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# Risk factors of bronchopulmonary dysplasia development in neonates with very low and extremely low birth weight

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***The study was aimed at*** *investigating the perinatal factors and levels of pro- (IL1α, IL6) and anti-inflammatory (IL1RA, IL10) cytokines in order to clarify their role in immune pathogenesis and prediction of bronchopulmonary dysplasia (BPD).* ***Patients and methods:*** *the prospective study involved 194 neonates with birth weight of 600-1,500 g and gestational age of 25-32 weeks. The primary perinatal risk factors and their role in BPD development in children with very low (VLBW) and extremely low (ELBW) birth weight were analyzed. A special screening program consisted in determining serum levels of IL1α, IL1RA, IL6 and IL10 in the $3^{rd}$-$5^{th}$ and the $7^{th}$-$10^{th}$ day of life with enzyme-linked immunosorbent assay using test systems manufactured by Cytokine, LLC. 40 neonates with and 40 neonates without BPD underwent immunological examination. The BPD incidence was 46.6% in neonates with ELBW and 10.5% in children with VLBW. BPD risk factors for BPD in VLBW neonates were low APGAR score in the $1^{st}$ minute ($p = 0.02$), severe respiratory distress syndrome (RDS; $p < 0.001$) and pneumonia ($p < 0.001$). Gestational age under 27 weeks ($p = 0.001$) had the primary role in children with ELBW; other risk factors included body weight ($p = 0.02$), low APGAR score in the $1^{st}$ ($p = 0.03$) and the $5^{th}$ minutes ($p = 0.002$), severe RDS ($p < 0.001$) and pneumonia development ($p < 0.001$). According to the comparative evaluation of prognostic significance of the level of pro-and anti-inflammatory cytokines in infants with BPD, IL1RA has the highest information value when its level > 670 pg/ml and IL6 ≥ 25 pg/ml.*
***Keywords****: bronchopulmonary dysplasia, risk factors, cytokines, neonates.*

Bronchopulmonary dysplasia (BPD) remains the most widespread chronic respiratory tract disease in neonates [1]. Thanks to the progress in the sphere of perinatal care and neonatal respiratory therapy, clinical description and natural course of BPD have changed significantly in the past decade [2-4]. Study of immunopathogenesis of the "new", or post-surfactant, form of BPD, which develops primarily in small premature infants, is an especially urgent problem. Formation of BPD is linked to pulmonary maldevelopment due to a large number of prenatal and postnatal factors in the setting of incomplete processes of alveologenesis and angiogenesis [2]. Conditions of intrauterine life of the child and early infancy are considered to be of primary significance for optimal growth and development of the respiratory system [3]. Literature features data on the connection of BPD with pre-eclampsia [5], chorioamnionitis [6], intrauterine inflammation [4, 7] and genetic predisposition [7]. Researchers focus attention primarily on the role of chronic inflammation of the pulmonary tissue, as the pronounced reaction to high oxygen concentration in the inspired mixture manifesting itself with an inflammation and, later, arrest of alveolar development and BPD development in small premature infants [3]. There have been more and more proofs that BPD results from the imbalance of pro-inflammatory and anti-inflammatory mechanisms [1]. However, the data on place and role thereof in BPD genesis

remain contradictory [8]. Several studies indicate the leading role of pro-inflammatory cytokines [7, 9, 10] and insufficient activity of anti-inflammatory cytokines [11]. According to different data, excessive anti-inflammatory effects of cytokines in the first week of life of neonates with respiratory disorders alter the natural antibacterial protection of lungs and are considered early BPD predictors [12, 13]. In this study, we made an attempt to evaluate significance of pro- and anti-inflammatory cytokines in combination with clinical-anamnestic data for the earliest possible BPD prognosis.

**The study was aimed** at analyzing perinatal factors and levels of pro- (IL1α, IL6) and anti-inflammatory (IL1PA, IL10) cytokines in order to specify their role in BPD immunopathogenesis and prognosis.

## PATIENTS AND METHODS

Prospective study involved 194 neonates with body weight of 600-1,500 g and gestational age of 25-32 weeks treated at the Clinical Maternity Hospital, Regional Perinatal Center and Pediatric Municipal Clinical Hospital No. 1 (all – State Budgetary Healthcare Establishments) of Astrakhan from 2009 to 2011. Mothers gave informed consent to examination and treatment in all cases.

By the end of the neonatal period, 47 neonates developed BPD. BPD diagnosis was established in accordance with criteria of the Russian classification of clinical forms of bronchopulmonary diseases in children [14]. BPD was diagnosed on the basis of anamnestic data, need in additional oxygen at the age of 28 days, physical examination data and characteristic alterations detected by means of chest X-ray examination. The main perinatal risk factors and the significance thereof for BPD development in children with very low (VLBW) and extremely low birth weight (ELBW) were analyzed. A special examination program consisted in determining serum levels of IL1α, IL1PA, IL6 and IL10 at the age of 3-5 and 7-10 days with enzyme-linked immunosorbent assay using test systems manufactured by Cytokine, LLC. 40 neonates with BPD and 40 neonates without BPD were immunologically examined. The control group was formed on the copy-pair basis.

Statistical data manipulation was performed using methods of descriptive statistics and correlation analysis by means of software package Statistica 6.1 and expressed as the arithmetic mean and standard deviation thereof (M±SD) in the event of normal parameter distribution. We used Student's test to reveal statistical significance of differences. In the event of abnormal distribution, the data were expressed as the median (25-75% quartile); non-parametric Mann-Whitney U-test and Spearman's correlation coefficient were used to reveal statistical significance of differences (r). $\chi^2$ test was used to determine differences between qualitative parameters. The critical error level $p$ was taken equal to 0.05 for all statistical calculations.

## RESULTS AND DISCUSSION

According to the study, the rate of BPD development in children with birth weight below 1,500 g and gestational age under 32 weeks is 19.5%. It is considerably higher in ELBW children (46.6%) than in VLBW children (10.5%). Comparative analysis of general clinical characteristics and data of perinatal anamnesis in children with and without BPD depending on body weight can be found in tb. 1. There are significant differences between male and female neonates indicating susceptibility of male persons to BPD development. VLBW children are the most susceptible ($p = 0.01$). It is known that male persons are susceptible to various undesirable complications and outcomes by default. Severe respiratory distress syndrome, BPD development and the fact that the child is male are possibly links of one pathogenetic chain. Comparative analysis of anthropometric data and gestational age in children with VLBW, who have or have not developed BPD, did not demonstrate any differences. Moreover, ELBW neonates featured a statistically significant difference in such parameters as body weight and gestational age. That is

why we performed an additional correlation analysis between body weight, gestational age and BPD development in ELBW children. The obtained results indicated inverse interdependence of the rate of BPD development and gestational age ($r = 0.4$; $p = 0.02$). It ought to be mentioned that 16 out of 28 children with BPD and only 4 out of 32 children without BPD and weight below 1,000 g were born in the $25^{th}$-$26^{th}$ week of gestation. No such interdependence of body weight and BPD development in children below 1,000 g was revealed. Thus, gestational age and, to a lesser extent, body weight were crucial to BPD development, especially in ELBW children.

APGAR score was of significance for determining BPD prognosis. According to the obtained data, the $1^{st}$ minute score was significantly lower in all groups of neonates, who had developed BPD. The $5^{th}$ minute score of ELBW children with BPD was also significantly lower than in children without BPD. Severe perinatal hypoxia extremely negatively affected general condition of neonates; this led to the use of a whole range of invasive BPD-associated measures (primarily, intubation of trachea with subsequent long-term artificial pulmonary ventilation [IPV]). Thus, severe RDS, long-term need in APV and development of pneumonia were associated with BPD development in both groups of neonates. It ought to be mentioned that artificial surfactant drugs were administered to an overwhelming majority of the neonates after the first 30 minutes of life; this affected the rate of BPD development neither in VLBW children nor in ELBW children. According to the currently available data, chorioamnionitis is not always associated with BPD development [6]. We did not observed any significant difference in the rate of chorioamnionitis between mothers of the children, who had or had not developed BPD.

The performed statistical analysis of perinatal risk factors revealed their significance for BPD development in VLBW neonates and ELBW neonates. In our opinion, the main significant difference is the absence of dependence of the rate of BPD development on the child's birth weight and gestational age in the group of VLBW children unlike in the group of ELBW children, the main risk factor of BPD development in whom initially was gestational age $< 27$ weeks ($p = 0.001$) and, to a lesser extent, body weight ($p = 0.02$).

One of the goals of the study was to analyze levels of pro- (IL1α, IL6) and anti-inflammatory (IL1PA, IL10) cytokines in order to determine their role in immunopathogenesis and prognosis of BPD. Retrospective evaluation thereof in this category of patients in the $1^{st}$ week of life is the most prognostically interesting and useful evaluation. Comparative general clinical description of the studied groups is given in tb. 2. Neonates in both groups were comparable in terms of the main anthropometric parameters and gestational age. The $1^{st}$ minute and the $5^{th}$ minute APGAR score were significantly different; these differences were taken into consideration for interpretation of the obtained data.

Considerable increase in the level of IL1PA was observed at the age of 3-5 days in the children, who would later develop BPD (tb. 3). We have previously established [15] that increase in this anti-inflammatory cytokine at the age of 3-5 days is characteristic of children with low APGAR score and severer course of RDS, as in the group of children with high risk of BPD development. According to D.K. Kakkera et al. [13], IL1PA increase in neonates with RDS was the earliest and permanent BPD development-associated symptom. Thus, severe perinatal hypoxia and RDS in combination with blood serum IL1PA increase at the age of 3-5 days may serve as BPD development predictors.

Levels of both pro- and anti-inflammatory cytokines in blood serum increased considerably at the age of 7-10 days in the children, who would later develop BPD (tb. 4). At that stage of examination it might have been caused by pneumonia in most children, who had developed BPD ($p < 0.001$). It is known that IL1 and IL6 cytokines are among the primary acute inflammatory process mediators. Blood serum IL1PA level increase at the age of 7-10 days might have partially been a compensatory mechanism. This supposition was confirmed by a revealed positive interdependence between the levels of IL6 and IL1PA at the age of 7-10 days in the neonates, who had developed BPD ($r = 0.5$; $p = 0.03$). At the same time, considerable increase in the serum level of this cytokine may disturb antibacterial lung protection and contribute to an inflammatory reaction [12]. Increase in the level of IL10 was less pronounced than in the case of

IL1PA. It might probably have been caused by peculiarities of generation of this cytokine in neonates, particularly with generation of an insufficient amount of these cytokines required for inhibition of anti-inflammatory cytokines [16]. S. Hikino et al. [13] had previously revealed the connection between IL10 level increase ($p < 0.01$) in children with RDS and BPD development.
Given the obtained results of examination of levels of pro- and anti-inflammatory cytokines in blood serum, we attempted to reveal the most informative of them for early BPD prognosis. We calculated prognostic value of positive and negative results obtained in the 1st week of life in the children, who had developed BPD (tb. 5). The obtained data on IL1α and IL10 levels indicated absence of BPD in the event of low amounts of IL1α and IL10 (below 300 pg/ml and 45 pg/ml, respectively). At the same time, the IL1α level was over 300 pg/ml in almost half of the children without BPD, the IL10 level – over 45 mg/ml in 70% of the children. IL1PA and IL6 levels over 670 pg/ml and 24 pg/ml in the 1st week of life indicated a 70-80% probability of BPD development and absence thereof in 90% of the children when these levels were below the aforementioned.

## CONCLUSIONS

1. Due to the use of artificial surfactant drugs and continuous improvement of respiratory support techniques, BPD develops primarily in the ELBW neonates born before the age of 28 gestational weeks. The rate of BPD development in this category of patients is 46.6%; in VLBW children – 10.5%.
2. BPD risk factors in VLBW neonates are low 1st minute APGAR score ($p = 0.02$), severe RDS ($p < 0.001$) and pneumonia ($p < 0.001$). The primary risk factor in ELBW children is gestational age under 27 weeks ($p = 0.001$); the other risk factors include body weight ($p = 0.02$), low 1st ($p = 0.03$) and 5th ($p = 0.002$) minute, severe RDS ($p < 0.001$) and development of infectious pulmonary complications ($p < 0.001$).
3. Comparative evaluation of prognostic significance of the level of pro- and anti-inflammatory cytokines in the neonates, who would later develop BPD, demonstrated that IL1PA > 670 pg/ml in blood serum and IL6 ≥ 25 pg/ml have the highest information value.

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**Table 1.** Comparative general clinical description of the children, who had or had not developed bronchopulmonary dysplasia (BPD), on the basis of birth weight

| **Symptoms (M±SD) Abs. (%)** | **ELBW children** | | **p** | **VLBW children** | | **p** |
|---|---|---|---|---|---|---|
| | **BPD (n = 28)** | **no BPD (n = 32)** | | **BPD (n = 19)** | **no BPD (n = 162)** | |
| Body weight, g | 859±108 | 918±84 | 0.02 | 1,312±121 | 1,340±136 | 0.36 |
| Body length, cm | 33.2±1.9 | 33.9 ±2.0 | 0.17 | 38.3±2.3 | 39.2±2.3 | 0.05 |
| Gestational age, weeks | 26.1±0.8 | 27.0±0.7 | < 0.001 | 29.5±1.1 | 29.8±1.0 | 0.24 |
| APGAR score:<br>- 1st minute<br>- 5th minute | <br>3.5±1.8<br>4.4±1.8 | <br>4.5±1.6<br>5.8±1.5 | <br>0.03<br>0.002 | <br>4.7±2.0<br>6.1±1.2 | <br>5.6±1.6<br>6.4±1.0 | <br>0.02<br>> 0.05 |
| Girls | 16 (57) | 26 (78) | 0.04 | 3 (16) | 81 (50) | 0.01 |
| Boys | 12 (43) | 6 (22) | 0.04 | 16 (84) | 81 (50) | 0.01 |
| Chorioamnionitis | 8 (29) | 5 (15.5) | 0.22 | 5 (25) | 19 (11.7) | 0.07 |
| Moderate RDS | 1 (4) | 18 (56) | < 0.001 | 4 (20) | 99 (62) | < 0.001 |
| Severe RDS | 26 (96) | 12 (37.5) | < 0.001 | 16 (80) | 63 (38) | < 0.001 |
| APV | 26 (96) | 14 (43.5) | < 0.001 | 19 (95) | 77 (47.5) | < 0.001 |
| Surfactant | 19 (68) | 18 (56) | 0.35 | 11 (58) | 83 (51) | 0.58 |
| APV-associated pneumonia | 18 (66) | 6 (19) | < 0.001 | 14 (70) | 24 (15.5) | < 0.001 |

*Note.* VLBW/ELBW – very/extremely low body weight, RDS – respiratory distress syndrome, APV – artificial pulmonary ventilation.

**Table 2.** Comparative general clinical description of the VLBW children and ELBW children, who had or had not developed bronchopulmonary dysplasia (BPD)

| **Symptoms (M±SD)** | **BPD (n = 40)** | **no BPD (n = 40)** | **p** |
|---|---|---|---|
| Body weight, g | 1,042 ± 251 | 1,066 ± 152 | > 0.05 |
| Body length, cm | 35.3 ± 3.2 | 36.2 ± 2.9 | > 0.05 |
| Gestational age, weeks | 27.5 ± 1.9 | 28.4 ± 2.4 | > 0.05 |
| APGAR score:<br>- 1st minute<br>- 5th minute | <br>4.1±2.0<br>5.2±1.6 | <br>5.2±1.8<br>6.2±1.2 | <br>**0.0008**<br>**0.0002** |

**Table 3.** Blood serum cytokine levels at the age of 3-5 days in the neonates with very low and extremely low birth weight, who had or had not developed bronchopulmonary dysplasia (BPD)

| **Parameters, pg/ml** | **BPD** | **no BPD** | **p** |
|---|---|---|---|
| IL1α | n = 40 | n = 40 | 0.18 |
| Median | 210 | 300 | |
| 25-75% quartile | 70-320 | 146-440 | |
| IL1PA | n=40 | n=40 | **0.005** |
| Median | 755 | 380 | |
| 25-75% quartile | 440-2,800 | 290-600 | |
| IL6 | n = 40 | n = 40 | 0.17 |
| Median | 19 | 14 | |
| 25-75% quartile | 11-54 | 10-24 | |
| IL10 | n = 40 | n = 40 | 0.38 |
| Median | 54 | 50 | |
| 25-75% quartile | 32-90 | 10-66 | |

**Table 3.** Blood serum cytokine levels at the age of 7-10 days in the neonates with very low and extremely low birth weight, who had or had not developed bronchopulmonary dysplasia (BPD)

| **Parameters, pg/ml** | **BPD** | **no BPD** | **p** |
|---|---|---|---|
| IL1α | n = 40 | n = 40 | 0.01 |
| Median | 440 | 210 | |
| 25-75% quartile | 280- 490 | 100-320 | |
| IL1PA | n = 40 | n = 40 | 0.001 |
| Median | 730 | 290 | |
| 25-75% quartile | 560-1,020 | 170-550 | |
| IL6 | n=40 | n=40 | 0.01 |
| Median | 17 | 8 | |
| 25-75% quartile | 11-30 | 2-15 | |
| IL10 | n = 40 | n = 40 | 0.04 |
| Median | 55 | 48 | |
| 25-75% quartile | 47-88 | 15-66 | |

**Table 5.** Comparative assessment of prognostic value (PV) of the level of pro- and anti-inflammatory cytokines in the neonates with very low and extremely low birth weight, who had developed bronchopulmonary dysplasia

| **Parameters, pg/ml** | **PV+, %** | **PV-, %** |
|---|---|---|
| IL1α > 300 | 49 | 88 |
| IL1PA > 670 | 72 | 91 |
| IL6 > 24 | 82 | 84 |
| Il10 > 45 | 33 | 91 |