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Quantitative changes of NK cells in umbilical cord blood of neonate in relation to the mode of delivery

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Introduction: *Natural killer (NK) cells are an important element connecting innate and acquired resistance in humans. They can be detected in fetal liver since the 6th week of pregnancy and in the second half of pregnancy the number increases to values observed in adults.*

The main aim of the study was the assessment of the NK cells and defining a relationship between the mode of delivery and NK cells quantitative changes in umbilical cord blood.

Material and methods: *The study included 72 neonates. Taking into consideration the time and mode of delivery, the children were divided into following groups. Group I included 40 full term neonates: 17 neonates born vaginally and 23 by cesarean section. Group II included 32 near-term neonates: 6 neonates born vaginally, 26 by cesarean section. Assessment of NK cells was performed with a flow cytometry technique.*

Results: *It was shown that the percentage NK cells' of in all full term neonates was statistically higher than in near-term ones. A higher mean percentage of those cells was also shown in full term and near-term neonates born by cesarean section. The mean number of NK cells lymphocytes in full term neonates born by elective cesarean section was statistically significantly lower than in neonates born by emergency cesarean section.*

Conclusion: *Cesarean section can be related to significant quantitative changes in NK cells in the umbilical cord blood. Near-term neonates, regardless of the mode of delivery, show lower values of NK cells in umbilical blood. Elective cesarean section carried out at term can be a cause of decrease of mean number of NK cells in neonate umbilical blood.*

Key words: *cord blood, lymphocytes, NK cells, caesarean section.*

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Introduction

Neonate immune system at the moment of birth is defended by non-specific immunological mechanisms and mother's antibodies provided by the placenta. The non-specific immunity (innate) programmed in advance, existing without previous contact with infectious factor till the moment when the immune system gets immunological experience, predominates in neonates. It is when the congenital immunological mechanisms get reinforced by specific (acquired) immunological response [1]. Natural cytotoxic cells NK (natural killers) are the important element combining processes of innate and acquired immunity, also by contact with dendritic cells. They are probably formed in bone marrow from $CD34^+$ progenitor cells with the participation of stem cell factor SCF, tyrosine kinase FLT3 and interleukin 2 (IL-2) and IL-15. They are detected in fetal liver in about 6th week of pregnancy, and in the second half of pregnancy their number increases to the values observed in adults [1, 2]. NK cells are described as the big granular

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lymphocytes which perform cytotoxic and immunoregulatory functions [2, 3]. NK cells are capable of killing target cells, including neoplastic and virus-infected cells [4]. The common feature for all NK cells is a lack of CD3 molecule, which distinguishes them from T $CD3^+$ lymphocytes. NK cells show CD56 and CD16 expression. Expression of those molecules is diversified depending on a function [2]. Defining NK cells phenotype allowed proving lower percentage in neonate blood in comparison to older children and adults. Special role of those cells depends on body defense against various microorganisms. It is also related to their ability to secrete pro-inflammatory cytokins, such as interferon γ (IFN-γ), tumour necrosis factor α (TNF-α) and factor stimulating formation of granulocytes and macrophage colony (GM-CSF) [5]. Proinflammatory and cytotoxic response is balanced by suppressor mechanisms, expression of autospecific receptors inhibiting anergy and modification on the surface of MHC cells of suppressing specific receptors [6]. Relation between low activity of NK cells, lowered cytotoxicity and reduced IFN production, can be one of the factors responsible for high proneness of neonates to infections and tendency to infection generalization [7]. The main aim of the study was a quantitative assessment of the percentage and number of NK cells and finding out if there is a relation between mode and time of delivery and quantitative changes of NK cells.

Material and methods

The study included 72 healthy neonates born in the years 1998-2003 in the Department of Perinatology and Gynecology in Zabrze Medical University of Silesia in Katowice. Taking into consideration pregnancy duration and mode of delivery, the children were divided into the following groups:

- Group I – 40 full term neonates, including:
  - I a – 17 neonates born vaginally,
  - I b – 23 neonates born to cesarean section, including:
    - I be – 15 with elective indications,
    - I bn – 8 with emergency indications;
- Group II – 32 near term neonates, including:
  - II a – 6 neonates born vaginally,
  - II b – 26 neonates born to cesarean section, including:
    - II be – 18 with elective indications,
    - II bn – 8 with emergency indications.

Description of the study group is presented in Table 1.

Assessment of neonate maturity was done in the first 12 hours of life with Ballard's scale, taking into account morphological and neurological criteria [8] and percentile grids worked out for Silesian neonates [9]. Neonates of body mass between 10^{th} and 90^{th} percentile were considered as appropriate for gestational age. Neonates included into the study fulfilled the following criteria:

- appropriate for gestational age neonates,
- neonates from pregnancies without any pathology,
- full term neonates born to elective cesarean section (status post cesarean section, status post infertility treatment, ophthalmologic indications),
- full term neonates born to emergency cesarean section (placental detachment, abnormal cardiogram record, imminent intrauterine fetal asphixia),
- near term neonates born to elective cesarean section (status post cesarean section, status post infertility treatment, ophthalmologic indications),
- near term neonates born to emergency cesarean section (placental detachment, abnormal cardiogram record, prolapse of the umbilical cord or part of the fetus).

The study excluded the neonates:

- with abnormal body mass in relation to fetal age (< 10 or > 90 percentile),
- with innate developmental anomaly,
- from mothers with chronic diseases, such as endocrinological system diseases, diabetes, hypertension, chronic inflammatory conditions, obesity,
- from mothers with HIV, HBV, HCV viruses,
- from teenage mothers (< 16 years of age),
- from mothers abusing alcohol and smoking cigarettes.

Immunological tests included single evaluation of the percentage and number of NK cells of $CD56^+/CD16^+$ phenotype in umbilical cord blood. The method of umbilical blood staining with sequential red blood cell lysis was applied in the study. 1 ml of umbilical blood was taken and was analyzed within 4 hours after sampling. Blood was incubated for 30 min with respective monoclonal antibodies from Becton Dickinson Company coupled with fluorochromes: fluorescein isothiocyanate (FITC), phycoerythrin (PE) and PerCP, and for the next 10 min with lysing solution FAClysis (Becton Dickinson) in order to eliminate erythrocytes. After rinsing two times with PBS buffer, stained cells were entered into flow cytometer FACScan (Becton Dickinson) and 10 000 cells were recorded. Analysis of morphological parameters and fluorescence

Table 1. Characteristics of studied newborns' groups

Group	*N*	Mass weight (g)			Week of pregnancy			Apgar score (5^{th} minute)	
		mean	± SD	range	mean	± SD	range	< 7	≥ 7
I	40	3213.13	429.08	2230-4320	39.23	1.158	38-42	3	37
II	32	2286.88	414.563	1800-3000	35.53	1.722	33-37	3	29

of the cells was done with Cell Quest software (Becton Dickinson).

Statistical analysis

Clinical data and the results of immunological tests were analyzed by Statistica software, version 6.0. After defining a variable distribution with the Kolmogorow-Smirnov test, *t*-Student test was applied for variables with normal distribution, however, for variables with distribution different than normal, U Mann-Whitney test was used. The results are presented in the form of mean value and standard deviation.

The level of significance was assumed to be $p < 0.05$.

Results

The analysis of studied immunological parameters in umbilical cord blood was performed in relation to the time of delivery. Full term and near term neonates were compared. It was shown that NK cells percentage in the full term neonate was 20.18 ±10.23% and it was significantly statistically higher ($p = 0.03$) in comparison with a mean value (14.69 ±10.10%) in near term neonates (Fig. 1A). No statistically significant differences were observed between mean values of NK cells in the both groups. On the other hand, mean percentage of NK cells in the full term neonates born to cesarean section was 19.43 ±7.70% and it was sig-

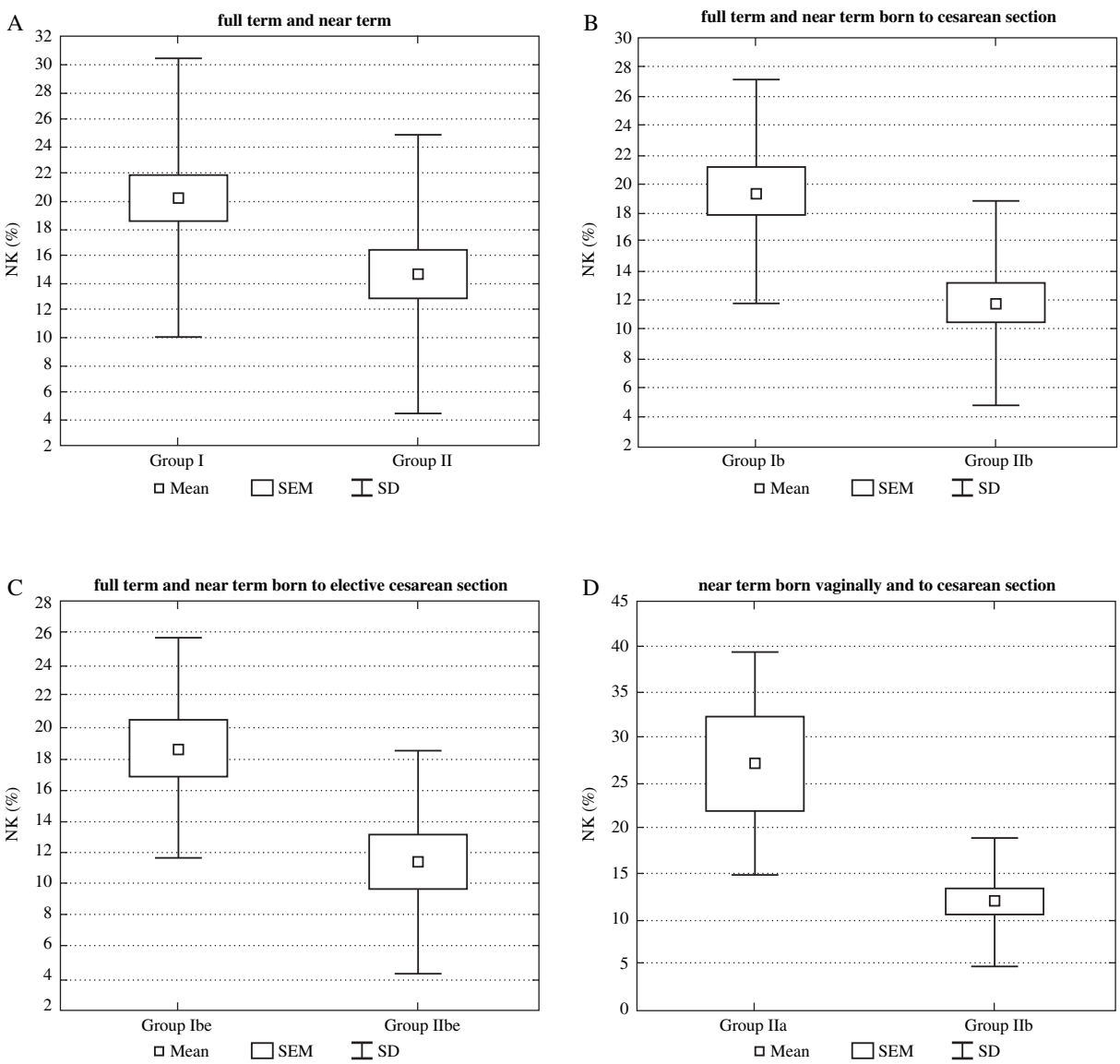

Fig. 1. Comparison of mean percentages of NK cells in the umbilical cord blood of neonates

nificantly statistically higher ($p = 0.0007$) than mean value (11.81 ±7.07%) received for near term neonates born to cesarean section (Fig. 1B). Mean percentage of NK cells (18.73 ±7.03%) in the group of neonates born at term by elective cesarean section was significantly statistically higher ($p = 0.006$) than the mean value (11.39 ±7.06%) found in the group of near-term born neonates (Fig. 1C). In addition, values of NK cells in the blood of full term neonates were analyzed depending on the indication to surgical delivery. It was found that mean number of NK cells (0.96 ±0.41 G/l) in neonates born to elective cesarean section was significantly statistically lower ($p = 0.002$) in comparison to the mean number (1.78 ±0.69 G/l) in neonates born to emergency cesarean section. Comparison of mean numbers and percentages of NK cells in the group of full term neonates born vaginally or to cesarean section did not show any significant differences. The percentage of NK cells in the group of near-term neonates born vaginally reached the mean value 27.17 ±12.38% and was significantly statistically higher ($p = 0.0003$) in comparison with the mean value (11.81 ±7.07%) in the group of near-term neonates born to cesarean section (Fig. 1D).

Discussion

NK cells are considered to be a vital element of innate immunity because they do not require specific gene stimulation and present the first defense line in infectious processes. In adults, a lower number of those cells in peripheral venous blood can reflect an excessive activation and intensive flow of NK cells into inflammatory tissues. In healthy neonates, the percentage of those cells is lower than in children and adults. Comans-Bitter *et al*. [10] working on reference values of various lymphocyte subpopulations for children and adults showed that in healthy neonates, percentage of cells in umbilical blood $CD3^-/CD16^+CD56^+$ fluctuates in the wide range from 6 to 50%, mean value is 20% and it is higher in adults (13%). Han *et al.* [11] showed that NK cells in umbilical blood do not have a receptor p55 for IL-2, which is of importance in the course of various inflammatory processes. Sanch *et al.* [12] suggested considering two forms of neonate NK cells differing in response to IL-2. The authors showed that population of natural cytotoxic cells go through two successive stages of maturation and as a result two cellular lines of different activity get formed. One has considerably lowered activity in comparison to NK cells in adults, while the other has properties similar to the observed in people with mature immunological system. Number of NK cells, both under the influence of infection and certain non-infectious risk factors, mainly hypoxia, undergo dynamic changes [13]. Neonates with infections, regardless to fetal age, had lower percentage of NK cells, it concerns both term and premature neonates. A lower number of those cells can confirm using up those cells in a battle against infection and prove a suppressor influence of a shock on the number of NK cells. Presently, el-Sameea *et al.* [14] believe that marking NK cells cytotoxicity is the important diagnostic marker of early infection, more sensitive and specific than marked protein C-reactive and IL-8. McDonald *et al.* [15] showed a correlation of NK cells number with fetal age finding that preterm babies have less NK cells showing lower activity and lower production of cytokines. In contrast, Kotiranta – Ainamo *et al.* [16] showed that the percentage of NK cells do not depend on neonate fetal age, infection occurrence, pregnancy complications or cesarean section, but on the applied betamethasone in mothers at risk of premature delivery. The studies showed that time and mode of delivery significantly influence the percentage and number of NK cells in umbilical blood of healthy neonates. It was found that in healthy full term neonates, a mean percentage of NK cells in comparison to near term neonates was significantly statistically higher. Similar results have been received by Mazur *et al.* [17] who found the highest values of NK cells in the term neonates born vaginally. In contrast, Georgeson *et al.* [18] evaluating cytotoxicity of NK cells in healthy full-term and pre-term neonates found that prematurity was related to considerable decrease of activity of natural cytotoxic cells. According to Behrendt *et al.* [19], the full term neonates with mild course of infection both general and one system without bacteria have higher percentage of NK cells than healthy neonates. It can be suspected that a prognosis to curing a full-term neonate from early severe infection depends also on the number of NK cells. Defensive effect of NK cells against infections depends on fast and intensive production of IFN-γ that takes part in intracellular killing of bacteria and destroying pathogens coated in antibodies via the process called cytotoxicity of antibody dependent cell immunity. NK cells after activation and starting specific immunological mechanisms are eliminated via apoptosis to prevent an excessive production of IFN-γ which can provoke uncontrolled activation of other immunological cells and release of excessive number of inflammatory cytokines [20]. It was also shown that the mode of delivery significantly influences the increase of mean number of NK cells, especially in neonates born at term by emergency cesarean section. Behrendt *et al.* [19] did not find significant quantitative changes with relation to the NK cell number in peripheral venous blood in neonates born to cesarean section, regardless to indications in comparison to neonates delivered vaginally. They showed higher value of mean percentage of NK cells in neonates born to emergency cesarean section with infectious risk factors. It can be explained by various indications for operation, different types of anesthesia, and also a long duration of delivery. Mazur *et al.* [17] also assessed risk factors resulting from pregnancy course and labour and their influence on the percentage and number of NK cells in umbilical blood. They showed the lowest values of percentage and number of NK cells in pre term neonates born to cesarean section. The

results of own studies indicate a presence of certain physiological variability of NK cells in umbilical blood in the first days of life of full term neonates. Mode and time of delivery are of vital importance for distribution of those cells.

Umbilical cord blood can be an excellent source for screening studies, especially, in cases of suspected immunological system disorders or for monitoring the cases of suspected intrauterine infection. Recognizing potential factors increasing impairment of immunological system is a particularly important issue. World Health Organization reports that 7.1 million neonates between 1 and 12 month of life die of infections caused by pathogenic micro-organisms every year in the world [7]. Evaluation of immunological system parameters in those groups of neonates and selecting those specially in danger of infections is well justified and enables monitoring its parameters and possibly correcting those disorders. Many authors suggest that immunological diagnosing with relation to lymphocyte phenotyping is supplementary for serologic and microbiologic diagnosing in patients with infections.

Conclusions

1. Cesarean section can be related to significant changes in quantities of NK cells in umbilical blood.
2. Near term neonates, regardless to the mode of delivery, show lower values of NK cells in umbilical blood.
3. Emergency cesarean section done at term can be a reason for increasing the mean number of NK cells in neonate umbilical blood.

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