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MARKERS OF BRONCHOPULMONARY DYSPLASIA IN ANALYZING INDUCED SPUTUM DURING PERIODS OF REMISSION   
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**Summary:** The 620 samples of induced sputum examination preterm infants aged 1 month to 36 months: 491 examinations of children diagnosed with bronchopulmonary dysplasia (study group) and 129 observations preterm who had respiratory disorders, but did not form bronchopulmonary dysplasia (group). For children with bronchopulmonary dysplasia in remission was characterized by thick-induced sputum (KW H (n = 620) = 37.14, rank - 5.2, p = 0.0001) with a significant number of extracellular bacteria (λUilksa = 0.701; F (4,61) = 65,46; p = 0.0001), white blood cells and alveolar macrophages (λUilksa = 0,767; F (2,67) = 93,49; p = 0.0001). Microbiological markers of BPD were mixt infection opportunistic pathogens and pathogens (KW H (n = 620) = 27.8; rank - 4.49, p = 0.0001). Mixt infection affect the high frequency of bronchial obstruction syndrome (r = 0,382; p <0,05) and severity of bronchopulmonary dysplasia (r = 0,600; p <0,05), and persistence of Pseudomonas aeruginosa, Klebsiella pneumonia correlated with a high mortality rate of bronchopulmonary dysplasia (r = 0,301; p <0,05). These data lead to the need for macro-microscopic and microbiological studies to children in remission for bronchopulmonary dysplasia to identify early markers of acute disease and adverse course of BPD.

**Keywords:** children, bronchopulmonary dysplasia induced sputum

Bronchopulmonary dysplasia - a chronic lung disease that occurs in premature, of course after mechanical ventilation [1]. Last 20 years have led to a significant improvement in survival of infants with very low birth weight, through the use of antenatal steroids, surfactant therapy and changes in ventilatory strategy [2,3]. At the same time the incidence of bronchopulmonary dysplasia among preterm remains high [4]. In the analysis of the etiology of BPD scientists have shown that a key factor behind the formation of the disease is inflammation. The role of nuclear factor (NF) -κB as the main determinants of induction of inflammatory lesions. NF-κB is located in the cell and is associated with inhibitor iκB as a complex NF-κB- iκB. Release of NF-κB contributes to a variety of mechanisms, including Hyperoxia, trauma and infection. Activation of nuclear factor promotes transcription of proinflammatory mediators. Yes, there is a lesion of the lung parenchyma prematurity [5,6]. Some authors point out that the majority of patients with BPD in sputum increased levels of white blood cells and epithelial cells in the postnatal period, and the function of the lungs for a long time abnormal [7]. Still examines the role of inflammatory lesions of the bronchi and lungs consequences of bronchopulmonary dysplasia. Not by the dynamics of their relationship elements sputum, depending on age, severity of illness, especially in sputum prognosis of bronchopulmonary dysplasia.

**Objective:** to improve the diagnosis of bronchopulmonary dysplasia by identifying marker as induced sputum in remission of the disease.

**Materials and Methods:** The study was conducted at the Department of Pediatrics and Neonatology №1 Kharkiv National Medical University (Head of department - G.S.Senatorova) in the regional center for diagnosis and treatment of bronchopulmonary dysplasia in children Kharkiv Regional Children's Hospital (chief doctor – G.R. Muratov). Analysis of induced sputum was performed prematurely aged 1 month to 36 months (620 cases): 491 examinations of children diagnosed with bronchopulmonary dysplasia (main group) and 129 observations preterm who had respiratory disorders, but did not form bronchopulmonary dysplasia (comparator group). For the analysis of the method of sputum induction sputum. Within 5 minutes was performed inhalation nebulized 3% salin followed by sputum aspiration catheter to the posterior pharyngeal wall. Aspiration was performed simultaneously with the cough impulse.

Analysis of induced sputum included: macroscopic examination (sputum determine the nature of its quantity, color, smell, texture, chemical properties), microscopic examination (determination of cellular elements and other elements sputum study of microbial flora in native and stained with Romanovsky Gimze smears), microbiological research (identifying and studying the properties of the pathogen). Statistical analysis of data was performed using the «Statistica-6".

**Results and Discussion:** We found that in children the main group detected significantly more yellowish and greenish sputum (p <0.001). In the examined group comparison - colorless sputum (p <0.001). Marker bronchopulmonary dysplasia was a yellowish color of sputum (KW H (n = 620) = 17.7; rank - 2.17, p = 0.0001). Greenish color of sputum was not characteristic of BPD (KW H (n = 620) = 7.24, rank - 1.01, p = 0.04). Significantly more frequently in patients of group revealed a dense (p <0.05) and viscous (p <0.001) consistency of phlegm. Thick (KW H (n = 620) = 37.14, rank - 5.2, p = 0.0001) and viscous (KW H (n = 620) = 16.78, rank - 2.06, p = 0.0001) were sputum features of bronchopulmonary dysplasia. The degree of viscosity of sputum correlate with the severity of the disease (r = 0,378; p <0,05). Excessive mucus viscosity probably led to inhibition evacuation of mucus obstruction of bronchioles, violations of drainage and elimination functions of the bronchial tree in patients of group and contributed to frequent exacerbations of disease with "oxygen depended" degrees of disease severity. Abundant nature secret diagnosed more frequently in children comparison group (p <0.001). All children born prematurely who had respiratory disorders in the early neonatal period, but not formed BPD detected mucous character of sputum (126 observations; 100%). In the sputum of children as the main group dominated sputum (p <0.001), but significantly more often in children with BPD than in the comparison group had sputum mucous-purulent character. Marker BPD was mucous-purulent nature of induced sputum (KW H (n = 620) = 19.04, rank - 2.31​​, p = 0.0001).

A considerable amount of solid residue was detected significantly more often in children of the main group (p <0.001). In the comparison group of patients often manifest low amount of solid residue (p <0.001). For patients with bronchopulmonary dysplasia was characterized by a moderate amount of fibrin in the sputum (KW H (n = 620) = 44.2; rank - 7.4, p = 0.0001). In the study of pH bronchoalveolar lavage revealed that children with bronchopulmonary dysplasia reaction secretion was 5,66 ± 0,52, which was significantly higher than in the comparison group observations 7,96 ± 0,97 (p <0.001). Increased acidity of sputum in children with bronchopulmonary dysplasia, we have regarded as excessive content of hydrogen ions, formed during metabolism of organic and inorganic substances, and as a result of the activity of microorganisms. Standard indicators of total protein in sputum according to MA Bazarnovoyi were 0,324 ± 0,29 g / l [8].

The level of protein in the core group of children stood at 0,352 ± 0,009 g / l. Patients comparison group - 0,231 ± 0,004 g / l. The level of total protein in the sputum in children with bronchopulmonary dysplasia directly correlates with the severity of the disease (r = 0,234; p <0,05).   
In the study group level cytosis in induced sputum was significantly higher than in the comparison group, indicating the presence of inflammation in the absence of exacerbation of bronchopulmonary dysplasia.

These data provided the basis for analysis of sputum cells. To adequately assess the composition of sputum us was determined the absolute number of effector cells, as due to a significant influx of neutrophils could be a disproportion between neutrophils and alveolar macrophages induced sputum, this estimate is unreliable migration of alveolar macrophages. Showed a significant increase in white blood cells in children with bronchopulmonary dysplasia (p <0.0001), which is regarded by us as the inflow of immune cells to the inflammatory focus. In children with bronchopulmonary dysplasia number of alveolar macrophages and neutrophils was also higher (p <0.0001). The number of eosinophils did not differ (P> 0.05).

Epithelial cells of the patients were submitted to bronchial epithelium and alveolar epithelium. The number of epithelial cells in induced sputum of children core group was significantly increased (p <0.001), which was due to degeneration and loss of epithelium, which along with the deterioration of the rheological properties of sputum resulted in inhibition mukotsiliarnoho transport. Given the significant differences for most measures the cellular composition of induced sputum conducted discriminant analysis (with the exception of the least significant factors).

Thus, the results of discriminant analysis of the most powerful marker of bronchopulmonary dysplasia were high absolute number of white blood cells and alveolar macrophages in induced sputum (Wilks Lambda = 0,767; F (2,67) = 93,49; p = 0.0001).

In both groups, degree of inflammation was determined by the results of the sum of three components (algorithm Corporal), such as analysis of the nature of the secret, the number of lymphocytes and alveolar macrophages. All children basic group showed signs of inflammation in induced sputum. Significantly more frequently in patients with BPD manifested moderate inflammation by Corporal (p <0.001). Drawn attention to the presence of minimal degree of inflammation in patients comparison group (32 observations; 24,8 ± 3,8%). This phenomenon we explain the release of inflammatory mediators under conditions of premature birth, mechanical ventilation in history, amid falling antioxidant capacity premature. These data may serve as a basis for further research on the characteristics of the respiratory system in preterm infants who had respiratory failure in history.

In the bacteriological examination of smears induced sputum bacteria count in patients of the main group was 23,8 ± 0,71 units. in sight. Observed in the comparison group the number of bacteria in sputum was significantly lower (6,01 ± 0,18 units. Under review; p <0.0001). Bacteria were located as separate colonies, and intracellularly in neutrophils macrophages, epithelial cells. In children, the main group dominated by extracellular bacteria (p <0.0001).

Also significantly higher in patients with BPD was the ratio of extracellular to intracellular bacteria (p <0.0001), indicating significant contamination by bacteria tracheobronchial tree against the backdrop of a possible weakened the ability of phagocytic effector cell phagocytosis of observations with bronchopulmonary dysplasia. Value extra- / intracellular bacteria was greater the greater the severity of the disease was (r = 0,181; p <0,05).

Discriminant analysis of micro- and macroscopic parameters showed that children with bronchopulmonary dysplasia characterized by thick sputum induced with a significant number of extracellular bacteria, white blood cells and alveolar macrophages (Wilks Lambda = 0,701; F (4,61) = 65,46; p = 0 0001).

Colonization by bacteria induced sputum was found in 95,5 ± 0,9% of patients with bronchopulmonary dysplasia. In patients of the main group in the sputum manifested more than one bacterium on average 1,47 ± 0,47 species of bacteria. Patients core group of Gram-positive bacteria accounted for 78,87 ± 1,48% of the total number of bacteria. Gram-negative bacteria in children with BPD was significantly less 21,19 ± 1,18%, p = 0.001. In the comparison group bacteria contamination was detected in the respiratory tract 19,14 ± 2,8%, which was significantly less (p = 0.001), of which 100 ± 0% were gram-positive opportunistic bacteria. Among patients with BPD was significantly more frequent than in the comparison group were found conditionally pathogenic Gram-positive bacteria. Contamination of opportunistic Gram positive bacteria was a characteristic feature of induced sputum of children with bronchopulmonary dysplasia Staphylococcus aureus (KW H (n = 620) = 27.8; rank - 4.49, p = 0,0001), Staphylococcus epidermidis (KW H (n = 620) = 25.89, rank - 4.06, p = 0.0001). Given the potential for opportunistic pathogens to violations of the ciliary epithelium and increased inflammation of the respiratory tract, induction of airway hyperactivity can assume their role in the sharp bronchopulmonary dysplasia. We have demonstrated that the severity of bronchopulmonary dysplasia (r = 0,475; p <0,05) and the frequency of bronchial obstruction syndrome (r = 0,463; p <0,05) depended on the number of opportunistic bacteria in induced sputum. Detection of pathogens in remission predictor of bronchopulmonary dysplasia was more frequent exacerbation of BPD (KW H (n = 620) = 129.88, rank - 8.64, p = 0.0001). Marker feature of induced sputum during bronchopulmonary dysplasia in children with BPD was to identify Pseudomonas aeruginosa (KW H (n = 620) = 33.92, rank - 3.81, p = 0.0001) and Klebsiella pneumonia (KW H (n = 620 ) = 34.69, rank - 3.88, p = 0.0001). Pseudomonas aeruginosa and Klebsiella pneumonia were probably derived vnutrishnohospitalno, taking into account the duration of the child with BPD in the neonatal department.

However, the Pseudomonas aeruginosa bacterium is unique to the "social" behavior, the ability to effectively receive general solutions for adaptation, protection by "signaling" molecules and toxin against a background of reduced immune reactivity. Klebsiella pneumonia producing endotoxin and membranotoksyn that strike the epithelium of the bronchi and contribute to bronchial hyperreactivity, inflammation and exudation of fluid. Contamination by pathogenic bacteria influence the frequency of bronchial obstruction syndrome (r = 0,382; p <0,05), the severity of bronchopulmonary dysplasia (r = 0,600; p <0,05) and mortality of children with BPD (r = 0,301; p <0,05) . Fungi of the genus Candida in induced sputum detected in 3,2 ± 0,8% patients of the main group. Significant difference in frequency of detection of Candida fungi in induced sputum is not proven (p> 0,05).

**Conclusions:**1. For children with bronchopulmonary dysplasia in remission characteristic thick phlegm induced with a significant number of extracellular bacteria, white blood cells and alveolar macrophages.

2. Microbiological markers of BPD can be considered mixt infection conditional pathogens and pathogens (Staphylococcus aureus, Staphylococcus epidermidis, Pseudomonas aeruginosa, Klebsiella pneumonia). Mikstinfikuvannya affect the high frequency of broncho-obstructive syndrome and bronchopulmonary dysplasia severity and persistence of Pseudomonas aeruginosa, Klebsiella pneumonia correlated with a high mortality rate of bronchopulmonary dysplasia.

3. We believe that the persistence of Pseudomonas aeruginosa and Klebsiella pneumonia was simultaneously indicator and inducer severity of BPD and fatal disease.   
4. These data lead to the need for macro-microscopic and microbiological studies to children in remission for bronchopulmonary dysplasia to identify early markers of acute disease and adverse course of BPD.

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