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## CC16 ROLE IN BRONCHIOLITIS IN YOUNG CHILDREN

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### Summary

**Introduction.** CC16 protein is secreted by Club epithelial cells of the bronchioles, maintains homeostasis of the airway epithelium and has anti-inflammatory effects in the lungs. It is important to study the level of CC16 protein in the serum in order to understand the integrity of the bronchial epithelium and the development of bronchial dysfunction in young children with bronchiolitis.

**Aim of the study.** Analysis of blood serum CC16 concentration in younger bronchiolitis patients.

**Material and methods.** We clinically examined 70 young children. The main group consisted of 35 non-allergic bronchiolitis patients. The comparison group included 25 young bronchiolitis patients with a history of allergies. The control group comprised 10 conditionally healthy children. The average age of patients was  $8.4 \pm 1.6$  months,  $6.2 \pm 1.4$ , and  $6.4 \pm 1.2$  months in the main, comparison, and control group, accordingly. The complex of clinical- and laboratory examination of children included CC16 serum tests. Serum CC16 content was determined by enzyme-linked immunosorbent assay according to the "Human CC16 ELISA Kit".

**Results of the study.** It should be noted that the most main group patients (22 (62.8 ± 6.4%) of all examined) had elevated CC16 readings. Whereas in the comparison group we did not find an increase in CC16 protein in any case. In contrast, 13 (51.8 ± 14.4%) children with bronchiolitis with a history of allergy had a decrease in CC16 protein, which may be a sign of endothelial dysfunction ( $p = 0.01$ ).

The study showed that young non-allergic bronchiolitis patients had the mean CC16 ( $38.9 \pm 4.5$  ng/ml) significantly higher than those with a history of allergies ( $22.9 \pm 3.3$  ng/ml), (OR=1,667; 0,854 - 3,250 95% CI;  $p < 0,05$ ). The control group patients had the mean CC16 within the reference interval ( $14.2 \pm 2.12$  ng/ml).

**Conclusions.** In young children, elevated CC16 may be considered a marker of respiratory failure in bronchiolitis patients. Bronchiolitis patients with a history of allergies had statistically significantly lower serum CC16 levels than those in children without a history of allergies.

**Key words:** Bronchiolitis; Young Children; Protein CC16.

### Introduction

Bronchiolitis is one of the most common forms of respiratory failure in young children. The results of clinical studies suggest that bronchiolitis remains a leading factor of hospitalization due to severity of the disease in young children [1].

The most commonly known cause of severe bronchiolitis in the first years of life is a respiratory syncytial virus (RSV) [2]. However, the causal relationship of pathophysiological changes in bronchial tree of bronchiolitis patients continues to be actively studied. Ciliated and club cells are highly susceptible to toxic substances released by viruses and bacteria. Damaging the epithelium in the late phase of inflammation, viruses provoke a release of a wide range of inflammatory mediators, such as histamine, bradykinin, leukotrienes, platelet and bronchial endothelium activating factors, which increase vascular permeability and mucosal edema. The release of elastase from damaged cells causes epithelial destruction, cell proliferation, lymphoid infiltration, and connective tissue matrix damage in the form of interstitial growth. [3]. In turn, inflammation also contributes to elevated viscosity of bronchial secretions, obstruction of bronchioles and small bronchi associated with the development of paralysis of the ciliary apparatus, and inhibition of phagocytic activity of alveolar macrophages, resulting in a failure of respiratory ventilation function [4].

In recent years, the medical community has strengthened its confidence in the fact that the question

of bronchiolitis pathogenesis is far from being finally resolved. The bronchiolitis genesis largely depends on interaction of immune mechanisms, failure of which may deregulate mucociliary clearance, cause defects of systemic and local immunity, macrophage system, trigger infection processes and exert influence on allergic status [5].

Clara cell protein (CC16) is secreted by club epithelial cells of bronchioles and is considered their specific marker. CC16 is the most common protein in normal airway secretions. CC16 is known to maintain airway epithelial homeostasis and to exert anti-inflammatory effects in lungs being targeted by allergens and viruses [6].

CC16 deregulation and deficiency contributes to increased susceptibility of respiratory system to viral infections and oxidative stress, which are often observed in the pathogenesis of respiratory diseases such as ARVI, obstructive diseases, and bronchial asthma. The results of recent scientific research suggest that CC16 is the protein that provides functional anti-inflammatory and antioxidant effects in various cells, including epithelial cells and leukocytes [7]. The anti-inflammatory activity of CC16 lies in its ability to inhibit the catalytic activity of secretory phospholipase A2 (sPLA2), a potent proinflammatory enzyme, by binding to cofactors required for achieving full catalytic activity of the enzyme [8].

Measuring the CC16 concentration makes it possible to assess a degree of Club cell damage, and, therefore, the magnitude of respiratory epithelium

damage and bronchial dysfunction [9]. Given the above, growing attention has been paid in the scientific literature to the research of new laboratory markers for diagnostics of bronchiolitis in young children.

### Aim of the study

Analysis of blood serum CC16 concentration in younger bronchiolitis patients.

### Material and methods of the study

We clinically examined 70 young children. The main group consisted of 35 non-allergic bronchiolitis patients. The comparison group included of 25 young bronchiolitis patients with a history of allergies. The control group comprised 10 conditionally healthy children. The average age of patients was  $8.4 \pm 1.6$  months,  $6.2 \pm 1.4$ , and  $6.4 \pm 1.2$  months in the main, comparison, and control group, accordingly.

Criteria for inclusion in the study were: children with bronchiolitis, full-term infants, children aged 0 to 12 months, informed consent of the child's parents to participate in the study. The exclusion criteria included congenital malformations of the bronchopulmonary system, cardiovascular system, bronchopulmonary dysplasia, gastroesophageal reflux disease, and prematurity. The complex of clinical-and-laboratory examination of children included: study of life anamnesis, anamnesis of the disease, perinatal and allergological anamnesis; conducting an objective examination according to generally accepted methods; CC16 serum tests. Serum CC16 content was determined by enzyme-linked immunosorbent assay according to the "Human CC16 ELISA Kit". The resulted data were statistically processed using the IBM SPSS "STATISTICA 12" StatSoft Inc. software package and Excel XP for Windows 10, parametric and nonparametric calculation methods.

The study was approved in terms of compliance with ethical and legal rules of medical scientific research of the Vinnytsya National Pirogov Memorial Medical University by the Commission on Biomedical Ethics. The study was found not contradicting basic bioethical standards and complying with the principles of the GCP (1996), the European Convention on Human Rights and Biomedicine (04 April 1997), the WMA Declaration of Helsinki on Ethical Principles for Medical Research Involving Human Subjects (1964-2008), basic provisions of Order of the Ministry of Health of Ukraine No.690 dated 23 September 2009 (as amended by Order of the Ministry of Health of Ukraine No. 523 dated 12 July 2012). All patients were informed about the purpose and possible consequences of the study procedures, and signed a written informed consent form for participation in the study before manipulations.

### Study results

The study showed that young non-allergic bronchiolitis patients had the mean CC16 ( $38.9 \pm 4.5$  ng/ml) significantly higher than those with a history of allergies ( $22.9 \pm 3.3$  ng/ml), (OR=1,667; 0,854 - 3,250 95% CI;  $p < 0,05$ ). The control group patients had the mean CC16 within the reference interval ( $14.2 \pm 2.12$  ng/ml).

It should be noted that the most main group

patients (22 ( $62.8 \pm 6.4\%$ ) of all examined) had elevated CC16 readings (mean -  $53.9 \pm 4.6$  ng/ml), while only 13 ( $37.2 \pm 16.4\%$ ) patients of the main group had this protein values within the reference interval (mean -  $12.15 \pm 2.1$  ng/ml), (OR=1,272; 0,435 - 3,717 95% CI;  $p < 0,05$ ). As for patients of the comparison group, most examined children (13 ( $51.8 \pm 14.4\%$ )) demonstrated low CC16 values (mean -  $8.53 \pm 1.69$  ng/ml). Other patients of the comparison group (12 ( $48.2 \pm 15.2\%$ )) had CC16 within normal limits (mean -  $19.2 \pm 2.62$  ng/ml), (OR=2,708; 0,925 - 7,927 95% CI;  $p < 0,05$ ).

It's worthy to note that although CC16 mean in the comparison group fell within the reference interval ( $22.9 \pm 3.3$  ng/ml), it was significantly higher than in the children of the control group ( $14.2 \pm 2.12$  ng/ml), (OR=1,153; 0,342 - 3,884 95% CI;  $p < 0,05$ ).

Subsequently, we analyzed the specificity and sensitivity of serum CC16 in allergic young bronchiolitis patients (figure 1).

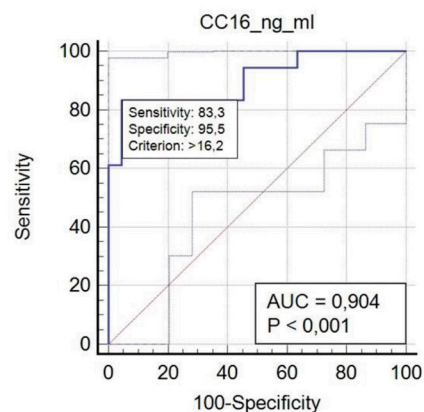


Figure 1. ROC-curve of the CC16 method

Analysis of specificity and sensitivity of serum CC16 readings in younger bronchiolitis patients showed that the AUC area under ROC curve was 0.904 [0.769-0.974, 95% CI]. The cut-off point was at the level of 16.2 ng/ml (sensitivity 83.3%, specificity 95.5%). At the same time, the CC16 sensitivity (55.0%) in allergic bronchiolitis patients was significantly lower with almost same specificity (82.0%); the AUC area under the ROC curve was 0.706 [0.344-0.706 95% CI], the cut-off point - 11.6 ng/ml.

### Discussion of the study results

As for today, it has been shown that blood serum CC16 may indicate the integrity of the bronchial epithelium and the development of bronchial dysfunction [10]. According to recent scientific studies, elevated blood serum CC16 as a response to toxic substances released by viruses, bacteria and harmful environmental factors suggests its potential role of reliable marker for early detection of acute respiratory failure, including bronchiolitis [11]. Current literature data show a trend of low blood serum CC16 in patients prone to early allergic sensitization and the development of allergic clinical manifestations [12].

Apparently, CC16 exerts anti-inflammatory and immunoregulatory effects and may be considered an endogenous anti-inflammatory protein for patients

with inflammatory respiratory failure [13].

In our study, we examined the role of CC16 as a diagnostic marker in younger bronchiolitis patients depending on a history of allergies. Taking into account prevalence of bronchiolitis in young children, the predisposition of children with allergy-burdened disease history to severe RSV infection and a risk of bronchial dysfunction, the use of biomarkers for early diagnosing and prognosis may be statistically clinically significant [14]. A mutation of CC16 gene was associated with an elevated risk of developing allergic diseases in the childhood, associated with a significant decrease of blood serum CC16 [15]. All in all, low serum CC16 readings were associated with allergic sensitization and asthma [16].

In the course of our study, we revealed that elevated CC16 tests in young bronchiolitis patients were associated with the inflammatory process in the bronchi. It is worthy to note that CC16 elevation in younger bronchiolitis patients not burdened with severe allergies was statistically significantly higher than in children with allergic history.

The study demonstrated an increase of serum CC16 in young in-patients. The study data regarding elevated serum CC16 in young bronchiolitis patients not burdened with allergies history was consistent with the literature [17].

In our study, we identified the CC16 role as a

diagnostic marker of bronchiolitis in young children. The study revealed high CC16 specificity and sensitivity in young bronchiolitis patients. In our opinion, the question of establishing a relationship between CC16, vitamin D levels, and indicators of endothelial dysfunction, remains topical issue, which predetermines the prospects for further research.

### Conclusions

In young children, elevated CC16 may be considered a marker of respiratory failure in bronchiolitis patients. Bronchiolitis patients with a history of allergies had statistically significantly lower serum CC16 levels than those in children without a history of allergies. The sensitivity and specificity of serum CC16 tests were statistically significantly higher in young bronchiolitis patients not burdened with a history of allergies (AUC 0.904 ( $p = 0.01$ )).

**Prospects for future researchers:** The study contributes to the bronchiolitis diagnostic potential, but the question of determining a relationship between CC16, vitamin D readings, and endothelial dysfunction remains a topical issue.

**Conflict of interests:** absent.

**Financing sources:** self-financing.

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## ЗНАЧЕННЯ БІЛКА CC16 ПРИ БРОНХІОЛІТІ У ДІТЕЙ РАНЬОГО ВІКУ

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### Резюме

**Вступ.** Білок CC16 секретується Club клітинами епітелію бронхіол, підтримує гомеостаз епітелію дихальних шляхів і має протизапальну дію в легенях. Важливим є вивчення рівня білка CC16 в сироватці крові з метою розуміння цілісності бронхіального епітелію та розвитку бронхіальною дисфункції саме у дітей раннього віку, хворих на бронхіоліт.

**Мета дослідження.** Провести аналіз рівня білка CC16 у сироватці крові дітей раннього віку, хворих на бронхіоліт.

**Матеріал і методи дослідження.** Проведене клінічне обстеження 70 дітей раннього віку. Основну групу склали 35 дітей із бронхіолітом без обтяженого алергологічного анамнезу. Групу порівняння становили 25 дітей, хворих на бронхіоліт, які мали обтяжений алергологічний анамнез. До контрольної групи було включено 10 умовно здорових дітей. Середній вік дітей основної групи становив  $8,4 \pm 1,6$  міс, групи порівняння –  $6,2 \pm 1,4$  міс та дітей контрольної групи  $6,4 \pm 1,2$  міс. До комплексу клініко-лабораторного обстеження дітей входило визначення рівня білка CC16 в сироватці крові. Вміст CC16 в сироватці крові визначали імуноферментним методом за набором “Human CC16 ELISA Kit”.

**Результати дослідження.** Встановлено, що рівень білка CC16 у сироватці крові був підвищений у більшості дітей основної групи (22 (62,8 ± 6,4 %) обстежених). Тоді як у групі порівняння нами не було виявлено підвищення рівня білка CC16 у жодному випадку. Натомість, у 13 (51,8 ± 14,4 %) дітей, хворих на бронхіоліт із обтяженим алергологічним анамнезом, спостерігалось зниження рівня білка CC16, що ймовірно може бути порушенням ендотеліальної дисфункції, ( $p=0,01$ ).

У ході дослідження нами також виявлено, що у дітей, хворих на бронхіоліт без обтяженого алергологічного анамнезу середнє значення білка CC16 ( $38,9 \pm 4,5$  нг/мл) було достовірно вищим, ніж у дітей, хворих на бронхіоліт із обтяженим алергологічним анамнезом, ( $22,9 \pm 3,3$  нг/мл), (OR=1,667; 0,854 - 3,250 95% CI;  $p<0,05$ ). Разом з тим, у дітей групи порівняння середній рівень білка CC16 в сироватці крові був достовірно вищим, ніж у дітей контрольної групи ( $14,2 \pm 2,12$  нг/мл), ( $p<0,05$ ).

**Висновки.** У дітей раннього віку підвищений рівень білка CC16 може розглядатися як маркер ураження дихальних шляхів при бронхіоліті. У дітей хворих на бронхіоліт, які мали обтяжений алергологічний анамнез, рівень білка CC16 в сироватці крові був достовірно нижчим у порівнянні із показником дітей без обтяженого алергологічного анамнезу.

**Ключові слова:** бронхіоліт; діти раннього віку; білок CC16.

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