

## 2017 № 4

### Contents

# Editorial Board Contents Review

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#### PROPHYLAXIS OF INFLUENZA IN THE ELDERLY. IS THERE ANY ALTERNATIVE?

6-12

**Grishyna O. I., Babinets O. M., Menkus O. V., Kalchenko G. R.**

The benefits of influenza vaccination in the elderly individuals are the subject of serious discussion. Evidence-based medicine can not boast of a large number of randomized clinical trials of the anti-influenza vaccine effectiveness in the elderly due to ethical issues. Over the past 20 years, the only large randomized clinical trial was to investigate an inactivated anti-influenza vaccine in adults aged  $\geq 60$  years, which was performed during one season and limited to healthy subjects. This trial demonstrated a 58% reduction in the risk of serologically verified uncomplicated influenza infection in the patients aged 60-69, but no conclusive findings were made for the individuals aged  $\geq 70$  years, because the capacity of this study was insufficient to investigate the vaccination efficiency in this age group. Moreover, an evidence of efficacy in healthy subjects aged 60-69 can not be related to the elderly at the age of 70, since elderly age and concomitant diseases are associated with an increased risk of complications and the immune system weakening. With respect to the lack of an evidence, based on randomized clinical trials, we use the results of observational, usually retrospective cohort trials that may be biased. We analyzed the results of randomized multicenter vaccine trials including Fluzone High-Dose Vaccine, meta-analysis data, and concluded that evidence for protection in adults aged 65 years or older is lacking. As an alternative, the results of clinical trials and a meta-analysis of the effectiveness of vitamin D3 for the prevention of influenza / influenza-like illnesses are considered. The extraskelatal effects of vitamin D are analyzed. The interest in vitamin D extraskelatal effects has rapidly grown over the last thirty years due to the identification of Vitamin D receptors (VDRs) in different systems, organs, and cell types. The effects of 1.25 (OH) 2D3 on regulation of both inherent and adaptive immune systems are string and their evaluation has been just started. VDR was detected in activated CD4+ and CD8 + T cells, B cells, neutrophils, monocytes, macrophages, and dendritic cells. The results of the meta-analysis twenty five randomized controlled trials (11,321 participants aged from 0 to 95 years) published by Adrian R. Martineau et al. were presented. The meta-analysis has found that adding vitamin D reduced the risk of acute respiratory infections among all the participants (0.88 corrected odds ratio, 95% 0.81-0.96 confidence interval, P for heterogeneity <0.001). Vitamin D did not affect a part of participants who experience at least one serious adverse event (corrected odds ratio of 0.98, 0.80-1.20, P=0.83). It was finally concluded that the vitamin D supplement was safe and generally protected against acute respiratory infections. A conclusion was drawn on the need for a large clinical trial comparing the efficacy and safety of a flu vaccine and vitamin D3.

**Key words:** influenza, flu-related illness, flu vaccine, elderly, vitamin D3, extraskelatal effects.

#### VITAMIN D3: RESEARCH BREAKTHROUGHS AND THERAPEUTIC USE

13-20

**Pohorila M.S., Martynov A.V., Romanova E.A., Igunnova N.I., Sidorenko T.A., Yukhimenko V.I., Shcherbak O.M.**

Vitamin D3 (cholecalciferol), the natural form of vitamin D, is produced in the skin from 7-dehydrocholesterol. The synthesis of vitamin D in the skin is the most important source of vitamin D. Vitamin D can also be taken through nutrition, in the diet, but it is present in only a few food sources, containing relevant levels of vitamin D. 1,25-dihydroxyvitamin D<sub>3</sub> [1,25(OH)<sub>2</sub>D<sub>3</sub>] is the hormonally active form of vitamin D. Novel researches show it generates a number of extraskelatal biological responses including inhibition of variety types cancer progression, effects on cardiovascular disorders and mediates a protection against a number of inflammatory, autoimmune and infection diseases. The biological actions of 1,25(OH)<sub>2</sub>D<sub>3</sub> are mediated by the VDR. The genomic mechanism of 1,25(OH)<sub>2</sub>D<sub>3</sub> action involves the direct binding of 1,25(OH)<sub>2</sub>D<sub>3</sub> activated VDR/RXR to specific DNA sequences in and around target genes resulting in either activation or repression of transcription [7]. VDR modulates the expression of genes involved in immune function and cytokine production. The VDR and CYP27B1, the enzyme located in kidneys and target organs, are present in immune competent cells, bronchial and pulmonary epithelial cells, among others, and is up-regulated following the ligation of specific toll-like receptors by extracellular pathogens, implicating vitamin D in innate immunity. By binding the VDR, calcitriol induces several endogenous antimicrobial peptides (AMP) in monocytes, neutrophils and epithelial cells including cathelicidin LL-37,  $\alpha$ -defensin,  $\beta$  defensin and neutrophil gelatinase-associated lipocalin and up-regulates nitric oxide (NO) synthase. Since the inflammatory response associated with infections such influenza, pneumonia and sepsis increases both clinical severity and mortality, the ability to reduce inflammation may allow vitamin D to decrease mortality and disease burden in certain infections. Notwithstanding the width of possible vitamin D application field, which being known now, large-scale clinical trials are still demanded. Our review has the aim to summarize current scientific understanding of Vitamin D<sub>3</sub> effects on the immunological field with the focus on its capacity to enhance the anti-infection and anti-inflammatory immune reactivity. **Vitamin D and Tuberculosis.** Vitamin D has been widely studied in the prevention and treatment of tuberculosis. Current studies were focused on how calcitriol enhances the antimicrobial effects of macrophages and monocytes – important effector cells, fighting against pathogens such as *Mycobacterium tuberculosis* (MBT). Several studies tracked the impact of vitamin D on cytokines that promote anti-MTB activity and the resolution of infection. Suppression of antigen-stimulated pro-inflammatory cytokines, attenuation of anti-inflammatory cytokines, and a more rapid treatment-induced resolution of lymphopenia and monocytosis associated with TB infection occurred following 100,000 IU doses of vitamin D<sub>3</sub> given monthly for 4 months. Conversion of sputum smear or sputum culture was used to measure response to treatment in several studies, though only sputum culture conversion is independently linked to long-term risk of treatment failure and relapse. Also it was found that 10,000 IU of vitamin D<sub>3</sub> given daily for 6 weeks to significantly increase sputum smear conversion (100 % in the treatment group vs. 76.7 % in the placebo group, p=0,002). IFN- $\gamma$  levels were impacted variably: 2 doses of vitamin D<sub>3</sub> (600,000 IU)) led to increasing of IFN- $\gamma$  expression, while a single 100,000 IU dose of vitamin D<sub>3</sub> showed no change. Negative results in some studies could be explained by variability of the Taq1 vitamin D receptor genotype polymorphism. It was shown that significantly accelerated conversion is appropriate of patients who have a *tt* genotype compared to those with the *Tt* or *TT* genotype. But these results were not confirmed by another study, where were founded no interaction between VDR genotype effectiveness of vitamin D. Several trials show vitamin D given largely as an adjunctive therapy with traditional

anti-tuberculosis regimens in a variety of dose and dosing schedule has some impact on clearance of MBT from sputum in the wide number randomized controlled multicenter trials of patients with active tuberculosis infection. Patients with infection of MBT with different strains of tuberculosis can take benefits from Vitamin D<sub>3</sub> consumption due to its effect on the clearance of MTB from sputum and on dampening the inflammatory response or anthropometric changes that may help tuberculosis patients recover. A significant microbiologic effect of vitamin D<sub>3</sub> was indicated in several trials that, also, sustained in vitro tests, where its antimycobacterial effects in cultured macrophages was shown. Antimycobacterial effect is provided enhances the expression of the anti-microbial peptide human cathelicidin (hCAP18) in cultured macrophages. The clinical benefit after high vitamin D<sub>3</sub> doses administrating to patients does not depend of their vitamin D<sub>3</sub> marked deficiency. The cause of this variation remains unexplained. The role of genetic polymorphisms in the vitamin D receptor, or in the multiple enzymes involved in its metabolism in vitD<sub>3</sub> effectiveness remains unproved. Measurement of calcitriol-induced antimycobacterial activity in ex vivo whole blood culture in future studies may help in understanding the functional effects of specific genetic polymorphisms. So, big attention will be required in future studies to determine mechanism of vitamin effect on patients with tuberculosis.

**Keywords:** Vitamin D<sub>3</sub>, cholecalciferol, VDR, infection, pulmonary disease, tuberculosis, innate immunity, adoptive immunity, antimicrobial effect

## Experimental papers

### DISEASE-ASSOCIATED HLA-DR POLYMORPHISM, CLINICAL AND IMMUNOLOGICAL CHARACTERISTICS OF MULTIPLE SCLEROSIS PATIENTS IN THE NORTHEASTERN REGION OF UKRAINE

21-25

**Kolyada T. I., Negreba T. V., Tupotilov O. V., Bilozorov O. P., Zelenska A. D., Kolyada O. M., Shvydka O. V., Belyavtseva O. I.**

The development of multiple sclerosis is the result of complex interactions between environmental factors, genetic factors that determine individual disease susceptibility, and immunological and physiological characteristics of the patient. Multiple sclerosis treatment requires obtaining information about the main pathological processes, therefore, the evaluation of biochemical, immunological, and genetic markers of such processes, and not just clinical indicators, can become the basis of improved approaches for diagnosis and monitoring of the disease. Polymorphism of the disease-associated genes is considered as one of the key factors in multiple sclerosis pathogenesis, capable of influencing the risk of development, clinical manifestations and nature of the course of the disease, treatment response. The HLA genes polymorphism was found to be the most important and playing an essential role in the development of autoimmune diseases. There are data on the population characteristics of these types of polymorphisms, which require conducting relevant research in Ukraine and its regions to identify relevant genetic markers. **The aim of the study** was to provide a comparative clinical and immunological characteristic of multiple sclerosis patients in the northeastern region of Ukraine, depending on the presence of the disease-associated HLA-DR polymorphism. **Materials and methods.** 39 patients with multiple sclerosis, inhabitants of Kharkiv and Kharkiv region, were examined, of which 7 men and 32 women of medium age of  $33.7 \pm 7.7$  and  $42.1 \pm 11.9$  years, respectively; control group was consisted of 27 practically healthy persons of both sexes with an average age of  $30.1 \pm 8.2$  years. Clinical characteristics of multiple sclerosis patients included the determining the form and the actual type of the disease, its duration, and disability assessment based on the EDSS scale. The rate of the disease progression was determined as the ratio of the EDSS score to the duration of the disease. Biological material was blood and buccal epithelium samples from multiple sclerosis patients and practically healthy people. In addition to assessing the clinical status of the patients, design of the study also included evaluation of the systemic immunity indicators, levels of nonspecific and antinuclear antibodies in the serum, and analysis of HLA polymorphism, in particular, detection of the HLA-DR15 haplotype for its specific marker SNP rs9271366. Isolation of high molecular DNA was performed on a magnetically sensitive sorbent using the NeoPrep100 DNA Magnet kit (NeoGene, Ukraine). SNP polymorphism rs9271366 A/G was typed by the allele-specific amplification method with subsequent electrophoretic detection of the results. Primer selection was performed using the method by Liu Jing et al. (2012) adding a mismatch in the third position at the 3' ends. The primers MS92AF 5'-CACGTAATATAA-ATGGTTGCAAAGGA-3', MS92GF 5'-CACGTAATATAAATGGTTGCA-AAGGG-3' and MS92R5' AACCTGATGTAACAGA(C/T)CTCTA-3' (Eurofins Genomics), as well as Taq-mut polymerase (Litech, Russian Federation), were used in the study. The amplification was performed on the multichannel amplifier Tercyc (Russian Federation). Amplification mode: denaturation at 96°C for 3 minutes and 35 cycles that included denaturation at 95°C for 30 seconds, annealing at 58°C for 30 seconds, and synthesis at 72°C for 30 seconds. The length of the amplicon was 233 bp. Electrophoresis was performed in 2% agarose gel in TAE buffer. Electrophoregram analysis was carried out on transilluminator UVT 1 (Biocom, Russian Federation). Serum IgM, IgG, IgA levels were evaluated by ELISA using a test system by NPL Granum (Ukraine) and the immunoassay analyzer Stat-Fax 303 (USA). The determination of peripheral blood lymphocytes subpopulations was carried out using the test systems by NPL Granum, Ukraine in accordance with the manufacturer's instructions. Circulating immune complexes in serum were evaluated by selective precipitation of antigen complexes with 3.5% PEG-6000 solution (AppliChem GmbH, Germany). The content of lymphocytotoxic autoantibodies was determined by a lymphocytotoxic macrotest. The complementary activity of the blood serum was evaluated by means of 50% of sheep erythrocytes hemolysis in the presence of homologous antibodies. The statistical treatment was performed using STATISTICA 11.0 (StatSoft, Inc). To determine the reliability of the differences between the indexes in the studied samples, the non-parametric Mann-Whitney's criterion was used, while the Student's T-criterion was used for the normal distribution. **Results.** At the stage of clinical examination of multiple sclerosis patients, 25 patients (64.1%) were diagnosed with RRMS, 4 patients (10.3%) with PPMS, and another 10 persons (25.6%) with SPMS. The familial form of the disease was established in 11 cases (28.2%), in 28 cases (71.8%) the form of the disease was classified as sporadic. The average disease duration of the examined persons was  $10.16 \pm 9.57$  years. The disability score assessed according to the EDSS scale was  $3.54 \pm 1.67$  points; the rate of progression of the disease was  $0.93 \pm 1.26$  and  $0.83 \pm 0.85$  points, respectively. Analysis of the HLA polymorphism indicated that the disease-associated (minor) allele G SNP rs9271366 was present in 43.6% of the subjects examined (haplotype AG, G+ group), and another 56.4% of the patients were homozygous by the major allele A (haplotype AA, G- group). The form of the disease, the EDSS score, and the rate of the disease progression in the examined patients did not have association with the allele G SNP rs9271366. Patients with familial and sporadic forms of multiple sclerosis did not have significant differences in immune status and clinical indicators. The average EDSS score was  $3.45 \pm 1.95$  points in G+ patients and  $3.65 \pm 1.26$  points in G- patients and the rate of disease progression was  $0.83 \pm 0.85$  points and  $0.93 \pm 1.26$  points, respectively. The study of cellular immunity indicators revealed a decrease in relative number of CD4+ and CD8+ lymphocytes in multiple sclerosis patients compared to that in control group, and it was more pronounced in subjects with the presence of the SNP rs9271366 minor allele. The relative content of CD4+ cells was  $28.00 \pm 3.45\%$  in heterozygous patients,  $30.94 \pm 4.42\%$  in patients homozygous for allele A, in the control group  $42.3 \pm 1.8\%$  ( $p < 0.05$ ), and the CD8+ cells content was  $18.55 \pm 4.08\%$ ,  $20.65 \pm 3.52\%$  and  $29.4 \pm 2.2\%$ , respectively ( $p < 0.05$ ). At the same time, the reduction in the relative number of CD4+ lymphocytes was observed in 90.9% of G+ patients, and in 64.7% cases in G- patients ( $p < 0.05$ ). Decreased CD8+ lymphocyte content was also more common in patients with G+ (77.3% of cases) than in G- (41.2% of cases),  $p < 0.05$ . Acute inflammatory process was determined in 38.5% of patients with multiple sclerosis, as evidenced by an elevated IgM level in 20.5% of cases, as well as an increase in complement activity in 62.5% of

subjects. The levels of lymphocytotoxic autoantibodies in patients with multiple sclerosis were significantly higher compared to the control group ( $18.33 \pm 3.67\%$  of subjects and  $5.82 \pm 3.12\%$  of subjects, respectively,  $p < 0.05$ ), and the levels of circulating immune complexes were elevated in 10.3% of patients. The study of the levels of antinuclear antibodies in multiple sclerosis patients revealed a diagnostically significant levels of antibodies to native DNA found in 92.3% of the cases, including all G + patients and 83.3% of G- patients. In this case, 16.7% of patients homozygous for allele A and 14.3% heterozygous patients were also positive for the presence of antibodies to denatured DNA. Similar results were also obtained concerning the presence of antibodies to formalinized DNA - 16.7% positive results in G- patients and 28.6% in G+ patients ( $p < 0.05$ ). At the same time, elevated levels of antibodies to native, denatured, and formalinized DNA were determined predominantly in patients with SPMS and PPMS (15.4% of the number of examined patients). The elevated levels of antinuclear antibodies may indicate an unfavorable course of the disease or its transition to the active phase. **Conclusion.** In the surveyed multiple sclerosis patients from the northeastern region of Ukraine, the prevalence of SNP rs9271366 and its association with a decrease in the relative number of CD4+ and CD8+ cells against the background of elevated levels of antinuclear and lymphocytotoxic antibodies were detected. **Keywords:** multiple sclerosis, disease-associated polymorphism of genes, immunopathogenesis.

## THE ANALYSIS OF THE THREAT OF REUSING PET BOTTLES FOR THE STORAGE OF DRINKING WATER

26-32

Manuilov A.M., Martynov A.V.

**Introduction.** According to a sociological survey of about 86% of Kharkiv (Ukraine) residents reuse PET bottles for a drinking water storing. This type of reuse of PET bottles isn't safe and the results of numerous research unequivocally confirm this assertion. The largest hazard of plastic bottles reuse for drinking water storage is biological film on the internally surface of bottle. This biofilm may contain pathogenic microorganisms which can migrate from biofilm to fresh water. Human, who drinking contaminated water, may drink microorganisms in common with this water. It's very dangerous, because the numerous strains of pathogens may migration in water and infect from gastric-bowel tract to the humans. Scientists from National technical university "Kharkiv polytechnic institute" in common with experts from Mechnikov institute of microbiology and immunology explored this problem and devised the apparatus, which can destroy a biofilm on polymer or another surface. **Materials & Methods.** The tested apparatus was the electrical device consisting of a block with electrodes, an electronic control, a water pump and a sprinkler for spraying the disinfectant. The electrode was made of 925° silver (sterling silver). Water for the preparation of a disinfectant was tap water and wasn't treated additionally. The sprinkler for spraying the disinfectant was placed in the neck of the infected bottle. Disinfectant solution was sprayed inside the bottle for 4 seconds. The water pressure was about 1.5 atmospheres. After that, the sprinkler was removed and the disinfectant was drained. A smear for microbiological composition was taken from three parts of the bottle - the neck, the middle part and the bottom. Growth of microorganisms and their detections was fixed by classic microbiological methods. **Results & Discussion.** In the article the scheme of the most probable and widespread way of infection of PET-bottles by pathogens and the way of minimization of this danger is given. Investigation of the contamination of the inner surface of the bottle by infected dust was carried out. It is determined that contaminated dust can cause a very serious infection contamination of inner surfaces of PET bottles and, subsequently, of water. In laboratory conditions and on the real object, a device for sanitizing surfaces was tested. It is established that the prototype of the device generates a disinfectant that destroys the most of known strains of microorganisms. This disinfectant is not toxic and is not dangerous to humans, the only product of evaporation of this product is water. With this disinfectant, the infection contamination on the inside of the PET bottle was completely eliminated. Thus, the use of a prototype device to minimize the threat of contamination of water consumers from recycled PET bottles is possible and very effective. **Conclusions.** 1. Potable water storage containers made of PET contain threats, the most serious of which is microbiological. 2. Without conducting regular disinfection or inactivation treatment of PET containers may be potential spreaders of human diseases. 3. The formed biological film cannot be destroyed or inactivated by means and substances that are at home. Potentially dangerous is the use of special, concentrated disinfectants at home. 4. Ions of silver are acknowledged in the world of practice antiseptic. 5. The use of silver ions to inactivate the biological film, while complying with state standards and methods of treatment, is safe and effective, which has been proved by research. 6. The developed method and apparatus are effective against the formed biological film and comply with the current legislation of Ukraine and some other countries, in particular the USA and Canada.

**Keywords:** threat, reusing pet bottles, drinking water

## ORGANIZATIONAL AND LEGAL REGULATION PROCEDURE FOR CIRCULATION OF EXTEMPORAL MEDICINES BASED ON PHARMACEUTICAL LAW

33-37

Shapovalov V. V., Zbrozhek S. I., Shapovalova V. O., Shapovalov V. V.

**Introduction.** The paper studied the situation regarding the production of medicines in pharmacies. Established that the presence of the pharmacy manufacture of medicines, patients entitled to receive medicines made to the needs of individuals. Goal – to study the organizational and legal procedure of regulation of extemporaneous medicines by developing of the algorithm for determining the legal act used in the event of conflict based on the law pharmaceutical law. **Materials and methods.** The materials of the study were legal acts of Ukraine: Laws of Ukraine, Decrees of the Cabinet of Ministers of Ukraine, Orders of the Ministry of Healthcare of Ukraine. The research methods were legal, documentary and comparative analyzes. **Results and discussion.** However, production of extemporaneous preparations in the pharmacy requires a production base and the appropriate staff. Therefore, the authors based on pharmaceutical law proposed organizational and legal procedure regarding the regulation of extemporaneous preparations by developing the algorithm for determining the legal act which should follow in the event of conflicts concerning the law. **Conclusions.** Based on pharmaceutical law held organizational and legal procedure for the regulation of circulation of extemporaneous medicines. Proposed the algorithm for determining of the legal act used in the practice of pharmacy professionals. Considered the professional status of an authorized person on the stage of quality control of extemporaneous medicines in their treatment in healthcare institutions of private property.

**Keywords:** the legal procedure, circulation, extemporaneous medicines, pharmaceutical law.

## MICROBIOLOGICAL PARAMETERS IN PATIENTS WITH INFLAMMATORY COMPLICATIONS AFTER KNEE AND HIP JOINTS ENDOPROSTHESIS REPLACEMENT AND THEIR DIAGNOSTIC EVALUATION

38-42

Shevtsova O.V., Marushchak O.P., Shapovalova O.V., Kuznetsova N.V.

**Introduction.** Presently in the inflammatory joint diseases diagnosis and treatment microbiological examination plays a leading role. This is due to the infectious diseases frequency general increase, the hospital infections incidence rise risk, the widespread use of antimicrobial agents in medical practice and the change in the infectious foci microbiocenosis structure. Microbiological and, if necessary, serological studies of articular fluid are fundamental components of diagnosis and inflammatory joint diseases effective treatment selection. Etiological agents of inflammatory processes in joints can be microorganisms of different groups. According to the literature, up to 80% of bacterial arthritis cases cause gram-positive cocci, among which *S.aureus* predominates. At the same time, the number of methicillin-resistant strains of this pathogen (MRSA) increases annually. Less commonly, from the affected joints  $\beta$ -hemolytic streptococcus group A and other groups

streptococcus, gram-negative rods, microscopic fungi and anaerobic bacteria are isolated. In view of the microorganisms biological nature characteristic, microbiological studies do not always make it possible to isolate the causative agent of infection. A major problem in bacteriological diagnostics is the periprosthetic and hematogenic infections low-grade causative microorganism, as well as subacute and chronic processes course presence. These include coagulase-negative staphylococcus (for example, *Staphylococcus epidermidis*) and anaerobic bacteria. Diagnostic and therapeutic difficulties can also be due to the pathogens ability to form antimicrobial therapy resistant microbial biofilms. It is reported that an antibacterial drugs uncontrolled intake, the biofilms formation, errors in the collection and transportation of biological material, can cause a situation when the joint infection infectious agent can not be detected in approximately (10-20) % of the cases. **Materials and methods.** The material for the studies, were synovial fluid samples collected from 64 patients of the SE "Sytenko Institute of Spine and Joint Pathology, NAMS Ukraine" clinic. The patients' diagnosis were status after knee and hip joints endoprosthesis replacement with inflammatory complications. The biological material was tested in the 2015-2017 period. The synovial material collection was conducted by the attending physician by the joint puncture method. The articular fluid withdrawn into the syringe was immediately got to a microbiological laboratory. The biological samples inoculation was carried out into a fluid thioglycollate storage medium, then to obtain the aerobic and facultative-anaerobic microorganisms pure cultures the isolate passage were conducted to Columbia blood agar, salt agar and Endo medium. Further isolated microorganisms identification was performed by standard methods in accordance with current guidelines. The microorganisms cultures were observed for 14 days. In the absence of microflora's growth, a preliminary negative result for all synovial material was given after 5-7 days. If there was a based on the disease anamnesis and clinic suspicion on the slowly growing pathogens presence the timing of the studies was increased. The isolates sensitivity to antimicrobial agents was determined by the disc-diffusion method. In determining the microorganism's sensitivity 29 antibacterial drugs from 8 chemical groups were used:  $\beta$ -lactams, fluoroquinolones, macrolides, aminoglycosides, tetracyclines, lincosamides, glycopeptides, oxazolidinones, glycylicyclines. **Results and discussion.** As a result of the microorganisms' identification, 68 cultures of facultative-anaerobic bacteria and microscopic fungi were isolated from the joint fluid. 82.3% of bacterial isolates were obtained in monoculture (n = 56). Of these, 25.0% of the cultures (n = 14) were staphylococcus species with ability to coagulate the blood plasma (*S. aureus* (n = 9), *S. intermedius* (n = 5)), other staphylococcus isolation rate was 60.7% (*S. epidermidis* (n = 21), *S. haemolyticus* (n = 9), *S. simulans* (n = 4)). Pathogenic streptococcus species was isolated from 5.4% of the samples (*S. pyogenes* (n = 3)). *K. pneumonia* cultures were isolated from 8.9% of biological material samples (n = 5). Mixed micrococenoses were detected in 6 samples of the biomaterial. The cultures associations consisted of two microorganisms species with the associations *S. intermedius* - *S. pyogenes*, *C. lusitania* and *C. neoformans* (n = 4) prevailing. Two other microbiocenoses were represented by *Candida* with *S. pyogenes* and *S. haemolyticus*. The bacterial cultures sensitivity to antimicrobial agents analysis showed that all *S. aureus* isolates were sensitive to linezolid, levofloxacin and the ceftriaxone-sulbactam combination. Generally it was determined that the most effective drugs for gram-positive cocci are linezolid, to which 88.1% of the studied isolates are sensitive, including all *S. aureus* and *S. haemolyticus* cultures, and tigecycline, which has activity against 78.0% of gram-positive cocci isolates. The estimated aminoglycosides efficacy is 73.7%. The fluoroquinolones, carbapenems and the third generation cephalosporins with sulbactam combinations antimicrobial activity is manifested for 50% of all isolates obtained. About 30% of cultures were sensitive to lincomycin and the third generation cephalosporins - ceftriaxone, cefixime and cefoperazone. **Conclusions.** 1. Microflora isolated from synovial fluid in case of the knee and hip joints is inflammatory diseases is represented by gram-positive cocci (86.8%) in most cases, gram-negative rods amount is 7.3% and fungi of *Candida* and *Cryptococcus* genera are made 5.9%. 2. The isolated microorganisms species antimicrobials sensitivity is characterized by individual diversity with a tendency to vancomycin resistance increasing in 44.4% of coagulase-positive staphylococcus isolates, of which 28.6% are *S. aureus* strains; 28.5% are other staphylococcus species cultures and 16.7% are *S. pyogenes* isolates. This indicates the exactly appropriate antibiotic therapy conducting necessity. 3. When choosing antibiotic therapy in patients in case of coxarthrosis and gonarthrosis it is recommended to take into account the bacterial isolates antibiotic resistance formation actual trends.

**Keywords:** periprosthetic infections, synovial fluid, microbiological examination

## THE STUDY OF SOME PROMISING PHARMACEUTICAL COMPOSITIONS WITH UREASE INHIBITORY ACTIVITY FOR THE TUBERCULOSIS REACTIVATION PREVENTION

43-45

**Bomko T.V., Martynov A.V., Nosalskaya T.N.**

**Introduction.** The aim of the study was to study the ability to inhibit urease by some pharmaceutical compositions that are promising in the prevention of tuberculosis reactivation. In particular, according to a number references sources, quercetin is able to successfully inhibit urease by a non-competitive mechanism. Another compound - dipyrone (metamizol sodium) according to preliminary molecular modeling has a pharmacophore structure similar to urea. **Materials and methods.** The biochemical studies (urease activity) of some substances effect on urease was carried out. As the leader substances - inhibitors was used quercetin and metamizole, another substances are not shown inhibition activity on the urease. As the substrate a 0.5% aqueous urea solution was used. The reaction was carried out at a temperature of 37 ° C, the incubation time was 10 minutes. The activity of urease was determined by the color reaction with the hypochlorite reagent. Photometry was performed on a FEK-3M photoelectric colorimeter at a wavelength of 590 nm. **Results and discussion.** Quercetin and metamizole sodium have the strongest inhibitory properties for urease, since the lowest values of semi-inhibitory concentrations are obtained for them. In the presence of chlorophyllipt, the inhibitory activity of quercetin against urease is not suppressed. Metformin showed no inhibitory activity against urease, and it was low for other substances. **Conclusion.** The highest anti-urease activity showed a composition based on metamizole sodium and quercetin, a little less - based on quercetin and vitamin D. Also interesting for further research is the composition of quercetin and chlorophyllipt.

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

46-52

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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. 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. The drug Ismigen 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. **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) were 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. Patient in group B had sublingual Ismigen postoperatively: once daily for a period for a period of 10 days. 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. A comprehensive assessment of the status of the oropharynx microbiocenosis was carried out in accordance with the criteria described earlier. According to these criteria, the status of the

microbiocenosis of the oropharynx was divided into: eubiosis, dysbiosis of the 1<sup>st</sup> degree, dysbiosis of the 2<sup>nd</sup> degree and dysbiosis of the 3<sup>rd</sup> degree. **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 1<sup>st</sup> 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  $\lg(4.1 \pm 0.2)$  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.