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Factors which can influence the quality related to cell viability of the umbilical cord blood units

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ABSTRACT

Cell viability is an important indicator for the quality of umbilical cord blood (UCB) units that can influence the transplant final outcome. Thus, it is particularly important to identify the factors that may affect the cell quality during the banking process. The present study is a first attempt to correlate the impact of exogenous factors (time from collection to processing, collected UCB volume) and endogenous factors (TNCC – total nucleated cell count, $CD34^+$cell count) on cell viability assessed before UCB units cryopreservation within a banking standardized process. Three thousand UCB units collected in 35 ml CPDA containing bags were processed by HES sedimentation within 48 h. TNCC, $CD34^+$ cell counts and total cell viability were determined after processing. Cell viability of 94.37 ± 4.67%, TNCC of $73.17 \pm 36.73 \times 10^7$ and $CD34^+$cell count of $2.61 \pm 2.29 \times 10^6$ was obtained after processing of units with UCB collected volume of 80.23 ± 28.52 ml. A significant negative correlation was found between cell viability and the time from collection to processing ($r = -0.7228$; $P < 0.0001$). The cell viability decreasing rate of 20.54%, 15.18% and 3–10% were achieved for units with collected UCB volume <40 ml, (40–80 ml) and >80 ml, to 48 h versus 12 h. There were no differences considering cell viability for the UCB units with similar collected UCB volume that had various $CD34^+$cell count or TNCC ($P > 0.05$). The extension of the time from collection to processing of UCB units can reduce the quality by decreasing cell viability. The cell viability decreasing rate owing to the time influence is determined by the collected UCB volume being inversely proportional to it. Endogenous factors do not affect the cell viability.

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1. Introduction

Umbilical cord blood banks are aimed to provide high quality units needed for clinical therapies, for hematopoietic and immune system reconstitution in order to treat malignant and non-malignant hematological disorders [1]. Because the cord blood stem cell number is limited, maintaining cell viability becomes a critical issue. Cell viability is an important indicator for the quality of umbilical cord blood units (UCB units) that can influence the final outcome of a transplant. It seems that extensive manipulation with significant variables could determine unexpected viability problems [2]. In this situation it is particularly important to identify the factors that may affect the cell quality during the banking process. It is essential to retain viability and functional integrity for the cells obtained by umbilical cord blood processing [3]. Studies accomplished to date show that the cell viability is influenced by storage conditions of UCB units before processing and cryopreservation. Thus, the storage temperature of UCB units from collection to cryopreservation seems to be an essential factor that influences the cell viability and contributes to maintaining the

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final product quality. The controversial results show that UCB units must be stored before processing at 4 °C [4–7] or at room temperature [8–10]. Other authors consider that there are no differences reflected in the quality of transplant units depending on the storage temperature [11–13].

The time from collection to processing and cryopreservation is another controversial factor, extensively studied, which seems to exert an important impact on cell viability. If some authors consider that significant loss of nucleated cells and $CD34^+$cells has occurred after 24 h of storage from collection [14], other authors consider that umbilical cord blood cells maintain their viability for a significantly longer period of 24 h [15], while other authors assume that the cells remain viable even after 48 h [9] or up to 72 h after collection [8,16]. Other studies show that umbilical cord blood storage for 72 h induces a high degree of cellular damage [17,18]. Although most authors recommend that processing and cryopreservation must be performed as soon as possible after umbilical cord blood collection to maintain the characteristics of immature cells, other authors [10] suggest that a storage time for 48 h should be preferred to 24 h for maintenance of progenitor cells with high capacity of proliferation, transmigration, with a low rate of apoptosis. In the issue, the maximum storage time for umbilical cord blood from collection to processing and cryopreservation may be 48 or 72 h [2,12].

The present study is a first attempt to correlate the impact of exogenous and endogenous factors on cell viability assessed before UCB units cryopreservation within a banking standardized process.

2. Materials and methods

2.1. UCB collection, transport and storage

A total of 3000 UCB units were collected by trained medical personnel at Obstetrics and Gynecology Hospitals in Romania. UCB samples were obtained by puncture of the umbilical vein, after normal or cesarean delivery, and collected into blood collection bags (Baxter, Fenwal), 250 ml capacity, containing 35 ml anticoagulant CPDA (citrate phosphate dextrose adenine). After collection, each UCB collection bag was folded into gel wrap (Therapak), inserted into insulated envelope foil (Therapak) and placed into a box for transport. Because of insulation system, the transport can be done in ambient temperature, between 15 and 30 °C, without affecting the temperature of UCB units. At the moment of reception, UCB collection bags revealed temperatures between 20 and 26 °C, measurements being done with Testo 830-T2 thermometer. After reception, the UCB bags were maintained at room temperature (22 ± 2 °C) until processing.

2.2. UCB processing

UCB units were deemed to comply if they have been processed within 48 h post collection. UCB units with collected UCB volume (excluding the amount of anticoagulant) less than 15 ml were not accepted for processing. Processing was achieved through volume reduction using hidroxietilamidon (10% HES Fresenius Pharma, Graz, Austria) sedimentation method as has been previously described [19,20]. After erythrocytes and partial plasma removal UCB units with a standard volume of 25 ml designed for cryopreservation were obtained and tested.

2.3. Total nucleated cell count

Quantification of nucleated cell number was performed by using Act Diff 5 Beckman Hematology Analyzer Coulter. The total nucleated cells count (TNCC) was established by testing a sample of the UCB units, using a calculation formula: $WBC \times 10^3/\mu l \times 25 \times 10^3\ \mu l$, where $25 \times 10^3\ \mu l$ represents the final volume of the UCB units obtained after processing.

2.4. Flow cytometry analysis: $CD34^+$ assay and cell viability assay

The flow cytometric analysis was performed after processing and before freezing on a flow cytometer Beckman Coulter FC500 equipped with CXP System Software (Version 2.0), using an automated method. We used Beckman Coulter Stem-Kit Reagents intended for "In vitro diagnostic use", including 7-aminoactinomycin D (7-AAD) – a cell viability reagent, CD34-phycoerythrin (PE; 580 clones)/CD45-fluorescein isothiocyanate (FITC; J33 clone) antibody, CD45-fluorescein isothiocyanate (FITC; J33 clone)/IsoClonic Control-phycoerythrin antibody, Stem Count Fluorospheres, NH4Cl Lysis solution. Two identical tests and an isomorphologic control to check the nonspecific binding of the CD34-PE were run for each sample. The software allowed the evaluation of the total cell viability percentage, the simultaneous identification and enumeration of viable $CD45^+$cells and the dual positive $CD45^+/CD34^+$cell population percentage or absolute counts and the $CD34^+$ total cells counts per UCB units.

2.5. Statistical analysis

The data were expressed as the arithmetic mean values ± standard deviation, median and intervals (minimum-maximum). Software Graph Pad Prism 5 (Graph Pad Software Inc, San Diego, CA) was used for data interpretation. Comparisons of quantitative variables were done by the *t* test, Mann–Whitney test, one way ANOVA Kruskal–Wallis test with Dunn's multiple comparison post test and two way ANOVA test with Bonferroni post tests. The Spearman's correlation coefficient was used to estimate the correlation between quantitative variables. All the reported *P* values represent two-tailed tests, with values of 0.05 or less considered statistically significant. All the statistical analyses were made with nonparametric tests.

3. Results

3.1. Cell viability of UCB units

Pre freeze cell viability of UCB units obtained after umbilical cord blood ($n = 3000$) processing was ranged from 61.86% to 99.5% with a mean value of 94.37 ± 4.67% and median of 95.62%. 95.14% of the UCB units had cell viability higher than 85%.

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