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# INDUCED SPUTUM ANALYSIS OF INTERLEUKINS SPECTRUM IN CHILDREN WITH PULMONARY DISEASES

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***Abstract**. This article has been performed the investigation of immunity system characteristics in children with bronchitis. There has been identified factors of unfavourable prognosis of bronchitis transformation in other chronic lung diseases, which include disorder of cytokines-synthesis. A study was undertaken to determine the airway and lung inflammation, by analysing cytokines in the induced sputum from 38 children with the acute bronchitis, 35 patients with the acute pneumonia, 15 children with the chronic lung disease, which had lung fibrosis, and 18 healthy children. Sputum levels of interleukin-4 (IL-4), interleukin-6 (IL-6), interleukin-8 (IL-8) and interleukin-10 (IL-10) in all groups were statistically significantly increased compared with normal controls. The increase in the interleukins concentration in induced sputum of patient with bronchitis and pneumonia we can use in clinical as the risk subgroup of patients with chronic lung diseases.*

***Key words:** bronchitis, pneumonia, interleukin*

The underlying mechanisms of lung inflammatory response in children with the chronic lung disease has not yet been completely elucidated [1-3]. We therefore used this technique to evaluate the presence of airway and lung inflammation in children with the acute bronchitis, pneumonia, chronic lung disease. Analysis of sputum induced by inhalation of hypertonic saline has recently been established as a useful non-invasive technique for measuring airway inflammation in patients [4-5].

**Aim and objectives**

Aim is to improve diagnosis of immunological disorders in children with respiratory diseases, which include the study of cellular, humoral immunity and levels of cytokine (IL-4, IL-6, IL-8, IL-10) in induced sputum.

**Material and methods.**

The 106 patients were recruited from Regional Children Clinical Hospital (RCCH), Kharkiv, Ukrainian. The Head of RCCH is Muratov G.R., the head of pediatric department of KNMU is DMedSci, Prof. Senatorova G.S. Children with the acute bronchitis (n=38) aged on average (6,9±2,4) years who had been admitted to the pulmonology department served as group 1. The distribution in the 1 group was as follows: 21(55,0±8,0%) boys and 17(44,7±8,1%) girls. The patient with the acute pneumonia (n=35) aged on average (8,0±2,3) years served as group The constituents average are 19 (54,3±8,4%) boys and 16 (45,7±8,4%) girls. Fifteen children with the chronic lung disease (n=15), aged on average (8,0±2,3) years, which had lung fibrosis, served as group 3. The distribution in the 3 group was as follows: 6 (40±13,1%) boys and 9 (60±13,1%) girls. Healthy controls (n =18) were negative for allergies and respiratory diseases. Respiratory diseases was defined according to the Ukrainian protocol of diagnosis and treatment lung diseases in children. After clinical evaluation and immunology blood testing, induced sputum was collected. To determine the biochemical analysis of sputum induced after inhalation of hypertonic saline, we analyzed sputum induced in children subjects. The sputum was induced with inhalation of ultrasonically nebulized hypertonic (2,7-5%) saline solution. The study was approved by the ethics committee of the Kharkiv national medical university and all parents of children gave informed consent to participate in the study. We performed IL-4, IL-6, IL-8, IL-10 "IFA-Best" as previously described using a monoclonal anti-human interleukins anti-body obtained by ( "IL-4-IFA-Best", "IL-6-IFA-Best", "IL-8-IFA-Best", "IL-10-IFA-Best", Russia) Statistical analysis was performed using „Stadia-6",version „Prof", „Statistica-6".

**Results and discussion**

We first compared IL-4 production of all groups from control subjects. Local IL-4 levels in sputum were higher in the samples of the all cases than in their controls. When compared with sputum from normal subjects sputum of patients with bronchitis (53,8 (32,3; 63,6) pg/ml, $p<0,0001$), with pneumonia (40,9 (20,4; 62,6)

pg/ml, p=0,0041), with chronic lung diseases (55,9 (53,3; 62,6) pg/ml, p<0,0001) contained a significantly higher levels of IL-4. There was statistically significant difference between sputum IL-4 levels of patients with pneumonia and with chronic lung disease (p=0,0431). This observation is inline with reports indicating a significant role of IL-4 in promoting inflammation in the lung. IL-4 increases the expression of other inflammatory cytokines from fibroblasts that might contribute to inflammation and lung remodelling in chronic respiratory diseases.

In patients the median level of IL-6 in sputum of children with bronchitis, with pneumonia and with chronic lung disease and control group were (69,1 (51,6; 85,9) pg/ml, p<0,0001), (49,4 (24,8; 79) pg/ml, p=0,0002), (55,1 (51,4; 60,7) pg/ml, p<0,0001) and 19,9(13,1; 26,3) pg/ml. There were significant differences in the sputum cytokine levels between the subjects of children with bronchitis and pneumonia (p=0,0040) and between the subjects of children with bronchitis and lung fibrosis (p=0,0415). The present data show that production of IL-6 indicating a significant role in the pathogenesis of acute inflammation.

In our study we compared IL-8 production in the sputum from control subjects and from all patients. The median and the interquartile range level of IL-8 in sputum are summarised for each age group: in the group 1 (78,1(60,5;86,5) pg/ml, p<0,0001), in the group 2 (79,4 (53,3; 88,3)pg/ml, p<0,0001), in the group 3 (90,1 (88,3; 93,8) pg/ml, p<0,0001) and in the control group (31,5(19,9; 43,3) pg/ml). Subjects of children with lung fibrosis had a significantly higher concentration of IL-8 in induced sputum than subjects of children bronchitis (p<0,0001) and subjects of children pneumonia (p<0,0001). The concentration of IL-8 in the induced sputum samples differentiated patients with bronchitis and pneumonia from patient with lung fibrosis, and indicated at risk for transformation acute diseases to chronic lung diseases.

IL-10 were significantly increased in induced sputum sample from patients of all groups compared with normal subjects. We found that induced sputum from subjects of patients with bronchitis (49,6 (38,9; 57,3) pg/ml, p<0,0001), with pneumonia (72,6 (59,4; 77,9) pg/ml, p<0,0001), with chronic lung diseases (81,5 (77,6; 85,4) pg/ml, p<0,0001) had a higher concentration of IL-10, compared to control (25,9(16,9; 30,2)

pg/ml). We found that induced sputum from subjects of patients with chronic lung diseases had a higher concentration of IL-10 compared to children with bronchitis ($p<0,0001$) and with pneumonia ($p<0,0001$), respectively. Increasing sputum levels of IL-4, IL-6, IL-8, IL-10 of all groups are indicating a role of cytokines in the remodeling process of the airways and lung.

**Conclusions**

Our results indicate that there is a predominant inflammation in the airways of patients with chronic lung diseases associated cytokines. The present data show that production of IL-4, IL-6, IL-8 and IL-10 in sputum, reflecting upper airway and lung inflammatory responses, was statistically significantly elevated in children with lung fibrosis, as compared to children with bronchitis and pneumonia.

**References.**

1. Strieter R.M. What differentiates normal lung repair and fibrosis? Inflammation, resolution of repair, and fibrosis/ R.M. Strieter // Proceedings of the American Thoracic Society. - 2008. - Vol.5, №3. - P. 305–310.

2. Mazur W. Comparison of 8-Isoprostane and Interleukin-8 in Induced Sputum and Exhaled Breath Condensate from Asymptomatic and Symptomatic Smokers/ W. Mazur, H. Stark ,VL. Kinnula // Respiration.- 2009.- Vol.№78. - P. 209–216.

3. Induced sputum in children with newly diagnosed mild asthma: the effect of 6 months of treatment with budesonide or disodium cromoglycate / P. Rytila, A.S. Pelkonen, T. Metso [et al.] // Allergy. - 2004.-Vol. 59, № 8. - P. 839–844.

4. Weiszhar Z. Induced sputum analysis: step by step / Z.Weiszhar, I. Horvath // Breathe. - 2013.- Vol.9(4).-P. 300-306.

5. Sagel S.D. Sputum Biomarkers of Inflammation in Cystic Fibrosis Lung Disease/ S.D. Sagel, J.F. Chmiel, M.W. Konstan // Proceedings of the American Thoracic Society.- 2007. – Vol. 4, №4 – P.406-417.

**Резюме.** У статті наведені результати дослідження показників імунної системи у дітей з бронхітами. Були відображені фактори трансформації бронхітів в хронічну бронхолегеневу патологію, включаючи порушення продукції

цитокінів. Дослідження визначило умови персистенціїї бронхолегеневого запалення, за допомогою вивчення концентрації цитокінів в індукованому мокротинні у 38 дітей з гострими бронхітами, у 35 дітей з пневмоніями, у 15 дітей з хронічною бронхолегеневою патологією та пневмофіброзом, та у 18 здорових дітей. При порівнянні з показниками групи контролю у пацієнтів всіх досліджуваних груп відзначалися статистично достовірно підвищені рівні інтерлейкінів (ІЛ-4, ІЛ-6, ІЛ-8, ІЛ-10) в індукованому мокротинні. Підвищення концентрації інтерлейкінов у індукованому мокротинні у дітей з бронхітами та пневмоніями, можливо використовувати в якості виявлення групи ризику у формуванні хронічної бронхолегеневої патології.
**Ключові слова:** бронхіт, пневмонія, інтерлейкін

**Резюме.** В статье представлены результаты исследования показателей иммунной системы у детей с бронхитами. Были выявлены факторы неблагоприятного прогноза и трансформации бронхитов в хроническую бронхолегочную патологию, включающие нарушение продукции цитокинов. Исследование определило факторы, способствующие персистенции бронхолегочного воспалительного процесса, с помощью изучения концентрации цитокинов в индуцированной мокроте у 38 детей с острыми бронхитами, у 35 детей с пневмониями, у 15 детей с хронической бронхолегочной патологией, имеющих пневмофиброз и у 18 здоровых детей. При сравнении с показателями группы контроля отмечалось достоверное повышение уровней интерлейкинов (ИЛ-4, ИЛ-6, ИЛ-8, ИЛ-10) в индуцированной мокроте во всех исследуемых группах. Повышение концентрации интерлейкинов в индуцированной мокроте у пациентов с бронхитами и пневмониями, можно использовать в качестве выявления группы риска формирования хронической бронхолегочной патологии.
**Ключевые слова:** бронхит, пневмония, интерлейкин.

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