CORD BLOOD BANKING AND TRANSPLANTATION

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Introduction

Hematopoietic progenitor cells (HPC) used for transplantation purposes have been traditionally procured by bone marrow collection and, more recently, from peripheral blood after HPC mobilization with recombinant cytokines. Towards the end of the 1980s, cord blood, i.e. the blood remaining in the placenta at the end of term deliveries was identified as an alternative source of HPC for transplantation (1–2). This discovery paved the way to the development of cord blood voluntary donation and banking programs for altruistic allogeneic transplantation. Several national and international organizations including the Italian GRACE (Gruppo per la Raccolta e l'Amplificazione delle Cellule Emopoietiche) cord blood network, the international NETCORD Foundation, the US National Marrow Donor Program (NMDP) and AsiaCORD are currently establishing professional standards and operative rules to ensure the offer of high quality cord blood to clinicians. Most efforts related to standardization and accreditation are being developed in conjunction with other organizations such as the Foundation for the Accreditation of Cell Therapy and the American Association of Blood Banks.

Current estimates that approximately 176,000 HLA typed cord blood donations are available at the Bone Marrow Donors Worldwide (BMDW) website, together with bone marrow donor registry data, for clinical purposes at 34 cord blood banks able to exchange units internationally (*Fig.1*), and that more than 3,500 unrelated cord blood transplants have been performed worldwide (3–4). Further interest was triggered by recent observations suggesting that hemopoietic progenitors can change their developmental program under strict environmental control, a cell capability termed 'plasticity' or 'transdifferentiation', which is currently the object of active investigation and hot debate (5–13).

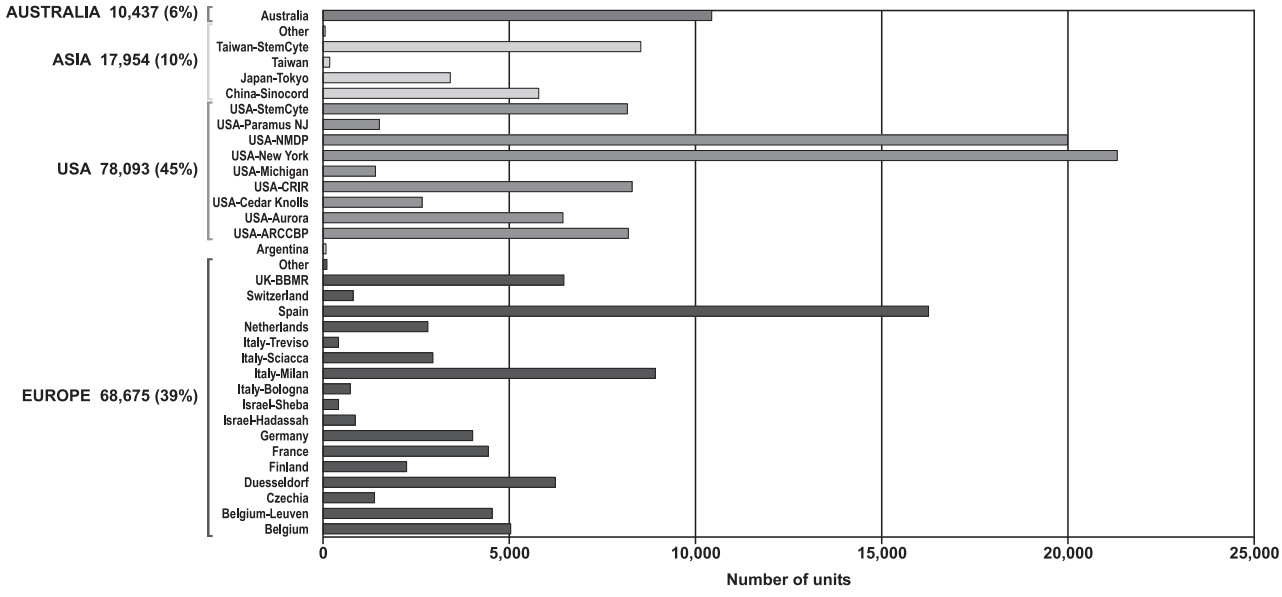

Figure 1: 175,159 Cord blood units in the BMDW, July 2004.

Cord blood banking programs

The CB banking process includes donor selection, CB collection, characterization, cryopreservation, CB unit storage, search and release for transplantation.

Clinicians who search compatible units for their patients need to easily access large inventories without repeating multiple searches at several hubs and to receive units of pre-defined, high quality levels regardless of the location of the procuring bank.

In turn, the banking and transplantation programs should be operationally connected so as to ensure optimal quality, effectiveness and efficiency. Furthermore, the banking programs should be operated within sound financial plans, as CB procurement and transplantation are associated with very high cost.

In this section we describe the main features of and the banking process at the Milano Cord Blood Bank. Furthermore, we present the Italian GRACE and the international NETCORD networks of CB banks, which were developed to satisfy the clinicians' needs discussed above.

The Milano Cord Blood Bank

The Milano Cord Blood Bank (MCBB) is located at the Centro Trasfusionale e di Immunologia dei Trapianti of Ospedale Maggiore, Milano, Italy (14). The current target of MCBB is to develop an inventory including 10,000 CB units for allogeneic unrelated transplantation and CB donations banked by family-related donors for patients suffering from conditions that may be treated with HPC transplantation. MCBB collections started in 1993 in three delivery suites. Activities were more recently expanded to include 19 collection sites located in Milano and in other cities at a maximum distance of 120 km.

During 1993-April 1999, a total of 14,955 unrelated and 149 related CB units were collected. Of these, 5,042 unrelated and 149 related units were banked. During the same period, 199 and 13 units were used in Europe, US, Australia and China for unrelated and related transplantation respectively.

MCBB operates with a Quality System developed in agreement with the ISO 9002 standard, which was described in detail elsewhere (15). The Quality System of MCBB was awarded a certificate of approval on July 18th, 1997, after having been assessed by a third party organization and found in accordance with the requirements of the quality standards EN ISO 9002: 1994. The certificate was confirmed at regular assessments performed every 6 months.

The CB banking process at MCBB is performed as follows. Before proceeding with CB collection, the midwife or the physician or an operator from MCBB checks the absence of the following donor exclusion criteria: duration of pregnancy less than 34 weeks; fever in the mother; malformations in the newborn; evidence or suspect of the presence of hereditary conditions in the newborn; risk behavior or positive serology in parents. Before CB harvest, the operator asks oral consent to the mother, while informed written consent is collected within 24 hours of delivery. By undersigning the consent, the mother agrees to CB harvest, banking and allogeneic transplantation for unrelated recipients, to serologic testing at delivery and at a later check performed six months after delivery, to biologic material storage, retrieval and testing if necessary, to personal data storage on paper and in electronic format and circulation in anonymous format.

CB is collected by midwives in a plastic bag containing CPD as an anticoagulant, by means of puncture of the umbilical vein after accurate disinfection of the cord. Midwives are trained to perform collections and regularly audited for this activity by MCBB staff. Collections are performed with the placenta in situ in vaginal deliveries and after placental delivery in cesarean sections. Time of

umbilical cord clamping varies in the delivery suites, due to the lack of a prevailing opinion in regard to the most correct time of clamping (early versus late).

CB units are stored at +4°C up to transportation to MCBB, and the cord blood units are transported by car. Transportation is performed in insulated containers similar to those used for transportation of red cell concentrates. Units are accepted at the bank if there is perfect match of data on sample tubes, bag and paper forms.

Units with volume below 60 mL and total white cell counts below 900×10^6 are not processed for banking. Processing consists of volume reduction with a bottom-and-top procedure and cryopreservation with DMSO at 10% final concentration. The latter is performed only with volume-reduced units showing white cell counts greater than 800×10^6 prior to cryopreservation. Units are stored in the liquid phase of liquid nitrogen tanks.

At time of banking, a sample of CB is used to perform a complete blood count, sterility tests for aerobic and anaerobic bacteria, CFU-GM and $CD34^+$ cell counts, ABO/Rh, HLA-A,B serologic typing, HLA-DRB1 molecular typing at low resolution. A maternal blood sample is used to perform the serologic screening, which includes detection of HBsAg, HCV/HIV-RNA, anti-HIV 1-2, anti-HCV, anti-HTLV I-II, anti-Hbc, anti-CMV (IgG and IgM), anti-toxoplasma (IgG and IgM) antibodies and TPHA. Serology is performed on the basal serum collected at delivery and on a new maternal sample collected 6 months after delivery. At this time, a physician from MCBB interviews the mother to collect the baby's medical history. Before unit release, cell viability, clonogenic potential and sterility are determined in a unit specimen obtained from a segment stored under the same conditions of the bag. Moreover, at unit release maternal HLA-A,B,DRB1 typing at low resolution is performed on a maternal sample stored in the frozen repository. Units are transported to the transplant center in an approved dry shipper. Patient's follow-up is performed at regular intervals by the EUROCORD Registry.

An analysis of economic issues performed at MCBB in 1996 showed that no less than 3% of the bank's inventory should be released per year at an individual fee of US$ 15,000 per unit released to fully recover the costs of bank implementation, unit processing and storage (16).

GRACE

In 1995 MCBB proposed the formation of GRACE, the acronym of the Gruppo per la Raccolta e Amplificazione delle Cellule Ematopoietiche – Group for the Collection and Expansion of Hematopoietic Cells, as a common forum for clinicians and investigators interested in CB banking and transplantation. Besides the cultural implications, GRACE was developed to harmonize banking procedures at high quality standards and to facilitate CB unit searches performed by clinicians through the development of a unique hub able to search the whole inventory of the member banks. Institutional GRACE members are the CB banks located in Florence, Milan, Pavia, Padua, Lazio, Turin. Other banks may apply for membership if compliant with the following rules: a) availability of a local inventory of at least 500 cryopreserved CB units; b) existence of a local quality system developed with ISO 9002 as a model of reference; c) use of procedures approved by the GRACE Board of Directors; d) FACT accreditation; e) formal recognition by the Health Authority; f) payment of annual membership fees.

The Milano Cord Blood Bank operates as the central hub of the GRACE network. The organizational flow-chart, the inventory, and the number of released cord blood units by the GRACE network are shown in *Figures 2, 3, 4, 5.*

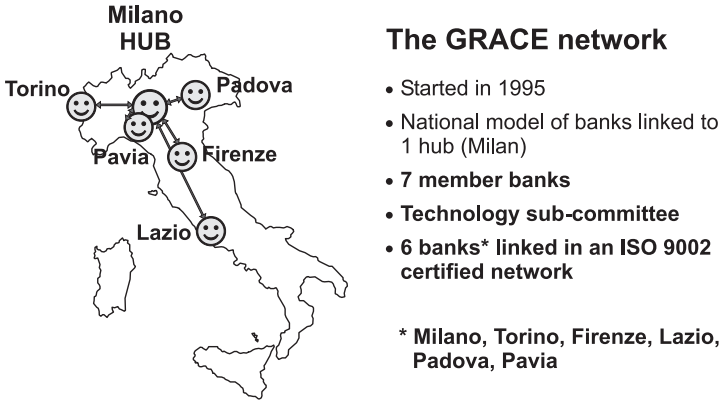

Figure 2: The GRACE network (The Italian Group for the Collection of Hematopoietic Progenitors)

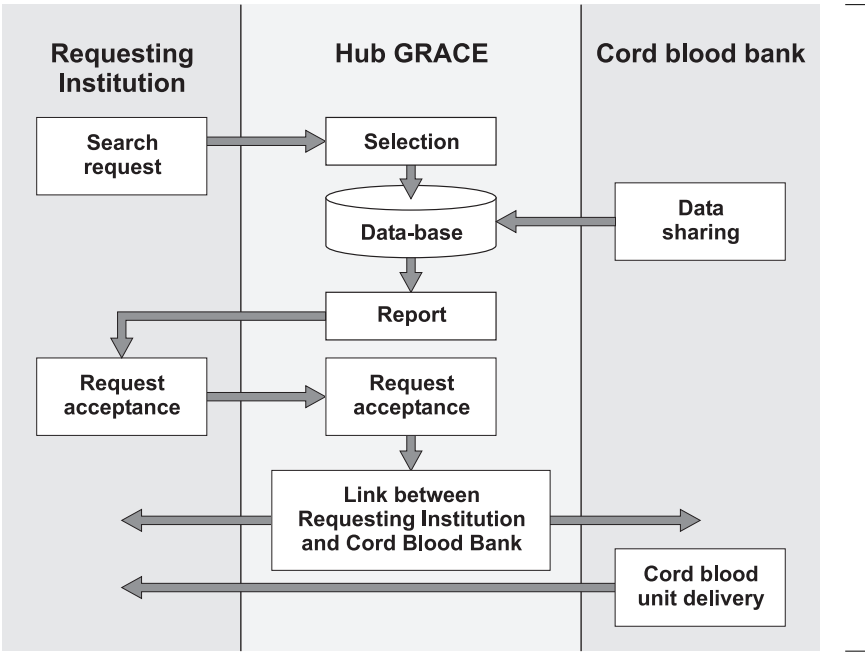

Figure 3: The GRACE organization flow chart

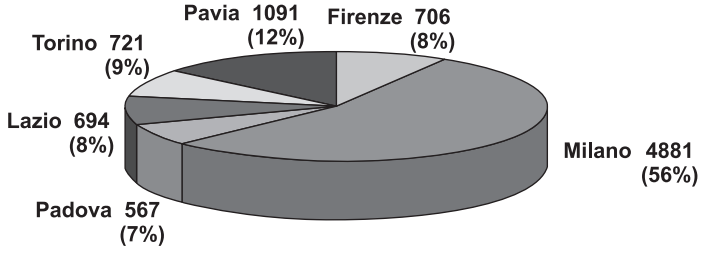

Figure 4: The GRACE inventory (August 2004)

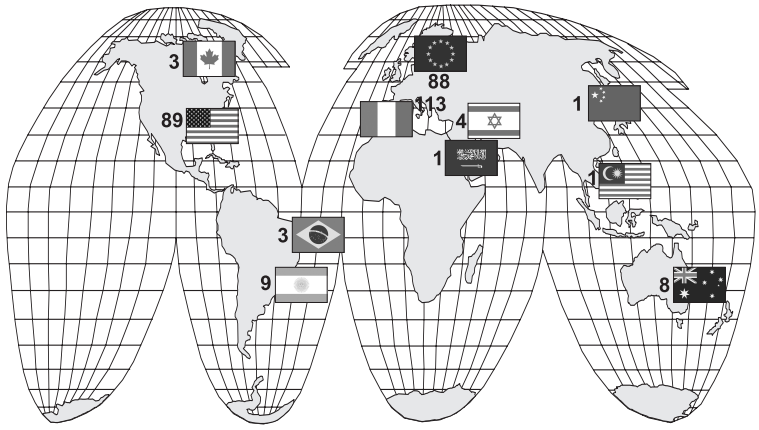

Figure 5: Number and transplant location of 320 unrelated cord blood units released by GRACE banks (July 31, 2004)

NETCORD

Following the positive experience collected with GRACE at the national level, in 1996 MCBB proposed a similar program at the international level, which involved, in addition to Milan, the CB banks in Düsseldorf and Barcelona. The program, which was named NETCORD, was formally constituted in 1998, at the end of a pilot phase spent to determine its feasibility (17). Approximately 70,000 cord blood donations are stored in banks linked in NETCORD (*Fig. 6*), which developed jointly with the Foundation for the Accreditation of Cell Therapy (FACT) the NETCORD-FACT standards for cord blood banking. Furthermore, NETCORD is currently implementing a real-time compatible unit search system capable of exploring the combined inventory of the member banks, which is termed the 'Virtual Office'.

NETCORD Inventory and Use July 2004

CB Bank	Inventory	Transplanted	Children	Adults
AusCord	10220	103	23	5
Barcelona	5893	167	94	73
Dusseldorf	8165	219	155	62
France Cord	4458	157	106	51
Helsinki	2236	1	1	0
Jerusalem	723	12	12	0
Leiden	2595	8	6	2
Leuven	4902	30	23	7
Liege	4157	56	35	19
London	5800	71	53	18
Milan (Grace)	8943	316	187	129
New York	21248	1449	1007	442
Prague	1303	5	5	0
Tel Hashomer	1320	4	4	0
Tokyo	3738	279	105	174
TOTAL	**85701**	**2877**	**1816**	**982**

Figure 6: The NETCORD inventory

Conclusion

Although a unique data source is not available at present, data from the International Bone Marrow Donor Registry, the EUROCORD registry (18, 19), reports from the New York Placental Blood Program (20), which has released the largest number of units as a single bank, and recent reports from AsiaCORD allow to estimate that more than 3,500 allogeneic unrelated transplantation procedures have been performed in the last 10 years.

Most frequent conditions treated with cord blood transplantation include leukemia, lymphoma and immune deficiencies. Approximately two thirds of cord blood recipients belong to the pediatric category, as the number of cells present in cord blood is insufficient in a proportion of adults. As compared with the traditional procurement of hemopoietic stem cells through the bone marrow donor registries, this disadvantage must be balanced with more prompt availability of cord blood, increased viral safety (virtual absence of CMV), less stringent requirements of the HLA match between cord blood donor and recipient and less frequent and severe GVHD in cord blood recipients.

Despite the disadvantage due to the limited number of cells present in cord blood, it is encouraging to note that recent analyses from the EUROCORD registry indicate that cord blood recipient survival after 1998 is significantly better than before. This observation suggests that, similarly to other therapeutic procedures, results improve in parallel to the learning curve of clinicians and biologists involved in the procedure. Moreover, it has been recently reported that cord blood can be a suitable hemopoietic cell source for transplantation not only of myeloablated recipients but also of patients conditioned with reduced intensity regimens.

The data from cord blood transplants so far performed worldwide indicate that the clinical outcome of allogeneic unrelated cord blood (CB) transplantation is significantly related to cell dose, being more effective in children than in adults, and – similarly to what is observed with other HPC sources - is highly dependent on disease stage at transplantation. Furthermore, clinical reports from the US and Europe show lower graft versus host disease (GVHD) frequency and severity and prolonged time intervals for platelet engraftment in patients transplanted with CB as compared to bone marrow and mobilized peripheral blood recipients.

The clinical data so far collected indicate that cord blood is a useful sources of hemopoietic progenitos for myeloablated recipients (21). Moreover recent evidence suggest that also adult patients treated with reduced – intensity conditioning regimens can benefit from cord blood transplantation.

In spite of its demonstrated clinical effectiveness, cord blood is an expensive resource. This requires careful planning of collection and banking programs. While the former need to be expanded so as to allow the collection of large-volume units, preferentially ethics groups with HLA phenotypes poorly represented in the current inventories, consolidation of the latter is of outmost importance, to ensure that the banking programs reach the critical mass necessary to release a number of units sufficient to recover the costs.

International efforts aimed at harmonising the national programs have been promoted to reach the above objectives.

Acknowledgments

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Note. This report includes elements from other manuscripts published from the Milano Cord Blood Bank.

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