Original article

Multi-component cord blood banking: a proof-of-concept international exercise

Dinara Samarkanova[1,2], Margarita Codinach[1,2], Tiziana Montemurro[3], Larysa Mykhailova[3], Giuseppa Tancredi[4], Pasquale Gallerano[4], Panagiotis Mallis[5], Efstathios Michalopoulos[5], Liam Wynn[6], Javier Calvo[7], Oscar M. Pello[8,9], Ioanna Gontica[10,11], Paolo Rebulla[3], Sergio Querol[1,2]; on behalf of the MultiCord12 Study Group[12] (Appendix 1)

[1]*Banc de Sang i Teixits, Barcelona, Spain;*
[2]*Transfusion Medicine Study Group, Vall de Hebron, Barcelona, Spain;*
[3]*Department of Transfusion Medicine and Hematology, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milan, Italy;*
[4]*Sciacca Cord Blood Bank, Sciacca, Italy;*
[5]*Hellenic Cord Blood Bank, Biomedical Research Foundation Academy of Athens, Athens, Greece;*
[6]*Anthony Nolan Cell Therapy Centre, Nottingham, UK;*
[7]*Banc de Sang i Teixits de les Illes Balears, Cell Therapy and Tissue Engineering Group (TERCIT), Balearic Islands Health Research Institut (IdISBa), Palma, Spain;*
[8]*HSC Processing and Cell Therapy Unit, Marques de Valdecilla Foundation, Santander, Spain;*
[9]*Hematologic Neoplasms and Hematopoietic Stem Cells Transplantation Group, Marques de Valdecilla Research Institute, Santander, Spain;*
[10]*Public Cord Blood Bank of Crete, Department of Hematology, University Hospital of Heraklion, Crete, Greece;*
[11]*Hemopoiesis Research Laboratory, School of Medicine, University of Crete, Crete, Greece;*
[12]*See Appendix 1*

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Correspondence: Dinara Samarkanova
e-mail: dsamarkanova@bst.cat

Background - Most public cord blood (CB) banks currently discard more than 80% of umbilical CB units not suitable for hemopoietic stem cell transplant due to low stem cell count. Although CB platelets, plasma, and red blood cells have been used for experimental allogeneic applications in wound healing, corneal ulcer treatment, and neonatal transfusion, no standard procedures for their preparation have been defined internationally.

Materials and methods - A network of 12 public CB banks in Spain, Italy, Greece, the UK, and Singapore developed a protocol to validate a procedure for the routine production of CB platelet concentrate (CB-PC), CB platelet-poor plasma (CB-PPP), and CB leukoreduced red blood cells (CB-LR-RBC) using locally available equipment and the commercial BioNest ABC and EF medical devices. CB units with >50 mL volume (excluding anticoagulant) and ≥150×10^9/L platelets were double centrifuged to obtain CB-PC, CB-PPP, and CB-RBC. The CB-RBC were diluted with saline-adenine-glucose-mannitol (SAGM), leukoreduced by filtration, stored at 2-6°C, and tested for hemolysis and potassium (K+) release over 15 days, with gamma irradiation performed on day 14. A set of acceptance criteria was pre-defined. This was for CB-PC: volume ≥5 mL and platelet count 800-1,200×10^9/L; for CB-PPP: platelet count <50×10^9/L; and for CB-LR-RBC: volume ≥20 mL, hematocrit 55-65%, residual leukocytes <0.2×10^6/unit, and hemolysis ≤0.8%.

Results - Eight CB banks completed the validation exercise. Compliance with acceptance criteria was 99% for minimum volume and 86.1% for platelet count in CB-PC, and 90% for platelet count in CB-PPP. Compliance in CB-LR-RBC was 85.7% for minimum volume, 98.9% for residual leukocytes, and 90% for hematocrit. Compliance for hemolysis ≤0.8% decreased from 89.0 to 63.2% from day 0 to 15. K+ release increased from 3.0±1.8 to 25.0±7.0 mmol/L from day 0 to 15, respectively.

Discussion - The MultiCord12 protocol was a useful tool to develop preliminary standardization of CB-PC, CB-PPP, and CB-LR-RBC.

Keywords: *umbilical cord blood, regenerative medicine, neonatal transfusion, cord blood banking.*

INTRODUCTION

Human umbilical cord blood (CB) collected after delivery of healthy, term newborns is a useful source of stem and progenitor cells for allogeneic hemopoietic stem cell (HSC)

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transplantation. The clinical use of CB was prompted by seminal studies by Broxmeyer and co-workers[1] and by the implementation of a large number of public CB donation and banking programs, which currently store more than 800,000 CB units[2]. About 55,000 of the latter (6.9% of the worldwide inventory) have been transplanted during the last three decades into patients suffering from blood cancers, immunodeficiencies, metabolic defects, and hemoglobinopathies[2-4].

The clinical efficacy of CB HSC is comparable to the traditional sources obtained from bone marrow and mobilized peripheral blood[4]. Moreover, CB is a preferred clinicians' HSC source in selected clinical conditions[5]. Despite the evidence of CB clinical efficacy, the economic sustainability of most public CB banking programs is challenged by the high cost of staff and cryogenic facilities, and by the limited revenues generated by the small proportion of HLA-compatible CB units released for transplantation[6,7]. Recently, the latter proportion has shown a progressive decline in parallel with the increasing success of HLA-haplo-identical transplants, which offer some attractive logistic and economic advantages.

Standard international quality criteria for CB banking and transplantation have been defined by FACT-Netcord, including CB donor selection, microbiological testing, HLA typing, unit cryopreservation, total nucleated cell (TNC) count (a proxy of HSC content), and $CD34^+$ cell count. Progressively increasing thresholds for TNC and $CD34^+$ cell counts have been adopted based on the direct relationship of cell dose with patient survival. The currently adopted high thresholds for CB banking cause a discard rate of collected units of over 80% in most programs[6-8].

The economic sustainability challenges, the ethical concerns about the high discard rate of generously donated CB units that do not qualify for HSC transplantation, and some unique biological characteristics of CB have all led to an increased interest in novel clinical applications of CB components, including red blood cells (RBC), platelets, and plasma.

Based on encouraging results from laboratory and clinical studies[9,10,11-18,19-21] 12 public blood banks co-ordinated by the Banc de Sang i Teixits ([BST] Barcelona, Spain) formed the international MultiCord12 network to develop a common methodological platform for the regular fractionation of CB into platelet concentrate (CB-PC), platelet-poor plasma (CB-PPP), and leukoreduced red blood cells (CB-LR-RBC), to be used in non-transplant clinical applications.

The aim of this article is to describe the methodology adopted and report the results of the first platform validation exercise carried out by the MultiCord12 network.

MATERIALS AND METHODS

Study aims and plan

The objective of the study was to standardize procedures to obtain: a) CB-PC with a pre-defined platelet count of 800-1,200×10^9/L, suitable for the preparation of CB platelet gel (CB-PG) and CB eye drops (CB-ED) to be used for the treatment of skin and corneal ulcers, respectively; b) CB-PPP suitable for the preparation of CB-ED to be used for the treatment of dry eye disease; c) gamma-irradiated CB-LR-RBC suitable for neonatal transfusion after maximum storage at 2-6°C for 15 days.

The platform validation exercise was carried out from November 2021 to May 2022. Twelve public CB banks accredited by their competent regulatory authorities in Spain (No.=5), Italy (No.=3), Greece (No.=2), the UK (No.=1), and Singapore (No.=1) were invited to participate by BST, which acted as study co-ordinator. Participation in the study did not require the mandatory use of a specific or unique set of laboratory instrumentation (e.g., centrifuges, sterile connection devices, automated hematology cell counters, flow cytometers, gamma irradiators) and procedures for the characterization of the blood components obtained from CB (e.g., methods for determining residual WBC count in CB-LR-RBC, bacterial contamination, electrolytes and hemolysis during storage), as the scope of the study was to determine the level of standardization that could be achieved in the preparation of CB components using a common centrifugation procedure in ordinary operational settings of public CB banks. All CB banks used the CE-marked BioNest ABC and BioNest EF (erythrocyte filtration) medical devices developed by WhiteNest Pharma S.r.l. (Milan, Italy) for closed system CB primary fractionation into CB-PC, CB-PPP, and CB-RBC, and for the preparation and storage of CB-LR-RBC in saline-adenine-glucose-mannitol (SAGM), respectively (**Figure 1**).

The co-ordinating CB bank developed a CB primary fractionation and CB-RBC leukoreduction protocol describing criteria for CB unit selection, time and g force

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for CB centrifugation (see below), which was discussed and approved within the MultiCord12 network. Each CB bank received 30 BioNest ABC and EF systems from the study sponsor (WhiteNest Pharma S.r.l.). In each CB bank, the processing staff was invited to use the systems for local training sessions following the above protocol. On completing training, the processing staff performed the validation exercise. Eight CB banks reported results from 4-27 CB units, based on local staff and CB availability during the study timeframe. Furthermore, 4 CB banks evaluated the quality of CB-LR-RBC during 15 day storage at 2-6°C. The data from each bank were collected using a common Excel spreadsheet.

All CB units (No.=105) were screened in compliance with local standard CB banking procedures for HBV (HBsAg, Ig anti-HBV core), Ig anti-HCV; Ig anti-HIV-1/2; NAT (RNA HCV, RNA HIV-1, DNA HBV); Ig anti-*Treponema pallidum* (syphilis); Ig anti-HTLV I/II; IgG+IgM anti-CMV; Ig anti-*Trypanosoma cruzi* (Chagas); HTLV I/II, and were negative by sterility testing with BacTec (Becton-Dickinson, Franklin Lakes, NJ, USA) or BactAlert (bioMerieux, Craponne, France) procedures. A flow chart of the validation exercise is reported in the *Online Supplementary Content* (**Figure S1** and **Table SI**).

Before starting the study, the MultiCord12 network agreed on a number of criteria to be proposed as provisional quality standards for CB-PC, CB-PPP, and CB-LR-RBC, the latter partly derived from standard criteria for adult donor RBC as reported in the 20th edition of the EU Guide to the preparation, use, and quality assurance of blood components[22]. With regard to CB-LR-RBC quality acceptance criteria, we proposed a hematocrit range of 55-65% to reduce variability, as compared with the 50-70% range reported in the EU Guide[22]. With regard to CB-PC, we set the minimum volume to 5 mL to ensure a minimum quantity suitable for the preparation of CB-PG[9,11] and of CB-ED[23]. Moreover, we defined as acceptable a platelet concentration in CB-PC ranging from

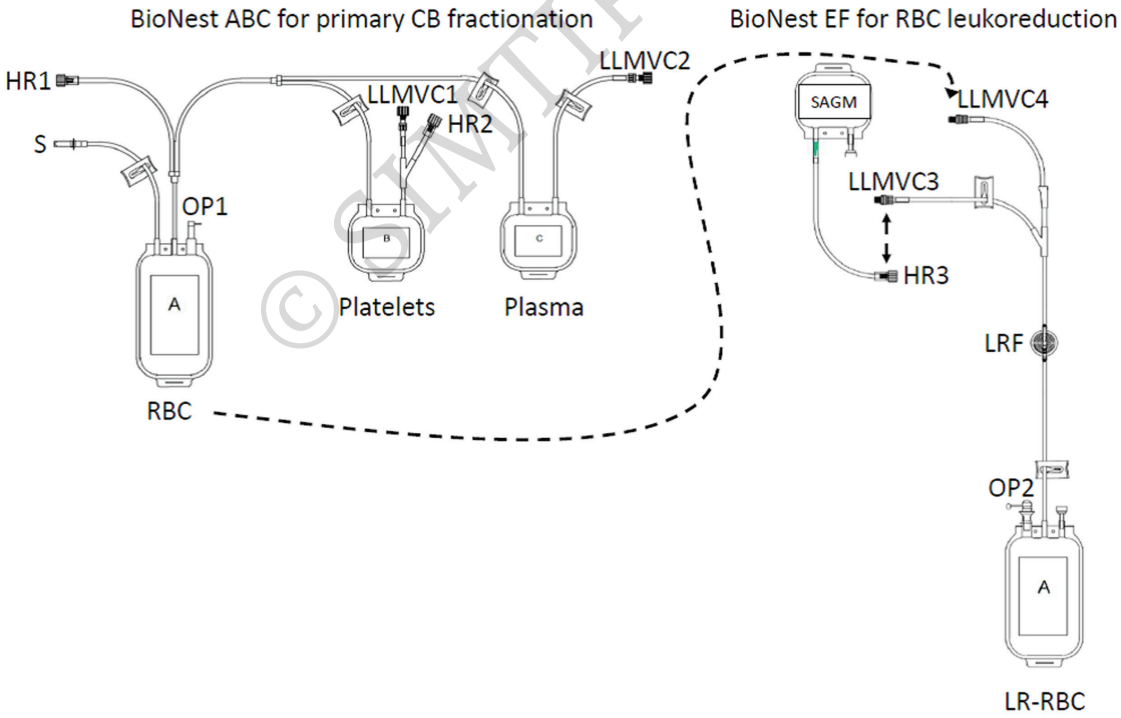


Figure 1 - BioNest ABC and BioNest EF systems. Nominal volumes of A, B, C and saline adenine glucose mannitol (SAGM) bags are 150, 60, 60 and 60 mL, respectively

SAGM bag is filled with 50 mL SAGM. CB: cord blood; HR: Halkey Roberts swabable valve; S: spike; OP: outlet port; RBC: red blood cells; LLMVC: Luer lock male vented cap; LRF: leukoreduction filter; LR-RBC: leukoreduced red blood cells.

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800 to 1,200×10⁹/L, based on early studies suggesting that a mean concentration of 1,000×10⁹/L is appropriate for wound healing applications[24].

Cord blood unit selection criteria

Cord blood units used in the validation exercise were selected with the following criteria: total nucleated and/or $CD34^+$ cell count below local minimum reference value for banking for transplant purpose; signed donor informed consent; CB collection <44 h; absence of visible clots; net CB volume ≥50 mL (excluding anticoagulant); platelet count ≥150×10⁹/L. All participating banks reported minimum umbilical cord clamping time of 60 seconds (sec).

Cord blood primary fractionation into CB-PC, CB-PPP and CB-RBC with BioNest ABC (see Figure 1)

1. Close all manual clamps along connecting tubes.
2. Seal and remove LLMVC1 on top of bag B.
3. Connect CB unit to S terminal using a sterile connection device and transfer CB into bag A. Seal connecting tube and remove original CB unit bag.
4. Insert bag A into a plastic protective envelope provided by WhiteNest Pharma S.r.l. (a flexible PVC tube to prevent bag damage during centrifugation).
5. Insert bags A, B and C into a rigid PVC cylinder provided by WhiteNest Pharma S.r.l. to maintain the bags in a vertical position during centrifugation. Flat or angled-bottom cylinders were provided to the MultiCord12 banks for optimal accommodation in flat or angled-bottom centrifuge buckets, respectively.
6. Set the protective cylinder containing bags A, B and C into centrifuge bucket and support it with folded empty transfer bags.
7. Centrifuge the unit at 250 × *g* for 10 min with acceleration and brake at 9 and 0, respectively.
8. Remove bags A, B and C from the cylinder and protective envelope, and set bag A into a manual plasma extractor.
9. Transfer the platelet-rich plasma into bag B.
10. Repeat steps 2-4.
11. Centrifuge bags A, B and C at 2,000 × g for 15 min with acceleration and brake at 9 and 3, respectively.
12. Set bag A into the plasma extractor and transfer the supernatant CB-PPP from bag A to bag C.
13. Remove bag A from the plasma extractor, set bag B into the plasma extractor, and transfer the supernatant CB-PPP from bag B to bag C. This step is started using the springed plate of the manual plasma expressor and completed by syphoning to allow complete transfer of CB-PPP from bag B into bag C without disturbing the platelet layer formed at the bottom of bag B.
14. Seal tube connecting bag A to B, and separate and store bag A containing CB-RBC at 2-6°C.
15. Aseptically connect a Luer lock syringe to LLMVC2 and remove a volume of CB-PPP from bag C, as determined with the formula:

CB-PPP volume (mL) = total platelets in CB unit (×10⁹) : 2

16. Aseptically connect the syringe to HR2 and transfer CB-PPP volume collected in step 15 into B bag.
17. Resuspend platelets in bag B by gentle manipulation and seal connection between bags B (CB-PC) and C (CB-PPP).
18. Store bag B without agitation for 30 min, followed by a gentle agitation on a platelet reciprocator for 60 min to facilitate platelet disaggregation.
19. Store bags B (CB-PC) and C (CB-PPP) at below –25°C.

CB-RBC leukoreduction with BioNest EF (see Figure 1)

1. Close manual clamps above and below LRF.
2. Determine the volume of SAGM to be added to CB-RBC before leukoreduction with the formula:

SAGM volume (mL) = 50% of CB-RBC volume

3. Swab HR3 from SAGM bag with validated disinfectant (70% isopropyl alcohol or equivalent) and aseptically connect it to a 20 mL Luer lock syringe.
4. Remove appropriate volume in order to leave the volume determined in the above step 3 in the SAGM bag. Remove and discard syringe.
5. Swab HR3 from SAGM bag with disinfectant and aseptically connect it to LLMVC4.
6. Swab HR1 from CB-RBC bag with disinfectant and aseptically connect it to LLMVC3.
7. Open manual clamp above LRF and transfer SAGM into CB-RBC bag. Close manual clamp above LRF.
8. Open manual clamp below LRF and perform leukoreduction by gravity filtration.
9. Seal tube below LRF and store CB-LR-RBC at 2-6°C.

CB-LR-RBC evaluation during 15-day storage at 2-6°C

Evaluations of hemolysis, pH, and extracellular K+ were performed with locally validated methods on CB-LR-RBC samples collected on days 0, 7, and 14 before and after 30 Gy gamma irradiation (14i) and repeated on day 15.

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Statistical analysis

Results are reported by descriptive statistics and presented as mean ± standard deviation (SD) or median, quartiles, and range. A comparison of data obtained in the different CB banks was performed with the Kruskal-Wallis test using GraphPad Prism 5 (Graphpad, San Diego, CA, USA).

RESULTS

All banks followed the same, established protocol, with the exception that 4 banks considered it unnecessary to use the rigid PVC protective cylinder provided by the BioNest ABC manufacturer to hold CB units vertically during centrifugation; this was replaced with traditional supports made of folded plastic transfer bags. Based on this evidence and on local experience, use of the rigid cylinder should be considered optional.

Eight banks reported the results of the fractionation of 105 CB units into CB-PC, CB-PPP, and CB-LR-RBC, each contributing data from 4-27 CB units. Thirty (28.6%) of the 105 units showed whole CB volume before fractionation (excluding anticoagulant) slightly below the 50 mL value required in the protocol. This was mainly due to reductions in CB collections during the COVID pandemic, which limited the availability of CB units for this exercise. Four banks were unable to comply with the platform validation exercise deadline of May 2022, mostly due to the above limitations. Details on laboratory devices and procedures used by each bank are reported in *Online Supplementary* **Table SI**.

Overall results and the proportion of units in compliance with the pre-defined acceptance criteria are reported in **Table I**. Cord blood volume (including anticoagulant), total hemoglobin, platelets, and leukocytes in CB units before fractionation were 90±21 mL, 10±4 g, 19±6×10^9,

Table I - *General results and compliance with pre-defined acceptance criteria (PDAC)*

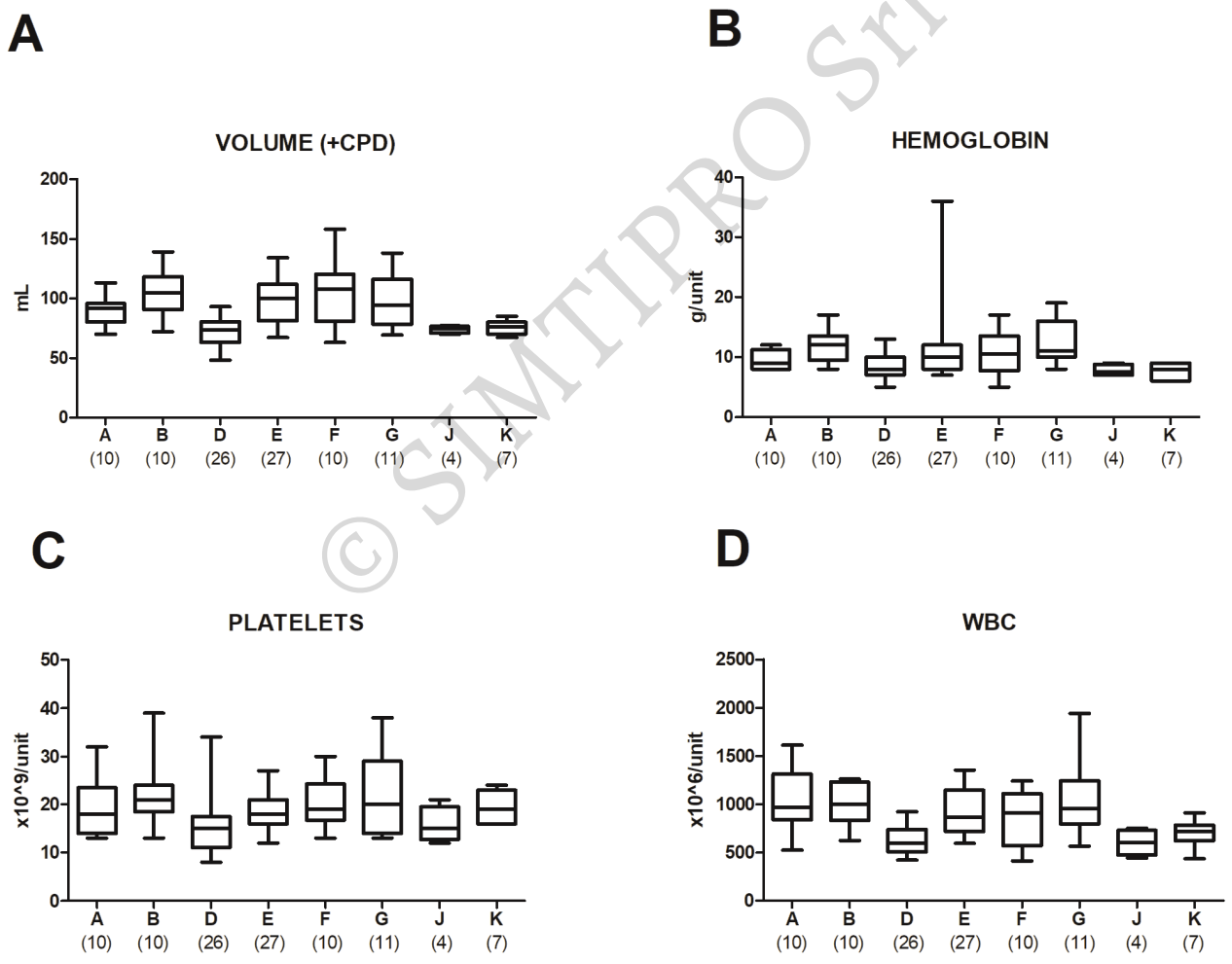
CB component	Parameter	No.	Mean ± SD	PDAC	% compliance	p*
Whole CB (excluding anticoagulant)	Volume (mL)	105	62±20	>50	71.5	<0.0001
	Hemoglobin (g/unit)	105	10±4	n.p.d.	n.a.	<0.0001
	Platelets (×10^9/unit)	105	19±6	>7.5	100	0.002
	Leukocytes (×10^6/unit)	105	847±287	n.p.d.	n.a.	<0.0001
CB-PC	Volume (mL)	105	9.6±3.4	>5	99.0	0.04
	Platelets (×10^9/L)	105	982±177	800-1,200	86.1	0.19
	Leukocytes (×10^9/L)	105	0.48±0.44	n.p.d.	n.a.	0.003
	Erythrocytes (×10^{12}/L)	105	0.04±0.05	n.p.d.	n.a.	n.d.
CB-PPP	Volume (mL)	105	29±14	n.p.d.	n.a.	<0.0001
	Platelets (×10^9/L)	105	29±21	<50	90	<0.0001
CB-LR-RBC	Volume (mL)	91	33±13	>20	85.7	0.0009
	Hematocrit (%)	91	57.5±5.3	55-65	90	0.0001
	Hemoglobin (g/unit)	91	6.5±2.8	n.p.d.	n.a.	0.01
	Leukocytes (×10^6)	91	<0.1	≤0.2	98.9	n.d.
	Hemolysis day 0 (%)	73	0.12±0.20	≤0.8	89.0	<0.0001
	Hemolysis day 7 (%)	73	0.35±0.41	≤0.8	92.0	0.05
	Hemolysis day 14 (%)	73	0.57±0.56	≤0.8	76.0	0.0005
	Hemolysis day 14** (%)	57	0.66±0.50	≤0.8	70.2	<0.0001
	Hemolysis day 15 (%)	57	0.75±0.59	≤0.8	63.2	<0.0001
	K+ day 0 (mmol/L)	73	3.0±1.8	n.p.d.	n.a.	0.2
	K+ day 7 (mmol/L)	47	14.6±6.2	n.p.d.	n.a.	0.0009
	K+ day 14 (mmol/L)	47	19.9±4.0	n.p.d.	n.a.	0.02
	K+ day 14** (mmol/L)	31	20.0±4.0	n.p.d.	n.a.	0.02
	K+ day 15 (mmol/L)	31	25.0±7.0	n.p.d.	n.a.	0.003

CB: cord blood; CB-PC: CB platelet concentrate; CB-PPP: CB platelet-poor plasma; CB-LR-RBC: CB leukoreduced red blood cells; K+: potassium; n.p.d.: not pre-defined; n.a.: not applicable; n.d.: not done. No.: number; SD: standard deviation. p value calculated for differences between centers. **After gamma irradiation.

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847±287×10^6, respectively. Anticoagulant volumes reported by the banks ranged from 25 to 30 mL. The target CB-PC minimum volume of >5 mL and platelet count of 800-1,200×10^9/L were achieved in 99% and 86.1% of the processed units, respectively. Platelet count <50×10^9/L was found in 90% of CB-PPP units. The target minimum volume of >20 mL, hematocrit of 55-65%, and residual leukocyte count of ≤0.2×10^6 of CB-LR-RBC were achieved in 85.7%, 90%, and 98.9% of the 105 processed units, respectively.

A post-hoc analysis carried out on the above 30 units enrolled in the exercise, despite a volume slightly below the 50 mL value required in the protocol, showed that two-thirds of such units did not fulfil acceptance criteria for CB-LR-RBC volume or CB-PC platelet count. The study of CB-LR-RBC during storage was completed by 4 CB banks, which reported a progressive increase of hemolysis and K+ release at days 7, 14, and 15, after gamma irradiation performed on day 14. The fraction of CB-LR-RBC units in compliance with the standard threshold of hemolysis below 0.8% progressively decreased from 89% on day 0 to 63.2% on day 15. CB-LR-RBC pH values on days 0, 7, 14 before and after gamma irradiation, and on day 15 were 6.78±0.16, 6.68±0.14, 6.56±0.09, 6.53±0.11, and 6.55±0.12, respectively. Data reported by individual banks are shown in **Figures 2-4**.



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Figure 2 - Whole cord blood (CB) unit volume
(**A**) Whole cord blood (CB) unit volume including CPD anticoagulant, (**B**) total hemoglobin per unit, (**C**) platelet count, and (**D**) white blood cell (WBC) count. Number of units per bank (codes A-K) is shown in brackets.

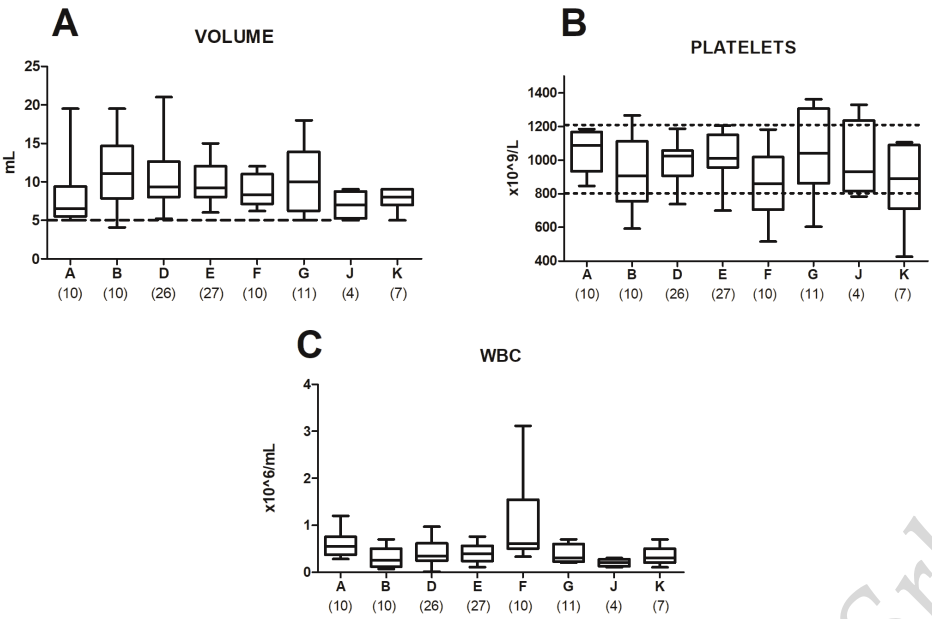


Figure 3 - Cord blood platelet concentrate (CB-PC) volume
(**A**) CB-PC volume, (**B**) platelet count, and (**C**) white blood cell (WBC) count. (**D**) Cord blood platelet-poor plasma (CB-PPP) and (**E**) platelet count. Number of units per bank (codes A-K) is shown in brackets.

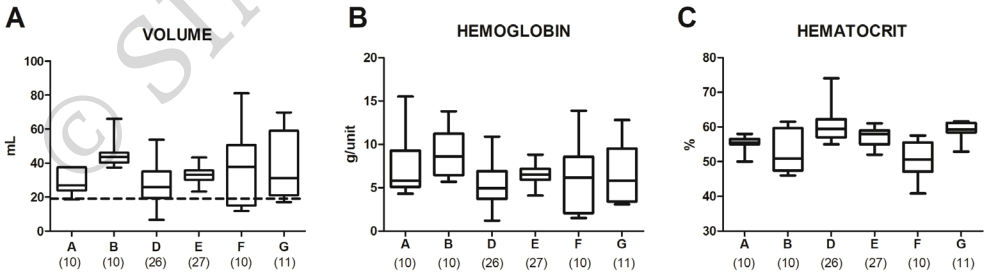
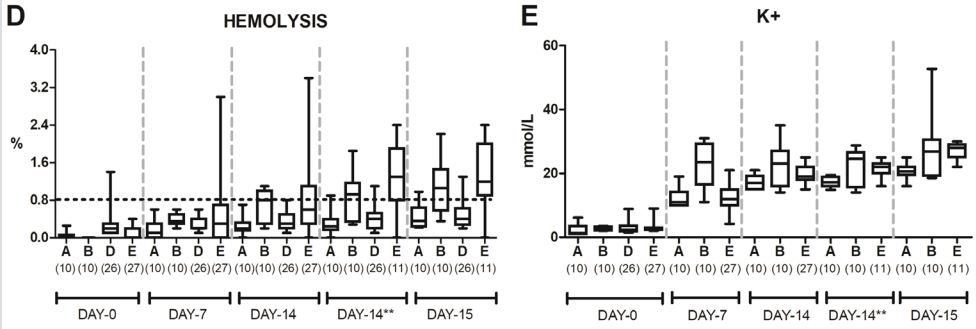


Figure 4 - CB leukoreduced red blood cells (CB-LR-RBC)
(**A**) CB-LR-RBC volume, (**B**) total hemoglobin, and (**C**) hematocrit. (**D**) CB-LR-RBC hemolysis and (**E**) potassium (K+) on day 0, day 7, day 14 before irradiation, and after irradiation (D14**) and 24 hours later (day 15). Number of units per bank (codes A-E) is shown in brackets.



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DISCUSSION

This study was carried out to promote international standardization and clinical use of CB-PC, CB-PPP, and CB-LR-RBC through collaboration between 12 public CB banks making up the MultiCord12 network. The name for this network was chosen to emphasize a shift from traditional "mono-component" CB banking, focussed on the cryopreservation and distribution of CB hematopoietic stem cells only, into a broader "multi-component" program[17,21].

The development of investigations into novel CB uses was initially triggered by ethical concerns caused by a very high discard rate of CB units generously donated to public banks for community use. In parallel, besides the well-known prevalent fraction of fetal hemoglobin in CB-RBC[23], basic studies showed that CB components display particular characteristics when compared to adult donor blood, including the presence of unique immunomodulatory factors related to feto-maternal immune tolerance[25,26] different concentrations of platelet-derived tissue regenerative factors[10,15,16], and differences in the platelet proteome[27,28].

These features meant that allogeneic CB units are not suitable for hemopoietic transplant purposes but are particularly attractive for non-transfusional applications to modulate excessive inflammation and promote tissue regeneration in skin and corneal ulcers with CB-PC and CB-PPP or serum. Allogeneic CB-PG obtained through activation of CB-PC with calcium gluconate and batroxobin[9,11] was successfully used for the treatment of skin ulcers in epidermolysis bullosa[29,30], diabetic foot ulcers[31], deep surgical site dehiscences[32], peri-anal fistulae[33], and oral mucositis[34,35]. Encouraging positive outcomes were obtained with CB platelet lysate and CB serum in experimental clinical trials in different pathological eye conditions including severe dry eye disease, corneal defects associated with autoimmunity, ocular burns, and ocular graft-versus-host disease[13,23,36-42]. Moreover, it was suggested that CB-LR-RBC could allow for a more physiologic approach to neonatal transfusion, particularly in premature newborns[43,44]. With regard to the latter, a hypothesis has been proposed for a causative role of high oxygen release by adult donor RBC transfusion and oxidative damage on premature newborns' immature organs in the development of severe complications of prematurity including necrotizing enteropathy, retinopathy of prematurity, and broncho-pulmonary dysplasia[45-47]. Although there is still no conclusive clinical evidence as to the possibility of reducing the frequency or severity of these complications with a transfusion of CB-RBC, encouraging studies and expert opinions on the feasibility of this approach have been reported[48-50]. Additional studies have documented the clinical efficacy and safety of allogeneic CB transfusion to treat anemia in countries with limited availability of safe adult donor blood[51-54].

Although encouraging, the above pre-clinical and clinical evidence of multiple novel therapeutic applications of allogeneic CB have for the most part been collected in small studies. Therefore, progress in this field requires the completion of laboratory investigations, and the development of large, preferably randomized, multicenter clinical trials with well-defined clinically relevant outcomes and sufficient statistical power. In this regard, CB component standardization is of paramount importance. The results of our proof-of-concept investigation show that an initial stage of standardization could be achieved with simple differential centrifugation and filtration procedures derived from those traditionally used for the preparation of blood components from adult blood donors[9] and with the use of two commercial disposable closed systems specifically designed for primary fractionation of low volume CB units and CB-RBC filtration leukoreduction.

With regard to CB-LR-RBC, a minimum volume of 20 mL and a target hematocrit of 55-65% (which were achieved in 85.7 and 90% of units, respectively) were chosen to ensure the provision of sufficient hemoglobin for one "small volume" or "top-up" transfusion episode with the standard requirement of 10-15 mL/kg body weight in most cases of premature newborn recipients[55,56]. We also made reference to the EU 0.8% threshold for hemolysis[22] and observed that this was shown only in 63.2% of the units at 15 days. This outcome was not totally unexpected given the well-known increased fragility of neonatal RBC and previous studies by Bianchi *et al.*[48] on CB-RBC leukoreduced by filtration reporting median and interquartile range values for hemolysis after 14-day storage of 1.3% (range 0.7-1.8%). However, the clinical relevance of the current <0.8% hemolysis standard could

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be questioned considering the absence of adverse events in CB-LR-RBC recipients of the clinical trials reported by Teofili, Bianchi and co-workers[49,57]. Further data in this regard are expected from the ongoing "umBilical Or Adult Donor Red Blood Cells in Extremely Low Gestational Age Neonates and Retinopathy of Prematurity (BORN)" clinical trial which plans to enrol 146 premature newborns (clinicaltrials.gov NCT05100212).

We also monitored K+ release in CB-LR-RBC supernatants during storage, as high K+ levels may cause transfusion-associated hyperkalemic cardiac arrest (TAHCA)[54,55,58,59]. We found K+ concentrations of 25.0±7.0 mmol/L at the maximum storage time of 15 days, 24 hours after gamma irradiation, a value that is considered safe for small-volume neonatal transfusions administered in 2-4 hours and is approximately one-half or one-third of that of RBC stored in additive solution for 42 days or irradiated and stored for ≥2 days[60]. As discussed in several publications, the absolute amount of K+ is less important than the transfusion rate (mL/min), and specific guidelines to reduce the risk of TAHCA have been reported for trauma and surgical recipients in need of large-volume transfusion (>25 mL/kg body weight) and rapid infusion rate[55,58-61].

The final assessment of statistically significant differences (**Table I**) between the CB banks which completed this proof-of-concept exercise yielded interesting information related to the possibility of achieving some degree of standardization of CB components. First, the detection of p values below the conventional 0.05 level for volume, hemoglobin, platelets, and WBC in CB units before fractionation indicates that the global data did not belong to a single distribution. This result confirms, not unexpectedly, that "not all CB units are born equal", due to local variations in biological characteristics and selection criteria of mothers donating CB, and in CB collection procedures used at the different CB banks. The quantitative differences between the CB units available at the different CB banks translated into quantitative differences in the components obtained after fractionation. Nonetheless, despite these differences, our protocol allowed the standardization of platelet concentration in CB-PC at 982±177×10^9/L, a value that showed no statistically significant variations between CB banks (p=0.19) and which was quite close to the 1,000×10^9/L value considered appropriate for clinical applications in wound healing[24].

Other parameters of practical and clinical relevance are volume, hemoglobin content, and hematocrit of CB-LR-RBC. Although for the latter parameters statistically significant differences were observed between CB banks, 85.7% of CB-LR-RBC showed a volume greater than 20 mL, which is usually considered sufficient for one "small volume" transfusion episode in premature newborns with average body weight of 1 kg. Moreover, the small variation between the observed 57.5±5.3% CB-LR-RBC hematocrit value and our pre-defined target of 55-65% is not expected to be clinically relevant and could be easily adjusted, if necessary, by slightly reducing the volume of SAGM used for CB-LR-RBC storage.

Our study has several limitations. First, not all banks of the MultiCord12 network were able to complete the validation exercise on the basic CB fractionation into CB-PC, CB-PPP and CB-LR-RBC (completed by 8 banks) and on CB-LR-RBC storage (completed by 4 banks). Second, a broad characterization of growth factors in CB-PC and CB-PPP is needed to determine reference values with a single, validated procedure in view of future clinical studies. Third, a number of classical RBC quality parameters like ATP, 2,3-DPG, glucose, and lactate dehydrogenase could not be determined due to economic limitations and operational difficulties; these will be tested in future exercises. Fourth, we acknowledge that a maximum CB-LR-RBC storage of 15 days limits the time in which RBC can be used to about one week after the completion of sterility testing. Progress in the development of a rapid, molecular sterility test is needed to help overcome this limitation[62].

Despite the above limitations, the proof-of-concept exercise carried out by the MultiCord12 network represents a first step towards promoting international standardization not only of CB-LR-RBC, but also of CB-PC and CB-PPP, three CB components with wide therapeutic application perspectives.

We firmly believe that the "multi-component" approach to public CB banking can contribute to reducing ethical concerns on the current high CB disposal rate, facilitating the development of large clinical trials testing the efficacy and safety of novel CB components, and improving the economic sustainability of public CB banks. We also

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believe that the "multi-component" approach will further motivate families to be involved in CB donation due to the potential of its broader application for the public good.

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AUTHORSHIP CONTRIBUTIONS

DS wrote the draft protocol and performed data analysis; MC performed data analysis; PR wrote the draft manuscript; SQ wrote the review protocol and final manuscript; all centers collected data. All Authors and Components of the MultiCord12 Study Group read and approved the study protocol and final version of the manuscript.

DATA AVAILABILITY

The data set for the current study is available on reasonable request from the corresponding Author with previous written approval from WhiteNest Pharma S.r.l.

DISCLOSURE OF CONFLICTS OF INTEREST

PR is co-inventor of patents on novel cord blood products, founder, equity holder and scientific director of Episkey S.r.l., and equity holder and scientific director of White Nest Pharma S.r.l. All other Authors have no conflicts of interest to disclose.

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APPENDIX 1: MULTICORD12 STUDY GROUP

- Dinara Samarkanova, *Banc de Sang i Teixits, Barcelona, Spain; Transfusion Medicine Study Group, Vall de Hebron, Barcelona, Spain* (Co-ordinator)
- Javier Calvo, *Banc de Sang i Teixits de les Illes Balears, Cell Therapy and Tissue Engineering Group (TERCIT), Balearic Islands Health Research Institut (IdISBa), Palma, Spain*
- Arantxa Cemborain, *Hematology and Cell Therapy and Foundation for Applied Medical Research, Division of Cancer, Clínica Universitaria, University of Navarra, Navarra, Spain*
- Margarita Codinach, *Cell Therapy Services, Banc de Sang i Teixits, Barcelona, Spain; Transfusion Medicine Study Group, Vall de Hebron, Barcelona, Spain*
- Irene Fragiadaki, *Hemopoiesis Research Laboratory, School of Medicine, University of Crete, Heraklion, Greece*
- Pasquale Gallerano, *Sciacca Cord Blood Bank, Sciacca, Italy*
- Antonio Gaya, *Banc de Sang i Teixits de les Illes Balears, Cell Therapy and Tissue Engineering Group (TERCIT), Balearic Islands Health Research Institut (IdISBa), Palma, Spain*
- Ioanna Gontika, *Public Cord Blood Bank of Crete, Department of Hematology, University General Hospital of Heraklion, Heraklion, Greece; Hemopoiesis Research Laboratory, School of Medicine, University of Crete, Heraklion, Greece*
- Roger Horton, *Anthony Nolan Cell Therapy Centre, Nottingham, United Kingdom*
- Panagiotis Mallis, *Hellenic Cord Blood Bank, Biomedical Research Foundation Academy of Athens, Athens, Greece*
- Maria Jose Martinez; *Blood and Tissue Bank of Aragon, Aragon, Spain*
- Stathis Michalopoulos, *Hellenic Cord Blood Bank, Biomedical Research Foundation Academy of Athens, Athens, Greece*
- Tiziana Montemurro, *Department of Transfusion Medicine and Hematology, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milan, Italy*
- Larysa Mykhailova, *Department of Transfusion Medicine and Hematology, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milan, Italy*
- Helen A. Papadaki, *Public Cord Blood Bank of Crete, Department of Hematology, University Hospital of Heraklion, Crete, Greece*
- Oscar Pello, *HSC Processing and Cell Therapy Unit, Marques de Valdecilla Foundation, Santander, Spain; Hematologic Neoplasms and Hematopoietic Stem Cells Transplantation Group, Marques de Valdecilla Research Institute, Santander, Spain*
- Cesare Perotti, *Immunohematology and Transfusion Service, Fondazione IRCCS Policlinico S. Matteo, Pavia Italy*
- Arun Prasath, *Women's & Children's Hospital, Singapore, Singapore*
- Daniele Prati, *Department of Transfusion Medicine and Hematology, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milan, Italy*
- Sergio Querol, *Banc de Sang i Teixits, Barcelona, Spain; Transfusion Medicine Study Group, Vall de Hebron, Barcelona, Spain*
- Paolo Rebulla, *Department of Transfusion Medicine and Hematology, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milan, Italy*
- Stefania Villa, *Department of Transfusion Medicine and Hematology, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milan, Italy*
- Giusi Tancredi, *Sciacca Cord Blood Bank, Sciacca, Italy*
- Sara Vallejo, *HSC Processing and Cell Therapy Unit, Marques de Valdecilla Foundation, Santander, Spain*
- Liam Wynn, *Anthony Nolan Cell Therapy Centre, Nottingham, UK*

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