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ANALYSIS OF EXHALED BREATH CONDENSATE IN PATIENTS WITH ASTHMA AND RECURRENT WHEEZING

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Wheezing is the most common clinical symptom of bronchial obstructive syndrome. The functions of pulmonary surfactant (PS) depend directly on the level of total phospholipids (TP). This can be used to assess the integrity of the cell membrane structure.

Objective. *To determine the level of total phospholipids and calcium in exhaled breath condensate in young children with recurrent wheezing and asthma.*

Materials and methods. *The study included 77 patients divided into 3 groups. Group 1 included 30 patients (20 girls and 10 boys) with no more than 2 episodes of recurrent wheezing in their lifetime; Group 2 – 10 patients (6 boys and 4 girls) with more than 3 episodes of recurrent wheezing, and 37 patients (15 girls, 22 boys) with asthma. The control group consisted of 20 conditionally healthy children (11 girls, 9 boys). All patients were aged from 3 months to 6 years. The determination of phospholipid levels and calcium (Ca) in the exhaled breath condensate was performed in dynamics - during the period of clinical manifestations and remission.*

Results. *The level of phospholipids in the exhaled breath condensate was the highest in patients of all groups at the stage of clinical manifestations compared with the period of remission, while its lowest level was noted in the control group. During the peak of the disease, the level of Ca in the exhaled breath condensate was significantly lower compared to the period of remission. The control group had the highest level.*

Conclusions. *The exhaled breath condensate's high level of phospholipids in the first days of the disease confirms cell damage in the presence of inflammation. Additionally, the exhaled breath condensate's Ca level is lowest during this period, possibly indicating its involvement in cell damage*

Keywords: *recurrent wheezing, asthma, exhaled breath condensate, phospholipids, children*

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1. Introduction

Bronchoobstructive diseases continue to be a pressing issue among paediatric patients. This condition is characterised by symptoms such as shortness of breath and wheezing, which can lead to complications, particularly in young children. Wheezing can present itself in various pathologies, including non-respiratory diseases [1, 2]. It should be noted that this syndrome is most commonly associated with asthma. This pathology is widespread globally and significantly impacts patients' quality of life. In Ukraine, for example, the prevalence of asthma in children can range from 5 % to 22 %, according to the results of uniform studies (ISAAC) [3]. We need to look at asthma in a broader context because of its high prevalence, the complexity of diagnosis in young children and the variety of triggers. For example, research has shown that there is an inverse correlation between serum vitamin D (25(OH)D3) deficiency and an increased risk of developing asthma [4, 5]. These changes are frequently accompanied by electrolyte metabolism disorders, particularly phosphorus-calcium metabolism. It is well known that bone development, metabolism and diet can affect blood

calcium and 25(OH)D3 levels [6, 7]. The use of glucocorticosteroids can also reduce these levels. However, some studies suggest that recurrent and chronic bronchopulmonary diseases may result in increased levels of total and ionised calcium in the blood [8]. Although the development of wheezing involves multiple mechanisms, it can be argued that inflammation and damage to pulmonary surfactants form the basis of this pathological condition [9, 10]. Pulmonary surfactant regulates the surface tension of the alveoli, and its functionality depends on the lipid composition [11]. The diagnostic significance of the levels of total phospholipids should be noted. Phospholipid levels can be used to judge the integrity of the cell membrane structure [9, 12]. The determination of such indicators is performed by analysing exhaled breath condensate. This method is non-invasive and widespread in pediatric practice. Expired breath condensate is a biological medium that can be used to assess the functional state of the bronchi-orespiratory tract. Studies have focused on detecting the amino acid composition, primary and secondary lipid peroxidation products, and the activity of antioxidant defence enzymes in the exhaled breath condensate [9, 13].

There is not much data in the literature on determining the level of phospholipid and calcium in the exhaled breath condensate and assessing their relationship with indicators of phosphorus-calcium metabolism in the peripheral blood of young children with asthma and wheezing, which prompted us to conduct this study.

The aim of the study: to determine the levels of total phospholipids and calcium in exhaled breath condensate of young children with asthma and wheezing over time.

2. Materials and methods

A cohort prospective study was conducted on the basis of the pulmonology department at Municipal Children's Hospital No. 16 Kharkiv City Council in the period from September 2021 to February 2022. The clinical data of 77 patients, who were divided into groups depending on the nosological form, were evaluated. Group 1 included 30 patients (20 girls and 10 boys) with no more than two episodes of asthma in their lifetime; group 2 included 10 patients (6 boys and 4 girls) with more than 3 episodes of asthma in their lifetime and 37 patients (15 girls, 22 boys) with an already established diagnosis of mild to moderate persistent asthma. The control group consisted of 20 conditionally healthy children (11 girls, 9 boys) who did not have any manifestations of upper respiratory tract disease and other acute pathology during the last month. All patients were aged from 3 months to 6 years.

The study included patients who met the following inclusion criteria: informed consent signed by the patient's parents; patient's age from 3 months to 6 years; diagnosis of recurrent wheezing and asthma. The diagnoses were made by a pediatric respiratory specialist and a pulmonologist in accordance with the protocol for the treatment of children with recurrent wheezing No. 18 of January 13, 2005, the protocol for the treatment of children with asthma No. 2856 of December 23, 2021, and GINA 2020–2022 recommendations.

All patients were diagnosed based on the presence of shortness of breath, dry rales, and cough. The analysis included the number of obstructions in the patient's medical history, acute respiratory diseases, the presence of allergic diseases and asthma in the patient and their first-degree relatives, as well as clinical and laboratory manifestations of bronchial obstruction. Specific therapy responses and clinical improvement within three months were also evaluated in detail. Patients were treated according to the protocols and global GINA 2020–2022 guidelines.

In addition to standardised protocols for the examination of children, the levels of total blood Ca were studied by the complexometric titration method and inorganic phosphorus (P) – by the molybdenum acid method (A. A. Pokrovsky, 1974).

The study of vitamin D (25(OH)D₃) levels in blood serum was performed by enzyme-linked immunosorbent assay (ELISA), according to the instructions for the use of reagent kits. Serum vitamin D levels below 10 ng/ml were considered deficient, 10 to 29 ng/ml were considered insufficient, and 30–100 ng/ml were considered normal, according to the World Health Organization (WHO) recommendations.

The determination of phospholipids and Ca in exhaled breath condensate was performed spectrophotometrically by thin-layer chromatography using a spectrophotometer SF-46. The sampling of exhaled breath condensate was performed using a modified device based on the Department of Pediatrics No. 2 of Kharkiv National Medical University (utility model patent No. 108795). This device consists of a portable U-shaped glass tube and a cooling mechanism using refrigerants that maintain a temperature of -10 °C. Exhaled breath was collected using a mouthpiece connected to a one-way exhalation valve to prevent inhalation of condensate. The exhaled breath was then converted to droplets in a glass tube and collected after disconnection from the device. The collected material was immediately stored at a temperature of -70 °C. The collection method is non-invasive, and depending on the temperature set in the device and the age of the patients, condensate was collected up to 0.5–1.0 ml in 10–15 minutes. The indicators were measured at the onset of recurrent wheezing and asthma and when clinical symptoms subsided, including cough, shortness of breath, and physical manifestations such as prolonged exhalation and wheezing in the lungs.

Statistical processing was performed using the package program EXCEL FOR WINDOWS, StatSoft STATISTICA version 8.0 (Tulsa, Oklahoma) and the statistical software MedCalc version 17.2. The Shapiro-Wilk test was used, and the histogram and q-q plot were examined to assess the normality of the distribution. If the distribution of the sample was different from normal, the median (Me) and interquartile range (Lq – lower quartile; Uq – upper quartile) were determined. The non-parametric Wilcoxon test (T) was used to compare two dependent samples. The nonparametric Mann-Whitney U test (MW) was used to compare two samples. The difference in parameters compared by two points was considered statistically significant at $p < 0.05$. When comparing indicators characterised by a comparison of more than 2 points, the Kruskal-Wallis (KW) test of analysis of variance was used, and differences were considered significant with the Bonferroni correction. The relationship between the series of indicators was assessed using Spearman's rank correlation (r).

All clinical examinations were approved by the Medical Ethics Committee of Kharkiv National Medical University (protocol No. 5 of October 7, 2020) and were conducted in accordance with the principles of the Declaration of Helsinki. All parents of children participating in the study gave written informed consent to participate in the study.

3. Results of the study

The statistical analysis of the patient groups showed that Group 1 had a predominance of girls in terms of gender, and the patients' age was Me 2.10 (0.80; 3.00) years. In patients Group 2 and Group 3, boys predominated in terms of gender, and the patients' age was Me 3.50 (1.10; 5.00) and Me 5.11 (4.00; 6.00) years, respectively.

The analysis of the anamnestic data showed that in patients Group 1, 9/30 had a history of allergies, and in 6 children, at least one parent had asthma. Allergic rhinitis

(AR) was diagnosed in 3/30 patients, including 1 patient with a seasonal (intermittent) course and 2 patients with a year-round (persistent) course. 6/30 patients had frequent cases of acute respiratory diseases (ARD) (more than 5 cases per year). Among the patients in Group 2, 4/10 had a history of allergies, and in 5/10 children, at least one parent had asthma. One patient was diagnosed with intermittent allergic rhinitis, and 3 patients in this group experienced frequent cases of acute respiratory infections.

Analysis of Group 3 patients revealed that 26/37 had a history of allergies, and 19 children had parents diagnosed with asthma. Allergic rhinitis was diagnosed in 13/37 children, of whom 6/13 had an intermittent

course, and 5 had a persistent course. Frequent cases of acute respiratory infections were noted in 11 patients.

No statistically significant difference was found when comparing the anamnestic data between the groups. However, patients in group 1 and group 2 differed in body weight. Additionally, a statistically significant difference was found between the following parameters in patients of group 1 and group 3: age, body weight, height, breathing rate, body temperature, and peripheral oxygen saturation levels. A statistically significant difference was found between the following parameters in patients of group 2 and group 3: age, height, breathing rate, peripheral oxygen saturation, and serum P (Table 1).

Table 1

Clinical and laboratory characteristics of patients with recurrent wheezing and asthma

Sign	Group 1 (2 episodes of recurrent wheezing) (n=30)	Group 2 (3 or more episodes of recurrent wheezing) (n=10)	Group 3 (mild and moderate asthma) (n=37)	p
Gender, male/female	10/20	6/4	22/15	p>0.05
Ages (years), Me (Lq; Uq)	2.10 (0.80; 3.00)	3.50 (1.10; 5.00)	5.11 (4.00; 6.00)	p ₁₋₂ >0.05, p ₁₋₃ <0.01, p ₂₋₃ <0.05
Weight (kg), Me (Lq; Uq)	12.75 (8.60; 14.50)	15.20 (11.00; 19.00)	18.04 (16.90; 19.00)	p ₁₋₂ <0.05, p ₁₋₃ <0.01, p ₂₋₃ >0.05
Height (cm), Me (Lq; Uq)	87.00 (70.00; 96.00)	100.00 (77.00; 110.00)	109.75 (107.00; 114.00)	p ₁₋₂ >0.05, p ₁₋₃ <0.01, p ₂₋₃ <0.05
Heart rate (per min.), Me (Lq; Uq)	108.00 (96.00;120.00)	112.00 (100.00;128.00)	102.91 (100.00; 106.00)	p ₁₋₂ >0.05, p ₁₋₃ >0.05, p ₂₋₃ >0.05
Breathing rate (per min.), Me (Lq; Uq)	26.00 (24.00; 32.00)	27.00 (26.00; 30.00)	23.67 (22.00; 26.00)	p ₁₋₂ >0.05, p ₁₋₃ <0.01, p ₂₋₃ <0.05
Body temperature (°C), Me (Lq; Uq)	37.00 (36.60; 37.50)	36.75 (36.60; 38.20)	36.75 (36.60;37.00)	p ₁₋₂ >0.05, p ₁₋₃ <0.05, p ₂₋₃ >0.05
Positive family allergic history	9/30	4/10	26/37	p>0.05
Positive allergic rhinitis in children	3/30	1/10	13/37	p>0.05
Intermittent allergic rhinitis	1/3	1/1	6/13	p>0.05
Persistent allergic rhinitis	2/3	–	7/13	p>0.05
Frequent acute respiratory diseases (more 5 per year)	6/30	3/10	11/37	p>0.05
Peripheral oxygen saturation (SpO ₂), (%)	98.00 (97.00; 98.00)	98.00 (97.00; 98.00)	96.83 (96.00; 98.00)	p ₁₋₂ >0.05, p ₁₋₃ <0.05, p ₂₋₃ <0.05
Ca in blood serum (mmol/L), Me (Lq; Uq)	2.10 (1.90; 2.30)	2.22 (2.00; 2.30)	2.10 (2.00; 2.25)	p ₁₋₂ >0.05, p ₁₋₃ >0.05, p ₂₋₃ >0.05
P in blood serum (mmol/L), Me (Lq; Uq)	1.40 (1.10; 1.70)	1.61 (1.40;1.80)	1.28 (1.20;1.40)	p ₁₋₂ >0.05, p ₁₋₃ >0.05, p ₂₋₃ <0.05
25(OH) D ₃ in blood serum (ng/ml), Me (Lq; Uq)	28.00 (26.62; 30.00)	27.50 (25.10; 29.00)	27.39 (26.00; 29.00)	p ₁₋₂ >0.05, p ₁₋₃ >0.01, p ₂₋₃ >0.05

Statistical analysis of the level of total phospholipids and Ca in exhaled breath condensate

The statistical analysis of exhaled breath condensate showed that the level of phospholipids was highest in patients of all groups during the stage of clinical manifestations compared to the period of elimination of clinical manifestations of the disease. The

lowest level of phospholipids was observed in the control group.

The statistical evaluation of Ca levels in exhaled breath condensate showed a significant decrease during the onset of the disease compared to the elimination of clinical manifestations. The control group had the highest level. A pairwise comparison indicated a significant difference between the indicators (Table 2).

Table 2

Statistical characteristics of exhaled breath condensate in patients with bronchial obstructive syndrome in dynamics, Me (Lq; Uq)

Sign	Group 1 (2 episodes of recurrent wheezing) (n=30)		Group 2 (3 or more episodes of recurrent wheezing) (n=10)		Group 3 (mild and moderate asthma) (n=37)		Control (n=20)
	The period of clinical manifestations	Period of elimination of clinical manifestations	The period of clinical manifestations	Period of elimination of clinical manifestations	The period of clinical manifestations	Period of elimination of clinical manifestations	
Phospholipids, mmol/L	112.64 (100.15; 133.49)	98.155 (90.03; 119.88)	125.86 (99.11; 134.26)	95.65 (91.13; 106.15)	157.48 (146.92; 170.17)	119.60 (110.55; 129.12)	81.59 (79.07; 86.05)
	p=0.000		p=0.004		p=0.000		
Ca, mmol/L	0.23 (0.19;0.27)	0.49 (0.43;0.54)	0.22 (0.19;0.25)	0.43 (0.41; 0.51)	0,25 (0,21; 0,28)	0,49 (0,47;0,51)	0.59 (0.55; 0.61)
	p=0.000		p=0.004		p=0.000		

Multiple comparisons revealed that the Kruskal-Wallis H criterion was highly significant for the phospholipids index both during the acute phase of the disease (H=72.309, p=0.000) and during the period of clinical manifestations elimination (H=58.722, p=0.000), and the level of this parameter depends on the patient's belonging to one group or another. When comparing independent groups, it was found that there was no statistically significant difference between the levels of phospho-

lipids in patients of Group 1 and Group 2 both during the period of clinical manifestations (p_{1,2}=0.573) and during the period of elimination of clinical manifestations of the disease (p_{1,2}=0.254). The highest level of this indicator was noted in patients of Group 3, both during the period of clinical manifestations and during the period of elimination of clinical manifestations of the disease, while its lowest level was noted in children of the control group (Fig. 1).

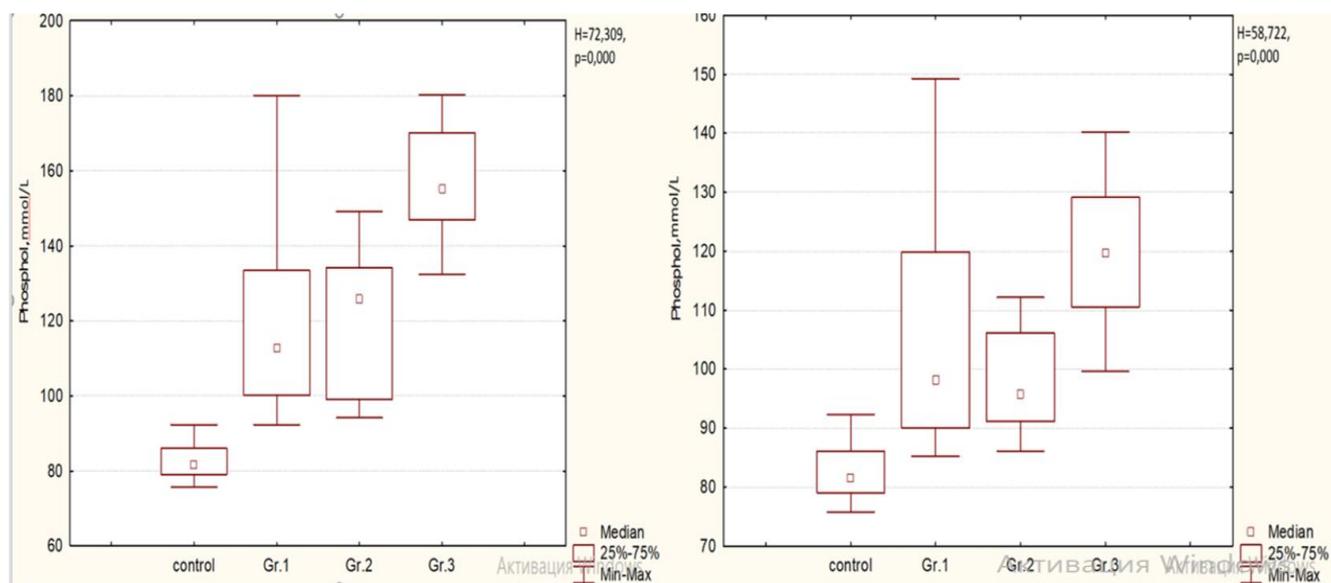


Fig. 1. Levels of phospholipids in the exhaled breath condensation of patients of all groups and children of the control group during the period of clinical manifestations and during the period of elimination of clinical manifestations of the disease

For the Ca indicator, the H criterion was significantly high both during the period of the disease ($H=47.995$, $p=0.000$) and during the period of elimination of clinical manifestations of the disease ($H=30.466$, $p=0.000$), and the level of this parameter depends on the patient's belonging to one group or another. When comparing independent groups, it was found that there was no statistically significant differ-

ence between the levels of Ca both during the period of clinical manifestations ($p_{1-2}=0.814$, $p_{1-3}=0.426$, $p_{2-3}=0.258$) and during the period of elimination of clinical manifestations of the disease ($p_{1-2}=0.472$, $p_{1-3}=0.668$, $p_{2-3}=0.135$). A statistically significant difference was found when comparing the index in patients from all groups and children from the control group (Fig. 2).

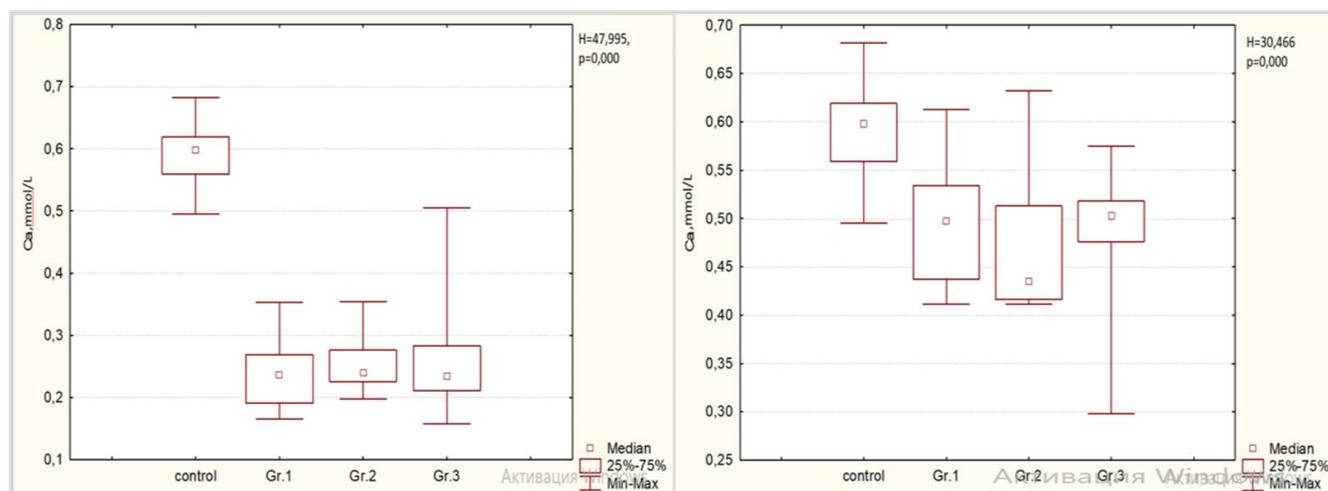


Fig. 2. Ca levels in the exhaled breath condensation in patients of all groups and children of the control group during the period of clinical manifestations and during the period of elimination of clinical manifestations of the disease

Correlation relationships

Significant correlations were found in patients in Group 1 and Group 2 with phospholipids of the exhaled breath condensation and patients' age ($r=-0.45$), height ($r=-0.46$), body weight ($r=-0.45$), heart rate ($r=-0.47$), breath rate ($r=-0.55$), and Ca level in the exhaled breath condensation ($r=-0.52$). In patients of Group 3, correlations were established between phospholipids and age ($r=0.45$), height ($r=0.53$); Ca in the exhaled breath condensation and serum potassium ($r=-0.46$), serum 25(OH) D3 ($r=-0.52$). In the control group, there was a correlation between phospholipids of the exhaled breath condensation and serum potassium ($r=-0.59$), Ca of the exhaled breath condensation and serum phosphorus level ($r=-0.69$).

4. Discussion of the study results

The study of exhaled breath condensation is increasingly used in paediatric practice due to the non-invasive method of sampling. By analysing the data obtained, it is possible to predict the course of certain lung pathologies and the results of their treatment. In our study, we focused on determining the levels of phospholipids and Ca in exhaled breath condensate as markers of cell wall damage and possible apoptosis and sought their relationship with phosphorus-calcium metabolism [9, 12, 14]. As one of the structural elements of the cell wall, phospholipids play an important role in its repair. In our study, phospholipids levels were significantly increased in all groups, both during the period of clinical manifestations and during the period of resolution of clinical manifestations of the disease, compared with the control group. The highest levels of this indicator were observed

in all groups during the peak of the clinical manifestations of the disease, which is obviously associated with damage to the membranes of the cells of the bronchopulmonary system.

The inflammatory process and the presence of hyperventilation contribute to the damage of the bilipid layer of cells, which leads to an increase in the level of phospholipids in the exhaled breath condensation both during the acute clinical manifestations and during the elimination of clinical manifestations of the disease. The highest levels of the index were in the group of children with the greatest number of obstructions. In our opinion, it indicates the formation of a chronic inflammatory process affecting the bilipid cell layer of the bronchopulmonary system. Our study confirms the fact that the level of phospholipids in the exhaled breath condensation depends on the age of the child and physical parameters (height, weight), as shown by direct and indirect correlations.

The analysis of Ca levels in the exhaled breath condensation shows that its level is significantly lower in the period of clinical manifestations than in the period of elimination of clinical manifestations of the disease. In our opinion, this is due to its possible accumulation in the cytoplasmic membrane of the cell during hypoventilation and inflammation in the tissues of the bronchopulmonary system, which probably leads to cell apoptosis. Obviously, when the bilipid layer is disrupted, extracellular calcium ions accumulate in the cell, disrupting electrolyte metabolism and affecting its further functional capacity. The existing indirect correlation between the level of phospholipids and Ca in the exhaled breath condensation supports this view. A decrease in Ca in the exhaled

breath condensation during the first days of the disease may also play a role not only in the development of inflammation and obstruction but also in the remodeling of the bronchial muscles. The existence of a probable indirect correlation between the levels of Ca in the exhaled breath condensation and the levels of potassium, 25(OH)D3 and P in the blood serum is essential for the metabolic function of the body's cells.

Practical significance. Our study showed that the level of total phospholipids in exhaled breath condensate is higher at the stage of clinical manifestations of the disease than at the stage of their elimination, and a steady increase in the index compared to patients in the control group. The obtained result allows us to determine the degree of damage to the cells of the bronchopulmonary system against the background of chronic and recurrent inflammation.

Limitations of the study: the small sample size is another limitation of this study, as it may make it difficult to determine the accuracy of the study findings.

The impact of martial law conditions: military actions prevented the involvement of more patients in the study.

Prospects for further research. The data obtained can be used to assess damage to the bronchopulmonary system in patients with frequent obstructions.

5. Conclusions

A high level of phospholipids in the exhaled breath condensation in the early days of the disease confirms the fact of cell damage against the background of their inflammation. An elevated level of the index when clinical manifestations of the disease are eliminated may indicate the presence of cell wall damage. A high fre-

quency of bronchial obstruction leads to a constant increase in the level of phospholipids in the exhaled breath condensation both during the period of clinical manifestations and during the period of elimination of clinical manifestations of the disease, which may indicate the formation of chronic inflammation. The level of Ca in the exhaled breath condensation is lowest in the first days of the disease, possibly confirming its involvement in cell damage.

Conflict of interest

The authors declare that they have no conflict of interest in relation to this research, whether financial, personal, authorship or otherwise, that could affect the research and its results presented in this article.

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Data availability

Data will be provided upon reasonable request.

Using artificial intelligence tools

The authors confirm that they did not use artificial intelligence technologies in the creation of the presented work.

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