splenic rupture due to peliosis, specially in patients with myeloproliferative disorders and/or receiving glucocorticoids. In these patients, prompt detection of enlargement of the liver or spleen and careful monitoring of liver function tests may be rewarding, as peliosis can be reversible after stopping these drugs.

Key words

Peliosis, splenic, immune thrombopenia, danazol

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Organization of an umbilical cord blood transplant program

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The development of human umbilical cord blood transplants with hematopoietic repopulating cells has enabled some problems associated with bone marrow transplants to be solved. Frozen umbilical cord blood banks should facilitate the finding of suitable stem cell donors. However, further experience is necessary to develop the optimal method for collection, separation, storage and cryopreservation of umbilical cord blood. We report our experience in the organization of a Cord Blood Bank.

Bone marrow transplants from related donors are the only alternative for some genetic, neoplastic, and non-neoplastic diseases, but they require an HLA identical donor, or with one mismatched antigen at most. Human umbilical cord blood (UCB) contains hematopoietic stem/progenitor cells and might be a clinically useful source of transplantable hematopoietic repopulating cells.[1]

In 1993 the *New York Blood Center* created the first cord blood bank for hematopoietic stem cell transplantation. In Spain there are two banks authorized by the National Transplant Organization, one in Barcelona and the other in Malaga. It is important to define the problems involved in organizing Cord Blood Banks.[2] The different international centers should be associated in an *International Cord Group*, such as *Eurocord*. The legislation should be similar to that applied to the transplantation of other tissues.[3]

We report our experience in the organization of a cord blood bank. UCB was collected from mothers of children who were candidates for a bone marrow transplant and from others for the constitution of an unrelated donor cord blood bank. Cord blood was not collected if there were obstetric complications. We had previously studied the organizational structure of the obstetrics unit which was co-ordinated with the cord blood bank. Subjects were recruited from the *Materno-Infantil Hospital* at the time of admission to labour and delivery. Human UCB samples were obtained from normal full-term vaginal deliveries. The mothers were from families with no known genetic disease and they gave written informed consent prior to delivery. Women with a history of a sexually transmitted disease, hepatitis, or other infectious disease were excluded from the study, even if an analysis was negative.

The method used by us for blood cord collection was the blood bag sterilized by betadine, to obtain the maximum volume for each collection in order to separate the cord blood mononuclear cell population. This is the method most commonly used.[4] It may be important to reduce the cryopreserved cord blood volume from 100 mL (±50) to 50 mL or less.

We used conventional 350 mL blood bags with CPD-A, reducing the anticoagulant volume to 25 mL in a sterile laminar flow hood. The modified bag and the samples for immunohematological controls, bacteriological and fungal cultures, flow cytometry and hematopoietic progenitor cell count were sterilized

and packed. The obstetrics staff kept the sterile packs awaiting collection by the UCB.

Immediately after delivery the umbilical cord was double clamped and transected 5 cm from the navel. Within 30 seconds the umbilical vein was catheterized, and the blood was collected by gravity into a 300 ml blood bag containing 25 mL of CPD-A, which was carefully shaken during collection to prevent blood clots. The doubly clamped blood bag was taken to the cord blood bank with two samples of maternal blood for standard controls. The umbilical cord blood was kept at 22°C in continuous agitation until processing within 24h to 48h.

HLA typing studies were made first for HLA class I antigens by serology, defining the split antigens DRB and DQB by generic DNA typing. Later, DNA typing for DRB, DQA, DQB and DPB HLA antigens was performed.

The personnel involved in this program is shown in Table 1. A total of 400 samples of umbilical cord blood were collected from full-term infants in 19 months, from January 1996 to July 1997. We examined the volume collected, the total mononuclear cells, the number of CD34^{+} cells, and checked for the presence of infectious diseases or bacteriological and fungal contamination.

The mean volume of the 400 samples of umbilical cord blood collected over 19 months was 71 mL (range: 31-128), the mean number of total cells was 8.46×10^8 (range: 1.60-29.10), the mean number of mononuclear cells was 5.44×10^6 (2.00-22.5), and the mean perccetage of CD34^{+} cells was 0.26% (0.10-0.90). Microbiological contamination was found in 35 samples (8.7%) (Table 2). Maternal blood tests for AgHBs, antiHCV, antiHIV or syphilis RPR were positive in 1.25% of the cases (2 HIV were initially reactive but Western-Blot negative, and 3 antiHCV were ELISA reactive).

Table 1. Umbilical cord blood program: human resources.

Department	Obstetrics	CBB*	Immunol	Serologic	Bacteriol	Hematol	Total
Physician	2	1	1	–	–	1	5
Midwife	25	–	–	–	–	–	25
Technician	–	1	1	1	1	1	5
Trainer	–	2	–	–	–	–	2
Total	27	4	2	1	1	2	37

CBB: Cord Blood Bank.*

Umbilical cord blood from a single birth contains a number of stem/progenitor cells within the range required for autologous and HLA compatible allogeneic transplantation in both the infant and the adult. The establishment of banks has been proposed in order to store frozen cord blood samples.[5] Umbilical cord blood can be used fresh, with minimal periods of cryopreservation, or after a long period of frozen storage. Several studies have attempted to determine the optimal collection, separation and cryopreservation techniques for cord blood banking and transplantation.[6,7]

An important question is the number of litres of nitrogen necessary to store a determinate number of UCB units. Many studies have been aimed at reducing the volume of the cord blood per unit to 24.1 mL, using a closed system and centrifuging the buffy coat, thereby reducing the storage requirements and resulting in a good recovery of MNC, lymphocytes, CD34^{+} cells and CFU.[8]

Table 2. Microbiological contamination in samples of cord blood.

Bacillus sp.	1
Citrobacter freundi	1
Candida albicans	1
Clostridium sp.	1
Corynebacterium sp.	4
Escherichia coli	6
Enterococcus durans	1
Escherichia faecalis	1
Staphylococcus	7 (1 *staphylococcus aureus*, 1 *staphylococcus warnieri*, 2 *staphylococcus hominis*, 2 *staphylococcus epidermidis*, 1 *staphylococcus haemolyticus*)
Streptococcus	8 (5 *streptococcus agalactiae*, 1 *streptococcus bovis*, 1 *streptococcus microaerofilico sp*, 1 *streptococcus lactobacillus sp*)
Proteus mirabilis	2

The staff involved in the program, belonging to two hospitals and six departments to a total of 37 persons including physicians, midwives, technicians and assistant staff, create an important organizational challenge, similar to that of the co-ordination of organ and tissue transplants.

The *National System of Public Health* pays the costs, which are distributed between each of the integrating centres of the program. The greatest workloads are in the *Umbilical Cord Blood Bank*, which requires a specific full-time technician for every 4 cryopreserved UCB units/day, and in the histocompatibility laboratory which carries out the typing study.

It is necessary to make a decision about the pre-cryopreservation period, that is whether the samples should be stored at 22°C or 4°C during periods of 24 or 48 hours. Results so far on pre-cryopreservation storage at 22°C show a higher significant post-thaw viability than storage at 4°C. No significant differences in cell viability or nuclear cell count were noted post-thaw when storage for 24 hours was compared with storage for 48h.[7]

The minimum acceptable volume was 30 mL. It was difficult to obtain a volume superior to 120 mL, though a mean volume around 70 mL is considered

acceptable for use in one blood bag. Our mean nuclear cellularity was 5.44×10^6, but this could possibly be increased by raising the collected blood volume. Checking for the existence of transmissible diseases must be performed in the maternal blood, the IgG antibodies levels are very low or undetectable in the newborn. Positive serological results invalidate the UCB for transplant. The serological tests are repeated in the mothers 3-6 months after the birth.

In conclusion, the establishment of Cord Blood Centers/Banks could open new fields in the procurement of donors for transplantation.

Key words

Program umbilical cord blood, cord blood bank, umbilical cord blood, blood donors

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Incidence and prognostic significance of idiopathic thrombocytopenic purpura in patients with Hodgkin's disease in complete hematological remission

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Idiopathic thrombocytopenic purpura (ITP) is a frequent and well recognized complication of lymphoproliferative diseases (especially chronic lymphatic leukemia),[1] but it is an unusual and poorly documented disease in the acute phase (1-2%).[2] We report on two female patients out of 812 patients with Hodgkin's disease (HD) followed between 1970 and 1995 at the Institute of Hematology and Medical Oncology "Seràgnoli" in Bologna, who developed ITP unrelated to bone marrow failure, 26 and 15 months after achievement of complete hematologic remission from HD.

Case #1. A 22-year-old woman presented in May 1991 with fatigue, fever and adenopathy in the right supraclavicular region. A chest radiograph defined the presence of intrathoracic adenopathy. Supraclavicular lymph nodes' biopsy revealed HD of nodular sclerosing type. A full blood count revealed hematocrit 44%, leukocyte count 0.54×10^9/L and a normal platelet count, described as being normal from the smear. The patient was placed in stage IIB, and chemotherapy was begun in June 1991. She received 3 cycles of MOPP protocol and 3 cycles of ABVD regimen.[3] At the end of chemotherapy, she began radiotherapy, receiving a total of 36 Gy to the mediastinal area.[4] The patient achieved complete remission (CR) in February 1992. Over the next 24 months she remained well and numerous complete blood counts were normal. In June 1994 she suddenly noted extensive purpura and easy bruising; examination revealed only ecchymoses and petechiae. The spleen was not palpable and there was no clinical evidence of recurring HD. The hematocrit was 44.8%, leukocyte count was 4.8×10^9/L with a normal differential count and the platelet count 4.0×10^9/L. Posterior iliac spine bone marrow biopsy revealed marrow in which erythroid and myeloid maturation appeared normal. No granulomas, tumor cells or any increase in fibrous tissue were seen. Roentgenograms of the chest and abdomen were negative. The diagnosis of ITP was suspected, and a trial of corticosteroids was begun. After 30 days, the platelet count was normal and corticosteroids were stopped. Over the next 14 months the full blood count remained normal. In September 1995 she again noted ecchymoses and petechiae. The blood platelet count was 5.0×10^9/L and an ITP relapse was diagnosed. After steroid therapy for two months without evidence of increased platelets, splenectomy was performed.[5] The platelet count rose immediately after splenectomy and remained normal. After two months, an autoimmune hemolytic anemia (AHA) appeared and the patient achieved a partial remission after prednisolone therapy. To date, there is no evidence of HD or ITP relapse.

Case #2. In December 1993, a 29-year-old woman noted enlarged lymph nodes in her right supraclavicular region. Lymph nodes biopsy revealed nodular sclerosing type HD. CT scanning of the chest, abdomen and pelvis revealed lymph node involvement of the anterior mediastinum without involvement of bone marrow. The patient was placed in stage IIIA and chemotherapy was started in January 1994. She received 6 cycles of ABVD regimen.[3] After completing chemotherapy she received the scheduled