Results of the Cord Blood Transplantation (COBLT) Study unrelated donor banking program

Joanne Kurtzberg, Mitchell S. Cairo, John K. Fraser, LeeAnn Baxter-Lowe, Geoff Cohen, Shelly L. Carter, and Nancy A. Kernan

BACKGROUND: The goals of the Cord Blood Transplantation (COBLT) Study banking program initiated in 1996 were to develop standard operating procedures (SOPs) for cord blood (CB) donor recruitment and banking and to build an ethnically diverse unrelated CB bank to support a transplantation protocol.

STUDY DESIGN AND METHODS: The program included collection centers, three banks, a steering committee, and a medical coordinating center (MCC) that developed and validated SOPs and a Web-based data collection system. External oversight was performed by the National Heart, Lung, and Blood Institute and the MCC.

RESULTS: A total of 34,799 potential donors were screened and 20,710 consented. A total of 17,207 ethnically diverse units were collected between 1998 and 2001. A total of 11,077 (64%) units were cryopreserved and quarantined. Of these, 79 percent met eligibility criteria and were HLA-typed and entered into the search registry. Higher CB volumes and cell counts were obtained from cesarean sections compared to vaginal deliveries. Units from African American persons contained lower cell counts per volume compared to other ethnicities. Birth weight correlated with volume and cell content. External oversight was accomplished through custom reports generated by the data collection system and periodic site visits. During maintenance, a breach in the SOPs was detected during a site visit at one of the banks. These units were designated for future use in nonclinical research.

CONCLUSION: The COBLT Study demonstrated that SOPs and data collection can be implemented in multiple banks coordinated by one MCC. Relationships between donor demographics and CB content may be useful in the development of other CB banking programs.

HEMATOPOIETIC STEM CELL TRANSPLANTATION (HSCT) is used to treat patients with aggressive malignancies, congenital immunodeficiency diseases, inborn errors of metabolism, hemoglobinopathies, and marrow failure syndromes. A major obstacle preventing access to transplantation is donor availability. More than 50 percent of patients in need of...
transplantation therapy cannot find a fully HLA-matched related or unrelated donor. Over the past decade, umbilical cord blood (UCB), the residual fetal blood in the placenta after delivery, has emerged as an alternative source of hematopoietic stem and progenitor cells for use in HSCT. Cord blood (CB) cells demonstrate increased immune tolerance and can be successfully transplanted without full HLA matching, making it an attractive source of cells for patients lacking a fully matched related or unrelated donor.

For more than a decade, CB has been collected and banked with maternal consent, from the placentas of healthy infants born after uncomplicated pregnancies and deliveries. The first public bank was established by Dr Pablo Rubinstein as a research project with support from the National Heart, Lung and Blood Institute (NHLBI) in 1991. Initial results of unrelated donor CB transplants (CBTs) utilizing units from this bank demonstrated successful engraftment despite partial HLA mismatching with tolerable levels of acute and chronic graft-versus-host disease (GVHD) in children.

To meet the ongoing needs of patients, particularly ethnic minorities, the Cord Blood Transplantation (COBLT) Study was conceived by the NHLBI. The COBLT Study is a Phase II, multicenter study designed to determine the utility of UCB for transplantation of patients with malignant and nonmalignant diseases. The initial goal of the study was to bank 15,000 cord blood units (CBUs) with common standard operating procedures (SOPs). The long-term goal of the study was to use these CBUs for transplantation of patients enrolled on a clinical protocol.

Three academic centers were awarded contracts from the NHLBI to create unrelated cord blood banks (CBBs). A medical coordinating center (MCC; The EMMES Corporation, Rockville, MD) was awarded a contract to create a Web-based banking database and search registry. A banking steering committee, composed of the bank directors, transplanters, obstetricians, medical technologists, ethicists, statisticians, and representatives from the MCC and NHLBI, met on a monthly basis to develop and validate SOPs for donor recruitment and education, donor screening, donor consent, CB collection, maternal and CB testing, processing, cryopreservation, long-term storage, thawing, shipping, transplantation, data capture forms, and an algorithm for the search registry.

Recruitment activities were initiated in October 1997 and were targeted toward hospitals serving ethnically diverse populations. CBB collections were initiated in December 1997 and were continued until June 15, 2001, when this phase of the study was completed. This report summarizes the results of this first national cooperative banking effort and describes banking activities, external quality assurance-quality control (QA-QC) oversight, and donor and CBU characterization, and identifies correlations between various factors that can be utilized for future modeling for larger scale CB banking.

MATERIALS AND METHODS

Establishment of the COBLT Study banking program

Three banks, each identifying a series of collection centers, participated in the COBLT Study program. Quality plans were developed and implemented. Current SOPs and data collection forms are available at http://spitfire.emmes.com/study/www_emmes_com/. A summary of the methods used on the study is described below.

Validation of processing and testing

The three banks developed, standardized, and validated methods by performing eight sample distributions for flow cytometry and CD34+ analysis after collection and before cryopreservation. Validation of assays to enumerate hematopoietic colony-forming units (CFUs) were attempted but not successful. For validation of time from collection to storage, samples were maintained on the bench top for 24, 48, and 72 hours and analyzed for cell count, viability, phenotype, and CFUs, and results were analyzed by the MCC. Samples were subsequently cryopreserved and thawed to evaluate survival and recovery of viable nucleated cells and CD34+ cells.

Donor recruitment and screening

Each CBB included trained nurse educators and collection specialists to inform potential donors about the COBLT program. Educational materials (study-specific brochures printed in English, Spanish, Chinese, Vietnamese, and Korean; a video tape describing the COBLT Study and CB collection process; and CBB newsletters and Web sites) that provided potential donors (pregnant women ≥18 years of age) were developed. Ethnically diverse communities were targeted, and materials were distributed through physician offices, prenatal classes, local news media, churches, and community organizations. Translators and validated translated informed consent documents were used to facilitate recruitment in Asian and Hispanic communities.

Potential donors, who were in their second or third trimester and with no known pregnancy complications or infectious disease (ID) history, were identified by clinical and obstetric office personnel who used a screening tool to exclude ineligible mothers without revealing their identity to the CB collection staff. The prescreening tool identified factors that would exclude mothers from the study including multiple gestation; premature delivery; active chorioamnionitis; mother being the recipient of an organ transplant; mother having been treated for cancer in the past; mother having high-risk behaviors or having
been previously diagnosed with human immunodeficiency virus (HIV), hepatitis, or syphilis; and mother having active vaginal herpes simplex virus or human papillomavirus and delivering vaginally. Potentially eligible mothers were then approached by the obstetric clinic, the office staff, or the CB collection staff to determine their interest in participating. The CB collection specialists obtained written informed consent from all donors after an in-person educational session that generally lasted 30 to 90 minutes any time after 28 weeks’ gestation. Before delivery or within 48 hours after delivery, all donors signed an institutional review board–approved informed consent document describing the procedures for CB collection, required blood samples, and confidentiality. The mother was required to demonstrate knowledge of the program and intent to donate before the onset of painful contractions requiring analgesic therapy. Verbal consent could be obtained before delivery, but written consent was required no later than 48 hours from the infant’s birth. When verbal consent was obtained before delivery, the CB collection specialist documented this on the written consent form that was later verified and signed by the mother. The informed consent process ensured that the donor understood 1) that the donation of her infant’s CB was voluntary and that she would not be compensated; 2) that she agreed to give a complete medical history for herself and the infant’s immediate family; 3) that she agreed to allow a sample of her blood to be taken to be tested for IDs that could be transmitted through the blood and that she understood that if any of these tests were positive, these results would be communicated to her and to the state agency for infection control as dictated by local jurisdiction; 4) that there would be no guarantee that the CB would be collected, processed, or banked; 5) that CBUs not eligible for banking might be used for anonymous medical research but not for development of a commercial product; 6) that the infant’s family would not be notified as to the status of the CB after collection; 7) that the CB might be tested in the future for a genetic disease (e.g., inborn error of metabolism) and that if positive, the donor’s family would be contacted and informed of that result; 8) that all identifiers linking the mother-infant pair with the CBU would be confidential; and 9) that the donor and her family would have equal but not preferred access to the CBB. The mother also agreed to notify the CB bank if, in the future, her baby developed a serious illness.

After obtaining consent, a medical history was obtained by a COBLT Study collection specialist. In addition to routine questions commonly used to screen blood donors for high-risk behaviors and IDs,21 specific questions addressed risks for transmission of hereditary or acquired blood-borne diseases that could be transmitted via HSCT. For example, a history of splenectomy or cholecystectomy as a child or young adult was a surrogate for congenital red cell (RBC) disorders and death of a first-degree relative in infancy or early childhood was used as a screen for heritable immunodeficiency diseases. Specific questions determined whether there was a family history of inborn errors of metabolism; hemoglobinopathies; congenital white blood cell (WBC), platelet (PLT), or RBC disorders; hemolytic anemias; or cancer in a first-degree relative. After delivery and collection of the CBU, the maternal donor reconfirmed her intent and consent to donate.

Strict criteria were developed to identify high-risk donors. The mother was excluded from the study if she was known to be infected with hepatitis B or C, human T-lymphotropic virus (HTLV) -1 and/or -II, HIV-1 and/or -2, or syphilis; was carrying more than one fetus; was the recipient of an organ transplant; delivered her infant before 34 weeks’ gestation; had sepsis; had chorioamnionitis; or had active venereal disease at the time of delivery. If the mother had a prior splenectomy or cholecystectomy, her complete blood count and RBC indices were reviewed by the medical director of the CBB to rule out the presence of a hereditary RBC membrane disorder (e.g., spherocytosis, elliptocytosis).

**CB and donor sample collection**

Preprinted cryogenic bar code labels were used to link the donor and the CB collection. Study label sets were prepared with each set having a unique bar code number formatted with the International Society of Blood Transfusion (ISBT)-128 coding system and an eye-readable number. Labels were sized to fit on study forms, blood sample tubes, and processing and cryopreservation bags and samples. Donor samples were distinguished from CB samples with the ISBT code “flag” characters and colored labels.

To protect the privacy of the donor, the CB collection team did not attend the delivery of the infant. CB collections were performed in a limited-access room within or proximal to the delivery unit. After delivery, hospital staff placed the placenta in a labeled container and handed the container to a CBB collector waiting outside the delivery room. The placenta was suspended in a collection stand, fetal side down, and the umbilical cord was stabilized below. The cord was sterilized with Betadine and alcohol, after which the umbilical vein was punctured with a 17-gauge needle connected to a sterile, bar code–labeled collection bag containing 25 mL of citrate-phosphate-dextrose anticoagulant. The fetal blood dripped by gravity into the collection bag that was agitated on a rocking scale to ensure mixing of the anticoagulant. Typically collections were initiated within 10 to 20 minutes of delivery and completed with 10 minutes. After collection, the collected CBU was stored at 15 to 25°C in a validated shipper and transported to the CBB processing laboratory.
Donor blood samples were collected for ID testing and retrospective HLA typing were collected within 4 days before and 14 days after CBU collection. ID screening of the sample included FDA-approved tests for cytomegalovirus antibody (anti-CMV), immunoglobulin M (IgM) antibody, hepatitis B core antigen, syphilis, anti-HCV, hepatitis B surface antigen, HIV-1 and/or -2, HIV p24 antigen, and HTLV-I and/or -II. If a screening test for hepatitis B or C, HIV, or HTLV was positive, even if a confirmatory test was later negative, the unit was excluded from the bank. If the screening test for syphilis was positive but the confirmatory test was negative, the unit was not excluded in accordance with standard FDA guidelines for blood donors. Hemoglobinopathy screening was obtained through the required newborn screening programs in each participating state laboratory. CBUs from infants with homozygous hemoglobinopathies were excluded from the inventory.

An immune screen for total immunoglobulin (including IgG and IgM) was used to test maternal plasma for antibodies against CMV. Positive samples were further screened for the presence of specific anti-CMV IgM by enzyme-linked immunosorbent assays. Units collected from these mothers testing positive for anti-CMV IgM were excluded. In one bank, as an additional research question, samples from maternal CBU plasma from IgM-positive mothers were further tested for CMV DNA via nucleic acid testing (NAT; Amplicor assay system, Roche, Indianapolis, IN). Additional samples of maternal whole blood, plasma, and DNA were stored to facilitate future ID or genetic testing or for resolution of HLA ambiguities in the CB donor.

**CB processing, cryopreservation, and characterization**

CBUs received at the processing laboratory within 48 hours of collection and with a collection volume (excluding anticoagulant) of at least 60 or at least 40 mL with at least 6 × 10^8 total nucleated cells (TNCs) were processed with a closed system to achieve volume reduction and RBC depletion. The CBUs were RBC-depleted with hydroxethyl starch (Hespan, Abbott Laboratories, North Chicago, IL) and gently centrifuged to isolate a WBC-rich, RBC-depleted fraction. The fraction was transferred to a third bag and pelleted. The remaining cell pellet was resuspended in cryoprotectant at final concentrations of 10 percent dimethyl sulfoxide in 5 percent dextran 40 (Protide Pharmaceuticals, St. Paul, MN), transferred to a 25-mL double-compartment cryopreservation bag (Medsep Pall, Covina, CA) with three attached segments, and maintained at 4°C in preparation for cryopreservation. The cryopreservation bag was overwrapped with soft plastic to provide a barrier to ID transmission. The overwrapped cryopreservation bag was placed inside a molded stainless-steel or aluminum cassette fitted for a high-density liquid nitrogen freezer. Units with a postprocessing recovery of at least 60 percent viable TNCs or at least 80 percent viable mononuclear cells (MNCs) or at least 6 × 10^9 total TNCs were cryopreserved by controlled-rate freezing before submersion under liquid nitrogen for long-term storage. Routine cultures for sterility were performed on the RBC pellet and extra plasma from the closed processing set used for volume reduction and RBC depletion.

Cryopreserved CBUs awaiting test results were stored in the vapor phase of a quarantine liquid nitrogen freezer or in the liquid phase of the primary liquid nitrogen freezer. CBUs meeting the COBLT eligibility criteria were stored in the liquid phase of liquid nitrogen. For all cryopreserved CBUs, sterility testing (aerobic and anaerobic) was performed with a microbial detection system (Bact/Alert 3D, bioMérieux, Inc., Durham, NC; BACTEC 9240, BD Diagnostic Systems, Sparks, MD). Testing was performed on samples of postvolume reduction WBC-poor plasma incubated for 7 days. CBUs with a positive sterility test were discarded.

CBUs were characterized by pre- and postprocessing TNC counts, percent cell viability by trypan blue staining, ABO blood type, and postprocessing CD34+ and CD3+ cell counts, as well as the presence of CFUs. Additional TNC counts, performed with automated cell counters, are reported in this article as viable TNC counts. CD34+ and CD3+ cells were enumerated by flow cytometric analysis. Enumeration methods varied between CBBs and over time. CD34+ cells, however, were enumerated with either a progenitor cell enumeration system (ProCOUNT, Becton Dickinson) or the ISHAGE gating strategy; CD3+ cells were enumerated with Trilight, Multitest, or the three-color CD4 fluorescein isothiocyanate–CD8 phycoerythrin–CD3 peridinin chlorophyll protein marker. Immunostaining markers and cell enumeration systems were supplied by Becton-Dickinson Immunocytometry Systems (San Jose, CA).

Molecular HLA typing was performed on the granulocyte-RBC–enriched pellet only for those units moved to permanent storage. This allowed 100 percent of the WBCs to be cryopreserved for future transplantation.

**HLA typing**

Aliquots of the granulocyte-RBC–enriched pellet were used to perform HLA typing at one of three COBLT laboratories (University of California at San Francisco, San Francisco, CA; University of California at Los Angeles, Los Angeles, CA; Navy Medical Research Laboratory, Kensington, MD).

HLA typing was performed with three DNA-based methods: sequence-specific probe hybridization, automated sequencing, and/or sequence-specific priming. The DNA was isolated from frozen aliquots of the granu-
locyte-RBC–enriched fraction. For the initial typing of each unit, HLA-DRB1 was typed at the allele-level and HLA-A and -B were typed at a minimum of low-intermediate resolution. Primary typing data were interpreted with a list of alleles that were recognized by the World Health Organization and readily available when the testing was performed. Low-resolution types were reported as the first two digits of the allele group with the exception HLA*B15, which was reported as 15AAA for alleles associated with B62, B63, B75, B76, and B77 or B15BBB for alleles associated with B70, B71, or B72. Intermediate resolution types were reported as a list of the potential alleles. High-resolution types were reported as a single allele. If only one HLA type was detected for a particular locus, the type was assumed to be homozygous.

Confirmatory typing was performed by at least one other COBLT Study laboratory and was required before shipping of a unit. Confirmatory typing was routinely performed as described above unless the transplant center requested typing of additional loci (HLA-C, -DQ, and/or -DP) and/or high-resolution typing. At the beginning of the study, QC of HLA typing involved typing of specimens at each of the three COBLT Study laboratories.

**Data collection**

**Screening data.** Between April 1998 and March 2001, each bank submitted a monthly recruitment report documenting the activities of the preceding month. The reports contained the number of potential donors who were screened, approached, and consented; consented and excluded before delivery; consented and delivered without a CBU collection; and approached but declined participation.

**CBU and donor data.** An Internet-based data system was developed to permit real-time data capture. The system was platform-independent, could be easily accessed through Netscape Navigator, allowed real-time data entry by multiple users, and gave CBB staff immediate access to the data. Only certified users with a valid user name and password combination could access the data entry system. All data transfers were encrypted with Secure Socket Layer technology.

Bar code validation routines, online calculations for real-time review of critical control points for collection and processing procedures and out-of-range checks were incorporated into the design. Additional checks for missing and inconsistent data were also performed.

The unique ISBT-128 number assigned at the time of collection identified each CBU. Donor names and contact information were maintained by the CBB in an independent, restricted access data system. No donor names or contact data were transmitted to the MCC.

Race or ethnicity of the infant’s mother and father was self-reported in five broad categories: Caucasian or white, black, Asian-Pacific Islander, Hispanic, and mixed or other. Data on collection, processing, and cryopreservation of the CBU; donor ID screening; current and past medical history of the donor and the donor’s family; hemoglobinopathy screening of the infant; and delivery information were completed for each CBU during a quarantine period. To be released from quarantine, data for each CBU had to pass an independent double review at the CBB and a computer algorithm review at the MCC. Eligible CBUs were moved to long-term storage, and a sample was sent for HLA typing. Data records for CBUs determined eligible for use as a stem cell source were locked and could only be modified after a request to the MCC. CBUs failing to meet eligibility criteria were “discarded,” and the primary reason for discard was entered into the data system. Discard reasons are described in detail in Fraser et al.20

**QA-QC**

The COBLT Study CBB QA-QC program was designed to monitor adherence to the COBLT Study SOPs and compliance with federal regulations across multiple banks. The program included internal and external QA-QC programs.

The internal QA-QC program for each bank was developed, implemented, and overseen by the CBB principal investigator (PI), medical director, and designated staff. Each bank developed site-specific SOPs to define and monitor facilities, staff training and competency, reagents, specimens, equipment, proficiency testing, and safety. CBBs were also required to document all procedures and implement a corrective action process. An overview of the quality plan is included in Chapter 7 of the COBLT Study SOPs at http://spitfire.emmes.com/study/www_emmes_com/.

External QA-QC oversight was performed by NHLBI and the MCC. As sponsor of the COBLT Study investigation new drug application, the NHLBI was responsible for the quality of the CBUs. Monthly conference calls were held with CBB PIs, NHILBI staff, MCC staff, and the steering committee chair during the CBU collection phase to discuss study progress and resolve issues. Site visits were performed throughout the study’s CBU collection and maintenance phases. Site visits included reviewing donor recruitment strategies, CBU collection, processing and cryopreservation procedures, and the maintenance of confidential files and CBU source documentation.

Site visit activities were developed at the MCC and based on FACT and AABB inspection procedures and modified to monitor specific COBLT Study requirements. Activities included observation of processing procedures by FACT-accredited inspectors, visits to collection centers, and data audits. Data audits compared source documentation in the CBU paper file with data submitted through the Internet-based data system. Written reports on the site
visit activities, results, and action items were prepared by
the MCC and distributed to the CBB PI and NHLBI. The
confidential contents of site visit reports were not shared
with other COBLT Study investigators.

Statistical analysis
Tests of associations were conducted with chi-square tests
and t tests for categorical and continuous outcomes,
respectively. Comparison of mean cell counts across eth-
nic groups was undertaken by linear modeling of the log-
transformed counts, with adjustment for demographic
variables and CBB. Analysis of variance (ANOVA) was
performed to examine the association of CD34+ cells and
CD3+ cells with several explanatory variables. Data anal-
ysis was performed with statistical computer software
(SAS, Version 8.2, SAS Institute, Cary, NC; STATA, Version
6.0, StataCorp, College Station, TX).

RESULTS
Establishment of the algorithm for
UCB banking
During the initial 18 months of the
study, the steering committee met on a
regular basis to establish the criteria for
banking, donor selection and screening,
and CBU testing. Procedures for CBU
collection, processing, storage, ship-
ment, thawing, and characterization
were established and validated by the
three banks.

Validation of CBU storage before
cryopreservation and CBU
characterization
Survival of viable TNCs and CD34+ cells
was studied as a function of the time
they were stored at room temperature
from collection to processing (Fig. 1).
Both TNCs and CD34+ cells remained
viable for more than 48 hours before
processing. Time from collection to ini-
tiation of processing up to 48 hours did
not negatively impact recovery of total
viable nucleated cells or CD34+ cell con-
tent after processing, before cryopreser-
vation (Figs. 1C and 1D). A small subset
of experimental samples was also cryo-
preserved and thawed for analysis of
recovery of viable nucleated cells and
CD34+ cells after thaw as a function of
time stored from collection to process-
ing (Fig. 2). Minimal losses of viable
nucleated cells and CD34+ cells were noted over the first
48 and 30 hours, respectively (Figs. 2A and 2B). These data
supported the algorithm for eligibility for CB banking that
was established to include units where processing was
initiated within 48 hours of collection.

Donor recruitment and screening
Between April 1998 and March 2001, 34,799 maternal
donors were screened for potential eligibility to partici-
pate in the study. Of these, 10,559 (30%) were not
approached to participate because of prospectively iden-
tified factors that would exclude them from study enroll-
ment. In the majority of these cases, the reason for
exclusion was pregnancy-related (63%) or because of a
variance in the mother's or family's medical history (23%)
(Fig. 3). An additional 0.6 percent of potential donors
(n = 212) did not participate because they elected to bank
privately. Of the 24,240 mothers deemed eligible to partici-
pate in the study after screening, 85 percent (n = 20,710

Fig. 1. Viable nucleated cells and CD34+ cells as a function of time from collection to
processing. CBUs (n = 7581) were collected and stored at room temperature for up to
72 hours before initiation of processing. CBU TNC content and cell viability were mea-
sured before (A) and after processing (B). (C) Recovery of viable nucleated cells. CD34+content was measured after processing (D). Losses of viable nucleated cells (A-C) and
CD34+ cells (D) were negligible over the first 48 hours.
mothers) agreed to participate. Efforts in each community to recruit donors with ethnic and racial minority backgrounds were specific to the individual community and found to be effective in reaching these donors. Translators as well as translation of educational materials and consents into the native language of the maternal donors were also very important.

Collection of the CBUs
CBUs (n = 16,764) were collected from 83 percent of the 20,278 mothers who consented for the study. The main reason for failure to collect from a consented mother was nonavailability of collection staff (44%) or a defect in the placenta (e.g., abruption, significant tear) or umbilical cord (one artery, tethered cord, clot in umbilical vein; 41%; Fig. 3). If the infant was found to have a congenital malformation or had evidence of congenital infection, the unit was not collected (7%). Collections were allowed from mothers with premature rupture of membranes as long as they were afebrile (<38.0°C) and had no signs of chorio-amnionitis at delivery.

A major goal of the COBLT program was to increase the ethnic diversity of the unrelated HSCT volunteer donor pool. The majority (61%) of units collected were donated by non-Caucasian mothers (Table 1). The availability of these units in the banks may increase access to transplantation therapy for patients of ethnic minority backgrounds.

Figure 4 shows data for a total of 17,207 collections made between December 1997 and August 2001 (including some collections not counted in the monthly recruitment and screening tallies used for Fig. 3). A comprehensive medical history was obtained from all maternal donors whose CB was successfully collected. After review of this medical history, 4 percent (n = 614) of the collections were excluded because of a risk factor identified in the pregnancy or mother or the infant’s family that was missed on the prescreening tool (Table 2).

Processing and cryopreservation of the CBUs
CBUs were eligible for processing and cryopreservation if they met certain criteria relating to volume, cell content, appropriate labeling, confirmation of maternal consent, successful acquisition of maternal test samples, sterility and sufficient recovery of nucleated or MNCs after RBC depletion, and volume reduction. With these criteria, 11,077 collected CBUs were cryopreserved (Fig. 4).

Banked units were thoroughly characterized. The mean unit contained $11.8 \times 10^8$ total viable TNCs in 80 mL before processing. After processing, a mean of $9.2 \times 10^8$ viable TNCs were recovered and cryopreserved. The mean CD34+ cell, CD3+ cell, and CFU contents were

Fig. 2. Recovery of viable nucleated cells (A) and CD34+ cells (B) as a function of time from collection to processing after cryopreservation and thawing. CBUs (n = 63) were collected and stored at room temperature up to 72 hours before initiation of processing. CBU nucleated cell content, cell viability were measured before cryopreservation, and after thaw for all samples and CD34+ content for 16 samples. Recovery of viable nucleated cells and CD34+ cells remained stable when cells were stored for no more than 48 and 30 hours, respectively, from collection to initiation of processing.
Testing of the CBUs

Units with low TNC content, low viabilities, failed sterility testing, unsuccessful CFU growth, positive maternal ID screening, positive hemoglobinopathy testing, insufficient lymphocyte counts, or technical problems during processing were excluded (Fig. 4). By these criteria, 53 percent of total collections (n = 8730 units) were moved to long-term storage and registered in the bank. The main reasons for discard were insufficient volume or TNC count (61%) or positive maternal ID screening test (10%; Fig. 4). Small numbers of units were excluded for failed sterility testing (2%, n = 194) or insufficient cell recovery (2%, n = 170). Units that were not excluded were subsequently HLA-typed as the final and most costly characterization step (Fig. 4).

Donors were tested for CMV via an immune screen that scored total immunoglobulin. Samples from donors with positive immune screens were tested for the presence of CMV IgM. CBUs from babies whose mothers had positive anti-CMV IgMs were excluded. One bank conducted a substudy to determine how many of the IgM-positive mothers and CBUs from the infants delivered to these mothers were positive for CMV NAT. Of the 892 such maternal-CBU pairs, this test determined that 2 maternal samples were CMV-positive and 8 CBUs were CMV-positive. Thus, less than 1 percent of the excluded units were actually viremic for CMV.

QA-QC

External oversight of the COBLT banking program proved challenging. The design of the COBLT data collection system enabled review of out-of-range data and form submission; a computer algorithm confirmed the CBU eligibility. Custom reports generated at the MCC detected potential problem areas within each bank. The reports generated by the computer algorithm enabled identification of potential problems with the CBU review process. The monitoring of missing forms reports detected inefficiencies in a bank's data collection process.

Adherence to the SOPs could also be identified by the computer algorithm. Not all compliance issues, however,
could be monitored centrally, and resolving compliance issues centrally proved to be difficult. Site visits with targeted data audits and reviews were found to be more effective. As the study progressed, CBB PIs were able to strengthen their internal QA-QC programs with customized data reports and data sets developed by the MCC.

Central coordination of CBB data was successfully accomplished with standardized data collection forms and an Internet-based data system. Key data elements were easily checked; CBUs and their associated samples were readily tracked. Noncompliance with study SOPs and federal regulatory issues could be partially identified centrally. The COBLT Study external monitoring program, however, relied on site visits to identify and resolve noncompliance issues.

Noncompliance issues that could not be resolved at a site visit or that could potentially compromise the integrity of stored CBUs were referred to the NHLBI for further review and action. The NHLBI was charged under the COBLT Study SOPs to determine what action should be taken when a major breach in the SOPs was identified. During the study, a major issue with the
maintenance of some CBUs in the COBLT Study search inventory was detected at a site visit. An information packet was prepared and reviewed by experts in stem cell transplantation, transfusion medicine, blood banking, biostatistics, and ethics. It was agreed that the CBUs were potentially compromised by the breach in the SOPs and that the units involved should be made available for non-clinical research only. This action plan was implemented by the NHLBI.

Factors influencing CBU characteristics

Relationships between the cellular and progenitor cell content of the stored CBUs and route of delivery, birth

### TABLE 3. Mean volume and cell counts of stored CBUs by ethnic group

<table>
<thead>
<tr>
<th>Variable</th>
<th>Ethnic group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Caucasian (n = 3631)</td>
</tr>
<tr>
<td><strong>Before processing</strong></td>
<td></td>
</tr>
<tr>
<td>Volume (mL)</td>
<td>80.6</td>
</tr>
<tr>
<td>Automated nucleated cell count (×10⁶/mL)</td>
<td>11.6</td>
</tr>
<tr>
<td>Total viable nucleated cells (×10⁶)</td>
<td>12.3</td>
</tr>
<tr>
<td>Total viable MNCs (×10⁸)</td>
<td>4.8</td>
</tr>
<tr>
<td><strong>After processing</strong></td>
<td></td>
</tr>
<tr>
<td>Automated nucleated cell count (×10⁶/mL)</td>
<td>45.5</td>
</tr>
<tr>
<td>Total viable nucleated cells (×10⁶)</td>
<td>9.6</td>
</tr>
<tr>
<td>Total viable MNCs (×10⁸)</td>
<td>3.9</td>
</tr>
<tr>
<td>CD34⁺ cells (×10⁶)</td>
<td>3.5</td>
</tr>
<tr>
<td>CD34⁺ cells/μL</td>
<td>163</td>
</tr>
<tr>
<td>Percent CD34⁺</td>
<td>0.34</td>
</tr>
<tr>
<td>CD3⁺ cells (×10⁸)</td>
<td>266</td>
</tr>
<tr>
<td>CD3⁺ cells/L</td>
<td>8621</td>
</tr>
</tbody>
</table>

* Based on two-sample t tests of African-American persons versus all other ethnic groups, African-American persons had significantly lower means on all variables except volume (p = 0.49 for volume, p = 0.02 for mean percent CD3⁺, and p < 0.0005 for all other variables).

### TABLE 4. Mean influence of type of delivery and infant sex on cell counts and volume of stored CBUs

<table>
<thead>
<tr>
<th>Variable</th>
<th>Vaginal (n = 6472)</th>
<th>Cesarean section (n = 2200)</th>
<th>Percent difference (cesarean section minus vaginal)</th>
<th>Male (n = 4541)</th>
<th>Female (n = 4131)</th>
<th>Percent difference (female minus male)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Before processing</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Volume (mL)</td>
<td>76.6</td>
<td>89.2</td>
<td>16.5</td>
<td>80.2</td>
<td>79.4</td>
<td>−0.9</td>
</tr>
<tr>
<td>Automated nucleated cell count (×10⁶/mL)</td>
<td>11.4</td>
<td>10.7</td>
<td>−5.9</td>
<td>10.9</td>
<td>11.6</td>
<td>6.5</td>
</tr>
<tr>
<td>Total viable nucleated cells (×10⁶)</td>
<td>11.6</td>
<td>12.3</td>
<td>5.7</td>
<td>11.4</td>
<td>12.1</td>
<td>6.1</td>
</tr>
<tr>
<td>Total MNCs (×10⁸)</td>
<td>4.6</td>
<td>5.0</td>
<td>4.2</td>
<td>4.6</td>
<td>4.8</td>
<td>4.7</td>
</tr>
<tr>
<td><strong>After processing</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Automated nucleated cell count (×10⁶/mL)</td>
<td>43.1</td>
<td>45.1</td>
<td>4.6</td>
<td>42.5</td>
<td>44.8</td>
<td>5.7</td>
</tr>
<tr>
<td>Total viable nucleated cells (×10⁶)</td>
<td>9.1</td>
<td>9.6</td>
<td>4.7</td>
<td>9.0</td>
<td>9.5</td>
<td>5.6</td>
</tr>
<tr>
<td>Total viable MNCs (×10⁸)</td>
<td>3.7</td>
<td>4.0</td>
<td>3.7</td>
<td>3.9</td>
<td>3.9</td>
<td>5.9</td>
</tr>
<tr>
<td>Total CD3⁺ cells (×10⁶)</td>
<td>3.3</td>
<td>3.6</td>
<td>3.5</td>
<td>3.2</td>
<td>−9.4</td>
<td></td>
</tr>
<tr>
<td>CD3⁺ cells/μL</td>
<td>153</td>
<td>169</td>
<td>10.5</td>
<td>164</td>
<td>149</td>
<td>−9.4</td>
</tr>
<tr>
<td>Percent CD3⁺</td>
<td>0.34</td>
<td>0.36</td>
<td>5.6</td>
<td>0.37</td>
<td>0.32</td>
<td>−14.1</td>
</tr>
<tr>
<td>CD3⁺ cells/L</td>
<td>227</td>
<td>247</td>
<td>9.2</td>
<td>232</td>
<td>232</td>
<td>0.0</td>
</tr>
</tbody>
</table>

* Based on two-sample t tests for each variable, all differences by type of delivery were highly significant (p < 0.001), and all differences by sex were significant (p < 0.005) except for mean volume (p = 0.18), total CD3⁺ cells (p = 0.92), and total CFUs (p = 1.00).

### TABLE 5. Proportions of stored CBUs suitable for transplant to recipients of given weights

<table>
<thead>
<tr>
<th>Recipient weight (kg)</th>
<th>Percent</th>
<th>Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>100</td>
<td>8728</td>
</tr>
<tr>
<td>20</td>
<td>92</td>
<td>8051</td>
</tr>
<tr>
<td>30</td>
<td>60</td>
<td>5219</td>
</tr>
<tr>
<td>40</td>
<td>33</td>
<td>2881</td>
</tr>
<tr>
<td>50</td>
<td>17</td>
<td>1499</td>
</tr>
<tr>
<td>60</td>
<td>8</td>
<td>727</td>
</tr>
<tr>
<td>70</td>
<td>4</td>
<td>365</td>
</tr>
<tr>
<td>80</td>
<td>2</td>
<td>183</td>
</tr>
</tbody>
</table>

* Suitability is defined as 2.5 × 10⁶ nucleated cells per kg. Thus 8728 of 8730 (100%) stored CBUs had more than 25 × 10⁶ nucleated cells; 8052 CBUs (92%) had more than 50 × 10⁶ nucleated cells; and so on.
weight, maternal age, infant sex, ethnicity, and gestational age were examined. Several important observations emerged.

Increasing gestational age was positively correlated with higher TNC content of the CBU (Fig. 5A). Both the volume and the TNC content of CB were strongly correlated with birth weight (Figs. 5B and 5D).

$CD34^+$ cell content of stored CBUs increased with birth weight but decreased slightly with gestational age and was lower in CBUs from African American infants (Fig. 6). There was no correlation of maternal age with TNC (data not shown) or $CD34^+$ cell content (Fig. 6B).

Units from African American donors exhibited significantly lower TNC counts, $CD34^+$ cell counts, and $CD34^+$ cell counts per $\mu$L (Table 3). Total viable TNC, $CD3^+$, and $CD34^+$ content per unit was also lower in this ethnic group when compared to other ethnic groups (Table 3; Fig. 6A). These observations remained significant even after adjustments for volume, birth weight, gestational age, type of delivery, sex, and bank (Fig. 7). Importantly, this resulted in permanent storage of a lower percentage of CBUs collected from these minority donors (Table 1, Fig. 8).

**DISCUSSION**

The COBLT Banking Study represents the first cooperative effort between multiple unrelated donor CBBs in the United States. The model consisted of a MCC, a steering committee, three CBBs, and a Web-based centralized data management system and search registry (Fig. 9). Common standards and operating procedures were developed by the steering committee to standardize the quality and specifications of the banked CBUs. Web-based data collection and search systems were developed by the MCC and validated and utilized by the banks and multiple transplant centers throughout the study.

Procedures for donor recruitment, education and consenting, donor testing, CB collection, testing, processing, cryopreservation, and long-term storage were developed, validated, and published. Procedures for cell counting, viability testing, RBC depletion and volume reduction, sterility testing, and $CD34^+$ measurement were standardized and validated by the banks. Attempts to standardize quantitative enumeration of CFUs between banks failed and it was decided that CBUs would only qualify for banking if CFU growth was present. Methods for HLA typing utilizing the granulocyte-RBC–enriched pellet were developed. The study demonstrates that it is feasible to validate and operationalize these activities among three banks and presents a possible model for other collaborative banking initiatives.
Although the initial request for proposal (RFP) for the COBLT banking study targeted banking of 15,000 CBUs, the banks participating in the study banked only 60 percent of this target. This was due to ongoing progress in understanding the factors influencing clinical outcomes in CB transplantation. In the 3 years that passed between the release of the RFP in 1996 and the initiation of UCB collections, new and compelling information emerged about the importance of higher TNC content of CBUs. The algorithm adopted by the COBLT Study banks incorporated this information and excluded units containing less than 60 mL and 600 million cells. There was also a more comprehensive algorithm developed for exclusion for medical history or positive ID screening results. Although the numbers of units collected were well within the initial goals of the RFP, the new algorithm excluded banking of smaller units and high-risk donors thus reducing the number of units actually banked.

This study demonstrates that over time, with ongoing and active collaboration, multiple banks can successfully adopt a uniform and efficient donor screening algorithm that allows the collection specialists to focus their time on eligible donors. Only 2 percent of screened and consented donors were later excluded for reasons covered in the screen. Furthermore, processing for RBC depletion and volume reduction in a closed-bag system maintained the sterility and integrity of the product. Only 2 percent of collections were excluded for positive sterility testing. The decision to store the CBUs under liquid nitrogen was reached to maximize maintenance of a continuous ultralow storage temperature. Each bag contained three attached segments for confirmatory HLA typing and other quality testing.

Assays for enumeration of CD34+ and T-cell subsets were validated between banks and obtained on all banked units. Standardization of these results between banks will allow for future studies to determine whether these variables correlate with engraftment, GVHD, immune reconstitution, or survival after CBT. The COBLT Study trial, which closed to enrollment December 31, 2003, is currently under active analysis and is addressing these and other clinical and correlative questions.

Screening for CMV presents unique problems in CBU donors. Some transplant physicians mistakenly interpret and presence of anti-IgG in the CB and/or mother as a risk factor for CMV infection in the recipient. This is not the case in CB where maternal IgG generated in response to a past infection crosses the placenta and subsequently tests positive in the infant. To address this problem, CMV screening was performed with sequential measurement of anti-CMV total immunoglobulin and, if positive, anti-CMV IgM. In one center, for IgM-positive mothers, CMV NAT was performed on the maternal plasma samples and CBU plasma and DNA samples. The results demonstrated that exclusion of CBUs from IgM-positive maternal donors grossly overestimated the rate of CMV viremia in the CBUs leading to unnecessary elimination of CMV-negative CBUs from the search registry (J.D. Roback, et al, unpublished data, 2005).22,23 FDA approval of the CMV NAT or other DNA-based tests would greatly benefit screening for CMV.

An important objective of the COBLT Study program was to increase the ethnic diversity of the unrelated HSCT donor pool. This was accomplished through several strategies. First, collection centers were established in hospitals delivering babies from ethnically diverse populations. Second, targeted strategies were utilized to recruit non-Caucasian donors. Third, it was hypothesized that mothers would be more likely to volunteer to donate their infant’s CB, a typically discarded substance, if there was no risk to themselves personally or to their infant. Also, the CB donation process requires a short-term commitment compared to listing as a marrow donor on the national registry. Less than 40 percent of the COBLT Study inventory is from Caucasian donors demonstrating success of these hypotheses and strategies.

Despite the increased representation of minority donors, a significant obstacle to increasing the pool of African American donors was encountered. CBUs from African American donors contained similar volumes but lower cell counts per milliliter and per CBU compared to all other ethnicities. Thus, a higher number of African American donors will need to be recruited to achieve equivalent representation in CB banking inventories.

Additional observations were used to create an algorithm to simplify the decisions about unit retention or discard at the collection center. Essentially, units from non-African American donors should contain 90 mL or greater of CB without anticoagulant whereas units from African American donors should contain greater than 100 mL to regularly achieve a TNC content of 1 billion cells or greater. In addition, larger term babies of any ethnicity are more likely to produce CBUs with higher volumes and nucleated and CD34+ cell content. Delivery by cesarean section is also associated with higher cell yields.

Conclusions useful for modeling of future banking efforts

Dosing of CB as a function of TNC per kilogram has been shown to strongly correlate with engraftment and overall survival in recipients of unrelated CBTs. Ideally a minimum dose of greater than $2.5 \times 10^7$ TNCs per kg, calculated from the precryopreservation count, should be delivered.25 The range of TNC content available from most CBUs is between $8 \times 10^8$ and $20 \times 10^8$ cells. For adults or larger children, only a fraction of units will be capable of delivering an acceptable minimum TNC dose. Of the 8730 units stored in the COBLT Study, 92 percent of units will deliver a sufficient TNC dose to a child weighing less than
20 kg whereas only 6 percent contain enough TNCs to deliver \(2.5 \times 10^7\) cells per kg to patients weighing more than 70 kg (Table 5).

To make efficient use of resources, a screening algorithm was developed to identify mothers who would not be suitable for CBU donation before significant resources were invested. The sequence of information acquisition and release from quarantine also was designed to perform more expensive tests (e.g., HLA typing) on units likely to be banked. It is recommended that units contain a minimum of \(1 \times 10^7\) TNCs, are collected and processed in a closed system, are RBC-depleted and volume-reduced, have attached segments for confirmatory typing, are stored under liquid nitrogen, and have additional samples of plasma, DNA, and viable cells stored for future testing. All units should be tested for hemoglobinopathies, and units with homozygous hemoglobinopathies should be excluded from the inventory. HLA typing should be resolved to intermediate resolution for HLA-A and -B and high resolution for HLA-DRB1.

Although the collection phase of the COBLT Banking Study was completed in 2001, there are ongoing banking activities supported through various grants and contracts in the United States and abroad. Information allowing for inclusion and/or exclusion decisions in the collection area would increase the efficiency and decrease costs associated with banking operations. Although TNC content is the final determinant of suitability for banking, most collection areas do not have access to automated cell counting on site. To this end, the relationship between volume collected and TNC count needs to be considered.

In summary, the COBLT Study CBBs demonstrate that a cooperative, unrelated donor CB banking effort is feasible with governance provided by a committee composed of bank directors, members of the sponsoring institution, and the coordinating center. Use of a centralized Web-based data collection system enhances operations, facilitates external oversight, and allows for detailed analysis of factors influencing CB donation, collection, and banking. The process of coordinating and ensuring high-quality operations is complex and challenging in this rapidly changing field. Factors associated with higher cellular yields included increasing birth weight, increasing gestational age, and delivery by cesarean section. African Amer-
ican donors were disadvantaged in that higher volumes of CB were required to reach targeted endpoints for TNC content. External oversight of QA-QC operations was found to be important to ensure adherence to the study SOPs. The authors believe that the coordinating center and sponsoring institutions must have sufficient resources to maintain oversight, conduct site visits, and facilitate communication and ongoing proficiency activities between banks.

ACKNOWLEDGMENTS
We acknowledge all of the mothers and infants who participated in the study. We also thank the CB collection specialists and medical technologists who recruited, educated, and consented in the study. We also thank the CB collection specialists and medical technologists who recruited, educated, and consented in the study. We also thank the CB collection specialists and medical technologists who recruited, educated, and consented in the study. We also thank the CB collection specialists and medical technologists who recruited, educated, and consented in the study. We also thank the CB collection specialists and medical technologists who recruited, educated, and consented in the study.

REFERENCES