Impact of cord blood banking technologies on clinical outcome: a Eurocord/Cord Blood Committee (CTIWP), European Society for Blood and Marrow Transplantation and NetCord retrospective analysis

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BACKGROUND: Techniques for banking cord blood units (CBUs) as source for hematopoietic stem cell transplantation have been developed over the past 20 years, aimed to improve laboratory efficiency without altering the biologic properties of the graft. A large-scale, registry-based assessment of the impact of the banking variables on the clinical outcome is currently missing.

STUDY DESIGN AND METHODS: A total of 677 single cord blood transplants (CBTs) carried out for acute leukemia in complete remission in centers affiliated with the European Society for Blood and Marrow Transplantation were selected. An extensive set of data concerning CBU banking were collected and correlations with clinical outcome were assessed. Clinical endpoints were transplant-related mortality, engraftment, and graft-versus-host disease (GVHD).

RESULTS: The median time between collection and CBT was 4.1 years (range, 0.2-16.3 years). Volume reduction (VR) of CBUs before freezing was performed in 59.2% of available reports; in half of these the frozen volume was less than 30 mL. Cumulative incidences of neutrophil engraftment on Day 60, 100-day acute GVHD (II-IV), and 4-year chronic GVHD were 87, 29, and 21 $\pm$ 2%. The cumulative incidence of nonrelapse mortality (NRM) at 100 days and 4-year NRM were, respectively, 16 $\pm$ 2 and 30 $\pm$ 2%. Neither the variables related to banking procedures nor the interval between collection and CBT influenced the clinical outcome.

CONCLUSION: These findings indicate a satisfactory validation of the techniques associated with CBU VR across the banks. Cell viability assessment varied among the banks, suggesting that efforts to improve the standardization of CBU quality controls are needed.

ABBREVIATIONS: ALL = acute lymphoblastic leukemia; AML = acute myeloid leukemia; CB = cord blood; CBB(s) = cord blood bank(s); CBT(s) = cord blood transplantation(s); CBU(s) = cord blood unit(s); CR = complete remission; CR1 = first complete remission; CR2 = second complete remission; CR3 = third complete remission; EBMT = European Society for Blood and Marrow Transplantation; HR = hazard ratio; HSC(s) = hematopoietic stem cell(s); LFS = leukemia-free survival; MAC = myeloablative conditioning; NRM = nonrelapse mortality; OS = overall survival; RI = relapse incidence; RIC = reduced-intensity conditioning; TNC(s) = total nucleated cell(s).

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Cord blood (CB) has been increasingly employed over the past 20 years as a source of hematopoietic stem cells (HSCs) for transplantation.[1] Favorable initial results of related cord blood transplantation (CBT), including a lower rate of acute and chronic graft-versus-host disease (GVHD) when compared to other sources of HSCs,[2] led to the creation of unrelated CB banks after 1992.[3-5] Due to both the availability of a large inventory of cord blood units (CBUs) and the improvement of transplant technology, the number of transplants using this source of stem cells increased significantly (Eurocord, personal communication).

Collection of CB is usually performed in a maternity unit linked to a cord blood bank (CBB). All other procedures (characterization, processing, freezing, and storage of the CBU) are performed at the bank. Thus, success of a CBT is strongly dependent on the quality of the CBB activity.[6,7] The definition of "high-quality units" is generally referred to large-size units that contain a high number of hematopoietic progenitors to ensure faster engraftment. However, the concept of quality in CB banking generally refers to the consistency of the CBU data reported by the banks to the registries. Discrepancies between CBU data reported by the bank and transplant center have been reported, which could possibly affect the selection of the most suitable CBU and therefore clinical outcomes.[8] Such discrepancies may also result from the variability of manipulation and the CBU characterization practice after thawing at transplant centers.[9,10]

Despite the development of CB banking standards and accreditation programs, a large variability in laboratory techniques still exists, with special reference to CBU characterization and volume reduction (VR). The latter represents a major issue for any allogeneic CBB as the low probability of a CBU being released results in a waste of cryogenic space and operational costs.[11] Techniques for reducing the CBU volume while preserving the majority of HSCs and their biologic properties result in a reduction of the storage-associated costs. VR methods are usually based on the centrifugation of the product and targeted to either collect the leukoenriched layer between plasma and red blood cells (RBCs; buffy coat) or to remove the plasma. The frozen product is usually stored in liquid nitrogen for years before clinical use. The different techniques of VR significantly modify the physical characteristics of the graft, such as the hematocrit and the total nucleated cells (TNC) and polymorphonuclear cells (PMNs) concentration, possibly modifying the viability of HSCs after the freezing-thawing process. The impact of VR technologies in the viability of HSC after thawing has so far been reported by single institutions,[12-14] but a large retrospective, registry-based analysis of VR on clinical outcome after CBT is still lacking. Indeed, other laboratory variables can influence the CBT process, including storage technology, the assessment of HSC content and, in particular, quality controls (QCs) upon release of the CBU.

The primary aim of this study was to retrospectively analyze the impact of the major variables associated with CB banking on the clinical outcome of an extensive set of patients who have undergone a CBT. Data concerning 677 unrelated CBUs delivered for single CBT, carried out from 1997 to 2010 in centers affiliated to the European Society for Blood and Marrow Transplantation (EBMT; www.ebmt.org) and reported to the Eurocord Registry, were collected from the banks that released the units. We report here the analysis of the correlation between such data and clinical endpoints.

MATERIALS AND METHODS

Study design

Major variables associated with the banking process, from collection to release, were listed. Only single CBU transplants were selected to avoid the overlapping of graft variables of multiple units. The inclusion criteria of transplant recipients focused on variables related to the quality of the banking process; therefore, patients with the same diagnosis (acute leukemia) and disease status (complete remission [CR]) at CBT were selected. TNC number at freezing $\geq 3\times10^7$ is associated with a better engraftment rate and overall survival (OS)[15,16] and therefore was selected as the minimum threshold for inclusion in this study. CBTs carried out between 1997 and 2010 were included, so as to be able to provide an adequate follow-up. Other cellular variables, such as CD34+ cell count and colony-forming units were not universally performed, especially in the older units, and therefore they were not considered mandatory data for inclusion in this study. A

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negative impact of the degree of HLA disparity was reported on both engraftment and nonrelapse mortality (NRM);[17] however, in the single-unit transplant setting, a disparity of not more than two of six HLA-A,B and DRB1 antigens is generally accepted.

Therefore, the selection criteria for this analysis included patients with acute leukemias in any CR who had undergone a single unmanipulated CBT from an unrelated donor in EBMT centers. Further inclusion criteria were TNC at cryopreservation of at least 3×10^7/kg and HLA-A,B (HLA typing at antigen level) and DRB1 (HLA typing at allele level) disparity of not more than two of six. Patients were classified as pediatric when their age was not more than 18 years. A minimum data set was also considered mandatory for inclusion in the study, such as follow-up with complete outcome data and bank identification.

Banking variables

Most banks did not concentrate the CBUs at the beginning of their activity and this started at a later stage; others began using one method and then switched to another. Indeed, concentration may well have been started with a manual method and subsequently carried out with an automated method. Therefore, we focused this study on the laboratory process instead of analyzing differences among individual banks.

The following variables of the banking process and disease and transplant characteristics were analyzed: VR of the CBU before cryopreservation, time interval between collection of the CBU and transplantation, recipient's age at CBT (adult vs. pediatric patients), diagnosis (myeloid vs. lymphoid leukemia), disease status at CBT, intensity of the conditioning regimen (reduced-intensity conditioning [RIC] vs. myeloablative conditioning [MAC] that was defined as regimens containing either total body irradiation with a dose of >6 Gy or busulfan with a dose of >6.4 mg/kg intravenous [8 mg/kg if oral] degree of HLA matching (number of mismatched HLA, defined as HLA-A,B by low-resolution typing and DRB1 high-resolution typing), cryopreserved TNCs (reported as 10^7/kg recipient weight), and cytomegalovirus (CMV) status of the patient.

The primary endpoint of this study was 100-day NRM. Secondary endpoints were neutrophil engraftment, acute and chronic GVHD rate, OS, and leukemia-free survival (LFS). The review board of Eurocord/EBMT approved this study.

Data search

Patients fulfilling the above inclusion criteria were selected from the Eurocord database. An invitation letter to participate in the study was sent to the banks that released CBU for transplant. Eurocord bank data collection forms were modified to integrate a larger set of laboratory variables. Data already reported to Eurocord were extracted and included in the updated data forms. All the banks that agreed to participate in the study received a form that only reported the selected patients' data. The list of participating banks is reported in Table S1 (available as supporting information in the online version of this paper).

Outcome definitions

The secondary endpoint of the study was neutrophil recovery, which was defined as achieving absolute neutrophil count of at least 0.5×10^9/L for 3 consecutive days. The diagnosis and grading of acute and chronic GVHD was assigned by the transplantation center using standard criteria.[18] Relapse and death from any cause were considered events. NRM was defined as death without prior relapse. LFS was calculated from the date of CBT until death, relapse, or last disease-free follow-up. OS was calculated from the date of CBT until death or last observation alive.

Statistical analysis

The analysis was carried out from October 2013. Median values and ranges were used for continuous variables and percentages for categorical variables. Patient, disease and transplantation characteristics were compared in CBUs that either underwent or did not undergo VR using the nonparametric Wilcoxon test for continuous variables and Pearson's chi-square test for categorical variables. For each continuous variable, the study population was initially divided into quartiles and in two groups by the median. The median value was found to be the best cutoff for analysis of outcomes. The probabilities of OS and LFS were calculated using the Kaplan-Meier method and the log-rank test for univariate comparisons. The probabilities of neutrophil engraftment, grade II to IV acute and chronic GVHD, relapse, and NRM were calculated with the cumulative incidence estimator using death or relapse as a competing event. Multivariate analyses were performed using Cox proportional hazards regression model for LFS and OS and Fine and Gray's proportional hazards regression model for other outcomes. Besides VR, we included in the univariate analysis clinically relevant variables related to the patient (age at CBT, CMV serostatus), to the disease (acute lymphoblastic leukemia [ALL] vs. acute myeloid leukemia [AML] and first CR [CR1] vs. second or third CR [CR2/CR3]), to transplantation technique (date of transplantation, conditioning), and to the graft (frozen TNC number, HLA matching). Variables that reached a p value of 0.15 in the univariate analysis were included in the initial models and variables were eliminated one at a time in a stepwise fashion to keep only the variables that reached a p value of 0.05 in the final model.

TABLE 1. Patients and graft characteristic according to VR of the CBU*

Variable	VR		p value
	No (n = 276)	Yes (n = 401)	
Year of transplant	2004 (97-10)	2006 (97-10)	<0.0001
Year of CBU collection	1998 (93-07)	2001 (94-10)	<0.0001
Recipient age (years)	9.3 (0.09-68.2)	6.8 (0.3-64)	0.004
Adults/children	80/196	98/302	0.23
AML/ALL	107/169	165/236	0.46
CR1 vs. CR2/CR≥3	167/109	206/195	0.02
RIC/MAC	54/213	53/337	0.03
HLA disparities <2/≥2	138/132	235/145	0.006
TNC count at freezing ($\times 10^7$/kg)	5.53 (3.03-26.1)	6.03 (3-29.3)	0.04

* Data are reported as median (range) or number.

p values were two-sided. Statistical analyses were performed with computer software (SPSS, SPSS Inc.; SPLUS MathSoft, Inc.; and R, https://www.r-project.org/).

RESULTS

Patient population

A total of 677 patients transplanted in 133 centers from 47 countries were selected for this analysis. CBU for transplant were provided by 38 banks in Europe, America, and Australia between 1997 and 2010 (median, 2005). Median age at CBT was 7.9 years (range, 0.1-68.2 years), with 73.6%/26.4% pediatric/adult ratio. This proportion reflects the diagnosis distribution: 405 (59.8%) were ALL while 272 (40.2%) were AML. Most patients (83.7%) received a MAC regimen. Median follow-up for survivors was 49 months (range, 1.4-168 months).

CBU characteristics

The median interval between CBU collection and transplant was 4.1 years (range, 0.2-16.3 years). Volume at collection (Fig. 1A), including anticoagulant, was 130 ± 34 mL (mean ± SD), containing a median of 5.86×10^7 TNCs/kg recipient weight (range, 3.02×10^7-29.28×10^7 TNCs/kg). Median frozen volume varied largely, due to the increasing use of concentrating the graft (Fig. 1B); overall the median (range) cryopreserved volume was 61 (10-430) mL. DMSO was the cryoprotectant used in all CBUs included in this analysis; data about the storage phase were available in 477 CBUs, being in liquid phase in 451 (94.5%) of them.

QCs at release

Control of the CBU identity at release on a reference sample is considered mandatory in the FACT-NetCord standards (4th Edition, 2010). A question about QC on cell viability in thawed CBU-associated samples was included in this survey and was reported to be carried out in 345 units. The most frequently reported methods included trypan blue (30.9%), 7-actinomycin-D (21.2%), and acridine orange (28.8%). The reported percent viabilities after thawing (mean ± SD) were 79.9 ± 17.2, 62.5 ± 22.8, and 89.5 ± 9, respectively.

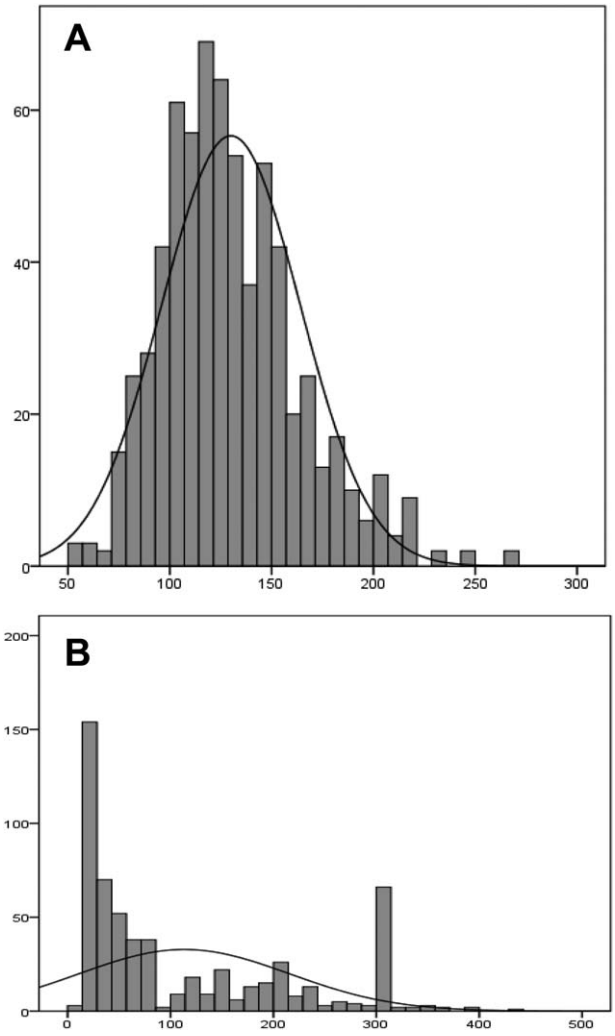

Fig. 1. (A) Distribution of CBU volume at collection (n = 677). (B) Distribution of CBU volume at freezing (n = 589). The bimodal shape reflects the impact of VR in 58% of the selected CBU.

VR and outcomes

A VR of the CBU before freezing was reported in 401 (59.2%) of the 677 selected transplants. The procedure was not routinely applied in the early years of banking; therefore, transplants carried out with volume-reduced CBUs have become frequent in more recent years, resulting in some differences between the two groups. Table 1 summarizes the characteristics of this subset of patients: overall, unmanipulated CBUs were used earlier and in younger patients, containing less TNCs/kg patient's body weight, with a better HLA matching and in a more advanced phase of disease. A further analysis was carried out in concentrated units (i.e., those with VR), according to the extent of CBU volume before freezing ($\leq$30 mL vs. $>$30 mL). VR below 30 mL was almost always (97%) achieved by adding HES as the sedimenting agent.

Results of univariate analysis are showed in Table 2. VR at any level was not associated with outcomes, either in univariate or in multivariate analysis (Fig. 2).The 60-day cumulative incidence of neutrophil engraftment was $87 \pm 1\%$. In multivariate analysis (Table 3), patients receiving TNCs of at least 5.86×10^7/kg presented a higher probability of neutrophil engraftment (hazard ratio [HR], 1.57; 95% CI, 1.30-1.88; $p < 0.001$).

Overall, 100-day cumulative incidence of acute GVHD and 4-year cumulative incidence of chronic GVHD were 29 ± 2 and $21 \pm 2\%$, respectively. Transplantation of CBUs with two or more of six HLA mismatches was associated with increased risk of chronic GVHD (HR, 1.70; 95% CI, 1.05-2.78; $p = 0.033$; Table 3). Relapse incidence (RI) was $27 \pm 2\%$ at 4 years. Disease status was the only factor associated with RI in multivariate analysis (Table 3; HR, 0.68; 95% CI, 0.48-0.96; $p = 0.030$ for patients transplanted in CR1). NRM was $16 \pm 2\%$ at 100 days and $30 \pm 2\%$ at 4 years. Diagnosis of AML and CMV-negative serology were both associated with decreased 100-day NRM in multivariate analysis (Table 3; HR, 0.56; 95% CI, 0.35-0.88; $p = 0.014$; and HR, 0.58; 95% CI, 0.38-0.90; $p = 0.016$, respectively).

The 4-year OSs in CBTs carried out with either concentrated or nonconcentrated CBUs were 46 ± 3 and $43 \pm 3\%$, respectively ($p = 0.43$; Fig. 2). In multivariate analysis, factors associated with increased survival (both OS and LFS) were transplantation in children, in CR1, and diagnosis of AML (Table 3).

DISCUSSION

This is a large-scale, retrospective registry analysis focusing on the clinical impact of the major variables associated to the banking of CBUs for allogeneic transplantation in an unrelated setting: namely, VR and CBU age at thawing. Such analysis had been previously reported in the form of internal analysis by the individual CBB.[4,19,20] Differences in CB banking were also analyzed within single CBT programs.[8]

QC programs have been implemented to ensure the quality of CBUs. FACT (Foundation for Accreditation of Cellular Therapy)-NetCord and the AABB programs rely on a list of procedures to be followed by CBBs to provide bank accreditation through an "on-site" inspection process. In some countries, local accreditation systems of the banks are in place. The accreditation systems aim at standardizing all the banking steps with the final goal of achieving better quality and homogeneity in the banks' CBU inventory, therefore improving the clinical outcomes of CBT.[21] Nevertheless, selection of a CBU from an accredited bank is not currently mandatory. Indeed, it should be mentioned that most of the major banks have been operating for 10 years or longer and that their operating procedures may have changed through such an interval, possibly raising further biases. Therefore, the influence of banking on the clinical outcome needs to be investigated through the analysis of all major variables associated with the banking process, from collection to shipping, rather than those associated with the CBB itself. In this regard, it should be noted that the duration of the storage did not have any negative impact on the clinical outcome. Interestingly, the number of frozen TNCs is higher in volume-reduced CBUs, due to the more recent attitude of CBBs to accept only high-quality units for storage.

This large, retrospective study shows that current methods aimed at reducing the CBU volume before freezing do not affect the clinical outcome of the CBT, therefore providing evidence of a satisfactory validation and reproducibility of such techniques. Methods are based on the removal of components of the graft that do not influence the engraftment and immune recovery, such as plasma and RBCs. Keeping the latter in the graft (RBC-replete units) results in a higher frozen volume and probably in a higher content of PMNs, compared to RBC-depleted units. In fact, PMNs tend to sediment faster than mononuclear cells, having a higher probability to be removed together with the RBC pellet after centrifugation. A correction factor was proposed for CBUs manipulated by plasma depletion,[22] but this approach is still controversial.[23] We show here that the outcome of transplants performed with CBU containing an adequate number of TNCs at freezing ($\geq 3 \times 10^7$/kg recipient body weight) is not influenced by the reduction of the graft volume before cryopreservation. Interestingly, this result was confirmed with any concentration and any method used; we adopted 30 mL to discriminate between RBC-depleted and RBC-replete units, respectively. A difference in the PMN content is expected in the two subsets, possibly too small to significantly influence the engraftment speed when combined with the other graft- and patient-associated variables.

It must be stressed that results derived by any registry analysis can be biased and their generalization should be

TABLE 2. Univariate analysis

Variable	Number	60-day neutrophil engraftment		100-day acute GVHD		4-year chronic GVHD		4-year RI		100-day NRM		4-year NRM		4-year LFS		4-year OS	
		%	p value	%	p value	%	p value	%	p value	%	p value	%	p value	%	p value	%	p value
All patients	677	87 ± 1		29 ± 2		21 ± 2		27 ± 2		16 ± 1		30 ± 2		43 ± 2		46 ± 2	
Age at CBT (years)																	
≤18 (children)	498	89 ± 1	0.51	32 ± 2	0.017	18 ± 2	0.031	27 ± 2	0.99	15 ± 2	0.021	28 ± 2	0.021	46 ± 2	0.032	48 ± 2	0.013
>18 (adults)	178	82 ± 3		22 ± 3		30 ± 5		27 ± 3		18 ± 3		38 ± 4		35 ± 4		40 ± 4	
CMV serology																	
Positive	346	87 ± 2	0.18	30 ± 2	0.50	23 ± 3	0.72	26 ± 2	0.11	18 ± 2	0.28	32 ± 2	0.27	42 ± 3	0.15	46 ± 3	0.13
Negative	289	89 ± 2		29 ± 3		20 ± 3		28 ± 3		12 ± 2		27 ± 3		44 ± 3		47 ± 3	
Diagnosis																	
ALL	405	85 ± 2	0.0035	31 ± 2	0.13	22 ± 2	0.44	29 ± 2	0.10	18 ± 2	0.12	32 ± 2	0.13	39 ± 2	0.005	42 ± 3	0.012
AML	272	90 ± 2		26 ± 3		20 ± 3		23 ± 3		12 ± 2		27 ± 3		49 ± 3		53 ± 3	
Disease status at CBT																	
CR1	304	86 ± 2	0.65	29 ± 3	0.90	21 ± 3	0.83	22 ± 2	0.008	16 ± 2	0.95	30 ± 3	0.95	49 ± 3	0.024	53 ± 3	0.011
CR2/CR3	373	88 ± 2		29 ± 2		21 ± 3		30 ± 2		16 ± 2		30 ± 2		39 ± 3		41 ± 3	
Median frozen TNCs ($\times 10^7$/kg)																	
<5.86	339	82 ± 2	0.00014	26 ± 2	0.025	22 ± 3	0.47	25 ± 2	0.23	18 ± 2	0.025	35 ± 3	0.025	40 ± 3	0.31	43 ± 3	0.069
≥5.86	338	92 ± 1		33 ± 3		20 ± 3		28 ± 2		13 ± 2		26 ± 2		46 ± 3		49 ± 3	
Number of HLA mismatches																	
0 or 1	373	88 ± 2	0.24	30 ± 2	0.55	16 ± 2	0.0006	26 ± 2	0.83	14 ± 2	0.07	28 ± 2	0.07	46 ± 3	0.12	48 ± 3	0.12
≥2	277	85 ± 2		27 ± 3		29 ± 3		27 ± 3		18 ± 2		34 ± 3		39 ± 3		44 ± 3	
VR																	
No	278	87 ± 2	0.22	30 ± 3	0.72	25 ± 3	0.078	32 ± 3	0.46	19 ± 2	0.45	27 ± 3	0.87	41 ± 3	0.37	45 ± 3	0.43
Yes	399	87 ± 2		28 ± 2		18 ± 2		29 ± 2		14 ± 2		26 ± 2		45 ± 3		47 ± 3	
Date of transplantation																	
≤2005	349	86 ± 2	0.007	30 ± 2	0.65	24 ± 3	0.13	27 ± 2	0.98	19 ± 2	0.15	33 ± 2	0.15	41 ± 3	0.15	44 ± 3	0.20
>2005	328	88 ± 2		28 ± 3		18 ± 3		26 ± 3		13 ± 2		28 ± 3		45 ± 3		48 ± 3	
Conditioning																	
MAC	550	89 ± 1	0.93	30 ± 2	0.51	22 ± 2	0.76	25 ± 2	0.11	16 ± 1	0.87	30 ± 2	0.87	44 ± 2	0.13	47 ± 2	0.23
RIC	107	79 ± 4		27 ± 4		19 ± 5		32 ± 5		16 ± 4		31 ± 5		36 ± 5		42 ± 5	

*Data are reported as mean ± SD.

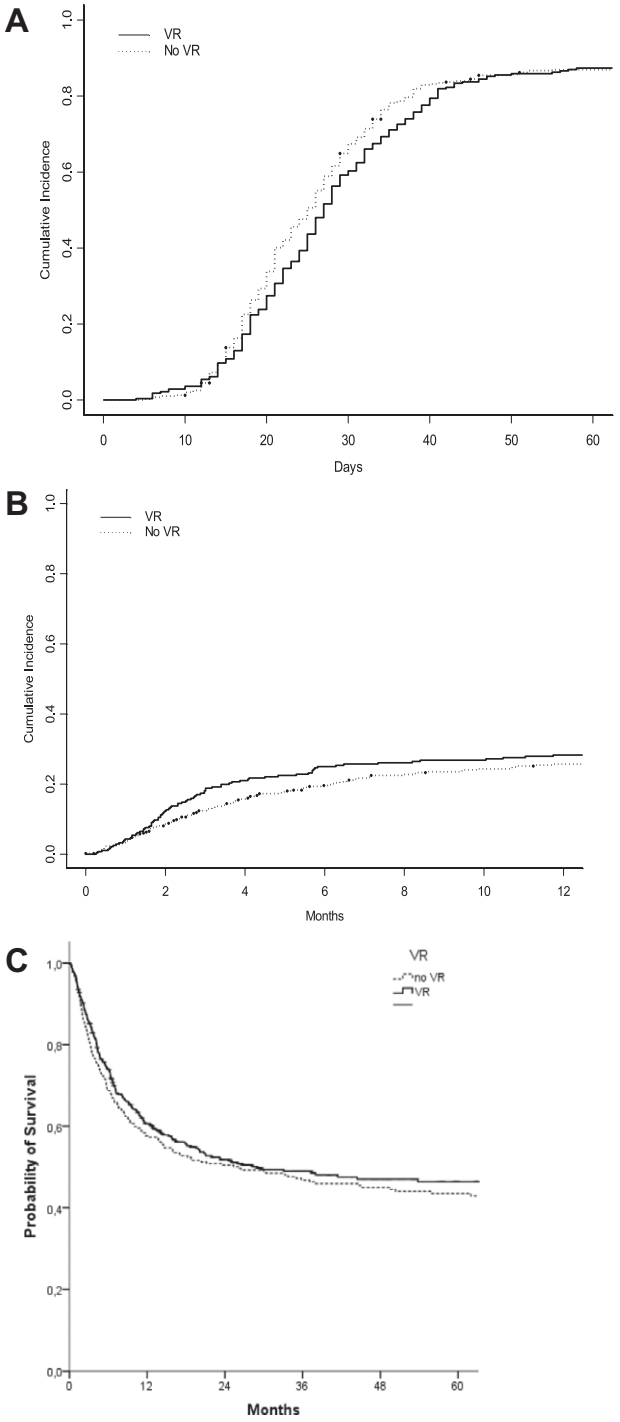

Fig. 2. Neutrophil engraftment (A), NRM (B), and OS (C) according to the VR of the CBU. (···) Unmanipulated CBU; (—) volume-reduced CBU.

validated through prospective studies; however, our data collected through a large, bank-independent analysis suggest that the cryogenic space saved by a high-fold reduction of the CBU volume does not have any major negative clinical impact, thus encouraging this increasingly used practice.

TABLE 3. Multivariate analysis

	Multivariate	
Variable	HR	p value
60-day neutrophil engraftment		
VR	1.1	0.88
Median year of CBT	1.2	0.12
Age at CBT (adult vs. children)	2.2	0.02
Diagnosis (AML vs. ALL)	1.8	0.62
Remission status at CBT	0.8	0.69
Conditioning (RIC vs. MAC)	0.7	0.45
Number of mismatches	0.3	0.6
TNC at collection	4.8	0.001
Patient CMV status	0.1	0.87
100-day NRM		
VR	0.7	0.19
Median year of CBT	0.8	0.37
Age at CBT (adult vs. children)	1.3	0.41
Diagnosis (AML vs. ALL)	0.6	0.014
Remission status at CBT	0.8	0.45
Conditioning (RIC vs. MAC)	1.0	0.83
Number of mismatches	1.0	0.8
TNC at collection	0.8	0.5
Patient CMV status	0.6	0.016
5-year OS		
VR	1.0	0.81
Median year of CBT	0.9	0.39
Age at CBT (adult vs. children)	1.6	0.009
Diagnosis (AML vs. ALL)	0.7	0.001
Remission status at CBT	0.7	0.007
Conditioning (RIC vs. MAC)	1.0	0.85
Number of mismatches	1.0	0.81
TNC at collection	1.0	0.95
Patient CMV status	0.8	0.16

As expected, all the selected CBUs were maintained in liquid nitrogen–based cryogenic systems. The current standards specifically require that the product is kept at a temperature lower than −150°C, thus using vapor-phase storage or mechanical freezers.[24] Concerns about the long-term storage of unrelated, allogeneic CBUs can probably account for the choice of the liquid phase for both the lower temperature and the longer maintenance of the optimal storage conditions even in the occurrence of inconveniences such as temporary lack of power or nitrogen supply.

The major lack of standardization in the banking process was found in the product characterization and especially in the QC on a reference sample. This is an important issue also due to the current practice of transplant centers to include cell viability in the CBU selection process, when different units with similar cellular content and HLA matching are available for one patient. CD34+ cell count at freezing is often lacking in the old units; furthermore, CD34+ cell viability assessment in the thawed sample needs further standardization. Most correlations between CD34+ content and engraftment were generated at the transplant center level on the thawed product at infusion,[25,26] while the count at freezing was not a better engraftment predictor than TNCs.[27] In this retrospective analysis the viability of nucleated cells was assessed by

different methods: a clear description of the method should be included on the unit report to enable the transplant center to perform a realistic evaluation of the QC result in the CBU selection process. Indeed, a cooperative effort in the bank's network should be devoted to improve the standardization of both characterization and QCs of the units exposed in the registries.

An international network of CBBs, transplant scientific societies, and registries have contributed in the past 20 years to make unrelated CB transplantation a clinical option for many patients missing a suitable donor. The current challenge is to improve the quality of the worldwide inventory by focusing collection of CBUs targeted at ethnic minorities and larger units. Improving the characterization of the CBUs by further standardization of the banking process will go a long way in improving outcomes after transplantation of this graft source. Finally it should be kept in mind that, apart from the graft quality, other clinical factors such as age, disease status, and conditioning regimen must be considered in the evaluation of CB as a stem cell source for transplantation.

CONFLICT OF INTEREST

The authors have disclosed no conflicts of interest.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's Web site:

Table S1. List of cord blood banks contributing to the study.