BIOLOGICAL MARKERS IN FUNDAMENTAL AND CLINICAL MEDICINE

COLLECTION OF ABSTRACTS

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The collection is intended for fast and systematic publication of abstracts, containing the results of author’s research, reviews highlighting major developments in the field of biological markers, short messages, new experimental and clinical studies, which use biological markers, as well as proposing new principles and methods for the study of biological markers.

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CONTENTS

Assessment of ischemia modified albumin serum level in isoproterenol-induced myocardial infarction
Timercan T., Timercan V.

Comparative evaluation of the dynamics of the size of the furuncle infiltrate of the maxillofacial area with different postoperative treatment
Flerjanovich M.S., Pohodenko-Chudakova I.O.

Elemental composition of hair as an objective indicator for diagnostics, prevention and treatment of microelementosis
Sharipov K.O., Bulygin K.A., Sharipov D.K., Dosymbekova R.S.

Effect of the antioxidant – herbal derivate on biomarkers of oxidative stress and antioxidant systems on the model of gastric ulcer
Zalyubovska O.I., Tiupka T.I., Minaieva A.O., Zlenko V.V., Litvinenko M.I., Avidzba Y.N.

Effect of freezing on anti-inflammatory efficiency of human placental extracts
Govorova Yu.S., Bobrova O.M., Repina S.V., Rozanova E.D., Nardid О.А., Schetinskey M.I.

Substantiation of the study on prediction of surgical dental treatment for women in the period of menstruation on the basis of the frequency of this risk factor detecting based on anamnesis
Sudakova S. E., Pohodenko-Chudakova I. O.

Vitamin D receptor gene polymorphism in patients with juvenile rheumatoid arthritis
Myslivets M., Paramonova N., Stepuro T.

Ceruloplasmin in experimental ovarian torsion/detorsion
Lazăr C.

Caries-preventive effect of lactobacillus reuteri in children with gastrointestinal pathology
Denysova E. G.

Loss of heterozygosity at the BRCA 1 locus as a marker of sensitivity for adjuvant chemotherapy in hereditary ovarian cancer

B-cell lymphoma-2 receptor expression in human breast cancer
Fulga V.

Correlation between molecular and clinical, histological characteristics of breast cancer in young women
Bacalîm L., Sofroni L., Corobcean N., Chimencedji I., Odobescu Ox., Ghidirim N.

Current aspects of odontogenic jaw cysts treatment
Stoyan E. Y., Denysova E. G.

Atom-absorptive spectrometry for determining of the elemental content of the glycyrrhiza glabra roots
Kasimova D.B, Abdukhalikova N.U, Gaibnazarova D.T.
FORWARD SESSION

SELECTED ABSTRACTS OF THE ON-LINE CONFERENCES “Modern Technologies of Diagnostics and Monitoring of Therapy in Experimental, Clinical Medicine and Pharmacy” and “Biological markers in fundamental and applied biology. From theory to practice”.

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ASSESSMENT OF ISCHEMIA MODIFIED ALBUMIN SERUM LEVEL IN ISO-PROTERENOL-INDUCED MYOCARDIAL INFARCTION

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Introduction: Acute coronary syndrome (ACS) remains the leading cause of mortality worldwide. Various epidemiologic studies demonstrate that the death rates due to cardiac diseases have been risen since 1990 from 28,9 % to 36,9 % (2020)[1]. Cardiac ischemia is known to be the key-mechanism underlying ACS [2]. Prolonged ischemia causes cellular necrosis and acute myocardial infarction (AMI)[3]. The everyday challenge for contemporary emergency medicine is to identify cardiac ischemia at the earliest stage before irreversible cell damage[4]. Ischemia modified albumin (IMA) is an early-rising serum biomarker of cardiac ischemia[5][6]. IMA is produced as result of the damage in the N-terminal aspartate-alanine-histidine-lysine sequence of serum albumin[4] in ischemic conditions due to hypoxia, acidosis, and oxidative stress[7].

Aim: The statistical analysis was performed to identify correlations between serum IMA and homogenate ischemia modified proteins (IMP) levels in experimental, isoproterenol induced myocardial infarction.

Materials and methods: Our study was carried out on 40 adult male rats (Ratta albicans) that were divided into five groups: L1 – intact (n=11); L2 – control animals which were administrated NaCl 0.9% (n=11); L3 (n=6), L4 (n=6) and L5 (n=6) included the animals with experimental myocardial infarction, which was induced by subcutaneous injection of isoproterenol 100 mg/kg (one dose) and sacrificed at 6 h, 24 h, and 7 days respectively. Serum IMA and homogenate IMP levels were assessed using the Gudumac et al. meth-
The data were represented by median, 25 and 75 percentile, and inter-quartile range. For result comparison, the Kruskal-Wallis nonparametric test was performed using “Statistical Package for the Social Science” version 23.

**Results:** The investigated groups have presented statistically significant difference in serum IMA levels ($\chi^2 = 9.918$ df = 4 p = 0.042) (Table 1), and no statistically significant difference in homogenate IMP levels ($\chi^2 = 6.524$ df = 4 p = 0.163) (Table 2). There was not any statistically significant difference in serum and homogenate IMA values in L1 and control groups.

**Table 1. Serum IMA levels (p < 0.05)**

<table>
<thead>
<tr>
<th>Groups</th>
<th>Median (25 percentile – 75 percentile)</th>
<th>%</th>
<th>Interquartile range</th>
</tr>
</thead>
<tbody>
<tr>
<td>L1</td>
<td>0.280 (0.263 – 0.288)</td>
<td>100</td>
<td>0.025</td>
</tr>
<tr>
<td>L2</td>
<td>0.291 (0.275 – 0.303)</td>
<td>104</td>
<td>0.104</td>
</tr>
<tr>
<td>L3</td>
<td>0.336 (0.276 – 0.362)</td>
<td>120</td>
<td>0.086</td>
</tr>
<tr>
<td>L4</td>
<td>0.304 (0.272 – 0.318)</td>
<td>108.6</td>
<td>0.046</td>
</tr>
<tr>
<td>L5</td>
<td>0.305 (0.286 – 0.342)</td>
<td>108.9</td>
<td>0.056</td>
</tr>
</tbody>
</table>

**Table 2. Homogenate IMP levels (p < 0.2)**

<table>
<thead>
<tr>
<th>Groups</th>
<th>Median (25 percentile – 75 percentile)</th>
<th>%</th>
<th>Interquartile range</th>
</tr>
</thead>
<tbody>
<tr>
<td>L1</td>
<td>64.68 (54.92 – 67.78)</td>
<td>100</td>
<td>12.86</td>
</tr>
<tr>
<td>L2</td>
<td>66.16 (57.32 – 70.23)</td>
<td>102.3</td>
<td>12.91</td>
</tr>
<tr>
<td>L3</td>
<td>69.67 (63.30 – 92.51)</td>
<td>107.7</td>
<td>29.21</td>
</tr>
<tr>
<td>L4</td>
<td>60.53 (58.59 – 66.31)</td>
<td>93.6</td>
<td>7.71</td>
</tr>
<tr>
<td>L5</td>
<td>69.99 (65.21 – 76.34)</td>
<td>108.2</td>
<td>11.13</td>
</tr>
</tbody>
</table>
Fig.1 Serum IMA and homogenate IMP content

Serum IMA value was higher in L3 by 120%, p < 0.05 (median 0.336; interquartile range 0.086) compared to L1 by 100%, p < 0.05 (median 0.280; interquartile range 0.025), L2 by 104%, p < 0.05 (median 0.291; interquartile range 0.104), L4 by 108.6%, p < 0.05 (median 0.304; interquartile range 0.046) and L5 by 108.9%, p < 0.05 (median 0.305; interquartile range 0.056). Homogenate IMP value was higher in L3 by 107.7%, p < 0.2 (median 69.67; interquartile range 29.21) compared to L1 by 100%, p < 0.2 (median 64.68; interquartile range 12.86), L2 by 102.3%, p < 0.2 (median 66.16; interquartile range 12.91), and L4 by 93.6%, p < 0.2 (median 60.53; interquartile range 7.71), but lower compared to L5 by 108.2%, p < 0.2 (median 69.99; interquartile range 11.13). Homogenate IMP content in L4 was the lowest compared to all the groups. Observed power for the tests varies between 0.2 - 0.25.

Conclusion: Oxidative stress generates high levels of free radicals in ischemic myocardium. As a result of the N-terminal oxidative damage of serum albumin, increased content of IMA can be assessed in blood. Our research demonstrated that serum IMA and homogenate IMP levels rise at the onset of ischemic attack. It confirms the utility of usage IMA as a marker for identification of cardiac ischemia. The results obtained in this study should be treated with caution, as a limited number of samples were assessed and the research is still carrying on.

References:

COMPARATIVE EVALUATION OF THE DYNAMICS OF THE SIZE OF THE FURUNCLE INFILTRATE OF THE MAXILLOFACIAL AREA WITH DIFFERENT POSTOPERATIVE TREATMENT

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Introduction: Questions of the etiology and pathogenesis of furuncles and carbuncles of the maxillofacial area are still relevant in dentistry, dermatology and general surgery [1].

The presence of severe complications, speaks about the need to improve the existing method of complex treatment of this pathology. The timely correction of the homeostatic parameters of patients, the stimulation of protective forces, and restorative treatment with the aim of maximizing the restoration of the aesthetics of the skin of the maxillofacial region, is of particular importance for patients with furuncles and carbuncles of this region [1, 2].

Electroneurostimulation, a long-established method of treating inflammatory processes, including the maxillofacial area, has a large number of advantages, in comparison with other methods and methods of therapeutic and rehabilitation effects. Its indisputable advantages include the absence of allergic reactions, ease of use, the minimum number of contraindications, and the possibility of application as early as the first day after the initial surgical treatment of the infectious-inflammatory focus [3].

The aim of the work is to compare the changes in the area of infiltration of boils of the maxillofacial area with standard complex treatment and complex treatment supplemented with the course of reflexotherapy.

Materials and methods of research. Eighteen patients with furuncles of the maxillofacial region were included into the study and divided into two groups. Group 1 included 10 patients who received standard treatment. Group 2 (8 patients) received standard course of treatment which was supplemented by a course of reflexotherapy.
Owing to the fact that in the vast majority of observations, the infiltrate has a shape approaching the circle. In this connection, the area of the inflammatory process was calculated according to the following formula: $S = \pi r^2$, where $S$ is the area of the circle (cm$^2$), the number $\pi$ is equal to 3.14 and $r$ is the radius of the circle (cm).

The measurements were carried out on the first, third and fifth days after the primary surgical treatment of the infectious inflammatory focus in dressings in patients with maxillofacial furuncles who received standard complex treatment in the dental department of the Vitebsk Regional Clinical Hospital. For this purpose, calipers had used, pretreated with an antiseptic solution.

The received data had processed using a personal computer and packages of applied “Statistica 10.0”.

**Results.** Measurements of infiltrates infectious inflammatory foci in patients who received only standard complex therapy were as follows. In the first day, the average value of the infiltrate area for furuncles of the maxillofacial region was $6.84 \pm 0.35 \text{ cm}^2$. The index on the third day of observation was $3.59 \pm 0.21 \text{ cm}^2$. The result on the fifth day of the study was $1.4 \pm 1.74 \text{ cm}^2$.

The results obtained indicate a decrease in the area of the inflammatory infiltrate from the first day to the third day by 47.51%. In the period from the third to the fifth, the area of the infiltration decreased by 61%. In general, the reduction in the area of infiltration from the first to the fifth day of the maxillofacial furuncles with standard complex treatment was 79.53%.

The following results were obtained in patients who underwent a standard complex treatment supplemented with a course of reflexotherapy. On the first day, the mean value of the infiltrate area for furuncles of the maxillofacial region was $6.31 \pm 0.42 \text{ cm}^2$. The index on the third day of observation was $3.11 \pm 1.52 \text{ cm}^2$. The average size of the infiltration on the fifth day of the study was $0.89 \pm 0.26 \text{ cm}^2$.

The results indicated decrease in the area of the inflammatory infiltrate from the first day to the third day by 50.7%. In the period from the third to the fifth, the area of the infiltration decreased by 71.38%. In general, the reduction in the area of infiltration of boils in the maxillofacial area with standard complex treatment was 85.9%.
Conclusions.

The obtained results indicated more efficient decrease in the average infiltrate area of the infectious-inflammatory lesion in the focus in patients with maxillofacial furuncles who underwent complex treatment supplemented with a course of reflexotherapy.

References:


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ELEMENTAL COMPOSITION OF HAIR AS AN OBJECTIVE INDICATOR FOR DIAGNOSICS, PREVENTION AND TREATMENT OF MICROELEMENTOSIS

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Introduction: Medicine of the XXI century is inextricably linked and increasingly uses biomarkers for the diagnosis and treatment of various diseases. The usage of biomarkers in clinic and biomedicine contributes to the formation of the innovative concept of an individual approach to each patient, i.e. personalized medicine [1].

A biomarker is a characteristic that can be objectively measured, and which can serve as an indicator of the metabolism or pharmacological responses to therapeutic intervention [2]. Nowadays, an increasing number of biomarkers are determined relatively easily, and they are part of routine medical examinations. However, there is still an urgent need in development of new biomarkers for detecting a number of diseases, their prevention and monitoring of the effectiveness of treatment. Contemporary researchers use omics technologies to develop new biomarkers, which mean an in-depth study of the genome, metabolome, metabonome, “metallome” etc.[3].

Metallomics is a quantitative measurement of the metallome components - the products of interaction of the ionic and atomic forms of metals with endogenous ligands (nucleotides, nucleosides, proteins, peptides, amino acids, carbohydrates and others). In recent time, the concept of metallomics includes not only the study of metals in the body, but also essential micronutrients [4,5].

Nowadays, the role of increasing number of macro- and micronutrients are identified in growth, differentiation, regeneration, apoptosis and necrosis of cells and in the pathogenesis of several diseases, accompanied by significant changes in the elemental status of the body [Oberlis, 2008, Panchenko, 2004, Skalnyi, 2003]. The imbalance of micro elements can be a triggering mechanism of the deregulate apoptosis. Either deficiency or
excess of some essential trace elements could have aggravated influence on the manifestation of genotoxic effects of some metals.

**The purposes** of the research are the determination of components of “metallome” i.e. micro- and macro elements in the hair of people and elaboration of biomarkers on the basis of revealed quantitative deviations of essential chemical elements from reference intervals.

**Methods and materials.** The object of investigations was the hair of the residents of Almaty city. The research were conducted by the methods of atomic emission spectrometry with inductively coupled argon plasma (ICP-AES) and mass spectrometry with inductively coupled plasma (ICP-MS). These methods are characterized by high informativeness, performance, sensitivity, and allows to identify simultaneously more than 25 chemical elements in the investigated objects (Aluminum (Al), Beryllium (Be) Boron (B), Vanadium (V), Iron (Fe), Iodine (I), Potassium (K), Cadmium (Cd) Calcium (Ca), Cobalt (Co), Silicon (Si), Lithium (Li), Magnesium (Mg), Manganese (Mn), Copper (Cu), Arsenic (As), Sodium (Na), Nickel (Ni), Tin (Sn), Mercury (Hg), Lead (Pb), Selenium (Se), Phosphorus (P), Chromium (Cr), Zinc (Zn)).

**Results.** The results of the instrumental study of the content of chemical elements in hair (element graph) were mostly in the normal range and all insignificant deviations had multidirectional character, as were to be expected. It should be noted that the contents of calcium, phosphorus and potassium were above normal on a 10%, while chromium and sodium were above by 12% in the studied groups of population. There were more specific abnormalities of essential trace elements such as cobalt, zinc and copper which had downward trend. In contrast to these, silicon had an upward trend. The cobalt’s content was below reference intervals of variation in 1/3 part of surveyed groups of people.

**Discussion of results and conclusions.** Elementogram of hair of particularly patient represents individual elemental status – the condition of the components of “metallome”. Elemental composition of hair reflects the regional specificity of environmental and nutritional factors. On a slight change of content of calcium, phosphorus, potassium and sodium, we did not pay special attention, as this may be associated with age, lifestyle, social status, nutrition and usually short-term. Multidirectional changes in the content of essential elements such as zinc, copper, chromium and espe-
cially cobalt may lead to certain orphan diseases or alarming signal which require comprehensive research and personalized approach.

Thus, the stable level of the “metallome” is a crucial factor of cellular homeostasis, whilst the individual element graph is a dynamic indicator of the MLH and could serve both for preclinical diagnosis and subsequent planning of personalized treatment and prevention of microelementosis.

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Accepted for printing on 30 Oct 2017
EFFECT OF THE ANTIOXIDANT – HERBAL DERIVATE ON BIOMARKERS OF OXIDATIVE STRESS AND ANTIOXIDANT SYSTEMS ON THE MODEL OF GASTRIC ULCER

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2 – National University of Pharmacy, Ukraine

Introduction: A definite value in the pathogenesis of peptic ulcer belongs to the activation of lipid peroxidation (LPO) processes. The activity of LPO processes is controlled by antioxidant defense factors, which should have cytoprotective properties. However, with their functional insufficiency, oxidative stress develops, in which LPO products “attack” cellular structures, are accompanied by damage to the lipids of cell membranes, increased permeability and destruction of cells, and contributes to the formation of a ulcerative defect [1, 2].

Phenolic compounds – natural synergists of ascorbic acid, are widely represented in the plant world, they have significant place among a large number of antioxidants. Polyphenols of vegetable origin have a number of biological effects, in particular antioxidant and anti-inflammatory effects. Polyphenols of grape seeds are recognized as one of the most powerful among all known natural antioxidants. By activity, they are almost 50 times higher than vitamin E and 20 times – vitamin C. At the same time, these secondary metabolism substances have low bioavailability [3, 4].

The purpose of our study was to study the effect of the liposomal emulsion of grape seed polyphenols (LEGSP) on the state of the system of LPO and antioxidant enzymes of erythrocytes on the model of chronic hydrocortisone gastric ulcer.

Materials and methods. Peculiarities of the therapeutic effectiveness of LEGSP were studied in white rats with chronic ulcer caused by intramuscular injection of hydrocortisone acetate 2.5% twice daily for 10 days at a dose of 15 mg/kg [5]. LEGSP was administered at a dose of 115 mg/kg with a prophylactic treatment regimen once daily intragastric.
The state of lipid peroxidation (LPO) and the activity of antioxidant defense enzymes were determined on the eleventh day of the experiment. In erythrocytes, the content of diene conjugates (DC), lipid hydroperoxides, glucose-6-phosphate dehydrogenase and catalase (CA) activity of malonic dialdehyde (MDA) and total antioxidant activity was determined; activity of superoxide dismutase (SOD) of glutathione peroxidase (HP) and the content of reduced glutathione (RG); activity of glutathione reductase (GR) \(^6\).

**Results.** In the course of this experiment, it was found that in the control group, the level of DC increased 2.5 times, lipid hydroperoxides increased in 3.5 times and MDA - in 1.4 times, which indicated activation of this destructive process in gastric ulcer.

ELPVN contributed to a decrease in the processes of intoxication with peroxides, as it was evidenced by a decrease in MDA content – 1.3, DC – 2.1, lipid hydroperoxides – 2.3 times, which was not significantly different from the indices of the intact group of animals and indicated a significant decrease in intoxication peroxides.

Increase of the antioxidant activity of blood, under the influence of LEGSP, was observed by 32.9% more in comparison with the control group.

All fat-soluble and water-soluble antioxidants neutralized free radicals either directly, or with the help of enzymes of CA, GR, HP, SOD. In the control pathology group, the most pronounced changes were revealed in relation to SOD and CA: the activity of SOD was significantly increased twice, and the activity of the CA decreased 1.85 times compared to the intact group of animals. The RH level decreased 3.1 times in comparison with the intact group, indicating a significant decrease in the antioxidant potential in erythrocytes. The use of LEGSP in the experimental gastric ulcer was accompanied by a tendency to normalize the activity of all antioxidant enzymes studied. The level of SOD increased 1.3 times, CA – 1.6 times; GP – 1,1 times, GR – 1,7 times. The increase in the level of GH leads to the restoration of glutathione, the activity of which increased 2.8 times, which indicates its increased use to reduce the peroxide intoxication of the organism and update the activity of the glutathione antiperoxidase system.

**Conclusions.** On the model of chronic hydrocortisone gastric ulcer, the ability of emulsion of liposomes from polyphenols of grape seeds to
reduce the activity of lipid peroxidation of red blood cells (decrease in malonic dialdehyde content, diene conjugates and hydroperoxide lipids) and increase the activity of enzymes antioxidant protection of red blood cells (increase in superoxide dismutase, catalase, glutathione reductase and reduced glutathione) was studied.

**Prospects for further research.** Determination of biomarkers of oxidative stress and antioxidant state in patients with peptic ulcer and study of the effectiveness of the use of plant antioxidants in complex treatment.

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*Accepted for printing on 15 Nov 2017*
EFFECT OF FREEZING ON ANTI-INFLAMMATORY EFFICIENCY OF HUMAN PLACENTAL EXTRACTS

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Introduction: It is known that placental tissues contain a large number of biologically active substances that determines a wide use of placental extracts in medicine. Due to its high anti-inflammatory efficacy, the extracts are used in the treatment of wounds of various origins, pressure ulcers, inflammatory processes of the oral cavity, etc [1, 2]. Placenta rapidly loses its unique properties during hypothermic storage. Low-temperature storage of placenta allows to ensure the availability of raw materials necessary for the preparation of extracts at any convenient time. In this work the influence of freezing on an anti-inflammatory efficiency of human placental extracts (HPE) was investigated.

An increase in platelet aggregation and a decrease in erythrocyte membrane resistance are the inflammation biomarkers in human body [3-5]. Therefore researchers often evaluate an anti-inflammatory effect of drugs of various origins in vitro using these biomarkers. The aim of the research was to assess the effect of freezing on the ability of placenta extracts to reduce the induced aggregation of platelets and to increase the thermal stability of red blood cells.

Materials and methods. Placental specimens were frozen in plastic bags either down to –196 °C (in liquid nitrogen) or down to –20 °C (in a freezer), then thawed in a water bath at 37 °C. Water-salt extracts were prepared by the standard method [1-3]. Fractions of HPE were obtained using gel chromatography.

The ADP-induced platelet aggregation in platelet rich plasma (PRP) was investigated in this work [3]. To study the effect of HPE on this index, PRP was incubated for 15 minutes with HPE and its fractions at 37 °C. The percentage of platelet aggregation was estimated by the change in the light scattering level at 650 nm spectrophotometrically.
To study the thermohemolysis, the washed red blood cells were resuspended with phosphate-buffered saline solution in a 1:1 ratio, incubated with HPE and fractions of different molecular weights for one hour. Then the erythrocytes were washed from HPE and fractions, resuspended and exposed to hyperthermia (20 min in an adjustable water thermostat at 55°C). Afterwards, the hemolysis was evaluated by measuring of the optical density in supernatant at a wavelength of 540 nm spectrophotometrically.

The temperature-dependent dynamics of the erythrocyte cytosol status was studied using the hydrophilic spin probe TEMPON (2,2,6,6-tetramethyl-4-oxo-piperidine-1-oxyl) in combination with a pericidal broadening agent potassium. After erythrocyte suspension heating in spectrometer’s resonator, there were also recorded the spectra at a fixed temperature of 55°C for 25-30 min every 5 min. Erythrocytes thermostability was evaluated by the rate of amplitude decrease of the mid-field component of TEMPON spectrum. The EPR spectra were recorded with EPR with a thermostatic device.

**Results:** The HPE were shown to modify the behavior of structural-dynamic state of erythrocyte cytosol within the range of + (40 ÷ 50) °C, and erythrocyte thermal stability, by reducing of the rate of barrier properties disturbance in erythrocyte membrane at + 55°C. Placental freezing down to -20°C and 196°C did not affect the HPE ability to modify the high-temperature changes in erythrocytes.

The HPE was found to reduce the ADP-induced platelet aggregation and the level of thermal hemolysis by (20.4 ± 2.1)% and (18.7 ± 1.9)%, respectively. The fractions obtained from HPE efficiently reduced the platelet aggregation and thermal hemolysis of erythrocytes. The highest percentage of inhibition of platelet aggregation was found in the high molecular weight fraction (32.3 ± 3.5)%. Fractions of <4 kDa and 12-20 kDa are efficient in decreasing the thermal hemolysis of erythrocytes and platelet aggregation.

Placental cooling both down to -20 and -196°C and following thawing did not lead to loss of the capability its extracts to reduce an induced platelet aggregation and thermohemolysis of red blood cells. Extract fractions from the frozen placenta preserved the ability to inhibit the platelet aggregation and thermal hemolysis of erythrocytes. After placental cool-
ing down to - 20 °C, the percentage of inhibition of thermal hemolysis by fractions of HPE <4 kDa and 12-20 kDa was (29.4 ± 3.1)% and (26 ± 2.8)%, respectively. The percentage of inhibition of platelet aggregation in this case was (43.8 ± 4.5)% and (38.7 ± 4.1)%, respectively.

Thus, the placental freezing did not result in a significant decrease in anti-aggregation efficiency of the extracts and extract fractions and their ability to increase the thermal stability of red blood cells. According to the presented in vitro model for assessing an anti-inflammatory effect, using the presented biomarkers, it can be concluded that the anti-inflammatory properties of the extracts are preserved after placental freezing. A high efficiency of fractions with weight <4 kDa and 12-20 kDa was demonstrated.

References:

Accepted for printing on 16 Dec 2017
SUBSTANTIATION OF THE STUDY ON PREDICTION OF SURGICAL DENTAL TREATMENT FOR WOMEN IN THE PERIOD OF MENSTRUATION ON THE BASIS OF THE FREQUENCY OF THIS RISK FACTOR DETECTING BASED ON ANAMNESIS

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Introduction: The success of surgical treatment, especially when emergency surgical interventions are performed, largely depends on careful collection of anamnesis and identification of risk factors in each individual patient. One of such factors is menstruation [3]. This condition can be complicated by diseases which may cause deficiency of vitamin K, by oral anticoagulant which are accepted because of the risk of developing thrombosis, in cardiovascular pathologies [4], and infectious and inflammatory processes of various genesis of the maxillofacial region and neck [1, 2, 9].

However, despite all of the above, at the moment there is no set of preventive measures aimed at reducing probability of development of complications in the patient, there is no system of measures for providing emergency outpatient surgical dental care for this category of persons.

The aim was to study prediction of the surgical dental treatment for women in the period of menstruation on the basis of the frequency of this risk factor detecting based on anamnesis.

Materials and methods of research. Questioning of 120 patients aged between 20 and 55 years was conducted as the part of the study. The level of women’s awareness of the possibility of performing surgical interventions during menstruation and possible complications was assessed. Additionally 120 dentists in Minsk, Belarus, were interviewed in order to determine how often they are interested in the cycle phase of the patients and correct the treatment they are carrying out in connection with this information they received.

The data obtained during the questioning were subjected to statistical processing using the “Statistica 10.0” software package [5].

Results. Analysis of the questionnaires of dentists showed that 13.3% of them are not interested in the presence of menstruation in the patient, as
they do not consider it necessary, 6.7% do not do it by reason of the lack of time and 40% are sure that patient must know necessary information and inform the doctor. At the same time, only 11.1% of patients do this.

Analysis of the questionnaires of the interviewed patients who applied for specialized medical care to the dentist surgeon found that the proportion of patients with an increased risk of complications from the total number of respondents, due to heavy menstruation was 26.7%. In addition, based on the data of laboratory studies, the proportion of people with the presence of associated pathological hemostasis disorders was 8.3%. This group of patients had pathology of the liver, biliary tract, gastrointestinal tract, in particular pathology of terminal ileum, autoimmune diseases. Also decline of fibrinogen, prothrombin index and an increase in thrombin time were diagnosed. It should be noted that 85.8% of the total number of respondents have a reduced coagulability of blood. It was found that 50% of interviewed women had taken estrogen-containing contraceptives, which reduce the risk of bleeding, this information is consistent with the reports of the special literature \[7, 8\]. At the same time, 10.8% of them used estrogen-containing drugs to control benign formations, this was confirmed by the data of D. Casertaetal. (2014) \[6\].

The analysis of the questionnaires also made it possible to identify the number of patients who are aware of the possible risks of performing operative outpatient dental interventions in the first phase of the menstrual cycle, it was only 15%. Frequency of possible risks of bleeding during emergency operations in the maxillofacial area in women during menstruation was 56.7%. And the total percentage of operations conducted in the first phase of the menstrual cycle was 22.5%.

Prospects for further research. In the future, it is possible to develop an educational program for work with the female population on the risks associated with performing interventions during menstruation, as well as creating a system for providing emergency outpatient and inpatient surgical care to patients during menstruation.

References:


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VITAMIN D RECEPTOR GENE POLYMORPHISM IN PATIENTS WITH JUVENILE RHEUMATOID ARTHRITIS

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Introduction: Juvenile rheumatoid arthritis (JRA) is the most common rheumatic disease developing in childhood; its onset occurs before 16 years of age [1]. It is supposed that the heterogeneity of this disease is due to different factors contributed to its pathogenesis. The central role in the pathogenesis of JRA has been attributed to dysregulation of the adaptive immune response, where regulatory T cells, effector T cells and antibody-producing B cells play the leading role. Further, activated B cells produce immunoglobulins, such as rheumatoid factor (RF) and antinuclear antibodies (ANAs). The information about the role of hereditary factors in the pathogenesis of juvenile rheumatoid arthritis has increased with the discovery of the growing number of genetic loci and single polymorphisms associated with RA susceptibility [2]. The most studied susceptibility locus for this disease is the major histocompatibility complex (MHC) located on chromosome 6p. Rheumatoid arthritis is the known risk factor of osteoporosis and bone fragility [3]. Vitamin D is one of the key points that interact with both the immune system and bone tissue. The biological effects of vitamin D are mediated by the functional activity of its receptor. More than 200 single nucleotide polymorphisms were detected in vitamin D receptor (VDR) gene that regulates its activity. VDR polymorphic alleles are the risk factor of such diseases as rheumatoid arthritis, celiac disease, systemic lupus erythematosus and many others. Thus, the study of molecular-genetic markers of JRA is highly required to reveal the hereditary predisposition and early diagnosis of this pathology.

Objectives: The aim of the study was to evaluate the distribution of VDR gene polymorphic alleles BsmI in patients with JRA.

Materials and methods: One hundred and four children were enrolled in this study. The patients were divided into three groups: 37 patients had...
JRA according to ILAR classification criteria, 33 children had articular syndrome and 34 hospital controls without any signs of autoimmune or inflammatory diseases. The method of patients genotyping: BsmI (c.IVS7 + 283 G>A) polymorphism of VDR was detected by allele-specific polymerase chain reaction method with the use of reagent kit and in accordance with manufacture’s instruction.

**Results:** It has been shown that VDR genotype GG was the most frequent among all three groups (18 (48.6%), 18 (54.5%), 20 (58.8%), respectively). In patients with JRA, it was revealed the increased frequency of allele A from 32.4% to 10.2% (p<0.05) as compared to the controls. In patients with articular syndrome frequency of allele A was 28.8%. In our study children with AA genotype had also earlier manifestation of articular syndrome, as compared to the patients with GA VDR gene polymorphism (8.0 (2.3–13.0) years and 13.0 (5.0–13.7) years, respectively (p<0.04).

**Conclusions:** Thus, minor A allele of polymorphic locus BsmI VDR gene is associated with JRA arising and with predisposition to earlier manifestation of the joint syndrome in patients with JRA.

**Prospects for further research.** It remains to be further investigated how genetic variants VDR gene may be associated with the activity of the inflammatory process in JRA patients. The finding of our future research will be allowed to answer the question if it is possible to predict complications of JRA and effectiveness of its treatment based on VDR gene polymorphism.

**References**

CERULOPLASMIN IN EXPERIMENTAL OVARIAN TORSION/DETORSION

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Introduction: Ceruloplasmin is a protein that has antioxidant properties [1, 2]. This characteristic is due to the capacity of ceruloplasmin to bind myeloperoxidase [3] and also to the possibility to convert the ferrous iron to ferric form [2-4], and in this manner to avoid Fenton reaction that produce a very reactive oxygen species as •OH (hydroxyl radical), that may damage cells components [2].

Ovarian torsion is a gynecological emergency. The treatment first implies the detorsion of the torsioned anexa, but reperfusion can aggravate the initial injuries due to oxidative stress [5]. We suppose that ceruloplasmin with its antioxidant properties may help the organ not to be damaged during the process of reperfusion.

The aim of our research was to examine ceruloplasmin levels variations in the serum samples of female rats which were exposed to different ovarian torsion/detorsion models. The Ethics Committee of the “Nicolae Testemitanu” State University of Medicine and Pharmacy, Republic of Moldova approved our study protocol.

Materials and methods.

Seventy healthy female rats (Rattus albicans), weighing approximately 180-265 grams, were divided into seven experimental groups (n = 10): Group 1: The rats underwent no intervention. Group 2: The sham group: The rats underwent only laparotomy. Experimental group 3: The rats were exposed to ovarian torsion for 3 hours (ischemia). Group 4: The rats were exposed to ovarian torsion for 3 hours and 1 hour simple reperfusion. Group 5: The rats underwent 3 hours ischemia and 1 hour controlled “on-off” reperfusion. This technique of reperfusion was performed by opening and closing the clips that were placed on the ovarian annexes in 10 seconds intervals, “on-off”, for 120 seconds, and then continued reperfusion up to 1 hour. Group 6: The
rats were exposed to ovarian torsion for 3 hours and 24 hours reperfusion. Group 7: The rats underwent 3 hours ischemia and 24 hours “on-off “ controlled reperfusion.

Ceruloplasmin levels were determined in blood serum samples by Colb V.G. and Camishnicov V.S. method (1982) [6].

The results were analyzed by Welch’s ANOVA with Games-Howell post hoc test.

**Results.**

Differences in ceruloplasmin levels registered in our torsion, torsion/detorsion groups were statistically insignificant (p>0.05) compared to control group. Statistically significant high levels of ceruloplasmin were registered in experimental groups compared to no-intervention group. This result indicates that our intervention determines the activation of the processes that increase concentration of ceruloplasmin in blood samples.

**The prospects for further research.**

Being an acute phase protein synthesized by the liver, ceruloplasmin levels were supposed to be statistically significant higher in torsion, torsion/detorsion groups compared to sham group. We suppose that ceruloplasmin was consumed during antioxidant protection of cells exposed to ischemia/reperfusion. Further research are required.

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CARIES-PREVENTIVE EFFECT OF _Lactobacillus Reuteri_ IN CHILDREN WITH GASTROINTESTINAL PATHOLOGY

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**Introduction:** Gastrointestinal diseases are the most common pathology compared with other diseases of the organs and systems in children; their incidence is 140 per 1000 children [1-4]. With such a somatic pathology, the risk of tooth caries is rather high. Thus, in patients with chronic gastroduodenitis, the prevalence of caries is 97%, and its intensity makes 5.80±0.54 [5]. Gastroesophageal reflux (GER) is one of the most frequent manifestations of motor disorders of the upper gastrointestinal tract. In 25% of cases, it accompanies and complicates digestive disorders in children [6]. Acid reflux at the oral cavity level can cause various pathological conditions leading to deterioration in the quality of life of these patients. They include erosion and caries of the hard tissues of the teeth, dryness in the mouth, burning sensation of the oral mucosa, erythema of the mucous membrane of the palate and uvula, halitosis [7].

The choice of the drug for local prevention and treatment of the pathology of hard tooth tissues in children against a background of gastrointestinal diseases is an urgent problem that is determined by compatibility of the drugs used to treat the underlying disease and caries and results in improvement in the quality of life of the patient. At present, a promising direction in dentistry is the use of probiotics in prevention and treatment of the oral cavity diseases, in particular tooth decay. This is determined by the fact that they are representatives of the normal microflora of the oral cavity, thus, do not initiate a carious process, but create an uncomfortable local environment for pathogens development [8-10].

**Purpose of the study.** To determine the effectiveness of pastilles containing _Lactobacillus Reuteri_ in the prevention and treatment of dental caries in children with gastrointestinal diseases.

**Methods of research.** The study involved 16 children, aged 10-15 years,
who were registered with a gastroenterologist for gastroesophageal reflux disease (GERD). The clinical examination included assessment of the intensity of tooth and surface involvement by caries (indices DMFT and DMFS, where D - decayed, M - missing, F - filled, T - tooth decay and S - caries surfaces).

The children were divided into 2 groups: basic (B) and controls (C). The treatment in the controls consisted of professional teeth cleaning, oral cavity sanitation, and individual hygiene. In addition to the above therapy, the children of the basic group were administered with pastilles containing *Lactobacillus Reuteri*, 1 - 2 pastilles for dissolving in the mouth after brushing the teeth. The drug reduces the number of bacteria that cause caries (*Streptococcus mutans*); selectively inhibits periodontal-associated bacteria (*Porphyromonas gingivalis, Fusobacterium nucleatum, Prevotella intermedia, Aggregatibacter actinomycetemcomitans*), reduces the plaque formation.

Statistical processing of the obtained data was carried out with the help of STATISTICA (StatSoft Inc. 1984 – 2011) software.

**Results.** The intensity of caries in the examined children was DMFT = 11 ± 0,65, DMFS = 14 ± 0,65, the difference between the controls and the basic group was statistically insignificant.

The carious process in the examined persons proceeded with the involvement of almost all teeth, including the immune zones. Such caries involvement of all functionally oriented groups of teeth indicated a very low level of resistance of hard tooth tissues.

Intensity of caries in the controls a year after the treatment and preventive measures (according to DMFT index) increased (12,57 ± 0,65), while caries increase was 1,29 ± 0,61. In the basic group, increase in this indicator (11,56 ± 0,62) was also observed, but the difference was not statistically significant, and intensity gain was only 0,22 ± 0,35. DMFS indices were somewhat better; despite the increase of this index in both groups, the difference was not statistically significant (C – 14,71 ± 0,98 and B – 14,22 ± 0,74). The increase in intensity according to DMFS in the controls was 0,85 ± 0,48, in the basic group – 0,22 ± 0,44, and this difference was statistically significant (p<0,05).

Thus, introduction to the complex of therapeutic and preventive of pastilles containing *Lactobacillus Reuteri* results in deceleration in the in-
crease in caries intensity according to both DMFT and DMFS, which makes it possible to talk about the prospects of the chosen tactics of managing patients with tooth decay against a background of the gastrointestinal tract pathology.

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LOSS OF HETEROZYGOSITY AT THE BRCA1 LOCUS AS A MARKER OF SENSITIVITY FOR ADJUVANT CHEMOTHERAPY IN HEREDITARY OVARIAN CANCER

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Introduction: It was shown that at the initial stages of treatment of BRCA1-associated (inherited) ovarian carcinoma (OC), neoadjuvant chemotherapy (NACT) is rather effective type of therapy. The exposure to cytotoxic drugs even for a short period leads to reduction of tumor size. However, in such patients after 1 - 3 years it is observed a relapse of the disease despite surgical treatment followed by the administration of long courses of platinum-based therapy. It is assumed that the likely cause of carcinoma resistance to NACT may be a change in the status of the BRCA1 gene in tumor cells.

The aim was to study loss of heterozygosity at the BRCA1 locus as a marker of sensitivity for adjuvant chemotherapy in hereditary ovarian cancer.

Materials and methods: BRCA1 germ-line mutation carriers were identified via the analysis of Slavic founder alleles in BRCA1 gene. Among them were the most frequently detected mutations 5382insC (c.5266dupC), 4153delA (c.4034delA) and C61G (c.181T > G).

Primary chemonaïve cancer cells were isolated from archival cytological slides (n = 21) or tumor biopsies (n = 2); post-NACT tumor samples were obtained from surgically removed material. The dissection of tumor cells and DNA extraction are described in Mitiushkina N.V. et al. [1].

BRCA1 loss-of-heterozygosity (LOH) was assessed by real-time allele-specific polymerase chain reaction (PCR) using mutation- and wild-type-specific primers [2]. Cases demonstrating the reversion of LOH status during neoadjuvant therapy were additionally analyzed by at least one independent method. QX100™ Droplet Digital PCR System (Bio-Rad, USA) was utilized for the validation of LOH results obtained for BRCA1 5382insC (c.5266dupC)
mutation carriers; the threshold for LOH was a two-fold difference in the count of wild-type and mutant-specific signals. Those samples, which failed droplet PCR amplification, were subjected to conventional allele-specific PCR with fluorescently-labeled primers; the intensity of peaks corresponding to the total amount of mutation-specific and wild-type-specific PCR products was measured by Nanophore-5 genetic analyzer (Syntol, Russia), and the ratio (R) between these values was calculated for pre-NACT (R1) and post-NACT (R2) tumors. LOH reversion status was assigned to the pairs with R1/R2 score equal or greater than 2. LOH reversion in the OC pairs obtained from BRCA1 4153delA (c.4034delA) or C61G (c.181T > G) mutation carriers was validated by Sanger sequencing. Search for single-nucleotide polymorphisms within BRCA1 gene was performed using high resolution melting analysis and Sanger sequencing [3].

**Results:** The loss-of-heterozygosity (LOH) at the BRCA1 locus was determined for 23 paired tumor samples obtained from BRCA1 germ-line mutation carriers before and after NACT. First, we determined that 17/23 (74%) pretreatment samples contained LOH at the BRCA1 locus. All instances of LOH involved a loss of the wild-type allele.

Next, we analyzed the material surgically removed after NACT and revealed the retention of the wild-type BRCA1 copy in 11 (65%) of 17 tumors that have shown LOH before NACT. Furthermore, we tested 3 of these reversion samples for LOH at intragenic BRCA1 single nucleotide polymorphisms and confirmed a complete restoration of the SNP heterozygosity in all instances.

Among 11 tumors with the restored BRCA1 heterozygosity, 8 (73%) were exposed to 3 or more cycles of NACT, while 3 (27%) underwent surgery after 2 cycles of systemic treatment. Patients with preserved LOH during NACT tended to have shorter duration of preoperative chemotherapy: 2 out of these 6 women received only 1 cycle of NACT and 1 additional patient underwent surgery after 2 cycles of treatment. An apparent gain of BRCA1 LOH after NACT was documented in 1 patient.

**The prospects for further research:** The neoadjuvant chemotherapy for BRCA1-associated OC is accompanied by a rapid expansion of pre-existing BRCA1-proficient tumor clones suggesting that continuation of the
same therapy after NACT and surgery may not be justified even in patients initially experiencing a rapid tumor regression.

It remains to be further investigated, to what extent the reversion of the LOH status in BRCA1-mutated tumors after chemotherapy may influence their sensitivity to a subsequent treatment. In theory, a somatic inactivation of the wild-type BRCA1 allele should correlate with the sensitivity to a platinum-based therapy, while restoration of the BRCA1 heterozygosity after NACT may call for alternative treatment options. In this respect, our study may be practice-changing and lead to a re-evaluation of post-NACT treatment options for hereditary BRCA1 mutation positive OC patients. Our results call for a separate clinical trial, in which the BRCA1 LOH status would be evaluated before and after NACT, on the one hand, and the efficiency of the same chemotherapeutic agents as before the surgery would be compared with alternative agents, to which the patients has not been exposed previously, on the other hand.

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B-CELL LYMPHOMA-2 RECEPTOR EXPRESSION IN HUMAN BREAST CANCER

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Introduction: BCL2 is a member of regulator proteins that regulate cell death. It is considered as an important anti-apoptotic protein, which apart from its well-known function, apoptosis inhibition, can also block progression of the cell cycle by delaying entry into the S phase and maintaining cells in the G0 phase. This marker can improve individualization of patient therapy management, by predicting response to chemotherapy, hormone therapy and radiotherapy. Due to its anti-apoptotic function BCL2 is considered an important factor in the modulation of hormonal/anti-hormonal responsiveness exhibited by tumors. Patients with elevated BCL2 expression have the greatest benefit from endocrine therapy.

The aim of this study was to highlight BCL2 expression according to ER, PR, HER2, basal cytokeratin CK5 status and molecular subtype of breast cancer. As result was determined an affinity of BCL2 expression to Luminal tumors.

Materials and methods. Patients. There were analyzed breast carcinomas of no special type (NST) from 84 patients of 33-86 years old (57.7±1.3, with a median of 58.5). In all cases patients underwent radical mastectomy and lymph nodes dissection, without prior chemo- and radiotherapy.

Specimen processing and immunohistochemistry. The specimens were fixed in 10% phosphate buffered formalin for 24-48h and paraffin embedded (Paraplast High Melt, Leica Biosystems). The immunohistochemical assessment were processed automatically with Leica Bond-Max autostainer and included 6 surrogate markers: ER (clone Er/6F11, Leica Biosystems), PR (clone Pr16, Leica Biosystems), human epidermal growth factor receptor 2 (HER2/polyclonal, DakoCytomation), marker of proliferation Ki67 (clone K2, Leica Biosystems), basal cytokeratin CK5 (clone XM26, Leica Biosystems) and BCL2 (clone BCL2/100/D5, Leica Biosystems). All cases were evaluated also by FISH as international rules recommended (PathVysion HER-2 DNA
Probe Kit II, Abbot). The Harris modified (HHS32, SigmaAldrich) hematoxylin solution was used for counterstaining.

**Microscopic evaluation.** The guidelines of ER and PR assessments proposed by Allred et al. were used \[1\]. The threshold of positivity was 10%. The HER2 assessments were done with LeicaBond Oracle HER2 IHC System (LeicaBiosystem). The HER2 status was interpreted in accordance with American Society of Clinical Oncology recommendations \[2\]. For Ki67 we used a 14% threshold. The basal cytokeratin CK5 was interpreted in accordance with Azoulay et al. recommendations \[3\]. Cases evaluated as +1 to +3 were considered positive. The BCL2 evaluation was based on Callagy et al. recommendations \[4\]. Cases scored as +2 and +3 were considered positive.

**Results.** The most frequent histological grade was G2, determined in 45 cases (53.6%), followed by G3 with 40.5%/34 cases and G1 (6%/5 cases).

In relation to BCL2 expression, the majority of positive scores were noticed in cases with G2 (36 cases/42.9%) and G3 grades (23 cases/27.4%).

BCL2 was positive in 62 cases/73.8%. The positive BCL2 highest score was determined in cases with high expression of ER (56 cases/66.7%) and PR (44 cases/54.8%) receptors, and vice versa increasing of the HER2 (10 cases/11.9%) and CK5 (4 cases/4.8%) expressions led to BCL2 score diminishing.

The proliferation marker Ki67 was considered positive (≥14) in 50 cases/59.52% of tumors. A positive BCL2 was followed by an elevated Ki67 (≥14) in 32 cases/38.10% and in 30 cases/35.71% with a Ki67<14. In 18 cases/21.43% of high Ki67 level, BCL2 was considered negative.

The most often determined subtypes were related to Luminal B group (45 cases/53.5%), structured as: 8 cases/9.5% of Luminal B/HER2 and 37 cases/44% of Luminal B/Ki67. Tumors described as Luminal A constituted 26 cases/31% followed by HER2 (8 cases/9.5%) and triple-negative subtypes (5 cases/6%).

By comparing the molecular profile with BCL2 score the highest rate of positivity was determined in Luminal B (39.29%) and Luminal A subtype (28.57%). The ratio of positive/negative BCL2 expression vs HER2+ subtype was 50/50 - (4.76% positive)/(4.76% negative). Appropriate ratio was determined also for Luminal B/HER2 – 4.76% positive/3.57% negative. A single triple-negative case of 5 expressed BCL2.
Discussion. Breast carcinoma is the most common cause of death among women. Failure to undergo apoptosis is one of mechanisms of cancerogenesis and chemoresistance.

BCL2 contributes to oncogenesis because in cooperation with c-myc, ras or viral genes it can transform and immortalize cells. Its expression was associated with a better differentiation of the cancers and particularly, G1 – 100% of BCL2-positive tumors, G2 – 81%, G3 – 60%. Contradictory, Binder et al. (1995) presented a significant inverse correlation between histological grading and immunoreactivity for BCL2, recently confirmed by Ermiah et al. too [5]. In the present study a statistical significant correlation between histological grade and BCL2 was not found.

BCL2 is considered as modulator of hormonal/anti-hormonal responsiveness exhibited by tumors. Binder et al. (1995) supposed that loss of BCL2 expression induce the loss of hormonal regulation, increased de-differentiation and deregulated proliferation. Later, Linjawi et al. (2004) determined that expressions of hormone receptors were strongly associated with BCL2 score, results which are in line with present study too.

BCL2 expression was inversely related to c-erbB-2 oncoprotein. Petry et al. (2010) purposed the concept that ERBB2 influences the expression of BCL2 family members to induce an anti-apoptotic phenotype. Authors indicated that ERBB2 alters the expression of BCL2 in a way that leads to adverse prognosis. In our assays BCL2 correlated negatively with HER2 expression.

The anti-apoptotic function of BCL2 should predispose tumor to high proliferation. No associations were observed with Ki67 proliferative status by some researches. More, a high proliferative activity assessed by Ki67 correlated inversely with BCL2 expression in primary tumor in Binder et al. (1995) experiments. The results of the present study are complementary to above mentioned.

CK5/6-positive breast carcinomas have a low BCL2 expression and highly proliferation rate. Same data arise from this study too.

Korsching et al. (2002) considered that different cellular subgroups in the female breast give rise to subgroups of breast carcinomas with different protein expression and cytogenetic alteration patterns that may be
related to clinical behavior. Approximately 80% of patients develop hormone positive tumors. Dawson et al. (2010) established that prognostic value of BCL2 was present across molecular subtypes (ER+/Luminal, HER2+, HER2− and triple negative), and was independent of tumor size, grade and stage. Cases with ER+/BCL2− pattern had a worse prognosis than those with ER−/BCL2+. The present study revealed the affinity of BCL2 expression to the ER, PR positive tumors.

**Conclusion**: The BCL2 expression is not dependent on carcinomas proliferative activity. The highest scores were determined in cases of BCL2 co-expression with ER and PR receptors. The HER2 and CK5 exhibition led to decrease BCL2 score. Luminal B and Luminal A carcinomas are leaders in BCL2 positivity.

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CORRELATION BETWEEN MOLECULAR AND CLINICOHISTOLOGICAL CHARACTERISTICS OF BREAST CANCER IN YOUNG WOMEN

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Introduction: The most common type of cancer among women is breast cancer, with over 1.7 million women worldwide diagnosed with the disease annually, ¹⁰ and of these women, 35% will lose their lives to the disease. ²,¹⁸,²⁰ Although, the breast cancer in young women is encountered more rarely, tend to have a more aggressive evolution and consequently a poorer prognosis than older women, with specific morphologic and molecular properties.

Molecular subtypes have been characterized in the last 20 years, than Perou and Sorlie proposed “Molecular Classification” terminology in breast cancer in 2000. ¹⁴,¹⁹,²¹-²⁴ Currently there are 20 major types and 18 minor subtypes of breast cancer that have been defined and included in the recently published WHO classification. ⁹ The current molecular classification divides breast cancer into five groups (Table 1) in depends of ER, PR, HER-2/neu, Ki-67, epidermal growth factor receptor (EGFR) and basal cytokeratins (CK14 and CK5/6, etc).

Table 1

<table>
<thead>
<tr>
<th>Molecular subtypes</th>
<th>Immunohistochemical profile</th>
</tr>
</thead>
<tbody>
<tr>
<td>Luminal A</td>
<td>ER+ and/or PR+, HER-2/neu- and Ki-67 &lt;14% (negative)</td>
</tr>
<tr>
<td>Luminal B</td>
<td>ER+ and/or PR+, HER-2/neu+ and Ki-67 &lt;14% (negative)</td>
</tr>
<tr>
<td>ER+ and/or PR+, HER-2/neu- and high expression Ki-67 &lt;14% (positive)</td>
<td></td>
</tr>
<tr>
<td>HER-2/neu +</td>
<td>ER-, PR-, HER-2/neu+</td>
</tr>
<tr>
<td>TNBC (triple negative breast cancer)/basal-like</td>
<td>ER-,PR-, HER-2/neu-</td>
</tr>
<tr>
<td>Normal-like</td>
<td>ER-,PR-, HER-2/neu-, EGFR+, CK 5/6+</td>
</tr>
</tbody>
</table>

The Prospective study of Outcomes in Sporadic and Hereditary breast cancer is the largest to investigate factors affecting breast cancer progno-
sis. One-third of them were hormone receptor (HR) negative, 20% had TNBC, HER2/neu overexpression, almost 60% had poorly differentiated or undifferen-
tiated tumors, anormal expression P53, and the presence of vascular inva-
sion compared with older women (Colleoni et al., 2002; Dubsky et al., 2002; Kothari and Fentiman 2002) [3-5,7,8]. Numerous other studies have also suggest-
ed more biologically aggressive cancers in younger women [6,10,11-17].

The aim was to estimate correlation between molecular, clinical and histological characteristics of breast cancer in young women.

Materials and methods. The study population consisted of 120 young patients with breast cancer treated in the Department of mamology Oncological Institute from 2012 until 2017 at the Oncological Institute in the Republic of Moldova.

Results. The distribution of patients by age classifications was as follows: 33 patients (27.5%) were younger than 35 years old, and 87 patients (72.5%) were 40 years and older (range: 26-47 years). Mean age at diagnosis in the younger women was 37.38 years. Molecular subtypes distribution of the tumors was as follows: luminal A: 43 patients (35.8%), luminal B: 35 patients (29.1%), HER-2/neu+: 27 patients (22.5%) and TNBC: 15 patients (12.5%). Tu-
mor characteristics in younger women tend to have a more aggressive evolution and consequently a poorer prognosis than older women, with specific molecular and clinicohistological properties. Tumor characteris-
tics are summarized in Table 2.

### Tabel 2

#### Correlation between molecular and clinicohistological characteristics

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Nr. (%)</th>
<th>Luminal A (%)</th>
<th>Luminal B (%)</th>
<th>HER-2/neu+ (%)</th>
<th>TNBC (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age (years)</strong></td>
<td></td>
<td></td>
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<tr>
<td>≤35</td>
<td>33(27.5)</td>
<td>5(11.6)</td>
<td>6(17.14)</td>
<td>15(55.5)</td>
<td>7(46.6)</td>
</tr>
<tr>
<td>&gt;35</td>
<td>87(72.5)</td>
<td>38(88.3)</td>
<td>29(82.8)</td>
<td>12(44.4)</td>
<td>8(53.4)</td>
</tr>
<tr>
<td><strong>Histology</strong></td>
<td></td>
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<td></td>
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<tr>
<td>invasive ductal carcinoma</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>neinvasive carcinoma</td>
<td></td>
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<tr>
<td>lobular</td>
<td>22(18.3)</td>
<td>7(16.2)</td>
<td>4(11.4)</td>
<td>6(22.2)</td>
<td>5(33.3)</td>
</tr>
<tr>
<td>tubular</td>
<td>2(1.6)</td>
<td>-</td>
<td>1(2.8)</td>
<td>1(3.7)</td>
<td>-</td>
</tr>
<tr>
<td>infiltrativ</td>
<td>4(3.3)</td>
<td>-</td>
<td>-</td>
<td>2(7.4)</td>
<td>2(13.3)</td>
</tr>
</tbody>
</table>
The most common histologic finding was invasive ductal carcinoma (especially poor differentiated or undifferentiated forms), involving 88 patients (73.3%), predominant with luminal A and B subtypes. In depends of tumor stages, pT1 in 81.3% cases there are luminal A than pT3 and pT4 – HER-2/neu+ and TNBC. The highest rates of lymph node involvement (pN0-3) also were observed in the HER-2/neu+ and TNBC subtypes. 42 out of 120 patients (35.0%) had lymph node involvement. In depends of degree of differentiation, 86.6% of the women with G3 was TNBC, but TNM stage were uniform distributed in the all molecular subtypes. In addition, 55.0% (66 out of 120) had lymphovascular invasion (Her-2/neu+ and TNBC) compared with 54 (45%) of 120 patients with negative lymphovascular invasion.
Future directions. Molecular subtyping developed in breast cancer emphasized biological heterogeneity, which has been histopathologically defined by pathologists for a long time. Our understanding of the correlation between molecular subtypes of breast cancer with clinicohistological characteristics has evolved exponentially since the discovery of the role of many new therapeutic targets specific to each tumor’s molecular pathogenesis.

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CURRENT ASPECTS OF ODONTOGENIC JAW CYSTS TREATMENT

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Introduction: In the structure of benign neoplasms of the maxillofacial area, the number of patients with odontogenic cysts does not have any tendency to decrease. According to the recent literature, the incidence of periapical granuloma varies between 9.3 and 87.1%, and development of radicular cysts ranges from 6 to 55% [6].

It should be mentioned that among operations performed by maxillofacial surgeons in outpatient settings, surgical intervention for presence of jaw cysts takes second place after tooth extraction [4]. Traditional methods of cystectomy involve mandatory resection of the root apex when the bone wound healing occurs under the blood clot [1-3, 7].

The difference in approaches to conducting such an operation is determined by the material used to fill in the cyst cavity. To improve osteogenic regeneration, the use materials with osteoinduction and osteointegration properties is growing [5]. But even with the perfect preliminary endodontic treatment, regression of the pathological cells, which greatly increases the indications for resection of the root apex, is not uncommon.

Therefore, one of the topical problems of modern dentistry is the search for the most effective means and methods for treating the bone cavity and tooth root during the stage of cystectomy under the conditions of maintaining its length.

The purpose of the work was to improve the method of cystectomy, which preserves the length of the tooth root, effects bone repair and minimizes the allergic action of the drugs used during and after the surgery.

Materials and Methods. We examined and operated 15 patients aged 30-56 years with radicular cysts of the alveolar process of the upper or lower jaw, who were divided into 2 groups: controls (C) - 7 persons and main group (MG) - 8 persons. Before the operation all patients underwent the necessary clinical and radiological examination. In C, the operation was carried out using the traditional method, with administration of a complex antibiotic therapy.
MG patients underwent the root canal filling in the causative tooth (teeth) with a self-curing paste under local anesthesia followed by serpentine or trapezoidal incision in the projection of the root, separation of the mucoperiosteal flap, trepanation of the cortical plate (in the presence of its defect, the bone defect was expanded to the required sizes), cystectomy. The cyst bed was treated with a 36% solution of polycresulenic acid for 1 - 2 minutes, washed with a physiological solution. The bone cavity and root apex were treated by ultrasound using a ball-shaped diamond bur through a 0.05% solution of chlorhexedine bigluconate for 5 minutes with ultrasonic apparatus UDS-L LED ULTRASONIC SKALER, an E9 handpiece with 3 - 20 W intensity and 28 ± 3 kHz frequency in the operating mode of 40-second action, 20-second pause. The bed of cyst (cystogranuloma) was filled with the gum “Stimul OSS” (solution of collagen, chlorhexidine, hydroxyapatite), the mucoperiosteal flap was put in place, the wound was sutured tightly by catgut. In the postoperative period, resorption of Septefril 0.2 mg was administered 6 times a day for 4 - 7 days; Brustan 1 tablet for 7 - 8 hours, was administered as an analgesic agent when necessary.

Results In the postoperative period, MG patients did not have any complaints, the body temperature was 37.2 ± 0.3 °C, only 2 of them took analgesics once. Objectively, on day 2-3 in MG patients, swelling was moderate, palpation of the region of the intervention projection was painless, the suture was complete, the mucosa in the area of intervention did not demonstrated any pathology.

The controls complained of an increased body temperature on the day of the intervention to 37.6 ± 1.6 °C, and the pain intensity required pain relief 2 - 4 times per day in all patients. Objectively, on day 2 - 3, swelling of some tissues was expressed, palpation of the area of the intervention projection was painful, the suture was complete, the mucosa in the area of the intervention was hyperemic, edematous. On day 4 - 5 in the controls the edema was moderate, palpation of the area of the intervention projection was painless, the suture was complete, the mucosa in the area of the intervention was without pathology.

Thus, MG patients on day 3 - 4 after the surgical intervention fully received food, resumed their work activities. In C only on day 7 the patients could lead a normal life.

Follow-up 3 months later consisted of computer tomography of the
intervention segment. The bone defect boundaries were determined in C patients, within which the bone tissue of the spongy structure with areas of heterogeneity was seen. In MG the bone tissue area had with a less distinct pattern compared with the surrounding tissue of the alveolar process, while there was no clear distinction between the structures, that is, it was not possible to determine the location of the operated radicular cyst.

**Conclusion** Thus, the proposed method of cystotomy allows to improve the efficacy of the treatment of odontogenic cysts and cystogranulomas and to maintain functional value of the tooth due to preservation of the length of the root and accelerate the wound healing process by ensuring complete osteointegration and angioosteogenesis of the implanted material, which is a promising aspect in the process of the subsequent orthopedic treatment and preservation of the physiological status of the dentofacial system.

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ATOM-ABSORTIVE SPECTROMETRY FOR DETERMINING OF THE ELEMENTAL CONTENT OF THE GLYCYRRHIZA GLABRA ROOTS

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Introduction: As it was previously proved that nutrients are biologically meaningful substances and elements of human body which are divided into macronutrients and micronutrients. Studies showed that number of essential nutrients, which is necessary for functioning of the human body cells, is about 600.

Derivate of the medicinal plant Glycyrrhiza is in the focus of the medical interest and, for instance, is used as expectorant for treatment of the respiratory tract diseases, and light laxative for treatment of the chronic constipation. Herbal drug which is obtained from Glycyrrhiza roots is named glycyram. Glycyram can be used for asthma, eczema, allergic dermatitis treatment. Other substance - glycerinate can be used for Trichomonas colpitis treatment. Glycyrrhizin and glycyrrhetin acids act as a regulator of water-salt metabolism and have effect similar to the deoxyxocorticosteron.

Materials and methods. Mass-spectral analysis with ICP MS (Inductively coupled plasma mass-spectrometer) was used for qualitative and quantitative element analysis of substances during determination of the element concentration.

Parameters of the apparatus: power of plasma was 1200Vt, integrating time was 0, 1 second, rotation speed of peristaltic pump was 0, 1 rot/second. The other parameters of the apparatus were determined in the tuning process and they were invariable during periods of making technical service. As a standard, there has been used multi-elemental (27components) standard solution with principal components 1, 0 mg/l.

Samples passed to the mass-spectrometer with a speed 1ml/min by the peristaltic pump into the pulverizer (nebulizer). Particles of dispersed sample were insimminated into the central channel of inductively coupled plasma, where they were evaporated. Ions from plasma fall into the
mass-detector through the cone series, where ions were separated based on the ratio mass to the charge. Mass-detector took signal, which was proportional to particle concentration with the same ratio. Concentration was determined by calibration with multi-element standards. ICP MS analysis permitted to determine elements with the atomic weight 7 to 250, that is Li to U.

**Results**

The range of the following elements were determined in the derivate from Glycyrrhiza glabra (table 1).

**Table 1**

<table>
<thead>
<tr>
<th>Name</th>
<th>Mass</th>
<th>Cons.</th>
<th>Counts (CPS)</th>
<th>Time (sec)</th>
</tr>
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<tbody>
<tr>
<td>Li</td>
<td>7</td>
<td>23.00 mg/kg</td>
<td>76,890.00</td>
<td>0.1</td>
</tr>
<tr>
<td>Be</td>
<td>9</td>
<td>0.1800 mg/kg</td>
<td>116.6667</td>
<td>0.1</td>
</tr>
<tr>
<td>B</td>
<td>1</td>
<td>56.00 mg/kg</td>
<td>38,400.00</td>
<td>0.1</td>
</tr>
<tr>
<td>Na</td>
<td>23</td>
<td>3.700 mg/kg</td>
<td>16,307.150</td>
<td>0.1</td>
</tr>
<tr>
<td>Mg</td>
<td>24</td>
<td>4.900 mg/kg</td>
<td>3,718.10</td>
<td>0.1</td>
</tr>
<tr>
<td>Al</td>
<td>27</td>
<td>2.100 mg/kg</td>
<td>2,915.867</td>
<td>0.1</td>
</tr>
<tr>
<td>Si</td>
<td>29</td>
<td>120.0 mg/kg</td>
<td>136,300.0</td>
<td>0.1</td>
</tr>
<tr>
<td>P</td>
<td>31</td>
<td>100.0 mg/kg</td>
<td>103,620.0</td>
<td>0.1</td>
</tr>
<tr>
<td>S</td>
<td>34</td>
<td>38.00 mg/kg</td>
<td>57,993.34</td>
<td>0.1</td>
</tr>
<tr>
<td>K</td>
<td>39</td>
<td>14.00 mg/kg</td>
<td>24,008,580</td>
<td>0.1</td>
</tr>
<tr>
<td>Ca</td>
<td>43</td>
<td>1.600 mg/kg</td>
<td>12,016.67</td>
<td>0.1</td>
</tr>
<tr>
<td>Sc</td>
<td>45</td>
<td>1.100 mg/kg</td>
<td>2,240,000</td>
<td>0.1</td>
</tr>
<tr>
<td>Ti</td>
<td>47</td>
<td>8.600 mg/kg</td>
<td>1,116,667</td>
<td>0.1</td>
</tr>
<tr>
<td>V</td>
<td>51</td>
<td>2.400 mg/kg</td>
<td>3,423.333</td>
<td>0.1</td>
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<tr>
<td>Cr</td>
<td>53</td>
<td>12.00 mg/kg</td>
<td>1,820,000</td>
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<tr>
<td>Mn</td>
<td>55</td>
<td>21.00 mg/kg</td>
<td>26,213.33</td>
<td>0.1</td>
</tr>
<tr>
<td>Fe</td>
<td>57</td>
<td>450.0 mg/kg</td>
<td>16,460.00</td>
<td>0.1</td>
</tr>
<tr>
<td>Co</td>
<td>59</td>
<td>3.600 mg/kg</td>
<td>3,616.667</td>
<td>0.1</td>
</tr>
<tr>
<td>Ni</td>
<td>60</td>
<td>15.00 mg/kg</td>
<td>3,940,000</td>
<td>0.1</td>
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<tr>
<td>Cu</td>
<td>63</td>
<td>210.0 mg/kg</td>
<td>112,970.0</td>
<td>0.1</td>
</tr>
<tr>
<td>Zn</td>
<td>66</td>
<td>35.00 mg/kg</td>
<td>3,576.667</td>
<td>0.1</td>
</tr>
<tr>
<td>As</td>
<td>75</td>
<td>2.500 mg/kg</td>
<td>233,333</td>
<td>0.1</td>
</tr>
<tr>
<td>Se</td>
<td>82</td>
<td>5.900 mg/kg</td>
<td>40,000000</td>
<td>0.1</td>
</tr>
<tr>
<td>Br</td>
<td>79</td>
<td>21.00 mg/kg</td>
<td>286,6667</td>
<td>0.1</td>
</tr>
<tr>
<td>Rb</td>
<td>85</td>
<td>8.000 mg/kg</td>
<td>6,153.333</td>
<td>0.1</td>
</tr>
<tr>
<td>Sr</td>
<td>88</td>
<td>35.00 mg/kg</td>
<td>54,700.00</td>
<td>0.1</td>
</tr>
<tr>
<td>Y</td>
<td>89</td>
<td>0.1600 mg/kg</td>
<td>176.6667</td>
<td>0.1</td>
</tr>
<tr>
<td>Zr</td>
<td>90</td>
<td>0.4200 mg/kg</td>
<td>220.0000</td>
<td>0.1</td>
</tr>
<tr>
<td>Nb</td>
<td>93</td>
<td>0.1000 mg/kg</td>
<td>100.0000</td>
<td>0.1</td>
</tr>
<tr>
<td>Mo</td>
<td>95</td>
<td>2.800 mg/kg</td>
<td>446,6667</td>
<td>0.1</td>
</tr>
<tr>
<td>Rh</td>
<td>103</td>
<td>0.05400 mg/kg</td>
<td>303,333</td>
<td>0.1</td>
</tr>
</tbody>
</table>
For detection of the metal admixtures, particles were decomposed from objects in the mixture of nitrogen and perchlorous acids (8ml: 2ml) in the microwave oven “Milestone” at four-graded programming of power 250 to 500Vt and at the temperature 180 to 220°C. Obtained solution was cooled, quantitatively carried into the measured flask with 100 ml volume and further there has been used for direct introduction into spray-camera of ICP MS apparatus (Inductively coupled plasma mass-spectrometer) Agilent Technology 7500.

**Conclusion.** 49 microelements were detected in the derivate from Glycyrrhiza glabra with the use of the mass-spectrometry. Further studies are needed to detect their co-interaction in vivo.

**References**


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