TRANSFUSION SAFETY STUDY

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Subject: Final Report

Attached is the Final Report covering the activities of the Transfusion Safety Study under National Heart, Lung, and Blood Institute Contracts N01-HB-4-7002, N01-HB-4-7003, and N01-HB-9-7074.

Thank you very much.

Distribution List:

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Final Report:
The TransFusion SafeTy Study (1984-1998)

A MULTICENTER APPROACH TO THE PROBLEM OF TRANSFUSION-ASSOCIATED ACQUIRED IMMUNODEFICIENCY SYNDROME

Funded by National Heart, Lung, and Blood Institute Contracts
N01-HB-4-7002, N01-HB-4-7003, and N01-HB-9-7074.

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TIME PERIOD: September 1984 through February 1998

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FINAL REPORT:
THE TRANSFUSION SAFETY STUDY (1984-1998)

A MULTICENTER APPROACH TO THE PROBLEM OF TRANSFUSION-ASSOCIATED ACQUIRED IMMUNODEFICIENCY SYNDROME

I. BACKGROUND

The etiology of acquired immunodeficiency syndrome (AIDS) was unclear in 1981 and 1982, but by the latter part of 1982 with cases in hemophiliacs, the suspicion was being considered that AIDS could be caused by a virus with epidemiologic behavior similar to that of hepatitis B virus. The other, and at that time more widely held view, was that blood transfusion as well as clotting-factor concentrates represented an antigenic challenge to the immune system. This challenge was thought to result in dysfunction that permitted latent infections to be activated and otherwise non-pathogenic microbial agents to cause serious infections leading to death. Studies at individual institutions in the United States and other countries were inconclusive.

The approach of the National Heart, Lung, and Blood Institute (NHLBI) was to organize a cooperative study, using a common protocol and immunologic tests standardized across institutions, to determine the components of etiology. The Transfusion Safety was developed as a response to an RFP entitled "Association of Blood Product Use with Immune Function Changes: Relation to Acquired Immunodeficiency Syndrome (AIDS)—A Prospective Study" issued in September 1983.

The approach was to investigate the incidence of immunologic changes suggestive of the pattern seen in persons with AIDS in four cities of very high prevalence, four cities of very low prevalence, and two cities in countries outside of the United States in which AIDS had not occurred. Differences in the prevalence of the relatively non-specific immunologic markers (as surrogates for AIDS) in cities with high and low AIDS prevalence would provide evidence for a virus in the community (if AIDS was largely or entirely due to a microbial agents). If the prevalence of immunologic markers was similar in cities of high and low AIDS prevalence, this would be evidence for immune stress caused by transfusion and pooled plasma products such as clotting factor concentrates. A large-scale cooperative approach was by far the most appropriate to obtain an answer as quickly as possible and minimize confounding differences between individual institutional observations.

Isolation of the human immunodeficiency virus (HIV-1) and its identification as the underlying cause of AIDS in May 1984 changed the laboratory emphasis. Almost as important, one of the systems for growing HIV-1 in cell cultures provided the very high yield of virus necessary to develop a commercial test in sufficient quantities to screen all blood and plasmapheresis donors.

With the anticipation of licensure and routine application, an additional RFP was issued to TSS to form a repository of serum from 200,000 donors in high AIDS prevalence areas of the United States. The purpose was to be able to determine later the outcome of the transfusion of blood components whose donors would be excluded when screening tests for anti-HIV-1 infection became available. A secondary consideration was to be able to determine the outcome of the transfusion of blood components from donors with positive tests for other viruses subsequently found to be of importance in transfusion medicine. Work under the "letter" contract (NO1-HB-4-7002) began in May 1984. From September 1984, through early February, 1985, the 200,000 donor specimens for the TSS/NHLBI Donor repository were collected in Greater New York, South Florida, San Francisco, and Los Angeles. Work under NO1-HB-4-7002 was completed in May 1985.

TSS itself (Contract No. NO1-HB-4-7003) began September 30, 1984, with a nine-month planning phase during which protocols were developed, forms and a data system designed, and laboratory procedures evaluated. Entry of subjects began in July 1985, and follow-up observations continued through May, 1993.

II. MAJOR GOALS OF TSS

The progression of the HIV-1 epidemic in the United States, and the rapid pace of technical advances in detecting and documenting HIV-1 and other retrovirus infections required continual revisions of goals and methods to keep both relevant to contemporary questions and the method of answering them. The result was some modifications of goals and, not unexpectedly, many changes in approach.

The following were the major goals:

1. To monitor transmission of HIV-1, HumanT-cell LymphotropicVirus Types I and II (HTLV-I/II), and other viruses by blood components and products.

2. To utilize the TSS/NHLBI Donor Repository specimens to determine the efficiency of new screening procedures for HIV-1 that are serious candidates for routine use by blood services.

3. To utilize the TSS/NHLBI Donor Repository specimens to determine prevalence of anti-HTLV-I/II among blood donors in metropolitan areas of the United States in 1984/5, and transmissibility of the agent to recipients of blood components.

4. To uncover any mechanisms of resistance to retrovirus infection among those exposed but not infected.

5. To observe the course of HIV-1 and HTLV-I/II infection and factors influencing it.

6. To observe any modifications of the pathogenicity of HIV-1 and/or HTLV-I/II due to infection with both, and modifications of either and/or both by activation of latent infection with herpesviruses and chronic infection with human hepatitis viruses.

7. To observe any modifications of the pathogenicity of herpesviruses and/or human hepatitis viruses attributable to infection with HIV-1 and/or HTLV-I/II.

8. To determine if blood components and products are themselves immunosuppressive and have any effect on the course of HIV-1 infection.

9. To assess the impact on the community reservoir of HIV-1 and HTLV-I/II infection acquired from blood components and products because of subsequent transmissions by other mechanisms.

10. To acquire and store serum and leukocytes from all subjects for the TSS/NHLBI Repositories.

III. THE TSS/NHLBI DONOR REPOSITORY

A. Development of the Donor Repository

From prior experience with other transfusion-transmitted viruses, NHLBI appreciated the fact that many donor-screening tests would be developed, among which conflicting claims concerning sensitivity and specificity would be made. Because differences in sensitivity particularly influence the ability of screening to protect blood and plasma recipients, NHLBI changed the direction of the incipient Transfusion Safety Study to one of collecting an adequate number of sera as a donor repository. Its major purpose was to answer the inevitably subsequent questions of sensitivity and specificity from different investigators and manufacturers.

It is important to appreciate that once the screening test for HIV-1 among donors was licensed and operative, it would no longer be possible to transfuse untested blood to determine the meaning of positive and negative laboratory tests. The goal, announced by Dr. Margaret Heckler, was to be screening all blood donors before the spring of 1985.

With great alacrity, therefore, NHLBI asked the Transfusion Safety Study to organize immediately the collection of 200,000 donor sera prior to the time any way of detecting contaminated units would be available. The Study carried out this part of its task in the four US cities with the highest incidence of reported AIDS--New York, Miami, San Francisco, and Los Angeles. Collections began in September 1984 and were completed by the first week in February 1985.

The Donor Repository is the property of NHLBI, and no part of these specimens is ever used without their explicit consent. It is the general policy of the TSS not to request specimens from

the Donor Repository unless the question is considered sufficiently important by the Group, NHLBI, and the TSS Protocol and Data Monitoring Committee. It has seemed appropriate, however, to apply new techniques as early as possible in their development whenever there is sufficient evidence of probable usefulness towards achieving the general objectives of TSS.

B. Anti-HIV-1 screening of the Donor Repository

The anti-HIV-1 screening test was licensed the first week of March 1985. Screening of the 200,000 donor repository sera began in late 1985 when the manufactured anti-HIV-1 kits became sufficiently available for this large research project, and confirmatory procedures were arranged. Among 196,684 admissible specimens, testing identified 307 positive donations from 287 donors. The overall rate of contaminated donations was 1.5 per 1,000, but it was 3.2 per 1,000 men 18-29 years of age. This prevalence varied less among the four cities than did the cumulative AIDS prevalence.

C. Anti-HTLV screening of the Donor Repository

The specimens from this donor repository are kept in long-term storage by NHLBI. Since the period of anti-HIV-1 testing, the entire group of specimens has been tested again to determine the prevalence rates of human T cell lymphotropic viruses types I and II for which there had been no large scale survey for the United States prior to beginning anti-HTLV-I/II testing. Fortunately, the prevalence of these two agents, which are etiologically associated with human T cell leukemia and progressive myelopathy, was only 0.9 per 1,000 donors. Apart from the question of donor prevalence, however, the study provided a survey of epidemiologic characteristics in the mid-1980's which differed considerably among the four cities.

D. HIV-1 p24 antigen screening of selected samples in the Donor Repository

Selected specimens (8,597) from the donor repository were used again in 1989 when the test for the core antigen (p24) was proposed as an additional donor screening procedure to prevent HIV-1 transmission by newly infected persons before they became anti-HIV-1 positive. The donors selected for this sample by their characteristics provided an answer essentially equivalent to screening 1.1 million donors in 1989. No instances of isolated p24 antigen-positivity were found, which was the same answer obtained simultaneously by a consortium of blood agencies throughout the US that tested some 500,000 donors.

E. Future uses of the Donor Repository

The donor repository remains a resource for answering questions about future tests for any agent capable of being transmitted by blood transfusion and plasma products.

IV. POPULATIONS UNDER STUDY AND RATIONALE

With HIV-1, the viral etiology of AIDS was established. There remained the question whether immune stress from transfusion and plasma products contributed to the highly variable rapidity with which infection proceeds to symptomatic disease and death. In addition, the natural history of HIV-1 infection, the best indices of progression, and other determinants of progression such as new or reactivated viral infections, have required large numbers of persons for adequate statistical evaluation. Thus, the study recruited a broad range of subjects in various categories related to transfusion medicine.

From July 1985, 4,084 persons were enrolled in the Study. The characteristics of these populations are as follows:

A. HIV-1 donor-recipient groups

1. Donors in four high AIDS prevalence areas who gave consent to have a serum specimen stored in the TSS/NHLBI Donor Repository and were subsequently found to be anti-HIV-1 positive. The 149 subjects were primarily recruited for further study of the characteristics of persons whose components were or were not associated with HIV-1 infection of recipients.

   Approximately 80% of the anti-HIV-1 positive TSS donors were found on follow-up to have probably acquired their infections as a result of homosexual contacts. The number of these homosexual encounters, however, was generally much smaller than those of subjects recruited into studies based on participation in gay organizations; approximately 40% of TSS anti-HIV-1 positive donors were bisexual. This circumstance permits an assessment of the role of less extensive exposure to other sexually transmitted agents on the course of HIV-1 infection.

2. Control donors (matched for sex, age, and AIDS prevalence in the area of their residence) subsequently demonstrated to be anti-HIV-1 negative at the time of the donation (s) represented in the Donor Repository. The 151 control subjects in this category were necessary for evaluation of the findings among anti-HIV-1 positive donors.

3. Recipients of blood components from donors subsequently found to have been anti-HIV-1 positive at the time of donation. Through establishment of the TSS/NHLBI Donor Repository, the 135 patients in this category represent the only large group of persons known to have been exposed through an anti-HIV-1 positive component. The probable date of infection can be established for almost all by the date of the implicated transfusion.

4. Recipients of blood components from a control donor known subsequently to have been anti-HIV-1 negative at the time of donation represented in the Donor Repository. The 60 patients in this group represent controls for the recipients of anti-HIV-1 positive blood components.

5. Spouses or spouse-equivalents of recipients found to be anti-HIV-1 positive or negative. Thirty-seven persons in these categories were enrolled. The frequency of sexual

transmissions beyond infections attributable to blood components and products has epidemiologic implications for the impact of transfusion-transmitted HIV-1 infections upon the total community reservoir.

B. HTLV-I/II donor-recipient groups

6. Donors who gave consent to have a serum specimen stored in the TSS/NHLBI Donor Repository and were found to have been anti-HTLV-I/II positive. The 111 subjects were primarily recruited for further study of the characteristics of persons whose components were or were not associated with HIV-1 infection of recipients.

7. Control donors (matched for sex, age, and ZIP code of their residence) who were found to have been anti-HTLV-I/II negative at the time of the donation(s) represented in the Repository. The 231 control subjects in this category were necessary for evaluation of the findings among anti-HIV-1 positive donors.

8. Recipients of blood components from donors now being identified as anti-HTLV-I/II positive at the time of donation, as well as recipients of more recent donations by the same anti-HTLV-I/II positive donors. 115 recipients in this group were enrolled.

9. Recipients of blood components from donors now being identified as anti-HTLV-I/II negative at the time of donation, as well as recipient of more recent donations by the same anti-HTLV-I/II negative donors. The 62 patients in this group represent controls for the recipients of anti-HIV-1 positive blood components.

10. Spouses and non-sexual household contacts of anti-HTLV-I/II positive and control donors and recipients. This group of 221 persons will be used to determine the extent of sexual and intrafamilial transmission in the United States.

C. Congenital clotting disorders (CCD)

Two other HIV-1 risk categories were also of concern. Proportional to their entire number in the US population, persons with hemophilia A and B have been the most heavily HIV-1-infected population. To have as wide a range of clotting disorders as possible, the study involved clinics not only in the four cities where donors and recipients were observed but also in Detroit and Seattle. We also extended recruitment to patients with other factor deficiencies and the rarer congenital clotting disorders. To provide a broad range of therapeutic needs (from none to several hundred thousand clotting factor units a year), we sought enrollment of all persons known to the clinics rather than those who attended frequently because of the severity of their illness.

Many patients with congenital clotting disorders have very large need for clotting factor therapy that usually must be met by high-risk products (i.e., those made from pooled plasma taken from paid plasmapheresis donors). The types of concentrates used varied among the clinics in the six areas, providing information on the effects of differences in manufacture. Most notably, however, was the inclusion of Puget Sound Blood Center in Seattle in the group. Their patients

with moderate to even severe hemophilia have long been treated with cryoprecipitate--a form of factor therapy that avoids the danger of pooling plasma from thousands of donors but carries a much larger admixture of proteins than concentrates. The ability to compare the characteristics of concentrate recipient in other cities with those of cryoprecipitate-treated individuals in Seattle was one of the most important advantages in the Transfusion Safety Study's approach to the HIV problem in hemophilia. Their incidence of transfusion-transmitted infection, therefore, cannot be translated into risk for persons receiving whole blood and unpooled components.

Persons in the congenital clotting groups were classified as follows:

11. Patients with all forms of congenital clotting disorders treated on at least one occasion from 1979 until Study entry with clotting factor concentrates and/or unpooled blood components. The 1,148 subjects include approximately 200 treated only with unpooled blood components during that time, a uniquely large group. It includes several patients known to have had an average of 100 to over 2,500 donor-exposures per year during the years 1981 through 1984.

    This group includes at least 250 patients treated with concentrates who remain anti-HIV-1 negative. The total also includes some 10 to 20 persons with clotting factor inhibitors sufficiently severe to make them candidates for immune tolerance induction, during which massive amounts of clotting factor concentrates are administered.

    Major points of interest among the observations being made on this group include the effect of allotypically heterologous proteins on the immune system, the effect of treatment on HIV-1 infection, and new instances of transmission of HIV-1 and other virus infections by blood components and products.

12. Persons with any form of congenital clotting disorder that have remained untreated with any blood component or product since 1979. The 100 subjects represent a group previously unstudied, and seem the ideal control for any variables influencing personal characteristics, physical findings, or laboratory values.

13. Household members of persons with congenital clotting disorders. The 890 subjects in this category include 335 primary sexual contacts, 343 parents, 84 offspring (regardless of age), 72 siblings, and 56 persons with other relationships.

    One of the major community anxieties about AIDS at the present time is the extent to which transfusion-transmitted HIV-1 infection spreads in the community beyond persons to whom blood components and products are given. This group of contacts is by far the largest among those under study, and should provide the best estimate of a very low-frequency event.

    The household members represent a large group of anti-HIV-1 negative persons, who also serve as controls for all observations of anti-HIV-1 positive persons.

D. Congenital anemias (CA)

The early cases of transfusion-associated AIDS, and then of HIV-1-like immune changes in transfused persons, similarly raised the question of viral infection and/or alterations in immunologic parameters due to underlying disease in persons with congenital anemias or induction of immune stress by whole blood or components such of red blood cells and platelets. Fortunately, the number of donor exposures for individuals with sickle cell disease and some other forms did not require so many exposures to single-donor components as to place them at a high risk of HIV-1 infection. The exception was the thalassemics, who did become infected and also have immune changes due to the effects of their disease. The number of thalassemics available for study, however, is limited by the relative infrequency of the homozygous form of the disease and the number of persons in any given area with the ethnic background that favors its occurrence. The collection of an adequate number of such patients would not have been possible at a single center.

Persons in the congenital anemia groups were classified as follows:

14. Patients with any forms of congenital anemias treated with blood components on at least one occasion since 1979. The 297 patients presently in the Study include at least 154 with sickle cell disease, 110 with various forms of thalassemia, and 16 with other congenital anemias. The total includes 23 patients who are anti-HIV-1 positive.

15. Persons with any form of congenital anemia untreated with any blood component from 1979 to Study entry. The 105 subjects represent a group previously unstudied, and have been an excellent control for any variables influencing personal characteristics, physical findings, or laboratory values.

16. Household members of persons with congenital anemias. The 163 subjects in this category include 13 primary sexual contacts, 99 parents, 12 offspring, 25 siblings, and 14 persons with other relationships.

V. SUBJECT OBSERVATIONS

Subject observations began in August 1985, and follow-up observations continued under the original contract through February 1989. Even early in its course, however, it became evident that longer term observation than had originally been proposed would be necessary. Accordingly, in November 1987, NHLBI solicited a new proposal. TSS responded on August 1989, and after scientific and programmatic review, NHLBI issued a new contract that began on February 15, 1989. It provided for continued clinical and laboratory observations through June 30, 1993.

The fact that essentially all available subjects in various categories were enrolled by TSS since late 1985 has offered two major advantages: 1) The population sizes were large enough to test validly some of the conclusions and conjectures offered from small series at individual medical

centers; and 2) infrequent to rare occurrences (e.g. seroconversion to HIV-1 and hepatitis viruses in HIV-1-infected persons) could be detected.

Subjects were seen at six month intervals. At each visit, a medical history and blood for serologic, hematologic, and immunologic evaluations. The same questions were asked of essentially all subjects, and the same laboratory tests were carried out.

VI. LABORATORY EVALUATIONS

Because of the need for as thorough standardization as possible of lymphocyte immunophenotypic counts for comparability across 6 clinical centers, it was decided that shipment of fresh blood specimens could introduce too large and uncontrollable a variable. Accordingly, six immunology laboratories were set up, each using the same model of flow cytometer, and each carrying out the same procedure in as closely standardized a manner as possible. In addition, the Coordinating Center Laboratory provided quality control samples for all procedures for itself and all laboratories testing any TSS samples. In view of the difficulties experienced in some other multicenter studies, this choice still seems the appropriate one.

Since the beginning of patient accession in August 1985, a sample of serum was sent to the Coordinating Center Laboratory for serologic and other tests. These sera continued to be accumulated and used in multiple evaluations as new procedures were (are) developed. Important results could be confirmed (e.g. seroconversion) by retesting earlier specimens with procedures having better sensitivity and specificity. Results that seemed erroneous could be validated by repeating the tests, and if needed, protein allotyping could be used to evaluate potential mislabeling. If precise serial quantitation is needed, all stored specimens can be run at one time using the same reagents and conditions to obtain maximum comparability.

At each visit, for all subjects, lymphocyte immunophenotyping was carried out by two-color flow cytometry using a large panel of monoclonal antibodies.

Virologic evaluations included routing testing of all subjects for anti-HIV-1, hepatitis B markers, and ALT. When the specific test for hepatitis C became available in 1989, all subjects still in follow-up were tested at each subsequent visit. For subjects not in follow-up, their stored sera were tested. Stored sera for selected groups of subjects were also tested for hepatitis A, D, and E, parvovirus B19, CMV and EBV.

VII. THE TSS/NHLBI SUBJECT REPOSITORY

At every specimen collection, 5 ml of plasma in 2 aliquots were stored at -70 degrees and 3 aliquots of buffy coat, each containing 1-3 million cells, in a medium containing DMSO were put into liquid nitrogen storage.

A total of 50,041 buffy coat specimens and 44,771 plasma specimens have been stored. These specimens constitute the TSS/NHLBI Subject Repository. The number of visits represented for each individual ranges from 1 to 23; the longest period of observation is 66 months.

As far as we are aware, TSS was the first to create a cell repository, and did so 10 years before other multicenter studies. Although the RFP stipulated that the Subject Repository include cells, it did not stipulate how they were to be stored. After extended discussions, it was decided to store them as buffy coat. The rationale was that testing that would be carried out an unknown number of years later could not be predicted, and that the important use could involve any subset of cells including nuclear debris from granulocytes.

The Subject Repository is the property of NHLBI (as is the TSS/NHLBI Donor Repository), and no part of these specimens is ever used without their explicit consent. It is the general policy of the TSS not to request specimens from the Subject Repository unless the question is considered sufficiently important by the Group, NHLBI, and the TSS Protocol and Data Monitoring Committee. It has seemed appropriate, however, to apply new techniques as early as possible in their development whenever there is sufficient evidence of probable usefulness towards achieving the general objectives of TSS.

The buffy coat specimens have been used both for PCR confirmation of anti-HTLV positivity and for typing as HTLV-I and HTLV-II. The cell repository, however, may have many possible applications beyond viral nucleic acids. It may, for example, be a possible resource for immune functional assays. We attempted to standardize immunologic tests for function during subject observations using fresh cells, but were never able to obtain reproducible results among laboratories. There is now interest in carrying out functional and new phenotypic assays on frozen cells. Preliminary work indicates that a sufficient number of frozen lymphocytes are intact and viable to be used in this type of assay.

DATA MANAGEMENT

TSS utilized a microcomputer-based distributed data management system to compile the clinical, virologic, and immunologic data obtained from the clinical centers and central laboratories. Until 1990, data were merged and processed on a mainframe computer at the University of Southern California. In 1990, to control costs and increase efficiency, TSS purchased its own workstation and related hardware and software to replace use of USC's mainframe.

All TSS data are stored as SAS files. The organization and contents of these files is described in the Appendices.

Because an investigator who wants to use the information either has to be able to program in SAS or obtain the assistance of a SAS programmer, the study identified the need for an investigator unfamiliar with SAS programming to have methods to easily access the data, to retrieve information quickly, to perform simple statistical procedures, and to depict the data in report and graphical formats. Because it could satisfy those complex requirement, the SAS System Version 6.12, operating on the PC-based Windows platform, was selected to develop this methodology. This choice provides the researcher with a rich and robust set of data management, statistical, and graphical tools in a cost effective setting.

The result is the **Repository Management System (RMS)**. Still in development, this system would integrate the demographic, clinical, laboratory and specimen information within one coherent database accessed by its software program. This system would provide an easy-to-use method for selecting subjects and specimens for additional analyses and to serve future investigators' specific research interests.

RMS is subdivided into two major subsystems: the Investigator Workstation (RMS/INVESTIGATOR) and the Inventory Workstation (RMS/INVENTORY). Each is implemented on a high-speed PC platform and presents a graphical user interface (GUI) for accessing the relevant information.

RMS/INVESTIGATOR provides the researcher with a structured view of the data categories and elements in the TSS database. By selecting variables of interest and specifying criteria for those variables, the researcher can create a dataset containing the subjects and information of interest. The researcher may review the data, create reports, perform analyses, save the dataset for future use, or export it to other platforms for further analysis. He/she may also identify particular subjects and their specific visits and blood draws for the purpose of requesting repository specimens.

RMS/INVENTORY allows the NHLBI repository contractor or qualified support organizations to manage the selection and allocation of the scarce resources of the specimen repositories themselves.

TSS has completed development of the RMS/INVENTORY for the TSS/NHLBI Donor Repository, and the Subject Plasma and Buffy Coat Repositories. These separate but similar systems interconnect with the Subject Data Repository. With specific subjects and visits of interest identified, the system:

- Provides the locations of the specimens, whether they have been aliquotted and the number of remaining aliquots
- Identifies the highest numbered aliquot for each specimen, codes it, and develops a shipping list. Informs if the original specimen has not been aliquotted
- Allocates locations for the new aliquots.
- Tracks the status of all requests and shipments
- Protects specimens that are being depleted.

IX. SUMMARY OF SELECTED MAJOR FINDINGS

A. Anti-HIV-1 screening of the Donor Repository

Of the 200,085 samples in the TSS/NHLBI Donor Repository evaluated for the presence of anti-HIV-1, 307 were found to be unequivocally positive. The rate of confirmed anti-HIV-1 positivity among tested donors to the four blood services in areas of high AIDS prevalence immediately prior to routine screening was 0.15 percent. The crude rate varied from 0.12 percent to 0.24 percent among the four areas.

The rates of confirmed anti-HIV-1 positivity among donors was calculated for residence within and outside of the major Standard Metropolitan Statistical Area (SMSA) in the service region of each of the four participating collection agencies.

- Anti-HIV-1 prevalence varied markedly among the predominantly suburban areas outside of the major SMSA's served by the participating collection agencies.
- The correlation was poor between anti-HIV-1 prevalence and CDC-reported AIDS cases in the same year (1984) and two years later (1986).
- The pattern of anti-HIV-1 prevalence among donors showed that some localities within SMSA's with high AIDS prevalence had a very high anti-HIV-1 rate among males under age 35 who served as blood donors (1.8 to 2.4 percent). Males in low AIDS prevalence localities, nonetheless, accounted for 42 percent and 75 percent of all anti-HIV-1 positive tested donations in two areas (San Francisco and Los Angeles) for which adequate data for this analysis were available.

The seropositive donors, who were detected before the institution of routine anti-HIV-1 screening, disproportionately were first-time donors and men with exclusively male sexual contacts. This suggests that test-seeking may have contributed to the high HIV-1 prevalence in the repository, and that implementation of alternative test sites when routine donor screening began in 1985 may have averted many high-risk donations.

Re-testing of 12,000 initially anti-HIV-1 negative samples in the TSS/NHLBI Donor Repository demonstrated a very low rate of false negativity. Compilation of results of repeat testing of quality control specimens by protein immunoblot showed that although this assay was generally reliable, it may sometimes be in error, and quality control checks are essential for all laboratories. Important decisions by the patient and physician should be based on positivity of more than one specimen.

B. Anti-HTLV screening of the Donor Repository

Screening of the Donor Repository in 1988-89 showed the prevalence of HTLV positivity to be 0.8 per 1,000 donations, about half that of HIV-1. The rates varied from 0.7 to 1.0 per 1,000 and showed no correlation with the prevalence of anti-HIV-1 among the same donors. Only one donor was positive for both.

The distribution of anti-HTLV positivity among donors showed it to be diffusely distributed in the four major metropolitan areas surveyed across both sexes and all age groups. This is consistent with the wide diversity of epidemiologic background found for enrolled donors.

Anti-HBc prevalence among in the four area ranged from 14 to 43 percent and anti-CMV prevalence ranged from 67 to 96 percent among anti-HTLV positive donors—generally well above the range of reactivity expected for blood donors in the U.S. Because both are indices of not only behavioral risks but also socioeconomic status, a correlation with anti-HTLV is not surprising.

C. HIV-1 p24 antigen screening of the Donor Repository

Testing of a subset of 8,597 Donor Repository specimens from young men, 18-44 years old, living in largely contiguous ZIP code areas with anti-HIV-1 prevalence greater than 1% showed p24 antigen positivity in 11% of the already confirmed anti-HIV-1 positive specimens, but in none of the seronegative samples. In view of these negative results in this enriched donor sample, we concluded that the yield of screening for p24 antigen in volunteer donors to identify – HIV-1 carriers would be negligible.

D. Anti-HIV-1 positive donors

From the repository of donors sera collected in late 1984 and early 1985, 289 donors who were unequivocally anti-HIV-1 positive were identified and 146 of these were enrolled for further study of the characteristics of persons whose components were or were not associated with HIV-1 infection of recipients.

Among the male anti-HIV-1 positive donors for whom sufficient data are presently available, 94 percent had a known risk factor, compared to 6 percent of donors found to be anti-HIV-1 negative and matched to the positive donors by sex, age, and area of residence. Among the anti-HIV-1 positive and negative females enrolled, four of eight (50%) and one of 12 (8%), respectively, had a known risk factor. Similarly large differences were observed when positive and negative donors with demographic characteristics similar to positive donors is reassuring about the sensitivity of present anti-HIV-1 screening.

Approximately 80% of the anti-HIV-1 positive TSS donors have been found on follow-up to have probably acquired their infections as a result of homosexual contacts. The number of these homosexual encounters, however, is generally much smaller than those of subjects recruited into studies based on participation in gay organization; approximately 40% of TSS anti-HIV-1 positive donors are bisexual. This circumstance permits an assessment of the role of less extensive exposure to other sexually transmitted agents on the course of HIV-1 infection.

The actuarial probability of progression to AIDS within 7 years was 40%; the probability of dying of AIDS was 28%. AIDS developed more often when the donor was p24 antigen-positive at donation. Over a 3-year period, significant decreases occurred in CD4+, CD2+CD26+, CD4+CD29+ and CD20+CD21+ counts, but not in CD8+ subsets, CD20+ or CD14+.

E. Transfusion recipients

The 133 enrolled recipients of blood components from donors subsequently found to have been anti-HIV-1 positive at the time of donation represent the only large group of persons known to have been exposed through an anti-HIV-1 positive component. The probable date of infection can be established for almost all by the date of the implicated transfusion.

Rate of infection. Follow-up observations for these 133 recipients showed that 91 percent were anti-HIV-1 positive when first seen. This was strong evidence that anti-HIV-1 positivity in the donor was a marker for presence of HIV-1 infection, and that recipients very rarely escaped infection.

Clinical and immunologic profiles of anti-HIV-1 negative recipients of positive blood indicated that they were not infected persons who failed to seroconvert. More recent evaluations showed negativity by DNA amplification.

Implications for look-back. The fact that not all recipients of blood components from anti-HIV-1 positive donors become positive had implications for the "look-back" policy generally accepted by blood collection agencies--the routine notification of recipients of components from prior donations by persons found to be anti-HIV-1 positive because of a donation after routine screening was implemented. Blood services were identifying, notifying, and testing recipients of successively earlier donations until an anti-HIV-1 negative recipient was found. The assumption underlying this procedure was that anti-HIV-1 negativity of one recipient indicates negativity of the donor at that time, and further "look-back" is unnecessary. TSS data showed that one anti-HIV-1 negative recipient does not permit the conclusion that the donor was not infected earlier.

Factors influencing infection in the recipient. Suggestions that a yet-unknown host factor was causing refractoriness of the CD4 cells preventing infection in the seronegative recipients of positive blood were investigated. For the five recipients from whom fresh lymphocytes could be obtained, the lymphocytes were infectible in vitro.

Infected recipients and those who remained negative after receiving a anti-HIV-1 positive units were similar with respect to sex and age. No association was found between seroconversion in the recipient and any impairment of immunologic competence suggested by the underlying medical indication for transfusion. Transmission was associated with all types of components with the exception of washed red cells, but no conclusion could be drawn about the effect of the washing procedure because red cells were processed in this way in only two instances. Duration of storage of red cells had a small effect on transmission, perhaps due to the release of degradative enzymes.

Nineteen pairs of recipients of components from the same anti-HIV-1 positive donor were enrolled. None had discordant anti-HIV-1 results: 18 pairs were anti-HIV-1 positive and one pair was anti-HIV-1 negative, suggesting an association between seroconversion in the recipient and donor characteristics. Donors linked to seropositive recipients and donors linked to seronegative

recipients did not differ by sex , age, HIV-1 p24 antigen positivity or HIV-1 risk factors. Nor did they differ by rate of change in CD4 count or progression to AIDS. Earlier qualitative and quantitative DNA PCR showed very similar results for transmitting and non-transmitting donors. However, an early PCR assay for RNA showed that 6 of 11 transmitters, but none of the 11 non-transmitters tested RNA positive.

A later more sensitive quantitative RNA assay detected RNA in all sera, but the median viral load was significantly higher in transmitting than non-transmitting donors. This difference in viral load between transmitting and non-transmitting donors remained significant even when component shelf life was taken into account. Level of viremia seemed to be an important determinant of HV-1 infection by transfusion.

TSS was the first to directly compare the incidence of transfusion transmission of anti-HIV-1, anti-HTLV-I, and anti-HTLV-II and the effects of refrigerator storage of the blood component on infectivity.

- Overall, 27% of recipients of components from anti-HTLV-I- and -II positive donors became infected.
- No recipient of acellular components became infected.
- There was no transmission by components stored more than 10 days.
- The rates of transmission were similar for HTLV-I and II. 74% for components stored 0-5 days; 44% for those stored 6-10 days; and 0% for those stored 11-14 days.
- In contrast, 89% of recipients of anti-HIV-1 positive blood were infected regardless of component type, and no effect on transmission occurred with storage for less than 26 days.

Recipients exposed to two seropositive components. TSS identified a case in which a premature infant was concurrently transfused with packed red blood cells from 2 different HIV-1 positive donors represented in the Repository. The 2 donors also each singly infected a second infant. Quasispecies analysis of the HIV-1 genomes in each of the two donors showed a close relationship to the strain in the respective singly-exposed recipient. Quasispecies analysis of the dually exposed recipient provided evidence of an individual simultaneously infected with two distinct HIV-1 strains, as well as recombination of the two *in vivo*.

Another case of dual exposure by transfusion in a 54 year-old male indicated an apparent lack of transmission by one of the units. We speculated that this case might represent a transmission bottleneck selecting specific genomes between different inoculated HIV-1 strains.

F. Sexual partners of recipients.

Nineteen long-term heterosexual partners of 18 HIV-1-infected transfusion recipients were prospectively observed. When first seen, 2 of the 19 partners were anti-HIV-1 positive. Four partners seroconverted during 23 person-years of observation.

- Interval after infection, type of sexual behavior, clinical status, CD4 count, and HIV-1 p24 antigen status had no definable relation to transmission.
- However, recipients who transmitted HIV-1 to their sexual partners, had a significantly higher mean viral RNA level than non-transmitting recipients.
- The data also suggested a threshold level of viremia (3.75 $\log_{10}$ copies/ml) below which sexual transmission is unlikely to occur.

G. Persons with congenital clotting disorders (CCD).

Prevalence of HIV-1 infection. Among persons with congenital disorders, the prevalence of anti-HIV-1 positivity at enrollment from mid-1985 varied with the major forms of therapy for the particular disorder. It was 83% for those treated with factor VIII concentrates, 57% for those treated with factor IX concentrates, and 24% for those treated with unpooled products.

HIV-1 seroconversion. TSS identified 6 instances of seroconversion to positivity among persons treated with plasma products for clotting factor deficiency. All could be explained by concentrates heated at 60 degrees for 24-30 hours; the preparations implicated in 3 cases were derived entirely from anti-HIV-1 screened donors.

Immunologic effects of therapy among HIV-1 uninfected hemophiliacs. TSS examined the effect of therapy on peripheral blood mononuclear cell immunophenotypic subsets in anti-HIV-1 negative persons with all types of CCD. Compared to controls, age-adjusted CD4+ counts were significantly lower in treated patients and in patients with all types of CCD who were seldom or never treated.

There was no difference attributable to type of clotting disorder or factor therapy. Significantly lower values among both treated and untreated CCD subjects were likewise found for total lymphocytes, several other T-cell subsets and the CD4/CD8 ratio. For most indexes including the CD4+ counts and CD4+/CD8+ ratio, the type of clotting deficiency was not a significant variable. Comparing persons who had no or minimal therapy with those having the most showed increases in CD8+ and CD20+CD21- counts and a lower CD20+CD21+/CD20+ ratio in the latter, but large amounts of treatment were not associated with a change in CD4+ count.

Immunologic effects of therapy among HIV-1 infected hemophiliacs. TSS also investigated the generally accepted view that low- and intermediate-purity clotting factor therapies accelerate the rate of CD4 decrease in HIV-1 infected hemophiliacs. TSS found no overall or dose-related deleterious effect of any form of treatment on CD4 trend among HIV-1 infected hemophiliacs. The CD4 decrease was less when cryoprecipitate was administered alone or combined with concentrate but not significantly so.

Prognostic usefulness of lymphocyte markers in HIV-1 infection. Examination of individual differences in rates of decline of CD4 counts in HIV-1 infected males with CCD showed that the rates of decline varied significantly among individuals, but that the short-term within- person variability was much greater than the long-term between person variability. The conclusion was an HIV-1 infected individual's CD4 counts over 6-12 months indicate little about his long-term rate of CD4 change, and that measurements of CD4 count over 2 years or more are required to provide a reliable estimate of his future rate of CD4 decline.

Interaction between HIV-1 and HCV. TSS investigated possible interactions between HCV and HIV-1 that alter the course of infection. Of the 1,243 persons with hemophilia enrolled in the study, 1,035 (83%) were anti-HCV positive throughout observation. Analysis of the effect of HCV on HIV-1 infection was limited by the very small number of HIV-1 infected persons who were anti-HCV negative (n=4). With respect to the effect of HIV-1 infection on HCV infection:

- Among HCV-infected persons, HIV-1 infection seemed to have no effect on ALT levels.
- The rate of chronic liver disease was significantly higher in HIV-1 infected than uninfected persons.
- The risk of death with liver disease as a cause (excluding those with AIDS mentioned as a cause) was signficantly higher for anti-HIV-1 positive persons than negative persons.

We concluded that coinfection with HIV-1 and HCV may exacerbate liver disease.

H. Household contacts of persons with congenital clotting disorders

Among sexual and non-sexual household contacts of anti-HIV-1 positive persons with congenital clotting disorders, anti-HIV-1 positivity was limited to sexual contacts and to children born to anti-HIV-1 positive wives of positive hemophiliacs. At entry, 10% of 201 sexual partners of HIV-1 infected persons with CCD were anti-HIV-1 positive; follow-up of 151 uninfected partners for a total of 351 person-years showed no seroconversions although there were 13 pregnancies. Among 304 nonsexual household contacts, none were seropositive at entry and none of the 263 household contacts followed for 605 person-years seroconverted.

I. Persons with congenital anemias.

Among persons treated since 1979 with unpooled blood components for congenital anemias, 4 percent were HIV-1 positive. The instances of transmission by red cell therapy were confined to New York, Miami, and Los Angeles, all of which had very high prevalence of AIDS prior to anti-HIV-1 screening of blood donors.

Studies of hematologic and lymphocyte indices in 173 HIV-1 negative subjects with sickle cell anemia (SCA) and 131 Black controls showed that that children 1-7 years with SCA had leukocyte counts and percentages of granulocytes, monocytes, natural killer cells, and T cell markers that were significantly higher than for control children. Percent total lymphocytes were decreased for this age but the total number of lymphocytes and T and B cell counts were similar

to controls. Platelets were not increased. Adolescents and adults with SCA had total leukocytes and monocytes that were increased, and lymphocyte counts that remained level instead of decreasing as did comparably aged controls. Lymphocyte subsets typically increased in count, but their percentages remained similar to controls. The exception was that CD56+ cell counts were increased in adolescents and adults. No lymphocytic subset change suggested impaired cellular immunity, and none could be related to transfusion. Prophylactically transfused patients had higher granulocyte counts, but these may arise from the complications of SCA itself.

J. Multi-group analysis and comparisons among risk groups.

CMV and EBV in relation to HIV-1 infection. Seroepidemiologic studies of cytomegalovirus (CMV) and Epstein-Barr Virus (EBV) infections among persons with and without antibodies to HIV-1 infection included donors, treated clotting disorder patients, recipients of components, and untransfused controls, including household contacts.

- A significantly higher proportion of anti-HIV-1 positive donors than anti-HIV-1 negative donors were anti-CMV positive, a finding associated with homosexual contact among some of the former.
- Among treated clotting disorder patients there was no difference in prevalence of anti-CMV or anti-EBV between anti-HIV-1 positive and negative persons.
- The prevalence of antibodies to EBV early antigens had no relation to anti-HIV-1 status.
- Subjects who developed AIDS after enrollment had no significant difference in median time from entry to diagnosis when analyzed by serologic evidence of CMV and EBV antibody status at entry.
- A few subjects had AIDS at entry without serologic evidence of prior CMV or EBV infection.

These results are consistent with acquisition and progression of HIV-1 independent of CMV or EBV infections.

Influence of HIV-1 infection on expression of chronic hepatitis B (HBV) and D (HDV) virus infections. Possible modifications of chronic HBV and HDV infections by infection with HIV-1 were examined among 47 subjects (blood donors, recipients of blood components and products, and household contacts) with chronic HBV without HDV infection, and 18 subjects with chronic HBV and HDV infections. The result did not suggest that HIV-1 infection alters the expression of chronic HBV and HDV infections.

Effect of age on progression of HIV-1 infection. TSS evaluated the extent to which age differences among risk groups, observed under a common protocol, account for rate differences in progression to AIDS.

- The actuarial risk for AIDS 7 years after infection was 51% among transfusion recipients, 27% among homosexual donors, and 19% among hemophiliacs.

- Risk group differences in AIDS progression were explained to a large extent by different age distributions in the cohorts and more rapid decline in CD4 counts among older persons.
- When both the CD4 value at each visit and age were used as covariates, however, homosexual donors had a more rapid progression than the other two groups.
- Omitting Kaposi's sarcoma as an AIDS-defining condition removed any significant differences among risk groups other than age.

Influences of age, viral load, and CD4+ count on rate of HIV-1 progression. In view of findings of TSS and others that age at time of HIV-1 infection is associated with more rapid progression to AIDS, and subsequent studies that showed that high plasma HIV-1 RNA levels are correlated with more rapid progression to AIDS, we investigated whether age is associated with viral load. Such an association could explain the accelerating effect of increasing age on HIV-1 disease progression. We found that viral load and age appear to be partly independent predictors of HV progression, and viral load seems to account for little of the effect of age.

Further analysis investigated the relation among CD4 level, age, and viral load. There was a strongly significant negative correlation between the CD4 count and viral load. Adjusting for viral load, the CD4 values for subjects younger than 40 years of age were moderately higher than those for subjects 60 year or older at the same level of viral load. These data suggest that younger persons tend to develop lower levels of viral load and to have less CD4 depletion at given viral loads.

It appears that younger persons may be able to mount a more effective immunologic response to HIV-1 infection, which is manifested by lower levels of viral load, higher levels of CD4 cells and less rapid progression to clinical AIDS.

Comparison of persons infected with the same or different HIV-1 strains. We compared rates of HIV-1 progression among persons infected by the same or different subtype B strains. Forty-three infection chain clusters were identified, each defined by an infected blood door, that donor's recipients, and the recipients' sexual partners. Analysis of level and rate of change in CD4 lymphocyte counts and viral load showed that members within a cluster were no more alike in their rate of change in CD4+ lymphocyte counts or viral RNA level than among clusters. Differences in entry viral RNA levels by cluster were marginal and markedly smaller than inter-individual differences. These results argue that, in general, host factors outweigh differences in viral strain in determining HIV-1 disease progression.

Transfusion transmission of human herpesvirus 8 (HHV8). TSS investigated whether there is evidence that HHV-8 (also known as Kaposi's sarcoma-associated herpesvirus) is transmitted by transfusion by evaluating stored specimens from blood donors, their specific recipients and persons treated with blood components and products for hemophilia. We found that the rates of HHV-8 infection in anti-HIV-1 positive individuals who acquired HIV-1 infection parenterally were similar to that of HIV-1 seronegative blood donors, but significantly lower than that for donors found to be infected with HIV-1 through homosexual contact. We concluded that current

epidemiologic evidence supports sexual activity as the primary route of HHV-8 transmission with little evidence for transmission by blood and blood derivatives.

Specific studies of recipients of anti-HIV-1 positive blood and their HIV-1 infected donors included 14 donors who were HHV-8 seropositive. Of their 14 recipients, ten became HIV-1 infected, but not one became HHV-8 seropositive even as long as 19 months after the transfusion. Thirteen of the 14 recipients from the HHV-8 seropositive donors received cellular components and yet remained seronegative. These data support the conclusion that the risk of both HHV-8 infection and development of Kaposi's sarcoma is mainly through sexual rather than parenteral routes.

Effect of a 32 base pair deletion with the CCR5 locus on HIV-1 infection and progression. After the report that individuals homozygous for a non-functional allele of CCR5, resulting from a 32 base pair deletion, resist HIV-1 infection, TSS focused on the importance of CCR5 during parenteral transmission of HIV-1 among individuals treated with HIV-1-contaminated blood and blood products. The distribution of the deleted CCR5 allele among 512 exposed individuals with hemophilia and 97 exposed transfusion recipients indicates that the loss of CCR5 expression resulting from homozygous deleted alleles protects individuals from infection. No difference in the survival rate of infected heterozygous versus homozygous wild-type individuals was observed. The latter finding is in contrast to previous studies principally focusing on sexual transmission among homosexual men, and suggests that disease progression following parenteral transmission may differ from progression following sexual transmission.

X. ORGANIZATIONAL COMPONENTS

Coordinating Center
- Director's Office
- Administrative Office
- Biostatistics Office

Central Laboratories
- Coordinating Center Laboratory (Central Processing Laboratory)
- Human Immunodeficiency Virus Testing Laboratory
- Human T-cell Lymphotropic Virus Testing Laboratory
- Herpesviruses Testing Laboratory
- Hepatitis Testing Laboratory
- Immunologic Standardization and Reagents Laboratory

Clinical Centers
- New York
- Miami
- Detroit
- Seattle
- San Francisco
- Los Angeles

Functional Components
- Steering Committee
- Publications Committee
- Forms Committee
- Immunology Working Group
- Virology Working Group
- Congenital Hematologic Disorders Working Group
- Working Group on Donor-Recipient Observations

The **Coordinating Center**, comprised of the Director's Office, the Administrative Office, and the Biostatistics Office, was at the University of Southern California (USC). It had overall responsibility for scientific planning and direction, administration, and data storage and analysis.

The six **clinical centers** were responsible for enrolling and observing subjects, carrying out laboratory tests that require fresh specimens, and entering data.

The **central laboratories** carried out specific testing required by the study if frozen specimens could be used.

The Coordinating Center Laboratory (Central Processing Laboratory) received and stored frozen aliquots of serum from every specimen taken as part of TSS (approximately 6,000 per year). It was responsible for EIA testing for anti-HIV-1, HBsAg, anti-HBs, anti-HBc, and anti-HAV. It also aliquoted serum for less frequently required procedures, including those at other central laboratories.

In addition, the Coordinating Center Laboratory provided quality control samples for all procedures for itself and all laboratories testing any TSS samples. One basis for quality control was a panel of more than 300 samples of plasma, in volumes of a few to several hundred ml. These were obtained as needed as "recovered" plasma from participating blood services or other sources, and from plasmapheresis of selected subjects. The entire plasma panel was tested using most assays for HIV-1, HTLV-I/II, hepatitis B virus (HBV), hepatitis D virus (HDV), cytomegalovirus (CMV), and Epstein-Barr virus (EBV). During anti-HTLV-I/II screening of the TSS/NHLBI Donor Repository, Coordinating Center Laboratory personnel prepared more than 10,000 aliquots of positive and negative specimens for training panels, certification panels, and plate controls (two per EIA plate).

The Human Immunodeficiency Viruses Testing Laboratory utilized procedures including the polymerase chain reaction (PCR) assay for HIV-1 RNA, and EIA testing for recombinantly derived HIV-1 epitopes.

The Human T-cell Lymphotropic Virus Laboratory carried out anti-HTLV-I/II screening by EIA, and confirmation by an HTLV-I p24 RIA and cell cultures.

The Herpesvirus Testing Laboratory carried out standardized EIA's for antibodies to cytomegalovirus (anti-CMV), antibody to the viral capsid antigen of Epstein-Barr virus (anti-EBV VCA), and antibody to EBV early antigen (anti-EBV EA).

The Hepatitis Testing Laboratory supplemented the routine HBsAg, anti-HBs, and anti-HBc screening in the Coordinating Center laboratory by anti-HAV, HBV DNA, anti-HBc-IgM, anti-HDV-IgM, and anti-HDV-IgG assays.

The immunologic tests carried out in TSS generally could not be performed centrally because lymphocyte function and phenotype change with storage. Accordingly, the approach to comparable data across six clinical centers was to provide each with the same flow cytometer, to develop standardized protocols for lymphocyte phenotyping, and to use quality control. The Immunologic Standardization and Reagents Laboratories were responsible for the development of the standardized procedures, and the distribution of interlaboratory quality control specimens. The Reagents Laboratories also provided quality control samples to all commercial and research laboratories testing any TSS samples, and monitored the results.

The **Steering Committee** was the executive advisory committee for the project. It was composed of the Project Director, the Director of the Biostatistics Office, and the directors of the six clinical centers.

The **Publication Committee** was responsible for planning and approving proposed papers and presentations. Consequently, it had a wide representation of investigators including the Project Director, the Director of the Biostatistics Office, the directors of the central laboratories, and the Chairperson(s) of each working group. Any clinical center not represented by one of these designations was represented by its director.

The **Forms Committee** was organized in 1988 to revise the forms in use since early in the Study. It was comprised of representative of the Director's and Biostatistics Offices and members chosen from the project coordinators, patient managers, and data managers at clinical centers.

The **working groups** are affiliations of investigators interested in particular specialty areas to evaluate incoming results, modify protocols, and determine future directions.

The following institutions and organizations participated in the Transfusion Safety Study:

XI. PARTICIPATING INSTITUTIONS

Coordinating Center

University of Southern California
Epidemiologic Research Laboratory, Department of Medicine
Division of Biometry, Department of Preventive Medicine

Central Laboratories

University of Southern California
Epidemiologic Research Laboratory, Department of Medicine
Department of Pathology facilities
SmithKline Laboratories
City of Hope Medical Center
University of Miami
Irwin Memorial Blood Centers
Abbott Laboratories

Clinical Centers

Mount Sinai Medical Center
Cornell University Medical College
New York Blood Center

University of Miami Medical Center
American Red Cross Blood Services, South Florida Region

Wayne State University
Children's Hospital of Michigan

Puget Sound Blood Center

University of California, San Francisco
Alta-Bates Hospital
Irwin Memorial Blood Bank

Orthopedic Hospital of Los Angeles
Los Angeles County-USC Medical Center
Cedars-Sinai Medical Center
American Red Cross Blood Services, Los Angeles-Orange Counties Region