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МИТСАНИ М., ОРТЕМЕНКА Е.П.

ХАРАКТЕР ВОСПАЛЕНИЯ ДЫХАТЕЛЬНЫХ ПУТЕЙ ПРИ ФЕНОТИПЕ ТЯЖЕЛОЙ БРОНХИАЛЬНОЙ АСТМЫ У ДЕТЕЙ ШКОЛЬНОГО ВОЗРАСТА

Буковинский государственный медицинский университет, г. Черновцы, Украина

Кафедра педиатрии и детских инфекционных болезней

Резюме. *На базе областной детской клинической больницы г. Черновцы у 43 школьника, страдающих персистирующей бронхиальной астмой, изучали характер воспаления дыхательных путей с целью индивидуализации выбора противовоспалительной базисной терапии. Первую (I) клиническую группу составили 24 ребенка с фенотипом тяжелой астмы, во вторую (II) группу вошли 19 детей со среднетяжелым течением. Цитологический анализ индуцированной мокроты проводился методом Pavord I.D.*

Больным тяжелой бронхиальной астмой присуща гиперэозинофильная реакция дыхательных путей. Так, значительная эозинофилия бронхиального секрета (эозинофилов ≥12% в мокроте) определялась у 29,2% пациентов с тяжелой астмой и только в 10,5% случаев (Рφ<0,05) во II группе. При этом, относительный умеренный лимфоцитоз дыхательных путей (лимфоцитов ≥11% в мокроте) зарегистрирован у 31,6% пациентов II группы и у 25,0% школьников с тяжелой астмой (Рφ>0,05). Количество цилиндрических эпителиоцитов в бронхиальном секрете ≥50% отмечено у 29,3% больных I и у 26,3% детей II групп сравнения (Рφ>0,05). Для школьников с тяжелой астмой характерен гиперэозинофильно-умеренно-лимфоцитарный вариант воспаления дыхательных путей, ассоциирующий с их более значительным ремоделингом, что, в целом, указывает на необходимость назначения высоких доз ингаляционных глюкокортикостероидов в комбинации с «антиэозинофильными» (антилейкотриены, блокаторы иммуноглобулина Е) противовоспалительными препаратами.

Ключевые слова: *бронхиальная астма, дети, фенотипы, цитологическая картина мокроты.*

MARIA MITSANI, YE.P. ORTEMENKA

CYTOLOGICAL PROFILE OF INDUCED SPUTUM IN SCHOOLAGE CHILDREN WITH SEVERE ASTHMA PHENOTYPE

Bukovinian State Medical University, Chernivtsi, Ukraine

The Department of Pediatrics and pediatric infectious diseases

Abstract. *On the base of the pulmonology department of the Chernovtsy Regional Children Clinical Hospital, the type of airway inflammation hah been studied in 43 school age children with persistent bronchial asthma with the aim to individualize basic anti-inflammatory therapy approach. The first (I) clinical group has been formed from 24 children with severe asthma phenotype. The second (II) clinical group included the remaining 19 patients with moderate bronchial asthma. A cytological analysis of induced sputum has been performed by the method of Pavord I.D.*

It has been observed that the hypereosinophilic response of airways has been more common for patients with severe asthma. Thus, a significant eosinophilia of bronchial secretions (number of eosinophilic granulocytes ≥12% in induced sputum) was determined only in 10,5% of cases in the II-nd group, however, was recorded in every third patient (29,2%) with severe asthma phenotype (Pφ<0,05). At the same time, a relatively moderate lymphocytosis of airways (lymphocyte counts in sputum ≥11%) has been observed in 31,6% of patients of the I-st clinical group and in 25,0% of school-aged children with severe asthma (Pφ>0,05). The number of cylindrical epithelial cells in bronchial secretion ≥50% was recorded in every third (29,3%) patients with severe asthma, and only in a quarter (26,3%) of patients of the II-nd comparison group (Pφ> 0,05). The severe asthma phenotype in school-age children characterized by mixed (hypereosinophilic response with moderate lymphocytic reaction) type of airways inflammation, associated with expressive damage of bronchial epithelial layer. Such changes require a combination of high-dose inhaled corticosteroids with anti-inflammatory drugs, action which is aimed at the additional suppression of the eosinophil-mediated inflammatory response (IgE blockers, leukotriene modifiers, chromones).

Keywords: *severe asthma, children, phenotypes, induced sputum.*

Introduction. An inadequate control of bronchial asthma (BA) is often associated with a severe, resistant to standard basic therapy, phenotype of disease [1,5,6].

Risk factors of severe asthma include some triggers action (smoking, exposure of allergens or pollutants, viral infection, stress), as well as a lack of compliance or the presence of concomitant diseases [2,4]. While, it is believed that the severe asthma phenotype caused by genetic determinism of the individual insensitivity to corticosteroids phenomenon [12].

At the same time, heterogeneity of the airways inflammation (eosinophilic, noneosinophilic, paucigranulicytic) is one of the aspects of inadequate asthma control [4,9].

Published data regarding the association of quantitative and qualitative characteristics of

induced sputum with the severity of the disease are still conflicting. Thus, according to some authors, the number of eosinophils in induced sputum has no correlation with the asthma severity and spirometric indices [3], but there was published the opposite position [7]. Researches of M. Zedan indicated an association of severe, resistant to the treatment of brittle-asthma phenotype with atopic form of the disease and eosinophilic pattern of bronchial inflammation [5].

Meanwhile, scientific sources accumulated data about the role of neutrophil leukocytes in the processes of bronchial remodeling [8] and associations of neutrophilic airways inflammation with decreased elasticity of the alveoli and goblet cells hyperplasia [11]. At the same time, increased count of neutrophils in the airways was registered in late phase of allergic reactions, [5] in patients with "fatal" and "nocturnal asthma" [10], as well as in steroid resistant asthma patients [8, 11].

It should be noted that most studies of airways' inflammatory subtypes were conducted among adults, but data about particularities of the chronic airway inflammation in children with severe asthma are limited [4, 8, 10, 11].

The aim. To study the particularities of the cytological profile of induced sputum in school-age children with severe bronchial asthma.

Material and Methods. In pulmonology department of the Chernovtsy Regional Children Clinical Hospital 43 school age children with persistent BA have been examined. A cytological analysis of induced sputum obtained by inhalation of serial dilutions of hypertonic solutions (3%, 5%, 7%) of sodium chloride has been performed to all children by the method of Pavord I.D. in modification of Pizzichini M.M. (1996). Viability of cells in sediment of induced sputum was detected by trypan blue exclusion method. A quota of epithelial cells was calculated from the total number of cells, the percentage of inflammatory cells (granulocytes, lymphocytes, alveolar macrophages) in induced sputum was detected by analysis of 200 cells, despite of epithelial cells [13].

The first (I) clinical group has been formed from 24 children with severe asthma phenotype. The second (II) clinical group included the remaining 19 patients with moderate BA.

The main clinical characteristics of the comparison groups were comparable. Thus, average age of patients of the 1st group was 12,2 years, and among them there were 66,7% of males and 66,7% of rural residents. The number of boys in the 2nd group was 68,4% (Pt >0,05), and mean age of patients was equal to 11,1 years (Pt>0,05), and at the same time the rate of rural residents was 63,2%, (Pt >0,05).

These survey results were analyzed by the methods of biostatistics and clinical epidemiology, using the software package "STATISTICA 7.0" StatSoft Inc. and Excel XP for

Windows on a PC, by parametric (Pt, Students' criteria) and nonparametric (Pu, Mann-Whitney U test; Pφ, Fisher's angular transformation) methods of calculation.

Results and discussion. The percentage of viable cells as a marker of the accuracy of obtaining sputum and its validity for further analysis did not differ significantly in clinical groups of comparison and corresponded to published data [13].

An analysis of induced sputum in children with severe asthma phenotype in comparison with patients with moderate disease course has been presented in Table 1.

Table 1

Induced sputum cell counts in asthma subjects, M±m (minimal – maximum data)

Indices of sputum analysis, %	I group (n=24)	II group (n=19)	P
Viability of cells	73,5±3,2 (37-96)	71,3±3,4 (37-95)	Pt >0,05
Eosinophils	7,3±1,4 (0-22)	4,1±1,8 (0-34)	Pu<0,01
Neutrophils	51,0±3,9 (12-82)	56,0±6,7 (5-96)	Pt =0,05
Macrophages	33,0±4,9 (2-80)	28,2±6,4 (1-93)	Pt >0,05
Lymphocytes	10,9±2,9 (1-51)	11,2±3,7 (0-68)	Pt >0,05
Epithelial cells	41,7±3,3 (20-80)	43,8±4,3 (20-89)	Pt >0,05

Comments. Pt - Students' criteria, Pu - Mann-Whitney U test

Based on the fact that in healthy children sputum a relative content of eosinophils does not exceed 2%, the percentage of neutrophils and lymphocytes an average is 46,4% and 3,1% respectively, but average share of pulmonary mononuclear cells is 50,3% [1], in children of both clinical groups has been defined a relative decrease of the cell pool of such natural pulmonary defense as alveolar macrophages with simultaneous increase of inflammatory cells (lymphocytes and granulocytes).

Despite numerous literature reports on the association of severe asthma phenotype in adult patients with neutrophilic type of airway inflammation [6, 7, 10], significant relative neutrophilia of bronchial secretions has been determined more often in children with moderate bronchial asthma. Thus, significant amount (>80%) of neutrophilic leucocytes in induced sputum has been registered in every forth (26,3%) patients with moderate asthma, but only in 8,3% cases in the I-st group of observation (Pφ<0,05).

The higher than normal (≥3%) count of eosinophils in induced sputum has been observed in the majority (70,8%) of patients with severe asthma, but only in a quarter (26,3%) of patients in the II comparison group (Pφ<0,01). However, the almost complete absence (0-1%) of eosinophilic granulocytes in induced sputum was recorded in the majority of patients with moderate asthma (58,0%) and only in two patients (8.3%)of a comparison group (Pφ<0,01).

In patients with severe asthma expressive hypereosinophilic response of airways has been observed. Thus, a significant eosinophilia of bronchial secretions (number of eosinophilic

granulocytes ≥12% in induced sputum) was determined only in 10,5% of cases in the II-nd group, however, was recorded in every third patient (29,2%) with severe asthma phenotype (Pφ<0,05).

There has been registered an expressive lymphocytic inflammatory reaction of airways among patients of both comparison groups. Thus, the relative content of lymphocytes in induced sputum was three times higher than the normal local rate (3,1±0,6%) as in children with severe asthma phenotype (10,9 ± 2,9%; Pt <0,05) and in patients with moderate bronchial asthma (11,2±3,7%; Pt <0,05). At the same time, a relatively moderate lymphocytosis of airways (lymphocyte counts in sputum ≥11%) has been observed in 31,6% of patients of the I-st clinical group and in 25,0% of school-aged children with severe asthma (Pφ>0,05).

Severe asthma phenotype has been associated with significant damage to bronchial epithelium that has been expressed by increased number of desquamated epithelial cell in cellular sediment of the induced sputum. Thus, the number of cylindrical epithelial cells in bronchial secretion ≥50% was recorded in every third (29,3%) patients with severe asthma, and only in a quarter (26,3%) of patients of the II-nd comparison group (Pφ> 0,05).

It has been observed that severe asthma phenotype in school-age children characterized by mixed (hypereosinophilic response with moderate lymphocytic reaction) type of chronic airways inflammation, associated with expressive damage of bronchial epithelial layer. These results not only confirm the presence of damage to bronchial epithelial layer as a result of chronic inflammation in asthma, but also suggested the possibility of expressive bronchial remodeling in patients with severe asthma with the formation of their rigidity and reduced hiperresponsiveness [11]. Such changes, in our opinion, require a combination of high-dose inhaled corticosteroids with anti-inflammatory drugs, action which is aimed at the additional suppression of the eosinophil-mediated inflammatory response (IgE blockers, leukotriene modifiers, chromones).

Moderate bronchial asthma in school-age children characterized by expressive neutrophilic-lymphocytic inflammatory reaction of airways, associated in a quarter of patients with high neutrophil count but insignificant eosinophilia. Such data, given into account the ability of inhaled corticosteroids (ICS) not only inhibit the activity of lymphocytes and accelerate apoptosis of eosinophils, but also extend the lifespan of neutrophils [7, 8], suggest the necessity to use low or medium doses of ICS combined with so-called "antineutrophil" drugs (sustained release methylxanthines, long-acting beta-2-agonists, macrolides, etc.) for partly controlled or uncontrolled moderate asthma.

Conclusion. The severe asthma phenotype in school-age children characterized by mixed (hypereosinophilic response with moderate lymphocytic reaction) type of airways inflammation, associated with expressive damage of bronchial epithelial layer, which require a combination of

high-dose inhaled corticosteroids with anti-eosinophilic drugs (IgE blockers, leukotriene modifiers, chromones).

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Сведения об авторах:
Митсани М. – студентка 42 группы 6 курса медицинского факультета №3 Буковинского государственного медицинского университета (Украина, 58002, г. Черновцы, Театральная площадь, 2).
Ортеменка Е.П. – к.мед.н., доцент, доцент кафедры педиатрии и детских инфекционных болезней Буковинского государственного медицинского университета (Украина, 58002, г. Черновцы, Театральная площадь, 2).