Impact of Maternal and Placental Pathology on Successful Umbilical Cord Blood Sampling and Cryopreservation

J.A. Marin[1], M. Calomfirescu[2], Roxana Bohâlțea[3], C. Ionescu Târgoviște[4]

1. PhD Student, UMF "Carol Davila", Bucharest
2. CMU, Bucharest
3. Obstetrics and Gynaecology Department, Emergency University Hospital Bucharest
4. PhD Coordinator, Institute of Diabetes Nutrition and Metabolic Disorders "N.C. Paulescu", Bucharest

Correspondence: Jan A. Marin
e-mail: andimarin@pulsmedia.ro

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Abstract

Umbilical cord blood (UCB) is an alternative for stem cell transplantation in the treatment of various diseases. ***Objective.*** *In order to find out whether the maternal, placental or fetal pathology before storage might have an impact on the quality of umbilical cord stem cells (UCBSC) and on the success of sampling and cryopreservation, viability and quantity of UCB were assessed.* ***Methods.*** *All 145 CB samplings with Biogenis Cord Blood Bank between 2007-2009 of 93 healthy pregnant women and 52 women with pregnancy pathology were investigated in a 3 years retrospective multicenter clinical study. Were analyzed: gestational age at delivery, birth weight, child sex, mode of delivery, pregnancy pathology, umbilical cord and placental pathology. Primary outcomes included umbilical cord blood (UCB) amount, white blood cells (WBC) quantity and viability.* ***Results.*** *Neither gestational age, mode of delivery, child sex, maternal, placental or umbilical pathology affected the WBC quantity. By contrast, cumulative associated maternal pathology negatively affected WBC vitality (p=0.038). The placental pathology significantly influenced both UCB amount and CD34+ concentration. The placental pathology and birthweight are independentely correlated to UCB mass. The unsuccessful collection of UCB was signifficantly correlated with placental pathology (p<0.0001).* ***Conclusions.*** *Data of this study show that perinatal factors are influencing the quality or quantity of UCB. Standard multiple regression models may prove useful for predicting successful UCB mass sampling. In addition, sampling conditions procedures are requisite for pursuing the banking and release of quality UCB for successful transplantation.*
Keywords: *stem cell, umbilical cord blood, placenta, CD 34+ CD 45+, viability*

Introduction

Umbilical cord blood contains both hematopoietic stem cells and pluripotent stem cells. The placenta has an important role as haematopoietic organ. The UCBSC is an alternative for stem cell transplantation, being one of the fields of stem cell research in the stem cell biology and regenerative medicine.

The first evidence of transplantable hematopoietic activity in the placenta can be found in the early transplantation studies by Till, Mc Culloch and Dancis and colleagues[1,2,3].

The new technological advances now permit cryopreservation and banking of UCB with high grade of functional recovery for at least 15 years[4].

Banked UCB may be available as an alternative superior tool to classical bone marrow and peripheral blood for hematopoetic transplant in treatment of various malignant and nonmalignant diseases[5,6]. Moreover, UCB may be a valuable resource of immature cells that can be engineered and used to obtain other advanced cell therapy products, EG for tissue repair, or gene therapy[7,8].

Various factors may be involved in the success of umbilical cord stem cells (UCBSC) sampling and cryopreservation.

In practice, CB preparations are usually collected in maternity wards under variable conditions and circumstances. Screening for bacterial contamination and for transmittable viral infections of the mothers is of crucial importance[9].

However, the characteristics of the mothers and circumstances of delivery it may be of clinical importance for collected CB preparations[10].

Also should be considered as well the potential impact of different processing methods on cellular composition and function of CB[11].

Deficiencies in quality have been found in a multicenter study in a rather high portion of CB samples[12].

Objective

This study was designed to find out whether the maternal, placental or fetal pathology before storage and conditions of sampling might have an impact on the quantity or quality of umbilical cord stem cells (UCBSC) and on the success of sampling and cryopreservation.

Method

Were analyzed all 145 deliveries that agreed with umbilical stem cell sampling to Biogenis Cord Blood Stem Cell Bank including 93 healthy pregnant women and 52 women with pregnancy pathology. The study was carried out in a retrospective multicenter clinical study between 2007-2009 in Clinical Hospital "Dr. I. Cantacuzino" and University Emergency Hospital Bucharest.

The samples were collected having the informed consent of sampling.

Were analyzed the distribution of umbilical cord blood amount (UCB), and flow cytometry was used to determine leukocytes, CD34/CD45+ cells, viability, and white blood cells (WBC) quantity and quality. The results were correlated to gestational age at delivery, birthweight, child sex, way of delivery, pregancy pathology (ie diabetes, hypertension, preeclampsia, oligoamnios, polyhydramnios), placental abnormalities (hematoma, infarctisations, umbilical cord pathology).

Were analyzed also the serological markers for Ag HBs, HCV, HIV, VDRL, TPHA, CMV. The data were provided by courtesy of Biogenis Cord Blood Stem Cell Bank.

Results

The main descriptive characteristics are summarized in table 1.

Regarding gestational age mean UCB amount was 52.2 ml before 37 weeks and slightly increased volume of 65.5 ml above 37 gestational weeks.

Was observed that in all of cases serology was negative, however in about 15 % of cases was noted an inadequate sample.

Neither gestational age, way of delivery, child sex, maternal, placental or umbilical pathology affected the WBC quantity.

By contrast, cumulative associated maternal pathology negatively affected WBC vitality ($p=0.038$). The placental pathology significantly influenced the UCB amount and CD34+ CD45+ concentration ($p<0.05$).

The placental pathology and birthweight are independentely correlated to UCB mass.

The unsuccessful collection of UCB was signifficantly correlated with placental pathology ($p<0.0001$).

When compared cumulative associated pathology, there is a significant difference between groups regarding vitality of UBC ($p=0.03$).

Regarding the placental pathology, the mean UCB amount was 46.5 ml compared to 66 ml, without a significant differences in WBC amount and vitality ($p>0.05$).

No significant differences were observed for viability - mean 96.3% irrespective to the moment and way of delivery, the presence of placental or other isolated parameter.

For assessing the contribution of some important factors on the quantity of UCB mass, a standard multiple regression model was used to assess the independent contribution of each marker to the success of sampling (table 1-5).

A second analysis was done to assess in a standard multiple regression model the unique contribution of five variables on the Umbilical cord blood mass: associated pathology placental pathology, umbilical cord pathology, gestational age, birthweight (table 6, figure 1).

The variables making a statistical significant unique contribution to the equation are represented by placental pathology and birthweight ($p<0.05$).

When comparing the contribution of each independent variable, the largest beta value is for placental pathology (-0.211), which makes the strongest unique contribution to explaining the dependent variable, when the variance explained by all other variables in this model is controlled for. Negative value indicate inverse relationship.

The Beta value for birthweight is slightly lower (0.194), indicating that it made less of a contribution.

Other regression models for analysis of contribution for WBC quantity and WBC quality failed to demonstrate a statistical significant contribution of the other markers. Although, cumulative maternal pathology influenced negatively the vitality of WBC ($p<0.05$).

The low number of 4 diabetic pregnancies did not showed differences either in quality or quantity of UCB, however the low number did not allowed conclusions. Further studies with wider number of diabetic pregnancies should be conducted in order to find out if diabetes has an influence on UCB quality and quantity.

Although umbilical cord pathology itself does not have a statistical significant contribution to this model, it is significantly correlated with the total Umbilical blood amount.

Other variables analyzed like child sex did not show an influence in any way either on the quantity or quality of umbilical stem cells ($p>0.05$).

Discussions

The objective of our study was to assess the influence of various pre-processing conditions on quality of CB donations collected during routine deliveries in an obstetrical unit.

Although in our analysis some markers like gestational age are correlated with UCB amount, birthweight is statistically significant correlated with the amount of UCB sample.

Table 1 Descriptive statistics

	Mean	Minimum	Maximum	Std. Deviation
Gestational age	38.91	31	42	1.448
Birthweight (gr)	3340.41	1400	5350	494.187
Bloodmass total (ml)	125.35	56	237	28.687
WBC quantity/µl	16.620	0	84.0	14.8665
WBC vitality	96.30	0	99	19.002

Table 2 Main characteristics of analyzed cases

Variable	Number	Percent
Delivery - natural	92	63.4
cesarean	53	36.6
Child sex - male	86	59.3
- female	59	40.7
Premature(<37 weeks)	9	6.2
Pathology associated cumulative	52	35.9
Diabetes	2	1.4
Obesity	30	20.7
Anemia	4	2.8
Thrombophylia	4	2.8
Oligoamnios	10	6.9
Hydramnios	2	1.4
Gestational hypertension	10	6.9
Placental pathology	12	8.3
Umbilical cord pathology	16	11
Cultures positive	24	16.6
Ag HBs, HCV, HIV, VDRL, CMV	0	0
Inadequate sample	22	15.2

Table 3 Statistical analysis of UCB amount, WBC quantity and quality, CD34 according to associated pathology. T student test

	Associated pathology N=52	No assoc pathol. N=93	p
UCB amount	62.58±31	65.49±23	0.53
WBC quantity	15.97±13	16.97±15	0.69
WBC vitality	84.79±28	93±10	**0.038**
CD34	69.9±101	32.26±89.5	0.63

Table 4 Statistical analysis of UCB amount, WBC quantity and quality, CD34 according to placental pathology; T student test

	Placental path present N=12	Placental path absent N=133	p
UCB amount	46.50±30	66.07±26	**0.016**
WBC quantity	13.09±16	16.77±14	0.39
WBC vitality	72±43	91.98±14	0.14
CD34	18.5±38	69.23±96	**0.001**

Table 5 Statistical analysis of UCB amount, WBC quantity and quality according to presence of umbilical cord blood pathology

	Umbilical cord path	No umbilical cord pathol.	p
UCB amount	53±30	65±26	0.072
WBC quantity	16.97±17	16.57±14	0.92
WBC vitality	76.13±37	92.09±14	0.11

Table 6 Standard multiple regression for Umbilical cord blood mass

Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.	95% Confidence Interval for B	
		B	Std. Error	Beta			Lower Bound	Upper Bound
1	(Constant)	87.363	65.228		1.339	.183	-41.605	216.331
	associated pathology	5.423	5.598	.091	.969	.334	-5.645	16.490
	Placental pathology	-21.856	9.249	**-.211**	-2.363	**.020**	-40.143	-3.568
	Umbilical cord path	-14.224	7.931	-.156	-1.794	.075	-29.904	1.456
	gestational age	.046	1.841	.002	.025	.980	-3.594	3.687
	Birthweight	.011	.005	.194	2.118	.036	.001	.022

a. Dependent Variable: Umbilical Blood mass *SPSS 15.0*

Thus prematurity is an important predictive factor for success of UCB sampling, having mainly a negative impact on the required minimal UCB amount. However the study results are limited by the small number of premature deliveries.

Although the gestational age at delivery did not correlate signifficantly in our study to CD34+ cell concentration, however other studies showed a decline of frequency of CD34+ cells in fetal blood from 17 to 41 weeks' gestation. This decline occurs during the transition from hepatic to bone marrow hematopoiesis. Therefore, early fetal blood, with a higher circulating frequency of progenitor/stem cells and proliferative capacity, probably may be a preferable target for gene therapy[13,14,15].

The influence of birthweight on the quality of UCB confirms the results of other studies: bigger babies had higher cell counts, more CD34+ cells, and more CFU-GM. Specifically, each 500 g increase in birth weight contributed to a 28% increase in CD34+ cell counts, each week of gestation contributed to a 9% decrease in CD34+cell counts[16,17,18].

At present the role of UCBSC in the treatment of various hematological and nonhematological diseases is analized in more than 300 clinical trials, that are currently listed on National Institute of Health Clinical Trials website. http://www.clinicaltrials.gov/

In a recent study assessing the quality parameters for Cord Blood Donations, concerning the conditions of

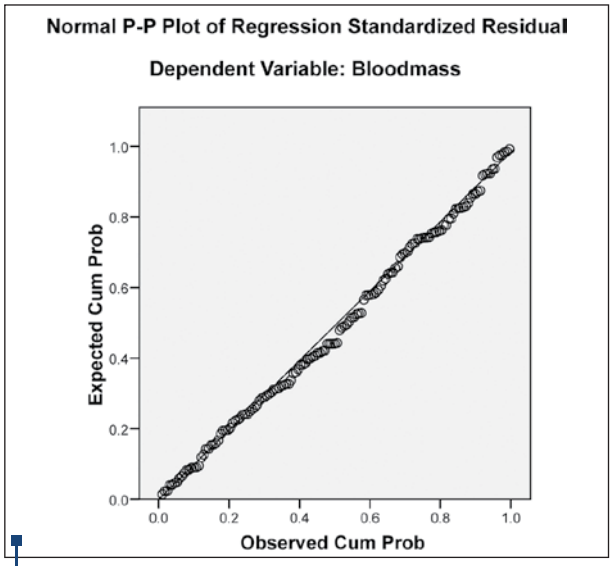

Figure 1

sampling, Ursula et al demonstrated that neither plasma citrate concentrations nor storage temperature (within standard 24h) affected cell viability or colony formation. In addition after storage for 49-80 h, leukocyte viability declined by about 16% compared to CB stored up to 24h. In contrast, the clonogenic activity and CD34/CD45+ cell content were not affected. A higher gestational age was

associated with a lower yield of clonogenic activity compared to midterm deliveries. Other parameters like number of red blood cells (NRBC) varied widely (median 7.3%; range 0.63-17.3%) without relation to gestational age or colony formation. There was a close correlation between the percentage of viable CD34/CD45+ cells and colony formation (r = 0.77 for CFU-GM; r = 0.75 for CFU-C).

Therefore, in this study the authors stated that the content of viable CD34/CD45+ cells represents the clonogenic activity of CB preparations, and thus the determination of viable CD34/CD45+ cells should be generally performed as a routine quality control assay[19].

Sterility testing in our study showed a higher positive cultures before further processing at about 16%, being much higher than literature 2.4%[19] or 6.4%[20].

These results emphasised that sampling conditions are important independent factors influencing the success of UCBSC sampling.

In a study of Cairo et al The Cord Blood Transplantation (COBLT) Study conducted on 8000 CB units, were correlated the hematopoietic progenitor cell (HPC) and lymphocyte subset (LS or WBC), with donor ethnicity, birth weight, gestational age, sex, and type of delivery. The results showed a significant correlation of CD34+ cell count with colony-forming unit (CFU)-granulocyte-macrophage, CFU-granulocyte-erythroid-macrophage-megakaryocyte, burst-forming unit-erythroid, and total CFUs. Male sex was associated with significantly fewer CD3+/CD4+, CD19+, and CD16+/CD56+ but increased CD3+/CD8+ LSs ($p<0.001$)[21].

The results of other studies are inconsistent regarding the influence of infant sex on the concentrations of CD 34+ cells. The male sex infants had significantly higher median CD34+ cell concentrations than female infants (31.8/microL vs. 30.2/microL, respectively; p = 0.03). Although the disparity in absolute concentrations was small, it was 5.3 percent. In multivariate linear regression analysis, the positive influence of male sex on the CD34+ cell concentration was significant ($p < 0.05$). Thus cord blood hematopoietic progenitor cell concentration was higher in male infants, even after correcting for birth weight.

However in our study there is no significant influence of infant sex either on quantity or quality of UCB, suggesting a variety in distribution of results in various studies.

Regarding the mode of delivery, there are inconsistent data in literature[22]. There are other studies were cesarean section was associated with significantly higher total CFU and CD16+/CD56+ but lower CD3+/CD4+, CD3+/CD8+, and CD19+ LSs[21,23]. However, our data showed that the way of delivery does not seem to affect either the quality or quantity of the cord blood content in CD34(+) cells.

Conclusions

Our data suggest significant statistical influence of placental pathology, birthweight on the UCBSC. Stress-related perinatal factors represented by maternal associated pathology, particularly are associated with CB unit and may improve unit selection. Multiple linear regression models may prove useful for predicting success of UCB sampling.

Mode of delivery did not affect model choice in our study.

Sampling conditions and transport are important independent factors that play a key role in sterility of samples influencing the success of UCB sampling.

UCB banks should emphasize selecting the heaviest infants and processing large-volume units with high WBCs to optimize hematopoietic potential.

These findings may be of relevance for adjusting the sampling technique ensuring successful sampling. ■

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