

PHENOTYPICAL CHARACTERISTICS OF THE BIOLOGICAL PROPERTIES OF STAPHYLOCOCCI WITHDRAWN FROM PATIENTS WITH ALLERGIC DERMATITIS

Svetlana Dzhoraeva

Laboratory and experimental department¹
idvnamnu@ukr.net

Nataliya Sobol

Laboratory of Clinical¹
idvnamnu@ukr.net

Helen Ivantsova

Laboratory of Clinical¹
idvnamnu@ukr.net

¹SE «Institute of Dermatology and Venereology of National Academy of Medical Sciences of Ukraine»
7/9 Chernyshevka str., Kharkiv, Ukraine, 61057

Abstract

Atopic dermatitis, eczema, allergic dermatitis occupy the main place among dermatoses, where the allergic component is leading in the onset and development of the disease. The most common complication of allergic dermatitis is the attachment of a secondary pyococcus infection, which is associated with a decrease in the antimicrobial resistance of the skin surface. Therapy of infectious lesions is complicated by the increasing resistance of the main pathogens of pyoderma – Staphylococcus aureus and Staphylococcus epidermidis – to widely used antibiotics.

The aim of the research: to determine the phenotypic features of staphylococci extracted from patients with allergic dermatitis to assess their pathogenic potential.

Materials and methods. The object of the study was 369 staphylococcus isolates removed from affected and intact skin sections of patients with allergic dermatitis, as well as from representative skin sections of healthy individuals undergoing inpatient treatment at the Department of Dermatology of “Institute of Dermatology and Venereology of NAMS of Ukraine”. Biochemical identification and biological properties of staphylococci were determined using methods of classical bacteriology.

Results. As a result of the conducted researches, it is established that the complex of phenotypic traits of the removed staphylococcus cultures indicates the presence in the pathogen of factors related to the resistance of the host protection mechanisms and determines the intensity of the alterative action of the infectant in relation to the host organism, the phenotypic manifestation of the studied factors was higher in the staphylococcus isolates removed from the affected skin areas of patients with allergic dermatitis.

Conclusions. The level and frequency of phenotypic expression of pathogenicity factors are more pronounced in microorganisms obtained from patients from affected and intact areas compared to controls, which confirms their pathogenetic role in the burden of the disease, which in turn can be used as an auxiliary differential diagnosis criterion.

Keywords: clinical strains of staphylococci, allergic dermatitis, infectious complications, pathogenicity factors.

DOI: 10.21303/2504-5679.2020.001125

1. Introduction

Atopic dermatitis, eczema, allergic dermatitis occupy the main place among dermatoses, where the allergic component is leading in the onset and development of the disease. Attention to these diseases is due to the high incidence; constantly recurrent course; increasing the number of trigger factors; insufficient effectiveness of traditional therapy [1].

According to a number of authors, about a tenth of the world's population is ill with eczema. In industrialized countries, this figure reaches 32–45 %, accounting for 51 % of all allergic dermatitis in some regions. According to various authors, the incidence of eczema in Ukraine is from 8 to 30 % of all skin diseases and is the most common pathology in the practice of a dermatologist.

venereologist [2]. According to the World Health Organization and national literature, the number of eczema patients has increased significantly in the last decade [1]. The eczema is characterized by the presence of acute inflammatory symptoms: intense itching of the skin, recurrent course, and increased sensitivity of patients to various exogenous and endogenous stimuli, tendency to spread and deepen the process. The most common complication of the eczematous process is the attachment of secondary urinary and fungal infections, which is associated with a decrease in antimicrobial resistance of the skin surface [3]. Treatment of infectious lesions is complicated by the increasing resistance of the main pathogens of pyoderma – *Staphylococcus aureus* and *Staphylococcus epidermidis*. Uncontrolled use of external antimicrobials, the sensitivity of which is lost, delays the process of rehabilitation of the infection and promotes the subsequent selection of resistant flora [4].

In atopic dermatitis (AD) also the course of the disease is often complicated by the adherence to a secondary infection. This feature reflects the anti-infective protection disorders inherent in patients with hypertension. In the case of *S. aureus* infection, the adverse effect of the microorganism is provided by T-lymphocyte-activating superantigens, stimulating the secretion of pro-inflammatory cytokines, and activating IgE antibody formation, which leads to the degranulation of caudal cells and the release of biologically active substances. In general, in addition to the classical scheme of appearance of IgE in the case of IgE-mediated AD, there is another mechanism of stimulation of its formation. This mechanism is associated with the action of superantigens, high molecular weight proteins, which include some antigens of bacterial or viral origin [5, 6]. Some studies have shown that in patients with AD whose skin was colonized with *S. aureus* containing the superantigen genes, the mean score on the AD severity scale – SCORAD – was significantly higher than in patients whose skin was colonized with *S. aureus* isolates without superantigen genes [7].

Taking into account the above data on the negative effect of *S. aureus* on the course of blood pressure, it should be noted that although it is not a commensal skin, but it is known that the place of its vegetation may be nasal passages, from which about 20 % of the population the microorganism is extracted, and its translocation to the skin may occur [8, 9].

In addition, in recent years, a great deal of attention has been paid to the study of microbiocenosis and penetration of microorganisms into the skin in patients with chronic allergic skin diseases. The skin microbiota is considered as part of a meta-organism that includes a macro-organism and a set of all microorganisms - symbionts. This implies the existence of complex relationships between the microbiota and the human body, and, first, with its immune system, which not only regulates the interaction of the organism with the microbiota, but also itself formed under its influence [10, 11]. At the same time, when investigating the microbiome of the skin of patients with allergic dermatitis, saprophytic species are often removed: *S. epidermidis*, *S. haemolyticus*, *S. saprophyticus*, *S. capitis*, *S. warneri*, *S. hominis*, *S. simulans* and *S. cohnii* harmful effects on the human body, but these properties are inherent in these microorganisms provided vegetation on healthy skin. However, it is often not possible to draw a clear line between saprophytes and pathogens that inhabit healthy human skin. Normal microflora play an important role in protecting the body against pathogenic microorganisms, although it may itself be a contributor to infectious disease [12, 13]. However, it is known that pathogens of coagulase-negative staphylococci such as proteases, hemolysins, lipases, etc. provide storage of these microorganisms on the damaged skin [7, 13]. That is why the study of the biological properties of coagulase-negative staphylococci with a definition of their pathogenic potential is a necessary diagnostic measure for the rational treatment of infectious complications of allergic dermatitis.

The aim of the research: to determine the phenotypic features of staphylococci extracted from patients with allergic dermatitis to assess their pathogenic potential.

2. Materials and methods

The object of the study were 369 isolates of staphylococci, removed from affected and intact areas of patients' skin for allergic dermatitis, as well as from representative areas of skin of healthy individuals undergoing inpatient treatment at the Department of Dermatology of "Institute of Dermatology and Venereology of NAMS of Ukraine" in 2017–2019 years. Biochemical identification

and biological properties of staphylococci were determined using bacterioscopic and bacteriological methods of investigation [14–16].

Statistical processing of the results was performed using the software package for Microsoft Excel 2003. The arithmetic mean values for a series of data (M) and the error of averages (m) were calculated. The reliability of the data obtained was evaluated by pairwise comparison and determination of the confidence interval based on the Student's t-factor calculation (t). The differences were considered statistically significant at $p < 0.05$. The degree of correlation was estimated using Pearson correlation analysis (R) to determine statistical significance (p). If the value of the correlation coefficient R per module is closer to 1, then it means that there is a strong connection, and if closer to 0 – the connection is weak or missing.

3. Results of the research

As a result of the analysis of bacteriological studies, comparative data were obtained regarding the staphylococcal component of the skin microbiocenosis of 114 patients with allergic dermatitis and 20 practically healthy persons. From these groups, 386 strains of microorganisms (346 strains from patients and 40 strains from practically healthy persons) were isolated, which were assigned to 4 genera: *Staphylococcus*, *Streptococcus*, *Micrococcus* and *Corynebacterium spp.*, with predominance of varieties of staphylococci – 369 strains (95.6%), which were removed both individually and in interspecies and intraspecific associations (predominantly).

In the beginning, the composition of the skin microbiota of patients with atopic dermatitis, eczema, and a group of practically healthy individuals was analyzed. In Fig. 1, 2 presents the ratio of the most common types of staphylococci in the skin biotopes of patients with allergic dermatitis.

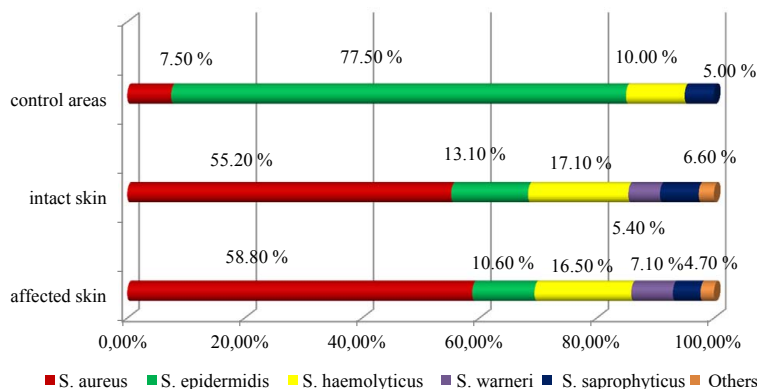


Fig. 1. Determination of the composition of staphylococcal component of the skin biotope of patients with AD and healthy individuals

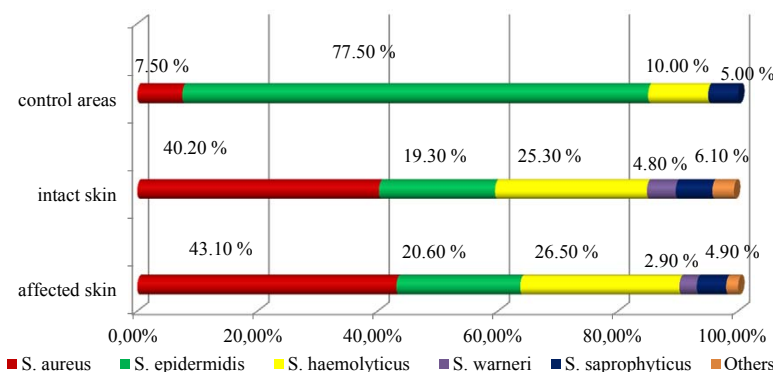


Fig. 2. Determination of the composition of staphylococcal component of the skin biotope of eczema patients and healthy individuals

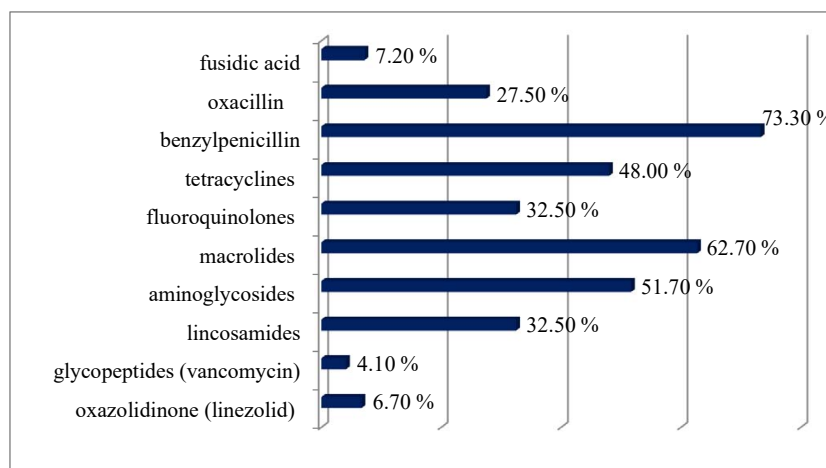
In the next phase of the study, the obtained data from pathogenic staphylococcal cultures were analyzed to determine the enzymatic activity of staphylococcus cultures (**Table 1**).

Table 1

Enzymatic activity of laboratory strains of staphylococci extracted from patients with allergic dermatitis

Staphylococcus type	Presence of activity			
	haemolytic	lipolytic	proteolytic	
			Fermentation of milk	Gelatin fermentation
n, abs. (M±m, %)	n, abs. (M±m, %)	n, abs. (M±m, %)	n, abs. (M±m, %)	
<i>S. haemolyticus</i>	75 (100 %)	62 (82.7±4.4 %)	37 (49.3 ±5.8 %)	18 (24.0±4.9 %)
<i>S. epidermidis</i>	31 (55.46.6± %)	43 (76.8±5.6 %)	41 (73.2 ±5.9 %)	11 (19.6±5.3 %)
<i>S. saprophyticus</i>	9 (47.411.5± %)	7 (36.8 %)	3 (15.8 %)	1 (5.2 %)
<i>S. warneri</i>	6 (35.3 %)	8 (47.1 %)	4 (23.5 %)	2 (11.8 %)
Total	121 (72.5±3.5 %)	120 (71.9±3.5 %)	85 (50.9±3.9 %)	32 (19.2±3.0 %)
<i>S. aureus</i>	170 (100 %)	127 (74.7±3.3 %)	162 (95.3±1.6 %)	21 (12.3±2.5 %)

The next stage of the study was to investigate the antibiotic resistance of the extracted laboratory strains of *S. aureus* to antibacterial preparations of different chemical groups, as the most pathogenic representative of the genus, to identify such antibacterial agents that led to the most complete elimination of the pathogen. The obtained data are shown in **Fig. 3**.

**Fig. 3.** Resistance rates of *S. aureus* strains isolated from patients with allergic dermatitis

4. Discussion

Staphylococci – representatives of normal microflora of the skin, mucous membranes of the respiratory and digestive tract, but they can cause severe infectious complications in patients with allergic dermatitis. The phenomenon of competitive relationships between *S. aureus* and *S. epidermidis* is also described in the literature. Substances that produce *S. epidermidis* (autoinducers) are known to block the formation of toxins in many strains of *S. aureus*, whereas enzymes synthesized by *S. aureus* do not inhibit the proliferation of *S. epidermidis* [13, 17].

According to the research results, the bacterial flora in persons with allergic dermatitis was significantly different from the microflora of healthy people and had its own characteristics, due to the formation of new microbial associations, which changed the habitat and the relationship between the associates. The study of microbial constituents of biotopes showed the dominance of microorganisms of the genus *Staphylococcus* in the skin cells of both patients and healthy individ-

uials. The difference was observed in the species composition of staphylococci and the degree of insemination of individual lesions. The appearance of non-resident staphylococcus species with higher pathogenic potential in affected and intact skin areas was a distinctive feature for most patients (**Fig. 1, 2**). As can be seen from **Fig. 1**, lesions of *S. aureus* strains (58.8 %) were observed in the lesions of the affected skin with a gradual decrease in the areas of intact skin and skin of healthy individuals (55.2 % and 7.5 %, respectively). The number of *S. epidermidis* strains found was inversely related to *S. aureus* strains (10.6 %, 13.1 %, and 77.5 %, respectively). No correlation relationships were found for *S. haemolyticus* strains depending on the extraction site.

As can be seen from **Fig. 2**, the prevalence of *S. aureus* strains (43.1 %) was observed in the lesions of the affected skin of eczema patients with a gradual decrease in the areas of intact and healthy skin (40.2 % and 7.5 %, respectively). The number of found *S. epidermidis* strains was inversely related to *S. aureus* strains (20.6 %, 19.3 %, and 77.5 %, respectively). The density of colonization in the centers of damaged skin of patients with allergic dermatitis averaged 10^7 – 10^8 CFU/ml, in intact areas and skin of healthy individuals – 10^3 – 10^5 CFU/ml.

The study of the biological properties of staphylococci showed that all isolates had typical morphology (gram-positive cocci) and biochemistry, in the catalase test – catalase-positive, all the extracted *S. aureus* strains had coagulase activity.

Haemolytic activity (β -type) was possessed by 72.5 ± 3.5 % of coagulase-negative staphylococci, of which 55.4 ± 6.6 % of *S. epidermidis* cultures (**Table 1**). Lipolytic activity was found in 71.9 ± 3.5 % of coagulase-negative staphylococci, more frequently in *S. haemolyticus* (82.7 ± 4.4 %) and *S. epidermidis* (76.8 ± 5.6 %). When determining this trait in *S. aureus* cultures, it was found that 74.7 ± 3.3 % of the strains were positive in this test. It is important to note that lipolytic activity was more commonly observed in strains of *staphylococci* extracted from affected lesions of patients with allergic dermatitis than from intact or control areas.

Staphylococci with proteolytic activity are more aggressive and, in the body, cause toxic tissue damage [11]. In the study of proteolytic activity, it was noted that about half of the coagulase-negative staphylococcus cultures fermented milk more often (50.9 ± 3.9 %), with *S. epidermidis* strains making up 73.2 ± 5.9 % and less frequently diluting gelatine (19.2 ± 3.0 %), regardless of the locus of crop selection. A similar trend was observed among laboratory isolates of *S. aureus*: most cultures fermented milk (95.3 ± 1.6 %) and infrequently diluted gelatine (12.3 ± 2.5 %).

A direct statistically significant relationship was established in *S. haemolyticus* and *S. aureus* between haemolytic activity and lipolytic activity ($R=0.99$, $p=0.0001$ and $R=0.9$, $p=0.0001$, respectively), and between haemolytic activity and fermentation of milk of designated varieties of staphylococci – $R=0.95$, $p=0$ and $R=0.99$, $p=0.0001$, respectively. For *S. epidermidis* cultures, these values were slightly lower – between haemolytic activity and lipolytic activity ($R=0.46$, $p=0.01$) and between hemolytic and fermentation of milk – $R=0.49$, $p=0.04$. The obtained data indicate the importance of individual pathogens of these microorganisms both in the development and maintenance of the inflammatory process of infectious genesis, and of the combined influence of these factors at a certain stage of the infectious process.

In determining the resistance of *S. aureus* to antibacterial preparations of different chemical groups (**Fig. 3**), the detection of 73.3 % of penicillin-resistant strains draws attention, with 27.5 % of them being so-called MRSA strains, which makes it impossible prescribing to the patient any β -lactam antibiotics. Isolated strains showed moderate sensitivity to aminoglycosides, lincosamides and tetracyclines – ranging from 49 % to 52 %, and quite low to macrolides – up to 37.3 %. Also noteworthy is the appearance in the structure of antibiotic resistance of vancomycin-resistant strains – 4.1 %, which indicates an increase in the aggressive potential of the pathogens obtained. The sensitivity of the extracted pathogens was highest for the preparations of fusidic acid, oxazolidinone and fluoroquinolones – 92.8 %, 93.3 % and 67.5 % of the strains, respectively. It is known that the use of one class of antibiotics may increase the risk of developing resistance to another class, so at the next stage of the study, a comprehensive assessment of the resistance of the extracted strains of *S. aureus* was made, taking into account the prevalence of polyresistant strains. Based on the monitoring, 54 % of MDR strains and 4 % of XDR strains were detected. Significant is the complete absence of PDR strains, i.e. microorganisms resistant to all classes of known antibiotics.

Study limitations. The study included adult patients with allergic dermatitis diagnosed with atopic dermatitis (L 20.0) and eczema (L 30.0 – other dermatitis) according to the International Classification of Diseases 10.

Prospects for further research. Further studies include the study of correlations between phenotypic and genotypic traits of extracted pathogen isolates in order to develop algorithms for predicting the severity of marked allergic dermatitis.

5. Conclusions

Thus, the complex of phenotypic features of the removed staphylococcus cultures indicates the presence in the pathogen of factors associated with the resistance of host protection mechanisms, on the one hand, and high pathogenic potential, on the other, which promotes active colonization of both affected and intact areas of the skin, which provides the conditions for long-term persistence and determines the intensity of the alterative action of the infectious agent against the host organism.

The level and frequency of phenotypic expression of pathogenicity factors are more pronounced in microorganisms obtained from patients from affected and intact areas compared to controls, which confirms their pathogenetic role in the burden of the disease, which in turn can be used as an auxiliary differential diagnosis criterion.

Conflict of interests

There is no conflict of interest.

References

- [1] Kantor, R., Thyssen, J. P., Paller, A. S., Silverberg, J. I. (2016). Atopic dermatitis, atopic eczema, or eczema? A systematic review, meta-analysis, and recommendation for uniform use of “atopic dermatitis.” *Allergy*, 71 (10), 1480–1485. doi: <http://doi.org/10.1111/all.12982>
- [2] Kutasevich, Ya. F., Ishcheykin, K. E., Zuban, I. V., Mangusheva, V. Y. (2018). Diferentchiyvanyi pidhid do diagnostiki ta zovnishnoi terapii ekzemy. *Dermatologiya ta venerologiya*, 1, 50–55.
- [3] Potekae, N. S. (2006). Ekzema: aspekty istorii I sovremennoe predstavlenie. *Klinicheskaya dermatologiya i venerologiya*, 4, 102–107.
- [4] Dzoraeva, S. K., Kutasevich, Ya. F., Oliynyk, I. O., Goncharenko, V. V. et. al. (2013). Vuvchennya faktoriv patogenosti staphylokokovoi mikroflory shkiry u khvorykh na poshireni dermatozy. *Dermatologiya ta venerologiya*, 1 (59), 20–25.
- [5] Belkaid, Y., Segre, J. A. (2014). Dialogue between skin microbiota and immunity. *Science*, 346 (6212), 954–959. doi: <http://doi.org/10.1126/science.1260144>
- [6] Rojo, A., Aguinaga, A., Monecke, S., Yuste, J. R., Gastaminza, G., España, A. (2013). Staphylococcus aureus genomic pattern and atopic dermatitis: may factors other than superantigens be involved? *European Journal of Clinical Microbiology & Infectious Diseases*, 33 (4), 651–658. doi: <http://doi.org/10.1007/s10096-013-2000-z>
- [7] Nada, H. A., Gomaa, N. I. M., Elakhras, A., Wasfy, R., Baker, R. A. (2012). Skin colonization by superantigen-producing Staphylococcus aureus in Egyptian patients with atopic dermatitis and its relation to disease severity and serum interleukin-4 level. *International Journal of Infectious Diseases*, 16 (1), e29–e33. doi: <http://doi.org/10.1016/j.ijid.2011.09.014>
- [8] Oh, J., Byrd, A. L., Deming, C., Conlan, S., Kong, H. H., Segre, J. A. (2014). Biogeography and individuality shape function in the human skin metagenome. *Nature*, 514 (7520), 59–64. doi: <http://doi.org/10.1038/nature13786>
- [9] Sergeev, A. U., Burtcheva, G. N., Sergeev, V. U. (2014). Staphylokokovaya kolonizatsiya kozhi, antibiotikorezistentnost i protivomirobnaya terapiya pri rasprostranennykh dermatozah. *Immunopatologiya, allergologiya, infectologiya*, 4, 32–45.
- [10] Kutasevych, Ya., Dzhoraeva, S., Shcherbakova, Yu., Bondarenko, G., Sobol, N. (2019). Studying the pathogenic properties of the skin's autoflora in patients diagnosed with atopic dermatitis. *Georgian Med News*, 7-8 (292-293), 113–117.
- [11] Grice, E. A., Kong, H. H., Renaud, G., Young, A. C., Bouffard, G. G. et. al. (2008). A diversity profile of the human skin microbiota. *Genome Research*, 18 (7), 1043–1050. doi: <http://doi.org/10.1101/gr.075549.107>
- [12] Murzina, E. A. (2009). Optimizatsiya patogeneticheskoi terapii pri atopicheskom dermatite, *Ukrainskii zhurnal dermatologii, venerologii, kosmetologii*, 2 (33), 16–19.
- [13] Granichnaya, N. V., Zaytcheva, E. A., Bondar, V. U. (2017). Fenotipicheskaya kharakteristika biologicheskikh svoystv koagulazonegativnykh staphylokokov, vudelennykh v kardiokhirurgicheskome stacionare. *Almanakh klinicheskoy meditsiny*, 45 (2), 127–132.

- [14] Ob unifikatsii mikrobiologicheskikh (bakteriologicheskikh) metodov issledovaniya, primenyaemih v kliniko-diagnosticheskikh laboratoriyah lechebno-profilakticheskikh uchrezhdeniy (1985). MZ SSSR, No. 535. Available at: <http://www.alppp.ru/law/zdravoohranenie--fizicheskaja-kultura-i-sport--turizm/zdravoohranenie/64/prikaz-minzdrava-sssr-ot-22-04-1985--535.html>
- [15] Baron, E. J., Miller, J. M., Weinstein, M. P., Richter, S. S., Gilligan, P. H., Thomson, R. B. et. al. (2013). A Guide to Utilization of the Microbiology Laboratory for Diagnosis of Infectious Diseases: 2013 Recommendations by the Infectious Diseases Society of America (IDSA) and the American Society for Microbiology (ASM). *Clinical Infectious Diseases*, 57 (4), e22–e121. doi: <http://doi.org/10.1093/cid/cit278>
- [16] Pro zatverdzhennya metodichnykh vkazivok “Vyznachennya chuvstvitelnosti mikroorganizmov do antibakterialnykh preparativ” (2007). MOZ Ukraine, No. 167. 05.04.2007. Available at: http://search.ligazakon.ua/1_doc2.nsf/link1/MOZ6809.html
- [17] Kozlovz, N. S., Barantchevich, N. E., Ivanova, L. V. et. al. (2015). Chuvstvitelnost k antibakterialnym preparatam stafilokokov, tchirkuliruushchikh v mnogoprophilnom statchionare. *Problemy meditchinskoy mikologii*, 17 (4), 58–62.

Received date 21.11.2019

Accepted date 14.01.2020

Published date 31.01.2020

© The Author(s) 2020

This is an open access article under the CC BY license

(<http://creativecommons.org/licenses/by/4.0>).

DYNAMICS OF FUNCTIONAL CONDITION AND QUALITY OF LIFE IN PATIENTS WITH ASTHMA-COPD OVERLAP AND CONCOMITANT ARTERIAL HYPERTENSION AGAINST THE BACKGROUND OF COMPLEX THERAPY

*Maryna Kochuieva*¹

kochuevamarina@gmail.com

*Hanna Tymchenko*¹

hanna.zaprovalna@gmail.com

*Ivan Hrek*¹

grek.ivan.md@gmail.com

*Yulia Zaikina*¹

julia.ua.h@gmail.com

¹*Department of Phthiology, Pulmonology and Family Medicine*

Kharkiv Medical Academy of Postgraduate Education

58 Amosova str., Kharkiv, Ukraine, 61176

Abstract

Patients with ACO have significant poorer health-related quality of life and more severe functional limitations compared to asthma and COPD alone. Most commonly, chronic respiratory disease is associated with cardiovascular disease, such as arterial hypertension. However, the impact of concomitant cardiac diseases on the quality of life and functional status of patients with ACO remains poorly understood.

The aim of the work was to study dynamics of functional condition and quality of life in with ACO and concomitant AH against the background of complex therapy.

Materials and methods. We selected for participating in the study 100 patients with ACO and concomitant AH. Examination of the patients included: clinical methods, spirometry, and questionnaires – mMRS, CAT, SGRQ, performing 6MWT.

Results. After 16 weeks of treatment there were no changes in lung functional status in patients on standard treatment, at the same time, in group of patients who had an active rehabilitation program, there was a significant improvement in the bronchial response to the action of bronchodilators, although other indicators of the functional status of the lungs didn't show significant changes. Patients