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# THE USE OF BACTERIAL LYSATES IN THE COMPLEX TREATMENT OF PATIENTS WITH CHRONIC DECOMPENSATED TONSILLITIS

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## Introduction

Chronic tonsillitis (CT) occupies a leading place in the structure of general Oto-rhino-laryngology (ORL) pathology which is characterized by a tendency to an increase in the number of patients with recurrent course, the development of complications and the diversity of etiopathogenetic mechanisms of onset. Among the medico-social tasks that determine the scientific and practical strategy in solving CT, the central place belongs to the search for adequate, safe and effective methods of treatment and rehabilitation of patients with CT. After all, an impressive arsenal of current therapeutic tactics used in CT therapy, cannot effectively alleviate this pathology [1, 2].

In the pathogenesis of chronic tonsillitis, the disturbance of the microbiota of the mucous membranes of the tonsils is of great importance since the oropharynx is an ecological system in which external factors interact dynamically with the internal ones while maintaining an equilibrium state. Indigenous microflora of a person has a number of vital functions, the main ones of which are colonization resistance, the stimulation of hereditary and adaptive immunity, detoxification, etc. Any stress factor that affects the microflora that normally forms the microbiocenosis of a particular biotope can lead to a loss of tolerance of the immune system to the microflora and development of the immune response [3, 4].

It has been established that the most acceptable tactic for the prevention of exacerbations of chronic tonsillitis is the use of various groups of drugs that enhance the humoral immunity of the mucous membranes, for example, ribomunil, imupret, which are applied orally, or by prophylactic vaccination with drugs lysates from bacteria (type IRS 19, imudon, bronhovax, bronchomunal) [5-7].

The drug Ismigen (certificate of state registration of a medical immunobiological drug in Ukraine No. UA/15678/01/01, Order of the Ministry of Health of Ukraine of December 22, 2016 №. 1391) is an immunostimulant based on a bacterial lysate, which increases the body's resistance to infections due to an increase in serum and secretory antibodies, activation of cellular and humoral factors of nonspecific immunity.

Bacterial lysates of 13 bacteria (*Staphylococcus aureus, Streptococcus pyogenes, Streptococcus viridans, Klebsiella pneumoniae, Klebsiella ozaenae, Haemophillus influenza, Moraxella catarrhalis* and 6 types of pneumococci (*Streptococcus pneumoniae*) that form part of Ismigen are obtained by mechanical lysis, which gives certain advantages over others immunomodulators, which include chemical lysates. With mechanical lysis, antigenic structures, in particular corpuscular antigens, are more fully preserved, which provides a long-term immune response. Evaluation of the use of Imigen/Respibron® compared to chemical lysate in patients with lower respiratory tract infections showed statistically significant differences in the efficacy of mechanical lysates [8]. Results of randomized clinical trials in 2557 patients showed that the use of mechanical bacterial lysates induces a significant reduction in recurrence of infections in both children and adults [9].

In previous studies, we showed that in patients suffering from chronic decompensated tonsillitis, micro-ecological disturbances of the normal biocenosis of the tonsil mucosa are observed. Elimination of the indigenous microflora leads to colonization of the biotope by pathogenic and opportunistic microorganisms, which aggravates the course of the underlying disease [10].

The foregoing study shows that the use of — Ismigen in the complex treatment of patients with chronic decompensated tonsillitis seems to be relevant.

**The aim of the study** was to evaluate the effectiveness of the use of Ismigen in the complex treatment of patients with chronic decompensated tonsillitis by studying the dynamics of microbiological indicators.

## Materials and methods

To achieve this goal, 31 patients with chronic decompensated tonsillitis (CDT) (according to I.B. Soldatov's classification, 1975) were examined. Patients involved in the study did not take antimicrobial drugs for three months and had no other medical pathology other than the one examined. Patients were divided into 2 groups depending on the proposed treatment regimen:

***Group A:*** patients with chronic decompensated tonsillitis who underwent laser tonsillotomy - 15 patients;

***Group B:*** patients with chronic decompensated tonsillitis who underwent laser tonsillotomy with further immunostimulation with the drug Ismigen - 16 patients.

The control group (CG) consisted of 17 practically healthy individual who had no chronic pathology of the upper respiratory tract in the anamnesis and did not receive any medical therapy within a month before the examination.

To conduct laser tonsillotomy, a high-energy laser semiconductor device "Lasermed-10-01" was used at a wavelength of 1.06 μm and a power density of 7-8 mW/cm$^2$. Patients underwent premedication and local infiltrative anesthesia in the paratonsillar tissue. A laser with preset parameters in the contact mode with the help of a light guide

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was used to cut off the lacerated surface of the tonsils. Duration of bilateral tonsillotomy with anesthesia was roughly 15-20 minutes.

Patient in group B had sublingual Ismigen postoperatively: once daily for a period for a period of 10 days [11].

Microbiological examination of the material from the mucous membranes of the tonsils or oropharynx was carried out in dynamics before the treatment, 7 days after the treatment and 1-2 months after the end of the course of treatment. Determination of the qualitative and quantitative composition of the microbiocenosis of the studied biotope was carried out in accordance with the current normative documents according to generally accepted methods. Identification of isolated bacterial cultures was carried out according to morphological, cultural, biochemical features in accordance with the «Determinant (identifier) of Bacteria Berdgi», identification of fungal strains - according to «The determinant of pathogenic and opportunistic fungi». The results of determining the number of microorganisms (colony forming units) were expressed in decimal logarithms per gram of clinical material – lg CFU/g.

A comprehensive assessment of the status of the oropharynx microbiocenosis was carried out in accordance with the criteria described earlier [12]. According to these criteria, the status of the microbiocenosis of the oropharynx was divided into: eubiosis, dysbiosis of the 1st degree, dysbiosis of the 2nd degree and dysbiosis of the 3rd degree.

Statistical processing of the data was carried out using the Statistics-8 software package, Microsoft Office Excel 2003.

**Results and discussion**

The main method of treatment of the decompensated stage of chronic tonsillitis for a long time was (and in the routine practice remains) the classical method: total removal of palatine tonsils. However, due to the expansion of knowledge about the fundamentals of immunophysiology of the tonsils, the tonsillar problem in its clinical aspect shifted towards a sparing attitude towards the lymphoepithelial structures of the mouth and nasopharynx, as special formations of the immune system called "border lymphoid tissue" associated with mucosal immunity [13]. Laser tonsillotomy just refers to organ-sparing methods of treatment of CDT.

To assess the effectiveness of the effects of various treatment regimens for patients with CDT on the state of the oropharynx microbiocenosis, an integrated approach to the assessment of microflora was used, which makes it possible to describe the communities that make up the microbiotic component of the studied ecosystem. The generalized results of the distribution of patients with CDT and controls of the above-mentioned degrees of dysbiosis and their dynamics under the influence of various treatment methods are presented in Table 1.

**Table 1. Dynamics of changes in the state of the oropharyngeal microbiocenosis in patients with chronic decompensated tonsillitis using various methods of treatment**

| Patient Groups | Terms of Examination of patients | Specificity (%) of patients in groups with | | | |
|---|---|---|---|---|---|
| | | Eubiosis | Dysbiosis 1-degree | Dysbiosis 2-degree | Dysbiosis 3-degree |
| Laser Tonsilotomy (gp. A, n=15) | Before treatment | 0 | 7,1 | 32,9 | 60,0 |
| | After treatment | 46,7 | 40,0 | 13,3 | 0 |
| | 1-2 months after treatment | 26,7 | 53,3 | 20,0 | 0 |
| Laser Tonsilotomy + Ismigen (gp. B, n=16) | Before treatment | 0 | 6,3 | 32,3 | 62,5 |
| | After treatment | 62,1 | 31,6 | 6,3 | 0 |
| | 1-2 months after treatment | 81,3 | 18,7 | 0 | 0 |
| Control group (n=17) | | 88,2 | 11,8 | 0 | 0 |

Before treatment, in the decompensated state of CT (59.7±2.8)% of the examined subjects was accompanied by dysbiotic manifestations and were classified to grade 3: 60.0% of patients in group A and in 62.5% of patients in group B. In no case pathology of the tonsils did not reveal the microbiological picture of eubiosis. In the control group, the microbial representatives of eubiosis colonized the surveyed biotope in 88.2% of practically healthy individuals.

Carrying out various types of therapeutic measures for patients with CDT positively affected the microbiocenosis status of the studied biotope in comparison with the initial data.

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In the first week after the treatment, all subjects surveyed had almost no dysbiotic condition of grade 3. The proportion of patients who had eubiosis or compensated dysbiosis a week after the end of treatment in groups A and B was 86.7-93.7%, respectively.

More clearly, the difference between different methods of treatment of patients with CDT manifested itself after 1-2 months. In patients who underwent laser tonsillotomy with further immunomodulation with bacterial lysates, the microbial communities of the mucous membranes of preserved tonsils did not statistically differ from the microbiota of the control group of practically healthy individuals: in 81.3% of patients Eubiosis was established in groups and 18.7% in dysbiosis 1st degree. In persons without pathology of the tonsils, these indicators were 88.2% and 11.8%, respectively.

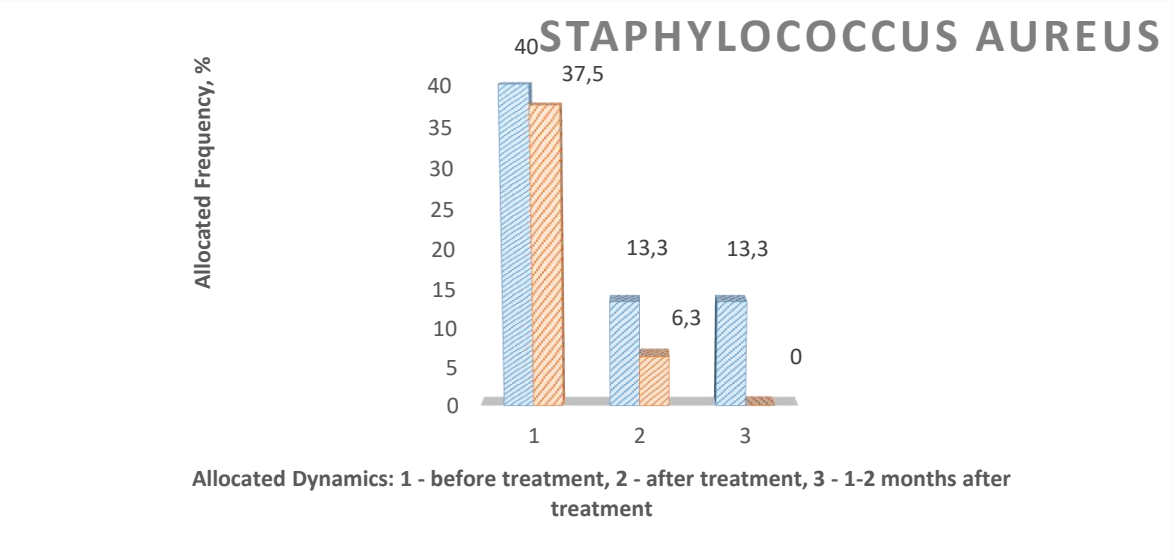
Comparison of the results of microbiological examination of patients in groups A and B showed the advantages of using immunocorrection after laser tonsillotomy. Thus, in 81.3% of the examined group B microbiocenoses of the mucous membranes of preserved tonsils are represented by eubiosis, compared with 26.7% in group A ($p<0.01$). The rest of the patients (18.7%) of group B showed dysbiotic phenomena of 1st degree, against 53.3% of persons in group A ($p<0.05$).

The study of the species composition of the oropharynx microbiota of patients with CDT before treatment and its comparison with bacterial antigens that are part of the preparation of Ismigen showed that practically every patient had persistent one or two respiratory pathogens, which are eliminated by bacterial lysates of Ismigen. Thus, in 46.7-50.0% of patients, pyogenic streptococci were isolated, the colonization density of which with mucous membranes was lg (6.2±0.4) CFU/g; Staphylococcus aureus was found in 37.5-40.0% of the patients examined in an average of lg (5.5±0.6) CFU/g; the presence of Klebsiella was found in 37.5-40.0% of patients (log (5.5±0.6) CFU/g); haemophilus and moraxelles in 20.0-33.3% of cases were excreted in the amount of lg (7.2±0.4) CFU/g and lg (4.9±0.3) CFU/g, respectively.

Immediately after the treatment, there were no significant differences in the incidence of the above-mentioned bacteria between groups of patients (Figures 1-5). Respiratory pathogens were detected in 5 patients of group A and 3 patients of group B. The density of seeding of the biotope decreased significantly compared to baseline values and averaged lg (4.1±0.2) CFU/g ($p<0.01$).

1-2 months after the treatment in none of the patients who received the preparation of «Ismigen», the indicated bacteria were detected in the microbiocenosis of the mucous tonsils, against 66.7% of the group A patients who did not undergo sublingual immunization ($p<0.01$) (Fig. 1-5). «Sanitizing», with respect to the microbial factor of CT, the effectiveness of performed tonsillotomy in a third of patients of group A was lost.

The aforementioned respiratory pathogens are most often encountered in chronic inflammatory processes in the upper respiratory tract. Long-term persistence of microorganisms is accompanied by aggravation of immunological disorders and an increased risk of recurrence of the disease [14]. Our studies confirmed the above for patients in Group A, who underwent adequate pat
hogenetic organ-sparing treatment of the tonsils, but without subsequent immunocorrection.




gr. A. Laser Tonsillotomy;

gr. B. Laser Tonsillotomy + Ismigen

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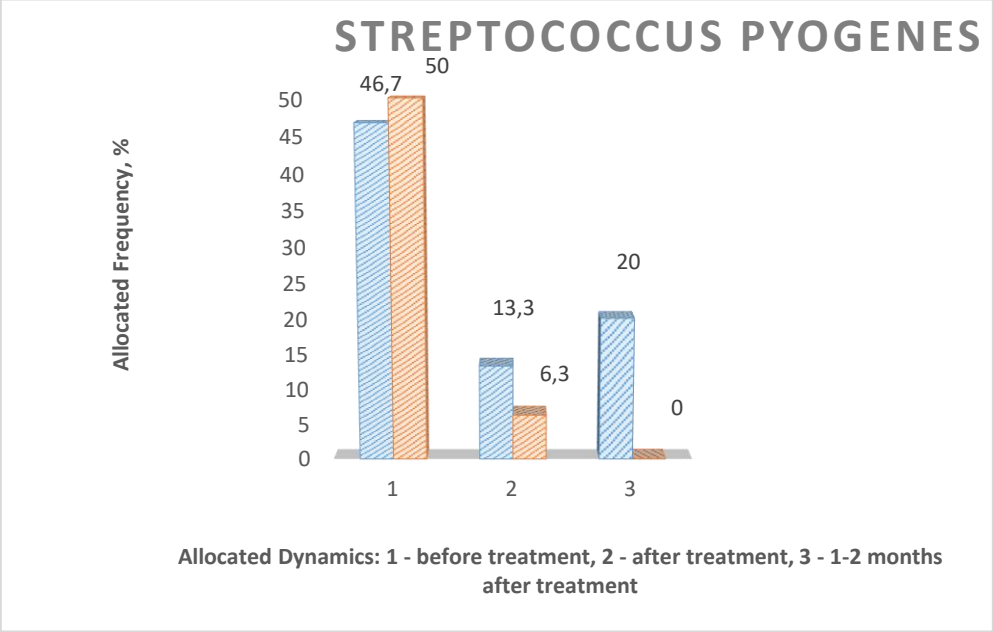
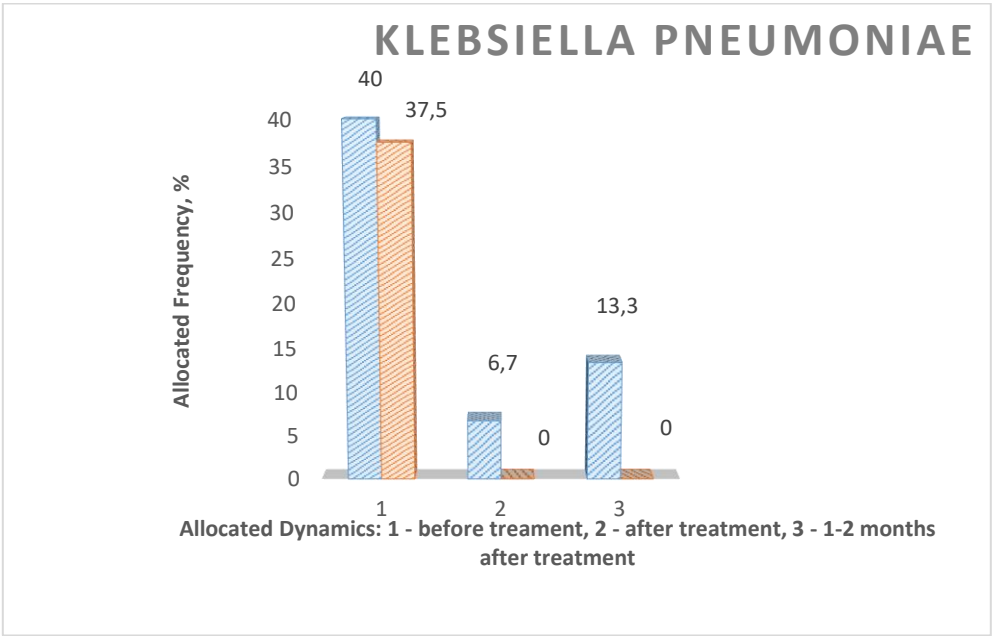
**Fig. 1. The frequency of allocation of *Staphylococcus aureus* from the mucous membranes of palatine tonsils in dynamics, depending on the ongoing treatment of patients with CDT.**




gr. A. Laser Tonsillotomy;

gr. B. Laser Tonsillotomy + Ismigen

**Fig. 2. The frequency of allocation of *Streptococcus pyogenes* from the mucous membranes of palatine tonsils in dynamics, depending on the ongoing treatment of patients with CDT.**




gr. A. Laser Tonsillotomy;

gr. B. Laser Tonsillotomy + Ismigen

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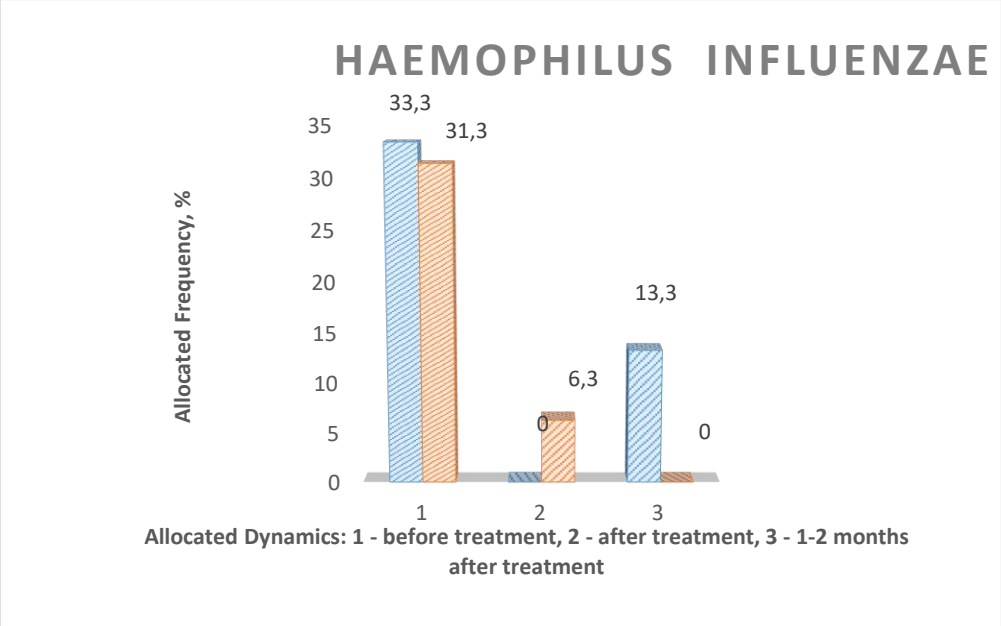
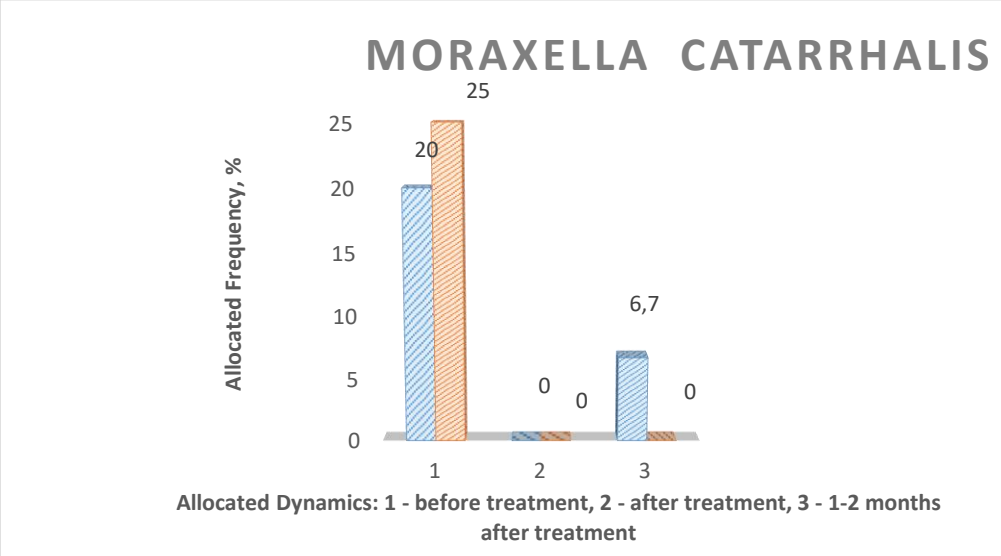
**Fig. 3. The frequency of allocation of *Klebsiella pneumoniae* from the mucous membranes of palatine tonsils in dynamics, depending on the ongoing treatment of patients with CDT.**




gr. A. Laser Tonsillotomy;

gr. B. Laser Tonsillotomy + Ismigen

**Fig. 4. The frequency of allocation of *Haemophilus influenzae* from the mucous membranes of palatine tonsils in dynamics, depending on the ongoing treatment of patients with CDT.**




gr. A. Laser Tonsillotomy

gr. B. Laser Tonsillotomy + Ismigen

**Fig. 5. The frequency of allocation of *Moraxella catarrhalis* from the mucous membranes of palatine tonsils in dynamics, depending on the ongoing treatment of patients with CDT.**

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**Conclusions.**

1. Ismigen, administered to patients with chronic decompensated tonsillitis as part of complex treatment provided a more pronounced antimicrobial effect and promoted restoration of the normocoenosis of the oropharynx.
2. Laser tonsillotomy performed with further immunomodulation with the help of immunostimulant on the basis of bacterial lysates Ismigen, allows to achieve the indices of microbial communities of the mucous membranes of preserved tonsils, which did not differ statistically from the microbiota of the control group of practically healthy persons: in 81.3% of patients, eubiosis and in 18.7% of the dysbiosis of the 1st degree. In persons without pathology of the tonsils, these indicators were 88.2% and 11.8%, respectively.
3. Ismigen has a selective antimicrobial effect against the most common respiratory pathogens, which is objectively manifested by the restoration of the eubiotic colonization profile of the oropharynx in patients with CDT 1-2 months after treatment.

**References**

1. Arefeva N.A. Tonsillar pathology. Current problem / N.A. Arefeva // Vestn. Otorinolar. – 2012. – № 6. – P. 10–13.
2. Chistjakova V.R. Angina tonsillaris and chronic tonsillitis / V.R. Chistjakova // Vestn. Otorinolar. – 2012. – № 1. – P. 68–76.
3. To be or not to be a pathogens: microbiocenosis of upper respiratory tract, physiological inflammation and pattern recognition receptors / Kamyshniy O. M., Voitovich O. V, Topol I. O., Degen A. S. // Annals of Mechnikov Institute. – 2011. – № 4. – C. 246-251. – http://www.imiamn.org.ua/journal/4_2011/PDF/5.pdf
4. Yankovskyi D.S. Dysbiosis and modern approaches to prevention / Jankovskij D.S., Shirobokov V.P., Moiseenko R.A. // Sovremennaja pediatrija. – 2010. – №3 (31). – C. 143-151.
5. Arefeva N.A. Peculiarities of immunotherapy of inflammation of the upper respiratory tract: the results of 30-year investigations / Aref'eva NA., Aznabaeva LF // Vestn. Otorinolar. – 2012. – № 6. – P. 30-32.
6. Palchun V.T. Classification and therapeutic strategy for chronic tonsillitis / Palchun V.T. // Vestn. Otorinolar. – 2013. - №3. – C. 8-11.
7. Luchikhin L.A. The use of immunocorrection therapy for the combined treatment of the inflammatory diseases of the pharynx / Luchikhin L.A. // Vestn. Otorinolar. – 2012. – № 5. – P. 48-51.
8. Rossi S. Efficacy and safety of a new immunostimulating bacterial lysate in the prophylaxis of acute lower respiratory tract infections. A randomised, open, controlled clinical trial / S. Rossi, R. Tazza // Arzneim-Forsch Drug Research. – 2004. – Vol. 54 (1). – P. 50-56.
9. Cazzola M. Polyvalent mechanical bacterial lysate for the prevention of recurrent respiratory infections: a meta-analysis / Cazzola M., Anapurapu S. // Pulm. Pharmacol. Ther. – 2012. – Vol. 25(1). – P. 62-68.
10. Zhuravlev A. S. Microbiocenosis of palatine of tonsils in patients with various forms of chronic tonsillitis / Zhuravlev A. S., Manee Hans, Demina E. V. // Journal of the ear, nose and throat diseases . – 2014 – № 1. – P. 58 – 62.
11. Instructions for use ISMIGEN / Order of the Ministry of Health of Ukraine of October 05, 2011 № 647.
12. Zhuravlev A. S. Features of antimicrobial effects of various methods of treatment chronic decompensated tonsillitis / Zhuravlev A. S., Manee Hans, Demina E. V. // Experimental and clinical medicine. – 2014. – № 1 (62). – P. 99 – 104.
13. Aznabaeva L.F. Immunological aspects of chronic tonsillitis / Aznabaeva LF, Aref'eva NA. // Vestn. Otorinolar. –. – 2013. – № 4. – P. 4-9.
14. Dynamics of microsymbiocenoses of upper respiratory tract in normal state and in pathology / Soboleva IuV, Usviatsov BIa, Khlopko IuA, Bukharin OV. // Zh Mikrobiol Epidemiol Immunobiol. – 2012. – № 3. – P. 55-61.

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***Results and discussion.*** Before treatment, in the decompensated state of CT (59.7±2.8) % of the examined subjects was accompanied by dysbiotic manifestations and were classified to grade 3: 60.0% of patients in group A and in 62.5% of patients in group B. In no case pathology of the tonsils did not reveal the microbiological picture of eubiosis. Carrying out various types of therapeutic measures for patients with CDT positively affected the microbiocenosis status of the studied biotope in comparison with the initial data. Comparison of the results of microbiological examination of patients in groups A and B showed the advantages of using immunocorrection after laser tonsillotomy. Thus, in 81.3% of the examined group B microbiocenoses of the mucous membranes of preserved tonsils are represented by eubiosis, compared with 26.7% in group A ($p<0.01$). The rest of the patients (18.7%) of group B showed dysbiotic phenomena of 1st degree, against 53.3% of persons in group A ($p<0.05$). The study of the species composition of the oropharynx microbiota of patients with CDT before treatment and its comparison with bacterial antigens that are part of the preparation of Ismigen showed that practically every patient had persistent one or two respiratory pathogens, which are eliminated by bacterial lysates of Ismigen. Immediately after the treatment, there were no significant differences in the incidence of the above-mentioned bacteria between groups of patients. Respiratory pathogens were detected in 5 patients of group A and 3 patients of group B. The density of seeding of the biotope decreased significantly compared to baseline values and averaged lg (4.1±0.2) CFU/g ($p <0.01$). 1-2 months after the treatment in none of the patients who received the preparation of «Ismigen», the indicated bacteria were detected in the microbiocenosis of the mucous tonsils, against 66.7% of the group A patients who did not undergo sublingual immunization ($p<0.01$) . «Sanitizing», with respect to the microbial factor of CT, the effectiveness of performed tonsillotomy in a third of patients of group A was lost. The aforementioned respiratory pathogens are most often encountered in chronic inflammatory processes in the upper respiratory tract. Long-term persistence of microorganisms is accompanied by aggravation of immunological disorders and an increased risk of recurrence of the disease [14]. Our studies confirmed the above for patients in Group A, who underwent adequate pathogenetic organ-sparing treatment of the tonsils, but without subsequent immunocorrection.

**Conclusions.** 1. Ismigen, administered to patients with chronic decompensated tonsillitis as part of complex treatment provided a more pronounced antimicrobial effect and promoted restoration of the normocoenosis of the oropharynx. 2. Laser tonsillotomy performed with further immunomodulation with the help of immunostimulant on the basis of bacterial lysates Ismigen, allows to achieve the indices of microbial communities of the mucous membranes of preserved tonsils, which did not differ statistically from the microbiota of the control group of practically healthy persons: in 81.3% of patients, eubiosis and in 18.7% of the dysbiosis of the 1st degree. In persons without pathology of the tonsils, these indicators were 88.2% and 11.8%, respectively. 3. Ismigen has a selective antimicrobial effect against the most common respiratory pathogens, which is objectively manifested by the restoration of the eubiotic colonization profile of the oropharynx in patients with CDT 1-2 months after treatment.

**Keywords:** chronic decompensated tonsillitis, complex treatment, Ismigen, microbiocenosis.

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