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Peculiarities of inflammatory response in schoolchildren suffering from bronchial asthma

Key words: *children, bronchial asthma, inflammatory blood pattern, induced sputum.*

Nowadays investigations of phenotypes and endotypes of bronchial asthma in children are being continued considering extension of this pathology in the whole world and topicality to search for new methods of monitoring the disease [2].

One of the most popular scientific areas is investigation of the character of inflammatory response of the respiratory tract to bronchial asthma phenotype by the character of inflammation [2,3]. It is due to implementation of examinations of bronchial secretion that distribution into eosinophilic and non-eosinophilic/neutrophilic phenotypes of bronchial asthma has become possible. Other distribution of the phenotypes of the disease have been suggested as well: eosinophilic, neutrophilic, poikilocytic (with normal interrelations of the cellular elements) and mixed – hypergranulocytic (with an increased content of neutrophilic and eosinophilic granulocytes) [7].

Investigation of the cytological content of induced sputum can be considered as a «gold standard» to detect the character of inflammation of the respiratory tract in patients suffering from bronchial asthma, although it is rather complicated and time-consuming process, especially among the cohort of children. At the same time, there are certain difficulties concerning interpretation of indices of the obtained bronchial secretion, as the standard indices in children are limited and disputable [1]. In clinical practice more available method is detection of inflammatory phenotypes by the content of circulating granulocytes in the peripheral blood. Since morphofunctional characteristics of eosinophils and neutrophils of the bronchi and blood are similar, a number of researchers suggest to carry out monitoring of the character of inflammation according to the indices of granulocyte content in the peripheral blood enabling to select an individual monitoring treatment for children suffering from bronchial asthma [3, 11].

Examination of the inflammatory blood patterns in patients with bronchial asthma enabled to detect that

frequent night attacks of the disease, severe course of the disease requiring administration of high doses of inhalation glucocorticosteroids is associated with hypergranulocyte inflammatory blood pattern [4]. On the one hand, indication of high doses of inhalation glucocorticoids for such patients results in apoptosis of eosinophils, and on the other hand, continues life of neutrophilic blood granulocytes [5]. Eosinophilic inflammatory phenotype of bronchial asthma (by the indices of granulocyte content in the peripheral blood) is characterized by positive skin tests with non-bacterial allergens, increased content of specific IgE in the blood, eosinophilia of the blood, decreased forced expiratory volume (FEV) per 1 second [4,8]. Neutrophilic inflammatory pattern of the blood in patients with bronchial asthma is characterized by more severe course of the disease, which is difficult to be managed by means of standard anti-inflammatory therapy, as well as less pronounced allergic reactivity, moderate results of skin tests with allergens, fixed obstruction of the respiratory tract, moderate response to broncholytic therapy, prevailing effect of environmental factors and development of inflammation mediated through macrophages and epithelial cells [9].

The area of our investigation was stipulated by the fact that interrelations between effector cells of allergic inflammation in induced sputum and granulocytes in the peripheral blood in case of bronchial asthma are disputable and insufficiently studied.

Objective. With the purpose to improve a comprehensive treatment of children suffering from bronchial asthma, to carry out a retrospective examination of cytological peculiarities of induced sputum in children depending on inflammatory patterns of the blood.

Materials and methods

To achieve the purpose a comprehensive clinical-immunological examination of I-II levels of 120 children

suffering from bronchial asthma (BA) was conducted. The following indices were studied: cellular and humoral immunity, the content of T-lymphocytes and their subpopulations in the peripheral blood, levels of A, M, G, E immunoglobulins and interleukin-4, -5, -8 in the blood serum. During the period of the disease free from attacks cytological analysis of sputum was made [10], obtained by means of induction method using serial dilution of hypertonic solutions (3 %, 5 %, 7 %) of sodium chloride according to the protocol suggested by I. Pin in modification of I.D. Pavord and M.M. Pizzichini.

Depending on the content of granulocytes in the peripheral blood four clinical groups were formed. The first group (I) included 34 children suffering from BA with hypogranulocytic pattern of inflammation (the content of eosinophils in the blood < 250 cells/mm³ and neutrophils < 5000 cells/mm³); an average age of children was 13,7±2,6, boys constituted 64,7 %. The second group (II) included 60 children suffering from BA with eosinophilic pattern of the peripheral blood (the content of eosinophils ≥250 cells/mm³) (an average age was 12,8±2,9, boys constituted 70,0 %). The third group (III) included 14 children with neutrophilic pattern (the content of neutrophils ≥5000 cells/mm³) (an average age 12,6±2,7, boys constituted 64,3 %), and the fourth group (IV) was formed with 12 children with hypergranulocytic pattern of inflammatory response (the content of eosinophils in the blood ≥ 250 cells/mm³ and neutrophils ≥ 5000 cells/mm³) (an average age – 14,9±1,9; a part of boys was 58,3 %). The groups of observation were comparable by the main clinical characteristics.

The results obtained were analyzed from the positions of biostatistics and clinical epidemiology by means of computer package «Statistica7» StatSoft Inc. and Excel XP for Windows, the difference of indices was considered statistically significant with $p < 0,05$. Relative risk (RR) and odds ratio (OR) of a certain event development were detected from the positions of clinical epidemiology. Selection and examination of patients correspond to the principles of biomedical ethics in pediatrics.

Results and discussion

Considering numerous controversial issues as to the choice of an optimal marker of respiratory tract inflammation, in addition to detection of inflammatory patterns of the blood, cytological analysis of induced sputum was conducted which results are presented in Table 1.

Considering the fact that maximal relative content of eosinophilic leukocytes in sputum of healthy children is no more than 2 %, and the main sputum cells are lung mononuclear leukocytes usually constituting 80-90 % [1], we have detected that in children of clinical groups of observation a relative pool reduction of normal lung protection cells was found – alveolar macrophages with simultaneous increase of cells associated with inflammatory reaction of the bronchi in case of BA: lymphocytes, eosinophilic and neutrophilic granulocytes.

A detailed analysis of the cytological content of sputum was indicative that the content of alveolar macrophages in sputum among the patients of the examined groups did not differ considerably, although the highest indices of a relative content of these cells occurred in patients with neutrophilic and hypergranulocytic inflammatory patterns of the blood. Thus, a relative content of alveolar macrophages in sputum over 33 % was found in half of the patients from IV group (50,0 %) and in 8,4 % ($p < 0,05$), 30,0 % ($p > 0,05$) and 38,1 % ($p > 0,05$) cases among the representatives of I, II and III clinical groups respectively. The results obtained are indicative of a prevailing expressiveness of inflammatory process in patients from III and IV groups and prove literature data concerning the role of macrophages in realization of remodeling processes of the respiratory tract in case of bronchial asthma [4].

Considering disputable evidence as to the involvement of neutrophilic and/or eosinophilic granulocytes in the development of bronchial remodeling in patients with bronchial asthma [9,11], we have evaluated appropriate indices in induced sputum of the patients from the examined groups. More pronounced neutrophilia of the bronchial secretion was found to be characteristic for the representatives of III clinical group. Thus, a relative content of neutrophils more than 65 % was registered in half (50 %) of the children with neutrophilic character of inflammatory pattern of the blood, while in patients from I, II, IV groups – only 14,3 % ($p < 0,05$), 13,5 % ($p < 0,05$) and 34,4 % ($p < 0,05$) cases respectively. The indices of registration risk over 65 % of neutrophils in induced sputum of children suffering from bronchial asthma associated with neutrophilic inflammatory pattern of the blood as compared to hypogranulocytic pattern were the following: relative risk – 2,1 [95 %CI:1,2-3,5] with odds ratio – 5,9 [95 %CI:3,0-11,9], and concerning eosinophilic inflammatory pattern of the blood: relative risk – 2,1 [95 %CI:1,3-3,7] with odds ratio – 6,4 [95 %CI:3,2-12,8].

Cytological content of sputum in children from clinical groups of comparison (M ± m)

Table 1

Clinical groups	Cytological content of sputum, %				
	Eosinophils	Neutrophils	Lymphocytes	Alveolar macrophages	Cast-off epithelium
I group	11,2 ± 4,2	49,9 ± 3,5	9,2 ± 4,0	27,5 ± 3,6	34,6 ± 6,8
II group	10,6 ± 2,3	42,7 ± 8,3	8,5 ± 1,9	18,1 ± 6,0	38,1 ± 2,4
III group	12,3 ± 3,4	58,4 ± 5,4	13,3 ± 5,2	30,6 ± 3,4	38,1 ± 6,7
IV group	15,1 ± 9,6	51,3 ± 4,0	10,3 ± 2,3	33,0 ± 10,6	46,4 ± 3,9
p	> 0,05	> 0,05	> 0,05	> 0,05	> 0,05

We have found that higher amount of eosinophils (> 3 %) in induced sputum was registered among representatives of I, II, III and IV clinical groups in 52,4 %, 65,9 %, 66,7 % and 75 % cases respectively. Although, the results of the studies were indicative that more pronounced eosinophilic of the respiratory tract was characteristic for patients with eosinophilic and hypergranulocytic inflammatory pattern of the blood. Thus, relatively high content of eosinophils (over 15 %) in sputum was found in more than one third of patients from IV group (37,5 %) and every fourth child from II group (24,4 %) and only in every sixth patient from I (14,3 %; $p < 0,05$) and III (16,7 %; $p < 0,05$) groups respectively. The indices of registration risk over 15 % of eosinophils in induced sputum of children suffering from bronchial asthma associated with hypergranulocytic inflammatory pattern of the blood as compared to hypogranulocytic pattern were the following: relative risk – 1,7 [95 %CI:0,9-2,9] with odds ratio – 3,6 [95 %CI:1,8-7,2], and concerning the neutrophilic inflammatory pattern of the blood: relative risk – 1,6 [95 %CI:0,9-2,7] with odds ratio – 2,9 [95 %CI:1,5-5,8].

A relative content of lymphocytes in sputum did not differ considerably as well in patients from the clinical groups of comparison, although it was 3-4 times higher than the regional normal index ($3,1 \pm 0,6$) % [1]. Therefore, irrespective of peculiarities of the blood patterns subpopulations of T-lymphocytes were involved in the development of respiratory inflammation in patients from the clinical groups, which is connected with reduced indices of the external respiration and forced expiratory volume (FEV) per 1 second [6].

Further analysis enabled to detect the tendency to an increased amount of cast-off epithelium in sputum of children with hypergranulocytic variant of blood inflammation which according to literature data [12] is a marker of activity of bronchial mucus damage due to their inflammation. Thus, the content of epithelium more than 46 % in children of IV group was registered in 52,4 % patients, and in I, II, III groups of comparison – only in 25 % ($p < 0,05$), 24,4 % ($p < 0,05$) and 33,4 % ($p > 0,05$) of cases respectively.

Therefore, in children suffering from bronchial asthma availability of hypergranulocytic inflammatory pattern of the blood was associated with the most pronounced indices of respiratory damage with involvement of eosinophilic-neutrophilic-macrophage inflammation and the signs

of an increased risk of bronchial remodeling due to more intensive damage of the respiratory mucous membranes, which in its turn requires an «aggressive» tactic of basic anti-inflammatory therapy. In patients with neutrophilic inflammatory pattern of the blood damage of the epithelial layer of the respiratory tract occurred due to their neutrophilic-macrophage-lymphocytic inflammation that can stipulate the requirement in administration of drugs which action is directed to inhibition of neutrophil-mediated inflammatory response. In schoolchildren suffering from bronchial asthma with eosinophilic inflammatory pattern granulocyte indices correspond to the similar ones in induced sputum with participation of eosinophilic-macrophage-lymphocytic mechanism of damage of the bronchial mucous membranes which promoted efficacy of a standard anti-inflammatory therapy directed mainly to eosinophilic variant of inflammatory process of the respiratory tract. Hypogranulocytic inflammatory pattern of the blood in children suffering from bronchial asthma was associated with the lowest indices of inflammatory response of the respiratory tract.

Conclusions

In children suffering from bronchial asthma availability of hypergranulocytic inflammatory pattern of the blood was associated with eosinophilic-neutrophilic-macrophage inflammation of the respiratory tract.

Registration chances over 15 % of eosinophils in induced sputum of children suffering from bronchial asthma in association with hypergranulocytic inflammatory pattern of the blood as compared to hypogranulocytic pattern were 3,6 times higher, as compared to neutrophilic pattern – 2,9 times higher.

In patients with neutrophilic inflammatory pattern of the blood damage of the epithelial layer of the respiratory tract was accompanied by the development of neutrophilic-macrophage-lymphocytic inflammation.

Registration chances over 65 % of neutrophils in induced sputum of children suffering from bronchial asthma associated with neutrophilic inflammatory pattern of the blood as compared to hypogranulocytic pattern become 5,9 times higher, and as compared to eosinophilic inflammatory pattern of the blood – 6,4 times higher.

In schoolchildren suffering from bronchial asthma with eosinophilic pattern of the blood damage of the bronchial mucous membrane occurred with participation of eosinophilic-macrophage-lymphocytic mechanism.

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