

БИОЛОГИЯ



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An assessment of the immunological status of patients with asthma–chronic obstructive pulmonary disease overlap syndrome

Abstract: Asthma–chronic obstructive pulmonary disease overlap syndrome (ACOS) has attracted great interest in recent years due to the poor knowledge of its mechanisms, difficulties in differential diagnosis and control. The purpose of this study was to investigate the characteristics of immunological changes in patients with ACOS. The study groups consisted of 22 people with COPD, 13 people with asthma, and 25 people with ACOS. We determined the content of lymphocyte subpopulations (CD3+, CD3+CD4+, CD3+CD8+, CD19+, CD3-/CD16+CD56+, CD3+HLA-DR), immunoglobulin levels (IgA, IgG, IgM, IgE) and assessed the phagocytic activity of neutrophils. Results revealed an increase of CD3+HLA-DR cells in patients with asthma, ACOS and COPD, an increase in the level of immunoglobulin E and a decrease in the phagocytic function to the NBT test in three groups of patients. Significantly high immunoglobulin G values were found in patients with COPD compared with patients with asthma and ACOS. Thus, changes in the immunological status of patients with ACOS are manifested in the form of an increase in activated T-lymphocytes, an increase in serum immunoglobulin E, and a decrease in the phagocytic activity of neutrophils.

Keywords: Asthma, immunological status, COPD, ACOS.

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Introduction. Asthma and chronic obstructive pulmonary disease (COPD) are widespread global diseases, causing significant health care costs. The base of these diseases is the chronic inflammation of the respiratory tract, which leads to the airways limitation. Bronchial obstruction in asthma is predominantly reversible either spontaneously or under the influence of bronchodilators, COPD is also characterized by airway restriction, which is irreversible and progressive. In recent years, a separate condition has been identified - the asthma–chronic obstructive pulmonary disease overlap syndrome (ACOS) [1]. The prevalence of this syndrome in COPD patients varies depending on the diagnostic criteria from 2.1 to 55% [2]. With the ACOS there is a constant airflow limitation with the presence of signs characteristic of both asthma and COPD. This category of patients is characterized by frequent exacerbations, poor quality of life, rapid deterioration of the function of external respiration and high mortality [3, 4, 5]. For patients with the syndrome of asthma and COPD, there is a high frequency of calls for emergency care and frequent hospitalization in intensive care units. In addition, the cost of health care for these patients is almost double the cost of patients with asthma. The adverse dynamics of morbidity and severe forms of asthma and COPD syndrome, resistance to therapy, increased mortality cause its important medical and social significance. In addition, the situation with its differential diagnostics and control remains unresolved [5]. Many interwoven pathological processes are involved in the development of asthma and COPD, the effects of which manifest at the local and systemic levels. In the pathogenesis of COPD, special attention is given to the Th1 type of immune response and the activation of the neutrophilic inflammation in the

airways [6]. The development of asthma is often associated with the prevalence of the Th2 response with the production of the appropriate cytokines, the formation of eosinophilic inflammation and hyperproduction of IgE [7]. How the interaction of immune mechanisms involved in pathogenesis both asthma and COPD is appeared, remains unclear. The aim of this study was to investigate the features of cellular and humoral immunity in patients with asthma–chronic obstructive pulmonary disease overlap syndrome.

Materials and methods. The study included 25 patients with ACOS, 22 patients with COPD and 13 patients with asthma. There were 36 males and 24 females, between the ages of 36 and 68 years. All the patients were hospitalized in Hospital № 2 of Astana with exacerbations of diseases. Diagnoses of asthma and COPD were established by the doctors of the Pulmonology Department in accordance with the Global Strategy for Asthma (GINA), and the Global Strategy for the Treatment and Prevention of COPD, respectively. The control group consisted of 30 healthy patients. The criteria for selecting healthy individuals were as follows: the absence of neurologic, autoimmune, allergic and chronic diseases in the acute stage, as well as the absence a family history of allergy. It was also excluded the presence of diabetes mellitus, systemic and local inflammatory diseases, recent hyperthermia or insolation (2-3 weeks before the tests). Data of study and control groups are presented in Table 1.

Table 1 – Clinical characteristics of patients

Parameters	Control group	COPD	Asthma	ACOS
Number of study participants, n	30	22	13	25
Age, SD	48.8±11.3	69.59±2.23	43.31±4.53	54.32±2.63
Male, n, %	19 (63.3%)	17 (77.3%)	4 (30.8%)*	15 (60%)
Nonsmokers, n, %	17 (56.7%)	8 (36.4%)	5 (38.5%)	11 (44%)
Index of smoking (PY), M±m	15.3±3.2	*32.7±12.8	*6.3±1.7	27.4±3.3
Baseline FEV1% predicted (SD)	95.1 (±7.3)	***52.3±4.05	89.4 ±17.3	67±12.4
FEV1/FVC (SD)	0.82 (±0,03)	**0.54 ±0.09	0.76 ±0.13	0.63±0.11
p <0.05 compared with the control group				

Immunological examination. To assess immunological changes, blood sampling was performed in an amount of 5 ml, anticoagulant - heparin. Lymphocytes were isolated on a density gradient of Ficol-Verografin $\rho=1.077\text{g/ml}$. Analysis of lymphocytes subpopulations was performed by indirect immunofluorescence using a panel of monoclonal antibodies to the surface antigens of lymphocytes: CD3+, CD4+, CD8+, CD19+, CD56+, HLA-DR+ with an evaluation of the results on the flow cytometer (Beckman Coulter Cytomics FC-500). The function of phagocytes was evaluated in the Park-test in the spontaneous mode (*E. coli* LPS) [8]. The serum levels of IgG, IgM, IgA, IgE were measured by immunoenzyme method assay (ELISA) with using the immunological kits (Vector-Best, Novosibirsk, Russia).

Statistical analysis. To assess differences between groups, a normal distribution was determined using the Kolmogorov-Smirnov test. Under normal distribution, Student's test was used, in the case of an anomalous distribution, the Mann-Whitney test was applied. Statistical analysis was carried out with using GraphPad InStat7 Software (Graphpad Software Inc., San Diego, CA).

Results. The clinical characteristics of 60 examined patients with asthma, COPD and ACOS are shown in Table 1. Among the asthma patients, 9 individuals (69.2%) had a partially controlled disease and 4 patients (30.8%) had uncontrolled disease. COPD patients were evaluated with using validated questionnaire the COPD Assessment Test (CAT) for assessment of symptoms and the modified British Medical Research Council (mMRC) scale for an assessment of breathlessness. GOLD II was diagnosed in 18 patients with COPD (81.8%) and GOLD III in 4 patients (18.2%). The group of ACOS patients included 6 patients with partially controlled asthma (24%) and 19 patients (76%) with uncontrolled asthma, all of which had GOLD II. Evaluation of immunological parameters of patients with asthma, COPD and ACOS showed a decrease in the relative lymphocyte count in patients with COPD and ACOS compared with the control ($p < 0.05$). A significant increase in

the indices of T-activated lymphocytes (CD3 + HLA-DR) in patients with ACOS, asthma and COPD was found in comparison with the control ($p < 0.05$). Significant differences were found between groups of patients with COPD and ACOS, COPD and asthma in serum immunoglobulin G ($p < 0.05$), and increase of serum immunoglobulin E was detected in patients with asthma, COPD and ACOS, without significant difference between groups. The spontaneous nitroblue tetrazolium (NBT) test showed the decrease of phagocytic activity in all groups of patients ($p < 0.05$).

Table 2 – Relative numbers of lymphocyte subpopulations in patients with COPD, asthma and ACOS

Parametres	Control group	COPD	Asthma	ACOS
Lymphocytes(%)	37 (29.67 -44.33)	*17.9 (7.6 -34.7)	27 (17 – 40)	*16.3 (8.1 – 49.6)
CD3+ (%)	64.83 (61.64 -68.02)	70.375 (79.6 -49.32)	64.4 (51.6 -79.6)	68.35(58.3 - 80.4)
CD3+CD4+ (%)	39.92 36.67-(43.17)	38.96 (8.4 – 50)	35.9 (17.5-52.6)	38.6(9.4-57.4)
CD3+CD8+ (%)	23.92 (21.14-26.7)	28.6 (22.6 -46.87)	27.1 (15.6 -36.7)	27.26(17.7 – 6.8)
CD19+ (%)	10.10(7.1-13.1)	13.71(3.38 -40.38)	14.5(6-29.8)	15.6(6-23.8)
CD3- /CD16+CD56+	10.90 (3,0-14.4)	14.45 (6.14- 21.85)	16.8 (8.6-25.5)	14.6 (8.6-21.4)
CD3+HLA- DR+(%)	8.4 (6.1-13.6)	**19.3 (8.7-47.2)	*12.8(6 – 33.3)	***23.4(7.8-38.9)

* p value <0.05 when compared to the control group

Absolute numbers of lymphocyte subpopulations in patients with COPD, asthma and ACOS

Parameters	Control group	COPD	Asthma	ACOS
CD3+	1148.15 (347.637- 1621.273)	1145.35(422.923- 2872.605)	1240.62 (715.292- 2423.82)	1105.007 (518.752- 4233.211)
CD3+CD4+	768.64 (143.273- 1029.384)	598.234 (211.466 -1054.944)	743.177 (392.94 - 1604.67)	597.618 (122.341- 2443.693)
CD3+CD8+	607.22 (132.734- 1029.39)	666.435 (124.073 -1486.826)	665.711 (214.812 -1101)	609.071 (297.84- 1746.267)
CD19+	256.11 (121.272- 634.83)	244.8009(133.47 -1385.779)	285.961 (189- 717.6)	297.8352 (173.0995- 850.2732)
CD3- /CD16+CD56+	273.96 (127.41- 637.27)	263 (43.4- 425.23)	306.14 (148.7- 790.5)	260 (37.2- 462.97)
CD3+HLA-DR+	240.0 (74.83- 523.77)	288.0385(112.2744 – 624.3224)	287.712(68.34 – 922.116)	288.49 (127.296- 1052.719)

* p value <0.05 when compared to the control group

Table 4 – Immunoglobulin content and NBT test in patients with COPD, asthma and ACOS

Parametres	Control group	COPD	Asthma	ACOS
Ig A, pg/ml	2.63 (1.1-4.3)	3.35 (0.9-5.7)	2.2 (1.4-3.6)	2.48 (0.96-4.7)
Ig G, pg/ml	13.41 (6.43-17.22)	*22.25 (16.4-36.8)	17.4 (6.5-22.6)	14.7 (5.1-26.7)
Ig M, pg/ml	1.93 (0.3-3.2)	1.71(0.6-4)	2.6(5.5-1.4)	2.7(0.46-4.6)
Ig E, pg/ml	45.60 (2.3-121.8)	*98.25 (5.2-913.3)	*124.5 (10.8-403.8)	*136.2 (8.9-735.4)
NBT test	8.20 (7.53-10.58)	*3.5 (1-28)	*4 (0-18)	*4 (0 - 13)

* p value <0.05 when compared to the control group

Discusslon. The asthma–chronic obstructive pulmonary disease overlap syndrome is diagnosed in patients with signs of both asthma and COPD, and, perhaps, due to this reason the immunological

indices of ACOS in our study predominantly have close values with these diseases. Significant differences were obtained in the relative content of T-activated lymphocytes in patients with COPD and ACOS compared with the control. Zhu and colleagues found an increase of peripheral CD8+ T lymphocytes expressing MHC class II (HLA-DR) in COPD patients than in healthy subjects [9]. It is assumed that activated T cells in COPD cause tissue damage, release and promote the production of pro-inflammatory mediators, attract and activate other immune and parenchymal effector cells. A higher level of immunoglobulin G was found in COPD patients compared with ACOS patients and asthma ($p < 0.05$). Its increase is probably associated with bacterial antigenic stimulation of the immune system, which is characteristic of COPD exacerbations. In addition, changes from humoral immunity were manifested as a significant increase in immunoglobulin E in all of three groups of patients compared with controls. However, the increase of IgE in COPD patients did not exceed the threshold. The increase in IgE in COPD may be due to the expression of Th2-inflammatory responsible genes that was found in 20% of COPD patients in the study of Christenson and colleagues [10].

Conclusion. Thus, in patients with ACOS there are changes in the cellular and humoral immunity, which resemble the immunological pattern of asthma and COPD. A distinctive immunological sign of COPD from ACOS can be considered a higher content of IgG in the blood serum. It is assumed that different types of inflammatory processes are the basis of stratification of ACOS phenotypes, therefore the study of immunological aspects of ACOS requires further extensive studies with the definition of cellular and molecular markers of immune inflammation and a large number of patients.

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Өкпенің созылмалы обструктивті ауруы және бронх демікпесі айқас синдромымен ауыратын науқастардың иммунологиялық жағдайын бағалау

Аннотация: Соңғы жылдарда Өкпенің созылмалы обструктивті ауруы және бронх демікпесі айқас синдромы (ӨБДАС) оның пайда болу тәсілдерін, дифференциалды диагностика мен бақылаудағы қиындықтарды зерттеудің жеткіліксіздігінен үлкен қызығушылық тудырып отыр. Зерттеудің мақсаты ӨБДАС -мен ауыратын науқастардың иммунологиялық өзгерістерінің ерекшеліктерін зерттеу болып табылады. Зерттеу барысында 22 науқас өкпенің созылмалы обструктивті ауруы (ӨСОА), 13 науқас бронх демікпесімен (БД) және ӨБДАС -мен ауыратын 25 науқас топтарынан тұрды. Осы мақалада лимфоцит субпопуляцияларының (CD3+, CD3+CD4+, CD3+CD8+, CD19+, CD3-/CD16+CD56+, CD3+HLA-DR) құрамы, иммуноглобулин (IgA, IgG, IgM, IgE) деңгейі анықталды және нейтрофилдердің фагоцитарлық белсенділігі бағаланды. Зерттеу нәтижелері бойынша ӨБДАС пен ӨСОА ауыратын науқастарда CD3+HLA-DR-клеткалары, Е иммуноглобулин деңгейі жоғарыласа, ал үш зерттеу топтарында НКТ тест нәтижесі

бойынша фагоцитарлық функциясының төмендегені байқалды. БД мен ӨБДАС науқастарымен салыстырғанда ӨСОА науқастарында G иммуноглобулиннің мәндрі айтарлықтай жоғары екендігі анықталды. Осылайша, ӨБДАС ауыратын науқастарда Т-лимфоциттердің белсенуінің, сарысу Е иммуноглобулиннің жоғарлауы мен нейтрофилдердің фагоцитарлық белсенділігінің төмендеуі ретінде иммунологиялық жағдайының өзгеруі көрінді.

Түйін сөздер: Астма, иммунологиялық жағдайы, ӨСОА, ӨБДАС.

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Оценка иммунологического статуса пациентов с синдромом перекрытия астмы и хронической обструктивной легочной болезни

Аннотация: Синдром перекреста бронхиальной астмы и хронической обструктивной болезни легких (СПБАХ) в последние годы вызывает большой интерес в связи с недостаточной изученностью механизмов его возникновения, трудностями в дифференциальной диагностике и контроле. Цель настоящего исследования заключалась в изучении особенностей иммунологических изменений у больных СПБАХ. В исследование было включено 13 больных бронхиальной астмой (БА), 22 больных хронической обструктивной болезнью легких (ХОБЛ) и 25 больных СПБАХ. Мы определили содержание субпопуляций лимфоцитов (CD3+, CD3+CD4+, CD3+CD8+, CD19+, CD3-/CD16+CD56+, CD3+HLA-DR), уровни иммуноглобулинов (IgA, IgG, IgM, IgE) и оценили фагоцитарную активность нейтрофилов. Результаты показали повышение CD3+HLA-DR-клеток у больных астмой, СПБА и ХОБЛ, повышение уровня иммуноглобулина Е и снижение фагоцитарной функции по данным НСТ-теста в трех группах больных. Обнаружены значимо высокие значения иммуноглобулина G у больных ХОБЛ по сравнению с больными астмой и СПБАХ. Таким образом, изменения в иммунологическом статусе больных СПБАХ проявляются в виде повышения активированных Т-лимфоцитов, повышения сывороточного иммуноглобулина Е и снижения фагоцитарной активности нейтрофилов.

Ключевые слова: Астма, иммунологический статус, ХОБЛ, СПБАХ.

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