Original article

Quality and stability studies of red blood cell concentrates from umbilical cord blood compared to their adult counterparts

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Background - Prematurity is a significant health issue due to its incidence and associated complications. Anemia is common in extremely preterm infants (EPI) and often requires transfusions. Red blood cells (RBC) from adult blood (AB) donors have been linked to oxygen-related complications in EPI, leading to the exploration of cord blood (CB) as an alternative source. However, standardization of CB-RBC manufacturing and comparison with AB-RBC characteristics are necessary before clinical studies can be conducted.

Materials and methods - This study investigated the quality and characteristics of leukoreduced, gamma-irradiated CB-RBC obtained using a commercial closed system from CB donations not meeting hematopoietic transplantation criteria. CB-RBC units were compared with AB-RBC units, both stored in saline-adenine-glucose-mannitol (SAGM). Various parameters, including hematological and biochemical characteristics, pH, 2,3-DPG levels, blood gases and potential toxicants, were evaluated during storage.

Results - CB-RBC units had acceptable initial quality parameters and a hematocrit (55±2%) comparable to AB-RBC. The main finding during storage was a faster rise in hemolysis compared to AB-RBC. Potassium (K+) significantly increased during storage in both sources. As expected, glucose levels decreased, and conversely, lactate levels increased, indicating similar patterns of anaerobic glycolysis during storage. pH decreased, affecting the oxygen dissociation curve due to reduced 2,3-DPG levels. After irradiation at 14 days of storage, CB-RBC were less stable as hemolysis and K+ significantly increased compared to AB-RBC at 24 hours. Phthalate concentrations, indicative of plasticizers, increased during storage, but significantly less in CB compared to AB-RBC. Most metals measured were within acceptable ranges.

Discussion - The quality of CB-RBC during storage is primarily influenced by levels of hemolysis and extracellular K+ content. Based on the analyzed parameters, we suggest that the expiration date for CB-RBC stored with SAGM should be set at 14 days, with transfusion occurring within <24 hours after irradiation.

Keywords: *cord blood, red blood cells, preterm infants.*

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INTRODUCTION

Anemia is a frequent problem in extremely preterm infants (EPI), often requiring the transfusion of red blood cells (RBC) from adult blood (AB) donors. Some prematurity complications, such as retinopathy of prematurity (ROP)[1], bronchopulmonary dysplasia (BPD)[2] and necrotizing enterocolitis (NEC)[3], have been linked to the transfusion of AB-RBC, mainly because of the high oxygen release by adult-type hemoglobin to the immature tissues of EPI. Allogeneic RBC from umbilical cord blood (CB) might be a better alternative[4,5], as their transfusion does not change the EPI hemoglobin profile, thus maintaining the physiological oxygen tension needs in those critical tissues.

Large quantities of CB-RBC could be readily available from public CB banks that routinely collect units for stem cell transplantation. In fact, a substantial amount of them, which are currently discarded due to the high cellular requirement for transplantation[6], could be used to prepare RBC concentrates[7].

Transfusing EPI with CB-RBC could reduce the frequency or severity of ROP[1,8], BPD[2] and NEC[3]. Before such studies are carried out, CB-RBC manufacturing needs to be standardized and their characteristics compared with those of AB-RBC.

The aim of this study was to investigate the quality of leukoreduced, gamma-irradiated CB-RBC prepared with a commercial closed system from CB donations not meeting transplantation criteria.

MATERIALS AND METHODS

Hematological and biochemical parameters were compared in CB-RBC and AB-RBC over three weeks, including gamma irradiation at day 14 of storage. Additionally, we carried out tests related to CB-RBC safety, including determination of plasticizers and heavy metal concentrations. The study was approved by the Ethics Committee for Research with Medicines of the Vall d'Hebron University Hospital, Barcelona, Spain - PR(BST)409/2019.

Samples

We used 34 CB units with their corresponding maternal samples and 20 leukoreduced AB-RBC concentrates, all provided by the Banc de Sang i Teixits Research Biobank (Barcelona, Spain).

RBC production

CB was collected with informed consent by healthcare professionals using conventional techniques and dedicated bags with 25 mL citrate phosphate dextrose (CPD) as anticoagulant (Grifols International, S.A., Barcelona, Spain). Acceptance criteria were: ≥50 mL net CB volume (without anticoagulant) and absence of visible clots.

CB was fractionated in a closed system using a commercially available kit (Solutran Hemo Bionest ABC, Meditalia Industriale, Lovero, Italy)[9] [see *Online Supplementary Content*, **Figure S1**]. After spin at 210 g × 10 min, platelet-rich plasma (PRP) was transferred to a satellite bag[10]. RBC and PRP were then centrifuged at 2,000 *g* for 15 min. The supernatant platelet-poor plasma (PPP) from RBC and PRP were transferred to a third satellite bag and 2 mL of buffy coat were extracted using a Halkey-Roberts luer for sampling. RBC were then connected to the leukoreduction kit (Solutran Hemo Bionest EF, Meditalia Industriale), which includes a small-volume polybutylene terephthalate filter, and a saline-adenine-glucose-mannitol (SAGM) bag was connected to the other end of the kit. SAGM was added to RBC in a 1:2 ratio to obtain a final hematocrit of 60±5%. Once diluted and filtered, CB-RBC were stored at 4°C. The final acceptable CB-RBC volume was established to be at least 20 mL, to target up to 1±0.5 kg newborns[11,12].

To carry out a comparative analysis, we used 20 group B AB-RBC units processed using the Reveos device (Terumo BCT, Bagshot, UK)[13].

Quality Control

PPP was used to determine antibodies against human immunodeficiency virus, hepatitis B virus, hepatitis C virus, cytomegalovirus, human T-lymphotropic virus I-II, *Trypanosoma cruzi* (Chagas disease) and *Treponema pallidum* (syphilis).

Complete blood counts were performed using the hematology analyser XN-550 (Sysmex Europe GmbH, Norderstedt, Germany). Residual WBC count in leukoreduced RBC was determined by flow cytometry (LeucoSure Enumeration Kit, Navios, Beckman Coulter, Brea, CA, USA). Glucose and lactate were evaluated with the Accutrend® Plus System (Roche Diagnostics, Basel, Switzerland), and pH was determined using the LAQUAtwin pHmeter (Horiba, Kyoto, Japan). pO_2 and pCO_2 as well as sodium (Na+) and potassium (K+) were evaluated on the EPOC blood analysis system using

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BGEM Test Cards (Siemens Healthineers, Erlangen, Germany). For the osmotic fragility test, RBC samples were exposed to varying dilutions of a saline solution (0-0.9%) for 30 minutes. After centrifugation, absorbance of supernatant samples was read at 540 nm to calculate the percentage hemolysis. For microbiology analysis, 1 mL of RBC was inoculated in iFA and iFN Plus flasks, which were incubated at 37°C for 14 days in the BACT/ALERT® system (BioMérieux, Salt Lake City, UT, USA). Intracellular 2,3 diphosphoglycerate (2,3-DPG) in CB-RBC was determined according to Beutler *et al.*[14]. Phthalate plasticizers in RBC were evaluated using ultra-high performance liquid chromatography coupled to tandem mass spectrometry. A fine chromatographic separation was optimized for separating isomeric compounds of di- and monophthalates. Control samples from three CB units were collected in glass vials containing the same proportion of anticoagulant as the plastic bags.

The concentration of heavy metals was measured by inductively coupled plasma mass spectrometry in 10 units of CB-RBC, AB-RBC and maternal whole blood samples using a panel of six metals: mercury (Hg), lead (Pb), cadmium (Cd), cobalt (Co), thallium (Tl), and manganese (Mn).

Quality acceptance criteria for the parameters assessed followed both the *Spanish Standards in Hemotherapy*[15] and *The Guide to the preparation, use and quality assurance of blood components* by the European Directorate for the Quality of Medicines and Healthcare (EDQM)[16].

Irradiation

RBC irradiation was conducted at 30 Gy using a Cs137 device (CIS bio international, Saclay, France). A blood irradiation indicator, affixed to the RBC bag, was employed to verify that the irradiation has been successfully performed for each CB-RBC. The CB-RBCs underwent irradiation on day 14, and samples were extracted before, immediately after irradiation, and 24 hours later for stability studies.

Statistical analysis

Results are reported as mean ± standard deviation (SD) unless otherwise specified. AB-RBC, CB-RBC and maternal blood samples were compared using the Mann-Whitney U test. The Wilcoxon test was utilized to compare CB-RBC at days 0 and 7 (0 *vs* 7), day 14 (7 *vs* 14) and 21 (14 *vs* 21), and pre- *vs* post-irradiation, as well as after 24 hours. GraphPad Prism was used for statistical analysis. Significance levels are indicated as follows: ***p<0.0001; **p=0.0001 - <0.01, *p=0.01 - 0.05, and not significant (ns) p>0.05.

RESULTS

CB-RBC and AB-RBC compliance with predefined quality parameters on day of manufacturing (day 0)

Table I shows a comparison of the main quality parameters for CB-RBC *vs* AB-RBC in relation to the acceptance criteria for clinical release. All CB-RBC had a final volume greater than 20 mL, which was the minimum defined value. The AB-RBC volume was in the normal range of units used in the blood bank. As expected, percentage fetal hemoglobin was the main difference between CB-RBC and AB-RBC (77 *vs* 0.5% respectively). Other critical parameters, including the time from collection to processing, residual leukocyte count after leukoreduction, and hematocrit, demonstrated comparability and fell well within the established acceptance range.

Other quality parameters are shown in **Figures 1-4** and **Tables SI-SII** in the supplementary material.

All RBC units were negative for infectious disease markers and for aerobic and anaerobic bacteria and fungi.

CB-RBC quality during storage

Detailed results from CB-RBC and AB-RBC during storage are shown in *Online Supplementary Content*, **Table SI**. Main outcomes are described below. Hemolysis increased significantly in CB-RBC during storage (**Figure 1A**) (p<0.0001 for day 14 *vs* day 7). No significant difference was found at day 21 *vs* day 14; nevertheless,

Table I - *Comparison of AB-RBC and CB-RBC characteristics at the beginning of storage*

Parameter	Acceptance criteria	Results	
		Day 0	
		AB-RBC	CB-RBC
Time between collection and processing (h)	<44	44 (40-46)	40 (14-42)
Volume (mL)	AB ≥150; CB ≥20	264±24	41±19
Residual leukocytes ($\times 10^6$/unit)	<1×10^6/unit	0.09±0.19	0.02±0.03
Hematocrit (%)	50-70 [35]	55.8±8	55±2
Adult hemoglobin (HbA2) (%)	n.d.	A2=2.5	A2=0.3
Fetal hemoglobin (HbF) (%)	n.d.	F=0.5	F=77
Hemoglobin (g/dL)	n.d.	18.5±1.4	17.7±0.8

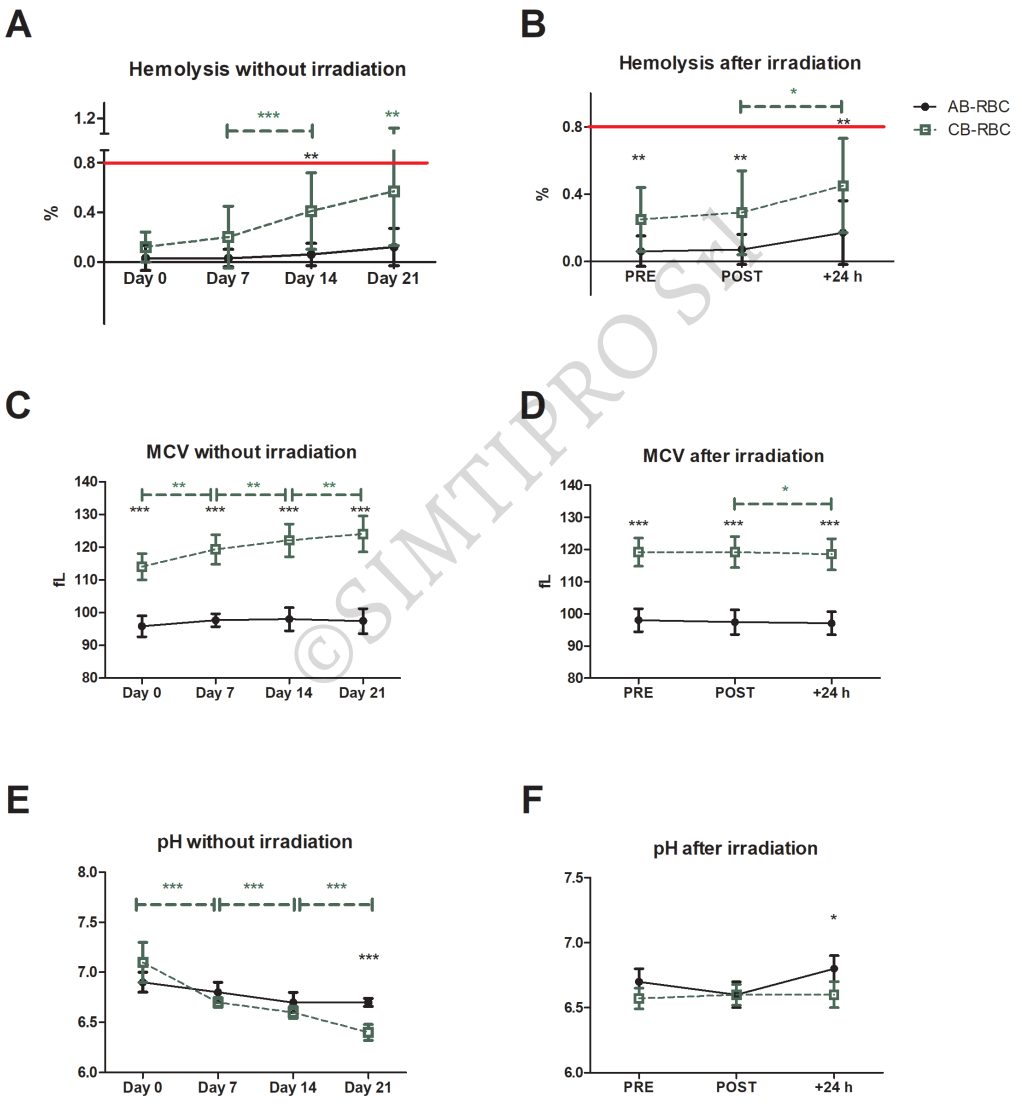
n.d.: not determined

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on day 21, hemolysis was above 0.8% in 42% (5/12) of CB-RBC. On days 14 (p=0.004) and 21 (p=0.005), significant differences were found between CB-RBC and AB-RBC.

A comparison of RBC indices is shown in *Online Supplementary Content* **Table SI**. No significant differences were found, except for mean corpuscular volume (MCV) in fresh CB-RBC *vs* AB-RBC (114±4 *vs* 95.8±3.2 fL, respectively, p<0.0001). During storage, MCV increased significantly in CB-RBC to 124±5 fL on day 21, p<0.0001 (**Figure 1C**). MCV, mean cell hemoglobin (MCH), red cell distribution width –standard deviation (RDW-SD) and red cell distribution width– coefficient of variation (RDW-CV) showed significant differences between CB-RBC and AB-RBC. No significant variations in RDW-CV amplitude were detected during CB-RBC storage.

The pH values in CB-RBC and AB-RBC were 7.1±0.2 *vs* 6.9±0.19 on day 0 and 6.4±0.08 *vs* 6.7±0.04 on day 21, respectively (**Figure 1E**).



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Figure 1 - Hematological parameters and pH in AB-RBC (No.=10) and CB-RBC (No.=14)
CB-RBC hemolysis without irradiation showed a significant increase during storage (**A**) and after irradiation (**B**). MCV showed significant differences between AB-RBC and CB-RBC (**C**) and after irradiation (**D**). pH showed significant differences at day 21 (**E**) and 24 hours after irradiation (**F**). Statistical significance of AB-RBC versus CB-RBC comparisons is shown above each time point by asterisks. Statistical significance of CB-RBC comparisons between each time point is shown with a cap line.

2,3-DPG levels in CB-RBC and AB-RBC were within the acceptance range throughout the storage period, with significant differences on day 21 (**Figure 2A**). No significant decrease was found in CB-RBC during storage.

CB-RBC osmotic fragility varied slightly during storage, within the acceptance range (data not shown).

pO_2 increased significantly (p<0.0001) during storage in CB-RBC *vs* AB-RBC, from 81±28 *vs* 55±20 mmHg on day 0 to 243±12 *vs* 127±63 mmHg on day 21 respectively. pCO_2 was 49±7 *vs* 66±9 mmHg on day 0 and 30±10 *vs* 112±7 mmHg on day 21 in CB-RBC *vs* AB-RBC, respectively (p<0.0001) (*Online Supplementary Content*, **Table SI**).

K+ concentration in CB-RBC *vs* AB-RBC increased from 3.5±1.7 *vs* 7.2±1 mmol/L (p=0.03) to 21±5 *vs* 17.6±1 mmol/L (p=0.1), respectively. CB-RBC showed significantly increasing K+ concentrations during storage on days 7 and 14 (p=0.002) and day 21 (p=0.02) (**Figure 2C**).

Na+ values on day 0 of 151±3 mmol/L in CB-RBC *vs* 141±2 mmol/L in AB-RBC significantly decreased on day 21 to 118±5 *vs* 112±2 mmol/L, respectively, with significant differences between products (p=0.01) (**Figure 2E**).

Lactate concentration in CB-RBC *vs* AB-RBC significantly increased from 2.9±1 *vs* 8.6±0.9 mmol/L on day 0 to 19.9±4 *vs* 21.4±3 mmol/L on day 21, respectively, with no significant differences between products (p=0.3) (**Figure 2G**).

Glucose concentration in CB-RBC *vs* AB-RBC significantly decreased from 503±87 *vs* 429±59 mmol/L on day 0 to 415±52 *vs* 255±43 mmol/L on day 21, respectively, with significant differences between products (p<0.0001) (**Figure 2I**).

CB-RBC compliance with quality parameters 24 hours after gamma irradiation carried out on day 14

Some parameters were altered after CB-RBC irradiation (*Online Supplementary Content*, **Table SII**). Hemolysis significantly increased from 0.25±0.19% on day 14 pre-irradiation to 0.45±0.28% (p=0.02) 24 hours after irradiation, with 20% of units above 0.8% on day 15 (see **Figure 1B**). Significant differences were found in CB-RBC *vs* AB-RBC at all-time points: pre-irradiation (p=0.004), post-irradiation (p=0.005) and 24 hours later (p=0.007).

MCV showed significant differences 24 hours after irradiation in CB-RBC (p=0.01) (see **Figure 1D**). The parameters MCV, MHC, RDW-SD and RDW-CV showed significant differences between CB-RBC *vs* AB-RBC (*Online Supplementary Content*, **Table SII**). For mean corpuscular hemoglobin concentration (MCHC), no significant differences were found.

pH showed a significant difference between CB-RBC and AB-RBC 24 hours after irradiation (6.6±0.1 and 6.8±0.1, respectively, p=0.02) (**Figure 1F**).

For 2,3-DPG, no significant decrease was observed at 24 hours post-irradiation. On day 15, significant differences were found (see **Figure 2B**).

Irradiation also significantly affected pO_2 and pCO_2 in CB-RBC (see *Online Supplementary Content*, **Table SII**).

K+ increased significantly in CB-RBC 24 hours after irradiation from 17.2±2 to 20.65±2.5 mmol/L (p=0.003) (see **Figure 2D**).

Na+ levels decreased in CB-RBC from 122.2±5 to 113±7 mmol/L (p=0.01) on day 14 before irradiation and after 24 hours, respectively, with no significant difference between CB-RBC and AB-RBC on day 15 (see **Figure 2F**).

There were no significant differences in lactate levels after CB-RBC irradiation. Additionally, when comparing pre-irradiated samples of AB-RBC and CB-RBC, no differences were found (p=0.1). However, immediately after irradiation, CB-RBC showed a lower lactate concentration of 16.8±3 mmol/L compared to 20.5±2 mmol/L in AB-RBC. Similarly, on day 15, lactate levels were 16.9±2 mmol/L and 10±2 mmol/L in AB-RBC and CB-RBC, respectively, demonstrating a significant difference (p=0.03) (see **Figure 2H**).

Glucose concentration on day 15 was 479±71 *vs* 284±43 mmol/L in CB-RBC *vs* AB-RBC, respectively (p<0.0001). No differences were found in CB-RBC after irradiation (**Figure 2J**).

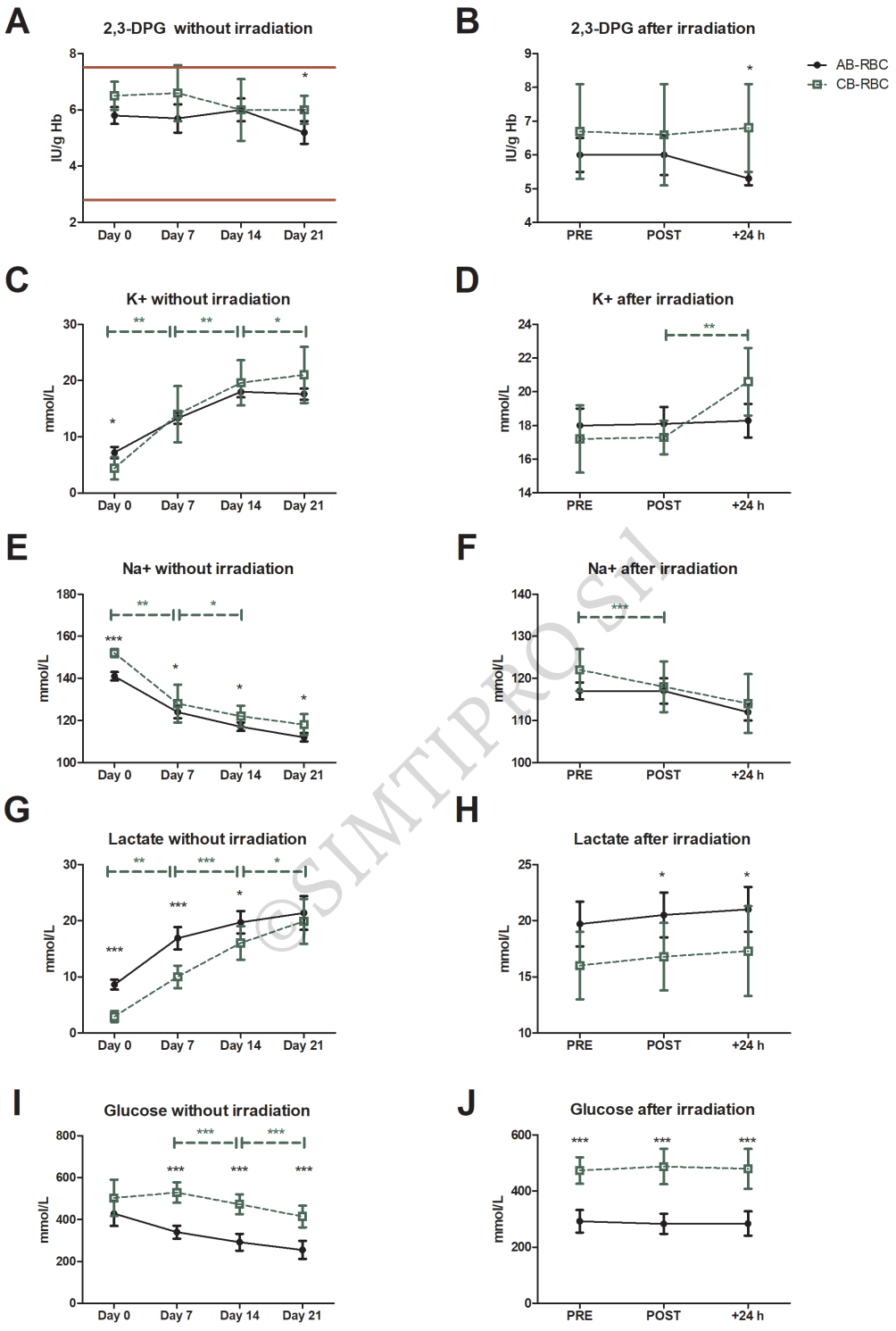
Evaluation of potential toxicants in CB-RBC and AB-RBC

On day 21 of storage (**Figure 3A-B**), CB-RBC had significantly lower contamination with residual plasticizers compared to AB-RBC. The concentration of DEHP in CB-RBC increased from 4,957±1,449 ng/mL on day 0 to 18,360±2,351 ng/mL on day 21 (p<0.0001). AB-RBC showed DEHP levels of 12,833±1,246 ng/mL on day 0, which increased to 32,565±4,905 ng/mL on day 21.

Initial MEHP concentrations of 177±70 and 621±133 ng/mL significantly increased on day 21 to 946±380 and 6,650±1,747 ng/mL in CB-RBC and AB-RBC, respectively (p=0.001).

CB-RBC irradiation on day 14 did not immediately affect the concentration of phthalates. However, after 24

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Figure 2 – 2,3-DPG, K+, Na+, lactate and glucose during storage (AB-RBC No.=10 and CB-RBC No.=14), before and after irradiation

2,3-DPG (**A**, **B**), K+ (**C**, **D**), Na+ (**E**, **F**), lactate (**G**, **H**) and glucose (**I**, **J**) showed significant differences during storage and after irradiation. Statistical significance of AB-RBC *vs* CB-RBC comparisons is shown above each time point by asterisks. Statistical significance of CB-RBC comparisons between each time point is shown with a cap line.

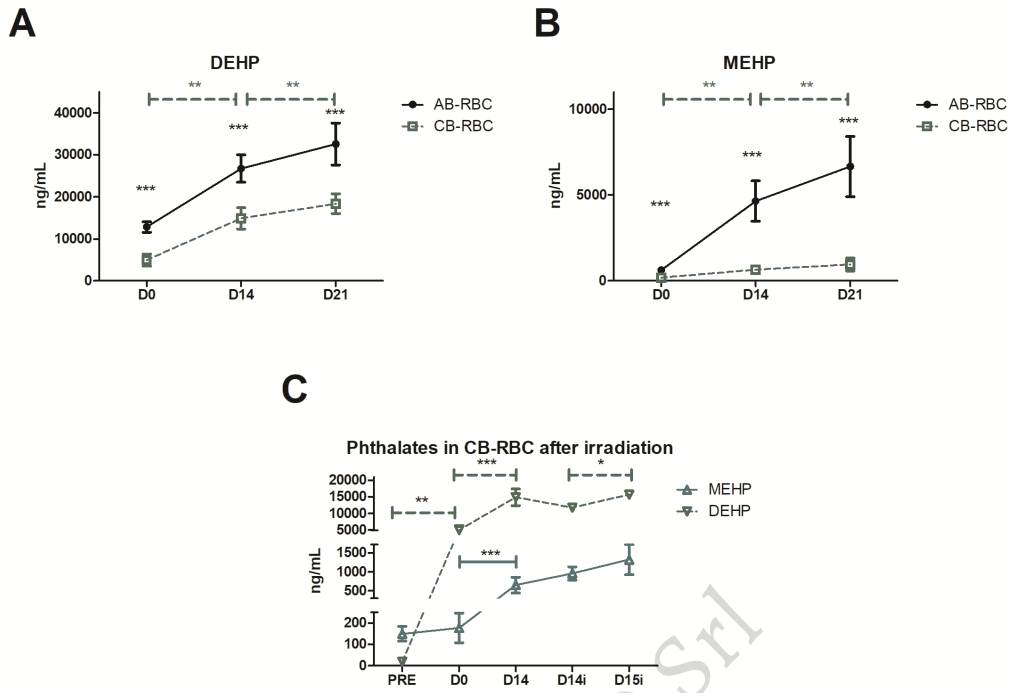


Figure 3 - Toxicants: phthalates
Significant differences of DEHP (**A**) and MEHP (**B**) were found during storage. Panel **C** shows DEHP and MEHP in CB-RBC during storage as compared with pre-storage whole CB samples collected in glass vials (No.=3).

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hours, DEHP significantly increased to 15,652±582 ng/mL (p=0.02). The concentration of MEHP after irradiation was 1,322±396 ng/mL, with no significant difference observed (p=0.1) (**Figure 3C**).

Study samples were analyzed for Hg, Pb, Cd, Co, Tl, and Mn (Figure 4)

Reference value for whole blood (R) and biological exposure index (BEI) for each metal were as follows: Hg R=<20, BEI=>50 µg/L; Pb R=<10, BEI=>30 µg/dL; Cd R=<2, BEI=>5 µg/L; Co R=<2, BEI=>5 µg/L; Tl R=<2, BEI=>5 µg/L; and Mn R=<20, BEI=>20 µg/L.

Hg levels did not show significant differences between CB-RBC (4.7±3.6 µg/L) and AB-RBC (5.4±3 µg/L) (p=0.2). However, they were significantly lower in maternal samples (2.5±1.7 µg/L), (p=0.02).

Pb concentrations were significantly lower in CB-RBC samples (1.1±0.5 µg/dL) compared to AB-RBC (2.1±1.6 µg/dL) (p=0.03). There was no significant difference in Pb levels between CB-RBC and maternal samples (1.3±0.7 µg/dL) (p=0.06).

Cd concentrations were significantly lower in CB-RBC (0.03±0.01 µg/L) compared to AB-RBC (0.3±0.2 µg/L) (p=0.0002) and maternal samples (0.2±0.1 µg/L) (p=0.001).

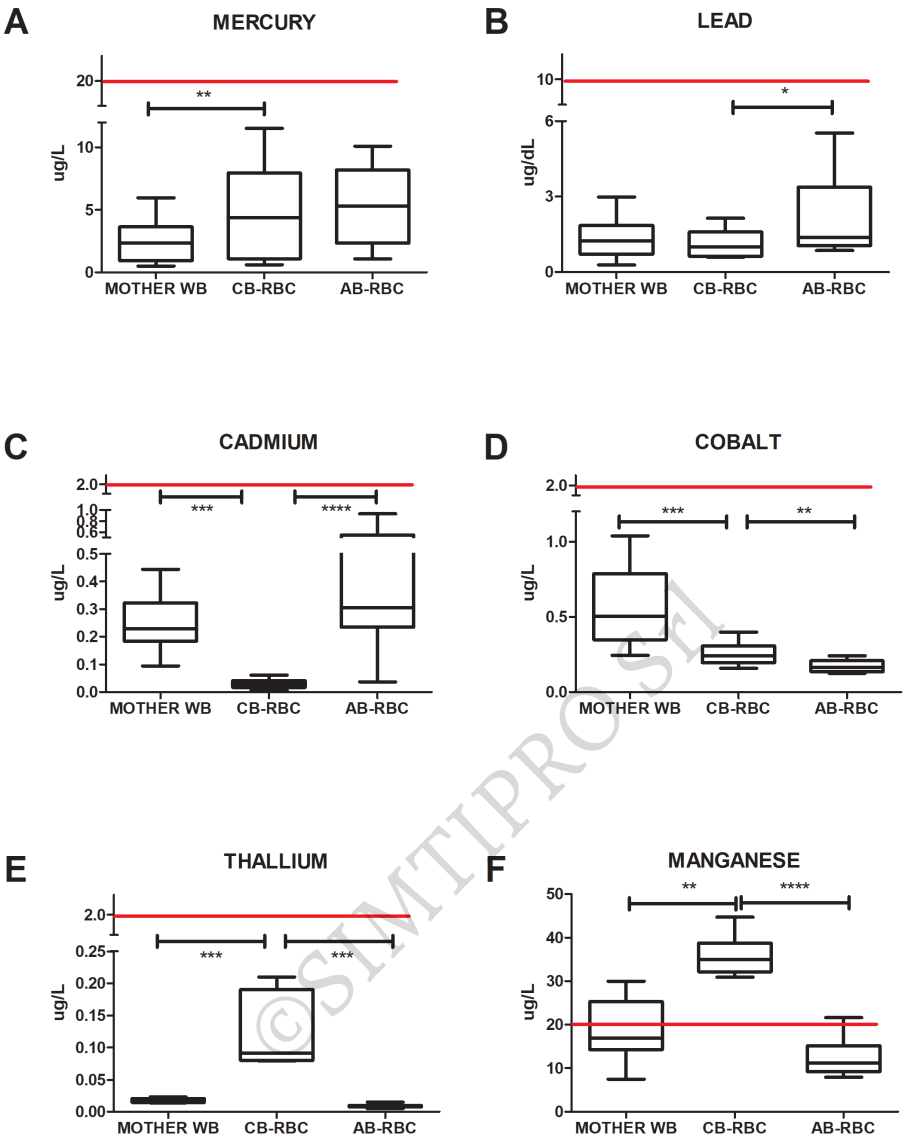
Co concentrations were below 1 µg/mL. CB-RBC showed significantly higher Co levels (0.2±0.07 µg/L) compared to AB-RBC (0.1±0.04 µg/L) (p=0.003). The highest Co concentration was observed in maternal samples (0.5±0.2 µg/L) (p=0.001).

Tl concentrations were very low (<1 µg/L) in all samples. CB-RBC had significantly higher Tl levels (0.1±0.05 µg/L) compared to AB-RBC (0.009±0.002 µg/L) (p<0.0001). CB-RBC also showed higher Tl levels compared to maternal samples (0.01±0.003 µg/L) (p=0.001).

Mn levels were higher in CB-RBC (36±4 µg/L) than in maternal samples (18±7 µg/L) (p=0.001). CB-RBC showed higher Mn levels compared to AB-RBC (12.4±4 µg/L) (p<0.0001).

DISCUSSION

We evaluated CB-RBC units obtained with a commercial closed-circuit system that prevents microbiological contamination and improves the efficiency of CB fractionation.



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Figure 4 - Toxicants: heavy metals
Mercury showed significant differences between maternal blood samples and CB-RBC and no differences between CB-RBC and AB-RBC (**A**); lead showed significant differences between CB and adult blood samples (**B**); cord blood had the lowest concentration of cadmium (**C**); adult blood had the lowest concentration of cobalt (**D**); CB-RBC had significantly higher concentration of thallium (**E**) and of manganese (**F**). The graphs are presented as mean, minimum and maximum error bars. The red line shows the reference value for whole blood.

At the beginning of storage, median MCV of CB-RBC was larger as compared to AB-RBC. Moreover, a sign of the enlargement of RBC during storage was the increase in MCV[17,18] and HCT at day 21, which is in line with the literature and was possibly caused by the hypertonic SAGM-solution used for RBC storage[4,17-19].

Hemolysis is an important indicator of progressive erythrocyte membrane damage during storage[4,17-23]. At day 15 (24 hours after irradiation on day 14), 20% of CB-RBC units exceeded the 0.8% hemolysis level established for AB-RBC release, which increased to 42% at day 21.

During CB-RBC storage, we noted a notable increase in RBC mass, hematocrit, and hemoglobin levels (see *Online Supplementary Content*). This augmentation appears in relation to the increase of MCV, and other events related to the storage lesions like lysis or rupture of RBCs during

storage that might contribute to hemoglobin increase, explaining the data shown in the validation. With regard to the well-known RBC metabolic slowdown during storage[23], our results showed a significant increase in K+ levels. Interestingly, fresh AB-RBC had a significantly higher K+ than CB-RBC, but after irradiation at the same time point no differences were found. Additionally, a significant decrease in extracellular Na+ levels at day 21 was observed in CB-RBC, confirming results from other studies[4,17,20,21,23].

CB-RBC K+ release is especially important, as EPIs are susceptible to hyperkalemia[20,24-26]. Despite its clinical relevance, a maximum concentration of K+ in CB-RBC for transfusion purposes has not yet been established. Prudentially, specialists do not recommend prolonged storage of the units[20,25] and propose to use potassium absorption filters with CB-RBC[25]. Nevertheless, it should be taken in account that this process can lead to an increase in hemolysis and, at the same time, clinicians should not forget that the reduction in K+ is accompanied by an increase in Na+ levels which may be clinically significant[26]. Reassuringly, Bianchi *et al.* reported no adverse events with products with 25 mmol/L K+. A slow transfusion technique may also be helpful[27].

Despite the metabolic rate reduction, during storage RBC use anaerobic glycolysis as the main metabolic pathway. Anaerobic glycolysis activation causes glucose consumption and production of lactate and protons (H+)[21,24,28]. All these changes have been reflected in the results of our analysis. In fact, glucose levels decreased and lactate concentration increased at day 21 in CB-RBC, confirming findings from other reports[4,17].

In this study, a statistically significant decrease in pH was detected, in agreement with other investigations[4,17-19,21,23,29]. Acidification of CB-RBC induces enzyme changes from diphosphoglycerate mutase (DPGM) to diphosphoglycerate phosphatase (DPGP)[28,29]. This leads to increased degradation of 2,3-DPG, the major hemoglobin regulator[28]. A statistically insignificant decrease in 2,3-DPG levels was detected in our analysis. In contrast, some studies have statistically confirmed the reduction of this metabolite in AB-RBC[29] and CB-RBC[21] during storage. Specifically, Bifano *et al.*[21] found that 2,3-DPG in CB-RBC decreased after 28 days of storage. At the clinical level, there are no studies demonstrating that the reported concentrations of 2,3-DPG have clinically significant effects in transfused patients[28,29]. In fact, it has been reported that after AB-RBC transfusion, 2,3-DPG levels increase to pre-storage levels[28]. In relation to blood gases, an increase in pO_2 and a decrease in pCO_2 were detected. Studies that have evaluated these parameters in whole blood suggest that the stability of gases is determined by the permeability of storage bags[30]. In this case, the increase in pO_2 may result from an exogenous influx and a reduced consumption of cellular metabolism.

In terms of microbiological contamination, CB units have a higher rate of contamination than adult donor blood[4,31]. However, the causes of CB microbiological contamination of CB units are highly variable, including type of delivery[20,32], collection technique, maternal clinical conditions and staff training.

The quality control parameters evaluated in the present study are considered essential for the quality and safety of the product. Nevertheless, recent studies evaluating RBC quality have incorporated new predictors in vitro, such as quantification of adenosine triphosphate (ATP), and determination of phosphatidylserine and CD47 in the plasma membrane[22].

Studies on the effects of irradiated CB-RBC are scarce, although the harmful effects of irradiation in AB-RBC are well described[26,28,32-34]. Gamma irradiation, a standard practice for the prevention of transfusion-associated graft-versus-host disease, has been reported to cause significant changes in AB-RBC, and these are correlated with the irradiation dose[26]. Loss of membrane integrity causes an accelerated decrease in CD47 levels and a progressive increase in hemolysis[28,32]. Furthermore, the increase in permeability of the erythrocyte membrane after irradiation favours the passage of ions through the lipid bilayer, an alteration that cannot be compensated with the activity of the sodium-potassium pump, since it is immobilized during storage[34]. This favours a significant increase in plasma K+ levels and a slight decrease in Na+ concentration in irradiated AB-RBC[26,28,33,34]. For this reason, the main transfusion guidelines[15,16] state that AB-RBC can be irradiated up to 28 days after collection, and that they should be transfused as soon as possible, but never later than 14 days post-irradiation. In this analysis, a significant decrease in pCO_2 and a significant increase in K+ levels were detected 24 hours after CB-RBC

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irradiation. Decreased pCO_2 is not considered relevant for transfusion, but since the risk of hyperkalemia in premature infants is evident, the high K+ content may be clinically significant. Considering the results obtained and the clinical relevance of this biochemical parameter in premature infants, additional studies are warranted to determine the maximum infusion flow of CB-RBC after irradiation.

Our analysis of hematological and biochemical parameters over the course of 21 days of storage, including the application of irradiation, reveals the presence of storage lesions in RBCs. These findings align with previous studies and underscore the potential clinical implications associated with such alterations[35]. The phenomenon of RBC storage lesions encompasses various detrimental effects such as hemolysis, potassium elevation, and metabolic impairments including lactate accumulation and 2,3-DPG depletion. These processes in transfused patients may lead to inflammatory responses and the onset of multiple organ dysfunction. Additionally, transfusion-related immune modulation further exacerbates these complications, as summarized in Yoshida's review[35]. To minimize these risks, and according to our validation exercise we limited expiration time to 14 days.

As a part of RBC stability, plasticizer degradation markers were measured. The results for phthalates indicate a significant increase at day 21. As irradiation also increases the degradation of phthalates, an elevated concentration was already expected after three weeks of storage. Notwithstanding, the kits for CB-RBC manufacturing showed decreased phthalates degradation compared to standard AB-RBC.

Heavy metals present in blood components can cause neurotoxicity in transfused preterm newborns[36]. Almost all measured metals were within the acceptance range determined by the Hospital Clínic de Barcelona's Biomedical Diagnostic Centre (CDB) laboratory. Mn was previously determined by Guan *et al.*[37], who described that its concentration in maternal and cord blood is higher than in adult blood and associated with birth size.

A limitation of our study is that ATP, which is an important marker of RBC ageing during storage, could not be validated by a luminescence method due to hemoglobin interference with luminescence. We plan to use other kits for further characterization of CB-RBC and determination of transfusion survival markers, such as CD47[22,32].

CONCLUSIONS

The quality of leukoreduced CB-RBC during storage is mainly determined by hemolysis levels and extracellular K+ content. Therefore, based on the quality control parameters analyzed, we propose that the expiration date of CB-RBC stored with SAGM should be 14 days and <24 hours after irradiation.

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ETHICAL CONSIDERATION

The research was conducted ethically, with all study procedures being performed in accordance with the requirements of the World Medical Association's Declaration of Helsinki.

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AUTHORS' CONTRIBUTIONS

SQ, GS, EA-N, DS, PR, MA. Processing: DS, EV. Determination of hematological and biochemical parameters: MC, GA, MG, ST. Analysis of results: SQ, MC, GA, EA-N, DS, MG, LR, CA, MR, EV, JFS, MA. Manuscript writing: DS, MC, MG, PR. Manuscript revision and discussion: all Authors.

CONFLICTS OF INTEREST

PR is co-inventor of the BioNest ABC and EF system and scientific director of Meditalia Industriale s.r.l. The other Authors declare no conflicts of interest.

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