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ORIGINAL PAPER

Umbilical cord blood CD34$^+$ stem cells and other mononuclear cell subtypes processed up to 96 h from collection and stored at room temperature maintain a satisfactory functionality for cell therapy

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Background and Objectives Umbilical cord blood (UCB) is a good stem cell source for cell therapy. We recently demonstrated that cord blood mononuclear cell (MNCs) subtypes were viable and functional until 96 h after collection, even stored at room temperature. Now, we analyzed the viability and functionality of the cells before and after cryopreservation.

Materials and Methods Twenty UCB units were analyzed at 24 and 96 h after collection, frozen for 6 months, thawed and re-evaluated. MNCs were analyzed by flow cytometry, viability by 7-AAD and clonogenic assays (CFU) were performed.

Results After 96 h of storage, no substantial loss of MNC was found (median $7{\cdot}320 \times 10^6 \times 6{\cdot}305 \times 10^6$). Percentage and viability CD34$^+$ cells, B-cell precursors and mesenchymal stem cells were not affected. However, mature B and T lymphocytes as well as granulocytes had a substantial loss. CFU growth was observed in all samples. Prefreezing storage of 96 h was associated with a relative loss of colony formation (median 12%). Postthaw, this loss had a median of 49% (24 h samples) to 56% (96 h samples).

Conclusion The delay of 96 h before UCB processing is possible, without a prohibitive impairment of CD34$^+$ loss in number and functionality.

Key words: cell therapy, cryopreservation, functionality, umbilical cord blood, viability.

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Introduction

Umbilical cord blood (UCB) is an essential therapeutic product for hematopoietic reconstitution and expansion and is being increasingly used for allogeneic hematopoietic stem cell transplantation with good results. UCB has also been under intense experimental investigation in regenerative medicine. Several cord blood subpopulations such as mesenchymal stromal cells, unrestricted somatic stem cells and endothelial cells have been studied in distinct assays [1–11]. Moreover, cord blood cells may be used for standardized induced pluripotent stem cell (iPSC) generation [12].

Major advantage of using UCB for transplantation includes the fact that the procedure may be carried out even without total compatibility of human leucocyte antigens (HLA) such as greater than or equal to 4/6 HLA (A, B,

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DRb) match. Also, two different UCB units may be infused in the same adult patient, due to the presence of naïve lymphocytes and natural killer cells, with a low incidence of graft-versus-host disease (GVHD) maintaining the graft vs. leukaemia effect (GVL) [13].

Cord blood processing and cryopreservation techniques have been improved during the last two decades, increasing the use of UCB volume reduction as a cost saving alternative to diminish the cryogenic space that is very expensive. There is an increasing demand for standardization of procedures for collection, manipulation and cryopreservation to fulfil the FACT/Netcord accreditation process [14, 15].

This quality assessment plan includes tests before freezing and assays performed on segments attached to the bags after thaw, to assure cord blood engraftment after transplantation [16, 17].

Recently, many investigations comparing different methods of $CD34^+$ stem cell enumeration, viability evaluation, and even a comparison among the different methodologies have been published [18–21]. Temperature and time of storage before freezing have been extensively studied, but several points have remained controversial. Most authors prefer to keep UBC units at 4°C until freezing [21–23], but others, including our previous study, have shown that UCB stem cells also remain viable and functional if kept at 20–22°C [21, 24, 25]. Concerning maximal time to delay for freezing, most authors have tested the maximal time of 72 h [22, 23, 25, 26], but in an earlier work, Pamphilon suggested that the cells could be satisfactorily be kept until 96 h [21], which we could confirm in our previous work [24]. All authors that analyzed samples postthaw observed that there was a larger loss of cells after cryopreservation than after delay in freezing, and so, they aimed to maximize the number of cells for cryopreservation to compensate loss postthaw.

In a previous study [24] using samples already volume-reduced for cryopreservation and stored at laboratory room temperature (RT) (20–22°C), we demonstrated that cord blood $CD34^+$ stem cells while mature T lymphocytes, immature B lymphocytes, monocytes and MSCs remained viable up to 96 h after collection, even when stored at RT. Only mature B lymphocytes and granulocytes decreased significantly over time. There were no differences detected between whole blood vs. blood volume-reduced units. We could confirm the findings of other authors [26–29] that $CD34^+$ stem cells maintained viability until 72 h and were present until 96 h. Clonogenic assays performed with samples stored fresh until 96 h showed that functionality was also maintained for this period [24].

Studies regarding postthaw $CD34^+$ stem cell viability have been also published. Xiao and Dooley [28] studied fresh and thawed UCB MNC and have shown that the non-viable cells in both samples were similar, revealing 2–3% of dead cells when processed 48 h after collection. Cell loss was larger in samples processed 72 h after collection.

Brazil is the largest Latin American country having a heavily mixed ethnic population with a great HLA diversity. Brazilian Public Cord Blood Bank Network was organized in 2006. However, to cover the entire HLA diversity, UCB units would necessarily have to be collected from all geographic regions. Sometimes these units could come from distant locations, rendering the cryopreservation process very difficult, as the end of procedure, including collection, manipulation and freezing should be carried out up to 48 h after collection, according standardized by international and national regulatory agencies.

The aim of this study was to evaluate whether $CD34^+$ stem cells and other MNC subtypes of UCB units processed up to 96 h after collection and submitted to cryopreservation could maintain good viability and functionality after thaw.

Materials and methods

The experiments were performed with twenty UCB units donated to the Public UCB Bank at the University of Campinas, which did not comply with the criteria for banking (UCB volume less than 70 ml, discounted the bag anticoagulant volume or initial TNCs below $8{\cdot}0 \times 10^8$), despite fulfilling all maternal criteria (absence of family history of genetic disorders, negative testing for viral or bacterial infections, absence of maternal fever during labour or delivery, gestation over 35 weeks and delivery occurring under 24 h after rupture of membranes). All mothers provided free informed consent before UCB unit collection. UCB units were processed according to the requirements of FACT/NetCord.

Umbilical cord blood collection

Collection of UCB was carried out without affecting the delivery by a staff submitted to continuous training (e.g. midwives and physicians). After delivery and with the placenta still 'in utero', the umbilical cord was clamped off. Thorough cleaning and disinfection was carried out to prevent contamination with maternal blood or germs. The umbilical vein was punctured under aseptic conditions, with a 16-gauge needle and cord blood was collected by gravity into a standard 250-ml collection UCB bag (Griffols, Barcelona, Spain) containing CPD as an anticoagulant. The procedure continued until the blood flow stopped.

Study design

From each collected UCB unit entering in the study, 24 ml was aspirated into a test-tube, with no volume reduction and fractioned into two aliquots of 12 ml. These samples were left at laboratory room temperature (20–22°C), and immunophenotyping was made 24 and 96 h after collection (prefreezing – PF). The cells remaining in the aliquots were frozen, stored for 6 months and then thawed and reanalyzed (postthaw – PT).

Flow cytometric analysis comprised quantification of $CD34^+$ stem cells, mature T lymphocytes (CD4 and CD8), mature B lymphocytes, monocytes, granulocytes, immature B-cell precursors and mesenchymal stem cells (MSCs) was quantified. Cell viability was tested in all aliquots (7-AAD exclusion technique).

In parallel, clonogenic assays of all samples were performed for BFU-E, CFU-GM, CFU-GEMM and CFU-E.

Flow cytometry analysis

Umbilical cord blood specimens were analyzed using a whole blood lysis technique. A six-colour combination of monoclonal antibodies (MoAbs) was used to identify the cell populations.

The antibodies used were purchased from BD Pharmingen, San Diego, CA, USA, except for CD34 (Becton Dickinson – San Jose, CA, USA) and CD105 (Invitrogen – Camarillo, CA, USA).

The combinations used at 24 and 96 h prefreezing and postthaw were as follows:

FITC-CD3/PE-CD8/7AAD/APC-CD4/APC-CY7-CD45
FITC-CD3/PE-CD19/7AAD/APC-CD34/APC-CY7-CD45
FITC-HLADR/PE-CD14/7AAD/APC-CD33/APC-CY7-CD45
FITC-CD90/PE-CD73/PE-Cy7-CD34/7AAD/APC-CD105/APC-CY7-CD45.

The specimens were diluted to a concentration of 5×10^6/ml. Cell suspensions were incubated with the conjugated MoAbs at room temperature in the dark for 20 min. Erythrocytes were lysed using BD Pharm Lyse (Becton Dickinson, San Jose, CA, USA), containing no fixative agent for 15 min in the dark at room temperature. Cells were centrifuged and the pellet was washed in phosphate buffered saline (PBS) (Merck KGaA, Darmstadt, Germany). The cells were resuspended in PBS until the analysis was performed.

Data acquisition was performed on a FACSCanto II (Becton Dickinson, CA, USA) flow cytometer using FACSDIVA software (Becton Dickinson). At least 100 000 non-gated events were collected and analyzed, selecting an appropriate gate on each cell population on the CD45/SSC plot and 7-AAD to exclude non-viable cells using the INFINICYT software (Cytognos S.L., Salamanca, Spain). The expression of each antigen was recorded as the percentage from the total cell number. The strategy for analysis is shown in Fig. 1. The same strategy of analysis was performed on the PF and PT samples.

Clonogenic assays

Clonogenic assays were performed on samples before freezing and immediately postthaw. Diluted UCB MNCs ($1{\cdot}0 \times 10^4$ cells/ml), obtained by Ficoll–Hypaque (Sigma Chemical Co., St. Louis, MO, USA) density 1077 procedure, were added to 3 ml of methylcellulose medium (Methocult GF H3343, StemCell Technologies Vancouver, BC Canada) and 1·5 ml plated into two separate wells of a six-well plate. The plates were incubated for 14 days at 37°C, in 5% CO^2/95% air. CFU (BFU-E, CFU-GM, CFU-GEMM, and CFU-E) with over fifty cells were counted and expressed as CFUs/10^5 TNCs.

Freezing and thawing procedure

Samples were centrifuged for 20 min at 1880 *g* for plasma reduction. An equal volume of buffy-coat and cryoprotectant solution (10% dimethyl sulphoxide (DMSO); Origen Biomedical, Austin, Texas, USA) was put into four polypropylene cyotubes (1 ml) through controlled-rate freezing in the Cryo-Med cooling chamber. The cells were cooled at 1°C/min to −30°C, 5°C/min to −70°C and 10°C/min to −90°C. Frozen cryotubes were then stored into liquid nitrogen containers. This procedure was performed with samples of 24 and 96 h after collection. Cells were kept frozen up to 6 months. The thawing procedure was carried out quickly in a water bath at 37°C and processed immediately to perform flow cytometry analyses and clonogenic assays.

Statistical analysis

A descriptive analysis was performed for all cell subsets, and the data were presented as the median percentages of positive cells. The changes occurring in each cell population throughout the observation period (24–96 h) for prefreezing and postthaw samples were analyzed by t-dependent test. The correlation among CFU number and the other cell subsets was performed by Spearman correlation WinStat 3.0 (Kalmia Company, Inc, Cambridge MA, USA. The results from the clonogenic assays were expressed as the arithmetical mean of the duplicates and submitted to t-dependent test analyses.

Results

Considering the number of MNCs, there was a small total mononuclear cell loss if samples were processed after

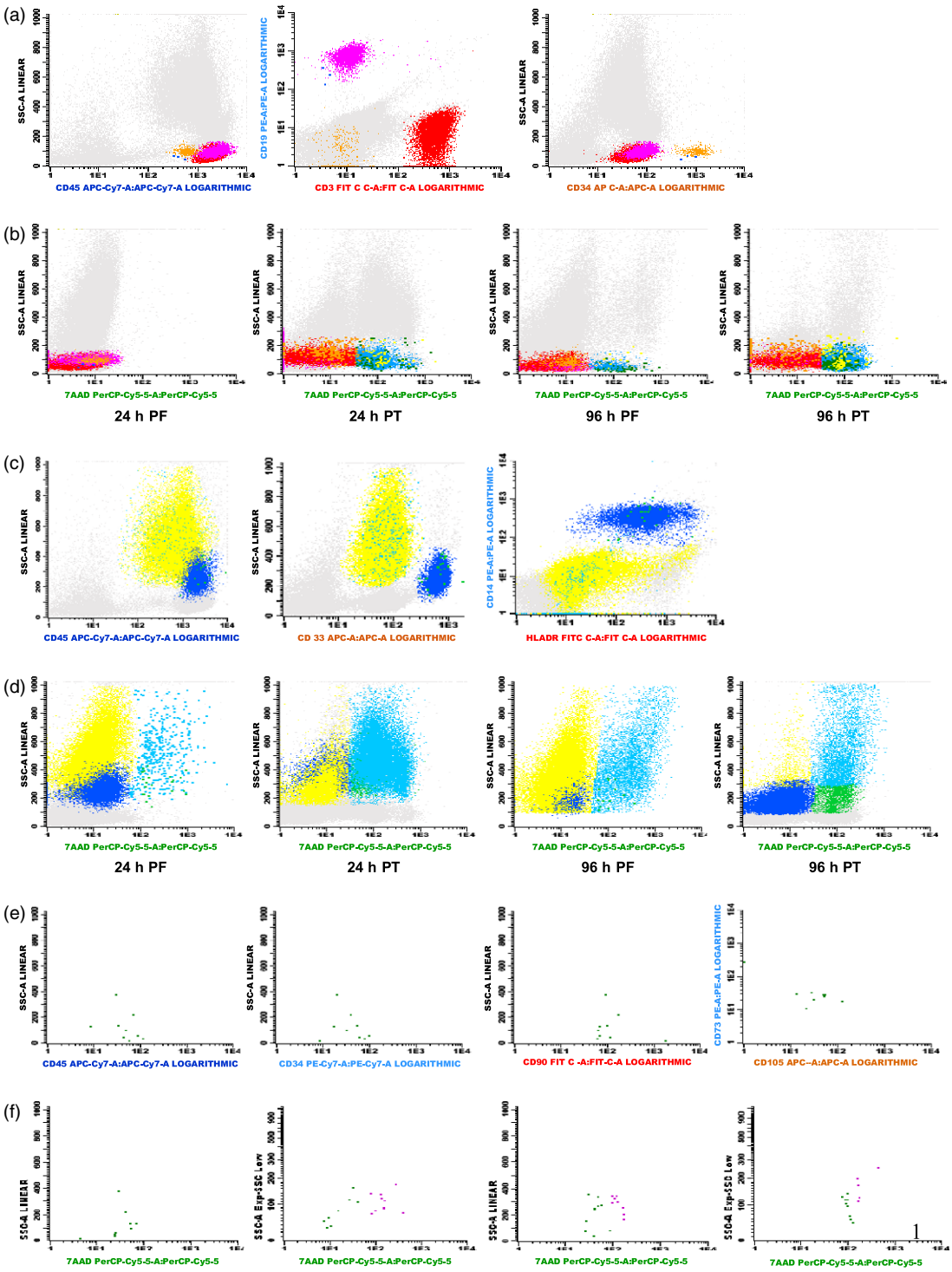

Fig. 1 Flow cytometry strategy of analysis of the distribution of mononuclear cells (MNC) subsets at 24 and 96 h prefreezing (PF) and postthaw (PT), using CD45 to separate the cell populations and 7-AAD for viability analysis at 24 and 96 h PF and PT. (a) Orange dots represent $CD34^+$ stem cells ($CD34^+C19^-CD3^-$); immature B ($CD34^+CD19^+CD3^-$) are identified as blue dots; B cells ($CD34^-CD19^+CD3^-$), pink dots; and T cells ($CD34^+CD19^-CD3^+$), red dots. (b) Live cells ($7\text{-}AAD^-$): $CD34^+$ stem cells are identified as orange dots; immature B, blue dots; B cells, pink dots; and T cells, red dots. Dead cells ($7\text{-}AAD^+$): $CD34^+$ stem cells, yellow dots; B cells, green dots; and T cells, cyan dots. (c) Monocytes ($CD33^+HLA\text{-}DR^-CD14^+$), blue dots; and granulocytes ($CD33^+HLA\text{-}DR^-CD14^-$), yellow dots. (d) Live cells: monocytes, blue dots; and granulocytes, orange dots. Dead cells: monocytes, green dots; and granulocytes, light blue dots. (e) MSCs ($CD45^-CD34^-CD90^+CD105^+CD71^+$), green dots. (f) Live cells: MSCs, green dots. Dead cells: MSCs, pink dots.

96 h instead of 24 h: median of $7{\cdot}320 \times 10^6$ (5·120–15·700) and $6{\cdot}305 \times 10^6$ (3·270–12·100), respectively. TNCs had a median of $4{\cdot}9 \times 10^8$ at 24 h and $3{\cdot}9 \times 10^8$ at 96 h. The median relative loss of TNCs for the delay of 96 h for processing was 17% (9–50).

Comparing the samples processed at 24 h and 96 h after collection, the percentages of $CD34^+$ stem cells increased after 96 h of storage and did not change significantly PT (Table 1, Fig. 2). The percentage of granulocytes decreased after fresh storage as well as PT (Table 1, Fig. 2).

Concerning cell viability (Table 2), only the percentage of mature T lymphocytes (especially and CD3/CD4 ones) and granulocytes showed a significant decrease ($P < 0{\cdot}001$).

Comparing the prefreezing and postthaw samples, granulocytes presented a significant decrease ($P = 0{\cdot}001$), followed by mature T lymphocytes (both CD3/CD4 and CD3/CD8) (Fig. 2). The proportion of all other subpopulations remained stable postthaw after the storage period of 6 months.

The absolute number of T lymphocytes fell from a median of 1·766 to 767 × 106/ml in samples processed at 24 h and from a median of 1·954 to 1·001 × 106/ml in samples processed at 96 h. Granulocytes fell from a median of 2·445 to 740 × 106/ml in samples processed at 24 h and from a median of 1·224 to 708 × 106/ml in samples processed at 96 h.

Concerning viability, PF and PT mature T lymphocytes and granulocytes showed a significant loss (Table 2). Despite of the decrease in percentage of mature B lymphocytes, the remaining cells viability did not decrease. Immature B cells, monocytes and MSCs maintained a viability of approximately 99%. $CD34^+$ cells viability PF and PT was approximately 99% (Table 2).

Clonogenic assays

The CFU growth was observed in all samples. However, fresh samples of 96 h after collection showed a significant decrease in colony number ($P < 0{\cdot}0001$), a larger decrease was found in samples postthaw (Fig. 3). The median CFU number in samples stored 24 h PF was 68 (32–121) and PT was 36 (17–69). For samples processed after 96 h, the median was 57 (31–110) and PT it was 27 (13–54).

The relative loss in CFU number in samples processed 96 h instead of 24 h after collection had a median of 12% (0–22%), demonstrated by '(CFU 24 h– CFU 96 h)/ CFU 24 h'. Comparing the number of colonies PT and PF, samples processed at 24 h had a median loss of 49% (22–57); samples processed 96 h after collection 56% (25–68).

The number of CFUs from samples processed at 24 h had a positive correlation with the newborns' weight ($r = 0{\cdot}53$; $P = 0{\cdot}008$ prefreezing CFUs and $r = 0{\cdot}39$; $P = 0{\cdot}04$ postthaw CFUs), the number of TNCs (0·42; $P = 0{\cdot}02$ prefreezing CFUs and 0·45; $P = 0{\cdot}02$ postthaw CFUs) and a negative one with the number of non-viable $CD34^+$ cells prefreezing ($r = -0{\cdot}52$; $P = 0{\cdot}008$ prefreezing CFUs and $r = -0{\cdot}51$; $P = 0{\cdot}009$ postthaw CFUs). For those processed at 96 h from collection, only the relation with TNCs was maintained ($r = 0{\cdot}49$; $P = 0{\cdot}01$).

Discussion

Umbilical cord blood-based therapies have been used with considerable success, mainly for transplantation of hematopoietic stem cells in the treatment of blood disorders. Recently, they have been increasingly used for novel applications such as cellular regenerative therapy, immunomodulation [30] and are also a promising stem cell source for induced pluripotent stem cells (iPSCs) procedures.

Brazilian Public Cord Blood Banks Network (Brasilcord) was founded in 2006. Until now, (December 2013) a total of 27·538 UCB units were collected but only 17·100 were cryopreserved (rejection rate of 38%). This is keeping with data reported from several countries all over the world

Table 1 Umbilical cord blood MNC subsets analyses in samples prefreezing and postthaw. Results were expressed as the median of the percentages

	Prefreezing (PF)		Postthaw (PT)	
UCB MNC subtypes	**24 h**	**96 h**	**24 h**	**96 h**
$CD34^+$ stem cells	0·21 (0·09–0·51)	0·40 (0·03–0·85)	0·18 (0·05–0·43)	0·32 (0·11–0·78)
Immature B cells	0·006 (0·00–0·05)	0·009 (0·00–0·09)	0·003 (0·00–0·02)	0·002 (0·00–0·02)
Mature B lymphocytes	5·43 (2·19–14·19)	1·27 (0·45–2·63)	6·36 (1·91–13·66)	0·99 (0·19–3·04)
Mature T lymphocytes	19·90 (6·94–39·77)	27·10 (6·36–51·89)	10·23 (2·77–27·40)	14·87 (2·52–34·15)
CD3/CD4 T cells	13·39 (5·10–27·13)	18·72 (6·16–41·59)	9·06 (3·26–18·96)	13·66 (2·73–30·88)
CD3/CD8 T cells	6·61 (1·91–12·02)	8·29 (1·65–13·81)	2·15 (0·54–7·20)	2·89 (0·86–11·08)
Monocytes	6·73 (2·01–12·18)	5·59 (1·51–10·50)	6·76 (1·21–14·09)	5·43 (0·14–14·23)
Granulocytes	30·72 (12·52–62·88)	18·50 (5·16–55·07)	7·73 (0·83–16·96)	11·45 (1·77–35·30)
MSCs	0·007 (0·001–0·02)	0·008 (0·001–0·05)	0·003 (0·00–0·02)	0·004 (0·001–0·02)

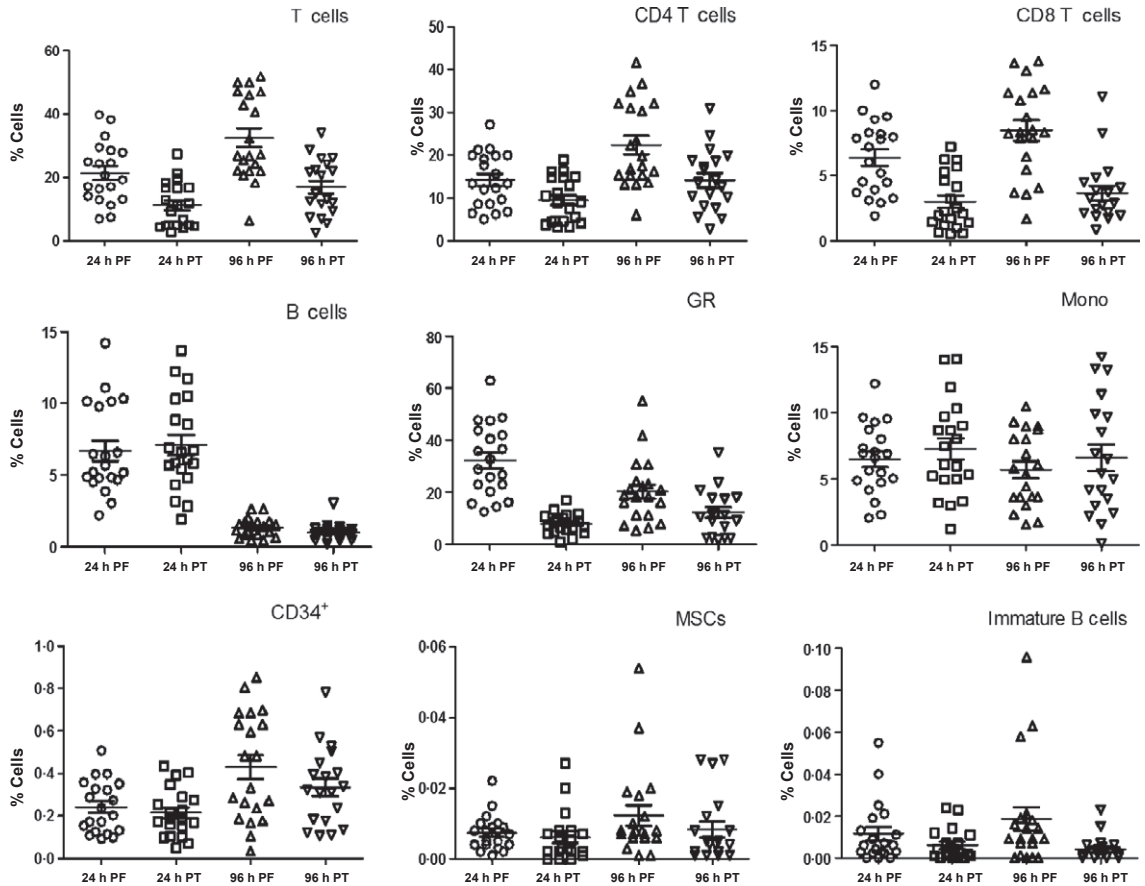

Fig. 2 Comparison of the percentages of the several subsets of mononuclear cells at 24 and 96 h prefreezing (PF) and postthaw (PT). T Cells = mature T lymphocytes; CD4 T Cells = T lymphocytes $CD4^+$; CD8 T Cells = T lymphocytes $CD8^+$; B Cells = mature B lymphocytes; GR = Granulocytes; Mono = Monocytes; $CD34^+$ = $CD34^+$ stem cells; MSCs = Mesenchymal stem cells; Immature B Cells = Immature B lymphocytes. Significant differences were found in T Cells, CD4 T Cells, CD8 T Cells and GR. Significant *P* values: 24 h PF – PT: Mature T lymphocytes (<0·001), CD3/CD4 (0·01), CD3/CD8 (<0·001), Granulocytes (<0·001). 96 h PF – PT: Mature T lymphocytes (<0·001), CD3/CD4 (0·005), CD3/CD8 (<0·001), Granulocytes (0·001) in the t-dependent test.

Table 2 Umbilical cord blood MNC subsets viability analyses at 24 and 96 h, samples prefreezing and postthaw. Results were expressed as the median of the percentages

	Viable cells Prefreezing		Viable cells Postthaw	
UCB MNC subsets	**24 h**	**96 h**	**24 h**	**96 h**
$CD34^+$ stem cells	99·99	99·99	99·96	99·96
Immature B cell	100·00	100·00	100·00	100·00
Mature B lymphocytes	99·99	99·96	99·53	99·73
Mature T lymphocytes	99·98	99·89	86·47	75·24
CD3/CD4 T cells	99·98	99·94	91·18	82·67
CD3/CD8 T cells	99·99	99·99	97·15	95·03
Monocytes	99·97	99·76	99·49	99·34
Granulocytes	98·43	96·39	86·78	94·97
MSCs	99·99	99·99	99·99	99·99

[31–33]. Only 167 units were transplanted (Brasilcord December 2013). The optimal number of UCB that should be banked to attend the country's necessity is difficult to calculate and depends on the HLA diversity of the population and the incidence of the different diseases to be treated. Brazil is a large country with a high HLA diversity, and logistic problems may hamper the collection of numerous units as specific HLA groups can be found in specific remote regions.

In a previous study, we analyzed MNC cord blood cells, including viability and functionality of $CD34^+$ stem cells up to 96 h after collection, stored at laboratory room temperature (20–22°C) [24]. In the present study, we studied viability and functionality of these cells, mainly $CD34^+$ stem cells, after a fresh storage without volume reduction for 96 h, cryopreservation and thawing after 6 months. This protocol better imitates a transport of UCB from the maternity hospital to the Cord Blood Bank.

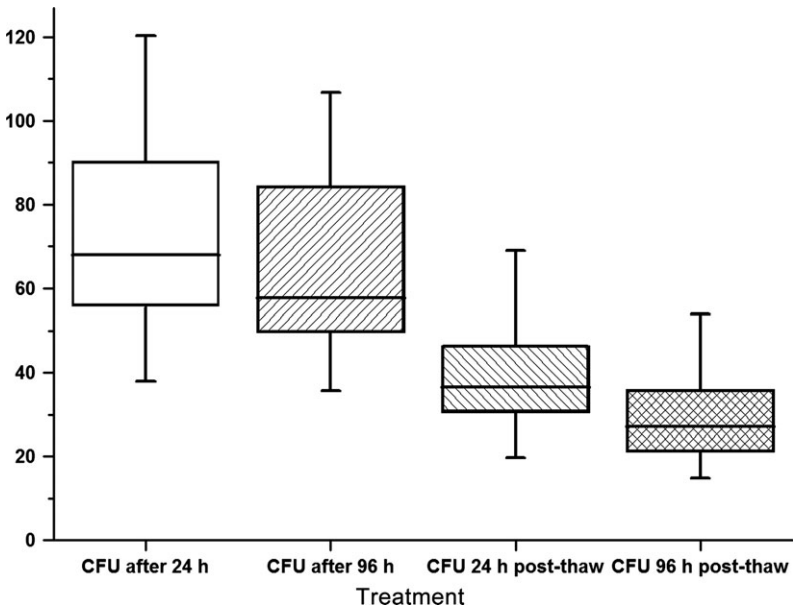

Fig. 3 Distribution CFU number of samples stored at room temperature at 24 and 96 h PF and PT. An important decrease was observed postthaw when compared with prefreezing samples.

We analyzed twenty samples stored at RT (20–22°C) and processed at 24 and 96 h after collection. Prefreezing and postthaw cell viability and functionality were analyzed and performed with whole UCB without volume reduction.

The percentage of $CD34^+$ stem cells maintained viability of approximately 100%, after a delay of 96 h for processing, similar to what was found by other authors [26, 27]. In the present study, although the number of colonies decreased in samples processed after 96 h, $CD34^+$ stem cells maintained a satisfactory viability and functionality even stored at room temperature. There was no significant loss of their percentage postthaw. Guttridge *et al.* [25] have analyzed UCB units stored until 72 h at RT and also demonstrated a good viability of $CD34^+$ stem cells. But the viability postthaw was impaired in samples with 72 h of delay for cryopreservation. Nevertheless, they did not examine their functionality by cell culture. Fry *et al.* [22] and Radke *et al.* [34] also demonstrated a good viability cells at RT or at 4°C during 72 or 48 h after collection.

Louis *et al.* [23] demonstrated that viability of $CD34^+$ stem cells and their capacity to grow in culture is not affected when processed until 72 h after collection, similarly if kept at RT or at 4°C. However, cell recovery in NSG mice was highly decreased for samples kept at RT, but unaffected when kept at 4°C. Yet, as it is generally accepted that CB stem cell engraftment in patients correlates well with CFU formation *in vitro*, the authors suggest that it should be examined if this influence of storage temperature is also observed in humans. Such a proposal, however, is very difficult to perform under the conditions of UCB banking, as in general, it takes a very long time to transplant a number of UCBs banked that would be suitable to render consistent results [35].

In our study, the colony number decreased more postthaw then after the delay in processing. This finding seems a consensus among several studies [22, 34, 36, 37]. Fry *et al.* [22] have shown losses in CFU number mainly after thawing. Radke *et al.* [34] analyzed whether the quantity of viable $CD34^+$ stem cells seeded affects CFU count, and they observed a strong dependence of clonogenic efficiency on the seeding density. They did not observe a significant difference in cell viability between samples of bag, aliquot and segment, but showed that manipulation of segments is more disadvantageous for cell viability. On the other hand, Lee *et al.* [36] showed that cryopreservation time (1–5 years) does not affect CFU number. Page *et al.* [38] analyzed 435 cases of CBT confirming that the clonogenic assays (total number of CFUs) are a strong independent predictor of neutrophil and platelet engraftment after unrelated UCB transplantation. The same group recently described a novel scoring system to optimize UCB unit selection for transplantation, analyzing TNCs, MNCs, CFUs, and $CD3^+$ and $CD34^+$ content. Page *et al.* [38] results corroborated with Migliaccio *et al.* and Prasad *et al.* All these authors demonstrated the same range of CFU units as our study [39, 40]. So, several features of prefreezing, freezing and storage conditions may affect the stem cell competence of engraftment *in vivo*, and results of several studies are hard to compare.

The proportion of mature B lymphocytes decreased during the period prefreezing but not postthaw. Kessel *et al.* [41] have shown that cord blood B cells present a higher apoptotic rate when compared to peripheral adult

B cells. These cells have a lower expression of Bcl-2 that plays a pivotal role in modulating membrane integrity and the release of apoptogenic factors from mitochondria. Cord blood B cells also show a low bcl-2/bax ratio. Lecoeur *et al.* [42] have shown that living $CD19^+$ B lymphocytes may have a low expression of this antigen, and consequently, apoptotic B-cells phenotyping could be misleading. $CD19^+$ B cells high sensibility to temperature variations, demonstrated by Paxton and Bendele [43], could be another hypothesis. B-cell precursors maintained similar proportions as found in our previous study.

Mesenchymal stem cells maintained almost the same percentages as in the prefreezing samples, showed a tendency to decrease postthaw maintaining their viability. Moreover, MSCs must necessarily be analyzed to verify whether they would be able to maintain their great capacity of proliferation and differentiation into other cell lineages.

Taken together, we can conclude that delay in processing UCB causes a small decrease of TNCs, but the cell loss was more pronounced postthaw. The proportion and viability of $CD34^+$ stem cells was maintained although colony formation decreased, especially postthaw. The number of colonies obtained postthaw in samples processed 24 h after collection could be predicted by the newborn's weight, TNCs and the non-viable percentage of $CD34^+$ stem cells prefreezing. No quality control performed by flow cytometry postthaw could predict this number. There was also a substantial change in the proportions of B and T lymphocytes as well as of granulocytes. How this could affect the immunological recovery after transplantation, the incidence of GVHD and relapses remains to be examined [44–46].

Concerning samples processed after 96 h, besides the number of TNCs prefreezing no other variables showed a relation with the number of CFUs postthaw, which could be considered as a gold standard of functionality of the UCB for cell therapy.

These data indicate that UCB could be processed up to 4 days from collection, which would increase the number of units collected with specific HLA phenotype, even in distant regions.

Conflict of interest

The authors declare no conflict of interests.

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