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ACTIVITY OF THE INFLAMMATORY PROCESS IN THE RESPIRATORY TRACT IN CHILDREN WITH BRONCHIAL ASTHMA

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Abstract

Aim of the study: To analyze the activity of the inflammatory process in the airways in children with bronchial asthma (BA) depending on the different onset of the disease.

Materials and methods: Keeping to the principles of bioethics a comprehensive retrospective examination of 319 children suffering from BA was performed. In 257 children (clinical group I) bronchial asthma developed on the background of chronic obstructive bronchitis. The second clinical group included 43 children, in whom asthma debuted after community-acquired pneumonia. The third (III) clinical group consisted of 19 children in whom asthma was first verified after inpatient treatment for asthmatic status.

Results: According to the severity of bronchial asthma, it was found that the representatives of the III clinical group, compared with other patients, significantly more often had a severe course of the disease. For patients of the I clinical group in the debut is characterized by increased eosinophils and decreased neutrophil counts in sputum, for patients of group II - increased eosinophils and epitheliocytes, but a decrease in lymphocytes, and for children of clinical group III - low eosinophils sputum with a simultaneous increase in neutrophils. In particular, a statistically significant increase in the content of VEGF, a decrease in the content of cationic proteins, MMP-9, and interleukins-6, and -13 in the sputum indicates the predominance of neoangiogenesis in children of clinical group III. Instead, in the representatives of the II clinical group the remodeling processes were mainly caused by the inflammatory process with the release of intracellular eosinophilic cationic proteins.

Conclusion: These data indicate the discrete nature of the type and severity of the inflammatory process of the respiratory tract in the dynamics of observation in children of clinical comparison groups, which suggests the presence of certain phenotypic differences due to the alternative onset of the disease, which in turn was determined by different triggers. Such deviations of the inflammatory process indicate that patients with asthma require a personalized approach to differentiated diagnostic monitoring and targeted anti-inflammatory treatment, taking into account the peculiarities of the onset of the disease.

Keywords: bronchial asthma, children, sputum markers.

INTRODUCTION

The doctor is always faced with the question of prescribing the necessary examinations and most effective tactics of treatment of pediatric patients, while trying to do it as non-invasively as possible for the child. In recent years, diagnostic non-invasive procedures have been actively developed. In particular, biomarkers of the inflammatory process of the respiratory system are very promising and attractive approach due to the need to study the characteristics to determining the type or nature of respiratory ways inflammation. These biomarkers are usually objectively measurable indicators of physiological or pathological processes, they are quite sensitive, reproducible and feasible in childhood[1,2].

One of the relatively non-invasive procedures for the study of cells and mediators from the lower respiratory tract is the method of sampling and analysis of induced sputum production. Sampling of sputum in this case is carried out after inhalation of nebulized hypertonic saline solution with a gradual increase in its concentrations to achieve the effect and sampling a certain amount of biomaterial[3,4]. The composition and number of cells in the sputum can detect various forms of airway inflammation: eosinophilic, neutrophilic, mixed, paucigranulocyte, etc.[5]. Sputum eosinophilia is usually a marker of the severity of allergic inflammation in bronchial asthma, and in such patients, as a rule, is higher efficacy of basic therapy with inhaled glucocorticosteroids (IGCS)[6], and at the end of the course of such therapy there is usually a decrease in bronchial hyperresponsiveness and in sputum eosiniphilia[7]. In these patients, in addition, there is always an increased number of epithelial cells in the sputum and the higher concentration of eosinophil cationic protein in the supernatant sputum[8,9]. Another common variant of bronchi inflammation is sputum neutrophilia, which is often accompanied by a reduced number of macrophages in the sputum and elevated levels of interleukin-8, and such children are less susceptible to standard basic therapy with inhaled corticosteroids[10]. However, some authors have proposed the concept of "divergent phenotypes" of asthma based on cellular phenotypes of sputum monitoring, to classify asthma patients as poly- or paucigranulocyte phenotypes and with a predominant cellular composition of sputum[11]. Based on the above, we considered it relevant and promising to study sputum markers in children with different onset of bronchial asthma, and analyze the features of the biomarkers content and the nature of the inflammatory process in children with chronic (chronic obstructive bronchitis) or sudden onset (asthma status, pneumonia) of the disease.

AIM OF THE STUDY

To analyze the activity of the inflammatory process in the airways in children with bronchial asthma depending on the different onset of the disease.

MATERIALS AND METHODS

Three hundred nineteen children were comprehensively examined in the Regional Children's Clinical Hospital in Chernivtsi by the method of "experiment-control" in parallel groups using a simple random sample. In 257 children (clinical group I) bronchial asthma developed on the background of chronic obstructive bronchitis (average age 11.7 ± 0.23 years, the proportion of boys 71.6%, the proportion of rural residents 55.6%). The second clinical group included 43 children (average age 9.9 ± 0.55 years, the share of boys 50.5%, the share of rural residents 72.1%), in whom asthma debuted after community-acquired pneumonia. The third (III) clinical group consisted of 19 children in whom asthma was first verified after inpatient treatment for asthmatic status (average age 7.7 ± 0.9 years, the proportion of boys and rural residents - 52.6%).

Diagnosis and treatment of bronchial asthma (BA) were based on the protocol and adapted clinical guidelines approved by the Ministry of Health of Ukraine on October 8, 2013, № 868 and recommendations of international harmonization guidelines (GINA)[12] and their subsequent versions. Comprehensive laboratory and instrumental examination of patients was performed in the exacerbation and remission of the disease. The duration of the disease in children with bronchial asthma at the beginning of the monitoring was on average 4.6 ± 0.24 years.

Asthmatic status according to the latest version of GINA is defined as a qualitatively new condition that accompanies a long and resistant course of a severe asthma attack (severe attack with the possibility of death), thus given the longitudinal nature of our observation, clinical group III will be conditionally marked as the one with the debut of the disease in the form of "asthmatic status".

To obtain sputum, a procedure was performed to induce its discharge by inhalation of serial hypertonic solutions of sodium chloride according to the method of Pavord I.D. and Pizzichini M.M.[13]. The eosinophilic type of inflammation is indicated by the presence of 3.0% or more eosinophilic leukocytes in the sputum. The non-eosinophilic nature of bronchitis was diagnosed with a relative content in the cytogram of cell sediment less than 3% of eosinophils or their absolute absence. Appropriate protocols were used to study the cytological composition of sputum[14,15]. The activity of oxygen-dependent metabolism of neutrophilic and eosinophilic granulocytes in peripheral blood was assessed by the histochemical method according to spontaneous and stimulated tests with nitro blue tetrazolium staining (NBT test) by the method of Park H.B. et al. The test results were evaluated by the percentage of pharmazan-positive cells (in%) and histochemical index.

The results of the study were analyzed by biostatistics and clinical epidemiology. In the normal distribution and large sample groups, parametric methods of analysis were used, and in small samples - non-parametric. Statistical analysis was performed using Statistica 8.0 StatSoft Inc. The population analysis assessed attributive (AR) and relative risk (RR), as well as the odds ratio (OR) with the calculation of confidence intervals for relative risk and odds ratio (95% CI).

RESULTS

It is shown that the allergic form of asthma occurred in 56.0% of patients of group I, in 32.6% of cases of group II and 57.9% of patients of group III (p I, III: II < 0.05), and mixed form - in 44.0%, 67.4% and 42.1% of children, respectively (p I, III: II < 0.05). However, the onset of the disease in children under 3 years of age (phenotype of early-onset asthma) was significantly more common in patients of group III, and after 6 years (phenotype of late-onset asthma) - in patients of clinical groups I and II. According to the severity of bronchial asthma, it was found that the representatives of the III clinical group, compared with other patients, significantly more often had a severe course of the disease, and the ratio of the chances of severe asthma in the future for these children compared with cohort group I was 6.8 (95 % CI: 3.59-12.81), relative risk 2.4, attributive risk 44.2% with a plausibility ratio of 3.1.

To assess the nature of bronchitis, a cytomorphological analysis of the cellular composition of induced sputum in children of the comparison groups was conducted (Table 1).

Table 1

The Cellular Composition of Sputum in Patients with Bronchial Asthma in the Dynamics of Observation (P ± m)

Patients group	Cellular Composition, %				
	eosinophils	neutrophils	lymphocytes	macrophages	epitheliocytes
Reference values	0,8 ± 0,06	56,0 ± 2,96	4,8 ± 0,89	38,3 ± 3,95	19,7 ± 5,44
At the beginning of observation					
I group	7,5 ± 1,14	53,4 ± 2,12	8,7 ± 0,90	23,9 ± 1,85	35,0 ± 1,99
II group	8,2 ± 1,18	60,9 ± 4,27	6,7 ± 1,55	22,8 ± 3,02	41,5 ± 5,16
III group	3,1 ± 1,25	68,2 ± 5,66	9,1 ± 3,30	17,1 ±2,92	35,9 ± 2,31
Pt	I,II:III < 0,05	I:III < 0,05	> 0,05	I:III < 0,05	> 0,05
At the end of observation					
I group	10,1 ± 2,62	55,3 ± 4,08	8,2 ± 1,58	18,4 ± 3,13	27,3 ± 3,55
II group	9,0 ± 3,44	58,9 ± 8,69	4,5 ± 1,72	10,5 ± 2,54	27,8 ± 6,07
III group	2,0 ± 0,89	51,8 ± 7,11	4,2 ± 1,69	6,8 ± 1,08	22,4 ± 10,53
Pt	I,II:III < 0,05	> 0,05	> 0,05	I:III < 0,05	> 0,05

* Note: Pt – Student's criterion

For patients of the I clinical group in the debut is charaeterized by increased eosinophils and decreased neutrophil eounts in sputum, for patients of group II - increased eosinophils and epitheliocytes, but a decrease in lymphocytes, and for children of clinical group III - low eosinophils sputum with a simultaneous increase in neutrophils.

Thus, the results indicate that in patients with pneumonia in the onset of asthma, the local inflammatory process of the bronchi was characterized by a hypergranulocytic inflammatory phenotype with the most pronounced desquamative processes of the respiratory epithelium. Patients in whom asthma debuted from asthmatic status were characterized by a predominance of the neutrophil variant of inflammation with lymphocytic infiltration and a decrease in the pool of macrophages, indicating a violation of the protective function of these cells [16]. Based on the data obtained, it can be argued that markers of the cellular composition of sputum in asthma patients can be used for diagnostic and prognostic purposes, in the first case - to exclude concomitant pneumonia, in the second - to verify deep inflammatory processes in the airways with their remodeling.

In the process of dynamic observation, it was found that as a result of the basic anti-inflammatory treatment there were reliable changes in the cellular composition of sputum of patients of clinical comparison groups. In particular, significant reduction in desquamative-inflammatory processes in the respiratory system was achieved, as evidenced by a decrease in the percentage of epitheliocytes in the mucosa of patients of group I by 1.3 times, patients of group II - by 1.5 times and in children with asthma debuted from asthmatic status - 1.6 times. This was accompanied by a decrease in the activity of macrophages, the severity of which was highest in representatives of the second clinical group (2.2 times decrease) and patients of the third comparison group (2.5 times decrease) and a decrease in lymphocyte content of 1.5 and 2.2 times.

According to the modern study [16], the process of persistent allergic inflammation in the bronchi is superseded by morphological changes in the form of remodeling and insensitivity to bronchodilator drugs. Based on the above, we believe it relevant to analyze the biomarkers for the remodeling in patients' sputum, to optimize the assessment of the effectiveness of the treatment and the prognosis of the BA for children with different onset of the disease. Based on the literature data on possible markers of such irreversible changes in the bronchi [17-18], we studied the content in the supernatant of sputum of the examined children of endothelial vascular growth factor (VEGF), eosinophilic cationic protein (ECP), cationic proteins of eosinophils, matrix metalloproteinase (MMP-9) and interleukins-6, -13 (Table 2).

Table 2

Sputum Biomarkers in Children of Comparison Groups (M ± m)

Clinical groups	VEGF, pg/ml	ECP, ng/ml	MMP-9, ng/ml	IL-6, pg/ml	IL-13, pg/ml
I group	123,9 ± 10,69	2,2 ± 0,29	5,7 ± 0,47	8,1 ± 0,74	29,4 ± 4,39
II group	110,4 ± 6,49	2,4 ± 0,56	4,5 ± 1,20	9,0 ± 2,42	40,3 ± 11,05
III group	174,5 ± 25,50	0,6 ± 0,06	1,8 ± 0,70	4,4 ± 0,60	3,3 ± 0,25
Pt	I,II:III < 0,05	I,II:III < 0,05	I,II:III < 0,05	I,II:III < 0,05	I,II:III < 0,05

* Note: Pt – Student's criterion

Note: VEGF - vascular endothelial growth factor, MMP-9 - matrix metalloproteinase-9, ECP - eosinophilic cationic protein, IL - Interleukin.

As follows from the presented data, in children with different onset of asthma, we can assume the presence of a multivariate nature of airway remodeling in patients of clinical comparison groups. In particular, a statistically significant increase in the content of VEGF, a decrease in the content of cationic proteins, MMP-9, and interleukins-6, and -13 in the sputum indicates the predominance of neoangiogenesis in children of clinical group III. Instead, in the representatives of the II clinical group the remodeling processes were mainly caused by the inflammatory process with the release of intracellular eosinophilic cationic proteins. At the same time, in patients in whom asthma debuted as a result of repeated episodes of obstructive bronchitis, airway remodeling was caused by structural rearrangements of the epithelial-mesenchymal unit with the accumulation of matrix metalloproteinase-9 in the sputum. Therefore, the identified changes may indicate the presence of phenotypic heterogeneity of the chronic inflammatory process in the airways in children with an alternative onset of asthma, which, in turn, justifies the relevance of the development of a personalized approach to basic anti-inflammatory treatment.

CONCLUSIONS

1. These data indicate the discrete nature of the type and severity of the inflammatory process of the respiratory tract in the dynamics of observation in children of clinical comparison groups, which suggests the presence of certain phenotypic differences due to the alternative onset of the disease, which in turn was determined by different triggers.
2. It was found that recurrent obstructive bronchitis in the onset of asthma is associated with the eosinophilic nature of the inflammatory process, the accumulation in the mucosa of sputum MMP-9 as a marker of bronchial remodeling. And the pneumonia process in the onset of bronchial asthma was accompanied by the hyper-granulocytic character of the inflammatory response. The phenotype of asthma, which debuted from the clinical manifestation of asthmatic status, is associated with the neutrophilic nature of the inflammatory process with a 2.2-fold increase in the concentration of endothelial vascular growth factor in sputum.

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