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Original Research

Maternal and neonatal predictors and quality of umbilical cord blood units

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Summary

Umbilical cord blood (UcB) is a rich source of stem cells which are used in the treatment of approximately different 80 disease units. Stem cells are stored in both state and private banks and it is crucial that optimal conditions for obtaining UCB are developed to ensure the highest viability of the preserved stem cells. The aim of the paper is to determine the correlation between the length of pregnancy, the number of deliveries, the newborn's birth weight, and selected properties of UCB stem cells. The study covers 50 pregnant females in whom either spontaneous labor or elective caesarean section were performed. UCB was collected immediately upon birth and the samples were analyzed in a Polish stem cell bank in Warsaw. The authors found that as the length of the pregnancy increases, so does the concentration of nucleated cells in UCB. However, tested parameters of UCB were not related to the number of deliveries or newborn's birth weight.

Key words: Stem cell; Umbilical cord blood; CD 34+ cells; Cord blood banking; Gestational age; Parity; Birth weight.

Introduction

For many years the umbilical cord blood (UCB) has been widely used as an alternative source of stem cells for transplantation purposes due to its large number of multipotent stem cells and their immunological incompetence, virological purity, accessibility, and ease of collection. The first UCB bank was established by Rubinstein in 1992 in New York [1]. A year later, the first allogenic transplantation was performed using previously frozen and banked UCB [2]. In 1996 it was proven that the number of stem cells present in a single unit of frozen UBC is enough to recreate the bone marrow of three recipient (a child) and also that UCB transplantation is associated with a lower risk of graft rejection [3]. These events led to the creation of consecutive UCB banks and various programs of financing them from public funds. As a result it became critical to establish standards and protocols for collecting, processing, and storing umbilical cord blood.

In 1998, the NETCORD foundation led to the creation of international register of UCB banks as well as to the establishment of procedures and standards for the safe exchange and clinical application of banked umbilical cord blood. Furthermore, the foundation defined and introduced international accreditation standards regarding UCB collection, processing, testing, and banking which were published in 2008 [4].

In 2012, as a result of the significant progress in the field of UCB research, the European Parliament issued a resolution regarding voluntary donating of tissues and cells, in which it urges member states to establish legal standards regulating public and private banking of UCB stem cells [5].

The transplantation quality of an UCB unit is determined by the total number of leukocytes translated into a unit of the recipient's body mass. Based on research, it was determined that to perform transplantation 3 ml of UCB are required for each kilogram of the recipient's body mass. The amount of collected material depends to a great extent on the quality of placenta, the pace of its detachment, the length of the umbilical cord, the newborn's birth weight, and the time from delivery to the clamping of the umbilical cord [6-8]. Much attention is paid to obtaining the best quality transplantation material and there have been a wide-scale research studies devoted to developing optimal conditions for obtaining UCB which will ensure the highest transplantation quality of UCB.

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Table 1. — *Evaluation of correlation between cell viability, concentration of CD34+ cells, and WBCs in umbilical cord blood and the number of deliveries.*

No. of deliveries	CD34+[number/µl]	% CD34+	Cell viability [%]	WBC [thousand/µl]
	Mean ± SD (Min-max)	Mean ± SD (Min-max)	Mean ± SD (Min-max)	Mean ± SD (Min-max)
3 and more	123.80±78.58 (46.00-256.0)	0.25±0.16 (0.09-0.51)	96.88±2.51 (92.88-99.02)	10.92±3.33 (7.80-16.20)
2	135.92±62.59 (52.0-245.0)	0.28±0.13 (0.10-0.49)	97.00±1.78 (94.04-99.87)	15.13±5.87 (8.30-27.20)
1	113.27±78.21 (10.00-352)	0.23±0.15 (0.03-0.70)	97.24±2.49 (86.71-99.88)	13.81±3.56 (8.00-22.70)
Total	119.76±73.97 (10.0-352.0)	0.24±0.15 (0.03-0.70)	97.15±2.30 (86.71-99.88)	13.84±4.27 (7.80-27.20)
p	NS ($p = 0.665687$)	NS ($p = 0.654744$)	NS ($p = 0.920044$)	NS ($p = 0.180049$)

Table 2. — *Evaluation of correlation between cell viability, concentration of WBCs CD34+ cells in umbilical cord blood, and pregnancy length.*

Pregnancy week	CD34+[number/µl]	% CD34+	Cell viability [%]	WBC [thousand/µl]
	Mean ± SD (Min-max)	Mean ± SD (Min-max)	Mean ± SD (Min-max)	Mean ± SD (Min-max)
<38	149.40±105.85 (49.00-316.00)	0.30±0.21 (0.10-0.63)	98.47±0.43 (98.20-99.23)	11.16±3.99 (7.80-17.10)
38-39	125.68±75.01 (34.00-352.00)	0.25±0.15 (0.07-0.70)	97.36±1.78 (92.49-99.88)	12.78±3.26 (8.30-19.10)
40 and more	104.16±65.98 (10.00-264.00)	0.22±0.13 (0.03-0.53)	96.48±3.01 (86.71-99.65)	16.08±4.74 (9.40-27.20)
Total	119.76±74.74 (10.00-352.00)	0.24±0.15 (0.03-0.70)	97.13±2.32 (86.71-99.88)	13.90±4.29 (7.80-27.20)
p	NS ($p = 0.421141$)	NS ($p = 0.4852720$)	NS ($p = 0.1834550$)	$p = 0.010214$

The aim of the study was to determine the effect of selected maternal and fetal factors on the "quality" of the obtained transplantation material. Therefore, the authors attempted to determine a correlation between the pregnancy length, parity, and birth weight of a newborn and selected properties of UCB cells.

Material and Methods

The study covered 50 pregnant females between 18 and 38 years of age. All the patients included in the study expressed a conscious consent to participate according to the protocol of the Bioethical Committee of the Medical University of Silesia in Katowice, Poland. All the participants of the project presented a normal course of pregnancy, had not suffered from any other non-gynecological chronic disorders, and had a spontaneous delivery or an elective cesarian section.

The UCB was collected directly after delivery, clamping, and cutting of the umbilical cord of a newborn. The material was collected into a plastic bag found in the collection kit containing 29 mg of anticoagulant citrate phosphate dextrose (CPD) solution. The blood was then placed in special stabilizing gels and within 24 hours it was transported at room temperature to the Polish Stem Cells Bank (PBKM) laboratory in Warsaw. At the laboratory, the material underwent the preparatory procedure in the course of which the cord blood volume was reduced via elimination of part of autological plasma and erythrocytes. To eliminate red blood cells, their sedimentation was performed in an HES 6% solution, and to eliminate the autological plasma, the material was centrifuged for 15 minutes at 20°C at 1420 × g. Approximately 0.5 ml was collected from the obtained suspension of nucleated cells for the study and the transplantation quality of UCB was then determined.

The count of nucleated cells was performed with the automated hematology analyzer. Quantification was performed twice, prior to the material preparation, and after completing it, which enabled to determine the proportional cell recovery rate.

CD34+ hematopoietic stem cells were enumerated with the flow cytometry unit FACS Calibur. In the course of material preparation, the cell suspension was labelled with phycoerythrin (PE)-conjugated monoclonal antibodies which were directed against HPCA-2 antigens and fluorescein (FITC)-conjugated monoclonal antibodies against CD45 antigens. The control group consisted of cells labelled with polyclonal antibodies. The total hematopoietic cells count was determined on the basis of the proportion of cells determined with the flow cytometry and the number of nucleated cells obtained with the hematology analyzer.

In order to evaluate their viability, the UCB stem cells were labelled with 7-amino-actinomycin D (7-AAD). 7-AAD is a compound with a strong affinity for DNA. This agent penetrates only dead cells and stains them. The cells were analyzed with the FACS Calibur flow cytometer and the CellQUEST Pro, BD software. Viability was assessed on the basis of the proportion of cells not binding with 7-AAD.

The obtained results were analyzed in the Statistica PL software and the ANOVA variance analysis was performed. Statistical significance was assumed at $p < 0.05$.

Results

Obstetrical history covering the total number of pregnancies, including stillbirths, was obtained from all mothers. In this study this number was between 1 and 4. In the studied patient population the majority of women, i.e. 33 women (66%), were primiparae; for 24% it was the second delivery, and only in five cases it was either the third or fourth delivery.

The WBC and CD34+ cell counts were performed together with cell viability and evaluated in terms of the number of experienced deliveries. As shown in Table 1, patients

Table 3. — *Evaluation of correlation between cell viability, concentration of WBCs CD34+ cells in umbilical cord blood, and birth weight.*

Body weight [g]	CD34+ [amount/µl]	% CD34+	Cell viability [%]	WBC [thousand/µl]
	Mean ± SD (Min-Max)	Mean ± SD (Min-max)	Mean ± SD (Min-Max)	Mean ± SD (Min-Max)
<2000	188.00±0.00 (188.00-18.00)	0.38±0.00 (0.38-0.38)	99.23±0.00 (99.23-99.23)	8.00±0.00 (8.00-8.00)
2500-2999	131.50±95.28 (49.00-316.00)	0.26±0.19 (0.10-0.63)	97.43±0.92 (95.94-98.27)	12.58±3.91 (9.20-18.00)
3000-3499	90.08±27.65 (34.00-147.00)	0.18±0.05 (0.07-0.29)	96.97±3.35 (86.71-99.88)	12.47±3.63 (7.80-19.10)
3500-3999	132.00±86.37 (10.00-352.00)	0.27±0.17 (0.03-0.70)	97.40±1.80 (94.04-99.87)	15.00±4.911 (8.30-27.20)
4000-4499	95.00±23.81 (68.00-113.00)	0.19±0.05 (0.14-0.23)	96.32±2.98 (92.88-98.05)	14.20±1.78 (12.80-16.20)
Total	117.47±73.19 (10.00-352.00)	0.24±0.14 (0.03-0.70)	97.26±2.27 (86.71-99.88)	13.84±4.38 (7.80-27.20)
p	NS ($p = 0.326131$)	NS ($p = 0.234789$)	NS ($p = 0.832502$)	NS ($p = 0.331118$)

with different number of deliveries did not differ in a statistically significant way in terms of the concentration of CD34+ cells, WBCs, and cell viability in the UCB collected in the course of labor. The tests only revealed that on average, the highest number of CD34+ cells and WBCs can be observed in UCB collected during the second labor. However, the highest mean cell viability can be observed in UCB collected from women giving birth for the first time.

In the studied female population, the mean gestational age at labor was 39 weeks. The large majority, i.e. 82% (41 women) gave birth between the 38th and 40th week of pregnancy. For 11% of women the pregnancy ended in the 41st week and for 7% it ended before the 38th week. Table 2 shows the statistically significant correlation between the WBC concentration in UCB and the gestational age. The highest mean WBC concentration in UCB ($p < 0.05$) was observed in full-term pregnancy patients (40th week and more). On the other hand, the lowest mean WBC concentration in UCB was found in patients who gave births before the 38th week of pregnancy. Pregnancy length did not have a statistically significant effect on cell viability or the concentration of CD34+ cells in the UCB. The highest mean cell viability and CD+ cells concentration were observed in samples of UCB of female patients who gave births before the 38th week of pregnancy. Furthermore, it can be observed that both parameters decrease as the pregnancy length increases.

Newborns' birth weights varied between 1,590 and 4,230 grams. The mean birth weight was 3,458 grams. The birth weight of the majority of newborns (98%) exceeded 2,500 grams. Only in 2% of cases the birth weight was lower than 2,000 grams. On the other hand, in 7% of newborns the birth weight was higher than 4,000 grams. The newborn's birth weight did not have a statistically significant effect on cell viability or concentration of CD34+ cells and WBCs in UCB. The highest mean WBC concentration was observed in samples of umbilical cord blood collected perinatally when the body weight of the newborn ranged between 3,500–3,999 grams (Table 3).

Discussion

For the last 28 years, UCB stem cells have found a wide application in the treatment of a wide range of diseases, for example, acute leukemia, myelodysplastic disorders, lymphoma, as well as in congenital immune deficits, metabolic disorders, congenital, and acquired anemia.

Labor is the greatest stress in life leading to the organism's mobilization and the ejection of stem cells from the bone marrow niche to the blood; unless banked properly, stem cells which end up in the umbilical cord and placenta are irretrievably lost [9].

There are two storage options for UCB: public and private (family) banks. As of today more than one million units of UCB are stored in the public and family banks worldwide [10]. The main difference between banking UCB for public purposes or storing it in commercial banks results from its sources of financing. However, due to financial difficulties, only a small portion of banked UCB units are at the disposal of the public banks. It is crucial to increase their resources and use them for the transplantation purposes in non-related patients in the allogenic model. In the process of UCB banking, it is also extremely important to obtain tissue material of the highest possible quality [10-12].

In the light of the above, it was analyzed whether, and if so – how the number of deliveries, the pregnancy length, and the birth weight of a newborn may affect cell viability, as well as concentration of WBCs and CD34+ cells in UCB. No statistically significant differences were observed in the concentration of CD34+ cells and WBCs or cell viability in UCB in relation to the number of deliveries. However, it was observed that the highest mean cell viability was in the UCB of women who had their first delivery. This viability was decreasing with each following delivery. On the other hand, the highest mean concentration of WBCs and CD34+ cells was observed in the material obtained in the course of the second delivery.

McGuckin *et al.* [13] and the Ballen *et al.* [14] showed that the concentration of CD34+ cells, WBCs, and CFU=GM in the UCB collected in the course of delivery decreases in a statistically significant manner with each

consecutive delivery. Similarly, Omori *et al.* [15] observed a statistically significantly higher count of WBCs in UCB obtained from primiparae as compared with the second or third delivery. It is believed that it is the first stage of delivery that may be responsible for this as it is often prolonged in the course of the first delivery. Under the influence of intensified oxidative stress, there comes to the "ejection" of stem cells from the bone marrow [16, 17]. On the other hand, Bonab *et al.* [18] observed a statistically significantly higher number of nucleated cells in the material obtained during the first and the second delivery.

Data regarding the effect of pregnancy length on the properties of UCB stem cells are not unequivocal. A positive correlation was revealed between gestational age and the count of nucleated cells in UCB [7, 13]. The highest WBC concentration was found in UCB obtained around the 40th week of pregnancy. Furthermore, a longer pregnancy period is connected with a higher concentration of CD34+ and lymphocytes in the studied material [13]. It is believed that the increase of CD34+ cells in UCB of pregnancy lasting more than 39 weeks is a defensive mechanism of the newborn's organism against intensifying hypoxia resulting from the process of placenta aging [19]. On the other hand, results of other studies show that the less mature a pregnancy, the higher percentage of CD34+ cells and more progenitor cells present in UCB, despite the fact the total count of nucleated cells is lower as compared to full-term pregnancies [7]. Similarly, the Ballen *et al.* assessed that the longer the pregnancy period, the higher number of nucleated cells can be found in the UCB obtained perinatally; however, it is characterized by a lower concentration of CFU-GM (statistically significant differences). In the study, the pregnancy length does not have a statistically significant effect on the concentration of CD34+ [14]. Only Nunes and Zandavali [20] did not reveal any statistically significant differences between the pregnancy length and the "quality" parameters of UCB (WBC, CD34+).

The present study revealed a statistically significant dependency only between the nucleated cells concentration and the pregnancy length. The highest number of nucleated cells were observed in the UCB obtained in the course of deliveries in the 40th or 41st week of pregnancy. Despite the fact that differences in the CD34+ cell count or cell viability were not statistically variable, it was observed that the longer the pregnancy, the fewer CD34+ cells were present and the lower viability of studied tissue material was shown.

Body mass of newborns was the last parameter to be assessed in the study. No statistically significant effect of this factor was observed on the concentration of CD34+ cells, WBCs or cell viability in the umbilical cord blood. However, various scientists have observed in their studies that UCB collected from newborns with higher body weight is richer in nucleated cells (a statistically significant dependence) [13-15, 19, 20]. Furthermore, in some scientific projects, a positive correlation was reported between the newborn's body weight and the HSC concentration in UCB obtained perinatally. These differences were also statistically significant [7, 13, 14].

UCB is an alternative source of stem cells to the bone marrow. The advantages of these cells lie in the fact that they are easier to collect, reveal a higher multiplication potential, the pre-transplantation procedure is easier, there is a lower number of severe complications, and the selection between a recipient and a donor is easier.

So far, more than 40,000 procedures of UCB stem cells transplantation have been performed both in children and adults. In recent years it has been shown that UCB stem cells may transform into different types of cells, including muscle and nerve cells. There are more and more studies aimed at the application of UCB stem cells in regeneration medicine [10].

Conclusion

As the pregnancy length increases, so does the count of nucleated cells in UCB. The number of deliveries as well as the newborns' body weight do not have any effect on the nucleated cells' viability or the number of nucleated cells and hematopoietic stem cells in the UCB.

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