The goal, therefore, should be to recruit a large enough number to maximize the probability that their characteristics *as a group* will be similar to those of study recipients.
3. To locate and observe donors whose sera give positive results during anti-HTLV-I testing, or whose sera require further study to define their antibody status.
4. To identify and observe as controls donors whose sera are negative for anti-HTLV-I by all available laboratory techniques. They should be matched for sex, age, and area of residence to permit interpretation of immunologic and serologic data with those for study subjects.
5. To establish the extent and epidemiologic characteristics of experience with HTLV-I in donor populations in New York, Miami, San Francisco, and Los Angeles.
6. To collect for long-term storage in a TSS-NHLBI repository plasma and cells from each person entered into the Study.
7. To collect from selected healthy persons with HTLV-I infection units of plasma to serve as a reference for quality control and other panels.
8. To assess the frequency of transmission of HTLV-I from recipients of anti-HTLV-I-positive blood to their household contacts.
9. To assess the frequency of transmission of HTLV-I between anti-HTLV-I-positive donors and their household contacts.
10. To assess the risk of progression from HTLV-I infection to clinical expression.

STUDY POPULATIONS

1. Persons in the geographic areas listed below who gave permission at the time of blood donation to have serum tested for infectious agents transmitted by blood transfusion and to be contacted concerning further participation if results of that testing indicate that additional observations would add to knowledge concerning the meaning of the test.
 - a. Donors to the Greater New York Blood Program's facilities in all four of its divisions (New York City, Northern New Jersey, the Hudson Valley, and Long Island).
 - b. Donors to the South Florida Region of the American Red Cross Blood Services (formerly, South Florida Blood Service).
 - c. Donors to the Irwin Memorial Blood Bank at its facilities in San Francisco and adjacent counties to the north.
 - d. Donors to the American Red Cross Regional Blood Services' facilities located in Los Angeles County.
2. Recipients of blood or blood components at hospitals in the areas served by the participating blood collection agencies.
3. Household contacts of donors and recipients.

The first population will consist of : (A) Donors whose positivity on EIA screening is confirmed by at least two additional tests (positive donors), and (B) matched donor controls whose negativity on screening is confirmed by other tests (negative donors). At this time there are no plans to follow donors whose EIA screening result is not corroborated by additional tests. If, at a later time, it appears that there would be scientific value in this, a subprotocol would be submitted.

The second population will consist of : (A) Recipients known to have received blood or components from identified positive donors, and (B) recipient controls known to have received blood or components from the matched donor controls.

The third population will consist of household members of enrolled donors and recipients in the above groups who have lived with the donor or recipient for at least six months and who are expected to do so for the duration of the study.

PROCEDURES

Testing of Donor Specimens

At each blood service performing anti-HTLV-I testing under this protocol, testing of serum specimens in the TSS Donor Repository will be performed by a specifically designated staff and handled separately from testing of routine blood donors. All test sites will use the same commercially manufactured EIA tests. This will allow prevalence determinations to be made and will aid in standardizing quality control approaches and data processing.

On each working day, the required number of specimens will be removed from the Donor Repository, thawed, and stored in a refrigerator at 4°C until the anti-HTLV-I screening result is known. Initially positive samples will be kept in the refrigerator for retesting in duplicate. Initially negative samples will be returned to their original positions in the freezer.

Initially positive samples will be retested in duplicate. If these samples are “positive” by the manufacturer’s criteria, an aliquot will be removed and the remaining serum in the original vial returned to its original position on the Donor Repository. The 0.5 ml aliquot will be immediately shipped frozen to the Central Processing Laboratory (CPL) for coding prior to confirmatory testing.

For each positive sample identified, two matched donor controls will be selected. The criteria for matching the controls with the anti-HTLV-I-positive (index) donor are: (1) same sex as the index donor; (2) same age group as the index donor; (3) residence in same, adjacent, or nearest census tract. To accomplish this, the CPL will notify the blood center and the Biostatistics Office when a positive donor sample has been confirmed by the central laboratories. The blood center will supply the Biostatistics Office with the characteristics of the index donor. The Biostatistics Office will then generate a list of potential controls ordered by repository location number from which the blood center will identify the two donors whose residence at the time of donation was nearest to that of the index donor. These samples will be tested as soon as possible after identification. Anti-HIV positive donors will be excluded from possible selection as controls.

The samples from potential control donors will be tested in duplicate at the time their sera are thawed. If the sample is negative by the manufacturer’s criteria, an aliquot will be removed, and the remaining serum in the original vial returned to its original position in the Donor Repository. The aliquot will be handled in the same way as described for sera from positive donors. If either result of duplicate testing is positive instead of negative, the above-described procedure for positive sera will be followed and another potential control evaluated.

For all sample retested in duplicate which are false-positive (i.e., the mean HTLV-I ratio is less than the respective cut-offs, or only one of three HTLV-I results is above the cut-off) no aliquots will be removed. The remaining serum will be returned in its original vial to its original position in the Donor Repository.

Additional Testing of Donor Specimens and Quality Control

Due to the probability of false positive screening results in a low prevalence population, it will be necessary to attempt to verify the specificity of EIA positivity. The procedure that presently seem appropriate for verification of anti-HTLV-I positivity include: repetition by a different EIA, and the identification of antibodies to specific viral antigens by immunoblot (PIB), radioimmunoassay (RIA), radioimmunoprecipitation (RIP), and dot blot hybridization.

Procedures based on evidence of viral latency or replication may be utilized at a later date in order to gain additional information about the initial donor sample. These procedures will not be used as a basis for selecting study subjects.

All samples sent for additional testing will be recorded at the CPL prior to such testing so that those performing the tests will not have any knowledge of EIA test results.

Quality control of the initial screening will be based primarily upon a proficiency panel to be used throughout testing. This panel will be prepared from anti-HTLV-I positive, negative and borderline plasma samples which will be coded and randomly ordered. An appropriate number will be assayed during the course of each day's work at each facility and the results reported to the Director's Office by electronic mail each day. This approach will permit comparison of test performance across different testing sites and, within each site, across different test runs.

A second method of quality control in screening will be re-evaluation by the central laboratory of a sample of donor specimens that are negative. A control group of donors will be selected (see below); these control donor specimens will provide a set of negative sera twice the size of the positive set. Confirmation of negativity will be performed prior to control donor follow-up. If one of the confirmatory tests is positive, then this donor will no longer be a potential control, but consideration will be given to enter such donors into the study.

The central laboratories responsible for confirmatory testing will report their results to the Central Processing Laboratory. The Director of the Central Processing Laboratory will provide the donor's Study identification number and results to the Project Director for review. If all findings are unambiguous and the person lives in a geographic area feasible for follow-up, then the person at the Clinical Center responsible for donor recruitment will be notified.

Throughout the identification and follow-up of donors, all specifically identifying information will be available only to those persons for whom it is absolutely necessary for making contact. Each collection agency will develop its own plan for accomplishing this purpose, which must be based on its system of keeping records. No donor will be contacted until the patient manager or TSS-blood bank clerk has located the informed consent signed by the donor. Both will make all possible efforts to protect the identity of these persons.

From the blood unit number, the patient manager or data clerk will obtain the donor's name and other information necessary to make contact. When the donor is first contacted, he will be reminded of his previously expressed willingness to be contacted if further participation may be helpful in evaluating new tests related to transfusion safety. The donor will be asked to present himself at a convenient facility available to the blood service to discuss further participation. The site and time will be one of mutual convenience. If the donor states that he is not interested in further participation but wants to know his test result, he will be told that, to ensure privacy, test results cannot be given over the phone or by mail. To ensure confidentiality, it is essential that the person satisfactorily be identified as the person who made the donation. He must, therefore, provide adequate identification in person at a facility available to the blood service in order to learn his result.

When the donor meets with a representative of the study, he will be reminded again that he had previously agreed to participate in a study of transfusion safety during a blood donation and will be asked to participate in another phase of study. A copy of his assigned consent form for the pre-screening phase will be agreeing to be interviewed and to have a blood sample taken for HTLV-I and HIV testing (antibody and possible virus isolation) and immunological tests including T-cell phenotyping. The informed consent must be discussed with the donor and not merely handed to him to read. The donor will be given the opportunity to ask the patient manager any questions about the study and the meaning of anti-HTLV-I tests, anti-HIV tests or any other tests. If the donor does not want to participate in the study, he will be given his test results at this time.

If a donor is positive for anti-HTLV-I, he will be given printed material concerning the possible significance of the findings and medical and mental health referral services in his community. The donor will be given a release form which would permit the blood center to release the test results to his personal

physician in a fashion which will protect the donor's confidentiality. A brief description of the research study will accompany these results.

After obtaining informed consent, the patient manager will conduct the interview, which will contain questions about medical history, present health, and patterns of behavior, including sexual practices (specifically homosexual contacts) and recreational intravenous drug use. The patient manager will also draw a 60 ml sample of blood.

At the time of the interview, a list of household members will be obtained for each donor. From this information and the personal history, appropriate household contacts for study can be identified.

The procedure for handling the completed questionnaires and blood specimens is described in the manual of operations.

Donor Household Contact Follow-Up

Household contacts eligible for study are persons who have lived with the enrolled donor for at least six months and who are expected to continue to do so for the duration of the study (three years). Household contacts of donors who choose not to enroll in study will not be contacted. Enrolled donors will be encouraged to ask their family members to participate in the study. If the donor agrees, the patient manager will request that the donor either: (1) Arrange for the family members to get in touch with the patient manager if they wish to participate, or (2) notify the patient manager of the family members' decisions about participation.

If no response is received within two (2) weeks, the patient manager will explain the study. After obtaining informed consent, the patient manager will conduct the entry interview as described above for donors. The informed consent must be discussed with the adult family member or parent of child family member and not merely given to him to read.

Recipient Follow-up

The two recipient groups will consist of the following: (A) Recipients known to have received blood or components from anti-HTLV-I positive donors and (B) recipient controls known to have received blood or components from matched donor controls.

Recipients in Group (A). Adult recipients in this group will be identified by reviewing blood center records and then contacting the blood bank director of the hospital to which the blood component was sent. Information will be obtained from the blood bank director concerning the patient who received the unit. The physician who ordered the blood component will also be identified from blood bank records and will be contacted by a physician on the study staff or the patient manager assigned to recipient follow-up (depending upon the written procedures of each clinical center). If he is not the physician currently responsible for the recipient, he will be asked to identify the physician who is responsible.

The responsible physician will be told about his patient's exposure to blood from a donor in whom antibodies to HTLV-I were detected and about the study. The physician will be asked to give permission for his patient to be informed about the exposure and to be recruited for the study. If the physician agrees, the recipient will be contacted according to the procedures established by each clinical center. They include the following options:

1. The physician will be asked to contact his patient, inform the patient of the exposure, and recommend that the patient participate in the study. If the recipient is willing to participate in the study, the physician will inform the patient manager, who will then contact the recipient. Or,

2. The patient manager will make the initial contact by telephone. Using a standardized script, an attempt will be made to be certain that the person on the telephone is actually the recipient. The patient manager will explain that a national study of transfusion safety is being conducted and that the recipient was selected to participate because he received blood or blood components. Or,
3. The recipient will be informed about the exposure and the study jointly by the physician and patient manager in the physician's office.

If the physician refuses permission to contact his patient, the recipient will not be contacted. However, this refusal will not be accepted until an attempt is made to inform the physician that he should be careful not to suppress information relating to his patient's health unless he has a very good reason why his patient should not be given the information, and that he should seriously consider cooperating with a study as potentially significant as this one.

When the recipient presents himself to the study staff member, he will be told by the appropriate person (the personal physician, a physician member of the study team, or patient manager) about his exposure to blood from a donor in whom HTLV-I antibody was detected and about the study. He will be given an opportunity to ask the staff member about the meaning of the exposure and the study, and will be asked to sign an informed consent to be interviewed and to have a blood sample taken for HTLV-I and HIV testing (antibody and possible virus isolation) and immunological tests including T-cell phenotyping. The patient will be told that these studies will be repeated at specified intervals for a period of three years. The informed consent must be discussed in detail with the recipient or with the parent of a recipient minor and not merely handed to him to read.

At the time of the interview, a list of household members will be obtained for each recipient. From this information and the personal history, appropriate household contacts for study can be identified.

Recipients in Group (B). Adult recipients of blood or components from negative donors and the physician who ordered the transfusion will be identified following the same procedure described above for recipients of components from positive donors.

The responsible physician will be contacted and told about the study. He will also be told that, although there is no evidence that his patient received anti-HTLV-I positive blood, we would like to evaluate the patient as part of the study. He will be asked for permission to contact the patient to enroll him in the study. The patient will not be contacted if the personal physician refuses permission.

If the physician agrees, procedures specified by each clinical center will be followed to contact the potential control recipient. They include the following options:

1. The physician will be asked to contact his patient, inform the patient of the study, and recommend that the patient participate in the study, the physician will inform the patient manager, who will then contact the recipient. Or,
2. The patient manager will make the initial contact by telephone and provide immediate assurance that the recipient is not being called because of known problems with any blood transfusions he has received. Nonetheless, the person's participation is asked because it is necessary to compare persons with no known risk to those with possible risk.

The potential recipient control will be told that this is a study of blood transfusion safety conducted to understand better the immunologic changes and the occurrence of infection, including HTLV-I, associated with transfusion. He will also be told that a sample of blood from a donor from whom he received a component was stored and tested for antibodies to HTLV-I when such a test became available and that no antibodies were detected. However, certain aspects of his health needs to be compared with those of a blood recipient who received blood from a donor later found to have HTLV-I antibodies.

The potential control recipient will be given an opportunity to ask any questions he may have about the study and will be asked to sign an informed consent agreeing to be interviewed and to have a blood sample taken for HTLV-I and HIV testing (antibody and possible virus isolation) and immunological tests including T-cell phenotyping. The informed consent must be discussed in detail with the recipient or parent of recipient minor and not merely handed to him to read.

If the recipient does not want to participate, there will be no attempts at further contact by any member of the study team. If the recipient signs the consent form., the patient manager responsible for recipient follow-up will conduct the interview and will draw the blood samples as described above for donors.

Minors. The recruitment procedures described for adults will be followed, with the legal guardian(s) acting as the recipient's representative(s). The legal guardian(s) will be asked to sign the informed consent form specifically designed for minor recipients. In addition, children over age 12 will be asked to sign an assent form. If the appropriate form(s) are signed, the patient manager will conduct the interview and will draw the blood sample. The parent/legal guardian specified by each clinical center, will also be interviewed.

Adult recipients for whom there is a legally appointed guardian will be approached in the same way as minors.

Recipient Household Contact Follow-up

Household contacts eligible for study are persons who have lived with the enrolled recipient for at least six months and who are expected to continue to do so for the duration of the study (three years). If recipients or parents of recipient minors choose not to enroll on the study their family members will not be contacted. Enrolled recipients will be encouraged to ask their family members to participate in the study. If the recipient agrees, the patient manger will request that the recipient either: (1) Arrange for the family members to get in touch with the patient manager if they wish to participate, or (2) notify the patient manger of the family members' decisions about participation.

If no response is received within two (2) weeks, the patient manager will contact the recipient by telephone or mail to identify the reason. If the recipient refuses to notify his family members, no further action will be taken.

When a family member agrees to participate, the patient manager will explaining the study. After obtaining informed consent, the patient manager will conduct the entry interview as described above for recipients. The informed consent must be discussed in detail with the adult family member or parent of child family member and not merely handed to him to read.

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