The relationship between selected labor-associated factors and the number of immature nucleated blood cells, including stem cells, in umbilical cord blood

MARTA KOSTRZEWA[1], JAROSŁAW KALINKA[1], WOJCIECH KAZIMIERAK[2]

Abstract

Umbilical cord blood collected during delivery contains stem and nucleated cells of the blood. In clinical practice these cells are increasingly often used for allogeneic graft, for example for older sibling suffering from leukemia. However, the number of nucleated and stem cells obtain from umbilical blood after delivery is not always sufficient for transplantation. *Objective:* The purpose of this study was to evaluate whether selected labor-associated factors such as: the mode of delivery, parity, duration of first stage of labor and pH of umbilical blood have an impact on the hematopoietic content of umbilical blood. *Material and method:* 30 smears of venous umbilical blood taken after delivery has been made and analyzed under the microscope. Mononuclear cells per 300 white blood cells such as: nucleated red blood cells(NRBC) and pool of unidentified Young Cells (YC), which contains immature hematopoietic and stem cells were counted. Samples were divided into two groups: smears obtained after spontaneous vaginal delivery (I group) and after elective caesarian section (II group). Only pregnant women with uncomplicated pregnancies were enrolled in the study. *Results:* There was no correlations between mode of delivery and number of nucleated cells. There was a positive correlation between parity and medium number of NRBC and YC in nulliparas ($p < 0.05$) only from group I, while in group II, the number of NRBC and YC was significantly higher in multiparas. Higher number of NRBC ($r = -0.76$) and YC ($r = -0.51$) were observed in samples obtained from children born with lower umbilical pH. *Conclusions:* Out of the analyzed factor only lower umbilical pH after the delivery correlates with higher number of nucleated hematopoietic cells in umbilical cord blood. The results of this study suggest that in order to obtain more valuable umbilical blood we should focus on its appropriate uptake after the delivery.

Key words: umbilical cord blood, stem cells, nucleated cells, pH of umbilical blood

Introduction

Nowadays collecting umbilical cord blood during delivery as important source of stem cells for children became relatively popular procedure. The interest of cord blood ensues from high content of young immature hematopoietic cells, including hematopoietic stem cells (HSCs). HSCs are the basis of the adult hematopoietic hierarchy that produces all the blood lineages throughout adult life. The concentration of hematopoietic stem cells in umbilical cord blood is approximately 10 times higher, compared to postnatal peripheral blood [1]. This fact has therefore became the trigger for making a transplantation of umbilical cord blood cells, obtained after the delivery, as a source of bone marrow repopulating stem cells. In primary conception of this procedure umbilical cord blood cells were supposed to be used for an autologic transplant. In clinical practice it is increasingly often used for allogeneic graft, for example for older sibling suffering from leukemia [2-4].

It is reported that to obtain a successful bone marrow transplant 200-400 million nucleated cells per kilogram of recipient weight is required, and the content of stem cells CD34+ as to exceed 1 million per kilogram of body mass of the recipient [1]. The mean single sample volume of umbilical cord blood, collected during routine work in obstetric ward, is 100 ml (ranges between 40-120 ml) and contains 47 million/ml nucleated cells [5]. The concentration of CD34+ cells in umbilical blood is 0,1-0,4% [1].

Taking everything into consideration the number of nucleated and stem cells from umbilical blood obtained after delivery contains enough hematopoietic stem cells to achieve transplant only for 30 kilograms weighing child [1-4].

The proportion of circulating white blood cells and hematopoietic progenitor cells is relatively constant during pregnancy [6]. During delivery, the amount of nu-

[1] Department of Perinatology, I[st] Chair of Gynecology and Obstetrics, Medical University of Łódź

[2] Department of High Risk Pregnancy, I[st] Chair of Gynecology and Obstetrics, Medical University of Łódź

cleated cells, CD34+ cells, and hematopoietic progenitor cells in umbilical cord blood changes [7]. These changes may be partially due to activation of cytokines during normal delivery [8-11]. It is known that several cytokines stimulate migration of progenitor cells into the peripheral circulation [12]. This process also causes the increase of the number of immature nucleated and stem cells in umbilical blood.

The purpose of this study is to estimate whether selected factors related to labor and delivery like: mode of the delivery, parity, duration of first stage of labor, and pH of umbilical blood have an impact on number of nucleated hematopoietic cells in umbilical cord blood.

Material and method

The study was performed in Department of Perinatology, 1st Chair of Gynecology and Obstetrics, Medical University of Łódź. The pregnant women with uncomplicated pregnancies were enrolled after giving informed consent. The research received an agreement from Bioethical Committee in Medical University of Łódź number RNN/18/10/KB. Umbilical cord blood has been collected after delivery, from the fragment of clamped umbilical cord. To analyze umbilical cord blood 0.1 ml of venous cord blood was obtained. Subsequently, smears of 0.025 ml of blood with the use of hematimeter were made. The smears were dried in room temperature, stained, perpetuated using May-Grunwald's and Giemza's method. Prepared blood smears were analyzed under the microscope using immersial oil and 1000 × zoom. The rest of umbilical blood was tapped to made acid-base balance with the use of Roche Cobas B221 analyzer.

Nucleated cells were counted in 2/3 of length of smear, in sinuous movements. Mononuclear cells per 300 white blood cells (WBC) such as: nucleated red blood cells (NRBC) and pool of unidentified Young Cells (YC), which contains immature hematopoietic and stem cells were counted. CD 34+ cells and hematopoietic cells can be clearly identified only by flow cytometry, with the use of monoclonal antibodies anti-CD34+ [16].

Samples of blood were divided into two groups: 14 smears obtained from spontaneous vaginal delivery (I group) and 16 from elective caesarian section (II group). Parameters taken into consideration were: mode of the delivery, parity, duration of first stage of spontaneous labor and acid-base balance parameters from umbilical blood.

Pregnant women with hypertension, diabetes, perinatal infections, IUGR, pPROM were excluded from the survey. Pregnant women who underwent caesarian section, were qualified due to following indications: state after caesarian section, ophthalmic and orthopedic indications and abnormal fetal presentation.

Statistical evaluation included the Pearson correlation coefficient for evaluation of the relationship between maternal and fetal variables and umbilical cord blood parameters, whereas for the assessment of significant differences between groups *t* test was used. Appointed statistical significance was $p < 0.05$.

Results

The both examined group were similar with respect to maternal and gestational age at the delivery. In first group most of pregnant women were multiparas in contrary to second group. Every newborn has 10 Apgar score and the neonatal birth weight was comparable. Pregnancy and delivery characteristics are shown in Table 1.

Table 1. Pregnancy and delivery characteristics of 30 cases

	Group I Spontaneus delivery (mean, range)	Group II Caesarian section (mean, range)
Number	14	16
Gestational age	39 (37-40)	38 (38-39)
Maternal age	30 (23-37)	31 (24-38)
Parity	2 (1-2)	1 (1-2)
Primigravidas	5 35%	9 56%
Multiparas	9 65%	7 44%
Apgar score	10	10
Neonatal birth weight (g)	3535 (2750-4000)	3550 (2400-4050)
Placental weight (g)		
Primigravidas	648 (500-680)	586 (400-710)
Multigravidas	666 (520-780)	622 (450-750)

Table 2. Relationship between mode of delivery and hematopoietic content of umbilical cord blood

		Neutrophils/300WBC (%)		Limfocytes /300WBC (%)		NRBC/300WBC		Young Cells/300WBC	
	No	Mean ± SD	Statistical significance	Mean± SD	Statistical significance	Mean ± SD	Statistical significance	Mean ± SD	Statistical significance
Group I	14	64.1 ± 8.26	$P > 0.05$	35.0 ± 8.0	$P > 0.05$	11.5 ± 6.31	$P > 0.05$	10.03 ± 9.82	$P > 0.05$
Group II	16	66.8 ± 5.19		31.9 ± 5.4		15.0 ± 15.47		6.8 ± 4.95	

Table 3. Relationship between parity and hematopoietic content of umbilical cord blood in both groups

			NRBC/300WBC		Young Cells/ 300WBC	
		No.	Mean ± SD	Statistical significance	Mean ± SD	Statistical significance
Group I	Primigravidas	5	13.7 ± 2.5	$P = 0.02$	13.97 ± 12.3	$P = 0.03$
	Multigravidas	9	7.72 ± 6.4		7.8 ± 6.8	
Group II	Primigravidas	9	8.57 ± 4.1	$P = 0.037$	4.34 ± 2.3	$P = 0.029$
	Multigravidas	7	23.47 ± 2.0		8.52 ± 5.1	

Table 4. Correlations between pH of umbilical blood and duration of first stage of labor and hematopoietic content of umbilical cord blood

			NRBC/ 300WBC		Young Cells/300WBC		Neutrophils /300WBC	
	No.	Mean ± SD	Mean ± SD	r	Mean ± SD	r	Mean ± SD	r
pH of umbilical venous blood	30	7.35 ± 0.074	10.87 ± 10.88	−0.76	7.11 ± 5.13	−0.51	190.4 ± 13.4	−0.48
Duration of I stage of labor (min)	14	217.2 ± 48.6	11.58 ± 6.31	−0.074	10.03 ± 9.82	−0.02	192.3 ± 22.6	−0.03

Indications for caesarian section were as follows: state after caesarian section in 56% (9/16), breech presentation in 25% (4/16), ophthalmic indications in 12.5% (2/16) and orthopedic indications occurred in 6.25% (1/16). Duration of first stage of labor and the pH of venous umbilical blood ranges in physiological values in both groups.

The results of the analysis of the cellular content of umbilical blood smears in group I and group II are presented in Table 2. There was no significant correlations between the mode of delivery and the content of neutrophils, leukocytes, nucleated red blood cells and number of young cells in umbilical cord blood smears.

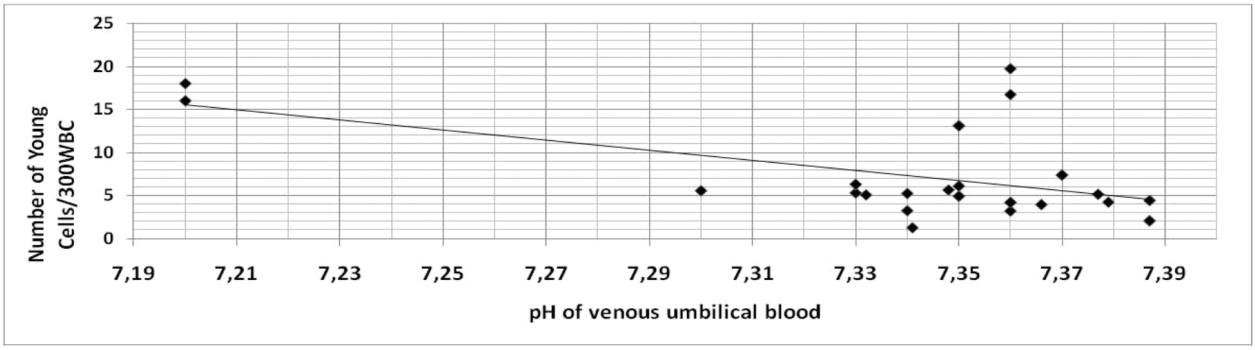

Fig. 1. Correlation between pH of umbilical blood and the number of Young Cells (YC)

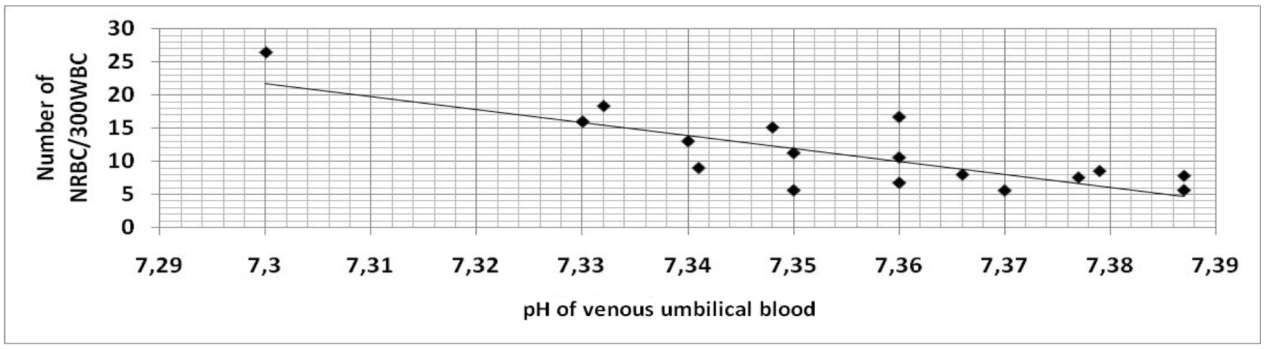

Fig. 2. Correlation between pH of umbilical blood and the number of NRBCs

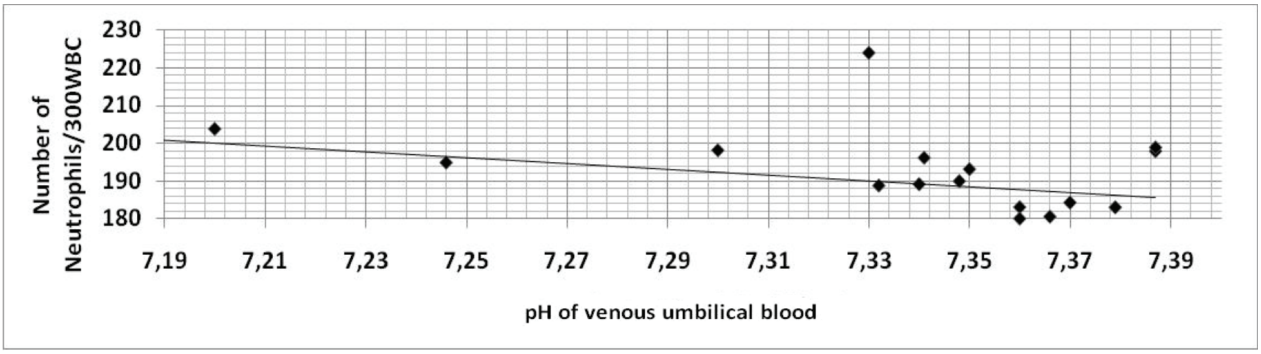

Fig. 3. Correlation between pH of umbilical blood and the number of netrophils

The analysis of the influence of the parity on the hematopoietic content of blood smears revealed higher number of young cells ($p = 0.03$) and nucleated red blood cells ($p = 0.02$) in primigravidas belonging to I group. Contrary, in group II there was significant correlations between parity and number of young cells ($p = 0.029$) and also nucleated red blood cells ($p = 0.037$) but only in multigravidas (Table 3).

The umbilical venous pH showed significant negative correlation with number of young cells ($r = -0.51$), nucleated red blood cells ($r = -0.76$) and neutrophils ($r = -0.48$) with a decrease in venous pH (Table 4). (Figure 1, 2 and 3).

As shown in Table 4 significant correlation between the duration of the first stage of labor and the number of young cells and nucleated red blood cells in umbilical cord blood smears was not observed.

Discussion

For the purpose of this study we decided to analyze only uncomplicated pregnancies and deliveries to verify whether the mode of delivery has any impact on the content of mononuclear cells in umbilical cord blood. It has been shown previously that umbilical blood of newborns from either spontaneous vaginal delivery or from emergency caesarian section contains higher levels of oxidation products than blood of newborns delivered by elective caesarian section [14, 15]. As the oxidative stress relates to the amount of released cytokines, so it is probably the reason for the higher number of immature nucleated cells in found in the umbilical blood in these cases. Though caesarian section performed in uncomplicated pregnancy could constitute strong stressogenic factor for fetus our results did not revealed the significant differences in the content of immature nucleated cells in umbilical blood as compare between group of cesarean section and vaginal delivery. This results suggests that the mode of delivery is not strong factor influencing the content of immature mononuclear hematopoietic cells in umbilical cord blood, as far as a healthy newborns are concern.

During prenatal period the relatively constant concentration of stem cells and hematopoietic progenitors in fetal circulating blood is demonstrated in literature [6]. It is also known, that the number of those cells increases during spontaneous labor. Lim et al. revealed that the increase of mononuclear hematopoietic cells and stem cells correlates with prolonged duration of first stage of labor [14]. The proposed reason of described phenomenon is that biochemical reactions mobilize ma-

ternal and fetal organism to make an enormous effort during labor. The most important factors influencing the increase concentration of hematopoietic immature cells and stem cells in umbilical cord blood are cytokines and hematopoietic growth factors released during labor [8-12, 13]. As it was said, the concentration of mononuclear cells and stem cells increase with prolonged duration of the labor. No significant correlation between duration of first stage of labor and number of nucleated cells in umbilical blood was not revealed in our study. This is probably because of the relatively short duration (mean 217 min) and physiologic course of the first stage of labor among analyzed women.

According to some studies nulliparity is consider as a factor influencing concentration of immature hematopoietic cells and stem cells in umbilical cord blood [13]. In our research such correlation was observed only in first group, where umbilical blood was drawn from newborns born by spontaneous delivery. This correlation is supposed to be an effect of a longer first stage of labor observed in primigravidas in comparison to multigravidas. Opposite results were found in our study in group II. Significant correlation between higher number of young cells, NRBCs and multiparity was revealed in pregnant women who underwent elective caesarian section. This fact may be explain by size and activity of the placenta. Placenta is supposed to be a source of hematopoiesis during prenatal period. HSCs are found in highly vascularised tissues of placenta. The human placenta contains a large variety of hematopoietic progenitors and HSCs. However, it is yet undetermined whether the placenta have the ability to generate HSCs [21, 22]. Providing that the amount of immature hematopoietic cells and HSCs is dependent from the size of vascularized placental tissue, the correlation between mass of placenta and the number of HSCs can be hypothesized. In our study average mass of placenta was higher in multiparas in group II. (Table 1). This fact may be the subject for future study.

Acid-base parameters of umbilical venous blood is thought to be objective exponent of stressogenic factors activity during labor, because as evidenced in the literature, the subjective interpretation of cardiotocography is less meaningful than the objective measurement of the acid-base status of the umbilical cord blood directly after birth [17]. For purpose of this study we performed venous umbilical acid-base analysis, because as it was demonstrated, low venous pH more than a low arterial pH indicates increased fetal stress of longer duration. Increased fetal stress occurring in the last 15 minutes before birth may result in a decrease of the arterial pH, but this period may be too short to induce mobilization of stem cells [13]. In our study negative correlation between number of young cells; including stem cells; nucleated red blood cells, neutrophils and venous umbilical blood pH was observed. Lower pH correlates to hypoxia of the fetus. Hypoxia induces cytokines and hematopoietic progenitors which mobilize bone marrow to produce stem cells and triggers hematopoiesis. This process results in the increase concentration of immature hematopoietic and also stem cells along with decrease of umbilical blood pH. This correlation was revealed in our study, although it should be stressed that the pH of umbilical blood ranges only the physiological values. Increased concentration of nucleated red blood cells in umbilical cord blood correlates with decrease of pH in cord blood or even in peripheral neonatal blood [18]. Our study revealed those correlation as the significant $r = -0.76$.

Mobilization of CD34+ cells is usually accompanied by leukocytosis. Neutrophils appear to be mediators of progenitor cell mobilization from the bone marrow, because IL-8-induced rapid mobilization of stem cells has been found to be dependent on the presence of neutrophils [19, 20]. Our finding suggest that lower pH of venous cord blood correlates with the number of neutrophils. Similar results in literature indicate that neutrophils may be concerned to be most important source of cytokines [13].

There are several methods to harvest and collect umbilical cord blood described in literature. In standard method, umbilical blood is drawn by continous intravenous line inserted to umbilical vein, resulting in 40-100 ml volume of harvested blood. However, Elchalal et al. show different methods, which conduct to harvest umbilical cord blood, where the collected volume of umbilical blood ranges between 60-273 ml. In these methods syringe is used to perform a sodium chloride solution flush and drain, which included withdrawal of cord blood by a syringe until the delivery of the placenta. It is followed by flushing through a catheter one of the umbilical arteries with sodium chloride solution and collection of the cord blood either into an open sterile container (method 1) or into a standard donation blood bag (method 2) [23]. New method as it is evidenced is literature doubles the total white blood cells collected with respect to current yields, which may make cord blood transplantation applicable for adults. However, there are serious disadvantages of these new methods. Firstly, it is dilution with sodium chloride of collected cord blood and secondly it is higher risk of bacterial contamination.

Our study revealed that analyzed factors, except pH of umbilical blood, do not have any significant influence on the concentration of immature nucleated hematopoietic cells in umbilical blood. Moreover, we should not expect worse quality of cord blood with lower pH. The results of this study suggest that in order to obtain more valuable umbilical blood we should focus on its appropriate uptake after the delivery.

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✉ Marta Kostrzewa

Department of Perinatology
I[st] Chair of Gynaecology and Obstetrics
Medical University of Łódź
ul. Wileńska 37, 94-029 Łódź
e-mail: j.kalinka@csk.am.lodz.pl