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Clinical, Immunological and Molecular Genetic Features of The Overlap of Bronchial Asthma and Chronic Obstructive Pulmonary Disease

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Abstract: *The aim of the study was to investigate the clinical picture and features of the immune status in patients with overlap of bronchial asthma and COPD in comparison with patients with isolated asthma or chronic obstructive pulmonary disease. During the period 2020-2021, 159 patients were examined. They were divided into 3 groups: the first group of 62 patients with bronchial asthma, the second group of 67 patients with COPD and 30 patients with ACO in the third group. The level of cytokines (IL-4, IL8, TNFa, INFy) was determined by the ELISA method. Genotyping was performed by the HRM-qPCR method. Clinical and anamnestic data, as well as the level of cytokines in each group, were analyzed. Analysis of the cytokine level revealed significant changes in the group of patients with ACO ($p \leq 0.05$). Based on the study of the immunological status, the most significant markers (IL-8 and IFNy) were identified. An index for predicting the course of the disease at the intersection of bronchial asthma and COPD has been developed, allowing to predict the course of the disease and the choice of adequate therapy. The heterozygous genotype G6986A of the CYP3A5 gene is a risk prognostic genotype in bronchial asthma and ACO.*

Keywords: *bronchial asthma, COPD, ACO, cytokines, IgE, CYP3A5, G6986A, polymorphism.*

Relevance: Due to the significant prevalence of chronic nonspecific lung diseases and the high level of disability, one of the most important tasks of healthcare is the prevention and effective diagnosis, as well as the treatment of patients with chronic diseases of the lower respiratory tract. Asthma and chronic obstructive pulmonary disease (COPD) are a major public health problem and are the leading cause of morbidity and mortality worldwide [1,2.7]. Asthma and COPD are the most common chronic respiratory diseases, each with a specific pathophysiology [1,11,12,20]. Usually, asthma is characterized by chronic inflammation of the airways with reversible symptoms, while COPD is characterized by persistent respiratory changes in the bronchopulmonary system [3,4,15,18]. However, patients can sometimes have clinical features of both diseases, and this condition is called asthma-COPD overlap (ACO), recommended by the joint guidelines of GINA (Global Strategy for the Management and Prevention of Asthma) and GOLD (Global Initiative on Chronic Obstructive Pulmonary Disease). According to this guide, ACO is characterized by "permanent airflow limitation with some features of asthma and COPD."

Due to the significant impact on health and reduced quality of life of the population, data on the incidence of overlap of asthma and chronic obstructive pulmonary disease are of critical importance for the strategic plan and health policy.

The significance of the ACO phenotype and the need for its further study are beyond doubt, since this pathology significantly reduces the quality of life of patients, being a serious medical and social problem. Most previous studies have shown that patients with ACO have more severe respiratory symptoms, frequent exacerbations, poor quality of life, high mortality, increased use of healthcare resources, and a higher prevalence of comorbidities than patients with isolated asthma or COPD [5,13,17,19]. That is why the study of clinical and laboratory parameters is a promising way of development in the field of innovative methods for diagnosing and

treating the combination of BA and COPD. It is important to emphasize that only dynamic clinical and functional monitoring of patients makes it possible to make a correct diagnosis.

A group of other scientists believe that ACO has a different combination of immune disorders, which is a consequence of the development of two separate pathologies. In some cases, Th2-type atopy and inflammation of the airways, eosonophilia, elevated IgE levels, with the participation of cytokines such as IL-4, IL-5 and IL-9, can be observed in patients with ACO. And other patients with ACO may have signs of COPD, neutrophilia and an imbalance of such cytokines as IL-6, IL-8 and tumor necrosis factor [4,7,20,25].

The study of cytokines shows their significant and diverse role in the development of immune, allergic and inflammatory reactions in respiratory diseases. Emerging data on the nature and functions of these mediators complement the understanding of the pathogenesis of pulmonary diseases. As the role of cytokines becomes clear, it becomes possible to control the inflammatory process and other pathophysiological consequences of lung damage [5, 6, 11,16].

A number of studies are being conducted around the world to study the prevalence, clinical and immunological molecular genetic aspects and risk factors for the development of overlap between bronchial asthma and chronic obstructive pulmonary disease [7, 9, 11]. The priority scientific direction in this area remains such key points as the study of the molecular genetic mechanisms of the development of the intersection of bronchial asthma and chronic obstructive pulmonary disease.

One of the priority modern directions is to identify genetic predisposition to the development of a particular pathology, including PBA. According to the literature, one of the genetic markers influencing the formation and development of lung disease is a gene belonging to the cytochrome 450 family CYP3A5, in particular its polymorphism 6986GA, due to its direct participation in the metabolism of corticosteroids [5, 14].

Purpose of the study: to study clinical, characteristics of the immune status, the role of polymorphism of the CYP3A5 gene in patients with bronchial asthma overlap COPD compared with patients suffering from bronchial asthma or chronic obstructive pulmonary disease.

Materials and methods of research:

To carry out this research work, 159 patients were examined in the period from 2022-2024 with chronic diseases of the lower respiratory tract. Clinical material was collected in the Bukhara Regional Multidisciplinary Medical Center, in the 3rd city hospital of the Mirabad district of the city of Tashkent and in the private clinic "Poytaxt med diagnostika".

The diagnoses were verified on the basis of a thorough medical history, clinical, laboratory (complete blood count, urine), biochemical blood tests, bacteriological examination of sputum, instrumental (chest x-ray, electrocardiography, spirography, peak fluometry) methods.

The following groups were formed to conduct clinical and laboratory studies:

– Group 1 - 67 patients diagnosed with bronchial asthma
– Group 2 - 67 patients diagnosed with chronic obstructive pulmonary disease
– 3rd group - 30 patients who had an overlap of bronchial asthma and chronic obstructive pulmonary disease (COPD)

Quantitative assessment of the levels of IL-4, IL-8, TNFα, IFNγ was carried out using test systems (LLC "Cytokin", St. Petersburg) by enzyme-linked immunosorbent assay.

An analysis of the polymorphism of the CYP3A5 gene (A6986G) was carried out in sick and healthy individuals; the studies were carried out in the laboratory of molecular genetics of the Institute of Human Immunology and Genomics of the Academy of Sciences of the Republic of Uzbekistan.

DNA extraction

The material for DNA extraction was venous blood from the cubital vein with a volume of 3-5 ml. (Beckton-Dickinson vacutainers with anticoagulant/preservative 15% tripotassium EDTA (Ethilen dianin-tetra acetic acid) were used for blood collection). Blood for further processing could be stored for up to 24 hours at a temperature not exceeding +40C.

To obtain genomic DNA, a two-step method of blood cell lysis was used. By double centrifuging the entire

volume of whole blood in RCLB buffer (Red cell lyses buffer) at a speed of 1500 rpm for 15-20 minutes, erythrocyte cells were lysed. The use of RCLB results in osmotic shock to the red blood cells, leading to their swelling and further destruction.

The supernatant containing destroyed red blood cells was carefully drained from the tube and the residue above the sediment was aspirated. The clot of leukocyte mixture remaining at the bottom was lysed in WCLB buffer (White cell lyses buffer, white blood cell lysis buffer) in an amount depending on the volume of the leukocyte mixture. WCLB is also a preservative even at room temperature.

Using the alcohol-salt treatment method, further purification of the leukocyte mass lysates took place (S. Milleretal, 1988) in a modernized form proposed by the Laboratory of Human Genomics of the Institute of Immunology of the Academy of Sciences of the Republic of Uzbekistan (currently the Department of Genome-Cell Technologies of the Russian Research Center of Immunology of the Ministry of Health of the Republic of Uzbekistan).

After the alcohol had dried, a diluted solution of TE (Tris-EDTA) 1:3 (TE: water) pH 8.0 was added to the test tube with dried DNA.

To perform polymerase chain reaction (PCR), DNA with an approximate concentration of 0.1 µg/ml, diluted with deionized water, and the corresponding primers with a concentration of 10 pmol/µl were taken. The reaction mixture and PCR conditions are as follows: deionized water - 4.6 µl; dNTPs mixture 10x: 2 mMdATP, 2 mMdTTP, 2 mMdGTP, 2 mMdCTP - 4 µl; 10x PCR buffer (16.6 µM (NH4)2SO4, 67 mMTris-HCl (pH=8.8)) - 4 µl; MgCl2: 25 mM - 4 µl (at the required concentration of 2.5 mM) or 2.4 µl (at the required concentration of 1.5 mM); Taq polymerase - 1.33 µl; template DNA - 4 µl; primer F(+) - 2.7 µl; primer R(-) - 2.7 µl. The reaction was carried out in 35-40 µl of the reaction mixture.

Q-PCR HRM PCR technology with melting curve analysis identifies DNA fragments by detecting changes in the fluorescence level of the fragment-probe complex (labeled fluorophore oligonucleotide probe) during its denaturation and then plotting the melting curve.

To type polymorphic variants of the candidate genes under study, DNA preparations obtained from 5 ml of venous blood and paraffin blocks were used. Genotyping was carried out using the HRM-qPCR method (Stratagene M*3005P, Agilent Technologies, USA; DT-Prime, DNA-Technology, Russia). The EMBL Nucleotide Sequence Database and NCBI were used as resources for population comparisons.

Statistical processing of the results was carried out using statistical software packages Arlequin 2006 (version 3.5.2.2.), Excell 2003, SISA.

Research results and their discussions: The average age of patients with bronchial asthma was 56.8 ± 9.94, patients with COPD - 60.3 ± 8.19, patients with ACO - 57.4 ± 8.48.

Age at the time of examination in patients with a combination of BA and COPD did not reveal significant differences.

Gender analysis revealed that women predominated in the BA and ACO groups, while there were more men in the COPD group. Perhaps this is due to the greater susceptibility to smoking in men.

First of all, the onset of the disease was observed in bronchial asthma at a young age (22.8 ± 8.86).

Isolated COPD was characterized by a shorter duration (15.2 ± 9.2) of the disease compared to ACO (18.8 ± 4.3) and bronchial asthma (34.1 ± 10.1).

When analyzing the clinical and pathogenetic variants of BA, the infectious type was observed in 12.7% (7) of patients, the allergic type in 73.2% (45). At the intersection of BA with COPD, 61.3% (18) of patients had a non-allergic type and 30 patients had an infection-dependent type of bronchial asthma.

A burdened allergic history was detected in 37.6% of patients with ACO and in all patients with isolated BA, while there were no signs of atopy in patients with COPD.

The main complaints of patients with the studied pathology were cough, of a different nature in 100% (159).

Shortness of breath was detected on average - in 69.9% (111), but was more observed in the group with ACO;

signs of intoxication (pallor, cyanosis of the nasolabial triangle, weakness, sweating, loss of appetite) were observed to a greater extent in ACO, as well as pain in the chest.
Recent studies indicate that obesity is a greater cause of disease than smoking and alcoholism. The main sign of obesity is the accumulation of adipose tissue in the body - body mass index (BMI). BMI is not only a diagnostic criterion for obesity, but also an indicator of the relative risk of developing diseases associated with obesity, in particular asthma and COPD. In this connection, we studied anthropometric parameters. When comparing the average indicators of anthropometric data, no static significant differences between the groups were found.

But when studying BMI in each group separately, the following was revealed; in the bronchial asthma group, patients with obesity prevailed 40.3% (25), while patients with normal and overweight were 22.6% (14) and 37.1% (23), respectively. In the COPD group, patients with normal body weight 67.2% (45) prevailed, overweight and obesity were 25.3% (17) and 7.5% (5), respectively. In the ACO group, overweight patients accounted for 43.3% (13), normal weight 20% (6) and obese 36.7% (11).
The analysis of somatic diseases revealed that in patients with COPD and ACO, hypertension was more common (92.4% and 80.3%, respectively). History of coronary heart disease in the group of patients with COPD was in 65.3%, which is significantly higher than in the group of bronchial asthma and ACO (32.5% and 38.6%). Diseases of the gastrointestinal tract (GERD, chronic gastritis, gastric ulcer and diseases of the hepatobiliary system) were detected in 70.4% of patients with ACO, which is significantly more common than in the group with isolated COPD (64.3%) and BA (20, 6%). The incidence of ENT diseases (allergic rhinitis, chronic tonsillitis and sinusitis) was higher in the isolated BA group (61.8%), which may be due to the presence of an allergic component in this group. Endocrine diseases were detected more in the COPD group, common in the ACO group.

Important among the risk factors for the development of chronic bronchopulmonary pathology is contact with dust, various paints and varnishes and abrasive materials, inhalation of pesticides and fuels and lubricants. Among the patients examined by us, occupational hazards in history prevailed in the group with ACO and COPD. Tobacco smoking is also one of the main risk factors for the development of ACO. Most of the patients examined by us, especially in the group with ACO (79.6%) and COPD (85.9%), had a long history of smoking. Among patients with AD, only three patients had smoking experience, and less than 1 pack/year.

Among the complications in the group of those examined with ACO, the percentage of cor pulmonale was higher than in the group with COPD (58.1%) and BA (2.6%), respectively. Respiratory failure of I and II degree was observed in 67.6% of patients with ACO, which was significantly higher than in the compared groups with COPD (54.5%) and BA (26.7%). Signs of chronic heart failure were more typical for patients in the COPD group (67.6%), as well as emphysema (87.6%).

Analysis of laboratory data revealed changes in the level of leukocytes (neutrophils, eosinophils, monocytes) in peripheral blood. Severe leukocytosis was observed in patients with COPD 67.6% (45) than in the group with ACO 56.2% (17), and leukocytes were normal in the BA group. There were no statistically significant differences in the level of eosinophils. The erythrocyte sedimentation rate was statistically higher in the group of patients with COPD, which is 2.35 times higher than in patients with ACO and 9 times higher than in isolated BA.
Fibrinogen levels were higher in the COPD group.
To study the role of innate immunity parameters in the development of ACO, we studied acute phase proteins.

The main function of the acute phase protein system is the excretion (elimination) of foreign cells and the regulation of the immune response.

One of them is C-reactive protein (CRP) - an acute phase protein related to non-specific protective factors produced by liver cells.

In the group of patients with BA, the level of CRP reached an average of 12.4 ± 0.7 mg/l, which is 1.63 times

higher than the values of the group with COPD, 1.16 times higher than the group with ACO and 3 times higher than the values of the control group (P<0.001). In the group of patients with COPD, the level of CRP reached up to 7.6 ± 0.4 mg/l, which is 1.85 times higher than in the control group (P<0.001). The level of CRP in patients with ACO was 2.6 times higher than the control values in (P<0.001).

In addition to CRP, complement components, APP also includes lactoferrin, the level of which is reduced in bronchopulmonary pathology, and a deeper deficiency was observed in patients with BA (365±12.2 ng/ml versus 445±9.8 ng/ml in control). (P<0.01).

. The level of lactoferrin in patients with COPD was significantly reduced compared with the data of the control group (P<0.05). In patients with ACO, the concentration of lactoferin was 1.2 times lower than the control values, averaging 372.4±24.3 ng/ml. The obtained results suggest that the reduced level of serum lactoferrin is due to the fact that it keeps neutrophils in the focus of inflammation, probably in connection with this, its content in the blood is reduced.

Hypovitaminosis D and excess accumulation of adipose tissue has a mutually negative effect, which results in the accumulation of inactive forms of vitamin D and a decrease in its bioavailability. Vitamin D in obesity has direct and indirect mechanisms of influence. BMI is not only a diagnostic criterion for obesity, but also an indicator of the relative risk of developing diseases associated with obesity, in particular asthma and COPD.
Since asthma and COPD overlap syndrome (ACO) is a controversial and multifaceted pathological process, the study of vitamin D concentration as one of the factors leading to the development of a vicious circle is relevant.
In all studied groups, obesity was observed in 41 patients, which is an additional risk of reducing the level of vitamin D, which in turn leads to aggravation of bronchopulmonary pathology. It was in these patients that the determination of the vitamin content in the blood serum was carried out.
Our data show that all obese and overweight patients are deficient in vitamin D, and its lowest level is observed in patients with overlapping asthma and COPD. Perhaps this is due to the long-term use of antibacterial, anti-inflammatory and glucocorticoid drugs, as well as the number of concomitant diseases, the duration of the process and the age of patients.
During the collection of anamnesis, 63 percent of the examined were found to be taking drugs containing vitamin D. But Unfortunately, most people find it difficult to reach the recommended levels of vitamin D intake, even if they consume a healthy and balanced diet, as rich food sources of vitamin D are rare.
Thus, the above data indicate the need for long-term administration of vitamin D for patients with AD, COPD, and ACO.
Next, we performed sputum analysis in 57 patients with COPD and 28 patients with ACO, which revealed an increase in the number of leukocytes more in patients with ACO than in patients with COPD (56 ± 2.1 and 11 ± 1.4 cells per field of view, respectively). The eosinophilic nature of inflammation was more characteristic of ACO compared to the group of patients with COPD (74.2% and 31.2% of individuals, respectively). (Pic. 2.)
Thus, our studies allowed us to draw the following conclusions: for patients with overlapping BA and COPD, an earlier onset of the disease was characteristic and, accordingly, a longer duration of the disease itself; occupational hazards and tobacco smoking were revealed to a greater extent; the predominance of the infectious dependent component. In the analysis of sputum for ACO, the eosinophilic nature of inflammation was more characteristic, as well as leukocytosis with neutrophilic inflammation in the blood test. Vitamin D deficiency was more pronounced in this group. Diseases of the gastrointestinal tract were more pronounced among comorbidities in the ACO group. Among the complications of the underlying disease for ACO, the formation of cor pulmonale and respiratory failure is more characteristic.
In our studies, we conducted a comparative analysis of pro- and anti-inflammatory cytokines in the studied groups (IL-4, IL-8, TNFα, IFNγ) (Table 1).

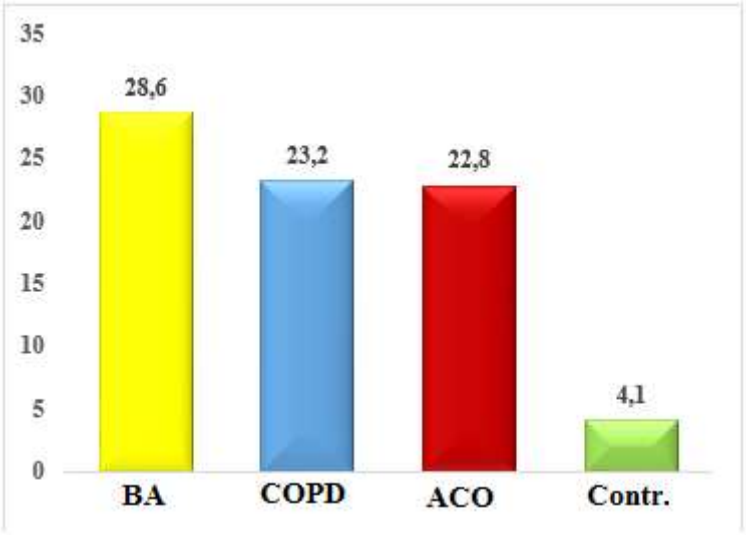
Table 1
The concentration of cytokines in the group of subjects

	BA (n=62)	COPD (n=67)	ACO (n=30)	Control
IL-4	28.6±1.7*	23.2±1.5	22.8±1.2	8.7±0.3
IL-8	18.7±1.4	27.8±1.3	39.6±1.1*	11.6±0.4
TNF-α	35.3±2.5	39.7±2.2	46.2±1.7*	21.4±0.1
IFN-γ	11.7±0.6	14.3±1.5*	12.4±0.2	19.1±0.9

Note: *Values are significant in relation to the control group

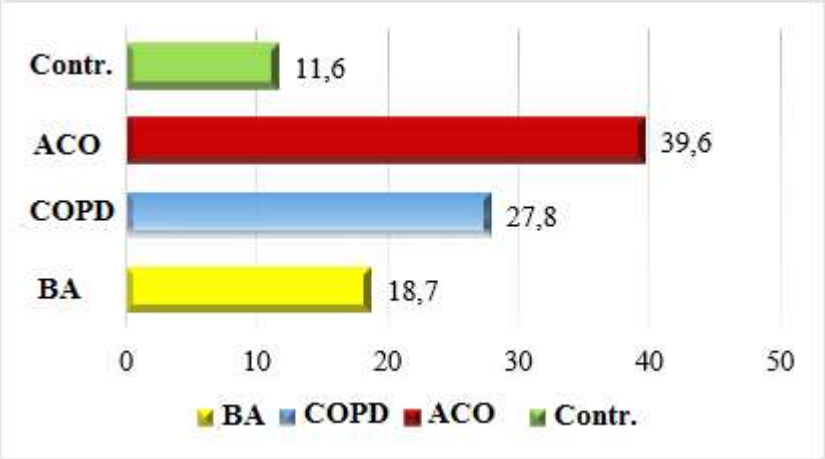
(P<0.05-0.001)

According to many authors, cells synthesizing Th2-type cytokines dominate in the airways affected by asthma. CD8+ cells, eosinophils, and mast cells produce IL-4, which, in turn, possibly causes bronchial tree hyperreactivity [10, 13, 19, 24]. Our data confirm that the levelIL-4 was the highest in the BA group - 28.6 ± 1.7 pg/ml, which was significantly higher by 3.97 times than in the COPD group and 1.25 times more than in the ACO group. (P<0.01) (Pic. 1.)



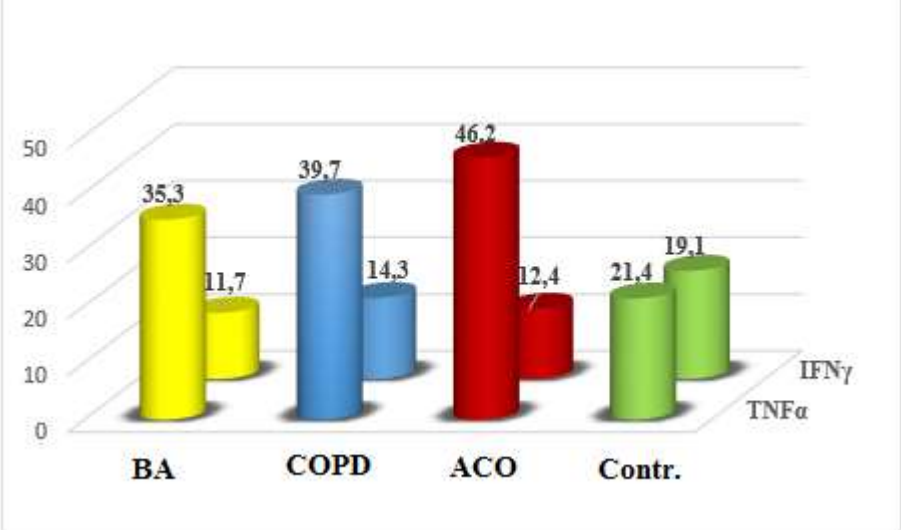
Pic. 1. The level of IL-4 in the groups of subjects, pg/ml (P<0.05)

In chronic obstructive pulmonary disease and ACO, an increase in the content of IL-8 in sputum is observed, which is associated with the involvement of neutrophils in the inflammation focus [12].In our study, the concentration of IL-8 was high in the ACO group - 39.6 ± 1.1 pg/ml, which was significantly higher by 2.1 times compared with the BA group and 1.42 times higher in COPD.(P<0.01)(Pic.2)



Pic. 2. The level of IL-8 in the groups of subjects, pg/ml (P<0.05)

When studying the concentration of tumor necrosis factor, there were no significant differences between the BA and COPD groups; in the ACO group, the concentration TNFα was increased 1.3 times compared with other groups (46.2 ± 1.7 pg/ml).(P<0.01).The increase in the level of TNFα in the ACO group is possibly associated with a more intense inflammatory process in the lungs.



Pic. 3. The level of TNFα and IFNγ in the groups of subjects , pg/ml (P<0.05)

Interferon gamma is an indicator of the Th1 immune response, which is more characteristic of a non-allergic inflammatory process. Level IFNγ was reduced in all the studied groups, but its lowest concentration was observed in the BA group 11.7±0.6.(P<0.01)(Pic.3.).

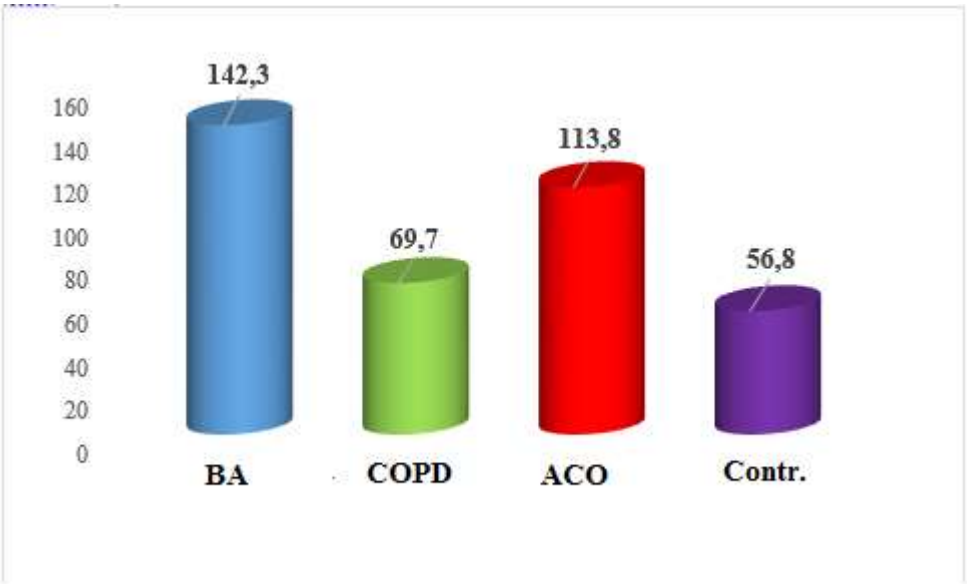
Thus, a comparative analysis of the indicators of pro- and anti-inflammatory cytokines in patients during exacerbation of BA, COPD and ACO revealed that IL-4 synthesis was the highest in the group of patients with bronchial asthma and was 3.97 times higher than in the group with COPD and 1 .25 times more than in the ACO group (P ≤ 0.01). The concentration of IL-8 was high in the ACO group - 39.6 ± 1.1 pg/ml, which was significantly higher by 2.1 times compared with the BA group and 1.42 times higher in COPD.(P<0.01). When studying the concentration of tumor necrosis factor, there were no significant differences between the BA and COPD groups; in the ACO group, the concentration TNFα was increased 1.3 times compared with other groups (46.2 ± 1.7 pg/ml).(P<0.01). Level IFNγ was reduced in all the studied groups, but its lowest concentration was observed in the group with BA 11.7±0.6 (P<0.01). The results obtained reflect the type and intensity of airway inflammation. The high values of the studied cytokines confirm their role in bronchial remodeling and contribute

to the irreversibility of obstruction in these pathologies. Perhaps this is due to the chronic course of both eosinophilic and neutrophilic airway inflammation. Undoubtedly, these cytokines play an important role in the pathogenesis of BA, COPD, ACO and can serve as markers of the severity of the pathological process.
The main biological role is the unique ability to bind to the surface of human mast cells and basophils [17, 21, 23].

IgE is synthesized mainly by plasma cells localized in the mucous membranes.

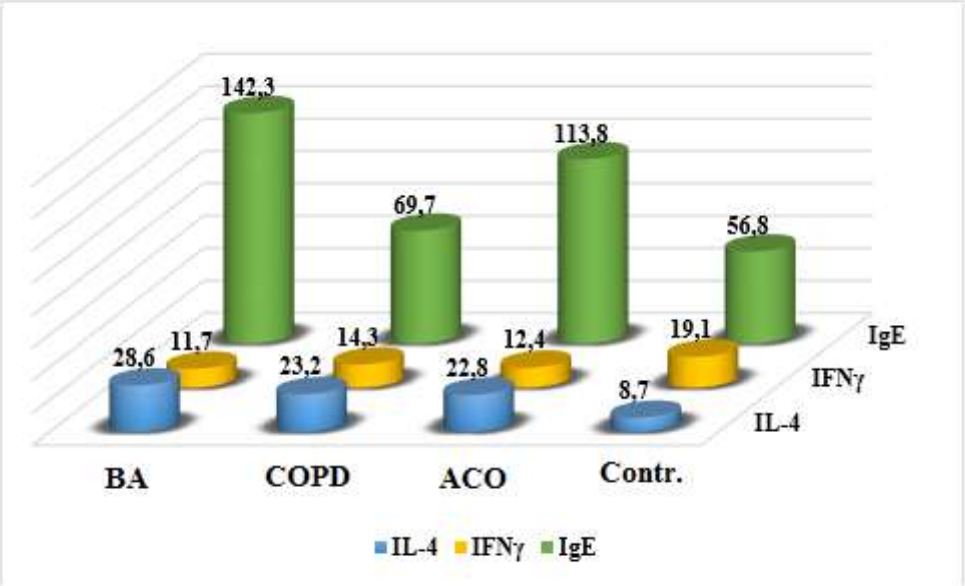
In an immediate hypersensitivity reaction, specific antibodies (reagins) are detected in the body that have the ability to sensitize their own tissues - IgE.

The results of our studies showed that in bronchial asthma there is a sharp tendency to increase the level of IgE (142.3±0.9 ng/ml versus 56.8±0.6 pg/ml in control). (P<0.01).



Pic.4. IgE level in examined patients (ng/ml) with BA, COPD, ACO

In patients with COPD and ACO, the level of IgE was increased by 1.2 and 2 times, respectively, averaging 69.7±1.3 ng/ml and 113.8±1.6 ng/ml versus 56.8±0 .6 ng/ml, (P<0.01) (Pic. 4.).



Pic.5. Level of IL-4, INFγ and IgE in examined patients (ng/ml, pg/ml) with BA, COPD, ACO (P<0.05)

The earlier study to determine the level of pro- and anti-inflammatory cytokines (IL-4, IFNγ) revealed a clear relationship with the synthesis of IgE in the examined groups, which was more pronounced in the group with bronchial asthma. This may be an evidence factor of the leading role of bronchial asthma in the development of ACO. (Pic.5.)

Interleukin-4 (IL-4) is leading in the formation CD4+ type of immunoreactivity, thus defining a completely different nature of inflammation. Despite the fact that this cytokine is determined to play a leading role in the formation of respiratory tract inflammation in bronchial asthma, it can also contribute to the pathogenesis of the inflammatory response in COPD. The formation of the CD4+ type of immune response is important in the development of the eosinophilic type of inflammation in the tissue of the respiratory tract, forming the eosinophilic phenotype of COPD. In addition, IL-4 activates the production of growth factors that contribute to the formation of airway remodeling. [18, 22] This once again proves that the violation of the mechanisms of immunological reactivity leads to the development of chronicity and aggravation of pathological processes in the bronchial tree.

Our studies have revealed that in patients with ACO, the levels of pro-inflammatory cytokines - IL-8 and IFNγ - undergo a sharp change, mainly. In this regard, we considered it appropriate to calculate an index that combines these indicators using the following formula:

$$IPCD = \frac{IL\text{-}8}{IFN\gamma},$$

where IPCD is the index of the prognosis of the course of the disease.

Previously, this ratio was used to predict the course of cystic fibrosis in children (N.Ya. Fayzullaeva 2017). Calculations showed that in practically healthy people (control group) the IPTI was less than 1 and amounted to 0.6±0.15 (Table 4.4).

This indicator increased in patients with BA, which amounted to 2.96±0.3, in patients with COPD = 1.61±0.12, and in ACO = 3.19±0.17. (Table 5)

Table 5
The content of IL-8 and IFNγ in the peripheral blood serum of the examined

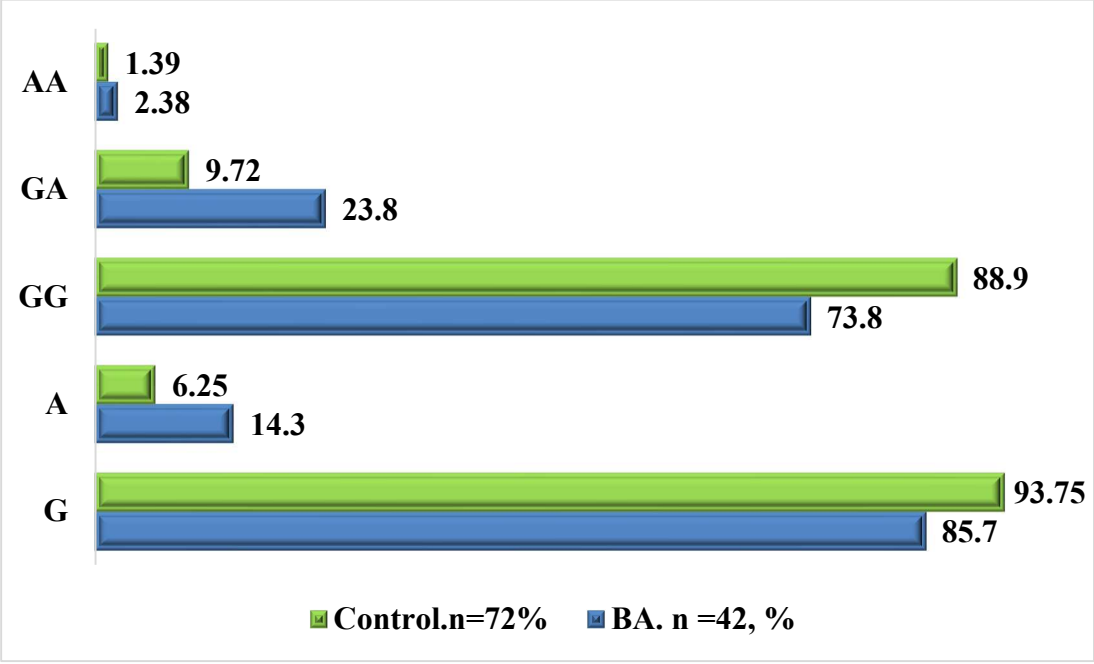
Indicators	Examined patients			
	C.gr.	BA	COPD	ACO
IL-8	11.6	34.7	27.8	39.6
IFNγ	19.1	11.7	14.3	12.4
IPCD	0.60	2.96	1.94	3.19
	(0.45-0.75)	(2.66-3.26)	(1.89-1.99)	(3.02-3.36)

Ratio IL-8 and IFNγ can serve as a reliable prognostic and diagnostic criterion for the course of this disease. Analysis of the result of the index of the prognosis of the course of the disease (IPCD) showed that among the examined, an increased index corresponded to a more severe clinical condition. So, for example, in patients with ACO with IPCD equal to 3.19 and higher, a higher percentage of complications, a severe protracted course, combined with symptoms of intoxication, were observed.

Our studies have shown that immunological parameters make it possible to predict the course of the disease with a fairly high accuracy.

Thus the ratio IL-8 and IFNγ provide important information about the state of the immune system not only at the time of the examination, but also allows predicting the further course of the disease. The study of these cytokines will help the doctor in determining the choice and duration of the necessary therapy.

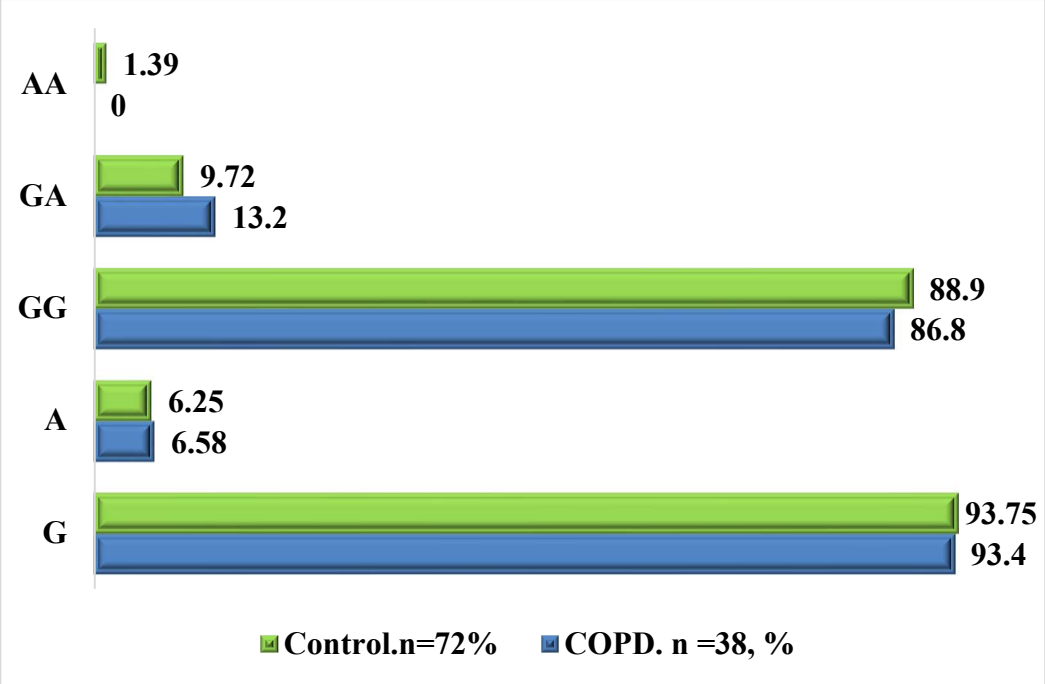
A comparative analysis of the data obtained from BA patients with the control group revealed that in the group the bronchial asthma genotype AA was found in 2.38% (n = 1), the GA genotype was found in 23.81% (n = 10), GG in 73.81% (n = 31) examined. The frequency of occurrence of the A allele was 14.29% (n = 12), the frequency of the G allele was 85.71% (n = 72). (pic. 6).



Pic.6.Frequency of occurrence of polymorphism

CYP3A5 (A6986G) in groups with asthma and healthy controls, (%), (P≤0.05)

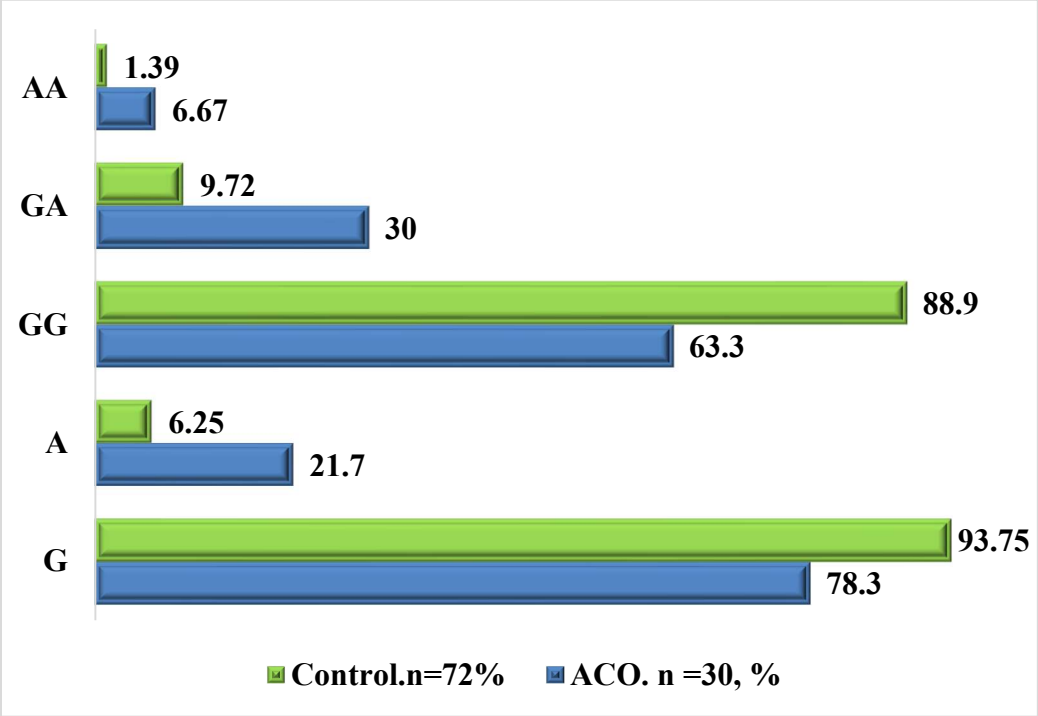
When analyzing the results obtained, it was revealed that allele A was a significantly significant marker of predisposition with a high score in this sample of patients (OR = 2.5, χ2 = 4.097, Wald 95% CI: 1.006> 2.5> 6.213). In turn, compared to practically healthy individuals, marker G was found less frequently in the group of patients (OR = 0.400, χ2 = 4.097, Wald 95% CI: 0.161> 0.4> 0.994). Compared to the group of patients with AD, the GG genotype was found more often in the healthy group (OR=0.352, χ2 =4.343 Wald 95% CI: 0.129 >0.352>0.964), while the GA genotype was found with the highest relative risk and reliability indicators in this group, which indicates its predisposing value (OR=2.902, χ2 =4.149, Wald 95% CI: 1.011 >2.902> 8.33). The AA genotype was more common in the AD group compared to the healthy group, but this indicator was not significant (OR = 1.732, χ2 = 0. 151, Wald 95% CI: 0.105 > 1.732 > 28.43).
Next, we determined the frequency of occurrence of the CYP3A5 polymorphism (A6986G) in the groups with COPD and in the control group. In the group of patients with COPD, the AA genotype was not detected, the GA genotype was found in 13.16%, the GG genotype in 86.84% of those examined, the frequency of occurrence of the A allele was 6.58%, the frequency of the G allele was 93.42%. (pic.7)



Pic.7. Frequency of occurrence of polymorphism CYP3A5 (A6986G) in groups with COPD and control group, (%), (P≤0.05)

In the studied sample of patients with COPD, genotyping of the A6986G polymorphism did not result in significant differences in the frequency of occurrence of alleles and genotypes. In a comparative analysis of the results obtained, it was revealed that in this sample of patients, allele A was a marker of predisposition to COPD (OR = 1.056, χ2 = 0.009, Wald 95% CI: 0.341> 1.056> 3.271). The genotype marker G was characterized by a distribution similar to the allelic distribution in the healthy sample of the studied individuals (OR=0.947, χ2 =0.009, Wald 95% CI: 0.306> 0.947> 2.932). Compared to the group of patients with COPD, the GG genotype was more common in the healthy group (OR = 0.825, χ2 = 0.1, Wald 95% CI: 0.25 >0.825 > 2.722), while the GA genotype had the highest relative risk in this group , which indicates its predisposing value (OR = 1.407, χ2 = 0.302, Wald 95% CI: 0.415 > 1.407 > 4.774).
Next, a comparative analysis of molecular genetic data was carried out in patients with PBA with the control

group (pic. 8).

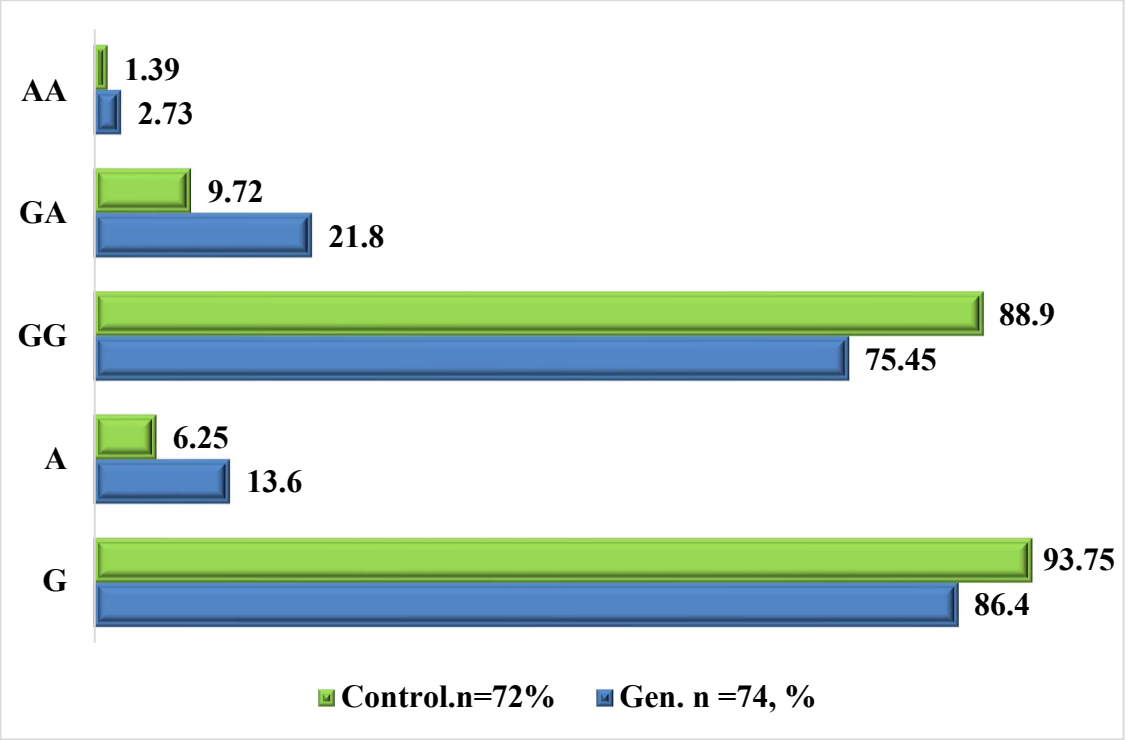


Pic.8. Frequency of occurrence of polymorphism CYP3A5 (A6986G) in groups with ACO and healthy controls, (%), (P≤0.05)

In the group with PBA, the AA genotype was found in 6.67% (n = 2), the GA genotype was found in 30.00%, and the GG genotype in 63.33% of those examined. The frequency of occurrence of the A allele was 21.67%, the frequency of the G allele was 78.33%.

When analyzing the results obtained, it was revealed that in this sample of patients, allele A was a significantly significant predisposition marker with a high significance index (OR=4.149, $\chi 2$ =9.478, Wald 95% CI: 1.666> 4.149> 10.332). In turn, compared to practically healthy individuals, marker G was found less frequently in the group of patients (OR = 0.241, $\chi 2$ = 9.478, Wald 95% CI: 0.097 > 0.241 > 0.6). Compared to the group of patients with PBA, the GG genotype was more common in the healthy group (OR=0.216, $\chi 2$ =9.124 Wald 95% CI: 0.076 >0.216>0.614), while the GA genotype was more common in the sick group (OR=3.980, $\chi 2$ =6.584, Wald 95% CI: 1.32 >3.98 > 11.997). The AA genotype has the highest relative risk, but this indicator is not significant (OR = 5.071, $\chi 2$ = 2.066, Wald 95% CI: 0.442 > 5.071 > 54.184).

Based on the data obtained, we can conclude that the GA genotype of the polymorphic marker CYP3A5 (A6986G) is a significantly significant marker of susceptibility to the development of ACO (pic. 9).



Pic.9. Frequency of occurrence of polymorphism CYP3A5 (A6986G) in the general group of patients and the control group, (%), (P≤0.05)

Summarizing the data obtained on the frequency of occurrence of the CYP3A5 gene (G6986A) in the general group of patients (BA, COPD, ACO) and in the control group, we found that allele A was a significantly significant marker of susceptibility with a high OR = 2.368, $\chi 2$ = 4.964, Wald 95 % CI: 1.089>2.368>5.151. Compared to practically healthy individuals, marker G was found less frequently in the group of patients (OR=0.422, $\chi 2$ =4.964, Wald 95% CI: 0.194>0.422>0.918). Compared to the healthy group, the GG genotype was more common in the patient group (OR=0.384, $\chi 2$ =5.056 Wald 95% CI: 0.164 >0.384>0.902). The GA genotype had the highest relative risk rates in the group of patients, which indicates its predisposing value (OR=2.591, $\chi 2$ =4.505 Wald 95% CI: 1.052>2.591>6.383). The AA genotype was more common in the patient group compared to the control group (OR=1.991, $\chi 2$ =0.363, Wald 95% CI: 0.203 >1.991>19.52), but did not reach true significance.

The heterozygous genotype G6986A of the CYP3A5 gene is a risk prognostic genotype in AD and ACO. According to the literature, the presence of the GA genotype and the presence of the A allele of this polymorphism dictates the need for additional doses of glucocorticosteroids [14, 16].

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