Creation of a new fundamental science: Cardiometry
EDITORIAL

Editor-in-chief

PROF. VLADIMIR ZERNOV
Russian New University
Moscow, Russia
phone/fax: +7 (495) 925-03-83
e-mail: zernov@cardiometry.net

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The performance of the human cardiovascular system and longevity: considerations from the standpoint of Cardiometry
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Not only protozoa being the simplest organisms have a single ventricle heart. Artificial heart systems used in cardiac surgery can be also of the single-ventricle design. Besides, there is a “single-ventricle heart condition”, i.e. a complex of defects in the structure of the heart that may occur in some human individuals.

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Impact of exercise on the activity of the autonomic nervous system among patients with acute myocardial infarction: A case-control study
Mojgan Haj Ahmadi Pour Rafsanjani, Samira Moghadam, Roghaiyeh Afshargharebagh

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This article discusses some regional features of the structure of dental morbidity in patients with cardiovascular diseases and diabetes mellitus. The relationship between these diseases is shown. It is stated that there is a lack of awareness of the existing dentition diseases in this sort of patients.

Impact of the academic performance grades on the blood picture in female students
Vakha A. Anzorov, Svetlana V. Moryakina

The paper presents the blood picture patterns in female students with different average academic performance grades in their record book. An improvement in the academic performance of female students is accompanied by a significant decrease in the concentration of neutrophils, a significant increase in the level of lymphocytes and minor changes in other blood parameters.

Cardiorespiratory system in female students when adapting to university studies
Vakha A. Anzorov, Svetlana V. Moryakina

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Motivational interactive prevention in outpatients with ischemic heart disease
Natalia A. Koryagina, Grigoriy N. Spasenkov, Aleksey V. Avdeev, Sofia G. Shulkina, Vladimir S. Koryagin

Working women suffering from ischemic heart disease (IHD), who receive ambulance cardiology treatment, have completed their training course at our Health School (HS), and their medical examination data have been compared with those in the reference group representing women with IHD, employed by the same enterprise, who have not attended HS.

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The one and only case in history of the modern civilization: creating CARDIOLOGY, a new fundamental science
Dear Reader!

The main focus of this issue of our journal is Cardiometry, which has been developed as a new fundamental science found nowadays in a widespread use. The concept of Cardiometry, integrating its original diagnostics capabilities and effective therapy possibilities, has been successfully translated into an advanced technology capable of changing the routine in public healthcare. We are proud to say that this advanced technology is already available on all continents across the globe.

This issue outlines exciting and numerous possibilities offered by Cardiometry. Our Readership can find some key reports stating the amazing aims formulated by Cardiometry: a fresh interpretation of the physiological mechanisms of ageing, which deteriorate quality of human life.

We also publish some unique results obtained in the COVID-19 diagnostics and therapy on the basis of the application of the advanced cardiometric technology. In this connection, it should be mentioned that the merits of this express-type technology are easiness in use and effectiveness.

There is one more topical issue which may attract your attention: the pioneering report to outline the basics of low-intensity electromagnetic field therapy. The developers of this pioneering technique and equipment have identified the required parameters thereof based on the ECG data processing, and, upon involvement of the theory of adaptation reactions by a human organism, they have converted the identified parameters into some specific EMF frequencies, which produce their favorable effects on the human organism in therapy in an absolutely safe manner.

We continue publishing other high-level research reports dedicated to revealing some mechanisms of responses by the heart and circulatory system to a variety of pathological processes.

We expect that this issue might be thrilling and that your reports will be submitted to our journal, why not?

Sincerely yours,

Editorial Board
Cardiometry
In Memoriam: Valery I. Orlov

On January 6, 2021, at the age of 77, Valery I. Orlov, a man who strived for the knowledge of the Great Mystery of Life, left us. He was a Great Scientist, endowed with the ability of gentle, delicate, communication and understanding of the unique language spoken by neurons, with the desire to understand the global role of the brain in the context of the Human Consciousness.

There is an opinion that everyone is replaceable: it might be true, but it might be applicable only to the most generalized productive or social skills. In fact, the uniqueness of each person covers all the levels: from the genomic to the mental one. A human individual can be happy if he is under those conditions where the phenomenon of an inner vision of giving the proper answers to unsolved challenging problems is found, and in this sense Valery I. Orlov was a happy man, who in his young years found himself in the center of the crystallization of fresh ideas in a new research area: Neurocybernetics.

Exactly 50 years ago, on April 20, 1971, guided by Professor A. B. Kogan, the Research Institute of Neurocybernetics at the Rostov State University was established (according to Resolution No. 224 by the Council of Ministers of the RSFSR). The Department responsible for research of the neural network organization was the key structure at the Research Institute, as it studied the organization of neural ensembles, which, according to Professor A. B. Kogan, were the basis of the mechanisms of information processing by the brain. In one of the laboratories, under the guidance of L. D. Karpenko, those studies were conducted with the participation of a young employee: it was V. I. Orlov. At that time, Valery I. Orlov had not only higher biological education, but also graduated with the engineering degree in radiophysics with a specialization in biophysics that made possible for him to focus on research studies on the neurons in the grape snail. The unique combination of his engineering expertise and biological experience, multiplied by his natural talent and skilled hands, was decisive for his involvement in upgrading and designing of the original lab equipment for conducting not only extracellular, but also intracellular research experiments, that was crucial for achievement of his goal to advance up his keen ideas of proto-ensembles, which were considered to be a new important link in the brain from the evolutionary point of view.

To carry out his excellent research work, focused on the neuron performance, Valery Orlov developed his own original method of preparation of the subpharyngeal complex in the grape snail Helix Pomatia central nervous system. He succeeded in designing a unique system to maintain the required physiological conditions for a long period of time to provide the normal neuronal performance using a unique non-magnetic chamber with a volume of 0.5 cm^3, equipped with devices for supplying a liquid medium and continuous recording of intracellular potentials (up to 3 days). For his original experiments, special ultra-thin microelectrodes with a tip diameter under 0.05 microns were manufactured, which easily penetrated the neuron without disturbing its background stability. The applied technique made it possible to record the full spectrum of the amplitude-frequency characteristics in a neuron, the recorded potentials were visualized and properly measured. At present, it becomes clear what a diligent, ultra-precise and profound work has been done. Valery Orlov worked hard and employed all his talents and energy, plunging in full into the mystery of the neuron network communication, which seemed to reciprocate the researcher’s fairness, diligence, tact, and a keen sense of responsibility to explore new worlds, showing no limit to what science might achieve: it would seem the neurons were prepared to disclose all their information about their performance to him…

No doubt, the new scientific knowledge gained by Valery Orlov has kindled a great interest in the research community, and a direct cooperation with the world-level scientists Kogan A. B., Sukhov A. G., and Vislobokov A. I. allowed properly identifying most promising theoretical and practical fields in neuroscience. In the collection of research publications by Valery Orlov we can find patented inventions, articles published both in Russian national journals and those abroad, reports submitted by him at many international conferences. The wide scope of his scientific interests was amazing. For the first time in the world, he recorded the electrophysiological parameters of the mycelium cells in filamentous fungi, investigated the effects produced by some medicinal substances and electromagnetic fields of various ranges. Unfortunately, many works were left...
uncompleted, although the results were statistically processed and needed to be generalized.

Perhaps it has been just the case when the anima and persona of this scientist has been complemented by his human qualities, which were really admirable. The main thing, decisive for that tall, slim and intelligent man, with the amazing softness of a deep baritone, was his open, honest, tactful and friendly attitude to people. He demonstrated the essence of the greatest human kindness and a desire to help students, graduates, employees, all acquaintances and friends everywhere. He loved his family so dearly, and he was able to enjoy the highest values of the spiritual world.

It is generally thought, as a sign of recognition, a delightful research work of a scientist may be compared with a masterpiece created by a brilliant composer. So, in the context of the Orlov’s fine-tuned way to delicately communicate with the neuron network, the depth of how he could read its signals, his understanding and empathy with the wonderful design component of living matter, Valery I. Orlov can be surely called a Mozart in Science.

We are grateful to the Lord God for giving us the opportunity to enjoy the breaking-through research work in joint efforts with the Great Scientist: Valery I. Orlov!

M.Y. Rudenko
Deputy Editor
CARDIOMETRY

Professor Alla I. Shikhlyarova
The performance of the human cardiovascular system and longevity: considerations from the standpoint of Cardiometry

Mikhail Y. Rudenko*, Vladimir A. Zernov¹, Olga K. Voronova¹, Konstantin K. Mamberger¹, Dmitry F. Makedonsky¹, Sergey M. Rudenko¹, Gennady A. Garbuzov¹

¹ Russian New University
Russia, 105005, Moscow, Radio str., 22
* Corresponding author:
email: cardiocode@mail.ru

Imprint

Considering a life cycle, we can find an interesting feature: it is linked to the performance of the cardiovascular system and it is determined by two factors as follows. They are on the first hand an internal factor and on the second hand an external factor. Both factors have their influence of the condition of the cardiovascular system and longevity. The internal factor depends on DNA mutations that may trigger the onset and progression of atherosclerosis, when an overexpression of connective tissue growth factor is induced, while the external factor includes nutrition, environmental conditions and life style of an individual. It is just the external factor that may provoke the onset and progression of atherosclerosis, which may lead to infarction, sclerosis, stroke etc. It should be noted that both factors govern the human individual life independently till the age of 40, and since this life milestone the same factors, interfacing with each other, begin exerting their synergetic effects. It is just the time borderline when biological activity of a human individual is declining, and, as a rule, upon completion approximately of the next forty years of life permanently, ceasing.

Figure 1 exhibits a characteristic curve referring to longevity. As evidenced by research, it is possible to extend the period of time before atherosclerosis is interfacing with sclerosis. In this case, we may expect that an active phase of life becomes longer, and, accordingly, longevity can be increased.

This brings up another point: what measures should be taken in order to extend longevity and make it healthy? Let’s discuss our opinion from the standpoint of Cardiometry.

In order to identify the time of atherosclerosis-sclerosis interfacing, required is a precise diagnostics
of the cardiovascular system performance. According to Cardiometry, the criterion for the sclerosis diagnosis is the appearance of the multiple P waves on an ECG curve that should be attributed to a loss of elasticity by the cardiac muscle fibers. As a consequence, the ventricles are filled with blood in diastole due to pumping supply provided by the atria only that results in the appearance of the multiple P waves on an ECG curve (see Figure 2 herein). Two or three P waves are recorded in this case on ECG, depending on the degree of loss of elasticity by the cardiac muscle fibers. Upon reaching the specified level of the pressure in the ventricles, the SA node is activated, and the QRS complex is formed.

An assessment of atherosclerosis is a composite subject. Primarily it is provided by the criteria “signs of thrombosis”, which may be revealed on a Rheo curve in its diastolic portion. Another marker is a three-peak Rheogram, when three vertices are found on a Rheo curve (see Figure 3 herein).

The respective prevention and treatment measures can be simple enough. It should be noticed that sclerosis is linked with a DNA mutation, and this is an age-related factor, which may be inhibited by inducing genome expression. Our studies have demonstrated that the latter can be successfully achieved with the use of medical therapy device EZh-2, applying low-intensity EMF effects, which has been developed and manufactured in Russia. The specific EMF parameters produced by the device have been defined on the basis of the theory of basic adaptation reactions discovered by Russian scientist L. Garkavi. The EMF frequencies generated by the above original therapy device have been selected from spectral characteristics of the normal human ECG. The applicable clinical trials have confirmed a high efficacy and absolute safety in use of the original device.

In regard to atherosclerosis, it should be mentioned there are a variety of factors responsible for its onset and progression. In this connection, we call attention to application of the EZh-2 device to effectively prevent atherosclerosis development. Another reasonable prevention measure can be use of the Diosklephyt product manufactured by VITAUCT: it is capable of normalizing the HDL and LDL levels and controlling calcinosis. In this case, respiration-related exercises to normalize the oxygen - carbon dioxide balance in blood are of importance. It is a prerequisite to provide the proper elasticity of blood vessels.

Some other means and measures can be involved for this purpose, but our idea is to highlight the most attractive tools. Our long-term studies have shown that it may result in an increase of longevity to target active healthy life expectancy of 95 years. Now we continue our investigations in this research area. However our obtained evidence data support our conclusion on efficacy of the above mentioned diagnostics and therapy techniques.
Cardiometric criteria for diagnostics, therapy, and prediction of COVID-19 virus infection development under hospital conditions

Mikhail Y. Rudenko*, Olga G. Dvorina¹, Vladimir A. Zernov¹, Olga K. Voronova¹

¹ Russian New University
Russia, 105005, Moscow, Radio str., 22
* Corresponding author:
email: cardiocode@mail.ru

Introduction
The topicality of the understanding and knowledge of the impact of the COVID-19 virus infection on the human organism is obvious. In this connection, all aspects are important: diagnostics, making decision on therapy, monitoring of the effectiveness of the administered therapy and prediction of the patient condition development. In the spring of 2020, the authors hereof obtained evidence data covering a group of patients diagnosed with the COVID-19 infection who were in the Red Zone of the inpatient treatment in one of the Moscow clinical institutions.

Materials and methods
For the purpose of the cardiometric diagnostics of the above mentioned group of the patients, we have employed cardiological PC-assisted device Cardiocode. The device has been designed to record an ECG and Rheogram in parallel in a patient that takes 30 seconds only. Then, in the automatic mode, the following data sets are calculated and displayed on the Cardiocode screen:
1. Hemodynamic parameters:
   - SV – stroke volume of blood (ml),
   - MV – minute volume (l / min),
   - PV1 – the volume of blood entering the ventricle during the early diastole (ml),
   - PV2 – the volume of blood entering the ventricle during the atrial systole (ml),
   - PV3 – the volume of blood ejected by the ventricle during the rapid ejection phase (ml),
   - PV4 – the volume of blood ejected by the ventricle during the slow ejection phase (ml),
   - PV5 – the volume of blood pumped by the ascending aorta in the systole (ml).
2. The above parameters are utilized to calculate the ejection fraction ratio RV1= PV1/SV (%). It is expressed as a percentage representing the ratio of the incoming volume of blood in the left ventricle in the early diastole to the blood volume ejected into the aorta. This parameter shows the capability of the heart to cover the actual blood demand by a human organism. Normally, this value is about 62%; higher values thereof are the indicators of hypertrophy of the heart, and the values below this limit value are markers of the heart hypofunction.
3. Another complete set of important indicators is formed by the metabolic characteristics in the heart muscle fiber cells, which are as follows:
   - the concentration of oxygen in the myocardial muscle fiber cells (O₂),
   - the concentration of lactate,
   - the concentration of phosphocreatine (PC).
4. The system parameters of the regulation are as listed below:
   - the indicator of heart rate variability, stress index SI,
   - the degree of mental concentration of attention,
   - the actual type of actual adaptation reactions.

The therapy was carried out with the use of noninvasive therapeutic device “EZh-2”designed to generate low-intensity electromagnetic field frequencies. The specific EMF parameters of the device are aimed at controlling the desired adaptation reactions in a human body. The produced EMF frequencies due to the bioresonance induce the genome expression. This brings the immune system performance to its optimal level that makes possible to achieve a pronounced therapeutic effect: the T-cell immunity is stimulated, and cell metabolism is activated. Also, the operation of enzymes is intensified.
Table 1
Results of the cardiometric diagnostics and therapy of patients with COVID-19

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>CT diagnosis</th>
<th>CRP, mg/l</th>
<th>Diagnosis &amp; Disease History</th>
<th>Cardiometric diagnosis when being in the COVID-19 Red Zone</th>
<th>Ejection fraction RV1, % (norm 62%)</th>
<th>Use of the EZh-2 device</th>
<th>O₂ (0.85...0.5 arb.u.)</th>
<th>SI (250 arb.u.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>3</td>
<td>317.54 / 3.86</td>
<td>Pneumonia</td>
<td>The slow ejection phase indicates weak respiration. The blood volume supplied to the coronary arteries is sufficient</td>
<td>47</td>
<td>+ at the beginning</td>
<td>0.6...0.4</td>
<td>80...200</td>
</tr>
<tr>
<td>2</td>
<td>1</td>
<td></td>
<td>Pneumonia</td>
<td>Cardiosclerosis</td>
<td>49</td>
<td>+ after resuscitation</td>
<td>0.7...0.6</td>
<td>60...80</td>
</tr>
<tr>
<td>3</td>
<td>2/3</td>
<td></td>
<td>CHD; spinal surgery. Pneumonia</td>
<td>Decreased blood flow in the coronary arteries</td>
<td>62</td>
<td>+</td>
<td>0.8...0.9</td>
<td>300...1000</td>
</tr>
<tr>
<td>4</td>
<td>3</td>
<td></td>
<td>Atherosclerosis, Stenosis of the left internal carotid artery 76%. Pneumonia</td>
<td>The slow ejection phase does not provide blood volumes for the coronary arteries. Weak respiration</td>
<td>39</td>
<td>+</td>
<td>0.2...0.2</td>
<td>100...100</td>
</tr>
<tr>
<td>5</td>
<td>4</td>
<td></td>
<td>Pneumonia</td>
<td>The slow ejection phase does not provide blood volumes for the coronary arteries. Weak respiration</td>
<td>38</td>
<td>+</td>
<td>0.3...0.2</td>
<td>2200...3000</td>
</tr>
<tr>
<td>6</td>
<td>-</td>
<td></td>
<td>Pneumonia</td>
<td>The slow ejection phase does not provide blood volumes for the coronary arteries. Weak respiration</td>
<td>50</td>
<td>+</td>
<td>0.8...0.8</td>
<td>2000...3000</td>
</tr>
<tr>
<td>7</td>
<td>3</td>
<td></td>
<td>Breast cancer. Radiation therapy. Pneumonia</td>
<td>The slow ejection phase does not provide blood volumes for the coronary arteries. Weak respiration. AVV prolapse. Atherosclerosis</td>
<td>49</td>
<td>+</td>
<td>0.5...0.5</td>
<td>4000...1500</td>
</tr>
<tr>
<td>8</td>
<td>3</td>
<td></td>
<td>Skin cancer. Polychemotherapy. Pneumonia</td>
<td>The slow ejection phase does not provide blood volumes for the coronary arteries. Weak respiration</td>
<td>20</td>
<td>+</td>
<td>0.6...1.4</td>
<td>500...80</td>
</tr>
<tr>
<td>9</td>
<td>3</td>
<td></td>
<td>Skin cancer. Radiation therapy</td>
<td>The slow ejection phase does not provide blood volumes for the coronary arteries. Weak respiration</td>
<td>52</td>
<td>+</td>
<td>0.4...0.6</td>
<td>150...250</td>
</tr>
<tr>
<td>10</td>
<td>1</td>
<td></td>
<td>Pneumonia</td>
<td>“The adrenaline heart”</td>
<td>30</td>
<td>-</td>
<td>0.8...0.5</td>
<td>90...400</td>
</tr>
<tr>
<td>11</td>
<td>3</td>
<td></td>
<td>Pneumonia</td>
<td>Problem of coronary blood flow. Signs of SCD</td>
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<td>112</td>
</tr>
<tr>
<td>12</td>
<td>½</td>
<td></td>
<td>Pneumonia</td>
<td>Problem of coronary blood flow</td>
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<td>-</td>
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<tr>
<td>13</td>
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<td>Pneumonia</td>
<td>-</td>
<td>-</td>
<td>+ from the 4th day</td>
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<td>-</td>
</tr>
<tr>
<td>14</td>
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<td>Pneumonia</td>
<td>-</td>
<td>-</td>
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</tbody>
</table>

As a rule, in the circumstances many patients demonstrate poor immunity and have a number of chronic diseases. Therefore, preventive diagnostics is important in the COVID-19 treatment.

For the purpose of the cardiometric diagnostics, the ECG and Rheogram recording technology has been used that is fundamental for cardiometry. Figure 1 herein shows an example of the recorded ECG and Rheogram curves corresponding to the norm.

Results
Under the clinical conditions, using the Cardiocode device, 14 patients have been examined. The obtained examination results are presented in Table 1 herein. The most typical ECG recordings of patients cardiometric data are shown in Figure 2, 3 and 4 herein. For 5 of the 14 patients the lethal outcome was reported. Of concern to us were the background indicators, when the above patients were admitted to the hospital. Upon a detailed examination from the point of view
Figure 2. Patient 1 was treated with EZh-2. CT diagnosis: lung damage degree CT3. Ejection fraction RV1=47%. Almost without any medication. A typical example of an under-development of the slow ejection phase that is identifiable according to the Rheogram. This patient has been cured.

Table 2. Blood biochemistry (CA-180)

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<td>Urea [mmol/L]</td>
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<td>ALT [units/l]</td>
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<tr>
<td>LDH [units/l]</td>
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<tr>
<td>Creatinine [mmol / L]</td>
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</table>

Figure 1. Typical normal ECG and Rheogram
of Cardiometry, we revealed a decline in the respiratory function during the phase of slow ejection. This phase of the cardiac cycle is responsible for the systolic blood pressure and the volume of blood required for normal blood filling of the coronary arteries [1]. As you can see, the actual blood filling conditions are not sufficient under COVID-19. Figure 2 and 3 present the cardiometric data of the cured patients, and Figure 4 exhibits the data recorded in a patient with the lethal outcome. In all the Figures, the slow ejection phase and an inclination of the Rheogram peak are marked with arrows.

In the given Table, the patients, who used the low-intensity EMF physiotherapy device EZh-2 in the course of treatment, are marked with the + sign. The device is commercially produced and widely used in practice as a therapy device for individual use.

For one female patient, the results of the CRP tests both before and after treatment are presented.

**Discussions**

Considering the presented data, it is important to highlight the main characteristic features which may
be decisive for the COVID-19 complications, if any, we should note the following:
1. The most informative indicators for cardiometric assessing the degree of influence made by the COVID 19 virus infection on the organism are the following three cardiometric criteria:
   - Weak respiration during the slow ejection phase;
   - The RV1 ejection fraction is less than 35 % versus the normal value of 62%;
   - The actual state of the coronary arteries.
2. All patients, who have used the EZh-2 device, designed for the low-intensity EMF therapy to induce an adaptation reaction of Calm Activation (the physiological norm), for their treatment immediately upon their hospital admission, have been cured.
3. Lethal outcomes have been reported for those patients in the above group, who have not been treated with the EZh-2 device.
4. For patient 1 the following CRP test data before and after treatment have been recorded (Table 2). It is evident from the above data that CRP value has decreased almost by a factor of 100, from 317.54 to 3.86 mg/l, and this effect should be attributed to the use of the EZh-2 device several times a day for the period covering 6 days.
5. In all cured patients, the ejection fraction has been reported to be below the normal level, however it has been found to be acceptable: 45% on average and greater (against the norm of 62%).

Figure 4. Patient 11. CT diagnosis: lung damage degree CT3. Ejection fraction RV1=36 %. Improper development of the slow ejection phase that is identifiable according to the Rheogram. Signs of possible SCD. The patient’s lethal outcome recorded.
6. The data on patient 3 should be separately discussed. Upon admission to the hospital in a severe condition, ejection fraction RV1=62% has been recorded. This is the normal value. The recorded Rheogram has been found also to be within the normal range. Despite the severity of the disease, the patient was able to recover quickly. Figure 5, the curve of anaerobic processes, exhibits the positive dynamics of oxygen saturation in the cardiac muscle fiber cells that reaches the maximum value within its normal range.

Conclusions
1. Cardiometric parameters are important for an effective development of preventive medicine, especially in terms of predicting further development of the state of a human organism and deciding on the most suitable method of therapy.
2. The most informative indicators for assessing the degree of the influence of the COVID-19 virus infection are found to be the following three cardiometric criteria:
- weak respiration during the slow ejection phase;
- the RV1 ejection fraction is less than 35 % versus the normal level of 62%;
- the actual state of the coronary arteries.

3. The high efficacy in treatment of COVID-19 has been established in case of the use of the low-intensity EMF therapeutic device EZh-2. The practical application of this specific EMF frequency range has been known for a long time. Russian scientist Dr. Georgy Lakhovsky in 1934 has already patented a similar device. Unfortunately, we can state that 90 years are not enough for the medical community to introduce this simple, very efficient, therapy device into a wide practice.
For the purpose of research of the cardiovascular system performance, various electromagnetic phenomena are widely considered. In particular, an ECG is treated as a result from the electrical activity by the myocardial cells. As to our research team, at the first stage of creating the cardiometric theory, we have focused on hemodynamics: our attention has been occupied in developing a fresh model of the human blood flow from the point of view of fluid mechanics [1]. Our mathematical concept of the cardiac cycle phase structure, which determines the cardiovascular system performance, has allowed achieving very good results in diagnostics. We have succeeded in making a number of scientific discoveries which have become the basis for a new science: cardiometry. But our experience has demanded to make further important steps in research, and we have concentrated our efforts on developing new methods of therapy to effectively solve the problems revealed by cardiometry.

Following this way, comprehensive studies of the methods and means of therapy have been lunched. At that stage, knowledge in the area of electromagnetic field (EMF) physics has been involved. When analyzing an ECG, the authors hereof have found that upon decomposition of every ECG into individual spectral components identified are 24 harmonics. This pattern is peculiar to all ECG curves (see Figure 1 herein). Further studies conducted by our team have shown that the detected harmonics are produced by the action potentials (AP) generated by the cells.

It has been detected that 24 harmonics form some groups, and there are 5 groups of harmonics in total. This is also certain regularity intrinsic thereto. We suggest that the five groups of harmonics may be linked with the five types of adaptation reactions made by a human organism, which are fundamental for human physiology and medicine and which have been discovered by outstanding Russian scientist Professor L. H. Garkavi (1976) [2]. These adaptation reactions are classified as follows: stress, reaction of training, reaction of calm activation, reaction of elevated activation...
Figure 2. The ECG energy is calculated using the power of the spectrum in each of the five groups. Each group corresponds to a specific frequency range. The average value of this range is the resonance frequency of the corresponding adaptation reaction. The power spectrum diagram is shown below the ECG curve.

and over-activation. So, the next challenging step for us has been to identify the respective criteria for relationship between the above adaptation reaction types and the actual ECG shape variants recorded. Our conceptual philosophy for establishing the criteria has been confirmed, and we have created an absolutely fresh method for diagnostics and therapy. It is well known that H. Selye has defined only the stress reaction, while L. H. Garkavi has described not only stress in more detail, but also discovered four other types of the adaptation reactions, which she has extensively investigated. This is evidence for the fact that the level of the Garkavi's discoveries is considerably higher than that demonstrated by H. Selye. In fact, the level of L. H. Garkavi's discoveries for medicine is equivalent to the value of the D. I. Mendeleev's discovery of his Periodical Table of Chemical Elements for chemistry. It is obvious that the key to designing a highly effective therapy is an application of the adaptation reactions theory by L. Garkavi. As it’s the case with chemistry which is unimaginable without the Mendeleev’ Periodical Table, so we cannot imagine with reasonable confidence the modern medicine without the scientific discovery made by of L. H. Garkavi.

We have assumed that the criteria for a non-invasive assessment of the adaptation reactions should be reflected in an ECG shape, since the reactions are based on the energy of metabolism, and the ECG spectrum is its mirror. The energy of any signal is calculated based on its spectrum. In case of an ECG, we have found that each of the five spectrum groups corresponds to a certain frequency range. In this range, one of the harmonics has the largest amplitude (see Figure 2 herein). Under different influences, the
Our device Cardiocode is capable of identifying the adaptation reaction type automatically with the use of our own original non-invasive technology. The relevant data and the curve are displayed in the upper right corner on the screen (marked with an arrow).

Figure 5. Shape of the cell action potential (AP) depends on the actual biochemical processes in the cell membrane.

harmonics energy in the range changes, but never disappears.

Upon calculating the power of the spectrum in each group, we can construct the respective diagram as illustrated in the lower part in Figure 2 herein. Previously, the type of the adaptation reaction has been determined according to the relevant blood test data, it has been just the percentage of lymphocytes which is a key marker of the actual type of the reaction. Upon completion of our studies to reveal the relationships and correlations between the blood test data and the ECG spectral power energy, which have been conducted together with Prof. A. I. Shikhlyarova at the National Medical Research Center of Oncology, Rostov-on-Don, Russia, we have succeeded in identifying five specific diagrams corresponding to the five types of the adaptation reactions (see Figure 3 herein).

Each adaptation reaction can be described by a complete set of its own specific signs or markers. The higher is the reaction level, the more active and excitable is a human individual in question. The norm corresponds to the calm activation reaction (see Figure 3b herein). Figure 3 shows that the ECG power curve rises under the over-activation reaction reaching its high frequencies.

Our device Cardiocode is capable of automatically identifying the actual type of the adaptation reaction with the use of our original non-invasive technology and displaying it on the screen in the manner as given in Figure 4 herein.

The diagnostic information value of the AP shape is beyond question. But it was necessary for us to clarify what biophysical process the AP shape depends on. Our studies have shown that the shape of the cell’s action potential, and, consequently, of the harmonics, depends on the actual biochemical processes, which take place in the cell membrane (see Figure 5 herein).

The authors hereof have completed their original experimental studies of the action potential recovery in the snail’s neurons. Figure 6 shows the data obtained in their experiments. Professor V. I. Orlov from
the Institute of Neurocybernetics, Southern Federal University, Russian Federation, has provided support to us in the above experiments. Upon exposure of the isolated CNS neuron in the grape snail Helix pomatia to some specific EMF, we have recorded the responses by the neuron due to initiation of some EMF mechanisms as assessed according to the parameters of the membrane potential (MP) level and the parameters of the recorded action potentials (APs).

The specific EMF exposure has induced reversible hyperpolarization of the neuron membrane, keeping it at the level of trace hyperpolarization under the EMF action. The restoration of the resting membrane potential is accompanied by an enhanced operation of the Na+/K+ ATPase, which removes the sodium ions from the intracellular space. In addition, there is an accelerated recovery of the concentration of the calcium ions due to the operation of the 3Na+/1Ca2+ antiporter and Ca2+ ATPase.

The specific EMF applied by us increases the rate of rise in the initial phase of the AP firing. This is facilitated by opening of a larger number of the sodium and calcium ion entry channels and, as a consequence, by increasing of the amplitude in the next phase and rising the rate of its repolarization with a steeper decline due to opening of a greater number of the potassium ion channels and a faster exit of the K+ via the respective leakage channels.

The regulation of the functional state of the neuron located in the neural network has been objectively recorded using a non-invasive, contact-free, regulation method. Our analysis has shown that the processes in the neuron are similar to the generation of a transmembrane potential by a cardiomyocyte and that they can be considered as a basic model of the neuronal cellular self-regulation in the phase-structured activity by the cardiovascular system.

Our research work has led to a discovery of a fine tool to control the neuron, the central nervous system and other cellular systems in a human organism. The specific EMF frequencies applied by us as stimulation can be accurately graded by its strength, duration and nature of their effects on living tissue; moreover the specific EMF frequencies applied and the electric current induced thereby are close to the natural mechanisms of the generation and propagation of excitation in living tissues.

It is important that the positive effect produced by our specific EMF therapy on the functional state of
the neuron has been experimentally evidenced. This is manifested in the "relaxing" dynamics of changes in the membrane potential of the neuron resulting in hypopolarization.

Having clarified the dependence of the influence of biochemical processes in the cell membrane on the AP shape, a new question arises: is it possible to normalize the ECG curve by an EMF stimulation targeted at the performance of cardiomyocytes?

The authors hereof have discovered that a contact-free exposure to the natural broadband carrier signals, modulated by some specific frequencies corresponding to the above types of the adaptation reactions, contributes to a pain relieving effect recorded not only for the myocardium, but also for soft tissues in a human organism. In our more detailed studies on the effects of some specific EMF frequencies with the parameters of the adaptation reactions in animals, we have found that immunity is rapidly and powerfully restored. The quantified assessment shows a 3...5-fold immunity enhancement. The assessment is based on the thymus/adrenal mass ratio. Table 1 herein exhibits that the coefficient characterizing the immune system performance, after the use of the EZh-2 device generating the specific EMF frequencies, on average reaches 3. It is significantly higher than that obtained with medication, and it has absolutely no side effects.

The results obtained by us experimentally have inspired us to carry out further investigations. As a consequence, another fundamental discovery has been made by us that is capable of explaining the phenomenal results upon exposure to the specific EMF with the parameters of adaptation reactions. It has been evidenced that the therapeutic effect lies not only in the bio-resonance recovery of the cell membranes, but also in the activation of DNA, namely, in inducing the genome expression. In particular, due to the latter, the intracellular factor HIF1α is triggered. As noted by Prof. E. I. Mayevsky from the Institute of Cell Biophysics, Russian Academy of Sciences, as a result of transcription of information from HRE genes, synthesis of a number of adaptive proteins is significantly accelerated. As a result, the supply of glucose into the cells increases, glycolysis as the main source of ATP under oxygen deficiency is activated, the synthesis of erythropoietin increases and the vascular growth in the hypoxia-affected tissue regions is initiated. This contributes to the stable activation of the anaerobic and aerobic energy-producing processes, provides for a more complete oxidation of all energy substrates and facilitates washout of under-oxidized metabolites from the tissues. Thus, the mobilization of the HRE genes promotes the long-term favorable effects.

In this connection, we should mention that the Nobel Prize in Physiology or Medicine in 2019 was awarded to Gregg Semenza and Peter Ratcliffe for their study of the erythropoietin gene, including how oxygen regulates the genome expression.

Our studies of the organism responses to the effects of the specific EMF frequencies with the parameters of the adaptation reactions allows us to prove the existence of the criteria for the interrelations between the central nervous system and the cardiovascular system performance. In particular, we have detected that it is possible to regulate some human emotions with the use of some specific EMF frequencies. It turned out that some AP shape abnormalities may be markers of some mental disorders like schizophrenia and autism. It has been found that the main problem in case of these diseases is an abnormally altered shape of the action potential that disables action potential firing by the sympathetic and parasympathetic sys-

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Table 1
Weight parameters of some organs of the endocrine and immune systems in male rats upon the use of specific EMF exposure with EZh-2 in the early post-immobilization period

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<th>thymus weight (mg)</th>
<th>adrenal gland weight (mg)</th>
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tems. And we have discovered that an ECG curve is a portrayal of the APs. The APs fingerprint appearance in the spectrum is strictly controlled by the synchronization time. Resynchronization disables feedbacks. Generally, it is reflected in an inadequate behavior of a human individual. The specific EMF frequencies discovered by us normalize the processes in the cell membranes, the action potential shape by recovering feedback in the central nervous system. Figure 7 depicts the curves of the ECG harmonics recorded in different human individuals. The first spectrum curve is recorded in a normal healthy human subject. The harmonics of low frequencies have their own intrinsic amplitude and width. The second spectrum curve is referred to an athlete, before winning the World Championship: the harmonics show lower amplitude and a larger width: this is typical for the high level of the attention concentration, when an individual is aimed at winning and focused on his/her activities. The third spectrum has been identified in a human subject before his suicide act. It is evident that there are very wide harmonics with very small amplitudes. The behavior of the human subject has been recorded to be inadequate, and as a result, the suicide tragedy has been reported.

The developers of the above device have succeeded in their clinical trials to eliminate the pathological APs in schizophrenics, similarly shaped as shown in Figure 7 herein and normalize the AP shaping in them. Regular exposure to the specific EMF frequencies with the characteristics of the adaptation reactions referred to the physiological norm has made it possible for two human individuals with severe schizophrenia to return to normal life. Upon the specific EMF therapy with our original device, individuals suffering from autism show better adequacy in their behavior.

And what about the immune system under COVID-19? The EZh-2 devices, which are now commercially produced, successfully cope with the virus infection problems. The favorable clinical outcomes are presented by us in the current issue of our journal. Those who had regularly used our therapy device EZh-2 several months before the pandemic had negative COVID-19 tests.

The device designed by our research team has been commercially manufacturing for several years in Russia [4]. We have closely analyzed the results of long-term effects made by the device on the human organism: the incidence rate of diseases has been sharply decreased, and many chronic problems have been solved. As to the elderly in this case, they remain very active over the age of 90. Reported are also favorable stable therapy effects on animals produced within the shortest time.

The miracle of the specific EMF frequencies with the characteristics of the adaptation reactions are not surprising for the authors hereof. We are just capable of explaining the mechanisms of their operation and their effects from the point of view of science. We continue our research work. First of all, we are aimed at studying the genome expression and its effect made on the cardiovascular system performance.

The results of the combined use of the diagnostics device Cardiocode and the therapeutic device EZh-2 have made it possible to make a number of discoveries in the field of physiology and medicine. It becomes possible to take a new look at the conventional, generally accepted, paradigms of knowledge, and it is very important that the achievements of this level have already become an integral part of the everyday life of many people. We are sure that cardiometry is already creating new accessible technologies that changes the life of the mankind.
Cardiometric assessment of toxicity of the experimental antitumor chemotherapy and the cardioprotective effect made by L-carnitine

Alla I. Shikhlyarova1*, Mikhail Y. Rudenko2, Yulia Y. Arapova1, Ivan A. Popov1, Galina V. Zhukova1, Elena M. Frantsyants1, Elena A. Dzhenkova1, Irina V. Kaplieva1, Valerija A. Bandovkina1, Irina V. Neskubina1, Natalya A. Maksimova1, Yulianna S. Shatova1, Elena A. Sheiko1, Botir A. Yuldashev1, Malika D. Murodova3

1 National Medical Research Centre of Oncology, Russia, 344037, Rostov-on-Don, 14 liniya, 63, building 8
2 Russian New University, Russia, 105005, Moscow, Radio str. 22
3 Samarkand State Medical Institute, Uzbekistan, 140100, Samarkand, Amir Timur str., 18
* Corresponding author:
phone: +7 (863) 200-10-00-(482)
email: shikhliarova.a@mail.ru

Abstract
The aim is to conduct a cardiometric assessment of formation of acute and late toxic disorders in experimental animals with Guerin’s carcinoma during carboplatin chemotherapy against the background of L-carnitine medication.

Materials and methods
The study was performed using 43 white outbred male rats weighing 200-230 g. of them intact (without a tumor) and 27 with inoculated Guerin’s carcinoma, who were administered chemotherapy drug Carboplatin-Teva (CP) intraperitoneally at a dose of 50 mg/kg of body weight twice at an interval of 5 days. L-carnitine was administered per os using a gastric tube in an amount of 0.3 ml of the solution. ECG was recorded using hemodynamic PC-assisted analyzer CARDIOCODE (ST LLC CARDIOCODE, Taganrog, Russia) in anesthesia-free animals fixed onto the operating table in the back position. The recording was performed for 15 seconds with skin surface mini-electrodes.

Results
24 hours after the administration of carboplatin to intact animals and tumor-bearing rats, early symptoms of cardiotoxicity appeared in the form of an initial P-wave response as a marker of an increase in the load on the atria and a slowdown in its pumping function. The further delayed effect of carboplatin demonstrated the smoothing of the P wave on the ECG, which indicated a stable effect of increasing the tonus of the atrial walls. A pronounced sign of cardiomyopathy was a decrease in the amplitude of the QR phase and an attenuation of QRS, reflecting a decrease in the energetic potential of cardiomyocytes. The corrective effect of L-carnitine in animals with tumor chemotherapy was manifested in the restoration of the P wave duration to the initial level of 0.019 s, and the duration of the PQ and QRS intervals began to correspond to intact animals without cytostatic administration, 0.03 s and 0.09 s, respectively. That was an evidence for the effect of the cardioprotector on the suppression of the carboplatin toxic action.

Conclusions
Studies with the use of the model of malignant tumor growth and antitumor therapy with carboplatin indicate that there are manifestations of early and late symptoms of cardiotoxicity in the form of pathological shifts in some ECG phase constants. The possibility of correcting disorders can be provided by cardioprotector L-carnitine as a participant in the energy supply to the cardiac mitochondria.

Keywords
Guerin’s carcinoma, Chemotherapy, Carboplatin, L-carnitine, Electrocardiogram, Hemodynamic analyzer CARDIOCODE

Introduction
The use of chemotherapy in oncology is one of the main methods of adequate and effective impact on excluding or preventing the growth of malignant tumors. Very often the effectiveness of antitumor drugs and their combinations competes with their side effects, which significantly worsen the quality of life of patients and force them to limit the possibility of
continuing drug treatment [1-4]. The cardiovascular and nervous systems are among the most sensitive to the toxic effects made by chemotherapy drugs (CD). As opposed to the tissues with intensive proliferative capacity, the cardiac muscle consists of myocardioocytes having a limited proliferative and regenerative potential that as a rule induces the development of persistent manifestations of toxicity. [4]. Highly specialized cellular elements of the heart respond subtly to toxic drug effects, as a result of which many vital organoids can be damaged, the processes of proliferation, repair, apoptosis, and various types of metabolic functions, including ion transport, can be disordered. For the cardiac activity, the complex of Na+, Ca++, and K+ ions determines the link between the action potential generated by the CA- and AV-nodes and the contractile function of the heart muscle fibers and the blood flow [5,6]. In this aspect, cardiotoxicity implies the effect of CD not only on cardiomyocytes, but also on the nervous system of the heart, abnormalities in conductivity of which serves as an important diagnostic criterion for the organ performance. In particular, platinum drugs affect neurons and cause denaturation of tubulin and, as a result, disordering in the structure and the function of intracellular microtubules. In other words, cytostatic metabolites, due to their direct diffusion, penetrate into nerve fibers, cells and accumulate in them, causing damage to tubulin and other proteins, which explains the manifestation of sensorineural symptoms [4].

According to the reference literature sources, the most pronounced manifestations of cardiotoxicity produced by platinum drugs include sinus bradycardia, changes in the ST-T waves, left bundle branch block, episodes of angina pectoris, and, rarely, myocardial infarction. These complications are associated primarily with hypomagnesemia, which is often found against the background of platinum-containing drug medication. In addition, at an early stage of their use, acute damage to the heart muscle is not excluded, and this acute cardiotoxicity can be recorded within 24-48 hours from the time of administration of the cytostatic agent. At the same time, the ECG shows sinus tachycardia, non-specific changes in the ST segment and the T wave, a decrease in the left ventricular ejection fraction, and some other adverse changes. The wide capabilities of the Cardiocode device are known: diagnostics based on the "here and now" principle, measurement of blood phase volumes, assessment of the heart life expectancy (the pioneering technique), an assessment and prediction of the functional performance status of the cardiovascular system (for the first time in the world), detection of signs of sudden cardiac death, monitoring of the population [5,6]. An express-method identification of cardiovascular disorders in the context of the phase analysis determines the unique possibility to adequately assess the damaging cardiotoxic effect of an antitumor therapy and the effectiveness of rehabilitation measures in the study of the tumor growth pathogenesis with experimental models and combination therapy [7-10].

Taking into account the analytical capabilities of CARDIOCODE, the new generation ECG recorder, a comparative study of both acute and dilated cardiomyopathy can be carried out in an experiment on tumor-bearing animals using platinum drugs. It is also interesting to study the capabilities of correcting effects of metabolic cardiotropic agents to improve energy supply in cardiomyopathy.

The aim herein is to conduct a cardiometric assessment of formation of acute and late toxic disorders in experimental animals with Guerin’s carcinoma during carboplatin chemotherapy against the background of L-carnitine medication.

Materials and methods

The study has been carried out using 43 white outbred male rats weighing 200-230 g. The experimental animals have been delivered to us by the Federal State Medical & Biological Institution “Research Center of Biomedical Technologies” (Branch Andreevka, Moscow Region) at the Federal Medical & Biological Agency. A cell line as a suspension of the transplantable Guerin’s carcinoma has been delivered by the Russian National Medical Research Center of Oncology named after N.N.Blokhin, Ministry of Health, Russia. The research study was conducted in accordance with the International Guiding Principles for Biomedical Research Involving Animals and Order No. 267 “Approval of the Rules of Laboratory Practice” dated June, 19, 2003 issued by the Ministry of Health of the Russian Federation. Manipulations with animals were performed in compliance with the generally accepted rules of asepsis and antisepsis.

The experiment included two stages: Stage 1. Preliminary stage; and Stage 2. Main stage.

Stage 1 of the cardiometric study has been carried out in 16 intact (without a tumor) animals, randomly
Figure 1. ECG of a reference group animal without tumor and carboplatin administration

divided into two groups: Group 1 (n=7) served as a reference group (without exposure), group 2 (n=9) animals were administered the chemotherapy drug Carboplatin-Teva (CP) intraperitoneally at a dose of 50 mg/kg of body weight twice at an interval of 5 days. To prepare the medical drug solution, a lyophilized powder of the drug from Teva Pharmaceutical Industries (Israel), produced by PHARMACHEMIE (the Netherlands), was used.

Stage 2 of the experiment was performed with the use of a model of tumor growth in 27 outbred male rats with transplanted Guerin’s carcinoma. The transplantation of the Guerin’s carcinoma cells to animals was performed by standard subcutaneous injection of the tumor suspension into the posterolateral surface on the back portion in a volume of 0.5 ml of cell suspension in a dilution of 1: 10 in saline solution. The dynamics of the tumor growth was estimated by calculating the volume using the Schreck’s formula for ellipsoids $a \times b \times c \times \pi/6$, where $a$, $b$, and $c$ are the maximum linear dimensions of the tumor in three mutually perpendicular planes. For the measurement, a caliper with a digital reading device (type SCHCC.01.001 PS) was used. By random sampling, all tumor-bearing animals were divided into three groups, 9 individuals in each: the reference group (without exposure), the comparison group with CP administration, and the main group with CP medication in combination with the use of cardioprotector L-carnitine (CP+LC) (Levocarnitine S. C. ROMPHARM Company, S. R. L., Romania). The first introduction of the chemotherapy drug was carried out on day 8 after the transplantation of the Guerin carcinoma cell line, when the tumor volume already reached more than 2.0 cm³. Simultaneously with the chemotherapy, the use of L-carnitine was started, which was administered per os with the help of a gastric tube in an amount of 0.3 ml of the solution daily for 10 days, starting from the 1st of chemotherapy.

At all stages of investigation ECG curves were recorded using hemodynamic PC-assisted analyzer CARDIOCODE (ST LLC CARDIOCODE RU Certificate of Registration No. FSR 2011/12126, Taganrog, Russia) in the anesthesia-free animals fixed onto the operating table in the back position. The recording was performed for 15 seconds with the help of skin surface electrodes designed for ECG recording in newborns. The EGC electrodes were fixed to the ventrolateral surface of the rat thorax with an arrangement that corresponded to the second standard Einthoven lead. At the points of the ECG electrodes placement, the hair was cut, and the skin was degreased with alcohol. At the first stage of the experiment in the intact animals of the reference group, an ECG was recorded once, while in the animals of the 2nd group, ECG recording was carried out before the administration of the chemotherapy drug, on the next day after its first and second administration, accordingly. At stage 2 of the experiment, ECG recording in the reference group of the tumor-bearing rats was performed on the 8th day after the tumor transplantation. In experimental rats with Guerin’s carcinoma from the CP and CP+LC groups, ECG recording was performed before administration, as well as the day after the first and second administration of the chemotherapy drug, as a monofactor and in combination with levocarnitine. Statistical data processing was carried out using the Statistica 10.0 software, and $p<0.05$ was taken as the level of statistical significance.

Results

At the first stage of the study, we analyzed the effect of the carboplatin chemotherapy drug on the actual ECG parameters in rats without tumors, i.e. in the conditionally intact animals. Our cardiometric study conducted in the intact animals without carboplatin administration (reference group 1) showed that on the rat cardiogram waves P, Q, R, S should be analyzed, while the T wave was absent (see Figure 1 herein).

At the same time, at point P, the wave duration was recorded to be 0.025 s, and the duration of the PQ interval was 0.03 s, which indicated the closure of the atrioventricular valves and the beginning of the interventricular septum contraction. As opposed to point
Table 1
The duration of the main components of the ECG in rats without tumor at the first stage of the study (in seconds)

<table>
<thead>
<tr>
<th>Groups of animals without a tumor:</th>
<th>Point P</th>
<th>PQ interval</th>
<th>QRS interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>1st reference group, n=9</td>
<td>0.025±0.002</td>
<td>0.03±0.002</td>
<td>0.09±0.004</td>
</tr>
<tr>
<td>Group 2, before the CP administration, n=9</td>
<td>0.02±0.002</td>
<td>0.03±0.003</td>
<td>0.07±0.002</td>
</tr>
<tr>
<td>Group 2, after the first CP administration, n=9</td>
<td>0.02±0.001</td>
<td>0.03±0.001</td>
<td>0.08±0.003</td>
</tr>
<tr>
<td>Group 2, after the second CP administration, n=9</td>
<td>0.02±0.001</td>
<td>0.03±0.001</td>
<td>0.1±0.005*</td>
</tr>
</tbody>
</table>

Note: in this table, N=450 (50 cardiac cycles were taken for analysis in each animal). These differences are statistically significant, since with a degree of freedom value greater than 120, the Student’s t-test value is 1.9652497 for a significance level of 0.05.

*Statistically significant differences (p<0.05) with the allowed for this volume Student’s t-test value for the QRS interval duration in group 2 animals before and after the 2nd administration of carboplatin (CP).

P, the Q point may not always clearly appear on the derivative graph, but just at the point Q the pressure becomes sufficient to excite the baroreceptors of the AV node, responsible for generation of an action potential, and the Na+ ions begin entering the cell. This completes the diastolic part of a cardiac cycle. Further dynamics of cardiometric parameters in group 1 animals is characterized by the length of the QRS interval equal to 0.09 s (see Table 1 herein). As is seen, the R point in the intact rats within the normal range is associated with the maximum amplitude on the ECG and reflects the end of the interventricular septum contraction and the beginning of the myocardial muscle contraction, which requires a sufficient level of oxygen and energy production in mitochondria. Of particular importance for the assessment of hemodynamics is point S, which has been found in 99% of the cases and which with a high degree of confidence and reliability has corresponded to the maximum of the derivative. Obviously, as it is the case within the human ECG, the contraction of the rat’s myocardial muscle fibers ends at this point, which corresponds to the end of the Na+ ions entry into the cell.

In animals without a tumor (group 2), before the administration of carboplatin, the duration of the P wave was recorded to be 0.02 s, the duration of the PQ interval was 0.03 s, and the duration of the QRS interval was measured to reach 0.07 s (see Table 1, Figure 2A). Upon expiration of 24 hours after the first administration of carboplatin, the ECG in experimental animals demonstrated some segments with high-point P waves and polymorphic R waves with unstable amplitude, having an average duration of 0.06±0.001 s (see Figure 2B), while the duration of the P wave remained within 0.02 s, the duration of the PQ interval was recorded to be 0.03 s, and the duration of the QRS interval increased to 0.08 s.

Upon expiration of 24 hours after the 2nd administration of carboplatin, the ECG picture in the tumor-free animals showed pronounced shape disorders: the smoothed P wave with the deep Q wave and the polymorphic R wave were determined (see Figure 2C herein). While the duration of the P wave and the PQ interval did not change, the duration of the QRS interval increased to 0.1 s. Taking into account the effect of reducing the amplitude and the R polymorphism after the second administration of carboplatin to the tumor-free rats, it was possible to identify a decrease in the function of interventricular septum contraction induced by the chemotherapy drug. That was confirmed by the lengthening of the QRS interval that was apparently caused by a lack of oxygen delivery components in the mitochondrial respiratory chain that contributed to the development of mitochondrial cardiomyopathy.

At the second main stage of our study, the cardio-toxic effect of carboplatin on ECG parameters was evaluated in rats with inoculated Guerin carcinoma. 8 days after the cell line was transplanted and the tumor node was manifested, an ECG was recorded in the rats of the 3rd reference group (see Figure 3 herein). The ECG phase analysis showed that the duration of the P wave was maintained at 0.02 s. At the same time, the duration of the PQ interval increased to 0.04 s in comparison with the respective indicator in the tumor-free animals, and the duration of the QRS interval decreased to 0.07 s (see Table 2 herein). This could be attributed to the pathogenic effect of the growing tumor, which weakened the metabolic supply of the heart and contributed to the compensatory acceleration of the interventricular septum muscle fiber contraction.

In the group 4 animals with the growth of Guerin’s carcinoma before carboplatin administration, the ECG showed the smoothed P wave with the duration of 0.01 s, the deep Q wave, the duration of the PQ interval was 0.04 s, and the duration of the QRS interval was 0.08 s (Table 2, Figure 4A).

After the 1st administration of carboplatin to the tumor-bearing animals, the ECG also demonstrated...
Figure 2. ECG of an animal without a tumor in the 2nd group.
Notes: (A) before the carboplatin administration, (B) 24 hours after the 1st use of carboplatin, (C) 24 hours after the 2nd use of carboplatin.

Figure 3. ECG of an animal with a tumor in the 3rd reference group

Figure 4. ECG of an animal with a tumor in group 4 at the stages of carboplatin chemotherapy.
Notes: (A) before the carboplatin administration, (B) 24 hours after the 1st administration of carboplatin, (C) 24 hours after the 2nd administration of carboplatin.
Table 2

Duration of the main ECG segments in tumor-bearing rats at the second stage of the study (in seconds)

<table>
<thead>
<tr>
<th>Groups of animals without a tumor:</th>
<th>Point P</th>
<th>PQ interval</th>
<th>QRS interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>3rd reference group (without CP), n=9</td>
<td>0.02±0.001*</td>
<td>0.04±0.001*</td>
<td>0.07±0.001*</td>
</tr>
<tr>
<td>Group 4, before the CP administration, n=9</td>
<td>0.01±0.001</td>
<td>0.04±0.002</td>
<td>0.08±0.001</td>
</tr>
<tr>
<td>Group 4, after the first CP administration, n=9</td>
<td>0.02±0.001*</td>
<td>0.04±0.001</td>
<td>0.09±0.003***</td>
</tr>
<tr>
<td>Group 4, after the second CP administration, n=9</td>
<td>-</td>
<td>-</td>
<td>0.1±0.001***</td>
</tr>
<tr>
<td>Group 5, before the CP administration, n=9</td>
<td>0.19±0.001</td>
<td>0.05±0.001</td>
<td>0.07±0.003</td>
</tr>
<tr>
<td>Group 5, after the first CP and L-K administration, n=9</td>
<td>0.02±0.001</td>
<td>0.04±0.001**</td>
<td>0.1±0.003***</td>
</tr>
<tr>
<td>Group 5, after the second CP and L-K administration, n=9</td>
<td>0.19±0.001</td>
<td>0.03±0.003**</td>
<td>0.09±0.007***</td>
</tr>
</tbody>
</table>

Notes: in this table, N=450 (50 cardiac cycles were taken for analysis in each animal). These differences are statistically significant, since with a degree of freedom value greater than 120, the Student’s t-test value is 1.9652497 for a significance level of 0.05.

*Statistically significant differences (p<0.05) with the allowed for this volume Student’s t-test value of the QRS interval duration in group 4 animals before and after the 2nd administration of carboplatin

**Statistically significant differences (p<0.05) with the acceptable for this volume value of the Student’s t-test in the PQ interval before and after administration of carboplatin and L-carnitine (L-K) to animals of group 5;

***Statistically significant differences (p<0.05) with the acceptable for this volume value of the Student’s t-test in the QRS interval before and after administration of carboplatin to animals of group 4 and carboplatin with L-carnitine to animals of group 5.

the smoothed P wave with its duration of 0.02 s; the deeper Q wave was noted, and a bifurcation of the R wave was observed. At the same time, the duration of the PQ interval did not change and was reported to be 0.04 s, and the duration of the QRS interval increased to 0.09 s (see Table 2, Figure 4B herein). Apparently, the smoothed front of the P wave, combined with a deep Q-wave while maintaining the overall duration of the interval, might reflect an increase in the tension of the atrial contraction function, the expansion of which can be inhibited by the Na+ ions, which enter the atrial muscle cells under the influence of the action potential. Since the smoothness of the P wave was observed, it can be assumed that the atrioventricular valve closure was slowed down due to the beginning of the Ca++ ions entry, a decrease in the level of which can affect the state of the heart’s nerve pulse generators. Besides, the duration of the P-Q phase depends on the elasticity of the ventricular muscle fibers. With a reduction in elasticity, the duration of this phase increases, but this is not observed in animals after the 1st administration of carboplatin: the recorded indicators coincide with the reference values that bears witness to the fact that there is a similar pathological effect of the tumor process itself on the state of the atria. It is suggested that the toxic effect of carboplatin has also manifested in relation to the phases of the ventricular systole, when, perhaps, under the influence of a toxic chemotherapy agent, the generation of the AV node action potential pulse is delayed, and consequently, the same is the case with the beginning of the interventricular septum contraction Q-R, because the contraction itself occurs before R. The subsequent aerobic process of the myocardial muscles contraction, associated with the entry of the Na+ ions into the cells, is also delayed, which has been confirmed by the prolongation of the QRS interval.

It is just the type of the cumulative cardiotoxicity of carboplatin that is more clearly manifested after the 2nd administration of the chemotherapy drug, when the duration of the QRS interval has increased even more and reached 0.1 s (see Table 2 herein). At the same time, the P wave was absent again, and the deep Q-wave was expressed, which indicated the functional load of the atria, and the R wave formed as a polyphase two-vertex pattern (Figure 3C). As is known, such a bifurcation of the R-wave is determined by changes in the quality of the interventricular septum muscle contraction, which are based on the biophysical mechanism of changing the muscle contraction of the IVS from its relaxation and to the recovery of the contraction, i.e. the contractility cycle in full [6]. This is also evidenced by our data on the decrease in the R-wave amplitude as it is approached the isoline. Besides, a decrease in the amplitude of the Q-R phase may indirectly indicate the state of aerobic processes in the interventricular septum muscle fibers. The greater the amplitude of the derivative, the more efficiently the muscle works and vice versa. It is known that the muscle contraction occurs due to the efficient work of mitochondria with their main function of ATP production, based on the capture of pyruvate, carbon chains of amino acids and, mainly, fatty acids, the transport of which can only be carried out when interacting with carnitine. Carnitine, regulating the metabolism of phospholipids, is actively involved in energy-de-
pendent reactions and is an indispensable link in the normal functioning of cardiomyocytes.

The question arises as to whether it is possible to use the active form of the cardioprotecting carnitine (L-carnitine) to correct the processes of energy functioning of cardiomyocytes and whether it is possible to prevent disorders in the mechanism of the heart muscle contraction in tumor-bearing rats with the use of the highly toxic antitumor chemotherapy drug carboplatin.

According to the record on our experimental studies, as it was the case with the previous series of our experiments, a cardiometric assessment of the initial state of the tumor-bearing animals in group 5 was first performed 8 days after the tumor transplantation before the use of carboplatin and L-carnitine (see Figure 5A herein). At the same time, it was found that the duration of the P wave was reduced to 0.019 s, while the duration of the PQ interval, on the contrary, increased to 0.05 s, and the duration of the QRS interval was reduced to 0.07 s (see Table 2 herein).

24 hours after the first administration of carboplatin and a single application of L-carnitine, it was not possible to record a pronounced corrective effect of the cardioprotector. At the same time, the ECG was characterized by the presence of the smoothed P wave with the duration of 0.02 s, reflecting the closure of the atrioventricular valves. Again, the formation of the polymorphic R wave with its reduced amplitude was recorded, indicating an increase in the load on the atria (see Figure 5B). The duration of the PQ interval was 0.04 s, and the QRS interval was 0.1 s, which differed from the initial state with a tendency to slow down the process of atrial pumping function and, especially, pronounced tension of myocardial muscle contraction (see Table 2 herein).

The further five-time use of L-carnitine against the background of the next (2nd) administration of carboplatin was characterized by the appearance of ECG segments of the P wave bifurcation, however, the shape of the Q and R wave began to correspond to the background ones (Figure 5C). The duration of the intervals characterizing the closure of the atrioventricular valves, as well as the duration of myocardial muscle contraction, also has experienced a normalizing effect made by L-carnitine: the P wave duration reached its initial level of 0.019 s, and the duration of the PQ and QRS intervals began to correspond to those found in the reference animals without a tumor, 0.03 s and 0.09 s, respectively (see Table 2 herein), which demonstrated a corrective effect of the cardioprotector in relation to the suppressive control of the toxic effect of carboplatin.

It should be noted that in comparison with the beginning of the use of L-carnitine, its further repeated action had a significant impact on the stabilization of the effect of increasing the amplitude of the R wave without the wave bifurcation. This was a confirmation
of overcoming the energy weakness in ATP production in the heart mitochondria, since a significant decrease in the amplitude of the QRS complex is evidence for the toxogenic nature of the medical drug which induces the weakening of energy processes in the mitochondria.

Discussion

In general, assessing the effect of carboplatin on the state of cardiac activity in the intact animals and in those with the tumor growth, we can note the manifestation of early symptoms of cardiotoxicity, consisting in the initial response of the P wave as a signal of an increase in the load on the atria, slowing down its pumping function. The further late effect of carboplatin demonstrated the smoothing of the P wave on the EEG that indicated a stable effect of increasing the tonus of the atrial walls. A pronounced sign of cardiomyopathy was a decrease in the amplitude of the QR phase and an attenuation of the QRS, reflecting a decrease in the energetic potential of cardiomyocytes, suppression of the electronic respiratory system and oxidative phosphorylation. It was just the link that needed the metabolic support that L-carnitine could provide.

Analyzing the experimental data obtained, it can be seen that even a relatively short-time 5-time administration of L-carnitine may have a suppressive effect on the manifestation of carboplatin cardiotoxicity. It was evidenced by the balanced closure of the atrioventricular valves with the normalization of the PQ and QRS intervals, which could indicate the restoration of myocardial contractile function.

At the same time, it is necessary to stress the advanced capabilities of cardiometry using analytical software CARDIOCODE [11-12]. This covers the involvement of predictive criteria for assessing hemodynamics in relation to the duration and shape of the P wave. Indeed, as shown by the above experimental study, after repeated administration of carboplatin, even against the background of the use of L-carnitine, a bifurcation of the P wave top was observed. In the context of the categories of cardiometric analysis, the physiological reason for this P wave bifurcation on the ECG may be an imbalance in the synchronization between the systemic and pulmonary circulatory systems, and the responsible biophysical mechanism implies different resistance to blood flow in the systemic and pulmonary circulatory systems. Such a bifurcated form of the P-wave may be associated with disorders of the hemodynamics in the lungs [6]. An alternative to these changes is the corrective effect made by L-carnitine, the stabilization of the effect of which is recorded with a systematic repetition of the drug use even against the background of carboplatin administration to animals with Guerin’s carcinoma. The presented method of verification of the ECG form in experimental animals by biophysical processes may become the basis only as a result of the creation of a complete theory of cardiac cycle phase analysis [13]. At the same time, the small amplitude of the QRS complex detected during the use of cytostatics in animals with and without a tumor demonstrated the weakening of the energy processes in the mitochondria. The therapeutic effect of L-carnitine, which intentionally targeted the state of the mitochondrial oxygen delivery chain-serotonin-L-carnitine, indicated the normalization of the ECG phase amplitudes as an electrophysiological equivalent of reducing the manifestations of mitochondrial dysfunction and cardiotoxicity under the influence of carboplatin.

Apparently, changes in the ECG during chemotherapy under clinical and experimental conditions are not specific in their nature, while, as is known, in radiation therapy of cancer it makes influence by a decrease in voltage, an expansion of the QRS complex, smoothness of the P wave, slowing-down of the intracardiac conduction [14]. Such a cardiometric assessment of the experimental antitumor chemotherapy toxicity can enhance the technological capabilities of the toxicity prevention upon the effect made by L-carnitine.

Conclusions

1. The use of the cytostatic carboplatin as a factor in systemic chemotherapy for Guerin’s carcinoma is characterized by early changes in the shape of the P, Q, and R waves, which may indicate an increase in the load on the atria, and in the subsequent period, and by disorders in the intraventricular conduction.

2. An involvement of L-carnitine as the cardioprotector in the scheme of experimental antitumor chemotherapy with carboplatin reduces the toxic effect of the latter on ECG parameters, which is manifested in the normalization of the Q and R waves; however the manifestation of the P wave bifurcation should be treated as a marker of dilated cardiotoxicity.

Acknowledgments

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Statement on ethical issues
Research involving people and/or animals is in full compliance with current national and international ethical standards.

Conflict of interest
None declared.

Author contributions
The authors read the ICMJE criteria for authorship and approved the final manuscript.

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Reverse techniques as a means of increasing the validity of the cardio-oculometric diagnostics

Elena V. Brodovskaya1,2, Anna Yu. Dombrovskaya1,2, Vladimir A. Zernov3, Elena V. Lobanova3, Elvira V. Likhacheva3, Lyubov P. Nikolaeva3, Aleksandr S. Ognev3, Mikhail Yu. Rudenko3

1 Financial University under the Government of RF
   Russia, 125993, Moscow, Leningradsky Avenue, 49
2 Moscow State Pedagogical University
   Russia, Moscow, 119991, 1/1 M. Pirogovskaya
3 Russian New University
   Russia, 105005, Moscow, Radio str., 22

* Corresponding author:
phone: +7 (962) 989-04-96
email: brodovskaya@inbox.ru

Abstract
The article presents empirical data confirming the validity of our assumption that the use of reverse modes in operations with visual stimuli presented on the eyetracker screen allows us effectively assessing the degree of respondents' honesty in work. It is shown that the combination of modes “authentic view” with the “reverse view” significantly facilitates performing all tests by the respondents, simplifies the procedure of their self-determination in their personal choice and makes their participation in research an interesting and attracting event. It is noted that a comparison of the gaze parameters obtained in these modes can serve as an additional indicator of the degree of subjective significance of various visual stimuli elements for respondents. It is also shown that the use of reverse modes, visual stimuli of personal orientation, active participation of respondents in the discussion of all oculometric and oculographic data obtained therewith increases the motivation of interviewees to validate various types of information content and confidential discussion of even unpopular questions.

Keywords
Eye tracking, Reverse technique, Accuracy of information, Socio-political research, Honesty of respondents

Introduction
Regular supply of reliable information about the attitude of citizens to certain socio-political and economic processes and events is the most important prerequisite for making informed decisions by any modern state and public institutions. Therefore, various surveys used for this purpose have become common practice. And the routine procedure carried out according to the rules of conducting such surveys becomes in itself a source of reducing the reliability of the information received. Due to repeated participation of respondents in surveys, which are conducted in the form of conventional questionnaires, instead of difficult understanding and expressing their true opinion, it leads for a surveyed person to nothing more than to reproduce certain social stereotypes. As a result, expensive and time-extensive surveys increasingly report common stereotypical responses, which differ dramatically from authentic information.

The problem of providing regular, timely, supply of such reliable information is aggravated by very consuming time required for the survey procedures themselves, the complexity of processing primary data and difficulties in choosing a user-friendly form of presentation of the obtained data. To all these difficulties added should be the growing fatigue of everybody involved due to the suddenly appearing annoying interviewers, due to the need, in case of consent to participate in the survey, to spend a lot of time searching for answers to boring questions, as well as due to the struggle with yourself when trying to give strangers honest answers to their sometimes rather sensitive questions.

As numerous recent studies [1-10] show, various variants of cardio-oculometric detection can be successfully used to overcome these difficulties. Serious reasons for this are provided by information about the successful use of eyetrackers in combination with computer-aided cardiographs in engineering-related and social psychology [7], in the study of various human ego states [10], in the diagnostics of personological characteristics of an individual [1, 2], in the assessment of human respondents to stimuli that differ in their
modality [3], in the study of various sources of stress [4], in identifying the degree of reliability and validity of information provided by respondents [5-10].

As exemplified above, the efficacy of obtaining primary data was achieved by using specially prepared visual stimuli. The presentation of the latter to the respondent took no longer than 3-5 minutes. At the same time, obtaining primary data did not require any special effort by the respondent. It was enough for him/her just to view a series of images, which appeared on the computer screen for 7-10 seconds. The efficacy and convenience of presenting the final results were provided by automatic processing of the recorded gaze parameters and convenient visualization of their integrated characteristics. The respondent's interest to the research and his/her willingness to provide authentic information were supported by the purposeful use of the phenomena of neurovisual programming and cognitive induction.

Our studies were mainly personological in character, and they were not directly aimed at assessing the viability and applicability of such methods in terms of studying public opinion on socio-political issues, and in this case, due to the above reasons, some additional indicators of the respondents' honesty are needed, when working with video content. According to our hypothesis, such an indicator can be a comparison of the respondents' gaze parameters when they work with the same set of visual stimuli in the following two modes: it is mode No. 1 ("authentic gaze") that implies direct contemplation of visual stimuli, when the respondent looks at where he wants within the screen, and it is mode No. 2 ("reverse gaze") when the respondent is offered to choose on the screen anything that, in his/her opinion, is diametrically opposite to his/her original (natural, authentic, true) choice.

Materials and methods

To test our hypothesis, the visual stimuli as described below were presented in mode 1 and mode 2 to a group, which included an equal number of the males and the females. In total, the group covered 48 respondents with an average age of 26 years. Along with these above images, the representatives of the group were presented with additional control visual stimuli. The following visual compositions, which were tested previously in the work in modes No. 1 and 2 with other groups of respondents (in total there were more than 800 people), were used as the stimuli:

- stimulus No.1 is a red line placed on a white background with inscription "no way out of line!";
- stimulus No.2 is phrase "where are you?" above the image of three runners at their finish line, the first of which has already reached the finish line, the second is one step away from the leader, and the third runs two steps away from the leader and closes the group of competitors;
- stimulus No.3 represents phrase "where are you?" above the image of two contour figures facing the viewer, one of which is an adult without any pronounced signs of belonging to any gender, wearing casual home dress, and the second is a child vigorously beating a drum;
- stimulus No.4 offers a contour image of a cow located in the center of the stimulus with word "lion" written on its body, as well as contour images of a lion (the upper right corner of the stimulus), a tiger (the lower right corner), a cow (the lower left corner) and a hippo (the upper left corner);
- stimulus No.5 exhibits the colored squares of the Lüscher color personality test arranged in two rows by four above and below the phrase "good mood" (the central segment of this visual stimulus).

To conduct a quantitative analysis of the obtained data using the AOI procedure (where AOI is a standard abbreviation for describing the working procedures of eyetrackers, referred to the word combination "Area of Interest", which denotes a parametric description of the gaze when a test subject is viewing separated areas of the stimulus image, i.e., calculation of the absolute and specific time of fixing the gaze on the elements of interest to the researcher, a count of the gaze returns to the various components of the image, etc.) provided by the GP-3 eyetracker software, we, as in all previous studies, used the following segmentation of the stimuli listed above:

- segment No.1: the entire part of stimulus No.1 is above the red line;
- segment No. 2: the red line depicted in stimulus No.1;
- segment No.3: the entire part of stimulus No.1 below the red line;
- segment No.4: the leading runner in the stimulus No.2 image;
- segment No.5: the lagging runners in the stimulus No.2 image;
- segment No.6: the contour image of an adult in the stimulus No.3 image;
- segment No.7: the contour image of a child beating a drum in the stimulus No.3 image;
- segment No.8: the contour image of a lion (the upper right corner of stimulus No.4);
- segment No.9: the contour image of a cow (the lower left corner of stimulus No.4);
- segment No.10: the green square in visual stimulus No.5;
- segment No.11: the blue square in visual stimulus No.5;
- segment No.12: the yellow square in visual stimulus No.5;
- segment No.13: the red square in visual stimulus No.5;
- segment No.14: the violet square in visual stimulus No.5;
- segment No.15: the grey square in visual stimulus No.5;
- segment No.16: the black square in visual stimulus No.5;
- segment No.17: the brown square in visual stimulus No.5;
- segment No.18: the 120 point size inscription "good mood" in visual stimulus No.5.

The following visual stimuli were also segmented as shown in Figure 1 herein:
- segments No.19 and 20 showing the rectangular standard-type national flags of Russia and Ukraine;
- segments No.21 and 22 containing words “Russia” and “Ukraine”;
- segments No.23 and 24 depicting the flying national flags of Russia and Ukraine;
- segments No.25 and 26 displaying the national state emblems of Russia and Ukraine;
- segments No.27 and 28 showing words “Moscow” and “Kiev”;
- segments No.29 and 30 depicting the national flags of Russia and Ukraine with their national emblems placed thereon;
- segments No.31 and 32 displaying the national flags of Russia and Ukraine with their national emblems and the names of the countries in their national languages shown thereon;
- segments No.33 and 34 exhibiting images of the national passports of Ukraine and Russia.

During the exposure of these stimuli on the GP-3 eyetracker screen, the examinees’ cardiograms were recorded in parallel using computer-assisted hemodynamic analyzers Cardiocode. Then, with some specific algorithms in the Cardiocode software, the Baevsky stress index (SI) values were calculated for each examinee.

After the test was completed, a post-test conversation was conducted with each respondent. At that time, records of the respondent’s eye movement, his/her condition, and possible interpretations of the obtained oculometric data were demonstrated and discussed. After completing the testing work with all the examinees, the general processing of the obtained statistics data covering the entire sampling was carried out using professional standard software package STADIA 8.0.

Results and Discussion

Based on the results of the completed individual oculographic studies, we determined the average indicators for the collected sampling data as a whole. The results of our work are presented in Table 1 herein.

Table 1 shows that during the transition from mode 1 to mode 2, significant changes in the duration of the fixation time (more than 1.5-2 times) occurred in 31 of 34 segments highlighted and described in detail above. As can be seen from the Table, all changes in the parameters of the gaze, without exception, when passing from one mode to another, are mirror-like, statistically significant and demonstrate the same logical pattern. At the same time, it should be noted that the gaze parameters for segments 1-18 in the specified group of 48 examinees and in the generalized group of respondents (in total, there were more than 800 individuals) were statistically indistinguishable.

When analyzing the data given in Table 1, attention should be drawn to the particularly strong differences in the parameters of the gaze between mode 1 and mode 2, which was fixed on the images of the depicted national state flags. No less significant differences between modes 1 and 2 were obtained in assessing the Baevsky index values. As to mode 1, the arithmetic mean value of the SI parameter reached 211 arbitrary units, while in mode 2 it was recorded to be 327 arbitrary units. It should be noted that the similar patterns were previously reported by us when comparing the SI values in the mode, when according to the preliminary instruction the respondents told the truth, against the mode, when they deliberately told a lie. Discussion of the obtained results with each respondent revealed that when working in mode 2, the respondents experienced noticeable physical and emotional discomfort that was not the case in mode 1. They also noted that the experience of working in the reverse mode significantly facilitated the implementation and understanding of their authentic choice.

Conclusions

The obtained results have confirmed the validity of the second hypothesis of our study that the use of the reverse modes of work by the examinees with visual stimuli presented on the eyetracker screen allows us effectively assessing the degree of honesty in
work both for each individual respondent and the entire group of the respondents. It has turned out that the combination of "the authentic view" and "the reverse view" modes significantly facilitates all tests for the respondents themselves, simplifies the procedure of the self-determination with their personal choice and makes their participation in research an interesting and exciting event. We have obtained additional evidence that the comparison of the gaze parameters recorded in these modes can serve as supplementary indicators of the degree of subjective significance of various visual stimuli elements for the respondents. Thus, it has been found that if the image includes both words "Russia" and "Ukraine" and the corresponding national flags of these countries, then in order to identify the preferences (where I want to live, who owns the Crimea, which is closer to me) the respondents usually twice longer fix their attention on the flag of the state appropriate for them.

The use of the reverse modes, the person-focused visual stimuli, active participation of the respondents in the discussion to interpret all oculometric and oculographic data obtained therewith increases the motivation of the interviewees to validate various types of information content. The personal context in the described study was effectively identified due to the fact that the oculometric and cardiometric analysis gave good results in determining the characteristic personal traits and the previous experience of the interviewees, which gave a sense to the content discussed.

The above research work has been completed within the framework of the RFBR Grant "Ukrainian information flows in the Crimean segment of social media: risks and technologies to overcome the negative effects of anti-Russian rhetoric in the online environment (No. 18-011-00937 for 2018-2020).

Statement on ethical issues
Research involving people and/or animals is in full compliance with current national and international ethical standards.

Conflict of interest
None declared.

Author contributions
The authors read the ICMJE criteria for authorship and approved the final manuscript.

References
Method for estimation of the pulse wave propagation velocity by a manual pneumatic arterial pressure sensor

Vyacheslav E. Antsiperov1,2*, Aleksandr S. Bugaev3, Mikhail V. Danilychev1,2, Gennady K. Mansurov1

1 Kotel’nikov Institute of Radio Engineering and Electronics of RAS
Russia, 125009, Moscow, Mokhovaya str. 11, build. 7
2 Russian New University
Russia, 105005, Moscow, Radio str., 22
3 Moscow Institute of Physics and Technology
Russia, 141701, Moscow region, Dolgoprudny, Institutsky per., 9
* Corresponding author:
email: antciperov@cplire.ru

Abstract

The paper discusses a new method for diagnosing atherosclerosis using a pneumatic arterial blood pressure sensor previously developed by the authors hereof. The possibility of applying a pneumatic sensor to measure the pulse wave transit time referring to a synchronous ECG is treated herein. The specification of the method consisting in the selection of the characteristic moment of the pulse wave as the timestamp, when measuring the signal transit time in relation to the R-peak of the synchronous ECG, is justified hereby. The averaged values of the wave transit time at different points of the artery, taking into account the variability of the front delay values, are used to directly determine the pulse wave propagation velocity in the area between the measurement points.

Keywords
Pulse wave, Arterial pressure sensor, Atherosclerosis, Diagnostics

Introduction

The most important factors in treatment of atherosclerosis are prevention and early diagnostics thereof. At the subclinical stage of atherosclerosis (before clear-cut clinical signs appear), the disease can be defined as a process of a gradual loss by the blood vessels of their main functions with the poorly detectable external manifestation. With time, vascular calcification occurs, the vascular walls lose their elasticity, and the progressive narrowing of the blood vessel diameter is observed. The distinctive features of this process are an increase in stiffness of the arterial walls and a rise in arterial blood pressure (AP). Contemporary practice suggests that the widely used method of the arterial stiffness assessment in terms of quantity is measuring of pulse wave velocity (PWV). The latter is the velocity of a higher pressure wave, traveling in the arteries, generated by ejection of blood by the left ventricle in the systole period. In the framework of the linear approximation of the theory of hydroelasticity for this value we can write [1]

$$V = \frac{\sqrt{\frac{Eh}{\rho D}}}$$

where E is the effective tangent Young’s modulus, h and D are non-disturbed wall thickness and diameter of the blood vessel, respectively, and \( \rho \) is blood density. From formula (1) it follows that an increase in the value of the elasticity modulus and a decrease in the blood vessel diameter result in a rise in the PWV value. It may be suggested that a reasonable method of measuring PWV is an immediate measurement of the pulse wave transit time (PWTT) between a pair of the specified cross-sectional areas in the artery to be examined. Theoretically, for this purpose, a pair of the sphygmometric sensing elements may be used, which are located proximally over the surface blood vessels (arteries) and distally referred to the heart (at the carotid, femoral, radial and some other accessible arteries). If distance d between the sensing elements (along the artery) is known and if the PWTT is determined as \( \Delta t = t_2 - t_1 \), evidently the PWV value can be represented by the ratio between the above values as given below:

$$V = \frac{d}{\Delta t} = d/(t_2 - t_1),$$

where \( t_2 \) and \( t_1 \) are the points in time of the successive traveling of a certain selected portion of the pulse wave, say, its front, when passing through each of the sensors. However, when implementing the method in practice, some difficulties in engineering and methodology emerge.
1 Measurement of PWV based on continuous monitoring of AP

Previously the authors hereof have developed and experimentally tested sensors of new types designed to trace the dynamics of the AP pulse wave, including a three-channel pneumatic sensor for noninvasive monitoring of arterial pressure (AP) [2-5]. The device developed by the authors hereof has an apparent advantage: it is capable of providing the mode of continuous measuring of the AP dynamics that makes possible not only to measure current systolic/diastolic pressure values, but also to trace the AP dynamics both within a cardiac cycle and considerable time intervals. The actual measurement of arterial pressure requires a certain position of the sensor above the artery, as well as a solid base under it, like the radius for the artery of the same name (see Figure 1 herein). Only in this case, the pressure in the operating chamber of the sensor can be considered to be equal to the blood pressure in the artery. It has been found experimentally that for arteries the location of which does not meet the above conditions, it is possible to observe a pulse pressure wave signal, the level and magnitude of which are noticeably damped by viscoelastic tissues lying both between the sensor and the artery under study, and behind the artery. However, in this case the time characteristics of the signal are retained that can be used to measure the wavefront transit time.

In order to solve the problem of assessing PWV with the use of the pneumatic sensors, a non-standard approach has been applied. The idea behind this approach is that if measurements for a pair of the points over the artery can be separated in time, too, we may try to use a single sensor technique only. It is evident that the location of the R wave on an ECG can be utilized as the periodical zero reference. For this purpose, an additional channel of synchronous measuring of an electrocardiographic (ECG) signal has been integrated into the pneumatic AP sensor designed by the above authors. In this case, in terms of radio-engineering, the above channel can be considered as a peculiar kind of reference signal generator. The ECG amplifier circuit was designed to use conductive-gel-free dry electrodes without a neutral electrode that required both analog and digital circuit filters. The simplified circuit design was chosen to minimize the inconvenience of placing and removing the electrodes, and it is currently used as a pilot version only at the development stage.

2 Methodology for measuring PWV with an integrated AP/ECG sensor

In cooperation with JSC NEUROCOM, a manual version design of the sensor has been developed that does not require to be fixed on the patient’s body surface (see Figure 2 herein). This is convenient for obtaining relatively short fragments of the dynamics of arterial pressure over the radial artery.

Moreover, it turns out that the offered design version with an applicator located remotely ahead has been found to be sensitive enough to record the time parameters of the pulse wave practically over any artery that can be detected by palpation, since the size of the applicator is equal to the area of the finger pad. Certainly, the pressure applied to the applicator plane surface at the working points is equal to the pressure in the arteries only under quite rigorous conditions referred to the position of the artery relative to the sensi-
The time relations of the pulse wave curve are quite suitable for an analysis and processing.

The existing methods for assessing the stiffness of the arterial walls by the pulse wave propagation velocity are usually based on measuring the pulse wave propagation time to, for example, the radial artery by the delay in the appearance of the wave on the wrist, relative to the R-peak of the respective electrocardiogram. It has been shown [6] that the moment of the pulse wave appearance in the aorta does not coincide with the R-peak of the ECG, but has a certain time lag that differs for certain age groups, and exhibits relatively little variation within these categories. However, even this small variability may quite strongly provide effects on the accuracy of measurements of the pulse wave velocity (PWV). The PWV measurement error mainly depends on the accuracy of the signal transit time measurement, since the length of the examined artery section can be measured quite accurately. By the aorta-to-the radial artery distance approximately of 0.8 m and the pulse wave velocity approximately of 8 m/s, we can estimate the signal transit time: it is 100 milliseconds. For example, with a given measurement accuracy of 5%, an absolute error in measuring the transit time should not exceed 5 ms. If the determination of the R-peak moment in the ECG with such an accuracy is not difficult, then for the pulse wave, the minimum in front of the wave, as is customary in some publications, does not seem to be the best place to fix the time of the wave passage.

Taking into account these considerations, employing the manual pulse wave sensor, developed has been a method for the PWV measurement using not the signal delay time from the ECG R-peak of the ECG, but their difference at specific locations over the artery.

3 Experiments on the measurement of PWV with AP/ECG sensor

In a full-scale experiment, the results of which are presented in this section, a pulse wave has been recorded at three points along the artery: over the subclavian artery, over the ulnar artery 0.35 m lower, and over the radial artery 0.27 m lower with the respect to the preceding point. Figure 3 shows a complete recording of the pulse wave signal and an ECG, and the sequence of measurements is as follows: from the beginning till 18 s, the sensor is positioned over the subclavian artery, from 50 till 75 it is placed over the radial artery, from 110 to the end it is located over the ulnar artery.

It should be recalled that only over the radial artery the pulse wave corresponds to the arterial pressure, while in other areas it is damped by soft tissues. Therefore, the amplitude and constant component of the pulse wave in these areas may differ significantly from the actual pressure in the artery.

Figure 4 displays a fragment of the pulse wave recording over the subclavian artery with a synchronous ECG signal, on which the wave fragments over the ulnar and radial arteries are overlapped, and the positions of the R-peaks of all three waves are made coincident in time (only one thereof is shown herein).

The graph in Figure 4 herein shows that the measurement of the signal wave transit time from the R-peak in the ECG according to passing by the derivatives the zero value without additional signal filtering is incorrect. However, since we are not bound by tabular reference values of “the pre-ejection time” [6], we can introduce a criterion for fixing the time of the wave passage, which is reliable and unified for all three signals. For this purpose, the leading and trailing edges of a signal front, which are well known in the pulse technology and which are fixed at the level of 0.1 and 0.9 of the signal amplitude, may serve as an eligible criterion. In our case, the threshold levels of the pulse wave edges are updated for each cycle, as shown in Figure 5 herein. Each positive edge of the signal has its own "personal" marks of the leading and the trailing edge, respectively. Of course, it is just the leading edge that should be taken as the timestamp of the wave propagation, since the shape of the pulse wave changes noticeably in the course of its travelling similar to the case with the edge time.

Figures 6-8 given herein exhibit three graphs, and, accordingly, Tables 1-3 show the quantitative values of the measured parameters of the AP wave edges for the subclavian, ulnar, and radial arteries, respectively. Designations used in the Tables are as follows: R, f01, f09 are the timestamps of the R-peak and the pulse.
Figure 4. Pulse wave for three positions of the sensor. Legend: 1. ECG signal; 2, 3, 4 – pulse wave, respectively, over the subclavian, ulnar and radial arteries; 5, 6, 7 – derivatives of curves 2, 3 and 4 (magnified by 20 times to make it more illustrative).

Figure 5. Timestamps at the level of 0.1 and 0.9 of the signal amplitude for each wave edge.

Figure 6. Timestamps of the AP wave and ECG for the subclavian artery.

Figure 7. Timestamps of the AP wave and ECG for the ulnar artery.

The wave edge at the level of 0.1 and 0.9 of the amplitude, from which follow the values of the edge duration \( f_{19} = f_{09} - f_{01} \), R-R are the time intervals between the successive R-peaks, f01-f01 are the intervals between successive edges of the AP waves, R-f01 is the transit time of the AP wave edge referred to the R-peak. All values are given in seconds.

It should be noted that the data are related to a specific measurement session and may vary depending on many factors that determine the state of the human body. The Tables herein exhibit variability of all time intervals. The average transit time of the wave edge referred to the R-peak in the ECG for the subclavian artery is 64 milliseconds (ms), for the ulnar artery it is 60 milliseconds (ms).

### Table 1.
Values of the measured parameters of the AP wave edges for the subclavian artery

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Mean squared error (MSE) 0.0002

### Table 2.
Values of the measured parameters of the AP wave edges for the ulnar artery

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Mean squared error (MSE) 0.0009

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radial artery can be used as a pulse wave sensor over the palpable arteries (without blood pressure verification). With an integrated ECG amplifier, it is possible to measure the transit time of the pulse wave edge at various points over the artery to determine the pulse wave velocity as one of the characteristics of the artery wall stiffness. It is important to note that in this case, the transit time is determined by the similar-shaped signals that significantly increases accuracy of measurements. Further development of the method may be an employment of interpolation methods for determining the pulse wave edge timestamps to improve accuracy.

The above research work has been completed within the framework of the Russian Governmental Task, supported by RFBR Grant 18-29-02108 mk.

Statement on ethical issues
Research involving people and/or animals is in full compliance with current national and international ethical standards.

Conflict of interest
None declared.

Author contributions
The authors read the ICMJE criteria for authorship and approved the final manuscript.

References

Research study on the efficiency of a defibrillating biphasic pulse of various durations based on mathematical modeling and simulation of a cardiomyocyte

Nikolay N. Chernov*, Aleksandr А. Bezverkhii

* Southern Federal University
Russia, 347900, Taganrog, Shevchenko str., 2

Corresponding author:
phone: +7 (928) 908-11-77
e-mail: nnchernov@sfedu.ru

Abstract
The paper presents the results of our research study on the impact of different durations of a biphasic rectangular pulse on the efficiency of the defibrillating action based on modeling and simulation of a cardiomyocyte in the framework of Cell Electrophysiology Simulation Environment. We used Luo-Rudy Mammalian Ventricular Model II (dynamic) as the mathematical model of the biochemical processes, which occur both on the surface and inside a cardiomyocyte in a guinea pig. An analysis of the obtained results allowed us to determine the optimal time of the defibrillating pulse. It has been shown that a pulse of 4 ms duration has the highest efficiency. The research has been carried out directly based on the mathematical model and simulation of the cardiomyocyte that makes it possible to minimize errors which may occur in experiments on animals.

Keywords
Transthoracic defibrillation, Biphasic pulse, Current stabilization, Cardiomyocyte repolarization, Ventricular fibrillation

Imprint

Introduction
According to numerous studies, 76% of the sudden cardiac arrests (SCA) are conditionally preventable cases, as they occur due to ventricular fibrillation (VF) [1-3]. Immediate electro-pulsed therapy (EPT) by external defibrillation is the main method of treating this type of arrhythmia [3-5]. The comparative study of modern forms of pulses carried out earlier made it possible to identify the concept of development of this field in science [6]. An analysis of the reference literature and the results of recent developments are conditions to define the key parameters of the pulse for an individual selection of the characteristics of the transthoracic defibrillation signal. The representation of the average current (I) and charge (Q) in the form of a certain function I (t) is the best way to dose the defibrillation pulse [7].

The calculation of the main parameters was carried out based on the work by Weiss and Lapicque [8], who proved the existence of the effect of cardiac tissue stimulation, and according to the calculations made by Blair, who described the existence of a rheobase [9, 10]. Studies by Irnich define in detail the effect of a pulse exposure time on cardiomyocytes as the key pulse parameter [11, 12]. Later, a large number of studies have proven in practice the high efficiency of a two-phase pulse against the single-phase one. There is still no theoretical justification for this evidence available, but there is a hypothesis that the second phase prevents the appearance of ectopic foci in the heart tissue that increases the effectiveness of defibrillation [13-17]. A detailed calculation of the ratio between the forward and the reverse pulse signals was conducted by Kroll, who demonstrated the possibility of minimizing the energy of the second phase [18], and many developers subsequently confirmed the large variation of the current amplitude tolerances [14]. The fact cannot be ignored that the phase switching time should not exceed 1 ms and be possibly minimized, otherwise the biphasic signal converts into two monophasic signals [19]. Bourlands in his paper in 1978 showed the comparative efficiency of pulses with a time constant, regardless of the chest impedance [20]. An unfairly neglected publication by Schuder in 1964 confirms the high efficiency of the rectangular pulse as compared with other pulse variants [13]. In 1993, Tucker has presented a graph derived from his analysis and proven that there is a delicate balance between an effectiveness of defibrillation and damage produced by each pulse [21]. In 1988 Wilson described the effect of charge building-up due to multiple defibrillations
Fishler summarized all the experiments in 2000 and determined the time interval for a rectangular pulse in the range from 3 to 6 ms and the ratio of the current amplitude of the first and second phases as 15A to 10A, respectively [23].

The aim of our research work is to study the efficiency of a biphasic rectangular pulse using mathematical modeling and simulation of a cardiomyocyte.

Materials and methods

An acceptable range of the time interval has been obtained by analyzing the reference literature and applying our own calculations. Experimental studies can be the next stage of optimizing the time interval of the pulse impact in question [7,23]. For the purpose of a humane approach to the study, we have completed signal modeling and simulation, and the obtained results have been objectified by with mathematical modeling in the Cell Electrophysiology Simulation Environment (CESE) [24]. Mathematical model Luo-Rudy Mammalian Ventricular Model II (dynamic) to simulate the biochemical processes occurring on the surface and inside the guinea pig cardiomyocyte has been applied. With the help of this model, it is possible to simulate an impact made by an electric pulse of a certain duration, shape and intensity by current strength on the cell membrane and obtain curves of the ion channels operation, changes in the concentration of ions on the surface and inside the cardiomyocyte.

Using the Java programming language, a rectangular signal of sufficient intensity to overcome the threshold of cardiomyocyte excitability was coded and implemented in the CESE. Taking into account the reference literature data and our own calculations [6,7], several rectangular signals with a duration of 3 to 6 ms with a step of 1 ms were selected for an analysis to identify the optimal pulse duration. The earlier experiments showed a difference in the duration of threshold pulses for the Luo-Rudy Mammalian Ventricular Model II (dynamic) cardiomyocyte and the results for the human cardiomyocyte model. The 11 ms pulse for the Luo-Rudy model was identical in its effects to the 4 ms signal in the human cardiomyocyte model [26, 27], i.e., the ratio of the pulse duration in the model used to that in the human cardiomyocyte model is 1: 2.75. In this regard, it was decided to introduce a correction factor for the pulse duration: $K_t = 2.75$.

Let us find the voltage on the surface of the membrane (membrane voltage (mV)) as the main parameter under consideration. The membrane resting potential (MRP) is constantly available on the surface of the membrane, and it is formed mainly due to the diffusion of the Na+ and K+ ions. The MRP value of a cardiomyocyte is approximately 86 mV [28]. When an electric pulse is applied to the cell membrane, depolarization occurs, and an action potential (AP) is generated on its surface. AP of the cardiomyocyte is fired at a voltage of 56 mV. In this case, the protein channels of the cell membrane are activated, and the Na+ ions enter the cell. The Na+ ions come into the cell according to the “all or nothing” principle, so the fact of AP generation can be clearly traced by the change in the velocity of the Na+ ions travelling through the membrane [29]. The given mathematical model allows us to construct a graph to depict the Na+ ion flow (Total Na ion flow (mM/ms)), and we can use it as a criterion for the efficiency of the signals under consideration.

In the repolarization phase, active cellular channels are involved, the operation of which is aimed at restoring the MRP value. The calcium ions play an important role at the repolarization stage. Their function is to maintain MRP at a certain level (86mV), and, with a decrease in their concentration, the excitability of the cell decreases. The maintenance of the Ca2+ concentration is regulated by the Na+/Ca2+ pump, and, as a rule, its operation is aimed at pumping Ca2+ out of the cell using the Na+/Ca2+ pump [30]. The peculiarity of the Na+/Ca2+ pump is the exchange of one Ca2+ ion for three Na+ ions. It follows from this that the overload in the operation of this mechanism, associated with an excessive entry of the Na+ ions, may lead to a reduction in the excitability of the cardiomyocyte [31]. To evaluate the efficiency of the defibrillation pulses in the model in our study, a plot was used that reflects the activity of the Na+/Ca2+ pump - Na-Ca exchanger current (uA/uF). A comprehensive analysis of the above parameters allows evaluating the efficiency of the pulses and assess their safety.

Results and Discussion

The pulse impact process was simulated in the framework of the CESE using the Luo-Rudy Mammalian Ventricular Model II (dynamic), with the proper rectangular signal of sufficient intensity to form an AP. The change in the duration of the rectangular pulse in CESE is performed by manual correcting of the required parameter: this function is called clamp of the parameter, namely, it is the stimulus duration. As de-
Figure 1. Mathematical modeling of changes in the parameters of the voltage on the cardiomyocyte membrane surface, the Na\(^+\) transmembrane current and the Na\(^+\) - Ca\(^{2+}\) pump activity, when exposed to an equivalent pulse with a duration of 3 ms.

scribed above, from earlier studies, in order to obtain results applicable to the human cardiomyocyte, it is necessary to introduce a correction factor $K_t$. After the correction, the pulse duration was the following: 8.25 ms (4ms), 11 ms (4ms), 13.75 (5ms) and 16.5 (6ms).

In the simulation environment, some summary plots were constructed showing changes in the membrane surface voltage (mV), the Na\(^+\) ion flow (Total Na ion flow(mM/ms)), and the Na\(^+\)/Ca\(^{2+}\) pump activity (Na-Ca exchanger current (uA/µF)) for each pulse separately.

When considering the plot produced by simulating a signal with a duration equivalent for a pulse of 3 ms (8.25 ms), as shown in Figure 1 herein, the following data are obtained: action potential (AP = -56 mV) develops at 0.35 ms, and the opening of the Na\(^+\) channels takes place at 0.4 ms. In this case, the reverse flow of the Na\(^+\) ions begins immediately after closing and reaches its peak at 4.2 ms, followed by a smooth decline. The operation of the Na\(^+\)/Ca\(^{2+}\) pump on the curve follows the dynamics of restoring the Na\(^+\) balance. In general, the effect made by a rectangular pulse with its duration of 3 ms can be characterized as sufficient to provide general repolarization. The pulse creates conditions for a standard contraction considering duration and activity of the ion channels [32].

In Figure 2 herein, obtained by simulating a pulse with a duration of 4 ms (11 ms under the simulation conditions in the context of the presented model), the formation of AP also occurs at 0.35 ms, and the opening of the Na\(^+\) channels is found at 0.4 ms. The respective curve shows the key difference between the 3 and 4 ms pulses: a plateau appears on the cell membrane from 0.9 ms till 10.1 ms with an average amplitude of 64 mV. Due to this plateau, the reverse current of the Na\(^+\) ions and their active transport from the cardiomyocyte cell are locked. The operation of the Na\(^+\)/Ca\(^{2+}\) pump shows a smooth start and shutdown, and it is a true illustration of the Na\(^+\) ions current dynamics. The formation of the locking plateau on the cardiomyocyte membrane surface reflects the very essence of defibrillation, which consists in creating general repolarization for every cell in the heart tissue.

When analyzing the pulse of the 5 ms duration (13.25 ms for the model in question), as illustrated by Figure 3, the formation of AP and the opening of the Na\(^+\) channels are similar to the previous results, which have been recorded to be 0.35 and 0.4 ms, respectively. Attention should be drawn to the absence of a plateau that is the same case with the 4 ms pulse and to the formation of the first peak on the membrane surface at 3.7 ms with amplitude of 238 mV. At the same time, the first peak in the velocity of the reverse flow of the Na\(^+\) ions is also noted. A detailed analysis of the data from the simulation revealed a pattern: the active current of the Na\(^+\) ions from the cell begins at the membrane surface voltage of more than + 80 mV. Further decrease and re-rise of the membrane voltage is accompanied by the synchronous activity of the Na\(^+\) ion current and the operation of the Na\(^+\)/Ca\(^{2+}\) pump. The total operating time of the Na\(^+\)/Ca\(^{2+}\) pump is 19 ms (from 1.35 ms to 30.35 ms). And if we address the fact that the duration of the Na\(^+\) channels opening is 0.6 ms for any pulse duration, and the maximum velocity does not have a significant difference (395 – 398 mmol/ms), we can conclude about the excess transport of Na\(^+\) from the cardiomyocyte cytoplasm. It should be mentioned that this involves the transport of Ca\(^{2+}\) ions into the
Figure 3. Mathematical modeling of changes in the parameters of the voltage on the cardiomyocyte membrane surface, the Na\(^+\) transmembrane current and the Na\(^+\)-Ca\(^{2+}\) pump activity, when exposed to an equivalent pulse with a duration of 5 ms.

Figure 4. Mathematical modeling of changes in the parameters of the voltage on the cardiomyocyte membrane surface, the Na\(^+\) transmembrane current and the Na\(^+\)-Ca\(^{2+}\) pump activity, when exposed to an equivalent pulse with a duration of 6 ms.

cell and, as a result, a decrease in the excitability of the cardiomyocyte [31]. From the results of modeling and simulation of the rectangular pulse with an equivalent duration of 5 ms, it can be concluded that prolonged exposure of a cardiomyocyte to a high-density current leads to excessive transport of the Ca\(^{2+}\) and Na\(^+\) ions inside the cell and on its surface, respectively.

The further increase in the pulse duration to 6 ms (16.5 ms in the model under study) confirms the conclusions drawn from the previous simulation. High voltage on the membrane surface induces an active ion substitution transport, which leads to a decrease in the concentration of the Ca\(^+\) ions on the cardiomyocyte membrane surface and, as a result, a decrease in the excitability of the cell, including for subsequent defibrillating pulses. Figure 4, which shows the curve of a pulse of the 6 ms equivalent duration, characterizes it as redundant for all the studied parameters.

As a result of modeling, we obtained the respective curves characterizing the response of a cardiomyocyte to rectangular pulses of different durations. Using this study, it is possible to evaluate the main processes of the cell excitability: the formation of AP under a short-term exposure to a defibrillating pulse, the repolarization of the membrane and the restoration of MRP.

Conclusions

The aim of our research study was to study the efficiency of a biphasic rectangular signal of various durations using the cardiomyocyte simulation in the framework of mathematical modeling system CESE. Using our analysis of the obtained results, we determined the optimal time of the defibrillating pulse extracted from the range produced by analyzing the data of some previous studies and calculations [7]. It has been shown that a pulse of 4 ms duration has the highest efficiency. Our research study has been carried out directly with the use of the mathematical model and simulation of a cardiomyocyte that makes possible to minimize errors that may occur during the experiment on animals. Differences in the thorax geometry in animals may result in a great number of difficulties in applying the results to human individuals, and the mathematical modeling for studying the time interval can be treated as a more preferable option. The next step in designing a biphasic pulse is to calculate the optimal current amplitude for the most efficient defibrillation, taking into account the varying chest impedance in a patient.

Statement on ethical issues

Research involving people and/or animals is in full compliance with current national and international ethical standards.

Conflict of interest

None declared.

Author contributions

The authors read the ICMJE criteria for authorship and approved the final manuscript.

References

The effect of diabetes mellitus under tumor growth on respiratory function and free radical processes in heart cell mitochondria in rats

Elena M. Frantsiyants¹, Irina V. Nes'kubina*, Alla I. Shikhlyarova¹, Natalia D. Cheryarina¹, Irina V. Kaplieva¹, Valeria A. Bandovkina¹, Lidia K. Trepitaki¹, Lyudmila A. Nemashkalova¹, Irina B. Lysenko¹, Polina S. Kachesova¹, Elena A. Sheiko¹, Maria I. Morozova¹, Inga M. Kotieva¹

¹ National Medical Research Centre of Oncology
Russia, 344037, Rostov-on-Don, 14 liniya, 63, building 8
* Corresponding author:
phone: +7 (928) 156-56-56
e-mail: neskubina.irina@mail.ru

Abstract
The aim is to study the effect of comorbid pathology, namely, diabetes mellitus, on free radical oxidation in the mitochondria in the heart cells in female rats with experimental Guerin carcinoma.

Materials and methods
The study has been performed in female outbred rats (n=32) weighed 180-220 g. The animals have been randomly assigned to the following experimental groups: intact group (n=8), control group with diabetes (n=8), comparison group (n=8) to include rats with standard subcutaneous transplantation of Guerin’s carcinoma, and the main group (n=8) to cover rats which were first reproduced with diabetes and which after 1 week of persistent hyperglycemia were transplanted with Guerin’s carcinoma. In the samples of the heart mitochondria, the following concentrations were measured by the ELISA method: cytochrome C (ng/mg protein) (Bioscience, Austria), 8-hydroxy-2’-deoxyguanosine (8-OHdG) (ng/mg protein); malondialdehyde (MDA) (mmol/mg protein); the Statistica 10.0 software was used.

Results
In diabetes mellitus (control group), an increase in 8-OHdG by 6.3 times, MDA by 1.9 times (p<0.05), and a decrease in cytochrome C by 1.5 times (p<0.05) were found in the mitochondria in the heart cells compared to the values in the intact group. The effect of Guerin’s carcinoma on the mitochondria of heart cells was expressed only as a change in the MDA level, which exceeded the intact level by 1.8 times (p<0.05). The combination of two pathological processes in the animal’s body, namely, diabetes mellitus and the malignant tumor, caused an increase in the level of 8-OHdG by 14 times, MDA by 1.7 times (p<0.05) and a decrease in cytochrome C by 1.46 times (p<0.05).

Conclusions
It has been found that the destabilization of the respiratory chain in mitochondria in female rats’ heart cells occurs as a result of the activation of free radical oxidation processes under the influence of diabetes mellitus, both in its independent variant and linked with the tumor process.

Keywords
Mitochondria, Heart, Free radical oxidation, Antioxidant defense, Diabetes, Guerin’s carcinoma, Female rats

Imprint

Introduction
Diabetes mellitus (DM) is one of the world’s biggest health problems in the 21st century. DM is also considered a major risk factor for cardiovascular disease, which is the most common cause of death among adults with DM [1]. In addition to the well-known microvascular complications triggered by DM, such as nephropathy and retinopathy, there is an increasing epidemic of macrovascular complications, including diseases of the coronary arteries, peripheral arteries and carotid vessels [2]. The diagnostics of such macrovascular complications becomes more precise and accessible owing to the development of a new fundamental science: CARDIOMETRY, created on the basis of discovery of the hemodynamics mechanisms involved therein [3]. A significant breakthrough has been made in the study of the pathogenetic mechanisms of combined pathologies, which allow us to evaluate multisystem complications and their correction in tumor growth models [4,5,6,7].
Pathophysiological changes in DM, including a disorder in the energy metabolism regulation (glucose, amino acids, and fatty acids), insulin resistance, oxidative stress, and inflammation lead to various complications, which contribute to damage to target organs, such as the heart, the kidneys, the liver, the eyes and others [8]. In particular, DM is most involved in the pathogenesis of various cardiovascular diseases, including chronic conditions such as atherosclerosis [9] and congestive heart failure [10] as well as acute processes: myocardial infarction and unstable angina [11]. Moreover, cardiovascular diseases, which include peripheral vascular diseases, coronary heart disease, and cerebrovascular diseases, make a significant contribution to the mortality of people suffering from diabetes. At the same time, a unique event is diabetic cardiomyopathy, which itself causes functional and structural aberrations of the heart, when there are no other concomitant diseases, such as IHD, dyslipidemia and hypertension [12]. Cardiovascular diseases in diabetes and diabetes itself contribute to the deterioration of the state of the body as a whole, so that they can be considered as factors responsible for high mortality risks. The pathogenesis of the diabetic complications involves many mechanisms; however, oxidative stress mediated by reactive oxygen species (ROS) is an important key component [13]. Under the normal conditions, ROS are necessary for maintaining homeostatic cellular signal communication, as well as for triggering antioxidant reactions in response to stress [13,14]. When the ROS levels become excessive, as in DM, there are dangerous consequences for the organism [13]. In DM, elevated and stable levels of ROS make a direct effect on the integrity of mitochondria and the ability of cellular signal transmission [13]. In the heart of a diabetic patient, the production of toxic products of lipid peroxidation can disorder the function of the myocardium and contribute to the progression both of acute and chronic heart diseases. DM is known to be associated with a malignant process, and patients with malignant tumors often suffer from diabetes [15]. Possible biological links between DM and malignancy include hyperinsulinemia, hyperglycemia, and chronic inflammation induced by obesity. Although the strongest association between these pathologies is found in the pancreas and the liver, there are many other organs involved in carcinogenesis in patients with DM, including the mammary gland, the endometrium, the bladder, and the kidneys.

In connection with the above, it is relevant to study the mitochondrial dysfunction of cardiomyocytes both in diabetes mellitus and in the malignant process combined with diabetes mellitus.

**Aims**

The aim is to study the effect of comorbid pathology, namely, diabetes mellitus, on free radical oxidation in the mitochondria in the heart cells in female rats with experimental Guerin carcinoma.

**Materials and methods**

The research has been carried out using female outbred rats (n=32) weighed 180-220 g. The animals have been delivered to us by the Federal State Medical & Biological Institution “Research Center of Biomedical Technologies” (Branch Andreevka, Moscow Region). The animals were kept under natural lighting conditions with no restrictions on their access to water and food. The research in animals was conducted in accordance with the Directive 86/609/EEC on the Protection of Animals Used for Experimental and Other Scientific Purpose, as well as in accordance with the International Guiding Principles for Biomedical Research Involving Animals and Order No. 267 “Approval of the Rules of Laboratory Practice” dated June, 19, 2003 issued by the Ministry of Health of the Russian Federation. The research record was approved by the Commission on Bioethics at the Federal State Budgetary Institution “National Medical Research Center of Oncology”, the Ministry of Health of the Russian Federation (Record No. 21/99 dated September, 1, 2020). Manipulations with animals were performed in the box in compliance with the generally accepted rules of asepsis and antisepsis.

We used the strain of Guerin’s carcinoma supplied by the Russian National Medical Research Center of Oncology named after N.N.Blokhin, Ministry of Health, Russia. The material for transplantation was obtained from donor rats on day 12-16 of the tumor growth. The Guerin’s carcinoma transplantation was performed by a standard subcutaneous injection of the tumor suspension under the right scapula in a volume of 0.5 ml of the cell suspension in a 1:10 dilution with saline solution. To reproduce experimental diabetes, the animals were once intraperitoneally injected with Alloxan at a dosage of 150 mg/kg of weight. Then, during a week, their blood glucose was measured. A high blood glucose level, in the range of 15-30 nmol
which the separation into 3 phases was observed; the coll gradient, centrifuged for 15 min at 21000 g, after
sion of subcellular structures was layered on the Per with the use of a 23% Percoll gradient. The suspen
fused from lysosomes, peroxisomes, melanosomes, etc.,
mitochondrial sediment was resuspended in the iso
of 0 - 2°C. Between the centrifugation procedures, the
ried out at 20000 g, for 20 minutes, at a temperature
10 minutes at a speed of 1000 g, at a temperature of
TRIS-HCL, 10 mm HEPES, pH 7.4). The tissues were
ed per gram of tissue, 10 ml of the isolation medium
nent rules of asepsis and antisepsis.
The animals have been randomly assigned to the
following experimental groups: the intact group (n = 8), the control group with diabetes (n=8), the compar-
ison group (n=8) to include rats with standard sub-
cutaneous transplantation of the Guerin’s carcinoma, the main group (n=8) to cover rats which were first re-
produced with diabetes and which after 1 week of per-
sistent hyperglycemia were transplanted with Guerin’s carcinoma in a volume of 0.5 ml of tumor cell suspen-
sion in a 1:5 dilution with saline solution. At the time
of transplantation of the Guerin’s carcinoma in the
imals of the main group (n=8), the average blood
glucose values were 25.4±1.2 mmol/l, whereas in the
group of the intact animals (n=8) the values were re-
corded to be 5.2±0.3 mmol/l.

Decapitation of the animals was performed with
the guillotine 14 days after transplantation of the
Guerin’s carcinoma or the reproduction of experi-
mental diabetes. After decapitation the animal hearts
have been quickly extracted with the use of refriger-
ants, and mitochondria were isolated by the method of
Egorova M. V., Afanasiev S. A. [16] (using refrigerants
and differential centrifugation on a high-speed refrig-
erated centrifuge Avanti J-E, BECMAN COULTER,
USA). The tissues were washed with an icy 0.9% KCl
solution. To destroy the intercellular bonds, the cell
wall and plasma membranes, we used mechanical
treatment of tissues with grinding with scissors and
homogenization in a glass homogenizer with a Teflon
pestle (the Potter-Elvehjem homogenizer). We add-
ed per gram of tissue, 10 ml of the isolation medium
(0.22 M mannitol, 0.3 M sucrose, 1 mm EDTA, 2 mM
TRIS-HCL, 10 mm HEPES, pH 7.4). The tissues were
homogenized and centrifuged for the first time for
10 minutes at a speed of 1000 g, at a temperature of
0 - 2°C, the second and third centrifugation was car-
rried out at 20000 g, for 20 minutes, at a temperature of
0 - 2°C. Between the centrifugation procedures, the
mitochondrial sediment was resuspended in the iso-
lation medium. Further the mitochondria were puri-
ified from lysosomes, peroxisomes, melanosomes, etc.,
with the use of a 23% Percoll gradient. The suspen-
sion of subcellular structures was layered on the Per-
coll gradient, centrifuged for 15 min at 21000 g, after
which the separation into 3 phases was observed; the
lower layer of mitochondria was left and resuspended
with the isolation medium. The next washing of the
mitochondria was performed by centrifugation for 10
minutes at 15000 g, at a temperature of 0 - 2°C. The
mitochondrial samples (protein concentration 4-6
g/l) were stored at -80°C in the isolation medium be-
fore their analysis. By the ELISA method determined
were the following concentrations: cytochrome C (ng/
mg protein) (Bioscience, Austria), 8-hydroxy-2’ - de-
oxoguanosine (8-OHdG) (ng/mg protein) (Enzo Life
Sciences, Switzerland), malonic dialdehyde (MDA)
(nM/g protein) (BlueGene Biotech, China); protein
(mg/ml) was estimated by the Biuret method (Olvex
Diagnosticum, Russia) with the ChemWell automatic
alyzer (Awareness Technology INC, USA).

The Statistica 10.0 software was applied for sta-
tistical analysis of the obtained data. The data were
analyzed for the compliance of the features distribu-
tion with the normal distribution law using the Sha-
piro-Wilk test (for small samples). The comparison
of the quantitative data in the groups (independent
sampling) was carried out using the Kruskal-Wallis
test (multiple comparisons). The table data are pre-
sented in the M±m form, where M is the arithmetic
mean, and m is the standard error of the mean; p<0.05
was taken as the level of statistical significance. The
obtained results were statistically processed in compli-
ance with the applicable general recommendations for
medical research.

Results

In the course of the experiment, first of all, it was
necessary to determine the effect made by diabetes
mellitus (control group) on the mitochondria in the
heart cells. Thus, as compared to the intact animal val-
ues, an increase in 8-OHdG by 6.3 times, MDA by 1.9
times (p<0.05) and a decrease in cytochrome C by 1.5
times (p<0.05) were detected (see Table 1 herein).

At the next stage of the experiment, the effect of
Guerin’s carcinoma on the mitochondria of the heart
cells was studied, and changes were recorded only in
the MDA level, which was 1.8 times (p<0.05) higher
than the recorded intact level.

The combination of two pathological processes in
the animal body, namely, diabetes mellitus and the
malignant tumor, caused the following changes in the
studied parameters: the level of 8-OHdG increased
by 14 times, MDA by 1.7 times (p<0.05), and cyto-
ochrome C decreased by 1.46 times (p<0.05), respec-
tively. Statistically significant differences between the groups of animals were determined only by 8-OHdG: the indicator, when compared between the control group and the main group, was 2.2 times higher in the main group, and, when compared with the values in the comparison group, was recorded to be 17.4 times higher in the main group. The MDA level in the main group exceeded the values in the comparison group by 1.53 times (p<0.05).

Thus, the most pronounced changes in the level of the studied parameters (8-OHdG, MDA, cytochrome C) in the heart cells mitochondria of female rats were revealed in the group of animals, where the malignant process developed against the background of diabetes mellitus as comorbid pathology.

**Discussion**

At present, due to its mutagenicity, 8-OHdG is the most studied product from nucleic acid oxidation. An accumulation of 8-OHdG is considered to be a sensitive marker of oxidative stress to DNA [17]. In addition, 8-OHdG is also treated as a risk factor for diabetes, correlating with the severity of vascular complications, suggesting the presence of oxidative stress involved in the pathogenesis of vascular complications caused by diabetes [18]. In animal experiments, hyperglycemia has been shown to increase the production of 8-OHdG and suppress antioxidant enzymes like MnSOD [18].

In the offered study, the data obtained on the content of 8-OHdG in the mitochondria of the heart cells in diabetes correlate with the reference literature data [18], and indeed, the accumulation of 8-OHdG has been revealed in diabetes.

The accumulation of 8-OHdG in nuclear DNA and mtDNA is a consequence of the accumulation process and the overproduction of ROS caused by a chronic disease such as diabetes [19]. Regarding the presented study, an increased content of the secondary product of lipid peroxidation (LPO), MDA, has been found in all experimental groups that is consistent with the concept of the above research work. MDA is considered as the most mutagenic product of lipid peroxidation [20]. MDA can directly affect DNA [21], and there is probably a relationship between 8-OHdG and MDA, and it is also suggested that the formation of 8-OHdG may be associated with lipid peroxidation [22].

We believe that the detected changes in MDA and 8-OHdG in this experiment indicate the ability of MDA to damage DNA through an increase in the 8-OHdG concentration as a biomarker of oxidative stress.

During the experiment, the investigations were conducted focused not only on the influence of diabetes and the malignant process on the mitochondria in the heart cells, but also on the tumor process linked with diabetes mellitus. As a result, changes in the same direction in the content of MDA and 8-OHdG in the mitochondria in the heart cells were recorded in diabetes and combination of comorbid pathology with the tumor growth. At the same time, the effect of the malignant process in its independent version led only to an increase in the level of MDA. Perhaps it is diabetes mellitus, i.e. hyperglycemia, that plays a leading role in triggering the cascade of reactions that lead to such an excessive production of reactive oxygen species and, as a result, damage to mtDNA.

It is known that hyperglycemia can induce some morphological changes in mitochondria [23], the hyperproduction of reactive oxygen species (ROS) [24] and the release of cytochrome C [25]. Cytochrome C is an important mitochondrial enzyme involved in the respiratory chain. Oxidative stress mediated by ROS increases the release of cytochrome C for cell apopto-

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**Table 1**

Changes in the parameters of free radical oxidation and respiration of mitochondria in heart cells in female rats with diabetes mellitus and Guerin's carcinoma

<table>
<thead>
<tr>
<th></th>
<th>8-OHdG (ng/mg protein)</th>
<th>MDA (nM/g protein)</th>
<th>Cytochrome C (ng/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intact group (n=8)</td>
<td>0.927±0.048</td>
<td>14.912±1.110</td>
<td>1.382±0.137</td>
</tr>
<tr>
<td></td>
<td></td>
<td>p1=0.0000</td>
<td></td>
</tr>
<tr>
<td>Control group - diabetes (n=8)</td>
<td>5.890±0.5291</td>
<td>27.866±0.8131</td>
<td>0.900±0.0481</td>
</tr>
<tr>
<td></td>
<td>p1=0.0000</td>
<td>p1=0.0000</td>
<td>p1=0.0053</td>
</tr>
<tr>
<td>Comparison group - Guerin's carcinoma (n=8)</td>
<td>0.748±0.058</td>
<td>16.992±0.599</td>
<td>1.215±0.101</td>
</tr>
<tr>
<td></td>
<td>p1=0.0000</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Main group – diabetes+Guerin’s carcinoma (n=8)</td>
<td>13.050±0.9421,2,3</td>
<td>26.092±0.6421,3</td>
<td>0.949±0.0411</td>
</tr>
<tr>
<td></td>
<td>p1=0.0000</td>
<td>p1=0.0000</td>
<td>p1=0.0093</td>
</tr>
<tr>
<td></td>
<td>p2=0.0000</td>
<td>p3=0.0000</td>
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</table>

Notes: p¹ statistically significant differences compared to the values in the intact group; p² statistically significant differences compared to the values in the control group; p³ statistically significant differences relative to the values in the comparison group.
sis [26] Under the action by ROS, the permeability of the mitochondrial pores increases, and cytochrome C is released into the cytoplasm from the inner space of the mitochondrial membrane, and Bax is translocated from the cytosol to the mitochondria that leads to cell apoptosis. Thus, ROS can indirectly induce apoptosis. In addition to the above, it is known that 8-OHdG affects mtDNA causing damage, then, damaged mtDNA is able to disrupt the transcription of most proteins of the respiratory chain, resulting in a vicious cycle of the ROS action [28].

In our study, we revealed a decrease in cytochrome C with an increase in MDA and 8-OHdG in the mitochondria of the heart in groups of animals with diabetes and a combination of diabetes with the tumor growth. We believe that diabetes plays a decisive role in destabilizing the mitochondrial respiratory chain and triggering apoptosis due to an indirect effect made by ROS on the proteins in the mitochondrial respiratory chain.

Conclusions

As a result of our experiment to study the effect made by diabetes mellitus in its independent variant and linked with the tumor process on the function of mitochondria in the heart cells in female rats, it has been found that the destabilization in the respiratory chain is mediated by free radical oxidation processes. Based on the results obtained, we can conclude that cardiovascular complications, both in diabetes mellitus and in the malignant process against the background of diabetes, are associated with the dysfunction of the heart cells mitochondria through the activation of the lipid peroxidation processes that causes local damage to mtDNA.

Statement on ethical issues

Research involving people and/or animals is in full compliance with current national and international ethical standards.

Conflict of interest

None declared.

Author contributions

The authors read the ICMJE criteria for authorship and approved the final manuscript.

References


Development of the single ventricle heart mathematical model based on the equation of forced oscillations

Aleksandr P. Chernyaev*, Elena R. Pavlyukova†, Alla Yu. Meyerson‡

1 Moscow Institute of Physics and Technology
Russia, 141701, Moscow region, Dolgoprudny, Institutsky per., 9
2 Kotel’nikov Institute of Radio Engineering and Electronics of RAS
Russia, 125009, Moscow, Mokhovaya str. 11, build. 7
3 Plekhanov Russian University of Economics
Russia, 117997, Moscow, Strelnyaniy per, 36

* Corresponding author:
phone: +7 (903) 558-85-69
email: chernyaev40@yandex.ru

Abstract
Not only protozoa being the simplest organisms have a single ventricle heart. Artificial heart systems used in cardiac surgery can be also of the single-ventricle design. Besides, there is a “single-ventricle heart condition”, i.e. a complex of defects in the structure of the heart that may occur in some human individuals and that may be found with different occurrence rate and severity. This defect should be attributed to an uneven development and malformation of some individual heart segments in the prenatal period. Single Ventricle belongs to the category of congenital heart defects. The latter greatly increases the scientific interest in studying the features of mathematical models of the single-ventricle heart condition. Herein, we offer our study on some issues of the smoothness of sewing of the exact solutions of a mathematical model of a single-ventricle heart based on the equation of forced oscillations.

Keywords
Active heart ventricle, Passive heart ventricle, Equations for free and forced oscillations, Artificial single-ventricle heart, Congenital defect

Imprint
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1. Simplified mathematical model of a single-ventricle heart

In [7], in addition to the problem of the single-ventricle heart performance, an equivalent electrical diagram of the circulatory system with one active ventricle is given. The hemodynamic model in [7] consists of one active and one passive chamber, respectively. This model describes the hemodynamic system found in proto-
zoa. Artificial hearts used in cardiac surgery can also be of the single-ventricle design type. Congenital heart defects (Single Ventricle) as a first approximation can also be described in a similar way. The hemodynamic model in [7] includes the following four equations:

\[
I_1 V_1^* (t) + R_1 V_1^* (t) + C_1^{-1} = P_1 (t) - F (t) \tag{1.1}
\]

\[
I_2 V_2^* (t) + R_2 V_2^* (t) + C_2^{-1} V_2 (t) = P_2 (t) \tag{1.2}
\]

\[
R_{12} V_1^* (t) = P_1 (t) - P_2 (t) \tag{1.3}
\]

\[
R_{21} V_2^* (t) = P_1 (t) - P_2 (t) \tag{1.4}
\]

In (1.1) – (1.4) \( V_1 (t), V_2 (t) \) are the volumes of the first active and the second passive chambers to be determined; \( P_1 (t), P_2 (t) \) are the pressures in these chambers; \( F (t) \) is an additional external pressure produced by the active wall of the first chamber; \( I_1, R_1 \) are expansion coefficients of inertia in the chambers; \( C_1, C_2 \) are expansion coefficients; \( R_s, R_c \) are chamber resistance coefficients, and \( R_{12} \) is the inter-chamber flow resistance coefficient. From (1.3) and (1.4) immediately follows

\[
V_1^* (t) = - V_2^* (t) \Rightarrow V_1 (t) + V_2 (t) = V_0 = \text{const}.
\]

2. Reduction of the single-ventricle heart model to a single equation of forced oscillations

It is shown in [8–10] that the problem (1.1) – (1.4) is reduced to finding a solution to a single equation of forced oscillations

\[
(I_1 + I_2) V^* (t) + \left( R_s + R_c + R_{12} \right) V_1^* (t) + \left( C_1^{-1} + C_2^{-1} \right) V_1 (t) = 0
\]

Equation (2.1) has three different solution modes depending on whether the roots of the characteristic equation

\[
(I_1 + I_2) \lambda^2 + \left( R_s + R_c + R_{12} \right) \lambda + \left( C_1^{-1} + C_2^{-1} \right) = 0 \tag{2.2}
\]

are different real numbers, coinciding the real ones, or complex conjugates.

We describe all solutions of equation (2.1). Let us introduce designations as follows:

\[
I = I_1 + I_2, R = R_s + R_c + R_{12}, \text{ and } I/C = 1/C_1 + 1/C_2.
\]

\[
\text{If}(t) = V_0/C_2 - F(t), \text{ and } V(t) = V_1 (t) \tag{2.3}
\]

Equations (2.1) and (2.2), taking into account (2.3), will have the following form:

\[
IV^* (t) + RV_1^* (t) + C_1^{-1} V_1 (t) = \text{If}(t), \tag{2.4}
\]

\[
I \lambda^2 + R \lambda + C_1^{-1} = 0. \tag{2.5}
\]

3. Three types of exact solutions to the forced oscillation equation with integral representation of partial solutions

We describe three types of exact solutions to equation (2.4) in other form than it is given in [11]. Let us introduce designations as follows:

\[
a = \frac{R}{I^2}, b = \frac{1}{CI}, y = V \tag{3.1}
\]

Due to (3.1), equation (2.4) is transformed to the following form:

\[
y'' + ay' (t) + by(t) = f(t), \tag{3.2}
\]

Referring to 2.36 (a) [11, p. 376] in case of \( \lambda_1 = a^2 - 4b > 0, \lambda > 0 \), the general solution to equation (3.2) has the following form:

\[
y(t) = \frac{2}{\lambda} \int f(s)e^{\frac{a}{2}t} ds + Ae^{\frac{a}{2}t} + Be^{\frac{a}{2}t}, \tag{3.3}
\]

where:

\[
A = \text{const}, B = \text{const}
\]

Since, for the roots of characteristic equation (2.5), the equalities as given below hold true:

\[
\lambda_1 = -\frac{a}{2} + \frac{\sqrt{a^2 + 4b}}{2}, \lambda_2 = -\frac{a}{2} - \frac{\sqrt{a^2 + 4b}}{2}, \lambda_2 = -\lambda_1,
\]

and formula (3.3) is transformed to the following form:

\[
y(t) = \frac{1}{\lambda_2 - \lambda_1} \int f(s) \left[ e^{\frac{a}{2}t} - e^{-\frac{a}{2}t} \right] ds + Ae^{\frac{a}{2}t} + Be^{\frac{a}{2}t}, \tag{3.4}
\]

where:

\[
A = \text{const}, B = \text{const}
\]

In case of \( \mu^2 = 4b - a^2 > 0, \mu > 0 \), the general solution to equation (3.2) according to 2.36 (b) [11, p. 376] will have the form:

\[
y(t) = \frac{2}{\mu} \int f(s) e^{\frac{a}{2}t} \sin \frac{\mu}{2} (t - s) ds + Ae^{\frac{a}{2}t} \cos \frac{\mu t}{2} + Be^{\frac{a}{2}t} \sin \frac{\mu t}{2}, \tag{3.5}
\]

where:

\[
A = \text{const}, B = \text{const}
\]

In this case, for the roots of characteristic equation (2.5), the equalities hold true as given below:

\[
\lambda_1 = -\frac{a}{2} + \frac{i \mu}{2}, \lambda_2 = -\frac{a}{2} - \frac{i \mu}{2}, \mu = \lambda_2 - \lambda_1,
\]

and formula (3.5) is transformed to form (3.4).

In case of \( 4b = a^2 \), according to 2.36 (b) [11, p. 376] the general solution to equation (3.2) will be the following:

\[
y(t) = \int f(s) (t - s) e^{\frac{a}{2}t} ds + Ae^{\frac{a}{2}t} t + Be^{\frac{a}{2}t}, \tag{3.6}
\]

where:

\[
A = \text{const}, B = \text{const}
\]

Since the formula \( \lambda_0 = \lambda_1 = -a/2 \) is valid for the two-fold root of the characteristic equation, formula (3.6) can be written as

\[
y(t) = \int f(s) (t - s) e^{\frac{a}{2}t} ds + A e^{\frac{a}{2}t} t + B e^{\frac{a}{2}t}, \tag{3.7}
\]

where:

\[
A = \text{const}, B = \text{const}
\]
Note that in formulas (3.3), (3.4), (3.5), (3.6) and (3.7) the first summand is a particular solution to equation (2.1), and it is represented by an integral, which depends on parameter $t$. The second and third summands represent the general solution to the equation of free oscillations [11, p. 375]

$$IV''(t) + RV'(t) + C^{-1}V(t) = 0, \tag{3.8}$$

which differs from the equation of forced oscillations (2.4) in the zero right part only.

Periodic solutions to case $\mu^2 = 4b-a^2 > 0$, $\mu > 0$ are well studied [12-14]. In the examples given in [8-10], the particular solutions of equation (2.4) are expressed by trigonometric functions, since the integrals in the integral representations of the particular solutions are taken explicitly.

4. Method for obtaining estimates of the order of smoothness at the point of change of amplitudes and frequencies of additional external pressure produced by the wall of the first chamber

Let us assume that an additional external pressure produced by the active wall of the first chamber at some point in time $t_a > t_0$ change the amplitude and frequency, that is,

$$F(t) = \begin{cases} a_0 \sin(\alpha_0 t + \phi_0), & t_0 \leq t \leq t_1; \\ a_1 \sin(\alpha_1 t + \phi_1), & t_1 < t < +\infty. \end{cases} \tag{4.1}$$

We impose a natural condition for the continuity of function (4.1) at point $t_1$:

$$a_0 \sin(\alpha_0 t_1 + \phi_0) = a_1 \sin(\alpha_1 t_1 + \phi_1) \tag{4.2}$$

For the time derivative of function (4.1), we can have

$$\dot{F}(t) = \begin{cases} a_0 \alpha_0 \cos(\alpha_0 t + \phi_0), & t_1 < t < +\infty; \\ a_1 \alpha_1 \cos(\alpha_1 t + \phi_1), & t_1 < t < +\infty. \end{cases} \tag{4.3}$$

We assume that function (4.3) at point $t_1$ is not smooth, and that means that the following inequality is valid:

$$a_0 \alpha_0 \cos(\alpha_0 t_1 + \phi_0) \neq a_1 \alpha_1 \cos(\alpha_1 t_1 + \phi_1) \tag{4.4}$$

We now show the smoothness of the equation (2.4) solution at point $t_1$, and also estimate the degree of this smoothness. Let us consider formulas (3.4) and (3.7). Let us compare the solutions according to formulas (3.4) and (3.7) with the initial disturbance specified by formulas (4.1) and

$$\dot{F}(t) = a_0 \sin(\alpha_0 t + \phi_0), t_0 < t < +\infty \tag{4.5}$$

Thus, for smoothness at point $t_1$, we need to consider the difference

$$V(t) - \dot{V}(t) \tag{4.6}$$

From the fourth formula (2.3) it follows that

$$f(t) - \dot{f}(t) = I^{-1}[\dot{F}(t) - F(t)] \tag{4.7}$$

It follows from (3.4) and (3.7) that only the particular solutions to the equation of forced oscillations need to be compared, because the second and third summands are the same and therefore are reduced. Further, from (4.7), (3.4) and (3.7), since the right part of (4.7) differs from zero only by $(t_1, t)$, then the smoothness should be considered as given below:

$$J_1(t) = \int_{t_1}^{t} [a_0 \sin(\alpha_0 s + \phi_0) - a_1 \sin(\alpha_1 s + \phi_1)] e^{\lambda_0(s-t)} - e^{\lambda_1(s-t)} ds, \tag{4.8}$$

$$J_2(t) = \int_{t_1}^{t} [a_0 \sin(\alpha_0 s + \phi_0) - a_1 \sin(\alpha_1 s + \phi_1)](t-s)e^{\lambda_0(s-t)} ds. \tag{4.9}$$

We omitted the non-zero constant multipliers before the integrals. To differentiate these integrals, we use the Leibniz formula [15]:

$$J_1(t) = \int_{t_1}^{t} \left[ a_0 \sin(\alpha_0 s + \phi_0) - a_1 \sin(\alpha_1 s + \phi_1) \right] \left[ \lambda_0 e^{\lambda_0(s-t)} - \lambda_1 e^{\lambda_1(s-t)} \right] ds, \tag{4.10}$$

$$J_2(t) = \int_{t_1}^{t} \left[ a_0 \sin(\alpha_0 s + \phi_0) - a_1 \sin(\alpha_1 s + \phi_1) \right] e^{\lambda_0(s-t)} + \lambda_0(t-s)e^{\lambda_0(s-t)} ds. \tag{4.11}$$

The Leibniz formula consists of three summands [15], among which only one remains in (4.10) and (4.11). This is because the lower limit in (4.8) and (4.9) is constant, and substituting the upper limit in the integrand for the integration variable results in zero. It is quite clear that in case of $t \to t_1$, both integrals (4.10) and (4.11) tend to zero that already indicates the desired smoothness. However, in addition, the integrands in (4.10) and (4.11) tend to zero at $t \to t_1$ that follows from (4.2). And this indicates increased smoothness. To obtain an estimate of the tendency to zero in (4.10) and (4.11), we apply the mean value theorem to the integrals in the right parts of (4.10) and (4.11):

$$J_1(t) = \int_{t_1}^{t} \left[ a_0 \sin(\alpha_0 s + \phi_0) - a_1 \sin(\alpha_1 s + \phi_1) \right] \left[ \lambda_0 e^{\lambda_0(s-t)} - \lambda_1 e^{\lambda_1(s-t)} \right] ds =$$

$$= \int_{t_1}^{t} \left[ a_0 \sin(\alpha_0 s + \phi_0) - a_1 \sin(\alpha_1 s + \phi_1) \right] e^{\lambda_0(s-t)} - e^{\lambda_1(s-t)} ds, \tag{4.12}$$

$$J_2(t) = \int_{t_1}^{t} \left[ a_0 \sin(\alpha_0 s + \phi_0) - a_1 \sin(\alpha_1 s + \phi_1) \right] e^{\lambda_0(s-t)} + \lambda_0(t-s)e^{\lambda_0(s-t)} ds =$$

$$= \int_{t_1}^{t} \left[ a_0 \sin(\alpha_0 s + \phi_0) - a_1 \sin(\alpha_1 s + \phi_1) \right] e^{\lambda_0(s-t)} + \lambda_0(t-s)e^{\lambda_0(s-t)} ds. \tag{4.13}$$

In (4.12) and (4.13) we have $\tau \in (t_1,t)$. Estimates (4.12) and (4.13) show the degree of smoothness in the solution to the equation of forced oscillations at the point of change in the amplitude and frequency of the additional external pressure produced by the active wall of the first chamber.
Conclusions

It should be mentioned that the single-ventricle hearts are found not only in protozoa as the simplest living organisms, but also as the design version of artificial single-ventricle hearts employed in cardiac surgery. Known are some congenital heart defects, which are referred to as the “single-ventricle heart condition” and which are the result from an uneven development or malformation of some individual heart segments in the prenatal period (a Single Ventricle Condition). A single-ventricle heart is easier to control than a multi-ventricle one. It gains even more attraction due to the exact solutions presented herein. Examples in [8 -10] describe the modes of the single-ventricle heart performance, when the amplitude and frequency demonstrate no changes with time. The processes of the single-ventricle heart performance, when the amplitude and frequency change at some point in time, are described by changing the amplitude and frequency of the additional external pressure produced by the active wall of the chamber. It has been shown that a non-smooth change in the external pressure leads to a sufficiently smooth change in the solution to equation (2.4). The obtained estimates of the degree of smoothness at the point of inflection of the external pressure show the absence of rhythm interruptions. The success of this study is based on the obtained herein solutions to equation (2.4) with an integral representation of the particular solution to the equation.

Statement on ethical issues

Research involving people and/or animals is in full compliance with current national and international ethical standards.

Conflict of interest

None declared.

Author contributions

The authors read the ICMJE criteria for authorship and approved the final manuscript.

References

Left distal radial artery approach versus conventional radial artery for coronary angiography

Mahmoud Shawky Abd El-Moneum1, Mohamed Asem Alam1,*, Hesham Khaled Rashid1, Mohamed Abd El-Shafy Tabl1

1 Department of Cardiology, Benha University Hospital
Egypt, 13511, Benha City, Fareed Nada Street, Benha, Qalubiya governorate
* Corresponding author:
phone: +201007779159, email: mohasemalam@yahoo.com

Abstract

We aimed to evaluate feasibility, contrast utilization and complications of trans-radial approach comparing the left distal trans-radial artery (lt. dTRA) access versus conventional right trans-radial artery (rt. TRA) access in coronary angiography.

Subjects and Methods: This study was conducted on (100) patients who underwent coronary angiography (50 patients via lt. dTRA & 50 patients via rt. TRA) and was performed in the department of cardiology, Benha University Hospital. All patients performed ECG, echocardiography, arterial doppler pre and post procedures.

Results

In comparison with conventional right trans-radial artery (rt. TRA), Our study revealed that performing coronary angiography via lt. dTRA had more difficulties at which it had significantly more failure rate to get puncture and high significantly more time to insert a sheath but with significantly less contrast volume required. Also, lt. dTRA approach had significantly less incidence to cause radial artery occlusion and less incidence to cause bleeding or infection without significant difference. Patients were more satisfied when procedures performed via lt. dTRA approach and they had less hospital stay time.

Conclusion

Compared to conventional right trans-radial artery (rt. TRA) access for coronary angiography procedure, left distal trans-radial artery (lt. dTRA) access has more failure rate to get puncture and more time to insert sheath but with less contrast volume required, less incidence of RAO and less hospital stay time.

Keywords

Left distal trans-radial, Radial; Snuff box, Coronary angiography

Imprint


Introduction

Since its first performance in 1929, cardiac catheterization has continued to develop [1]. The advancement in technology and understanding the physiological properties of the vascular system have contributed to new vision into coronary angiography procedures. In concerning with arterial entry sites, a variety of research studies have been undertaken to establish which entry site is better suited for individual patients and situations. Notwithstanding the ease of access of femoral artery to coronary angiography, vascular-related complications and bleeding have resulted in increased morbidity, deaths and hospitalization, especially when anticoagulation and antiplatelet therapy are implemented [2]. The Femoral approach was compared in several randomized and observational trials with radial approach for both coronary angiography and interventional procedures. The established results were the safety of the patient and satisfaction, as well as reduced bleeding complications and immediate post-procedural mobilization as significant advantages of radial access. [3]. Based on the findings of these randomized trials, trans-radial access is taken as the default coronary access technique [4]. The majority of operators choose the right radial artery, as they operate on the patients’ right side. On the other hand, the occlusion of the radial arteries, the underdeveloped right radial artery, extreme tortuosity, sclerosis, calcification, past or potential use of the proper radial artery as a free arterial graft leads to change the operator decision to use left radial artery. [5]. Otherwise, left radial access may be exhausting for the operator as he should bend over the patient to insert the sheath and deal with it. This annoying location could make the catheterization process uncomfortable and it is probable that he moves to another artery access site. A possible way to provide a convenient position for both the patient and the operator is to reach through left distal radial artery situated on the anatomical snuff-
box or "fossa radialis" [5]. Anatomical snuffbox (AS) is an area of depression within the radial portion of the wrist. It is seen when you stretch the thumb. It is laterally surrounded by the tendons of the abductor pollicis longus and extensor pollicis brevis muscles and medially by the extensor pollicis longus muscle tendon. The base of this triangular region is created by the distal radius, scaphoid, trapezium and base of the first metacarpal bone. [6].

Aim of the work
The aim of this study was to evaluate feasibility, contrast utilization and complications of trans-radial approach comparing the left distal trans-radial artery (lt. dTRA) in the anatomical snuffbox versus conventional right trans-radial artery (rt. TRA) approach in coronary angiography procedures.

Study design and population
This study was conducted over one year period from July 2019 to July 2020 and was performed in the department of cardiology, Benha University Hospital, Benha City, Egypt on one hundred (100) patients who underwent diagnostic coronary angiography (50 patients via left distal trans-radial artery (lt. dTRA) approach who represented group A & 50 patients via conventional right trans-radial artery (rt. TRA) approach who represented group B). All patients were indicated for coronary angiography. Exclusion criteria were refusal of patients, patients in whom radial approach were contraindicated, Patients who suffer from previous unsuccessful or complicated Radial approach, in this case the femoral approach may be more suitable for the operator and in patients with moderate to severe renal impairment or coagulopathy. The protocol was approved by the hospital’s ethics committee.

All participants included in our study had been subjected to:
- Informed written consent for coronary angiography via right trans-radial or left distal trans-radial Approach.
- Complete history taken: included history of hypertension, diabetes mellitus, dyslipidemia, peripheral vascular disease, smoking habit and analysis of chest pain.
- physical examination: included:
  1) General examination e.g. heart rate and blood pressure.
  2) Local examination of heart e.g. heart sounds and cardiac murmurs.
- Investigations:
  - 12 lead ECG: A 12-lead surface ECG was done for each patient on admission. The electrocardiograms were recorded at a paper speed of 25 mm/s and an amplification of 10 mm/mV with special assessment for heart rate, rhythm, ST-T changes.
  - Laboratory investigations: blood samples were dragged from all participants and the following investigations were performed: Serum creatinine, PT, PTT, INR, CBC, virology markers and Hba1C.
  - Echo-doppler study: A conventional transthoracic echocardiographic evaluation was performed in all patients after hospital admission with special assessment for the left ventricular systolic function, valve assessment and regional wall motion.
  - Arterial doppler pre and post procedure to detect blood flow and complications.
- Procedure:
  After gaining approval from the institutional review board and all consents and pre-procedural tests, patients were put in a typical supine position on the catheterization lab table. Their arm was kept immobilized and the wrist was hyperextended and then wrapped in a sterilized way, access was made to the right radial or left distal radial artery by:-

- A- Left distal trans-radial approach technique (Anatomical Snuff Box):
  - The patient had been wrapped with a sterilized drape during disinfection. The operator bring the patient’s left hand to his right iliac area and took a nearby site, then subcutaneous 3 ml Lidocaine administered at the Snuff Box area.
  - The patient was pinched his thumb inside the other four fingers to bring the artery superficially. The radial artery was penetrated by a 21 G needle with angle of 30-45 degrees.
  - The needle was pointed proximally to the direction of the strongest pulse in snuff box triangle.
  - Following the succeeded puncture in the anterior or wall of the radial artery, the guide wire was easily advanced through the needle and used to direct the sheath through the artery, accompanied by a slight incision in the skin, followed by the insertion of a 6F radial sheath.
  - Thereafter, 0.2 mg of nitroglycerin and 500 IU heparin were administered. At the level of the patient’s thigh, operator took a position to advance the diagnostic guide wire and the diagnostic catheters (left and right) to proceed the coronary angiography.
- The sheath had been removed after the angiography and pressure was placed over the arteriotomy site to maintain hemostasis with implementation of TR – band.

B- Conventional right trans-radial approach technique:
- The patient had been wrapped with a sterilized drape during disinfection. The operator took up a position near right upper limb of the patient then subcutaneous 3 ml Lidocaine injected at the site of radius bone styloid process.
- The radial artery was penetrated at an angle of 45 degrees, 1 cm proximal to the stylized radius process with a 21 G needle.
- Following the succeeded puncture in the anterior wall of the radial artery, the guide wire was easily advanced through the needle and used to direct the sheath through the artery, accompanied by a slight incision in the skin, followed by the insertion of a 6F radial sheath.
- Thereafter, 0.2 mg of nitroglycerin and 5000 IU heparin were administered. At the level of the patient’s thigh, operator took a position to advance the diagnostic guide wire and the diagnostic catheters (left and right) to proceed the coronary angiography.
- The sheath had been removed after the angiography and pressure was placed over the arteriotomy site to maintain hemostasis with implementation of TR – band.

Follow up
Arterial Doppler had been performed post procedural for all the patients in the two groups to assess the flow through the artery and post procedural complications.
Outcomes of the procedures in the two groups had been reviewed including:
- Success and failure rate of cannulation.
- Post Catheterization Radial Artery Occlusion (RAO) and thrombosis.
- Bleeding or hematoma.
- Infection.
- Total duration of the procedure.
- Discharge time and satisfaction between groups.

Statistical analysis
Using an IBM compatible personal computer with SPSS statistical package version 23 (SPSS Inc. Released 2015. IBM SPSS statistics for windows, version 23.0, Armonk, NY: IBM Corp.), results were collected, tabulated and statistically analyzed by an
There were two types of statistical analysis:
a) Descriptive statistics e.g. g. Number (No), percentage (%), mean (X¯) and standard deviation (SD).
- Arithmetic mean (x): The measure used for central tendency.
- Standard deviation (SD): The measure for dispersion.
- Percentage (%).
- Median: was used as a measure of central tendency.
- Range: was used as a measure of dispersion

b) Analytic statistics:
- Chi-squared test (χ²): a parametric test used to find the correlation between two or more qualitative variables.
- Fischer exact test: for 2 x 2 tables, in case the predicted cells count of more than 25 percent of cases were less than 5 and the significant p-value < 0.05.
- Student t-test: is a significant test used for comparing with independent parametric data between two classes of quantitative variables
P-value at 0.05 was used to indicate the significance:
  - P-value > 0.05 means non-significant statistically.
  - P-value ≤ 0.05 is used to mean significant statistically.
  - P-value ≤ 0.001 means of high significant statistically.

Results
Demographic features of studied patients
Our study included one hundred (100) patients who underwent coronary angiography (50 patients via left distal trans-radial approach (lt. dTRA) which represented group A & 50 patients via conventional right trans-radial approach (rt. TRA) which represented group B).

Risk factors of studied patients
As regarding risk factors, there was no significant difference between groups. Out of the 100 patients, 11 (22%) were diabetics in group (A) whereas 10 (20%) members of group (B) (p=0.806). 30 (60%) patients had dyslipidemia in group (A) whereas 29 (58%) members of group (B) (p=0.839). 13 (26%) were hypertensives in group (A) whereas 15 (30%) members of group (B) (p=0.656). 11 (22%) were smokers in group (A) whereas 10(20%) members of group (B) (p=0.806) (table 2).

Procedural aspects of studied patients
As regarding procedural aspects of the groups, there was a significant difference between the groups
Table 1
Distribution of studied patients according to demographic features (n = 100)

<table>
<thead>
<tr>
<th>Demographic features</th>
<th>Group A No=50</th>
<th>Group B No=50</th>
<th>Test of sig.</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>51.34±8.53</td>
<td>51.46±8.47</td>
<td>t</td>
<td>0.944</td>
</tr>
<tr>
<td>Range</td>
<td>35-65</td>
<td>36-65</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median</td>
<td>54</td>
<td>54</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>35(70%)</td>
<td>36(72%)</td>
<td>χ²</td>
<td>0.826</td>
</tr>
<tr>
<td>Female</td>
<td>15(30%)</td>
<td>14(28%)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

$t = \text{Student’s t test}$  \  $\chi^2 = \text{chi-square test}$  \  $\text{NS} = \text{non-significant}$

Table 2
Distribution of the studied patients according to risk factors (n = 100)

<table>
<thead>
<tr>
<th>Risk factors</th>
<th>Group A No=50</th>
<th>Group B No=50</th>
<th>χ²</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>DM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>11</td>
<td>10</td>
<td>0.060</td>
<td>0.806</td>
</tr>
<tr>
<td>No</td>
<td>39</td>
<td>40</td>
<td></td>
<td>NS</td>
</tr>
<tr>
<td>HTN</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>13</td>
<td>15</td>
<td>0.198</td>
<td>0.656</td>
</tr>
<tr>
<td>No</td>
<td>37</td>
<td>35</td>
<td></td>
<td>NS</td>
</tr>
<tr>
<td>Dyslipidemia</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>30</td>
<td>29</td>
<td>0.041</td>
<td>0.839</td>
</tr>
<tr>
<td>No</td>
<td>20</td>
<td>21</td>
<td></td>
<td>NS</td>
</tr>
<tr>
<td>Smoking</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>11</td>
<td>10</td>
<td>0.060</td>
<td>0.806</td>
</tr>
<tr>
<td>No</td>
<td>39</td>
<td>40</td>
<td></td>
<td>NS</td>
</tr>
</tbody>
</table>

$\chi^2 = \text{chi-square test}$  \  $\text{NS} = \text{non-significant}$

Table 3
Comparison between studied groups as regard procedural aspects (n=100)

<table>
<thead>
<tr>
<th>Procedural aspects</th>
<th>Group A No=50</th>
<th>Group B No=50</th>
<th>Test of sig.</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Failure to get puncture</td>
<td></td>
<td></td>
<td>χ²</td>
<td>0.03</td>
</tr>
<tr>
<td>Yes</td>
<td>7</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>43</td>
<td>49</td>
<td>4.9</td>
<td>S</td>
</tr>
<tr>
<td>Time to insert sheath (minutes)</td>
<td></td>
<td></td>
<td>t</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>5.08±0.75</td>
<td>4.02±0.55</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Range</td>
<td>4-7</td>
<td>3-5</td>
<td>8.03</td>
<td>S</td>
</tr>
<tr>
<td>Median</td>
<td>5</td>
<td>4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total procedure duration (minutes)</td>
<td></td>
<td></td>
<td>t</td>
<td>0.124</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>19.34±1.81</td>
<td>19.90±1.79</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Range</td>
<td>17-23</td>
<td>17-23</td>
<td>1.55</td>
<td>NS</td>
</tr>
<tr>
<td>Median</td>
<td>19</td>
<td>19</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Contrast volume</td>
<td></td>
<td></td>
<td>t</td>
<td>0.002</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>56.50±8.93</td>
<td>63.20±11.68</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Range</td>
<td>40-80</td>
<td>40-100</td>
<td>3.22</td>
<td>S</td>
</tr>
<tr>
<td>Median</td>
<td>60</td>
<td>60</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

$\text{FXT = Fisher’s exact test}$  \  $t = \text{Student’s t test}$  \  $S=\text{significant}$  \  $\text{NS}=\text{non-significant}$  \  $\text{HS}=\text{High significant}$

as regarding failure rate to get puncture at which operators failed to get puncture in 7 (14%) of group (A) while there was 1 (2%) failed puncture in group (B) (p=0.03). As regarding time to insert sheath, there were 5.08±0.75 minutes for sheath insertion in group (A) and 4.02±0.55 minutes in group (B) (p<0.001) with highly significant difference between groups. As regarding total duration of procedure, there were 19.34 ±1.81 minutes in group (A) and 19.90±1.79 minutes in group (B) (p=0.124) with no significant difference between groups. As regarding contrast volume used in procedure, there was a significant difference between both
groups at which operators used about 56.50 ± 8.93 mL of contrast in group (A) and 63.20 ±11.68 mL of contrast used in group (B) (p=0.002).(Table 3)

Complications of studied patients
As regarding post-procedural complications, there was bleeding in 3(6%) of cases of group (A) and in 4(8%) cases of group (B) (p=0.500) without no significant difference between the groups. Infection occurred in 2(4%) of cases of group (A) while that occurred in 1(2%) case of group (B) (p=0.558) without no significant difference between groups. Thrombosis and radial artery occlusion (RAO) occurred in 3(6%) of cases of group (A) while that occurred in 10(20%) cases of group (B) (p=0.040) with a significant difference between 2 groups (Table 4).

Satisfaction of studied patients
Regarding patient satisfaction, there was no significant difference between the 2 groups, as there were 4(8%) patients from group (A) were not satisfied while there were 10(20%) patients from group (B) (p=0.084).

Discussion
Coronary artery disease (CAD) is one of the most prevalent causes of morbidity and mortality worldwide [7]. Trans-radial arterial technique (TRA) is a relatively easy way of intervention for coronaries [8]. Recently, interventional cardiologists have begun to follow a modulation of the trans-radial approach, the left distal trans-radial technique (lt. dTRA) for coronary procedures [9].

In our study we aimed to evaluate feasibility, contrast utilization and complications of trans-radial approach comparing the left distal trans-radial (lt. dTRA) versus conventional right trans-radial (rt. TRA) approach in coronary angiography procedures.

This study showed that out of the 100 patients, 35 (70%) were males and 15 (30%) were females in (group A) whereas 36 (72%) members of the (group B) were males and 14 (28%) were females. No significant variation between groups regarding gender. The mean ± SD ages were distributed between both groups as 51.34±8.53 and 51.46±8.47 respectively with no sig-
significant difference between groups. Our results were in agreement with the study conducted by Roghani-Dehkordi et al. which reported that in 159 patients, men were 76% with age 58.1 ± 10.5 years and women were 24% with age 61.2 ± 9.6 years [10]. But our results were in disagreement with Brunet et al. regarding sex of patients who reported that male participants comprised only 21.2% of the recruited participants [11].

This study showed that regarding risk factors, 11 (22%) were diabetics in group (A) whereas there were 10 (20%) members of the group (B), 30 (60%) had dyslipidemia in group (A) whereas there were 29 (58%) members of the group (B), 13 (26%) were hypertensives in group (A) whereas there were 15 (30%) members of the group (B) and 11 (22%) were smokers in group (A) whereas there were 10 (20%) members of the group (B). Our results were in disagreement with Soydan & Akın who documented that the most common risk factor for patients who experienced coronary angiography via left distal radial artery was hypertension with rate of 61.1 percent [12].

This study showed that regarding procedural aspects, there was a significant difference between the groups as regarding failure rate to get puncture at which operators failed to get puncture in 7 (14%) of group (A) while there was 1 (2%) failed puncture in group (B) (p=0.03). Our results were in agreement with the study conducted by Brunet et al. which reported that failure rate to get puncture via distal radial approach was 8%, with the majority of failed cases converted to trans-femoral artery for convenience [11]. Our results were in disagreement with the study conducted by Mizuguchi et al. which reported that failure rate for initial puncture was 0.4%, with conversion to contralateral distal radial artery [13].

This study showed that contrast volume used for coronary angiography in group (A) was significantly less than used in group (B) at which it was 56.50±8.93 mL and 63.20±11.68 mL respectively, P = 0.002. This was in disagreement with Coughlan et al. who showed that there was no statistically significant difference in contrast dose used for coronary angiography in distal trans-radial versus conventional trans-radial artery access (82.93 ± 23 vs 92.1 ± 33 mL respectively, P = 0.1215) [14].

This study showed that total procedure time in group (A) in comparison with group (B) was non significantly shorter as time distributed was 19.34±1.81 min and 19.90±1.79 min between groups respectively. This was in agreement with Coughlan et al. who showed that Procedural length did not vary significantly between groups (28.95 ± 5.89 vs 29.76 ± 8.16 min, P = 0.5824) [14].

Arterial Doppler had been performed pre-procedural and post-procedural for all the patients in the two groups to assess the flow through the radial artery and to evaluate the post-procedural complications especially radial artery occlusion (RAO).

This study showed that the rate of RAO was significantly higher with group B (20%) than group A (6%) (P=0.040). Our results were in agreement with a study of 1320 patients who subjected to dTRA for coronary intervention, late RAO was observed in few cases about 0.61% [15]. Our results were in disagreement with Sinha et al. who showed that the rate of RAO in conventional radial artery was low (1–6%) [9].

Regarding bleeding, there was no significant difference between the 2 groups, as there were 3(6%) patients from group (A) had bleeding while there were 4(8%) patients from group (B) had bleeding (p=0.500). Our results were in agreement with Wretowski et al. who documented that only single patient on oral anti-coagulation with DAPT had minor bleeding and had treated conservatively [16].

Regarding patient satisfaction, there was no significant difference between the 2 groups, as there were 4(8%) patients from group (A) were not satisfied while there were 10(20%) patients from group (B) (p=0.084). This was in agreement with Koutouzis et al. who found that slightly higher rates of patient satisfaction in the distal trans-radial group than in the conventional trans-radial artery group, although this difference was not significant [17].

Our study showed that rt. TRA was associated with highly significant increase in hospital stay time after procedure in comparison with lt. dTRA (5.56±0.951 hours vs 3.92±1.06 hours respectively, P<.001). This was in agreement with Coughlan et al. who showed that from the other benefits of snuff box access was shorter discharge time due to statistically significant decreases in the time required for radial artery compression [14].

**Conclusion**

In conclusion, compared to conventional right trans-radial artery (rt. TRA) access for coronary angiography procedure, left distal trans-radial artery (lt. dTRA) access has more failure rate to get puncture and more time to insert sheath but with less contrast volume required, less incidence of RAO and less hospital stay time.
Limitations
- This study is a single-center study.
- Small sample size.
- Short duration of study.

Financial support and sponsorship
Nil

Statement on ethical issues
Research involving people and/or animals is in full compliance with current national and international ethical standards.

Conflict of interest
None declared.

Author contributions
The authors read the ICMJE criteria for authorship and approved the final manuscript.

References
Impact of exercise on the activity of the autonomic nervous system among patients with acute myocardial infarction: A case-control study

Mojgan Haj Ahmadi Pour Rafsanjani1, Samira Moghadam1, Roghaiyeh Afsargharehbagh1

1 Department of Cardiology, Seyyed-al-Shohada Heart Center, Urmia University of Medical Science, Iran, Urmia

* Corresponding author: email: afsargharehbaghr@gmail.com

Abstract
Considering the diversity of proposed programs, various studies yielded different findings in this regard. The aim of the present study was to evaluate different types of exercise as a method of rehabilitation after acute myocardial infarction on the activity of the autonomic nervous system.

Methods
In this case-control and prospective study, a total of 60 patients with the first acute myocardial infarction were randomly selected. Controlled treadmill exercise was performed for 15-20 minutes three days a week for three consecutive weeks and then 5 weeks of home-based exercise, including 30-min walking 3 times a week based on heart rate in the two groups (n= 20 people per group). The control group performed home-based exercise for 8 weeks.

Results
In A, B, and control groups, the mean changes in SDNN (28.30, 29.28, and 15.40, respectively), LF (57.10, 198.8 and -47.70, respectively), HF (-11.70, 120.60, and -58.10, respectively) TP (192.80, 1251.20, and -0.225, respectively), pNN50 (0.80, 4.60, and -0.40, respectively), SDNN index (90.20, 13.4, and -0.20, respectively), and SDANN (80.80, 22.24, and 16.20, respectively) were significantly higher in the intervention groups, but there was no statistically significant difference between the two intervention groups (A and B).

Conclusion
The present study showed that in-hospital exercise-based rehabilitation can have a more favorable effect on the activity of the autonomic nervous system after acute myocardial infarction. It seems necessary to establish rehabilitation centers in hospitals, because uncontrolled home-based rehabilitation is probably less effective for various reasons, such as lack of strict adherence to the instructions.

Keywords
Rehabilitation, Exercise, Acute myocardial infarction, Activity of autonomic nervous system, Heart rate variability

Imprint

Introduction
Cardiovascular diseases are the leading cause of death among people over 30 years of age in the world, and acute myocardial infarction (AMI) accounts for almost 10% of these deaths. Daily lifestyle and exercise have been shown to be a risk factor for cardiovascular diseases, therefore, exercise are recommended both at workplace and during leisure time. Changes in cardiac biomarker values, along with at least one of the following: ischemic symptoms, abnormal electrocardiogram changes, or abnormalities in the structure or wall motion abnormalities detected by imaging techniques, are considered as criteria used to diagnose AMI in a clinical context. According to the results of some studies, patients with AMI demonstrate increased sympathetic activity in the autonomic nervous system (ANS), which predisposes them to fatal ventricular arrhythmias and sudden death. HRV is a relatively simple, non-invasive, reproducible, and low-cost method for determining cardiac autonomic regulation. HRV measures variations between normal heart rate intervals or R-wave peaks (RR or NN intervals). Changes in heart rate and R-R interval are measured to assess sympathetic and parasympathetic changes in the sinus group and diagnose patients at risk for fatal cardiovascular events. Cardiac rehabilitation can lead to improvement of these unpleasant changes in patients. Time domain indices in-
clude standard deviation of all NN intervals (SDNN), mean standard deviation of NN intervals calculated for each 5-minute segment (SDNN), and percentage of successive RR intervals that differ by more than 50 ms (pNN50). Frequency domain indices include low frequency peak (LF) and high frequency peak (HF), which are expressed as millisecond squares or normalized units (nu) 5, 9. It has been shown that time domain variables are generally interrelated and are affected to varying degrees by parasympathetic block. Accordingly, these are indices of parasympathetic cardiac regulation. However, the HF peak is the most important HRV index of vagal efferent activity (vagal regulation of heart rate). Although there is no complete agreement on the interpretation of the LF component, this parameter is generally considered as an index of both sympathetic and parasympathetic regulation 10, 11, 12. One of the most important results of exercise after myocardial infarction (MI) is a decrease in the activity of the sympathetic nervous system, which changes the sympathetic-parasympathetic balance and increases the activity of the parasympathetic system by reducing the sympathetic dominance. Diastolic dysfunction is an important factor as a result of complications from MI and unlike systolic function, no treatment or intervention has led to a significant improvement in the diastolic function. Data from animal studies and patients with diastolic heart failure show that exercise can have a positive effect on diastolic function parameters 13. Other factors (other than HRV), which predict the occurrence of post-MI arrhythmia and increased subsequent mortality include heart rate turbulence, change in QT size, T-wave alternation, and baroreceptor sensitivity (BRS) index, which do not have many clinical aspects 14,15,16. So far, there have been few articles on the effect of various types of exercise on the activity of ANS among patients with AMI. The aim of the present study was to evaluate the effect of different types of exercise on the activity of ANS among patients with AMI by measuring HRV indices.

Materials and methods

This case-control and prospective study was performed on 60 patients with acute ST elevation in 2019. Patients underwent initial examination after initial recovery and when they were able to walk 10 days following MI. Inclusion criteria included a normal sinus rhythm, consent to participate in the study, left ventricular ejection fraction (LVEF 30-40%), exercise tolerance in the initial test. Exclusion criteria included patients aged over 75 years, systolic blood pressure above 200 mm Hg or diastolic blood pressure above 100 mm Hg, ventricular block / acute systemic disease, unstable angina / peripheral neuropathy (according to medical records and evidence), chest pain during exercises, valvular heart disease requiring surgery, severe renal failure (serum creatinine>2.5 mg / dL), diabetes, and thyroid disease. Pre-study assessments (first visit) included heart murmurs, 12-lead ECG, eco-doppler, CBC, and measurements of serum Na, K, BUN, and CR levels. Information such as age, sex, location of MI, medications, and medical history in patients were also recorded. Patients underwent a 24-hour holter and HRV indices were recorded. Then, patients were divided into two groups of 20 and 40 of people based on their desire to be in the hospital and do exercises or the desire to do these exercises at home. The 40-person group was randomly divided into A and B groups (n= 20 people per group). In the intervention group, the exercises included 5-10-min body warm-up and treadmill-based cooling and endurance exercises for the same period, which included complete control of the ECG and heart rate for 15 to 20 minutes, three days a week, for 3 consecutive weeks based on Karvonen formula. Later on, home-based exercises (30-min walking) were performed 3 times a week for 5 weeks. These patients were divided into two groups, i.e. group A (40-50% of maximum heart rate) and group B (60-80% of maximum heart rate). All patients referred for re-examination one week after exercise, underwent a 24-hour holter, and HRV indices were recorded again. Finally, the obtained data were compared between 3 groups in a pairwise comparison manner. This study has been approved by the Ethics Committee of Urmia University of Medical Sciences with the Ethics Code of IR.UMSA.REC.1393.5. Written consent was obtained from all participants prior to the study. Participants were assured that their information would be kept confidential. STROBE checklist was used to report the study 19. Data analysis was carried out using descriptive (mean, percentage, frequency) and analytical (chi-square) tests in SPSS ver. P-value<0.05 was considered as the significance level.

Results

A total of 60 patients with AMI were studied in 3 groups of 20 people. Mean age of participants was 54.41 (29-73). Most of participants in all groups were male (n=50, 90%), the most prevalent comorbidity was
### Table 1
Demographic characteristics of participants and baseline variables in three groups

<table>
<thead>
<tr>
<th>Group</th>
<th>Variables</th>
<th>Group A (N=20)</th>
<th>Group B (N=20)</th>
<th>Control group (N=20)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Gender</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Male</td>
<td>16(80)</td>
<td>16(80)</td>
<td>18(90)</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>4(20)</td>
<td>4(20)</td>
<td>2(10)</td>
</tr>
<tr>
<td></td>
<td>Age</td>
<td>54.60±13.39 (29-73)</td>
<td>53.70±4.52 (46-59)</td>
<td>54.3±6 (48-63)</td>
</tr>
<tr>
<td></td>
<td>Comorbidity</td>
<td></td>
<td></td>
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</tr>
<tr>
<td></td>
<td>HTN</td>
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<td>0(0)</td>
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<tr>
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<td>Hyperlipidemia</td>
<td>0(0)</td>
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<td>0(0)</td>
</tr>
<tr>
<td></td>
<td>Smoking</td>
<td>8(40)</td>
<td>6(30)</td>
<td>10(50)</td>
</tr>
<tr>
<td></td>
<td>Anatomical location of occurred MI</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Anteroseptal</td>
<td>10(50)</td>
<td>8(40)</td>
<td>6(30)</td>
</tr>
<tr>
<td></td>
<td>Lateral</td>
<td>2(10)</td>
<td>-</td>
<td>2(10)</td>
</tr>
<tr>
<td></td>
<td>Inferior</td>
<td>4(20)</td>
<td>6(30)</td>
<td>4(20)</td>
</tr>
<tr>
<td></td>
<td>Anteroseptal and Inferior</td>
<td>2(10)</td>
<td>-</td>
<td>4(20)</td>
</tr>
<tr>
<td></td>
<td>Postero Inferior right ventricular and Inferior</td>
<td>-</td>
<td>-</td>
<td>2(10)</td>
</tr>
<tr>
<td></td>
<td>Lateral posterior inferior</td>
<td>-</td>
<td>2(10)</td>
<td>-</td>
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</table>

### Table 2
Baseline and final values of HRV parameters in three groups

<table>
<thead>
<tr>
<th>Group</th>
<th>Variables</th>
<th>Group A</th>
<th>Group B</th>
<th>Control group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SDNN (ms)</td>
<td>Mean±SD</td>
<td>Mean±SD</td>
<td>Mean±SD</td>
</tr>
<tr>
<td></td>
<td>Before</td>
<td>(49-125)</td>
<td>94.30±21.40</td>
<td>(38-140)</td>
</tr>
<tr>
<td></td>
<td>After</td>
<td>(80-194)</td>
<td>122.60±33.50</td>
<td>(48-161)</td>
</tr>
<tr>
<td></td>
<td>LF (ms²)</td>
<td>Mean±SD</td>
<td>Mean±SD</td>
<td>Mean±SD</td>
</tr>
<tr>
<td></td>
<td>Before</td>
<td>(59-1059)</td>
<td>388.10±308.24</td>
<td>(68-664)</td>
</tr>
<tr>
<td></td>
<td>After</td>
<td>(60-969)</td>
<td>445.20±75.19</td>
<td>(23-1826)</td>
</tr>
<tr>
<td></td>
<td>HF (ms²)</td>
<td>Mean±SD</td>
<td>Mean±SD</td>
<td>Mean±SD</td>
</tr>
<tr>
<td></td>
<td>Before</td>
<td>(10-528)</td>
<td>126.60±151.88</td>
<td>(17-185)</td>
</tr>
<tr>
<td></td>
<td>After</td>
<td>(12-204)</td>
<td>144.90±73.58</td>
<td>(23-1826)</td>
</tr>
<tr>
<td></td>
<td>TP</td>
<td>Mean±SD</td>
<td>Mean±SD</td>
<td>Mean±SD</td>
</tr>
<tr>
<td></td>
<td>Before</td>
<td>(400-3714)</td>
<td>2036.30±1699.99</td>
<td>(299-2557)</td>
</tr>
<tr>
<td></td>
<td>After</td>
<td>(323-5272)</td>
<td>2229.40±1316.24</td>
<td>(203-1130)</td>
</tr>
</tbody>
</table>

### Table 3
Changes in baseline and final values of HRV parameters in three groups

<table>
<thead>
<tr>
<th>Variables</th>
<th>Group A (N=20)</th>
<th>Group B (N=20)</th>
<th>Control group (N=20)</th>
<th>Value P</th>
</tr>
</thead>
<tbody>
<tr>
<td>SDNN (ms)</td>
<td>28.02±28.30</td>
<td>5.53±29.40</td>
<td>34.13±15.40</td>
<td>0.03</td>
</tr>
<tr>
<td>LF (ms²)</td>
<td>165.45±57.10</td>
<td>5.53±198.80</td>
<td>235.63±47.70</td>
<td>0.03</td>
</tr>
<tr>
<td>HF</td>
<td>129.56±11.70</td>
<td>5.53±120.60</td>
<td>111.94±58.10</td>
<td>0.02</td>
</tr>
<tr>
<td>TP</td>
<td>1319.78±192.80</td>
<td>234.16±1251.20</td>
<td>1253.3±225.2</td>
<td>0.03</td>
</tr>
<tr>
<td>PNN50 (%)</td>
<td>3.61±0.80</td>
<td>10.86±4.60</td>
<td>2.56±0.40</td>
<td>0.05</td>
</tr>
<tr>
<td>SDNN index (ms)</td>
<td>14.02±0.40</td>
<td>17.94±13.90</td>
<td>14.32±0.20</td>
<td>0.02</td>
</tr>
<tr>
<td>SDANN (ms)</td>
<td>23.33±24.80</td>
<td>33.37±22.80</td>
<td>33.26±16.20</td>
<td>0.04</td>
</tr>
<tr>
<td>LF/HF (%)</td>
<td>1.65±0.03</td>
<td>2.08±0.79</td>
<td>2.98±0.21</td>
<td>0.36</td>
</tr>
</tbody>
</table>
The most common location of MI in all patients was Anteroseptal. Patient characteristics and baseline variables of the three groups are summarized in Table 1. The mean HRV parameters of baseline and final values in three groups are summarized in Table 2 (Table 2).

Results of one-way ANOVA test showed a statistically significant difference between the three groups in all cases except for LF / HF. Tukey post hoc test was used to compare the two groups in a pairwise manner and the results showed a statistically significant difference between both A and control (P = 0.03) groups and B and control (P = 0.04) groups in terms of mean changes in SDNN. There was a statistically significant difference only between the B and control groups in terms of mean changes in LF (P = 0.02). There was no statistically significant difference between A and control groups in this regard (P = 0.46). There was a statistically significant difference only between the B and control groups in terms of mean changes in HF (P = 0.02). There was no statistically significant difference between the A and control groups in this regard (P = 0.75). There was a statistically significant difference only between the B and control groups in terms of mean changes in TP (P = 0.02). There was no statistically significant difference between A and control groups in this regard (P = 0.72). There was a statistically significant difference only between the B and control groups in terms of mean changes in SDNN (P = 0.02). There was no statistically significant difference between A and control groups in this regard (P = 0.65). There was a statistically significant difference between both A and control groups (P = 0.02) and B and control groups (P = 0.02) in terms of mean changes in SDNN. There was no statistically significant difference between the A and B groups terms of mean changes in of the above-mentioned indices (P=0.99, p= 0.25, p= 0.10, p= 0.13, p= 0.19, p=0.13, and p=0.98, respectively). The mean changes of HRV parameters in the three groups are summarized and compared in Table 3 (Table 3).

Discussion

Heart rate variability (HRV) refers to the continuous changes in heart rate that depend on the continuous modulation of the sympathetic and parasympathetic branches of the autonomic nervous system. HRV is effective as a predictor of post-MI risk and an early warning sign of diabetic neuropathy and is currently a useful risk classification tool in cardiology after admission and discharge. Recent evidence suggests that HRV analysis may predict complications even among patients undergoing heart surgery 17, 18. The present study investigated the effect of different types of exercise on the activity of ANS among patients with AMI based on HRV indices. HF peak is the most important HRV index of vagal efferent activity (vagal regulation of heart rate). Although there is no complete agreement on the interpretation of the LF component, this parameter is generally considered as an index of both sympathetic and parasympathetic regulation. The present study demonstrated that both protocol A (in terms of SDNN and SDANN variables) and protocol B (in terms of all studied protocols except (LF / HF)) had more optimal rehabilitation outcomes compared to the control group (home-based exercises). So far, some studies have investigated the effect of exercise-based rehabilitation on impaired autonomic function in patients with coronary artery disease and MI. Kleiger et al., showed the prognosis of this group of patients in cases with SDNN values below 50 ms 5. In another study, however, La Rovere et al. reported a 70-second cut-off point as a predictor of 1- and 2-year mortality rate after MI (19). As noted earlier, previous studies showed that exercise activity can have positive effects on patients with MI. Kasargod Prabhakar and Stewart reported that cardiovascular disease mortality rates were lower among people who perform moderate-intensity exercises on a regular basis than those who exercised less frequently or did not exercise at all. However, more intense exercises may lead a relatively lower reduction in mortality rate and may even increase the risk of mortality. A meta-analysis of rehabilitation experiments in the Kasargod Prabhakar’s and Stewart’s study showed lower mortality among people who performed controlled exercises, which is consistent with the present study 20. Consistent with the present study, some studies have shown that exercises significantly improve HRV parameters in patients with MI. Consistent with the present study, Fujimoto et al. showed a significant increase in mean SDNN, SDANN and HF after exercise 14. Iellamo et al. 10, Stahle et al. 21, Tsai et al. 22, and Malfatto et al. 23 also reported an average increase in SDNN, SDANN and HF after exercise, which is consistent with the present
study. Results of previous studies were inconsistent with the present study regarding some parameters, especially between B and control groups). Different reasons can be used to explain the differences in the results of studies, including low sample size, different inclusion and exclusion criteria for different groups in terms of cardiac status, different study times in terms of interval between inset of MI and exercise, lack of information about the size and location of MI, and lack of information about other potential interfering factors such as diet status and other physical activity. Besides, the use of different exercise protocols and different ways of recording HRV parameters may also be effective in this regard. The same different findings between A and B groups compared to the control group also confirm that the parameters involved in the regulation of exercise activities are one of the most important factors in obtaining different results in various studies.

In this study, patients were divided into two groups based on their exercise capacity. That is, the exercises were performed up to 40-50% of the maximum heart rate in one group and 60-80% of the heart rate in the other group. Although there was no statistically significant difference between the two groups, HRV indices showed better improvement in the second group. According to the results, it seems that establishment of in-hospital rehabilitation centers in patients after AMI is of high importance considering its positive effect on the autonomic system.

Limitations

It seems that future studies with a larger sample size can help to achieve more definite results in this field. In addition to the sample size, the present study has other limitations, such as not using a non-home-based exercise group. If a non-home-based exercise group was used in future studies, a more comprehensive and complete conclusion can be achieved.

Conclusion

The present study showed that in-hospital exercise-based cardiac rehabilitation can have a more favorable effect on the activity of ANS after AMI as compared to home-based exercise. Therefore, it seems necessary to establish rehabilitation centers in hospitals, because uncontrolled home-based rehabilitation is probably less effective for various reasons, such as patients’ lack of strict adherence.

Financial support and sponsorship
None

Statement on ethical issues
Research involving people and/or animals is in full compliance with current national and international ethical standards.

Conflict of interest
None declared.

Author contributions
The authors read the ICMJE criteria for authorship and approved the final manuscript.

Ethics approval
This study has been approved by the Ethics Committee of Urmia University of Medical Sciences with the Ethics Code of IR.UMSA.REC.1393.5. Written consent was obtained from all participants prior to the study. Participants were assured that their information would be kept confidential. All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

References


Dental status of patients with cardiovascular diseases and diabetes mellitus

Adana R. Nakhusheva, Farida Kh. Temirzhanova, Alisa S. Akmeeva, Darina F. Matsuhova, Melena M. Iusupova, Aishat I. Ibueva, Mareta L. Muligova, Rina M. Aramisova, Zareta A. Kambachokova

1 Federal State Budgetary Educational Institution of Higher Education “Kabardino-Balkarian State University named after H.M. Berbekov”
Russia, 360000, Nalchik, Chernyshevskogo str., 173
* Corresponding author:
phone: +7 (963) 168-52-21
email: k.zareta.7@mail.ru

Abstract
This article discusses some regional features of the structure of dental morbidity in patients with cardiovascular diseases and diabetes mellitus. The relationship between these diseases is shown. It is stated that there is a lack of awareness of the existing dentition diseases in this sort of patients.

Keywords
Cardiovascular diseases, Diabetes mellitus, Dental status


Topicality of the problem
Changes in the oral mucosa (COM) may be the first symptoms of cardiovascular diseases (CVD) and diabetes mellitus (DM) [1, 2, 5]. Diseases of the cardiovascular system (CVS) can manifest as nonspecific changes in the oral mucosa (COM) [2]. The development of pathological processes in the oral cavity is associated with microcirculatory disorders. So, with myocardial infarction, there is a change in the color of the oral mucose (cyanosis, crimson color), reported is the appearance of some cracks, erosions, hemorrhages in the papillae of the tongue. Patients suffering from PM cardiosclerosis show a high incidence rate and intensity of dental caries. In patients with chronic heart failure, severe ulcerative-necrotic changes in the oral mucosa are often found and trophic ulcers develop. Human individuals suffering from hypertension and atherosclerosis often have hemorrhagic blisters in the oral cavity [4].

It has been established that the DM patients show an increased prevalence of dental diseases. In the DM patients, the first signs of diabetes are very often found in the oral cavity. Moreover, the DM disease affects the rapid progression of dental diseases: there is a more severe course of the disease, and a significant slowdown in regenerative processes is observed [3,6].

According to the data of numerous studies, changes in the maxillofacial area under the MD conditions depend on the level of glycemic load control and the duration of this pathology. A high level of glycated hemoglobin is associated with such changes in dental status as increased susceptibility to caries, an increase in the risk of development of secondary adentia [7, 8].

Among the nonspecific changes and diseases of oral mucosa under DM, there are the following: swelling of the mucous membrane of the cheeks and surfaces of the tongue along the line of closing teeth, atrophy of the papillae of the tongue, cheilitis, recurrent aphthous and ulcerative stomatitis, lichen planus and leukoplakia [9, 10]. Neurological disorders are also observed, which are manifested in the oral cavity in the form of burning sensation in the mouth and tongue, and a perversion of taste.

Structural changes in the salivary glands, impaired salivation and biochemical changes in saliva are noted, which, in its turn, causes xerostomia and the development of complications such as multiple caries, candidiasis and halitosis [1, 4].

A change in the microbial landscape in the oral cavity increases tissue resistance to insulin and contributes to the deterioration of metabolic control of diabetes, and a high concentration of glucose in the gingival fluid is favorable to the reproduction and persistence of the subgingival microflora. Periodontal disease under the DM conditions leads to the development of systemic inflammation, as a result of which the risk of subclinical atherosclerosis and coronary artery disease...
is increased, and as a consequence, the risk of heart attack and stroke increases [11,12]. In this regard, an intense cooperation to involve cardiologists, endocrinologists and dentists seems to be highly reasonable.

In modern studies, it has been shown that the DM patients have an insufficient level of awareness of the relationship between their primary, general, disease and their dental health condition. Considering the essence of the issue, it becomes quite obvious that the relevant measures aimed at improving their oral cavity state are of great importance [1].

Thus, the high prevalence of dental diseases among patients suffering from CVD and DM dictates the need for continuous improvement of dental care for this contingent of patients.

The aim of the study was to study some regional features of the prevalence of dental diseases in patients with CVD and DM, depending on the duration of the progression course, the severity of the underlying disease, the state of compensation in carbohydrate metabolism, age- and gender-related specificity.

Material and research methods

To assess the state of the oral cavity in patients with CVD, 202 patients in total were examined: 96 male inpatients and 106 female inpatients in a hospital cardiology department. Their age ranged from 36 years (1 patient) to 81 years (3 patients). All patients underwent clinical and instrumental examination of the oral cavity. The intensity of caries was determined using an assessment index, which represents the sum of carious, filled and extracted teeth in an examined patient (CFE index). The classification of dentition defects was carried out according to E.I. Gavrilov scale (1968). An assessment of the state of the oral mucosa was carried out using visual examination.

Also, we have examined another cohort, covering 45 patients (20 males and 25 females), with type 1-2 diabetes, hospitalized in an endocrinological department of the city clinical hospital in Nalchik in September 2020, due to DM decompensation, at the age of 45-60 years, upon their informed consent on the study. The degree of the diabetes compensation was determined by the level of glycated hemoglobin.

Research results

The results of our study show a high prevalence and intensity of caries among the patients with CVD diseases. The intensity of caries according to the above mentioned assessment index in men was higher than that found in women: it was reported to be 23.2 teeth, while that recorded in women was 20.2. It should be noted that in the structure of this index the main place is occupied by component "extracted teeth", which is recorded to be 14.8 in men against 13.5 in women. Significantly lower is the contribution made by component "teeth treated for caries and its complications" that is reported to reach 5.22, which is found in men and women almost the same level: 4.92 against 5.52, respectively. As to the contribution of component "caries", we have identified its level of 4.0 in male patients in contrast to the significantly lower level of 1.1 in female patients.

Caries was detected in 20.5% of the examined patients who needed dental treatment. The caries associated complications in the CFE index structure were reported to reach 3.6 with 3.0 in male patients and 4.2 in female patients, respectively. It should be mentioned that the teeth in question were subjected to endodontic treatment and were included into assessment component F.

Our analysis of the study results demonstrated that dental treatment was required for 24.8% of the patients. All examined patients had defects in dentition and needed prosthetics of various orthopedic design versions. One- and two-sided end defects were observed in 20.5% of the patients; 17.6% of the patients had included lateral defects on one or both sides; in 8.8% of the cases found was the complete loss of teeth only in the upper jaw, and single teeth on one or both jaws were recorded in 17.6% of the patients.

Full removable plate prostheses for one or two jaws were used by 44.1% of the examinees, partial removable plate prostheses were found in 38.2% of the cases, and various fixed structures (artificial crowns, bridges) in combination with partial removable plate prostheses were reported to be in 27.7% of the patients in the cohort. New prosthetics upon primarily used prostheses of various designs were required for 13.3% of the examined individuals.

In 53.2% of the patients, poorly manufactured or worn out orthopedic structures were identified, which required repeated prosthetics. Thus, 66.5% of the surveyed individuals needed prosthetics.

Our examination of the DM patients revealed a complex of oral symptoms: coated tongue (33.1%), gum edema and hyperemia (86.3%) and fissured tongue (43.2%).
When examining patients with diabetes, the following structure of inflammatory and destructive periodontal diseases was established:
- generalized gingivitis – in 25.9% of the cases;
- mild chronic generalized periodontitis – in 31.6% of the cases;
- chronic generalized periodontitis of moderate degree - 17.4% of the cases;
- chronic generalized periodontitis of severe degree -15.8% of the cases.

At the same time, with age and an increase in the experience in the diabetes patients, a rise in the prevalence of periodontitis was noted.

Upon our visual assessment of the level of oral hygiene, we identified in 83.3% of the subjects abundant pigmented soft dental plaque, covering the crowns of all teeth completely or half. Dry mouth was found in 80% of the examined subjects.

Dental caries was revealed in 87% of the DM patients. In general, the caries incidence rate, both in terms of its prevalence and intensity, tends to increase with age. Moreover, in patients with decompensated diabetes, the indicators of the intensity of caries were higher. The indicators achieved their highest level in individuals aged 50-59 years (95.1%), the smallest value was reported for the group of patients 60 years of age or older.

At the same time, the degree of tooth decay in the DM patients was distributed as follows: a high and very high degree of tooth decay was noted in 96.3% of the cases, and the average degree of damage was only 3.7%.

All patients were diagnosed with chronic generalized periodontitis: the initial stage- grade I periodontitis was noted in 56.4% of the cases; in 32.8% of the cases we found periodontitis grade I-II, and in 10.8% of the cases we reported periodontitis grade II-III.

Attention should be drawn to the high need for treatment of the dentition, prosthetics and restoration of teeth. The need for prosthetics was recorded in 79.1% of the cases.

Our analysis of the awareness of the presence of dental problems in the DM patients shows a low level of their awareness thereof. Thus, according to a lot of evidence data from the relevant reference sources, considering the results obtained from our own pilot study at the regional level, under the diabetes mellitus conditions, there is a high prevalence of dental morbidity as well as a poor level of oral hygiene available.

Conclusions
Upon completion of our study, we arrive at the following conclusions:
1. The prevalence of dental diseases in patients with CVD and DM depends on the duration and severity of the disease course.
2. Patients with CVD and DM need not only follow-up care for their disease, but they also require treatment and prevention of dental diseases.
3. Management and implementation of scheduled oral cavity sanitation in patients with CVD and diabetes mellitus is an extremely necessary measure to be widely applied in practice.
4. Education of patients with CVD and DM covering oral hygiene, prevention of gum and dental diseases can improve the level of dental care in general.

Statement on ethical issues
Research involving people and/or animals is in full compliance with current national and international ethical standards.

Conflict of interest
None declared.

Author contributions
The authors read the ICMJE criteria for authorship and approved the final manuscript.

References


Impact of the academic performance grades on the blood picture in female students

Vakha A. Anzorov¹, Svetlana V. Moryakina¹

¹ Chechen State University
Russia, 366007, Chechen Republic., Grozny, pr. Bulvar Dudaeva, 17
* Corresponding author:
email: mail@chesu.ru

Abstract
The paper presents the blood picture patterns in female students with different average academic performance grades in their record book. An improvement in the academic performance of female students is accompanied by a significant decrease in the concentration of neutrophils, a significant increase in the level of lymphocytes and minor changes in other blood parameters. Thus, the level of neutrophils experiences a gradual decrease, reaching its minimum of 57.7% (P <0.02) in girls who has performance grade A and 66.2 in case of grade C. The number of lymphocytes in girls with the highest academic performance grades in the record book is recorded to be 33.6% (P <0.05), while in those with the minimum acceptable performance grade it is found to be 26.1%

The level of platelets increases up to 320.8 thousand per microliter (mCL) of blood in the girls with academic performance grade B, decreases to 296.4 in case of grade A, and it is reported to reach 287.0 for those with academic performance grade C.

Keywords
Academic performance, Erythrocytes, Hemoglobin, Leukocytes, Platelets

Imprint

Introduction
The quality of life of students is the main indicator of their health status. Higher educational institutions are responsible not only for training highly qualified graduates, but also for maintaining and improving their health.

The results of studies carried out in recent years demonstrate an increase in occurrence rates of various health disorders among students. Thus, the percentage of students with unsatisfactory health status is recorded to be in the range from 10 to 40% [1]. Studying at the university, new knowledge is acquired.

Acquiring of new knowledge by students stems from the formation of skills and abilities. The factors that determine the career growth of a higher school graduate are the level of his/her expertise, skills and abilities.

Academic success of a student is determined by the depth of the knowledge and skills he/she has acquired within the framework of the required educational standard. The main goal of the university’s activities today is to raise the level of educational services. Due to the fact that the main objective of the individual’s activity is the full realization of his/her capabilities, the biggest challenge in our time is to achieve the highest academic performance in the students.

The academic performance is the student’s capability to acquire the training material and use, if necessary, of the skills and knowledge acquired in mastering the educational program.

On o f the studies on issues of the academic performance has been undertaken by T.V. Gadaya, N.F. Talyzina, B.G. Ananyeva, Yu.V. Bratchikova and Yu.A. Samarina. They have demonstrated that the complex of the factors like the functional state of the body systems and the conditions created in the educational process are decisive for the success of the student’s educational activity.

It is known that mental work has the following features: high tension of the central nervous system and a high rate of the mental processes.

The state of the human intelligence is the main marker, which is deciding for a success both in education and in any kind of activity.

The results of studies both of Russian and foreign researchers demonstrate that the academic performance depends on the characteristics of the individual, the level of the initial background knowledge and adaptive features of the human organism. Researchers have established a direct relationship between the person’s mental abilities and his/her active state. The
person’s abilities determine his/her capabilities in the implementation of any activity, and at the same time his/her active state is a factor, which forms and develops his/her skills.

The educational activity is one of the variants of the mental work, the essence of which is the proper acquisition, storage and use/implementation of a considerable amount of the relevant information.

Exposure to a high information load for a long time can reduce the functional reserves of the human body and affect the human health condition [2].

The learning environment determines both the mental success and the actual academic performance. The state of the functional systems in the student’s body also depends on the conditions of the learning environment.

When organizing the educational activities, universities shall decide on those means and methods that would best contribute not only to improving the mental performance, but also to strengthen health in their students.

Only the presence of good memory and high-focused attention is the key to success in mental activity. The fact that there is a direct relationship between the mental work and the state of the body physiological systems has been noted in [3, 4]. The increased physical and mental loading taken by the central nervous system and the cerebral cortex has a significant impact on such mental processes as attention, memory, thinking and others.

The state of the student’s body systems is influenced by such factors as prolonged sitting position, the impact of stresses of various origins, negative emotions, a high pace of work with a shortage of time under anxiety because of the final result to be achieved.

Since the value of the graduate training on the labor market is determined by his/her successful academic performance in his/her studies at the university, which depends on the state of the body systems, investigations devoted to the blood system state in female students with different academic performance grades should be treated as a topical issue.

Therefore, the purpose of our study is to identify and assess the blood picture in female students with different academic performance grades.

Materials and methods

To study the blood picture in female students with different academic performance grades, we conducted our research in the Human Physiology Laboratory with involvement of the Research Testing Equipment Sharing Center at the Chechen State University.

32 clinically healthy female students of the full-time education participated in our research. Their age ranged from 18 to 21 years.

Depending on their academic performance, the girls were divided into several groups. Each group consisted of 7 to 14 female students. The reference group consisted of the girls with average grade C in their record book.

Full blood count was performed in every participant in our research using MEK7222K Hematology Analyzer manufactured by Nihon Kohden (Korea).

Statistical processing of the obtained research data was carried out with the Biostatistics software.

Results and discussion

The results of the conducted study of blood count data in the female students with different academic performance grades at the university are shown in Table 1 and in Figures 1 and 2 given herein.

From the data given in the above Table and Figures, it can be concluded that the values of the average indicators in the female student groups are within the physiological norm.

It may be deduced that with an improvement in the academic performance of the female students, the concentration of neutrophils in blood significantly decreases, the level of lymphocytes significantly increases, while the other indicators in question do not undergo significant changes [5, 6].

Thus, the level of erythrocytes in blood of the girls having “good” and “excellent” grades is lower by 0.4 and 0.2 million per mcL, and that of hemoglobin decreases by 12.5 and 10.1 g/L, respectively, against those with the academic performance grade “satisfactory”.

The concentration of leukocytes in blood of the girls with the highest average grade in the record book is lower by 0.5 thousand in mm³ of blood, and that recorded in the girls with the “good” grades is higher by 0.6, than in those who have grade “satisfactory.”

The concentration of neutrophils in blood in the female students having grade B is found to be lower by 1.7%, and that in the female students with the A performance grade is recorded to be reduced by 8.5 (P <0.02). The level of eosinophils slightly fluctuates in both directions. Their spread between groups is reported to be 0.7%. The concentration of basophils in
the best grade students is recorded to be 0.2% higher as against in those who have the "satisfactory" grades.

The blood count of the girls with average grade A in the record book shows 7.5% (P <0.02) more lymphocytes than it is the case with the female students with academic performance grade C.

The difference between the maximum and minimum concentration of monocytes is found to be 0.3%. Their highest level is detected in the students with the excellent performance grade.

With an improvement in the academic performance, the level of platelets in the blood slightly fluctuates in both directions. So, the concentration of platelets is reported to be higher in the female students having grade B by 33.8 thousand in microliters, and it is recorded in those with grade A to be higher by 9.4 as compared with the value of 287.0 found in the girls with academic performance grade C.

The detected blood picture in the female students with different academic performance grades shows its distinctive features. The differences in the blood count data are probably caused by different conditions available in each group. An increase in the academic performance is accompanied by a rise in the mental load and a decrease in physical activity. Low physical activity initiates a decrease in the performance of the body systems.

The working capacity, the academic performance, mood, health and life expectancy of a student depend on his/her physical activity.

Metabolism rate and tissue oxygen supply demonstrate their decline with insufficient physical activity and abuse of tobacco smoking [7-9].

Obviously, a slight decrease in the level of erythrocytes and hemoglobin with an improvement in the academic performance of the female students is determined by hypodynamia and high fatigue, the causes of which are high mental stress and disturbed daily schedule.
Insufficient physical activity is the reason for a decrease in the performance of the brain, the organism systems and the organism as a whole. Kalyuzhnaya R.A. [10] believes that high mental stress leads to dysfunction of the main body systems on which health depends.

According to the concept by A.A. Guminsky [11], the performance of the organs and systems in the human body is governed by the state of the muscles. Gorinevsky V.V. [12] reports that physical inactivity inhibits development and weakens the health condition in students. The main factors triggering a drop in the functional activity of the body’s vital systems, according to A.A. Artemenko [13], are low physical activity, high nervous and mental stress and disturbed daily schedule. According to J.J. Rousseau [14], movement is the fundamental condition for cognition of the surrounding world.

Low physical activity not only worsens the health condition, but also inhibits the mental performance [15], therefore high physical activity is recommended for the proper mental development.

Conclusions
We can conclude that a rise in the average grade level in the student’s record book leads to a significant decrease in the number of neutrophils, a significant increase in the level of lymphocytes and minor fluctuations in both directions of other blood parameters in the female students, when analyzing the obtained blood picture patterns in the above mentioned cohorts.

In terms of our quantitative analysis we have identified the following:

- The concentration of erythrocytes in blood in the female students with academic performance grade B is lower by 0.4 million per microliter and in those with academic performance grade A by 0.2 million per microliter, as compared with the C grade group.

- The level of hemoglobin in blood in the female students having the B grades falls to 122.5 g/l, while in the A grade female students it is recorded to reach 124.9 against 135.0 in those having the C grade.

- The number of leukocytes in the groups ranges from 5.4 to 6.5 thousand per mm3.

- The concentration of neutrophils in blood in the female students with the highest average grade in the record book is 8.5% lower (P <0.02) than that recorded in the girls with the minimum positive academic performance grade.

- The blood data found in the female student with the A grade show 6.5% (P <0.02) more lymphocytes than it is reported in case with the C grade female students.

Statement on ethical issues
Research involving people and/or animals is in full compliance with current national and international ethical standards.

Conflict of interest
None declared.

Author contributions
The authors read the ICMJE criteria for authorship and approved the final manuscript.

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Cardiorespiratory system in female students when adapting to university studies

Vakha A. Anzorov¹, Svetlana V. Moryakina¹

¹ Chechen State University
Russia, 366007, Chechen Republic., Grozny, pr. Bulvar Dudaeva, 17

* Corresponding author:
email: mail@chesu.ru

Abstract
The article describes the state of the cardiovascular and respiratory systems in female students in the process of their adaptation to educational activities. The process of study of female students at a higher educational institution is accompanied by a decrease in the indicators of the cardiovascular system performance, so that the value of systolic blood pressure in the 2nd year female students is roughly significantly lower than that recorded in the 1st year of their study. The minimum pressure level, 116.8 mm Hg (P <0.05), is detected in the 2nd year female students against 122.88 mm Hg revealed in the first year group of the female students.

In the process of the girls' adaptation to their educational activities, there is a gradual increase in the vital capacity of lungs (VCL) with the maximum level of 3.11 l (P <0.02) in the fourth year of the study, while the lowest value, 2.66, is found in the first-year female students. The value of the inspiratory reserve volume (IRV) significantly increases by the third and fourth year of the study. IRV is recorded to reach 1.47 l (P <0.05) in the 3rd year of study, 1.46 (P <0.01) in the 4th, and 1.24 in the 1st year of the study, respectively. Studying at a university reduces the expiratory reserve volume to 0.76 l by the second year, by the third and fourth years it increases to 0.94 and 1.08 (P <0.001), while in the first year it is reported to be 0.80.

Keywords
Study, Adaptation, Heart rate, Arterial blood pressure, Tidal volume and lung capacity

Introduction
An adaptation is finding a balance between the organism state and the environment, which should allow the body to favorably exist in the environment. Upon matriculation, an adolescent experiences a change in his/her usual living conditions and lifestyle. As a result of the educational activities, a student at a university is forced to accept new forms and methods of teaching, a new mode of work, sleep, rest, nutrition, and, perhaps, climatic conditions.

Going of higher education institutions to applications of more intensive methods and the introduction of innovative technologies into the educational process may produce a rise in stress loading on the mental and physiological systems of the body in the yesterday's schoolchildren and require strengthening of their activity.

Educational activity at the university places heavy demands on the state of the body's systems and on the health of an adolescent, as it is a long-term complex process.

According to T.N. Semenkova et al. [1], the conditions of study determine the state of mental work and the academic performance of a student. It is also found that the functional state of the body's systems depends thereon.

The functional activity of the body's systems provides for its adaptation to the surrounding conditions and the available educational activities. This means that the conditions, under which the training is carried out, have an impact on the performance of the cardiovascular and respiratory systems in the body, which are responsible for oxygen supply to the human organism.

Some indicators like the health status level, the academic performance and the mental performance govern the adaptability of the student's body to learning activities.

An analysis of the data upon completed research study shows that in recent years the students' adaptation to educational activity has resulted in weakening of the state of the body systems in students.

The peculiarities of studying at a university are high workload, increased emotional and mental stress and insufficient physical activity, which not only hinder the adaptation process, but also inhibit the development in general. As a result of the mental work, which
continues for a long time under the conditions of insufficient physical activity, disordering in the body’s functions occurs.

In the initial period of study, a high mental load under the influence of a shortage of time makes it necessary to master the culture of the mental work.

The process of an adaptation of a student to studying in each year has its own characteristics. Thus, today 30% of the first-year students have a health disorder: in this case, disorders of the digestive, respiratory and cardiovascular systems are most commonly found in the adolescents who begin studying at a university.

A high training load leads to an increase in the tension of the organism systems that may result in disordering of the functional activity of blood circulation and respiration. In this connection, the study of the state of these systems in the process of the educational activity is of great importance.

Therefore, the purpose of our research is to assess the state of the cardiovascular and respiratory systems in female students in the process of their studying at a higher educational institution.

Materials and methods

To identify the state of the cardiorespiratory system in the female students in the process of studying at the university, we conducted our research study in the laboratory of Human Physiology at the Department of Human and Animal Physiology and Anatomy.

The subject of our study was a cohort of 40 full-time female students who participated in our research work. They were all found to be clinically healthy. Their age ranged from 18 to 21 years. In accordance with the years of study, 4 groups 10 female students each were composed. The collection of experimental data was carried out upon the examination of the female students with the use of the Alton-03 electrocardiograph and the OMRON M3 Expert tonometer.

Metering of the respiratory system parameters was carried out using the Diamant-S spiromograph.

For statistical processing of the experimental data obtained, the Biostatistics software was employed.

Results and discussion

The dynamics of the indicators of the performance of the cardiovascular system in the female students during their adaptation to studies at a higher educational institution is shown in Table 1 and Figure 1 given herein.

The results of our conducted study show that during the adaptation by the female students to university studies, only systolic blood pressure undergoes considerable changes. Thus, the level of the maximum blood pressure in the second-year girls is 6.0 mmHg lower (P <0.05), and the third and the fourth year female students are recorded to have a decrease in this blood pressure value by 2.2 and 3.1 mmHg, respectively, against that recorded in the group of the first year girls (122.8 mmHg).

The minimum blood pressure in the 2nd, 3rd and 4th year female students are reported to be reduced by 1.4; 0.8 and 1.8 mmHg, respectively, as compared with 69.0 mmHg in the first year girls.

The girls’ educational activity leads to slight fluctuations in the heart rate values in both directions, not reaching the values recorded in the first year. Thus, the heart beats less frequently in the second-year female students by 5.7 beats per minute; in those of the
third year it is reduced by 1.4 and in the fourth year female students by 1.8 beats per minute as against the first year group. Depletion of the adaptive reserves leads to dysfunction of the student's body systems [2, 3]. According to E.S. Gevorkyan et al, [4], an adaptation to the 1st and 2nd year courses is difficult. Disorders in the cardiovascular system performance in young females may be a consequence of tobacco smoking [5].

The average level of systolic pressure in the first and third year female students and the heart rate values in the girl students in the first, third and last years of study exceed the upper limit of the normal value.

In the process of the adaptation of the students, the academic performance load is the main factor affecting the state of the nervous, cardiovascular and respiratory systems, as it is believed in [6, 7].

The student's educational activity is accompanied by a decrease in the performance of the cardiovascular system, according to L.A. Proskuryakova and E.N. Lobykin [8]. In the process of studying at the university, according to A.A. Artemenko [9], in 46.7% of the boys and 41.7% of the girls, the heart rate is found to be above the normal value.

Under the rest conditions, 42.2% of the young males and 69.1% of the young females show high heart rates [10].

The average values of all indicators characterizing the electrocardiogram curves in the female students, when adapting to the university education, are within the physiological norm.

The time of the atrial contraction in the female students during their educational activities demonstrates minor fluctuations in both directions.

So, the difference between the extreme values of this indicator in the groups is reported to be 0.007 seconds.

The average time of conduction of excitation from the atria to the ventricles in all groups of the female students corresponds to the physiological norm. However, in the process of education, in the females it declines. So, its greatest decline, 0.006 seconds, occurs in the fourth year female students, against 0.165 seconds in the first year girls. During the period of study of the female students at the university, the time of ventricular excitation and contraction coverage recorded remains within the norm.

While the above time is subjected to minor fluctuations in both directions, the range of the QRS time fluctuations between the groups is reported to be from 0.066 to 0.072 seconds.

The range between the extreme average QT values during the study period is recorded to be 0.010 seconds.

The study of the cardiovascular system state in the female students during their adaptation to the university educational conditions shows that the educational activity is accompanied by a significant decrease in their systolic blood pressure and a deviation from the upper normal limit of the maximum blood pressure in

### Table 1

<table>
<thead>
<tr>
<th>Indicator</th>
<th>Year in the study</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1st year</td>
</tr>
<tr>
<td>APs, mm Hg.</td>
<td>122.8±1.99</td>
</tr>
<tr>
<td>APd, mm Hg.</td>
<td>69.0±2.02</td>
</tr>
<tr>
<td>Heart rate, beats per minute</td>
<td>83.4±2.42</td>
</tr>
<tr>
<td>P, s</td>
<td>0.112±0.0023</td>
</tr>
<tr>
<td>PQ, s</td>
<td>0.165±0.0066</td>
</tr>
<tr>
<td>QRS, s</td>
<td>0.072±0.0027</td>
</tr>
<tr>
<td>QT, s</td>
<td>0.345±0.0033</td>
</tr>
</tbody>
</table>

Note: **P < 0.05

### Table 2

<table>
<thead>
<tr>
<th>Indicator</th>
<th>Year in the study</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1st year</td>
</tr>
<tr>
<td>Respiratory movements per minute</td>
<td>17.4±2.02</td>
</tr>
<tr>
<td>Vital capacity of lungs, l</td>
<td>2.66±0.086</td>
</tr>
<tr>
<td>Inspiratory reserve volume, l</td>
<td>1.24±0.036</td>
</tr>
<tr>
<td>Expiratory reserve volume, l</td>
<td>0.80±0.041</td>
</tr>
<tr>
<td>Respiratory volume, l</td>
<td>0.62±0.059</td>
</tr>
</tbody>
</table>

Notes: *P < 0.05; **P < 0.01; ***P < 0.001
the 1st and 3rd year female students, and a deviation from the normal heart rate value in the 1st, 3rd and 4th years, respectively.

Currently, new non-invasive methods are used to measure the parameters of metabolic processes in the cardiac tissue during the initial ECG processing, which can be employed to detect the degree of the adaptation to the educational process of the university students [11, 12].

The respiratory system state in the female students in the process of their adaptation to training at the higher educational institution is shown in Table 2 and Figure 2 herein.

From the data in the above mentioned Table and Figure, it follows that the adaptation to the university studies among the female students leads to a significant increase in the vital capacity of the lungs, the inspiratory and expiratory reserve volumes, to an insignificant increase in the number of respiratory movements and to a minor fluctuation in of the tidal volume in both directions.

The level of the indicators of the respiratory system in the females in the 1st and 2nd year of study is below the lower limit of their physiological norm. In the process of the educational activity, the respiratory rhythm in the 2nd year students decreases by 1.7 movements per minute, and in the female students of the 3rd and 4th year it increases by 2.0 and 3.2, respectively, against the data recorded in the 1st year female students. The value of the vital capacity of the lungs during the adaptation to learning demonstrates a gradual increase. Thus, the VCL value in the females in the fourth year of study is 0.54 liters higher (P <0.01) than that found among the students in the first year. The adaptation of the female students to the educational process leads to a gradual and significant increase in the inspiratory reserve volume parameters.

It increases by 0.27 l (P <0.05) in the 3rd year students, and by 0.31 (P <0.001) in the 4th female students as compared to the 1st year girls. Learning activity leads to a decrease in the reserve expiratory volume in the 2nd year by 0.04 liters and to an increase in the 3rd and 4th years by 0.14 and 0.28 (P <0.001), respectively, as compared to the 1st year students (0.80 liters).

The tidal volume in the females adapting to the university education is subjected to slight level fluctuations in both directions. So, in females of the 2nd year it is 0.05 liters higher, and it is recorded to be 0.04 and 0.05 lower in the 3rd and 4th year, respectively, than it is the case in the first year (0.62).

Obviously, a gradual increase in the indicators of the respiratory system performance in the process of educational activity should be attributed to a rise in the excitability of the sympathetic part of the autonomic nervous system; it is also an age-related factor. Nifontova O.L. and R.K. Nasrullaev [13] note that in young males aged 20-22, the activity of the parasympathetic nervous system becomes higher in the regulation of body functions, while in young females observed is an elevated activity of the sympathetic part.

Zakharina E.A. [10] believes that students do not complete their adaptation even by their fourth year. So, it may be concluded that stresses induced by the educational activity lead to significant changes in the indicators of the performance of the respiratory and cardiovascular systems in students [14].

From the results of the conducted research we can arrive to a conclusion that the low level of the physical fitness is the reason for insufficient adaptation of students [15].

Based on the results of their research, I.N. Pushkareva, S.V. Kumskov and S.A. Novoselov [16] state that students have a low level of physical fitness and poor physical health.

Batrymbekova S.A. [17] reports that only 28.1% of the students reach a high level of the adaptation to learning, 52.2% of them demonstrate an average level and 19.75% a low level thereof.

The results of our research show that the adaptation by the female students to study at a higher educational institution leads to a significant increase in the vital capacity of lungs, the reserve volume of inspiration and expiration and to insignificant fluctuations in both directions in the number of respiratory movements per minute and the tidal volume values.

Conclusions

- The mean values of systolic and diastolic pressure between the groups of female students range from 116.8 and 67.2 mm Hg up to 122.8 and 69.0 mm Hg.
- The electrocardiogram indicators in the female students in the process of study are subjected to insignificant fluctuations in both directions.
- The increase in the vital capacity of lungs in the 4th year female students is 0.54 l (P <0.01), in the reserve volume of inspiration 0.31 l (P <0.001) and that
of exhalation 0.28 l (P <0.001), as compared to the respective data obtained in the first year female students.

The range of fluctuations in the number of respiratory movements between the groups is recorded to be from 15.7 to 20.6 movements per minute, and the tidal volume ranges from 0.57 to 0.67 l.

Statement on ethical issues
Research involving people and/or animals is in full compliance with current national and international ethical standards.

Conflict of interest
None declared.

Author contributions
The authors read the ICMJE criteria for authorship and approved the final manuscript.

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Motivational interactive prevention in outpatients with ischemic heart disease

Natalia A. Koryagina1*, Grigoriy N. Spasenkov1, Aleksey V. Avdeev1, Sofia G. Shulkina1, Vladimir S. Koryagin1

1 Perm State Medical University named after Academician E. A. Wagner
Russia, 614000, Perm, Kuibysheva str., 39
* Corresponding author:
phone: +7 (342) 217-20-20

Abstract
Working women suffering from ischemic heart disease (IHD), who receive ambulance cardiology treatment, have completed their training course at our Health School (HS), and their medical examination data have been compared with those in the reference group representing women with IHD, employed by the same enterprise, who have not attended HS. The training course program has included 5 training lessons, 60 minutes each. The educational training material was presented in the interactive form. To monitor the state in the trained outpatients within one year, used were telehealth technologies including teleconsultation sessions held every week. The clinical effect was monitored and assessed upon expiration of 12 months with the use of the respective closing medical examination. It has been found that in the HS group outpatients the levels of arterial pressure, total cholesterol and scores of anxiety depression syndrome are much lower than those identified in the reference group. The adherence to the administered therapy in the reference group was recorded to be less than 50%. It is reported that the implementation of the motivational prevention program has demonstrated its feasibility for the Employer: the cost effectiveness has been assessed as the return rate at the level of Rbl. 1,4 per Rbl of investment not only due to reduction in paid sick leaves, but also due to an increase in labor productivity.

Keywords
Health School, Ischemic heart disease, Women, Cost effectiveness, Motivational interactive prevention

Materials and methods
We have conducted an observation of 200 women with IHD, employed by the same enterprise. Among the whole cohort, 100 female outpatients, receiving the relevant cardiology therapy, have attended and completed their training courses at our Health School (HS) according to the Methodological Management Record [6], while the other 100 non-attending women, suffering from IHD, have been considered as the reference group of the observed outpatients.

Training provided by our HS is a method of rational psychotherapy, which represents a health management program consisting of 5 lessons to cover the basic topics connected with IHD. In order to complete the training program for the employed women, we have additionally involved the following medical staff: a Doctor of Physical Therapy/Sports Medicine and an Endocrinologist. The duration of every lesson has been scheduled to be 60 minutes. The training material has been offered in the interactive form; electronic presentations have been displayed; the training scenarios implied switching from every information module to a discussion of the presented information, treating
of some clinical cases or role-playing. To monitor the current state in every of 200 female outpatients, a dedicated electronic resource has been developed that has been used every week for online consultation within the one-year period, furnished with an option of feedback and an option to schedule an appointment at a doctor’s office, if required, for the purpose of the office visit consultation.

Monitoring of the clinical effect upon expiration of 12 months have been provided in accordance with the assessment procedure of clinical efficacy & cost effectiveness of the given interactive prevention program. Upon expiration of 12-month-observation period, all participants of the motivational prevention program versus the reference group have been subjected to the proper closing medical examination. Our assessment of the clinical effect made by the HS motivational training versus the reference has included the following:

- the dynamics of arterial pressure (AP) in the above groups;
- the levels of blood total cholesterol (total cholesterol) and blood glucose;
- body mass index (BMI) and waist circumference values;
- psychoemotional risk factors (RF) (acc. to the Hospital Anxiety and Depression Scale (HADS));
- PROCAM risk scoring.

Results

All the above outpatients have been under observation for the one-year period. We can notice that the dynamics of the data on the clinical metabolic and neuropsychological status in the outpatients has been recorded (see Table 1 herein). So, upon expiration of 12 months, the group of the HS motivated outpatients has demonstrated in their screening that the levels both of systolic and diastolic AP are significantly lower than those recorded initially, i.e. the effects established by the administered therapy and the HS preventive measures have been maintained for a long period of time. As to the reference group, upon expiration of the 12-month period, we have not identified any significant dynamics of the systolic and diastolic AP values. Recorded has been a pronounced change pattern of lowering of total cholesterol within the one-year period due to the respective 3-month hypolipid medication as well as due to the high adherence to the statin therapy.

We have also recorded that the HS group outpatients have permanently used the special electronic resources, particularly e-mailing, where the consultation options have been available in order to discuss the biochemical screening data, including those on their values of cholesterol and transaminases. The reference group outpatients, who have been administered with hypolipid drugs, have demonstrated their low adherence to the medication. Upon their closing medical examination, it has been reported that less than 50% of them have maintained their adherence to the statin treatment. As to the recorded BMI values in the HS group, we have noted a significant reduction in their body mass and the maintenance of the tendency for 12 months; in this case we have observed the hypocaloric diet keeping level 20% higher against that recorded in the reference group. For the female outpatients in the motivated HS group we have reported a significant lowering of their anxiety and depression scores within the 12-month observation period as against insignificant growth of the same parameters in the reference group. To summarize the above, the motivated HS outpatients have shown in most cases the significantly decreased parameters in question (the AP level, total cholesterol and the level of the anxiety / depression syndrome) as compared to their respective initial values recorded.

A decrease in the total number of the hospital stay days for circulatory diseases (CD) has been identified in the HS group. We can also highlight that the number of the office visits to a cardiologist, an endocrinologist and a physician have been reduced. The frequency of the outpatients’ visits to primary care offices in the reference group has been reported to be lower, but to a much lesser degree than it has been the case with the HS group: it might be attributed to the primary diagnostics, to revealing the IHD risk factors at an early stage and making the working women aware thereof. As to the motivated HS group, the number of the ambulance calls before their participation in the motivational interactive prevention program had reached 3,5, and after their participation no ambulance calls in total have been reported. It should be mentioned that the number of the recorded emergency calls in the reference group has decreased, too.

The HS outpatients have demonstrated a considerable reduction in paid sick leave days related to CDs, while for the reference group we have identified more paid sick leave days for these diseases that has involved a substantial growth of costs associated with the sick leave payment volume. It may be explained by the fact
that the reference group outpatients, who have not attended the HS training lessons, but who have been aware of possible CVD risks, had more often to use medical services.

An analysis of the paid sick leave data on effects produced by Health School training and treatment in social service teams supervised by the Employer is one of the most useful approaches to assessing the prevention effectiveness by the primary healthcare institutions.

Generally, the total annual costs connected with CVDs in the HS group outpatients have been found to be lower as compared with those in the reference group, and their total volume has been recorded as Rbl. 172 084,5 in the HS group versus Rbl.445570,5 in the reference group, respectively. During the HS group observation, we have recorded the following significant changes: a decrease in the SAP values by 6,0 mmHg, a decrease in the DPA values by 5,8 mmHg, and lowering risks according to PROCAM score by 10-15 due to an improvement in the HS outpatients’ lipid profile.

### Discussion

Decisions on visiting a health care office are more often made by women, and this category is known to use more intensively the health care resources. As to our observations, we can state that in the reference group the SAP values and PROCAM risk scoring have been increased, but no considerable DAP changes have been identified. When reviewing the effectiveness of the prevention program in the HS group, we have ob-

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**Table 1**

Dynamics of the obtained data in the HS group versus the reference group

<table>
<thead>
<tr>
<th>Values, data</th>
<th>HS group</th>
<th>Reference group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Initially</td>
<td>Upon exp.of 12 months</td>
</tr>
<tr>
<td>SAP, mmHg</td>
<td>131,4±16,3</td>
<td>124,6±14,3</td>
</tr>
<tr>
<td>DAP, mmHg</td>
<td>90,3±23,9</td>
<td>81,3±13,7</td>
</tr>
<tr>
<td>Total cholesterol, mmol/L</td>
<td>7,6±2,4</td>
<td>5,2±1,4</td>
</tr>
<tr>
<td>Glucose, mmol/L</td>
<td>5,4±1,2</td>
<td>5,0±1,2</td>
</tr>
<tr>
<td>BMI, kg/m2</td>
<td>29,7±7,6</td>
<td>27,8±5,4</td>
</tr>
<tr>
<td>Anxiety, total score</td>
<td>9,0±3,4</td>
<td>6,5±3,4</td>
</tr>
<tr>
<td>Depression, total score</td>
<td>8,6±3,4</td>
<td>4,9±2,3</td>
</tr>
<tr>
<td>Menopause index, score</td>
<td>24,5±3,4</td>
<td>19,5±3,5</td>
</tr>
<tr>
<td>PROCAM scoring (%)</td>
<td>46±8,5</td>
<td>34±7,5*</td>
</tr>
</tbody>
</table>

p < 0,05 (intra-group differences, Mann Whitney U test)

**Table 2**

Costs analysis in the HS group vs. the reference group within the one-year period

<table>
<thead>
<tr>
<th>Cost item</th>
<th>HS group (n=100)</th>
<th>Reference group (n=100)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Initial</td>
<td>Upon exp. of one year</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hospital stay days</td>
<td>22,1</td>
<td>12,1</td>
</tr>
<tr>
<td>Hospital stay costs, Rbl.</td>
<td>41 523</td>
<td>21 640</td>
</tr>
<tr>
<td>Outpatient appointments, number of visits to outpatient office</td>
<td>24,3</td>
<td>4,3*</td>
</tr>
<tr>
<td>Ambulance calls, number</td>
<td>3,5</td>
<td>0</td>
</tr>
<tr>
<td>Volume of ambulance call costs, Rbl.</td>
<td>9837,4</td>
<td>0</td>
</tr>
<tr>
<td>Initial prevention examination, Rbl.</td>
<td>93 085</td>
<td>93 085</td>
</tr>
<tr>
<td>Motivational interactive prevention, Rbl.</td>
<td>75 025</td>
<td></td>
</tr>
<tr>
<td>Closing medical examination, Rbl.</td>
<td></td>
<td>93 085</td>
</tr>
<tr>
<td>Direct costs, total, Rbl.</td>
<td>22 5540,4</td>
<td>115 950,5</td>
</tr>
<tr>
<td>Indirect costs</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Paid sick leave days</td>
<td>258,4</td>
<td>43,2</td>
</tr>
<tr>
<td>Paid sick leave days, volume of costs, Rbl.</td>
<td>234,056</td>
<td>56,134</td>
</tr>
<tr>
<td>Costs, total</td>
<td>45 9596,4</td>
<td>172 084,5</td>
</tr>
</tbody>
</table>

p < 0,05 (intra-group differences, Mann Whitney U test)
tained the data revealing that the specific cost of reducing the SAP level by each 1 mmHg per 100 employees has amounted to Rbl.12 152, and in case of DAP reducing under the same conditions the specific cost has reached Rbl.11 350, while for the reference group the specific cost under the same SAP reducing conditions has been reported to be approximately Rbl.25 000, with one or more drugs changed; as to the specific cost to reduce DAP in the reference group, they have been found ineffective in general. The specific cost volume to decrease hypercholesterolemia by 0.1 mmol/L of the entire group has amounted to Rbl. 21 083. The costs to reduce the CVD risk in the motivational interactive group have been reported to be rather high: they have run as high as Rbl. 60 450 in order to cover all major modified risk factors: arterial hypertension, obesity, high cholesterol level and hypodynamia. It should be mentioned, that at present no benchmarking criteria for assessing acceptable costs to achieve the specified clinical effect or the relevant cost effectiveness analysis can be found in the reference literature. Taking into account all the above, the outcome deduced from our analysis of the cost effectiveness in the context of the motivational interactive prevention can be considered as profitable, while in the reference group the total costs connected with rendering medical services and losses due to paid sick leave days have been found as substantially uncompetitive: the expenditures incurred have not led to any improvement in the predicted health condition, i.e. the use of the cost resources can be regarded as inefficient. The total costs have amounted to Rbl. 447 365 per annum. When analyzing the costs connected with the motivational prevention program from the point of view of the Employer, we make an attempt to calculate the return on investment (ROI). The total input cost of the investment has covered the costs of the initial prevention examination and the closing medical examination (the dynamics of the initial versus final data) focused on an identification of the major IHD risks, including the metabolism profile, plus the Health School training costs, so that the volume of which has reached Rbl. 261 195 per 100 outpatients in the HS group. Upon our analysis of the data on the paid sick leave days per Rbl. 100 000 at the enterprise, considering therewith the costs of the prevention investment, is has been found that, a total decrease in the number of the paid sick leave days by 88.4 days has been recorded for the full operating year. The detected tendencies in the paid sick leave data dynamics bear witness to the favorable effect produced by of the implemented Health School prevention program revealed for the working women. In general, the completion of the motivational interactive prevention program has been found as expedient and cost-effective from the point of view of the Employer, since we have recorded per Rbl.100 000 invested in the program a gross product output of Rbl. 140 314,2 not only due to the reduction in the paid sick leave days, but also due to an increase in labor productivity.

Conclusions

Our evidence data have shown that an effective prevention can be provided not only on the basis of the complete technological cycle of the relevant measures, and it shall not be limited to preventive examinations only: it also shall be accompanied by motivational interactions addressing actual demands, taking into account some gender- and team-related specificity. Our research has proven the feasibility of the implementation of the motivational interactive prevention program for a team of employees that is targeted at reduction of the VD exacerbation events from the point of view of the Employer.

Statement on ethical issues

Research involving people and/or animals is in full compliance with current national and international ethical standards.

Conflict of interest

None declared.

Author contributions

The authors read the ICMJE criteria for authorship and approved the final manuscript.

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Mortality from circulatory system diseases in the Kabardino-Balkarian Republic in 2015-2019


1 Federal State Budgetary Educational Institution of Higher Education "Kabardino-Balkarian State University named after H.M. Berbekov" Russia, 360000, Nalchik, Chernyshevskogo str., 173
2 Rostov State Medical University of the Ministry of Health of the Russian Federation Russia, 344022, Rostov-on-Don, Suvorova str., 119

* Corresponding author:
phone: +7 (866) 242-25-60

Abstract
Aim of the study is to analyze the mortality rates of the population of the Kabardino-Balkarian Republic (KBR) from circulatory system diseases (CSD) for the period 2015-2019 to determine measures to reduce the mortality of the population of the Republic.

Material and methods
The data of Rosstat on the number of deaths classified by statistically recorded causes were used according to official “Brief Classification of Causes of Death”. The level and dynamics of total mortality and mortality from CSD of the population of the Republic for the above five-year period, the nosological structure of the causes of death of the CSD class, the share of CSD in the structure of the total mortality of the population of KBR were studied herein. Non-standardized mortality rates were utilized.

Results
Mortality from CSD in 2019 was reported to be 384.3 per 100 thousand population (46.1% of deaths from all causes). The share of cerebrovascular disease (CVD) (I60-I69) of all deaths from CSD was 34.5% and that from ischemic heart disease (IHD) (I20-I25) reached 21.7%. Over the five-year period, the mortality rate from CSD decreased by 103.2 per 100 thousand of the population. There was an increase in mortality from hypertension (23.8 per 100 thousand population in 2015 against 26.1 per 100 thousand population in 2019) and from myocardial infarction (15.7 per 100 thousand population in 2015 against 16.5 per 100 thousand population in 2019).

Conclusion
Despite the recorded decrease in mortality, CSD retains a leading position in the structure of mortality in the Republic. The revealed features of the structure and dynamics of mortality from CSD dictate the need to develop a set of measures to improve medical care for patients with CSD. Conducting an epidemiological study seems to be an important step towards a comprehensive analysis of the situation and an introduction of targeted prevention programs.

Keywords
Mortality, Causes of death, Diseases of the circulatory system, ICD-10, Kabardino-Balkarian Republic

Introduction
Circulatory system diseases (CSD) are the leading cause of death both worldwide and in the Russian Federation. Despite the trend in the last decade towards a decrease in the share of deaths from CSD, the mortality rates for this class of diseases in the Russian Federation exceed the corresponding indicators of all economically developed countries [1, 2]. Ischemic heart disease (IHD) and cerebrovascular diseases (CVD) occupy the main positions in the structure of mortality from CSD among cardiovascular diseases leading to death [3-5]. It is known that the level and dynamics of mortality from CSD have some regional characteristics. It is also known that the structure and dynamics of mortality can be influenced by two groups of causes. The first group thereof includes the frequency of CSD risk factors, the implementation of preventive programs, the development of modern medical technologies, the quality and availability of medical care, and the patient adherence to treatment. The second group thereof is associated with the sys-
tem of official registration of deaths from CSD, which includes the knowledge and ability of doctors to use the proper coding rules to properly select the initial cause of death and methods of formulating a post-mortem clinical diagnosis [5]. The CSD prevention issues are the subject of close attention paid by the regional authorities in the Kabardino-Balkarian Republic. Currently, the Kabardino-Balkarian Republic (KBR) is implementing its regional projects “Combating cardiovascular diseases (Kabardino-Balkarian Republic)” and “Forming a system of motivating citizens to a healthy lifestyle, including healthy eating style and rejection of bad habits (Kabardino-Balkarian Republic)” within the framework of the relevant Federal Projects. There is no doubt that the planning and execution of interventions aimed at reducing CSD mortality in KBR requires an assessment of the actual mortality levels and dynamics, a thorough analysis of the nosological structure of mortality and the factors influencing these indicators.

Purpose of the study is to analyze the mortality rates of the population of the Kabardino-Balkarian Republic (KBR) from circulatory system diseases (CSD) for the period 2015-2019 to determine measures to reduce the mortality in the population of the Republic.

Material and methods

The study was carried out in the Kabardino-Balkarian Republic, which occupies an area of 12,470 km² (the 79th region of 85 regions). The resident population of the Republic as of January 1, 2019 is 868,350 people. The structure of the Republic's population at the beginning of 2018 is represented by 21.7% of the people under the working age, 57.4% of the people of working age and 20.9% thereof over working age [6]. For our analysis, we used the official Rosstat data on the number of deaths according to causes of death based on the applicable “Brief Classification of Causes of Death”. The level and dynamics of mortality of the population of the KBR, the share of these deaths in the total number of deaths in 2015 and 2019, the share of CSD in the structure of the total mortality of the population of the KBR, the nosological structure of causes of death of the CSD class, the share of each of the causes of death in the number of deaths from CSD, the level and the dynamics of mortality from CSD over a five-year period were studied. The study used non-standardized mortality rates. The mortality rates for 2019 are analyzed based on preliminary data for January-December 2019 (excluding final medical death certificates), due to the lack of updated data at the time of the study.

Results

In 2019, 7231 people died in the KBR (the mortality rate was recorded to be 833.8 per 100 thousand of the population), among which 3333 people died from CSD (the mortality rate was recorded to be 384.3 per 100 thousand population, and the share of deaths from all causes was 46.1%). From 2015 to 2019, there was a decrease in mortality from CSD (see Figure 1 herein), which led to a decrease in the share of the latter in the structure of all-cause mortality from 55.6% in 2015 to 46.1% in 2019.

The nosological structure of the causes of death of the CSD class in 2015 and 2019 and the share of each of the causes of death as the numbers of deaths from CSD are presented in Table 1 herein.

CVD (I60-I69) and IHD (I20-I25) made a significant contribution to mortality from CSD. The share of CVD in the structure of mortality from CSD remained high throughout the analysis period with a slight decrease from 38.1% in 2015 to 34.5% in 2019. At the same time, the share of acute disorders of cerebral circulation (I60-I64) in the structure of mortality in different years was 45.3-38.8% of CVD (see Figure 2 herein), and most of the deaths were attributed to other cerebrovascular diseases. Thus, the number of deaths from CVD (I41-I50) was 1151 people in 2019 (34.5% of all CSDs), and acute cerebrovascular accidents (I60-I64) caused the death of 475 people, which was 14.3% of all deaths from CSD and 41.3% of all deaths from CVD.

The share of deaths from chronic atherosclerotic and other lesions of cerebrovascular vessels, which included “Cerebral atherosclerosis”, “Other specified lesions of cerebral vessels”, “Cerebrovascular disease, unspecified”, “Sequela of cerebrovascular diseases”, “Other cerebrovascular encephalopathy” accounted for 20.3% of deaths from CSD and 58.7% of deaths from CVD. In 2015, acute disorders of cerebral circulation (I60-I64) caused the death of 724 people that accounted for 17.3% of all deaths from CSD and 45.3% of all deaths from CVD. The percentage of deaths from chronic atherosclerotic and other lesions of cerebrovascular vessels was 20.8% of all deaths from CSD and 54.8% of all deaths from CVD. In 2019, compared to 2015, there was an increase in mortality from cerebral infarction (I63), hypertensive
Figure 1. Dynamics of total mortality, mortality from CSD and other death causes of the population in the Kabardino-Balkarian Republic for the period 2015-2019

Table 1  
The numbers and shares of deaths from CSD in 2019 compared to 2015

<table>
<thead>
<tr>
<th>Brief classification of causes of death</th>
<th>ICD 10</th>
<th>Causes of death</th>
<th>The absolute number of deaths in 2019</th>
<th>Share (%) of deaths from CSD in 2019</th>
<th>The absolute number of deaths in 2015</th>
<th>Share (%) of deaths from CSD in 2015</th>
</tr>
</thead>
<tbody>
<tr>
<td>121</td>
<td>I00-I02</td>
<td>Acute rheumatic fever</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>122</td>
<td>I05-I09</td>
<td>Chronic rheumatic heart diseases</td>
<td>16</td>
<td>0,48</td>
<td>9</td>
<td>0,21</td>
</tr>
<tr>
<td>123</td>
<td>I11</td>
<td>Hypertensive heart disease</td>
<td>203</td>
<td>6,09</td>
<td>161</td>
<td>3,83</td>
</tr>
<tr>
<td>124</td>
<td>I12</td>
<td>Hypertensive chronic kidney disease</td>
<td>1</td>
<td>0,03</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>125</td>
<td>I13</td>
<td>Hypertensive heart and chronic kidney disease</td>
<td>5</td>
<td>0,15</td>
<td>3</td>
<td>0,07</td>
</tr>
<tr>
<td>123-125</td>
<td>I11-I13</td>
<td>Hypertensive heart and CKD with heart failure and/or CKD</td>
<td>209</td>
<td>6,27</td>
<td>164</td>
<td>3,91</td>
</tr>
<tr>
<td>126</td>
<td>I10</td>
<td>Essential (primary) hypertension</td>
<td>17</td>
<td>0,51</td>
<td>41</td>
<td>0,98</td>
</tr>
<tr>
<td>123-126</td>
<td>I10-I13</td>
<td>Hypertensive diseases</td>
<td>226</td>
<td>6,78</td>
<td>205</td>
<td>4,88</td>
</tr>
<tr>
<td>127</td>
<td>I21</td>
<td>ST elevation (STEMI) and non-ST elevation (NSTEMI) myocardial infarction</td>
<td>137</td>
<td>4,11</td>
<td>114</td>
<td>2,71</td>
</tr>
<tr>
<td>128</td>
<td>I22</td>
<td>Subsequent ST elevation (STEMI) and non-ST elevation (NSTEMI) myocardial infarction</td>
<td>6</td>
<td>0,18</td>
<td>21</td>
<td>0,50</td>
</tr>
<tr>
<td>Brief classification of causes of death</td>
<td>ICD 10</td>
<td>Causes of death</td>
<td>The absolute number of deaths in 2019</td>
<td>Share (%) of deaths from CSD in 2019</td>
<td>The absolute number of deaths in 2015</td>
<td>Share (%) of deaths from CSD in 2015</td>
</tr>
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<td>----------------------------------------</td>
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<td>---------------------------------------------------------------------</td>
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<td>--------------------------------------</td>
</tr>
<tr>
<td>127-128</td>
<td>I21- I22</td>
<td>Myocardial infarction</td>
<td>143</td>
<td>4,29</td>
<td>135</td>
<td>3,21</td>
</tr>
<tr>
<td>129</td>
<td>I25.1</td>
<td>Atherosclerotic heart disease</td>
<td>236</td>
<td>7,08</td>
<td>444</td>
<td>10,57</td>
</tr>
<tr>
<td>130</td>
<td>I25.0</td>
<td>Atherosclerotic cardiovascular disease, so described</td>
<td>24</td>
<td>0,72</td>
<td>67</td>
<td>1,60</td>
</tr>
<tr>
<td>131</td>
<td>I25.9</td>
<td>Chronic ischemic heart disease, unspecified</td>
<td>173</td>
<td>5,19</td>
<td>185</td>
<td>4,40</td>
</tr>
<tr>
<td>132</td>
<td>I25.2-.8</td>
<td>Other forms of chronic ischemic heart disease</td>
<td>81</td>
<td>2,43</td>
<td>82</td>
<td>1,95</td>
</tr>
<tr>
<td>133</td>
<td>I20, I24.1-.9</td>
<td>Other forms of acute ischemic heart disease</td>
<td>67</td>
<td>2,01</td>
<td>128</td>
<td>3,04</td>
</tr>
<tr>
<td>127-133</td>
<td>I20- I25</td>
<td>Coronary heart disease</td>
<td>724</td>
<td>21,72</td>
<td>1041</td>
<td>24,79</td>
</tr>
<tr>
<td>134</td>
<td>I26-128</td>
<td>Pulmonary heart disease and diseases of pulmonary circulation</td>
<td>295</td>
<td>8,85</td>
<td>288</td>
<td>6,86</td>
</tr>
<tr>
<td>135</td>
<td>I42.6</td>
<td>Alcoholic cardiomyopathy</td>
<td>23</td>
<td>0,69</td>
<td>96</td>
<td>2,29</td>
</tr>
<tr>
<td>136</td>
<td>I42.9</td>
<td>Cardiomyopathy, unspecified</td>
<td>63</td>
<td>1,89</td>
<td>1</td>
<td>0,02</td>
</tr>
<tr>
<td>137</td>
<td>I51.5</td>
<td>Myocardial degeneration</td>
<td>8</td>
<td>0,24</td>
<td>52</td>
<td>1,24</td>
</tr>
<tr>
<td>138</td>
<td>I50.9</td>
<td>Heart failure, unspecified</td>
<td>83</td>
<td>2,49</td>
<td>91</td>
<td>2,17</td>
</tr>
<tr>
<td>139</td>
<td>I46.1</td>
<td>Cardiac arrest</td>
<td>31</td>
<td>0,93</td>
<td>50</td>
<td>1,19</td>
</tr>
<tr>
<td>140</td>
<td>I30-141, I42.0-.5, I43-145, I46.6-9, I47-149, I50.0, I51.0-.4, I51.6-.9</td>
<td>Other heart diseases</td>
<td>73</td>
<td>2,19</td>
<td>67</td>
<td>1,60</td>
</tr>
<tr>
<td>141</td>
<td>I60</td>
<td>Subarachnoid hemorrhage</td>
<td>29</td>
<td>0,87</td>
<td>41</td>
<td>0,98</td>
</tr>
<tr>
<td>142</td>
<td>I61-162</td>
<td>Nontraumatic intracerebral hemorrhage and other and unspecified nontraumatic intracranial hemorrhage</td>
<td>95</td>
<td>2,85</td>
<td>212</td>
<td>5,05</td>
</tr>
<tr>
<td>143</td>
<td>I63</td>
<td>Cerebral infarction</td>
<td>295</td>
<td>8,85</td>
<td>241</td>
<td>5,73</td>
</tr>
<tr>
<td>144</td>
<td>I64</td>
<td>Stroke not specified as hemorrhage or infarction</td>
<td>56</td>
<td>1,68</td>
<td>230</td>
<td>5,47</td>
</tr>
<tr>
<td>145</td>
<td>I67.2</td>
<td>Cerebral atherosclerosis</td>
<td>283</td>
<td>8,49</td>
<td>700</td>
<td>16,67</td>
</tr>
<tr>
<td>146</td>
<td>I67.4</td>
<td>Hypertensive encephalopathy</td>
<td>11</td>
<td>0,33</td>
<td>2</td>
<td>0,05</td>
</tr>
</tbody>
</table>

| 127-128 | I21- I22 | Myocardial infarction | 143 | 4.29 | 135 | 3.21 |
| 129     | I25.1   | Atherosclerotic heart disease | 236 | 7.08 | 444 | 10.57 |
| 130     | I25.0   | Atherosclerotic cardiovascular disease, so described | 24 | 0.72 | 67 | 1.60 |
| 131     | I25.9   | Chronic ischemic heart disease, unspecified | 173 | 5.19 | 185 | 4.40 |
| 132     | I25.2-.8 | Other forms of chronic ischemic heart disease | 81 | 2.43 | 82 | 1.95 |
| 133     | I20, I24.1-.9 | Other forms of acute ischemic heart disease | 67 | 2.01 | 128 | 3.04 |
| 127-133 | I20- I25 | Coronary heart disease | 724 | 21.72 | 1041 | 24.79 |
| 134     | I26-128 | Pulmonary heart disease and diseases of pulmonary circulation | 295 | 8.85 | 288 | 6.86 |
| 135     | I42.6   | Alcoholic cardiomyopathy | 23 | 0.69 | 96 | 2.29 |
| 136     | I42.9   | Cardiomyopathy, unspecified | 63 | 1.89 | 1 | 0.02 |
| 137     | I51.5   | Myocardial degeneration | 8 | 0.24 | 52 | 1.24 |
| 138     | I50.9   | Heart failure, unspecified | 83 | 2.49 | 91 | 2.17 |
| 139     | I46.1   | Cardiac arrest | 31 | 0.93 | 50 | 1.19 |
| 140     | I30-141, I42.0-.5, I43-145, I46.6-9, I47-149, I50.0, I51.0-.4, I51.6-.9 | Other heart diseases | 73 | 2.19 | 67 | 1.60 |
| 141     | I60     | Subarachnoid hemorrhage | 29 | 0.87 | 41 | 0.98 |
| 142     | I61-162 | Nontraumatic intracerebral hemorrhage and other and unspecified nontraumatic intracranial hemorrhage | 95 | 2.85 | 212 | 5.05 |
| 143     | I63     | Cerebral infarction | 295 | 8.85 | 241 | 5.73 |
| 144     | I64     | Stroke not specified as hemorrhage or infarction | 56 | 1.68 | 230 | 5.47 |
| 145     | I67.2   | Cerebral atherosclerosis | 283 | 8.49 | 700 | 16.67 |
| 146     | I67.4   | Hypertensive encephalopathy | 11 | 0.33 | 2 | 0.05 |
encephalopathy (I67.4), other specified cerebral vascular lesions (I67.8), and unspecified cerebrovascular disease (I67.9). Of practical importance was the decrease in the share of deaths from stroke not specified as hemorrhage or infarction (I64) among CSDs from 5.47% in 2015 to 1.68% in 2019.

As to IHD, chronic rather than acute forms of this pathology were recorded as the cause of death (see Figure 2 herein). The number of deaths from IHD in 2019 was 724 (21.7% of all CSD deaths), including 143 deaths from myocardial infarction (acute MI and recurrent AMI) and 67 deaths from other forms of acute ischaemic heart disease (6.3% of all CSD deaths). The percentage of deaths from the causes “Atherosclerotic heart disease” (I25.1) and “Atherosclerotic cardiovascular disease so described” (I25.0), “Chronic ischaemic heart disease, unspecified” (I25.9) and “Other forms of chronic ischaemic heart disease” (I25.8) in 2019 was 15.4% of all CSD deaths. The vast majority of the IHD deaths (74.7% of all IHD deaths in 2015 and 71% of all IHD deaths in 2019) should be attributed to nonacute coronary events. Compared to 2015, an increase in deaths from chronic coronary heart disease, unspecified (I25.9), other forms of coronary heart disease (I25.2-6,8) and acute myocardial infarction (I21) were recorded in 2019 against a background of decreasing mortality from IHD (I20-I25) and most causes of that group.

All other causes of death from CSD accounted for 1,458 deaths in 2019 (43.7% of all CSD deaths) and 1,559 deaths in 2015 (37.1% of all CSD deaths). Among those, an increase in the number of deaths in 2019 compared to 2015 was recorded from hypertension with heart failure and CKD, chronic rheumatic heart disease, pulmonary heart disease and pulmonary circulatory disorders, unspecified cardiomyopathy, unspecified heart failure, other heart diseases, atherosclerosis, other arterial, arteriolar and capillary
diseases, phlebitis and thrombophlebitis, thrombosis and embolism and other diseases of veins and lymphatic vessels. There was a decrease in the number of deaths in the other cases.

The dynamics of mortality from CVD, IHD and hypertension for the period 2015-2019 is shown in Figure 2 herein. Over the five-year period of our analysis, a decrease in mortality from acute cerebrovascular accidents (I60-I64) was revealed. Moreover, the greatest decrease in mortality took place in 2017 compared to 2016. There was a decrease in mortality from hypertension (I10-I13) from 23.8 per 100 thousand population in 2015 to 12.2 per 100 thousand population in 2018 with a subsequent increase to 26.1 per 100 thousand population in 2019. The lowest death rate from myocardial infarction was recorded in 2017 (15.3 per 100 thousand population) with an increase to 16.5 per 100 thousand population in 2019.

Discussion

As can be seen from the data presented herein, mortality from CSD and some selected causes, included in the CSD class, decreased in the Republic in the period between 2015 and 2019. The decrease in mortality is certainly a favorable factor and may indicate the effectiveness of prevention and treatment programs. However, attention should be paid to the increase in mortality for a number of reasons. Of particular practical importance is the increase in mortality from hypertensive heart and CKD with heart failure and/or CKD or ERSD hypertension. It can be assumed that the multidirectional dynamics in the causes of death from CSD is associated with an approach to determining the leading cause of death and coding the causes of death. Among the possible reasons for the decline in mortality from CSD, the possibility of the effect of distortion of statistical data on mortality rates is widely discussed. S.A. Boytsov, A.G. Vishnevsky, O.M. Drapkina, E.P. Kakorina, I.V. Samorodskaya, V.Yu. Semenov repeatedly pointed to the distortion of the statistics of mortality from CSD and individual causes of the CSD class by “transferring” deaths from one class of causes to another [1, 3-5, 7, 8]. The decrease in the mortality rates from CSD (-103.2 per 100 thousand population) in our analysis was proportional to the increase in mortality from the class of diseases according to the code ICD-10 R00-R99 “Symptoms, signs abnormal clinical and laboratory findings, not elsewhere classified” took place due to the diagnosis “Old age” (R54). Thus, the share of deaths according to the code R54 ICD-10 “Old age” among all deaths was only 3.3% in 2015 (28.9 per 100 thousand of the population) and 13.3% in 2019 (82.4 per 100 thousand population).

It is known that mortality rates can be influenced by the age structure of the population. The low rates of mortality from CSD in KBR and its decrease over a five-year period, as well as an increase in mortality due to “Old age” (ICD-10 code R54) can be explained by changes in the demography of KBR, which has resulted in an increase in the number of people over working age by 65, 7 thousand people from 1990 to 2018 and by 16.1 thousand people from 2015 to 2018 [6]. Despite the negative changes in the demography of KBR, the share of the population over 65 years old at the beginning of 2018 was recorded to be 11%, and the share of people over working age was 20.9%. The impact of approaches to coding the original cause of death cannot be ignored. O.M. Drapkina and co-authors explain the widespread increase of mortality due to “Old age” by the introduction of the relevant recommendations of the Russian Ministry of Health on the use of this coding as the cause of death (Letter No 14-
Full assessment of the cardiological health state in the population of the Republic, the identification of the priorities of targeted prevention is possible only based on the results of epidemiological studies. The study of the prevalence of CSD and CSD risk factors is the most important step towards an objective assessment of the situation, identification of the regional features of public health and management of medical care followed by the subsequent development and implementation of targeted preventive measures.

Limitations of the study

The paper have used data supplied by the official medical statistics, which do not fully reflect the nosological structure of mortality. This is due to the fact that when the initial cause of death is selected, only one of the present diseases is coded in the patient suffered from. The mortality statistics system in use does not take into account comorbidities, does not allow assessing the actual contribution of certain individual diseases and risk factors to the level and structure of mortality, therefore it does not allow properly evaluating the effectiveness of regional programs for the prevention and treatment of CSD to be assessed. In other words, the data from official medical statistics can only, with certain limitations, reveal the problems on which the efforts of the health care system and other services need to be focused in order to reduce mortality rates. Recording of the initial cause of death only and the omission of comorbidities from the accounting system make mortality analysis unsuitable for assessing the management of medical care at all stages of its provision and its resource allocation and assignment. At the same time, the lack of the possibility of obtaining the real picture using data from official statistics justifies the need for an epidemiological study in the Republic, the results of which will give the actual situation with CSD and make it possible to implement preventive measures more effectively and use resources rationally when providing specialized medical care.

Conclusion

The mortality rate from CSD in 2019 decreased by 103.2 per 100 thousand people compared with 2015, while maintaining the leading position in the mortality structure of the Republic’s population. The identified regional peculiarities of mortality from CSD can be used in the development and implementation of a set of measures to improve medical care for patients with CSD, as well as in improving approaches to properly determining the causes of death. An epidemiological study in KBR, the results of which will provide a realistic picture of the prevalence of CSD, appears to be an important step towards the development and implementation of preventive programs properly adapted to the needs of the region.
Statement on ethical issues
Research involving people and/or animals is in full compliance with current national and international ethical standards.

Conflict of interest
None declared.

Author contributions
The authors read the ICMJE criteria for authorship and approved the final manuscript.

References
Comparative evaluation of the effectiveness of a local hemostatic agent modified with a bio-organic composition

Elena V. Budko¹, Leonid M. Yampolsky¹, Darya A. Chernikova*, Anatoly A. Khabarov¹

¹ Kursk State Medical University
Russia, 305000, Kursk, Karl Marx str, 3
* Corresponding author:
email: darlachernikova@yandex.ru

Abstract
Constant attention to the study of the experience of using hemostatic products proposed for local bleeding arrest encourages new developments in this field. Modern hemostatics are porous multilayer systems with an inclusion of active coagulants. The results of the assessment of hemostatic activity obtained with the help of clinical and laboratory methods often do not lend themselves to cross-checking and statistical processing, and do not allow us to study objects with different physical and chemical properties. Methods of chemometrics, namely planimetry, allow you to visualize the parameters of sorption and hemocoagulation activity. A comparative planimetric study of commercial local hemostatic agents like Celox powder, sponges and napkins of various companies, zeolite powder, as well as new hemostatic compositions, which were given provisionally label A52 and A58, was carried out. It is shown that the hemostatic composition labeled as A52 leads to the formation of a stable volumetric primary and secondary thrombus. A comparative evaluation of the effectiveness of a new hemostatic agent in an acute experiment shows a high expression of hemostasis (the time of primary hemostasis is 15-20 seconds) and no recurrence of bleeding for the studied samples compared to the reference.

Keywords
Local hemostatic agent, Hemostatic sponge, Hemocoagulating activity, Rabbits, Venous-arterial bleeding, Planimetry

Introduction
According to statistical data, the need for local hemostatic agents (LHA) increases exponentially every year. Modern LHA by the mechanism of activity are vasoconstrictive and proaggregational drugs, plasma clotting factors, fibrinolysis inhibitors, aggregation and adhesion stimulators, agents that promote protein denaturation [1], as well as coagulation factor concentrators and mucoadhesive (able to remain on the mucosa) agents [2]. Hemostatic activity is observed in various classes of substances: protein compounds (collagen and gelatin); polysaccharides (cellulose, chitosan, starch and their derivatives); acrylic acid derivatives; aluminosilicate minerals; heavy metal compounds [3]. To date, complex preparations are being actively developed: matrix, fibrin and / or thrombin and combined products (matrix + fibrin and / or thrombin hemostatic agents) [4]. An example is gauze impregnated with procoagulants such as zeolite and kaolin [5], hemostatic spongy products produced by MedTrade (UK), Etikon and Z-Medica (USA), “Green Oak Grove”, Belkozin (Russia), Nycomed (Norway) etc.

Modern versions of LHA are based on the development and production of new materials, for example, polymer sponges and foams with shape memory [6], alginate calcium microspheres [7], nanostructured fibrin in agarose hydrogel [4], magnetically controlled hemostatic preparations of thrombin with gamma-Fe2O3 nanoparticles [8] and other nanoscale agents [9]. These materials differ from each other in their composition, shape, and the mechanism of action. They have shown outstanding performance and versatility compared to commercially available hemostatics, but many problems have remained still unresolved.

Recent trends are aimed at a rapid and effective hemostatic action along with a beneficial contribution to the healing and restoration of tissues. At the same time, any interference with the body's functions carries a potential risk. In particular, allergic reactions are possible as a result of exposure to exogenous collagen, thrombin and prothrombin, high exothermic reactivity and poor bio-degradability of inorganic hemostatic materials can easily cause thermal damage and inflammatory reactions [10]. Some important bio-safety
issues and the high cost of new hemostatics significantly limit their medical use. The reference source [11] defines the main requirements for a modern hemostatic agent. High hemostatic activity should be accompanied by such specific characteristics as no sudden changes in pH and a temperature in the wound, bio-degradability, bio-compatibility, mechanical strength; not excessive, but sufficient swelling coefficient; apyrogenicity, stability, adjustable adhesion. Accordingly, the ideal LHA should simultaneously have the capability to quickly provide hemostasis, sound bio-compatibility, properly selected degradation; it shall exclude or avoid adverse effect on the tissues and contribute to the acceleration of the healing process. In addition, important issues of quality, production cost, stability, swelling rate, safety, and regulated recoverable volume should also be considered and addressed [11]. Efficiency, safety, usability, cost, and regulatory approval are required as the five main conditions for commercial use of a hemostatic agent [12]. Therefore, all the developments of hemostatic agents are accompanied by numerous and multi-directional studies in vivo and in vitro formats.

In vitro experiments should mainly focus on the assessment of hemostatic activity. The mechanism of hemostatic activity is determined by measuring four main parameters of blood clotting (activated partial thromboplastin time (APTT), thrombin time (TT), prothrombin time (PT), fibrinogen content (FIB), and platelet count (PLT)) [9]. The few methods of laboratory research [13] are often limited to visual examination and some other subjective criteria, depending on the experience and professionalism of the experimenter. The most effective method for comparing hemostatic activity is considered to be thromboelastography [14]. Tests are performed in contact with human donor blood after recalcification [15] or freshly collected rat arterial blood [9].

An assessment of the characteristics of the hemostatic agent, in addition, should be carried out in two more areas: the description of the technical object and its impact on the biological substrate.

We will not dwell on the need to understand the chemical structure of materials and composites. All possible methods are used here, including infrared spectroscopy [15, 16], fluorescence spectroscopy, liquid and other types of chromatography [9] etc. The scope of application of these methods includes the assessment of the main toxicity profile for plant-derived hemostatics [17], antioxidant activity [15] and other tests based on the study of chemical structures and physico-chemical interactions between them.

For LHA in the form of nanoparticles, the micro-structure and morphology, and the particle size distribution are studied [7, 9]. Hemostatic sponges are evaluated by porosity (number of pores per cm2 of cross-section) and density [15]. Sponges and sorbents mainly work as aggregation and adhesion promoters, and it is desirable for them to determine the surface type of the adsorbent (hydrophobic, hydrophilic) for further evaluation of the interaction with the surgical site and the external organs. Sorption activity is more often evaluated, and swelling coefficients are calculated [6]. Micrographs allow us to visualize the processes of adhesion of blood cells to the surface of the biomaterial [15].

In our opinion, the most important properties of hemostatic products are their pH of a water extraction and heat of adsorption. They are often defined as auxiliary parameters in testing of hygroscopicity (swelling). Issues on the proper selection of the medium for an extract product are topical. Possible is use of water and blood or serum; in [16] described is a technique of application of a 0.9% sodium chloride solution and cotton seed oil. Another very important property, which is found only in a few of the products, is bio-degradability, which is assessed according to data on swelling, especially in the presence of human lysozymes [15] or blood and serum.

The following biological tests are widely used in the in vitro studies: hemolysis test [16], bio-compatibility with human tissues, for example, with human dermal fibroblasts (HDFs) [15], cytotoxicity test (for instance, in testing of gel-type materials [16,7]) and nanoparticles [9]. Antibacterial properties of hemostatic products are desirable [15,6], but they are not always achievable.

To characterize the process of hemostasis of biological objects, in vivo experiments are used: a test for skin sensitization, acute systemic toxicity (pyrogenicity) [16], toxic effects and tissue reactions [7]. And most importantly is to provide an assessment of hemostatic efficiency [16], [7], [6], [9] using acute (invasive) experiments [10, 18].

Thus, modern LHA are, to some extent, multicomponent application systems. At the same time, the use of each of them in practical surgery has certain limitations. Improvement of clot formation and, consequently, the quality of coagulation can be achieved by...
the following mechanisms: introduction of additional amounts of thrombotic elements, concentration of endogenous coagulation elements in the wound through rapid absorption of fluid from blood, stimulation of the non-platelet coagulation pathway. To improve the efficiency of the development and visualization of results, the method of thromboelastography is not always applicable, especially if the hemostatics are dense composites. Hemostasis is not always caused by porosity: high porosity may not ensure the formation of a blood clot. The results obtained with the help of clinical and laboratory methods often do not lend themselves to cross-checking and statistical processing (due to a small number of parallel experiments). On the other hand, a wide access to specialized computer programs allows you to use them for processing results of an analysis, makes it possible to combine various methods to assess the effectiveness of hemostasis. The development of chemometrics has led to the creation of new, more accurate methods for assessing the specific biological activity of drugs and substances [19], for example, the planimetric method, which is quite clear and accurate, has great capabilities and does not require complex equipment. JMicroVision v1.27 is an application that allows you to calculate the exact area, for example, of the wound and sorption surface, etc. The quantitative data obtained with its use are subject to interpretation and visualization (Corel Draw, Microsoft Excel, Statistica, Mathematica, Statgraphics, etc.). Therefore, the development and subsequent verification of methods for evaluating the effectiveness of existing hemostatic drugs and the resulting new hemocoagulating compositions is a promising field in modern medical research.

Materials and methods

Research objects. Powdered preparation Celox (MedTrade, Great Britain), zeolite (size about 1 mm of the Sakhtpinskye field of the Krasnoyarsk Territory), new bio-organic hemocoagulating compositions (developed by the Department of General and Bio-organic Chemistry at the Kursk State Medical University) under the provisional label. Hemostatic planar materials (see Figure 1): bio-degradable collagen hemostatic sponge produced by LLC “Luzhsky Zavod “Belkozin” (50.0±2.0×50.0±2.0×7.0±2.0 mm), hemostatic collagen sponge with silver produced by CJSC “Green Oak Grove” (50.0±2.0×50.0±2.0×7.0±2.0 mm), hemostatic agent “EversLife-Hemo” napkins (13x18 cm), atrumatic hemostatic dressing (PAG) non-woven with aminocaproic acid sterile “APPOLO-Hem” (6x10 cm). All materials were cut into square pieces of the same size (volume 1x1x1 cm, and planar size 1x1 cm).

Determination of the sorption rate. The time of complete absorption of 30 µl of blood into the material was measured.

Determination of the level of hematocrit. Venous blood samples were taken by venopuncture of the marginal ear vein of male Chinchilla rabbits with a body weight of 4.0 – 4.6 kg. 10 ml of venous blood was collected from each animal in tubes containing a preservative (sodium citrate). Then blood was mixed and divided into reference and experimental groups, each of them had 4 test tubes. For the convenience of determining the volume of platelet clots, we used measuring tubes with a graduated scale. After mixing, the samples were centrifuged for 30 minutes at a speed of 3000 rpm, and in the transmitted light, the height of the platelet clot was noted. To calculate the hematocrit value, the obtained data were processed using the formulas given below:

\[ HTC = \left( \frac{V_{blood\ total}}{V_{sediment}} \right) \times 100\% \]

where:

HTC is hematocrit (%);

\( V_{blood\ total} \) is the volume of blood placed in a test tube;

\( V_{sediment} \) is the volume of sediment.

After calculating the hematocrit value for each series, a graphical interpretation was performed using the Microsoft Excel software.

Planimetric studies of hemocoagulating properties. The area occupied by one drop of blood was determined at its free fall on the following items: standard carriers made of 100% cellulose (acc. to GOST R 52354-2005 filter paper and acc. to GOST R 57641-2017 printing paper), on nonwovens before and after their modification with dry powders. Native blood was slowly applied to each sample of planar material by the drip method (using a standardized drip gauge), waiting for the fluid movement to stop. Before applying each drop of blood, the sample was photographed, observing the perpendicular optical axis. To calculate the area of the run-up zones, photos of the samples were uploaded to the JMicroVision v1.27 software application; the area of the blood run-up zones was determined. When studying the effect of powders on the absorbency at the preparatory stage, the same weight lots (0.3–0.5 g) of dry hemocoagulating substances and compositions were weighed. Then the suspended
weights of substances and compositions were placed evenly over the area of the lower layer of the carrier.

An experiment on animals. To evaluate the effectiveness of a new hemostatic composition on a model of a stab wound in an acute experiment, the authors' team based on the developments of N. Alam (2003) proposed his own method for evaluating the effectiveness of local application hemostatics with subsequent dynamic observation of surviving animals. The essence of the method is to record the dynamics of blood pressure and heart rate (HR) values in animals in the absence of the stage of wound revision for animals of both groups with their subsequent mandatory euthanasia.

For the experiment, 10 male Chinchilla rabbits with a body weight of 4.0 - 4.6 kg were selected. The experiment was performed in a veterinary operating room at the laboratory of “Experimental Surgery and Oncology” of the Research Institute of EM at the Kursk State Medical University in compliance with the requirements of the local Ethics Committee and the applicable Helsinki Declaration on Animal Experimentation. In the course of the study, in accordance with the ethical requirements of the Helsinki Declaration, only those animals whose condition was assessed as critical by the operating surgeon were removed from the experiment.

At the preparatory stage, the experimental animals were divided into two groups (the reference group and the experimental group) and deprived of food with free access to water. Each group consisted of 5 animals. As a method of general anesthesia, endotracheal anesthesia with “Isoflurane” was chosen at a dosage calculated based on the weight of each animal and the duration of the operation (on average, 4 ml per rabbit to provide anesthesia for 40 minutes) [20]. For invasive monitoring of blood pressure, a 5Fr introducer was installed in the carotid artery. Liquid media were infused through a 6Fr catheter into the external jugular vein of the animal [21]. A scalpel was applied to an oblique linear incision 5 cm long in the upper third of the right thigh below the inguinal fold in the projection of the femoral artery. After cutting the skin in a blunt and sharp way, the femoral artery and the femoral vein were isolated from the muscles for 2-3 cm (Figure 2), which was simultaneously excised by a transverse incision, resulting in intense arteriovenous bleeding. After a latent period of 30 seconds, each animal lost 1/3 of the BCC, which caused a decrease in blood pressure by 25 mmHg.

Before applying hemostatic agents to the wound surface, the wound bed was drained with sterile wipes to determine the volume of blood loss. After that, a
prototype LHA or a standard reference agent (a bio-degradable hemostatic collagen sponge produced by the Luhzhsky BELKOZIN Plant) was applied and tamponed into the wound. Finger tamponade was performed for 1 minute. If necessary, the tamponade was repeated up to 3-5 operations, each for no more than 1 minute. Simultaneously with the temporary stop of bleeding, the lost BCC was replenished with 0.9% sodium chloride solution. After 1 minute, tamponade was stopped, and the result was evaluated. When the bleeding resumed, the LHA was re-compressed into the wound for 1 minute. A similar scheme of actions was performed on each animal as needed, until the complete stop of arteriovenous bleeding was achieved (on average, 3-5 procedural operations) [22]. Blood pressure and heart rate readings were recorded throughout all stages of the operational procedure. After achieving hemostasis, the LHA was left in the wound bed or sewn to the edges of the wound if additional fixation was necessary. Then the skin was sutured, and each animal was transferred to a separate cage, where it was observed for 3 hours.

The general condition of the animal was evaluated, namely, the wound area was subjected to visual examination with an assessment of the flow of blood through the stitches in the wound. The evaluation of the effectiveness of LHA was carried out according to the following indicators: the number of animals that managed to achieve primary hemostasis; the volume of blood loss and survival. The entire study was recorded using video recording technique, photographs, and blood pressure and heart rate readings recorded throughout all stages of the operation, using the MR-150 BIOPACK.

Study of the heat of dissolution. The tested liquids (water, blood) were sequentially introduced into the calorimeter measuring vessel, and the system equilibrium temperature was recorded. The temperature of the media was 250°C. Then the samples of the studied compositions were added within the range of mass ratios from 0.05 to 0.5 g of liquid, and the temperature of the mixture was measured at regular intervals until its change became insignificant (thermodynamic equilibrium was established). The temperature increase corresponded to the heat of dissolution of the sample in question.

Determination of the acidity of media. The pH values of the solutions were studied using standard laboratory ionometer ETAN (Tom Analyte, Russia). The measurement was carried out with the magnetic stirrer switched on until the pH values were stabilized. The test liquids (water or blood) were successively poured into a measuring cup, the initial pH value was fixed, then a sample of the composition was added and mixed until they were dissolved and parameters stabilized, and the pH value was measured again. The temperature of the media was 250°C.

To measure the mass, we used laboratory electronic scales VK-150.1 (manufactured by JSC “MASSA-K”, Russia), accuracy class II (acc. to GOST R 53228-2008), the absolute error of weighing was (± 5 - ± 10 mg).

In the course of the study, purified water was used (water distillation system AE-5 “LivaM”, Russia).

A mechanical pipetator was used as a standardized drop gauge.

Every measuring procedure was performed in at least 3 times.

**Results and Discussion**

We have selected various porous materials and powders as the objects of our research. The area of a single drop of blood, as well as the rate of absorption of blood depends on the adhesive properties of the surface and its absorbency. The minimum suction time was shown by loose materials: paper, a dry EversLife-Gemo napkin, and the maximum suction time was reported for the Green Oak Grove sponge.

When applying a drop of blood to a cellulose carrier acc. to GOST R 52354-2005 (filter paper), it is absorbed in the area occupied by a free-falling drop and then the blood penetrates into the adjacent and underlying layers of the carrier (see Figure 3). Replacing the cellulose carrier with a non-woven material or a porous sponge logically leads to a decrease in the area and an increase in the absorption time.

When applying powders between the layers of the cellulose carrier, the capability to move blood along the plane of the carrier is sharply reduced. We can only partially to talk about the sorption of the liquid part of blood due to the properties of granules/crystals. An introduction of sorbent powders (Celox, zeolite) leads to a reduction in the area, but the new hemocoagulating compositions of a powdery form under the temporary labels A52 and A58 do not have the sorption capacity, create an almost “non-absorbent” surface (see Figure 3B). In this case, the introduction of powders of acetylsalicylic acid and hexamethylenetetramine (blood-thinning substances) leads to blood spreading...
Figure 3. The increase in the area occupied by the first (A, B) and seventh (C, D) drops of native blood (reference A, B on the cellulose carrier acc. to GOST R 52354-2005 (filter paper) and after applying hemostatic powder A52 – B, D between the layers of the cellulose carrier); E, F – the reference and the test sample on a glass substrate.

Figure 4. The average area (indicating scattering of values) occupied by 1 drop (30 µl) of blood on surfaces with different absorbency and hemocoagulation capacity. The lines show the range and the average of the obtained values.
over the surface. Volumetric materials (sponges) help to reduce the area occupied by the first drop of blood, but when introducing powders between their layers, the drop area decreases (see Figure 4) comparable to the experience with thin layers.

If the volume occupied by a drop of blood is represented in the form of a virtual cylinder proportional to the area occupied by this drop, then a comparison of the heights of the cylinders in different porous systems allows us to estimate the “depth” of blood penetration into the material (see Figure 5). Based on Figure 3, the first drop on a thin cellulose carrier occupies the maximum area, being distributed in a single absorbent volume. The heights of the cylinders calculated by experimental and theoretical values (Figure 5 cellulose carrier in 1 and 3 layers) are comparable that confirms the reasoning. Napkins made of non-woven material are soaked to the entire thickness of the material, which we take for the height of the cylinders. The sponge does not get wet to the full depth from a single drop of blood, and its surface is heterogeneous, the formation of the height of the virtual cylinder in the sponge is influenced by multidirectional factors, but the obtained values for the porous system of the Belkozin sponge significantly differ from both a well-absorbing surface and a non-absorbing one. Due to the component composition of the sponge “Green Oak”, the blood penetrates deeply into its body, without spreading horizontally. At the same time, the sponge quickly gets wet in the place of contact with the blood, remaining dry in the rest of the area. The values of the heights of the virtual blood cylinders in the cellulose carrier after its modification are specifically located. The apparent height of the cylinder is much greater than the thickness of the pulp layer and even the apparent height of the cylinder in the sponge. In terms of the range of height values, the cellulose modified by hemostatics differs little. The virtual cylinder of the composition A58 is located in the negative region, since the blood formed a stable drop that is practically not absorbed by the cellulose carrier.

The kinetics of the increase in area with an increase in the number of blood drops was studied planimetrically. The increase in the area on the cellulose carrier acc. to GOST R 52354-2005 (filter paper), the Belkozin sponge and the non-woven napkin was expected to be observed in an almost linear relationship. When placing hemocoagulating powders between the carrier layers, the area of the zone soaked in blood is stabilized by adding a certain number of drops (see Figure 3 herein). The number of blood drops forming a “stable zone” depends on the activity of the powder: the greater the sorption-coagulating ability it has, the less the area of the blood drop on the outer layer of the carrier changes. The type of kinetic curve depends on the mechanism of powder activity: an almost lin-
Table 1. Results of planimetric estimation of the area of the blood zone on the film carrier when introducing powders with hemocoagulating activity Celox and composition A52 between its layers

<table>
<thead>
<tr>
<th>Blood drop number</th>
<th>Average area of the blood run-up zone, ( \text{mm}^2 )</th>
<th>The Mann-Whitney test criterion*</th>
<th>Blood drop number</th>
<th>Average area of the blood run-up zone, ( \text{mm}^2 )</th>
<th>The Mann-Whitney test criterion*</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>58.9</td>
<td></td>
<td>1</td>
<td>119.2</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>104.75</td>
<td>0 ( p \leq 0.01^{**} )</td>
<td>2</td>
<td>204.8</td>
<td>0 ( p \leq 0.01^{**} )</td>
</tr>
<tr>
<td>3</td>
<td>142.4</td>
<td>4 ( p \leq 0.01^{**} )</td>
<td>3</td>
<td>191.3</td>
<td>0 ( p \leq 0.01^{**} )</td>
</tr>
<tr>
<td>4</td>
<td>138.4</td>
<td>0.05 ( \leq 3 ) ( p \leq 0.01^{***} )</td>
<td>4</td>
<td>197.8</td>
<td>65 ( p \geq 0.05 )</td>
</tr>
<tr>
<td>5</td>
<td>138.1</td>
<td>69 ( p \geq 0.05 )</td>
<td>5</td>
<td>193.5</td>
<td>0.05 ( \leq 34 ) ( p \leq 0.01^{***} )</td>
</tr>
<tr>
<td>6</td>
<td>135.7</td>
<td>25 ( p \leq 0.01^{**} )</td>
<td>6</td>
<td>193.2</td>
<td>72 ( p \geq 0.05 )</td>
</tr>
<tr>
<td>7</td>
<td>271.9</td>
<td>0 ( p \leq 0.01^{**} )</td>
<td>7</td>
<td>193.7</td>
<td>72 ( p \geq 0.05 )</td>
</tr>
</tbody>
</table>

* Compared to the previous drop, ** The data is in the area of significance, *** The data is in an area of uncertainty, **** The data is in the area of insignificance

Ear increase in area was observed when zeolite powder was introduced into the carrier, but if Celox was placed between the layers of the absorbent carrier, the curve became stepwise. An almost linear increase in the area was observed when the A58 composition was introduced into the carrier, while the A52 composition led to the formation of a step graph. The planimetric method made it possible to conduct a comparative analysis of the hemocoagulating and sorption properties of 6 objects in 4-12 series of experiments without resorting to in vivo methods. The reliability of the quantitative data obtained in the course of the study was confirmed by statistical processing using the nonparametric Mann-Whitney test (Table 1).

To test the capability of the powders to hold the blood flow, their identical masses were placed in measuring cylinders, compacted, and the same volumes of blood were placed on top. Figure 6 shows that A52, A58 and zeolite absorb blood. Celox is not saturated with blood, but when the closing valve is removed in the measuring cylinders, Celox continues to create a plug, which is well comparable with the experience of using Celox series hemostatics [2]. The zeolite has passed through blood and has been washed away by the stream that also corresponds to the recommendations for the use of zeolites in the composition of dressings and other carriers. In contrast to Celox, the composition A52 retains a column of blood despite getting wet, and the composition A58 in the form of a gel clot has been washed out of the cylinder. This experiment visualizes the behavior of powders at excess and average blood pressure, the result of the experiment is well comparable with conclusions of many authors. The observed processes are associated with various mechanisms of coagulation processes of the studied powdered hemostatics.

Hematocrit was studied in blood of animals. Its value for the reference samples was 37.5-39.0 %. The powders were injected into the blood samples and mixed, sponges and napkins were placed in test tubes before blood was injected, and the samples were centrifuged. When zeolite was injected into the blood sample, the hematocrit value did not change, while blood was separated into three fractions (platelet clot, suprathrombic fluid, and powder layer). When Celox powder was injected into blood, the entire volume of blood formed a primary blood clot, which was not stable with time (Figure 4 A, B). The increase in hematocrit to 100% in all samples was revealed due to the capability of the drug to swell as a result of the absorption of the liquid part of blood. The developed compositions A52 and A58 initiated stable blood coagulation (Figure 4 C, D), and the hematocrit level reached 64.7%. The difference between them was in the amount of suprathrombic serum released and the degree of its transparency. The resulting clots thickened with time. The suprathrombic serum released during coagulation was examined for the protein content. For this purpose, qualitative reactions were performed according to known methods. Negative test results showed the almost complete absence of peptides over the clot.

One of the most significant characteristics of LHA is the heat of absorption and the change in the pH value of the wound. Acidification of the medium is a negative factor when using oxidized cellulose, heavy metal salts, etc. Commercial LHA products eliminate this disadvantage by introducing neutralizing components. To evaluate the new hemostatic compositions, the pH values of blood and the aqueous medium were studied depending on the mass ratios of the composition and the liquid. As can be seen from the graph
Figure 6. Measuring cylinders with hemostatic powders (0.3 g) before (A) and after (B) filling with blood (3 ml). In the measuring cylinders, the closing valve is removed (B).

Figure 7. Appearance of platelet clots (immediately after centrifugation and after a day) obtained by adding Celox powders (A,B) and composition A52 (C,D) to native blood.

(Figure 8), the medium is acidified, but the experimental data in water and blood do not coincide with each other and with the calculated pH values. Lower pH values were not detected due to a deep change in the structure and properties of blood (coagulation).

The visual and parametric examination of the onset of primary hemostasis was performed by our operating surgeon according to the following signs: tight fit of LHA to the excised vascular bundle; no need for an additional fixation of LHA in the wound bed (suturing, etc.); no leakage of blood under LHA into the wound bed with its subsequent filling; achievement of primary hemostasis by 1 LHA unit; time of onset of primary hemostasis less than 1-1.5 minutes; restoration of the initial indications of blood pressure and heart rate after installing the LHA in the wound bed; no relapses of postoperative bleeding through sutures applied to the wound. When using the A52 composition to stop bleeding, primary hemostasis was achieved within 15-20 seconds in all animals of the experimental group (Figure 9, 10). All test parameters fully corresponded to the onset of primary hemostasis. All the animals from the experimental group were able to stand up 30 to 40 minutes after the operation, and no blood leaking through the sutures was observed in any of the animals in the group. Within 3 hours of the observation, all the animals of the experimental group remained alive.

In contrast to the experimental group, the reference animals required additional suturing of several (on average 3 – 4) units of bio-degradable hemostatic collagen sponge to achieve primary hemostasis. After
Figure 8. Results of correlation changes in acidity and heat effect when the composition A52 is introduced into water and blood.

Figure 9. Appearance of the wound after applying the sponge with the new hemostatic composition A52: immediately after application (A) and 20 seconds after application (B).

Figure 10. Dynamics of blood pressure readings and heart rate at all stages of the experimental study of the new hemostatic composition A52.
reaching the primary hemostasis, the blood pressure and heart rate readings were found to be not restored to the initial level. At the same time, the surviving animals of the reference group did not try to get to their feet even after 180 minutes after the operation, during the first 60 minutes after the operation they had blood leaking through the sutures on the wound. The results of the study for each group are presented in Table 2 given herein.

### Conclusion

Our analysis of scientific publications on the problem under consideration demonstrates the constant attention to the study of the experience of using hemostatic products proposed for local bleeding arrest, as well as to new developments in this field. Most LHA products are combined, and their hemostatic activity is synergistic, but it is always based on the capability to form mesh matrix structures and the possibility of forming ionic complexes, which leads to an effective adhesion of third-party macromolecules. Dehumidification of the impact site leads to an increase in the viscosity of the adjacent liquid and the concentration of coagulation factors. These characteristics bring together napkins and sponges, sorbents, aluminosilicates and polymers of protein nature, cellulose and chitosan materials. For the comparative in vitro analysis of powders and planar systems with hemostatic activity, we used the method of planimetric analysis employing cellulose carriers, which allowed us to visualize the parameters of the sorption and hemocoagulating activity of powders, sponges and napkins. Comparative planimetric analysis shows that Celox powder retains quite large volumes of blood, due to swelling and creating a plug. Zeolites absorb the liquid with the entire mass and when the mass ratio is exceeded in favor of blood, they do not cope with hemostasis. Sponges and wipes are blotted according to the absorbed volume, and the introduction of a hemostatic agent into the volume of the porous material reduces the effectiveness of the material. However, the introduction of the new hemostatic compositions under the provisional label A52 and A58 leads to the activation of absorbent materials, the formation of a stable volumetric primary and secondary blood clot. A comparative evaluation of the effectiveness of a new hemostatic agent in an acute experiment shows a high expression of hemostasis (the time of primary hemostasis is 15-20 seconds), and there is no recurrence of bleeding for the studied samples compared to the reference samples.

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### Statement on ethical issues

Research involving people and/or animals is in full compliance with current national and international ethical standards.

### Conflict of interest

None declared.

### Author contributions

The authors read the ICMJE criteria for authorship and approved the final manuscript.

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Influence of standard and stimulated growth of B16/F10 melanoma on AIF levels in mitochondria in cells of the heart and other somatic organs in female mice

Oleg I. Kit1, Elena M. Frantsiyants1, Irina V. Neskuibina4*, Natalia D. Cheryarina1, Alla I. Shikhlyarova1, Yuriy V. Przhedetskiy1, Viktoria V. Pozdnjakova1, Ekaterina I. Surikova1, Irina V. Kaplieva1, Valerija A. Bandovkina1

1 National Medical Research Centre of Oncology, Russia, 344037, Rostov-on-Don, 14 liniya, 63, building 8
* Corresponding author:
phone: +7 (928) 156-56-56, email: neskubina.irina@mail.ru

Abstract
Aims: studying AIF levels in mitochondria in cells of the heart and various organs in female mice with the growth of experimental melanoma B16/F10 and comorbid pathology.

Material and methods
The study included female mice of the C57BL/6 strain. The experimental groups were composed as follows: an intact group, n=21; animals with chronic neurogenic pain (CNP), n=21; a group with an isolated growth of B16/F10 melanoma, n=63; and a group with B16/F10 melanoma growing in the presence of CNP, n=63. The levels of AIF (ng/mg protein) were determined in mitochondria by ELISA (RayBiotech, USA). Statistical analysis was performed by the Statistica 10.0 software.

Results
CNP upregulated the levels of AIF in mitochondria in the brain by 7.2 times, in the heart by 2.3 times, in the liver by 1.5 times (p<0.001), and in the skin by 1.4 times (p<0.001). The AIF dynamics in mitochondria of the internal organs depended on the stage of the isolated melanoma development, while AIF in the melanoma itself was lower than that in the intact skin: after 1 week by 6.5 times, after 2 weeks by 8.7 times and after 3 weeks by 3.5 times, respectively. The development of melanoma in the presence of CNP was accompanied by a decrease in high AIF levels almost in all internal organs; low AIF levels were recorded in tissues of melanoma growing in the presence of CNP, compared to the levels in the skin of mice with CNP.

Conclusions
Stimulated melanoma growth at the terminal stage is characterized by low AIF levels in the heart and in most organs, the skin and the tumor, which indicates suppression of the electron transfer chain.

Keywords
Mitochondria of cells, AIF, Chronic neurogenic pain, Experimental B16/F10 melanoma, Female mice

Imprint

Introduction
In the international reference literature on cardiology and oncology, many common reference marks can be found, which are related to pathogenesis that characterize disorders and abnormalities in the energy metabolism in mitochondria and in the processes of programmed cell death. The promotion of digital technologies with the possibility of simultaneous recording of hemodynamics and calculated parameters of heart metabolism (PC-assisted hemodynamic analyzer "CARDIOCODE", STC CARDIOCODE RU No. FSR 2011/12126, Taganrog, Russia) is supplemented by analog biochemical models for studying mitochondrial factors of cellular regulation in the tumor growth and comorbid conditions. In this context, the factors of apoptosis attract special attention.

About 20 years ago, the apoptosis-inducing factor (AIF) was identified as the first caspase-independent mitochondrial effector of cell death [1].

With an increase in the permeability of the outer mitochondrial membrane that occurs during the activation of most apoptotic pathways AIF is released from the mitochondria and translocated to the nucleus, where it promotes chromatin condensation and DNA degradation [2]. The contribution of AIF to cell death depends not only on their histology, but also on
the nature of the apoptotic pathway [3]. In addition to its apoptotic activity, AIF plays an indispensable physiological role in cell survival, proliferation, and differentiation by regulating the optimal functioning of the respiratory chain of complex I in mitochondria [4].

Recently, the first mammalian AIF mitochondrial interactor, a protein called the coiled–coil-helix-coiled-coil-helix domain and containing 4-CHCHD4 was isolated [5]. This sheds new light on the mitochondrial survival function by linking AIF to an evolutionarily conserved redox-regulated CHCHD4-dependent transport mechanism, which operates in the intermembrane space of this organelle [5, 6]. Taking into account the diversity of mitochondrial processes, AIF cannot be considered solely as a regulatory factor for the respiratory chain complex I [7]. The emerging idea is more likely to be that AIF in mammals is a key component of the transport mechanism, which, in addition to its role in the biogenesis of respiratory chain subunits, also has the capability to regulate complementary actions, ranging from protein transport to intramitochondrial lipid homeostasis, an antioxidant response, calcium accumulation, mitochondrial translation, and mitochondrial membrane mobility [6, 8]. Establishing the fact that AIF regulates the biogenesis of protein subunits of the respiratory chain opens up a new area of research, which will undoubtedly help to clarify the specific mode of action of AIF for each type of tissues [1]. Experimental studies of this sort are essential to solve a variety of issues in the context of translational medicine [9, 10, 11].

The aim hereof is to study the level of AIF in the mitochondria in the heart and other somatic organs in female mice under the growth of experimental B16/F10 melanoma and comorbid pathology.

Materials and methods

Our research work has been carried out using female mice of line C57BL/6 (n=168), 8 weeks of age with a mass of 21 to 22 g. The animals have been delivered to us by the Federal State Medical & Biological Institution “Research Center of Biomedical Technologies Andreevka” at the Federal Medical & Biological Agency; mouse melanoma line B16/F10 have been supplied by the Russian National Medical Research Center of Oncology named after N.N.Blokhin, Ministry of Health, Russia. Our research study has been conducted in accordance with the International Guiding Principles for Biomedical Research Involving Animals and Order No. 267 “Approval of the Rules of Laboratory Practice” dated June, 19, 2003 issued by the Ministry of Health of the Russian Federation, and it has been approved by the Commission on Bioethics at the Federal State Budgetary Institution “National Medical Research Center of Oncology”, the Ministry of Health of the Russian Federation (Record No. 2 dated May, 31, 2018).

In this study, the model of chronic neurogenic pain (CNP) has been used as comorbid pathology in the growth of experimental melanoma. The animals were randomly divided into groups as follows: the group of intact mice (n=21); the CNP reference group to reproduce CNP (n=21); group M with a standard growth of melanoma B16/F10 (n=63); group CNP+M with a subcutaneous growth of melanoma B16/F10 against the background of chronic neurogenic pain (n=63). CNP was reproduced in animals by ligation of the sciatic nerves on both sides under xyl-zoletil anesthesia: as premedication used were xylazine (Xyl) intramuscularly at a dose of 0.05 ml/kg of body weight (according to the instructions), then, 10 minutes later, Zoetil-50 at a dose of 10 mg per 100 g of body weight [12]. Melanoma was transplanted under the skin of the right scapula in the volume of 0.5 ml of tumor suspension in a 1:10 dilution with saline solution, and in the CNP+M group 3 weeks after the reproduction of CNP.

Decapitation of animals was carried out using the guillotine. Animals from groups M and CNP+M were decapitated after B16/F10 melanoma transplantation in the following time periods: 1 week, 2 weeks and 3 weeks after transplantation; mice from the CNP group were decapitated 3 weeks after the nerve ligation. The skin and tumors were excised in each animal, and their brains, livers, kidneys, and hearts were harvested. Conditionally healthy skin was excised at the maximum distance from the tumor node. Mitochondria were isolated by the method of Egorova M. V., Afanasiev S. A. [10] (using refrigerants and differential centrifugation with high-performance refrigerated centrifuge Avanti J-E, BECMAN COULTER, USA). The tissues were washed with an ice-cold 0.9% KCl solution. To disrupt the intercellular bonds, the cell walls and plasma membranes, mechanical treatment of tissues with grinding using scissors and homogenization in a glass homogenizer with a Teflon pestle (Potter-Elvehjem homogenizer) was employed. For each gram of tissue, 10 ml of the isolation medium was added (0.22 M mannitol, 0.3 M sucrose, 1 mM EDTA,
2 mM TRIS-HCL, 10 mM HEPES, pH 7.4). The tissues were homogenized and centrifuged for the first time for 10 minutes at a speed of 1000 g at a temperature of 0–2 °C, the second and third centrifugation was carried out at 20000 g for 20 minutes at a temperature of 0–2 °C. Between the centrifugations, the mitochondria sediment was resuspended in the isolation medium.

Mitochondria were further isolated from lysosomes, peroxisomes, melanosomes, etc. utilizing the Percoll 23% density gradient centrifugation. The suspension of subcellular structures was layered on the Percoll gradient, centrifuged for 15 min at 21000 g, after which the separation into 3 phases was observed; the lower layer of mitochondria was left and resuspended with the isolation medium. The next mitochondria washing procedure was performed by centrifugation for 10 minutes at 15000 g, at a temperature of 0–2 °C.

Mitochondrial samples (protein concentration 4–6 g/l) were kept at -80 °C in the isolation medium before analysis. In mitochondrial samples, the AIF concentration in ng/mg of protein was determined using ELISA (RayBiotech, USA), and the protein concentration in mg/ml was identified with biuret method (Olvex Diagnosticum, Russia) utilizing automatic analyzer ChemWell (Awareness Technology INC, USA).

The Statistica 10.0 software was used for our statistical analysis. The obtained data were analyzed for the compliance of the features distribution with the normal distribution law using the Shapiro-Wilk test. The comparison of quantitative data in the above groups was carried out employing the Kruskal-Wallis test. The table data are presented in the M±m form, where M is the arithmetic mean, and m is the standard error of the mean.

**Results**

In our study, first of all, it was necessary to investigate an effect of CNP on the functional state of the mitochondria in the heart cells and other organs to be examined. CNP caused an increase in the level of AIF in the mitochondria as indicated below: by 2.3 times in the heart, by 7.2 times in the brain, by 1.5 times in the liver and 1.4 times in the skin (see Table 1 given herein). No change in the mitochondria in the kidney cells under the influence of CNP was detected.

Upon expiration of the one week of the standard melanoma growth (M), as compared with the values in the intact group, the content of AIF in the mitochondria in the heart and the skin remained stable, while it increased in the mitochondria in the brain by 17.6 times and by 2.2 times in the liver; it was found that it decreased in the mitochondria in the kidneys by 1.3 times, respectively (see Table 1, Fig. 1-4 herein).

After 2 weeks in mice from the M group, the AIF value in the mitochondria in the heart and the kidneys for the first time decreased by 1.4 times as compared to the intact animals. As to the AIF level in the brain, the liver and the skin, not affected by the malignant process, it declined to the normal values in the first two organs, and in the skin it was 2.3 times lower than that recorded in the intact group (see Table 1 herein). After 3 weeks of the standard melanoma growth, the content of AIF demonstrated its decrease in the mitochondria in the heart, the liver and in the skin areas not affected by the malignant process against the background of comorbid pathology, i.e. a chronic neurogenic pain, a completely different oscillatory dynamics of AIF in the mitochondria of the studied organs was noted (see Table 1, Figures 1-5 herein).

Thus, it is obvious that by the terminal stage of the standard tumor growth (3 weeks), a decrease in the level of AIF was found in the mitochondria in the heart and in almost all the studied organs, as well as in the tumor (see Figures 1-5 herein).

With the growth of melanoma against the background of comorbid pathology, a completely different oscillatory dynamics of AIF in the mitochondria of the studied organs was noted (see Table 1, Fig. 1-5 herein). Thus, after 1 week of the malignant growth against the background of CNP, in relation to the values of the reference group, the level of AIF decreased in the mitochondria as given below: in the brain by 10.2, in the liver by 29.3, in the kidneys by 1.3, and the skin by 3.7 times, respectively. The exception was the mitochondria in the heart, where the level of the marker in question, on the contrary, increased by 3.7 times. After 2 weeks, the level of AIF increased in relation to the previous study period in the mitochondria: in the brain by 3.8, liver 24.5 and in the skin by 3.7 times. At the same time, in the liver and the skin mitochondria, the marker did not demonstrate statistically significant differences from the values in the R group, and in the brain mitochondria it was decreased by 1.3 times.
2.7 times lower. In the mitochondria in the heart and the kidneys, the level of AIF, in contrast, decreased, as compared to week 1, by 3.2 and 1.5 times, respectively, and, at the same time, the level in the heart mitochondria did not differ from the corresponding reference, and in the kidney mitochondria it was 1.9 times lower than the reference values (see Table 1 herein).

After 3 weeks, in the CNP+M group, the level of AIF decreased in relation to week 2 in the mitochondria in the heart by 6.1 times, in the brain by 3.4 times, in the liver by 5.5 and in the skin by 3.5 times, respectively. The exception was the kidneys, where the level thereof in the mitochondria increased by 2.6 times. As a result, the concentration of AIF was lower than that in the animals of group R: in the brain by 9.3 times, in the liver by 5.5 and in the skin by 3.5 times, respectively. Only in the mitochondria of the kidneys, the level of the marker was 1.4 times higher than the corresponding reference values (see Table 1 herein). In the melanoma tissue, growing against the background of CNP, lower values of the AIF were recorded than those in the skin in the mice with CNP: after week 1, it was reported by 49.3 times lower, after week 2 - by 16.6 times, and after week 3 - by 2.0 times (see Table 1, Figure 6 herein).

### Table 1. Dynamics of AIF levels (ng/mg protein) in mitochondria of organs in female mice with standard and stimulated growth of 816/F10 melanoma

<table>
<thead>
<tr>
<th>groups</th>
<th>brain</th>
<th>liver</th>
<th>heart</th>
<th>kidneys</th>
<th>skin</th>
<th>tumor</th>
</tr>
</thead>
<tbody>
<tr>
<td>intact</td>
<td>22.044±2.092</td>
<td>341.53±15.038</td>
<td>40.533±1.25</td>
<td>81.64±47.42</td>
<td>399.22±7.9</td>
<td>-</td>
</tr>
<tr>
<td>CNP (C)</td>
<td>159.37±6.039</td>
<td>511.41±36.67</td>
<td>91.238±3.72</td>
<td>834.18±49.53</td>
<td>577.95±15.5</td>
<td>-</td>
</tr>
<tr>
<td>M week 1</td>
<td>388.6±13.89</td>
<td>753.84±32.52</td>
<td>36.622±1.74</td>
<td>609.1±38.76</td>
<td>645.19±108.8</td>
<td>-</td>
</tr>
<tr>
<td>M week 2</td>
<td>22.22±2.196</td>
<td>278.96±26.06</td>
<td>29.511±1.38</td>
<td>580.42±35.08</td>
<td>171.39±25.2</td>
<td>61.159±1.84</td>
</tr>
<tr>
<td>M week 3</td>
<td>31.289±2.668</td>
<td>47.82±4.283</td>
<td>19.911±1.48</td>
<td>629.26±46.77</td>
<td>78.078±78.08</td>
<td>45.689±2.14</td>
</tr>
<tr>
<td>CNP + M week 1</td>
<td>15.57±2.556</td>
<td>17.446±2.677</td>
<td>340.82±11.64</td>
<td>633.86±39.62</td>
<td>157.56±27.3</td>
<td>11.71±1.08</td>
</tr>
<tr>
<td>CNP + M week 2</td>
<td>58.903±4.415</td>
<td>427.88±27.37</td>
<td>107.03±4.26</td>
<td>428.24±39.15</td>
<td>584.32±25.3</td>
<td>34.84±1.86</td>
</tr>
<tr>
<td>CNP + M week 3</td>
<td>17.212±2.238</td>
<td>77.326±4.774</td>
<td>17.68±1.51</td>
<td>1129.7±58.26</td>
<td>168.98±26.62</td>
<td>290.98±10.62</td>
</tr>
</tbody>
</table>

Note: *statistically significant compared to the values in the intact group; †statistically significant compared to the values in the corresponding reference group; ‡statistically significant compared to the values in the previous period.

### Discussion

Under the physiological conditions, AIF plays a vital role in mitochondrial bioenergetics, as it supports the normal oxidative phosphorylation of a cell. Mitochondrial AIF make its influence on a variety of the catabolic and anabolic pathways as well as epigenetic processes, which depend on mitochondrial metabolites [14]. Our studies have shown that AIF can regulate the mitochondrial function by participating in the assembly and/or stabilization of the respiratory complexes and contributing to the normal cellular functions [15].

According to the supercomplex organization model, changes in the architectural structure of the electron transport chain lead to a disorder in or abnormality of the regulation of the oxidative phosphorylation, along with respiratory defects, to a reduced capability to produce ATP and altered production of reactive oxygen species [16]. The important role of AIF in the biogenesis and maintenance of the function of mitochondrial respiratory chain complexes has been evidenced [17].

AIF levels have been found to increase dramatically in response to CNP in the mitochondria of the heart, the brain, the liver, and the skin. Probably, CNP being a stress factor stimulates the activity of the respiratory complex of the mitochondria in these organs. It is known that AIF can transfer equivalents to the electron transport chain and, as a result, maintain a pool of NAD + / NADH and/or the NADH-dependent proton pump through the inner mitochondrial membrane [18]. On the other hand, AIF can make an influence on the mitochondrial function by regulating other cellular processes, taking into account the large other cellular processes, taking into account the large.
Figure 1. The level of AIF in the mitochondria in the brain in the context of dynamics of the standard growth of melanoma (-) versus that stimulated (--).

Figure 2. The level of AIF in liver mitochondria in the context of dynamics of standard growth of melanoma (-) versus that stimulated (--).

Figure 3. AIF level in the heart mitochondria in the context of dynamics of standard growth of melanoma (-) versus that stimulated (--).

Figure 4. AIF level in kidney mitochondria in the context of dynamics of standard growth of melanoma (-) versus that stimulated (--).

Figure 5. AIF level in skin mitochondria in the context of dynamics of standard growth of melanoma (-) versus that stimulated (--).

Figure 6. AIF level in melanoma mitochondria in the context of dynamics of standard tumor growth (-) versus that stimulated (--).
number of the AIF partners. For example, AIF interacts with the g subunit of eukaryotic translation initiation factor 3 (eIF3g) and, as a result, inhibits protein synthesis during apoptosis [19].

In our study, it has been found that the standard growth of melanoma also contributes to an increase in the level of AIF at the initial stages (week 1) in the mitochondria of the brain, the liver and the skin, but it is not the case with the heart and the kidneys. However, later, the level of the marker drops in all the examined samples. A number of researchers have determined that a tissue-specific deletion of AIF in the liver and a global decrease in the AIF activity in mutant Harlequin mice with Aifm1 deficiency are responsible for a disorder in or an abnormality of the oxidative phosphorylation and a change in glucose metabolism, typical for stress [20]. As a consequence of the AIF deficiency in mutant Harlequin mice, higher oxidative stress is noted. Inactivation of AIF in the neurons leads to an impaired activity of the mitochondrial respiratory chain complex I. This disorder in the mitochondrial oxidative phosphorylation reduces the activity of the neurons. The AIF-deficient neurons show an increased capability to use fatty acids for the mitochondrial respiration and the formation of oxygen radicals [21]. Loss of AIF in fibroblasts induces defects in the mitochondrial electron transport chain (ETC) and an inhibition of proliferation [22].

Knowing that the absence of AIF causes only moderate changes in the expression of nucleus-encoded mitochondrial oxidative phosphorylation genes [4], post-transcriptional regulation of these subunits seems to be a more likely scenario. According to this point of view, AIF regulates the biogenesis of the electron transport chain through its physical interaction with the CHCHD4 domain [5]. Previously, we detected not only the stimulation of melanoma growth under the influence of chronic neurogenic pain [12], but also the cancellation of the genetically determined inhibition of malignant tumor growth [23]. In this regard, we believe that the mitochondrial dysfunction in the growth of melanoma against the background of comorbid pathology, CNP, may be other than the standard tumor growth. In this study, the growth of melanoma against the background of CNP has resulted in the reciprocal changes in the level of AIF in the mitochondria in the brain and the liver after 1 week of the tumor growth.

Let us separately focus on the dynamics of the marker in question in the heart mitochondria. It is obvious that, in case of the standard tumor growth, it smoothly decreases from week 1 to 3, and in case of the stimulated one, a sharp rise is noted, followed by a similarly sharp drop. It is known that the release of AIF from the mitochondria in cardiomyocytes is observed after sodium-induced heart failure and post-ischemic-reperfusion injury [24]. This bears witness to the role of AIF in the process preceding the death of cardiomyocytes. In the AIF-knockout mice the skeletal muscle atrophy and cardiomyopathy, secondary to the complex I deficiency, develop. In addition, the AIF deficiency leads to a higher sensitivity to oxidative stress and necrotic-like cardiomyocyte death, that is observed in the mutant mice, which are also more prone to heart damage after ischemia-reperfusion [25]. In the mitochondria of the kidneys, some reciprocal changes are noted upon expiration of 3 weeks only.

In the mitochondria of the skin not affected by the malignant process, the reciprocal changes were found both in week 1 and 2. The dynamics of the AIF level in the tumor mitochondria was similar.

At present, it turns out that many of the genes and proteins involved in mitochondrial apoptosis may acquire properties, which cause cell death as secondary rather than primary factors. Since the mitochondrial function make its influence on numerous major cellular and organismal functions, it is not surprising that AIF is a representative of the system of the apoptotic factors, a marker of mitochondrial stress, and it is involved in the adaptation of organelles to pathological impacts like malignant growth. The fluctuations in the AIF level revealed in the mitochondria of the heart, the studied organs, and the tumor itself, most likely, should be considered from the point of view of the participation of this agent in the functioning of the mitochondrial respiratory chain complexes. The data on the chemoluminescence of cardiac mitochondrial conglomerates under the development of malignant neoplasms with chronic neurogenic pain are also consistent therewith [26].

Conclusions

Studying AIF only within the mitochondria without an assessment of the cytosolic fraction of this flavoprotein, we believe that the revealed dynamics of the AIF level characterizes the functions of the com-
plexes in the mitochondrial respiratory chain. A clear change in the AIF content has been revealed not only in the heart, but also in all the studied somatic organs and tumor tissue, both under the standard tumor growth and that stimulated. A distinctive feature of the malignant growth against the background of comorbid pathology is that the significantly lower levels of the AIF concentration in the heart and in the most organs are recorded that indicates the suppression of the electron transport chain. The rare abrupt rises in the AIF level in the mitochondria of some organs at the stages of tumor growth associated with comorbid pathology can be explained by the difference in the function and/or morphostructure of the organs, as well as their capability to activate protective and adaptive pathways. In the process of adaptation by cells to various pathological processes (for example, metabolic and transcriptional alterations), a certain cell resistance is produced, despite their high loading due to disorders occurring in the mitochondria. In some cases, adaptation can trigger a prolonged epigenetic response that activates protective signaling pathways up to a certain threshold. We believe that further study of the complex of factors involved in the mitochondrial apoptosis and the bioenergetics of the heart and all organs under the tumor growth conditions will be useful to establish the systemic response by the body as a whole at the stages of the development of the malignant process linked with comorbid pathology.

Statement on ethical issues
Studies in animals have been conducted in accordance with the principles of humanity set out by European Community Directive 86/609/EEC and the Helsinki Declaration. The study was approved at the meeting of the Bioethics Commission for Research in Animals at the Federal State Budgetary Institution NMRC of Oncology, the Ministry of Health, Russia, May, 31, 2018, Ethics Commission Record No. 2. All authors signed their informed consent to participate in the study in accordance with the above bioethics rules and regulations.

Conflict of interest
None declared.

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Author contributions
The authors read the ICMJE criteria for authorship and approved the final manuscript.

References
Content of apoptosis factors and self-organization processes in the mitochondria of heart cells in female mice C57BL/6 under growth of melanoma B16 / F10 linked with comorbid pathology

Elena M. Frantsiyants1, Irina V. Neskubina1*, Alla I. Shikhlyarova1, Marina A. Yengibaryan1, Larisa N. Vashchenko1, Ekaterina I. Surikova1, Lyudmila A. Nemashkalova1, Irina V. Kaplieva1, Lidia K. Trepitaki1, Valeriyia A. Bandovkina1, Yulia A. Pogorelova1

1 National Medical Research Centre of Oncology
Russia, 344037, Rostov-on-Don, 14 liniya, 63, building 8
* Corresponding author:
phone: +7 (928) 156-56-56
email: neskubina.irina@mail.ru

Abstract
The aim is to study some mechanisms of regulation of apoptosis and self-organization in the mitochondria in the heart cells in female mice during the growth of experimental melanoma B16/F10 linked with chronic neurogenic pain as comorbid pathology.

Materials and methods
The work has been performed in female mice of the C57BL/6 strain (n=168). The following experimental groups have been presented: the group of intact mice (n=21), the control group (n=21) to reproduce chronic neurogenic pain (CNP), the comparison group (n=63) with a standard growth of melanoma B16/F10 and the main group (n=63) with CNP + melanoma B16/F10. In mitochondria the following concentrations have been determined by the ELISA method: cytochrome C (ng/mg protein), caspase-9 (ng/mg protein), Bcl-2 (ng/mg protein), AIF (ng/mg protein) and calcium (mmol/g protein). The Statistica 10.0 software was used for statistical analysis.

Results
As against the intact values, the standard growth of melanoma reduced in the mitochondria of the heart cells the level of calcium in week 1-2 by 2.9 and 6.7 times, respectively, the AIF content in week 2-3 by 1.4 times (p < 0.05) and 2 times, respectively; Bcl-2 after 1-2 weeks by 1.7 and 2.9 times, while cytochrome C and caspase 9 remained stable throughout the entire period of the melanoma growth. CNP induced a decrease in the level of Ca2+ by 3.2 times, that in Bcl-2 by 1.3 times (p <0.05), in caspase 9 by 1.5 times and an increase in the AIF content by 2.3 times compared with the intact values. After 1 week of the melanoma growth against the background of CNP, the levels of Ca2+ and caspase 9 increased by 5.3 times and 2.4 times relative to the control values, in the subsequent periods of the melanoma growth, Ca2+ decreased almost to undetectable values. AIF, Bcl-2, and cytochrome C changed abruptly, but by the end of the experiment they were at a low level with a 5.2-fold decrease in AIF, and a 2.2-fold decrease in Bcl-2 and cytochrome C content.

Conclusions
The standard growth of B16/F10 melanoma in female mice is accompanied by a decline in the respiratory and energy function of the heart cell mitochondria. The growth of melanoma, stimulated by CNP as comorbid pathology, exacerbates the mitochondrial dysfunction, suppresses the activity of apoptosis factors and leads to the development of myocardial infarction in the vast majority of animals, accompanied by compensatory mitochondrial aggregation and chemiluminescence.

Keywords
Mitochondria, Apoptosis, Self-organization processes, Heart, Chronic neurogenic pain, Melanoma B16/F10, Female mice

Imprint

Introduction
In the myocardium, that is one of the most energy-consuming tissue types in the body, mitochondria occupy 30-40% of the cell volume against 6-8% found in slow and 2-3% in fast muscle fibers. Mitochondria in oxidizable tissues, such as the heart, are numerous, located mostly near myofilaments, and are capable to use fatty acids for energy production. They are adjusted to the continuous supply of energy needed to provide the muscle endurance and the heart function...
tor SIRT1 is suppressed that allows the acetylation of actions caused by angiotensin II, the cytoprotective fac For example, under the hypertensive heart condi also contribute to apoptotic responses in the heart. uli [6]. Changes in the mitochondrial morphology activation even in the presence of the apoptic stim nuous apoptosis inhibitors in cardiomyocytes, which is due to the high level of expression of the endoge The integrity of the outer mitochondrial membrane is regulated both by the anti-apoptic (Bcl-2, Bcl - XL) and pro-apoptic loci (Bax, Bak) of the Bcl-2 protein family. In general, the ratio between the levels of the apoptotic and proapoptotic proteins in the cell is determined by the permeability of the outer mitochondrial membrane and the release of cytochrome C into the cytosol and the activation of caspases with the cleavage of various cellular proteins, causing rapid cell death [5].

Unlike other cell types, cardiomyocytes are highly resistant to the caspase-dependent apoptosis, hence, the death of cardiomyocytes shall involve some caspase-independent apoptotic mechanisms. This is due to the high level of expression of the endogenous apoptosis inhibitors in cardiomyocytes, which prevents Apaf1 from its activity and caspase from its activation even in the presence of the apoptic stimulus [6]. Changes in the mitochondrial morphology also contribute to apoptotic responses in the heart. For example, under the hypertensive heart conditions caused by angiotensin II, the cytoprotective factor SIRT1 is suppressed that allows the acetylation of p53 and the Drp1-dependent mitochondrial division, due to which mitochondrial fragmentation ultimately leads to the cardiomyocyte apoptosis [7]. In addition, the suppression of protein phosphatase 1 in acute ischemic injury activates mitochondrial division and cardiomyocyte apoptosis [8]. Damage to the heart muscle during reperfusion can also cause a collapse in the heart microcirculation, facilitating the expression of mitochondrial division factor Mff, which promotes the apoptosis of the endothelial cells in the heart microcirculation through increased opening of the mitochondrial transition pore and release of cytochrome C into the cytosol [9]. Taken together, the morphological changes in the mitochondria are important reasons for the stimulation of the cardiomyocyte apoptosis in heart diseases. Studies of some pathophysiological features of cardiomyopathies at the subcellular level can be conducted with the involvement of experimental models, which are beneficial for resolving issues in the context of translational medicine [10, 11, 12].

The aim hereof is to study the parameters of apoptosis in the mitochondria in the heart cells of female mice of the C57BL/6 strain with the growth of experimental melanoma B16/F10 linked with chronic neuropathic pain as comorbid pathology.

Materials and methods

Our research has been carried out using female mice of strain C57BL/6 (n=168), 8 weeks of the age, with an individual body mass of 21 to 22 g. The experimental animals have been delivered to us by the Federal State Medical & Biological Institution “Research Center of Biomedical Technologies” (Branch Andreevka, Moscow Region) at the Federal Medical & Biological Agency. The animals were kept under natural lighting conditions with no restrictions on their access to water and food. The research in animals was conducted in accordance with the Directive 86/609/ EEC on the Protection of Animals Used for Experimental and Other Scientific Purpose, as well as in accordance with the International Guiding Principles for Biomedical Research Involving Animals and Order No. 267 “Approval of the Rules of Laboratory Practice” dated June, 19, 2003 issued by the Ministry of Health of the Russian Federation. The research record was approved by the Commission on Bioethics at the Federal State Budgetary Institution “National Medical Research Center of Oncology”, the Ministry of Health of the Russian Federation (Record No. 2 dated May, 31,
Manipulations with animals were performed in the box in compliance with the generally accepted rules of asepsis and antisepsis.

We used the line of mouse melanoma B16/F10 supplied by the Russian National Medical Research Center of Oncology named after N.N.Blokhin, Ministry of Health, Russia. The material for transplantation was obtained from donor mice on day 12-16 of the tumor growth. The B16/F10 melanoma transplantation was performed by a standard subcutaneous injection of the tumor suspension under the right scapula in a volume of 0.5 ml of the cell suspension in a 1:10 dilution with saline solution. Tools, dishes, and hands were disinfected in a generally accepted way in compliance with the generally accepted rules of asepsis and antisepsis.

The model of chronic neurogenic pain (CNP) was reproduced by applying a ligature to the sciatic nerve on both sides under xyl-zoletil anesthesia [13]. The anesthesia medication was employed as follows: xyl-zoletil, 10 minutes before the main anesthesia; premedication: xylazine (Xyl preparation) intramuscularly, at a dose of 0.05 ml/kg of body mass (according to the instructions), then after 10 minutes, Zoletil-50 was administered at a dose of 10 mg per 100 g of body mass.

The animals were randomly assigned to the following experimental groups: the intact group (n=21), the control group (n=21) to reproduce the model of chronic neurogenic pain (CNP), the comparison group (n=63) with mice which were reproduced with the model of CNP and which 3 weeks after that were transplanted with the B16/F10 melanoma.

Decapitation of the animals has been performed with the guillotine, in the main and the comparison group, in the following periods of time: week 1 is day 7 of the melanoma growth, week 2 is day 14 of the melanoma growth, and week 3 is day 21 of the melanoma growth. After decapitation, the heart was quickly extracted with the use of refrigerants; mitochondria were isolated by the method of Egorova M. V., Afanasiev S. A. [14] (using refrigerants and differential centrifugation on a high-speed refrigerated centrifuge Avanti J-E, BECMAN COULTER, USA). The tissues were washed with an icy 0.9% KCl solution. To destroy the intercellular bonds, the cell wall and plasma membranes, we used mechanical treatment of tissues with grinding with scissors and homogenization in a glass homogenizer with a Teflon pestle (Potter-Elvehjem homogenizer). We have added per gram of tissue, 10 ml of the isolation medium (0.22 M mannitol, 0.3 M sucrose, 1 mm EDTA, 2 mM TRIS-HCL, 10 mm HEPES, pH 7.4). The tissues were homogenized and centrifuged for the first time for 10 minutes at a speed of 1000 g, at a temperature of 0 - 2 °C, the second and third centrifugation was carried out at 20000 g, for 20 minutes, at a temperature of 0 - 2 °C. Between the centrifugation procedures, the mitochondrial sediment was resuspended in the isolation medium. Mitochondria were further purified from lysosomes, peroxisomes, melanosomes, etc., with the use of a 23% Percoll gradient. The suspension of subcellular structures was layered on the Percoll gradient, centrifuged for 15 min at 21000 g, after which the separation into 3 phases was observed; the lower layer of mitochondria was left and resuspended with the isolation medium. The next washing of the mitochondria was performed by centrifugation for 10 minutes at 15000 g, at a temperature of 0 - 2 °C. The mitochondrial samples (protein concentration 4-6 g / l) were stored at -80 °C in the isolation medium before their analysis. By the ELISA method determined were the following concentrations: cytochrome C (ng/mg protein), caspase-9 (ng/mg protein) (Bioscience, Austria), Bcl-2 (Thermo Fisher Scientific, Austria), AIF (ng/mg protein) (Ray-Biotech, USA); the concentration of calcium (Ca2+) (mmol/g protein) was determined by the method of arsenazo III (Abris+, Russia), and protein (mg/ml) was estimated by the Biuret method (Olvex Diagnosticum, Russia) with the ChemWell automatic analyzer (Awareness Technology INC, USA).

In parallel with the study of the apoptosis factors, a visual examination of the supramolecular processes in the mitochondrial self-organization in native mitochondrial suspension smears made on slide glasses was performed for the general assessment of the state of the mitochondrial energy. Smears were stained according to Pappenheim, and we used also an alcoholic solution of fuchsin with the addition of a drop of ammonia applied to the smear under microscopy, photo and video recording. Applied were the techniques of light, dark and polarizing microscopy with the Leica DM LS2 microscope with the x40, x90, x100 magnification, by capturing photo and video images utilizing digital camera OLYMPUS Camedia C-5050 (Germany) and image input device OLYMPUS C-3040 ADU (Japan).

The Statistica 10.0 software was applied for statistical analysis of the obtained data. The data were
analyzed for the compliance of the features distribution with the normal distribution law using the Shapiro-Wilk test (for small samples). The comparison of the quantitative data in the groups (independent sampling) was carried out using the Kruskal-Wallis test (multiple comparisons). The table data are presented in the $M\pm m$ form, where $M$ is the arithmetic mean, and $m$ is the standard error of the mean; $p<0.05$ was taken as the level of statistical significance. The obtained results were statistically processed in compliance with the general recommendations for medical research.

**Results**

The results have shown that the reproduction of chronic neurogenic pain (CNP) caused by bilateral ligation of the sciatic nerves makes a stimulating effect on the growth of experimental melanoma in female mice and promotes early tumor formation and metastasizing, as well as shortening of the life expectancy span of animals and canceling the genetically determined inhibition of the malignant process [15].

In the dynamics of two variants of the melanoma growth, i.e. an independent melanoma growth and that with comorbid pathology (CNP), the state of the heart muscle in female mice was examined and assessed macroscopically and morphologically; the results thereof are presented in Table 1 herein.

The morphological examination of the areas of the heart muscle damage has demonstrated that in the myocardium there are hemorrhage, foci of necrosis, ruptures of some individual cells, focal infiltration by leukocytes, fibrinous necrosis of the vascular walls, enlarged heart cavities, blood clotting in the lumen.

Firstly, it was of interest to study changes in the dynamics of the apoptosis factors in the mitochondria of the heart cells during the standard growth of melanoma (1, 2, 3 weeks) (see Table 2 herein). It was found that the level of calcium in the mitochondria of the heart after 1 week of the tumor growth decreased by 2.9 times compared with the same indicator in the intact animals, by 1.7 times, and after 2 weeks it was recorded to be 2.9 times lower, remaining the same upon expiration of 3 weeks. The level of cytochrome C and caspase 9 in the mitochondria of the heart cells did not have statistically significant changes in the dynamics of the standard melanoma growth.

In addition, it was important to study the indicators of apoptosis in the mitochondria of the heart cells after the reproduction of CNP before the tumor inoculation. It was found that under the influence of CNP, in the mitochondria of the heart, the level of calcium decreased by 3.2 times, the level of Bcl-2 by 1.3 times ($p <0.05$) and caspase 9 by 1.5 times ($p <0.05$) compared with the respective indicators in the intact mice. At the same time, the AIF content, on the contrary, increased by 2.3 times, and the level of cytochrome C did not significantly differ from the intact level (see Table 2 herein).

1 week after the inoculation and the growth of melanoma against the background of CNP as the comorbid process, the level of calcium in the mitochondria in the heart cells in the female mice increased by 5.3 times as compared with the control values. In the subsequent periods of the study, the level of calcium in the mitochondria in the heart cells during the tumor process against the background of CNP decreased to almost undetectable values. The level of the AIF concentration in the mitochondria in the heart cells in the dynamics of the melanoma growth linked with CNP changed abruptly: 1 week after the tumor transplantation, it increased by 3.7 times, then after 2 weeks it fell to the level of the control values, and after 3 weeks it became 5.2 times lower than that recorded in the control animals and 2.3 times lower as against that in the mitochondria of the heart cells in the intact ani-

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**Table 1.**

<table>
<thead>
<tr>
<th>Groups of animals</th>
<th>% of animals with heart muscle damage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Standard melanoma growth B16/F10</td>
<td>-</td>
</tr>
<tr>
<td>Stimulated melanoma growth (CNP + B16/F10)</td>
<td>14.3%</td>
</tr>
<tr>
<td></td>
<td>57.1%</td>
</tr>
<tr>
<td></td>
<td>71.4%</td>
</tr>
</tbody>
</table>

Note: The table shows % of the animals with heart muscle damage.

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The levels of Bcl-2 and cytochrome C were similarly altered. Thus, the level of Bcl-2 after 1 week of the melanoma growth against the background of CNP increased by 1.7 times (p<0.05) as compared with the control level, and then after 2-3 weeks it decreased and became on average 2.2 times lower than that in the mitochondria of the control group. The level of cytochrome C after 1 week had no statistically significant differences from the values in the control group, and after 2-3 weeks it decreased by 2.2 times. The level of caspase 9 in the mitochondria in the mouse heart cells during the growth of melanoma linked with CNP was high, on average, 2.4 times higher than the control value.

Under microscopy of the heart cells mitochondria suspension smears in week 2-3 of the melanoma growth against the background of CNP, a process of high-brightness cold light (chemiluminescence) was recorded. During the photo and video examination, the phenomenon of luminescence was manifested both in the form of a spot-type chemiluminescent reaction by the substrate, representing vast areas of small granules of mitochondrial associates, and in the form of the formation of enlarged complexes-associates of the linear filamentous shape, located in the field of view. The luminescence of the heart mitochondrial samples was accompanied by bright flashes, as well as 10-15 seconds of intense white glow with its gradual fading and sedimenting a large filamentous aggregation of mitochondria on the substrate layer (see Figure 1 herein).

The luminescence effect was not observed in preparations of other organs: it was detected only in the associates of the mitochondria of the heart in animals showing the tumor growth against the background of chronic neurogenic pain. Concomitant comorbid pathology, CNP, contributed to the deployment of the processes of disabling of the energy supply systems in the energy systems of cardiomyocytes. Apparently, the disabling process occurred through an elevation of a low-energy shift, an increase in acetic-oxalic restriction (degree 1-4), and an increase in free-radical activity, as was previously shown in chemical carcinogenesis [16]. It can be assumed that structuring of the mitochondrial associates of cardiomyocytes is associated with the formation of long electron transport systems, the suppression of respiration against the background of a drop in the level of cytochrome C and the depletion of endogenous succinic acid reserves [16].

All of the above factors contributed to the production of the effect of the bright luminescence chemical reaction. Obviously, the revealed effect of the induced chemiluminescence of the heart mitochondria is directly related to a significant increase in the concentration of calcium in week 1, which is also one of the key conditions for the regulation of apoptosis. In this case, we can note a single complex path of self-organization of the mitochondrial processes in the implementation of the program of cardiomyocyte death, including the detected changes in Bcl-2, AIF and caspase content as a natural result from the influence of chronic neurogenic pain.

### Table 2
Content of apoptosis factors in the mitochondria in the heart cells in female mice with standard versus stimulated by chronic neurogenic pain growth of melanoma B16/F10

<table>
<thead>
<tr>
<th></th>
<th>Ca&lt;sup&gt;2+&lt;/sup&gt; mmol / g protein</th>
<th>AIF ng / mg protein</th>
<th>BCL-2. ng / mg protein</th>
<th>Cytochrome C ng / mg protein</th>
<th>Caspase 9 ng / mg protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intact</td>
<td>0.19±0.015</td>
<td>40.53±1.25</td>
<td>62.00±2.85</td>
<td>4.61±0.57</td>
<td>0.266±0.028</td>
</tr>
<tr>
<td>Control group (CNP)</td>
<td>0.06±0.004&lt;sup&gt;1&lt;/sup&gt;</td>
<td>91.24±3.72</td>
<td>46.66±3.87&lt;sup&gt;1&lt;/sup&gt;</td>
<td>6.18±0.59</td>
<td>0.174±0.018&lt;sup&gt;1&lt;/sup&gt;</td>
</tr>
<tr>
<td>B16/F10 melanoma growth (comparison group)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>week 1</td>
<td>0.067±0.015&lt;sup&gt;1&lt;/sup&gt;</td>
<td>36.62±1.74</td>
<td>36.85±1.92&lt;sup&gt;1&lt;/sup&gt;</td>
<td>5.11±0.59</td>
<td>0.238±0.019</td>
</tr>
<tr>
<td>week 2</td>
<td>0.029±0.002&lt;sup&gt;1&lt;/sup&gt;</td>
<td>29.51±1.38&lt;sup&gt;1&lt;/sup&gt;</td>
<td>21.38±1.56&lt;sup&gt;1&lt;/sup&gt;</td>
<td>3.81±0.47</td>
<td>0.204±0.02</td>
</tr>
<tr>
<td>week 3</td>
<td>0.024±0.002&lt;sup&gt;1&lt;/sup&gt;</td>
<td>19.91±1.48&lt;sup&gt;1,4&lt;/sup&gt;</td>
<td>21.6±1.55&lt;sup&gt;1,4&lt;/sup&gt;</td>
<td>4.92±0.43</td>
<td>0.221±0.018</td>
</tr>
<tr>
<td>CNP + B16/F10 melanoma growth (main group)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>week 1</td>
<td>0.32±0.016&lt;sup&gt;1,2&lt;/sup&gt;</td>
<td>34.8±11.64&lt;sup&gt;2,3&lt;/sup&gt;</td>
<td>80.99±1.6&lt;sup&gt;2,3&lt;/sup&gt;</td>
<td>7.62±0.47</td>
<td>0.388±0.022&lt;sup&gt;2,3&lt;/sup&gt;</td>
</tr>
<tr>
<td>week 2</td>
<td>0.01±0.001&lt;sup&gt;2,3&lt;/sup&gt;</td>
<td>107.03±4.26&lt;sup&gt;2,3&lt;/sup&gt;</td>
<td>24.03±1.38&lt;sup&gt;2,3&lt;/sup&gt;</td>
<td>2.93±0.47&lt;sup&gt;2,3&lt;/sup&gt;</td>
<td>0.457±0.034&lt;sup&gt;2,3&lt;/sup&gt;</td>
</tr>
<tr>
<td>week 3</td>
<td>0.01±0.001&lt;sup&gt;2,3&lt;/sup&gt;</td>
<td>17.68±1.5&lt;sup&gt;1,2,4&lt;/sup&gt;</td>
<td>19.35±1.09&lt;sup&gt;2,4&lt;/sup&gt;</td>
<td>2.74±0.36&lt;sup&gt;2,4&lt;/sup&gt;</td>
<td>0.420±0.026&lt;sup&gt;2,4&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Note: statistically significant differences: <sup>1</sup> - in relation to the level in the intact group; <sup>2</sup> - in relation to the level in the CNP group (control); <sup>3</sup> - in relation to the level at the previous period of the study; <sup>4</sup> - in relation to the level in week 1.
genic pain under the growth of melanoma B16/F10. The common structural and functional features of the degradation in the mitochondria of the heart cells demonstrate the leading role of these organelles in the triggering mechanisms of apoptosis.

Discussion and conclusions

A number of authors have shown that the mitochondria in the heart cells in female rats show their greater capability to retain calcium than it is the case with the mitochondria in the male rodents [17]. The calcium kinetics also differs between the heart mitochondria in the male and female rats [18]. Mitochondria in females are more resistant to mitochondrial swelling at high concentrations of calcium [19]. The influx of calcium into the mitochondria is necessary for their activation [20].

In our study, it was found that in the dynamics of the standard growth of melanoma B16/F10 in female mice, the level of calcium in the mitochondria of the heart cells progressively decreased, but still remained at the level of the determined values of the indicator.

It is known that calcium is an important secondary messenger responsible for linking of the contractile function of the heart and mitochondrial metabolism [21]. The authors hereof have shown a correlation between the mitochondrial calcium content and the cardiac dysfunction under chronic stress. However an exact amount of calcium absorbed by mitochondria in the normal and weakened hearts has not been determined.

Taking into account the above, we can assume there is a disorder in the contractile function of the heart in the dynamics of the standard growth of melanoma, as a manifestation of tumor stress, or rather, a systemic effect produced by the tumor on the organism. However, our macroscopic and morphological examination revealed no signs of damage to the heart wall. Interestingly, CNP as an independent pathogenic factor also led to a decrease in the level of calcium in the mitochondria of the heart, but without disordering the structural integrity of the myocardial cells.

Only under the conditions, when the initial growth of melanoma occurred against the background of CNP as comorbid pathology, a sharp increase in the content of calcium in the mitochondria in the heart cells was observed (week 1). Only in 14.3% of the cases, myocardial damage was detected (see Table 1 herein).

It should be mentioned that mitochondrial metabolism is stimulated by calcium, but under pathological conditions, calcium overloading can trigger opening of the mitochondrial permeability transition pore (mPTP). The release of the mitochondrial content, such as cytochrome C, causes apoptosis or loss of membrane potential and, as a result, prolonged opening of mPTP, which causes ATP deprivation and necrosis [22]. Most of the evidence data on calcium toxicity in mitochondria comes from experiments using genetic approaches. Indeed, calcium overloading in a number of animal models leads to mitochondrial phenotypes similar to those observed in heart failure, such as mPTP opening, increased mitochondrial oxidative stress, mitochondrial membrane potential collapse, impaired ATP production and cardiomyocyte necrosis [23,24].

At a later stage of the study, the further growth of melanoma against the background of CNP (week 2 and 3) was characterized by a sharp decrease in the level of calcium in the mitochondria of the heart almost to zero that can be regarded as a dramatic disorder in the heart rhythm. Just in these periods of the study we
have detected 57.1-71.4% of the cases of heart attacks in female mice (see Table 1 herein).

It should be noted that the most obvious connection between myocardial infarction caused by myocardial ischemia and necrotic cell death is mPTP. Cardiomyocytes, as terminally differentiated cells, have an extremely limited ability to regenerate, and excessive death of heart myocytes caused by stress and their pathological effects leads to the development of various heart diseases, including myocardial infarction [25]. Using a similar probe, Hamilton S. and Terentyev D. [26] recently have shown that increased mitochondrial calcium accumulation increases the mitochondrial ROS production and increases proarrhythmic spontaneous calcium release.

In our experiment, it was recorded that with the standard growth of melanoma, along with a decrease in the calcium content, starting from the second week after the tumor transplantation, a decrease in the level of AIF was observed in the mitochondria of the heart in female mice. This fact should probably be considered as a gradual transition to the hypoxic type of respiration and the production of ATP. A slightly different situation occurs when studying the dynamics of AIF in the mitochondria of the heart during the growth of melanoma stimulated by CNP. First of all, CNP itself induces a more than twofold increase in the level of this factor and an even greater increase therein upon expiration of 1 week after tumor transplantation, which also correlates with the level of calcium.

It should be noted that AIF is an evolutionarily old mitochondrial flavoprotein involved in the embryonic development and survival of the heart cell [27]. Healthy mitochondria contain a mature form of AIF consisting of two FAD-binding domains, the NADH-binding domain and the C-terminal domain. AIF is attached to the inner mitochondrial membrane (IMM), where it plays a bioenergetic role, regulating mainly the activity of complex I of the mitochondrial respiratory chain [28]. It has been found that human or mouse cells deprived of AIF accumulate lactic acid and show an increased dependence on the formation of ATP by glycolysis due to a serious decrease in the activity of the respiratory chain complex I. Further studies have shown that without AIF complex I demonstrates the most serious damage. So, AIF is required for the normal process of oxidative phosphorylation [3]. In addition, AIF deficiency leads to a higher sensitivity to oxidative stress [3]. A rise in the ROS production may be directly related to an increase in the calcium content. The mitochondrial respiratory chain complexes I-IV transfer electrons to oxygen, producing superoxide radicals as a by-product of this process due to incomplete oxygen reduction. At low concentrations, ROS may mediate some physiological effects, but the ROS overproduction is involved in the pathogenesis of coronary heart disease. Elevated mitochondrial calcium levels were shown in a mouse model of mitochondrial cardiomyopathy [29]. The authors note that this increase in calcium may contribute to a deficiency in oxidative phosphorylation. A deficit of oxygen and respiratory substrates stops oxidative phosphorylation, leading to the collapse of the mitochondrial membrane polarization, calcium overloading, the cytochrome C release, impaired membrane permeability, and, finally, cell necrosis. Thus, mitochondria play a central role in both types of cell death: necrosis and apoptosis [30].

The best-characterized function of the Bcl-2 family consists in controlling the permeability of the mitochondrial outer membrane [5].

In the present study, no increase in the level of Bcl-2 was found in the mitochondria of the heart during any period of the study with standard tumor growth. On the contrary, in the dynamics of the tumor growth, its content in the mitochondria of the heart progressively decreased. With the growth of melanoma linked with comorbid pathology after 1 week of the growth, an increase in the level of Bcl-2 was detected in the mitochondria of the heart, along with an increase in the content of calcium and AIF, and their further decline in the dynamics of tumor growth was recorded.

The drop in the level of AIF could also be interpreted from the standpoint of intracellular stress, which results in the depolarization of the mitochondrial membrane, followed by the release of the apoptogenic form of AIF from the mitochondria into the cell nucleus, where it induces chromatin condensation and large-scale DNA fragmentation through a mechanism independent of caspase activation [27].

As to caspase 9 in the mitochondria of the heart, studied herein, its content in case of the standard growth of melanoma in mice did not have statistically significant differences from the indicators in intact animals throughout the experiment. However, in the mitochondria of the mice heart with the growth of melanoma linked with CNP, the level of caspase 9 remained at high values throughout the study period.
Similarly to AIF, cytochrome C is an important component of the mitochondrial respiratory chain, responsible for the transfer of electrons from complex III to IV, and plays an important regulatory role in oxidative phosphorylation due to its highly dynamic interactions with redox targets [31].

In the context of our study, it was shown that the level of cytochrome C in the mitochondria of the heart with standard growth of melanoma did not experience significant changes during all the study periods, while with the growth of melanoma against the background of comorbid pathology after two weeks, its sharp decline was noted.

The mitochondrial function is known to be closely related to cardiomyopathies and coronary heart disease. Although the exact underlying mechanisms are not fully understood, it is clear that mitochondrial metabolism is intensively involved in both heart damage and protection [22]. We should also mention a unique discovery in the heart muscles: it is a continuous mitochondrial communication [32]. It is noted that there are between the neighboring mitochondria “kissing molecules” available, which provide the exchange of proteins and ions. In addition, nanotunnels are found between the neighboring organelles to control the mitochondrial response, especially under changes in the calcium dynamics [33]. Indeed, in the preparations of the mitochondria of the heart in the animals with the tumor growth against the background of chronic pain, it was possible to see significant areas of associates forming complexes and a spatially complicated, self-organized, network structure. All the above points to the important role of the surface charge in establishing intermitochondrial contacts. Because of the above, permanent pain stimulation, modifying the pathogenesis of the tumor, determines the only way of energy supply through the self-organization of long electron transport pathways, transmembrane movement of protons and an increase in the potential difference at the membrane, followed by photo effects of luminescence in a reactive medium activated by NH4OH. The enlargement of the sizes of the moving in space luminous aggregations is evidence for their complex multidimensional organization. Obviously, the critically high load on the heart under the conditions of an integration of chronic pain and the tumor growth is realized due to the compensatory reaction of the mitochondriome self-organization, as a key factor of life support under the conditions of prolonged stress, when protection is provided by destruction and high energy consumption.

Taking into account the above, we consider it possible to draw the following conclusions:

1. The standard growth of B16/F10 melanoma and the development of CNP as independent variants of pathology in female mice were accompanied by a decrease in the respiratory and energy function of the heart cell mitochondria.

2. The combination of both factors, namely, the growth of melanoma against the background CNP as comorbid pathology, significantly aggravated the mitochondrial dysfunction and led to the development of myocardial infarction in the vast majority of animals.

3. The mitochondrial mechanisms of apoptosis and self-organization of subcellular energy structures under the conditions of extreme functional loads linked with the growth of a malignant tumor against the background of CNP as a comorbid state are mediated by disordering the poly-enzyme systems of the apoptosis regulation, by a high level of oxidative stress that induces chemiluminescence of mitochondrial associates in cardiomyocytes.

Statement on ethical issues
Research involving people and/or animals is in full compliance with current national and international ethical standards.

Conflict of interest
None declared.

Author contributions
The authors read the ICMJE criteria for authorship and approved the final manuscript.

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3. Bano D, Prehn J. Apoptosis-Inducing Factor (AIF) in Physiology and Disease: The Tale of a Repented
State of free-radical processes in the heart cell mitochondria under melanoma B16/F10 growth against the background of chronic neurogenic pain as comorbid pathology


1 National Medical Research Centre of Oncology
Russia, 344037, Rostov-on-Don, 14 liniya, 63, building 8
* Corresponding author:
phone: +7 (928) 156-56-56, email: neskubina.irina@mail.ru

Abstract
The aim hereof has been to investigate the dynamics of processes of free radical oxidation and antioxidant protection in the heart cell mitochondria in female mice of strain C57BL/6 at different stages of the B16/F10 melanoma growth under the comorbid pathology conditions, namely, chronic neurogenic pain.

Materials and methods
Our research work was conducted in female mice of strain C57BL/6 (n=168). The animals were randomly distributed in separate groups as follows: the group of intact mice (n=21); the reference group (RCNP) (n=21) to reproduce the model of chronic neurogenic pain (CNP); group M (n=63) with melanoma B16/F10 upon subcutaneous transplantation of the tumor; group CNP + M (n=63), where the B16/F10 melanoma was transplanted 3 weeks after CNP modeling. In the heart cell mitochondria samples, using ELISA tests, we have determined concentrations of superoxide dismutase 2 (SOD 2) (pg/mg protein), 8-hydroxy 2’ deoxyguanosine (8-OHdG) (ng/mg protein); malone dialdehyde (MDA) (mcM/mg protein); the total SOD activity (units/mg protein), the Mn SOD activity (units/mg protein) and the Cu-Zn SOD activity (units/mg protein). The obtained statistics data have been processed with software Statistica 10.0.

Results
Under the CNP conditions, we have revealed in the female mice in the heart cell mitochondria that the SOD 2 level has decreased by 2.9 times, the total SOD activity has been diminished by 1.54 times (p<0.05), and the Cu-Zn SOD activity has been recorded to be 2.7 times lower, as compared with the respective data in the intact animals. 1 week after the melanoma growth stimulated by CNP, as against the reference values, an increase in the SOD 2 level by 3.2 times has been identified, and it has demonstrated high values upon expiration of 2 and 3 weeks of the tumor growth. Considering the same period of the tumor growth under the CHP conditions, we have observed an elevation of the activity of the total SOD and the Mn SOD by 1.7 and 2 times, respectively (p<0.05). On the contrary, 1 week after the stimulated tumor growth, the Cu-Zn SOD activity has been lowered reaching its undetectable values; upon expiration of 2 weeks it has been found to be at the level of the respective reference values, but after 3 weeks its decrease by 3 times has been recorded. The 8-OHdG concentration has been revealed to be increased after 1 week by 4 times and after 2 weeks by 6.6 times, respectively. The MDA level in week 1 and 2 has exceeded the reference levels on the average by 2 times. Upon expiration of 3 weeks of the stimulated tumor growth, both indicators have been found to reach the level of the reference values.

Conclusion
The accompanying chronic neurogenic pain has contributed to the functional re-setting of subcellular structures in the organs not affected by the tumor. In the heart cell mitochondria, which are considered as the most sensitive mechanisms in the cell regulation, we can observe surges in the prooxidant activity followed by further dynamics of its normalization. The initial suppression of the activity of the antioxidant system elements gives the way to a considerable surge and maintenance of a permanent or a variable high level of the activity that bears witness to the fact that stress or tension develops accompanied by depletion of energy resources of the heart under the CNP conditions.

Keywords
Mitochondria, Heart, Free radical oxidation, Antioxidant protection, Chronic neurogenic pain, Melanoma B16/F10, Female mice

Imprint
Introduction

The heart always requires a large quantity of energy to be used primarily to maintain the cardiac muscle contractility and blood circulation. But unexpectedly the heart demonstrates its low-level capability of accumulating energy. Therefore, in order to keep up the required high-density energy flux by the heart, the ATP molecules should be synthesized rapidly, without any interruptions [1]. In cardiomyocytes, the mitochondria take up about one third of the entire volume of the cells and produce more that 95% of ATP that reflects a great demand of this cell type for energy. Therefore, it is not surprising that the mitochondrial dysfunction has much to do with development of cardiomyopathy and a higher risk of heart insufficiency [2]. Disorders in functioning of mitochondria may be attributed to the following factors: an imbalance in the mitochondrial dynamics [3], an aberrant mitochondrial lipid homeostasis [4], vitamin- and co-factor-related deficiency of metabolism and altered redox processes [5]. Many aspects of the mitochondrial dysfunction are also treated as cancer contributors [6].

At present, a break-through high-tech system is available that makes possible to apply a fresh digital approach to a phase-related analysis of pathological changes in the cardiovascular system performance. The above approach offers to non-invasively estimate the mitochondrial dysfunction in cardiomyocytes by delivering computed data on concentrations of metabolites like oxygen, lactate and phosphocreatine with the use of the original proprietary analytical software employed by hemodynamic analyzer CARDIOCODE (PC-assisted hemodynamic analyzer CARDIOCODE, designed and manufactured by STC Cardiocode, Certificate of Registration RU No. FSR 2011/12126, Taganrog, Russia). This high-tech system shows much promise for quantified assessment of functional loading and actual pathological processes including those in case of oncopathology. An extra option of the offered technology is establishing of laboratory biochemical relationships and parallels when determining the mitochondrial dysfunction of cardiomyocytes, especially with respect to levels of reactive oxygen species (ROS), in case of significant functional loading, under the normal conditions and pathology, including the tumor growth and accompanying comorbid states. In the framework of translational medicine, an application of experimental models can be a help in solving a variety of problems revealing dysfunctional factors [7, 8, 9].

An accumulation of ROS in cells is the primary cause of the damage and the dysfunction of mitochondria. In fact the mitochondria are the main cellular source of ROS. Electrons from coferments (NADH and FADH 2) are closely linked with oxidative phosphorylation to form ATP. However about 0,2-2% of the electrons escape the electron transport chain, and they are aberrantly accepted by O2 that results in formation of a superoxide anion radical. The electron escape can take place in every of the three complexes, i.e. in complex I, II and III. The superoxide is released from complex I and II into the matrix and from complex III into the intermembrane space and the matrix [2]. Long electron transportation pathways in mitochondrial associates in cardiomyocytes can be detected by photo-induced effects as chemiluminescence [10].

In the mitochondria, the ROS production is balanced by an effective detoxification protection. In the matrix layer, superoxide may be transformed by mitochondrial antioxidant manganese SOD – SOD2, and H2O2 can either freely move through the mitochondrial membranes or be further detoxified by other mitochondrial antioxidant ferments like glutathione peroxidase (GPx) [11]. At its lower levels, ROS may serve as a signal molecule for modification of the signal proteins [2]. But an excess of the ROS production is accompanied by a decline in antioxidant capacity and an elevation of oxidative stress. An excess of ROS leads to an interruption in energy generation, an increase in death of the cells and an irreversible oxidative damage of the mitochondrial DNA (mtDNA) and alterations in gene expression.

The aim of our study was to investigate indicators of free radical oxidation and antioxidant protection in the heart cell mitochondria in female mice of strain C57BL/6 under growing melanoma B16/F10 against the background of comorbid pathology, namely, chronic neurogenic pain.

Materials and methods

Our research work was conducted in female mice of strain C57BL/6 (n=168), at the age of 8 weeks, with an initial body mass of 21-22 g. The animals were randomly distributed in separate groups as follows: the group of intact mice (n=21); the reference group (RCNP) (n=21) to reproduce the model of chronic neurogenic pain (CNP); group M (n=63) with melanoma B16/F10 upon subcutaneous transplantation of the tumor cells; group CNP + M (n=63), where the
B16/F10 line melanoma was transplanted 3 weeks after the completion of CNP modeling.

The experimental animals have been delivered to us by the Federal State Medical & Biological Institution “Research Center of Biomedical Technologies” (Branch Andreevka, Moscow Region) at the Federal Medical & Biological Agency; mouse melanoma line B16/F10 have been supplied by the Russian National Medical Research Center of Oncology named after N.N.Blokhin, Ministry of Health, Russia. The tested animals have been kept under natural light conditions with a free access to water and food. Our research study has been conducted in accordance with the relevant regulations stated by EU Directive 86/609 EEC, the Declaration of Helsinki (DoH), the International Guiding Principles for Biomedical Research Involving Animals and Order No. 267 “Approval of the Rules of Laboratory Practice” dated June, 19, 2003 issued by the Ministry of Health of the Russian Federation; our study has been approved by the Commission on Bioethics at the Federal State Budgetary Institution “National Medical Research Center of Oncology”, the Ministry of Health of the Russian Federation (Record No. 2 dated May, 31, 2018). All researchers have signed their written informed consent thereon.

Melanoma B16/F10 was transplanted in a standard manner under the skin of the right scapula in the volume of 0.5 ml of tumor cell suspension in a 1:10 dilution with saline solution. The chronic neurogenic pain (CNP) model was reproduced in animals by ligation of the sciatic nerves in both hind limbs under xyl-zoletil anesthesia [12]. For preanesthetic premedication, 10 minutes before anesthesia, used were xylazine (Xyl) intramuscularly at a dose of 0.05 ml/kg of body weight (according to the instructions), then, 10 minutes later, Zoletil-50 at a dose of 10 mg per 100 g of body weight. Manipulations with animals were carried out in the box in compliance with the generally accepted rules of asepsis and antisepsis.

Decapitation of the animals was performed with the use of the guillotine. Animals from groups M and CNP+M were decapitated after the B16/F10 melanoma transplantation in the following periods: 1 week of the melanoma growth, 2 weeks and 3 weeks of the melanoma growth. The CNP group mice were decapitated 3 weeks after the CNP model reproduction: their decapitation was performed in parallel with that of the intact mice. Mitochondria were separated by the method of Egorova M. V., Afanasiev S. A. [13] (with the use of refrigerants and differential centrifugation with high-performance refrigerated centrifuge Avanti J-E, BECMAN COULTER, USA). The tissues were washed with an ice-cold 0.9% KCl solution. To disrupt the intercellular bonds, the cell walls and the plasma membranes, mechanical treatment of tissues with grinding using scissors and homogenization in a glass homogenizer with a Teflon pestle (Potter-Elvehjem homogenizer) was utilized. Per gram of tissue, 10 ml of the isolation medium was added (0.22 M mannitol, 0.3 M sucrose, 1 mM EDTA, 2 mM TRIS-HCL, 10 mM HEPES, pH 7.4). The tissues were homogenized and centrifuged for the first time for 10 minutes at 1000 g at a temperature of 0-2 °C, the second and third centrifugation procedures were completed at 20000 g for 20 minutes at a temperature of 0-2°C. Between the centrifugation procedures, the mitochondria sediment was resuspended in the isolation medium. Mitochondria were further purified from lysosomes, peroxisomes, melanosomes, etc. using the Percoll 23% density gradient centrifugation. The suspension of the subcellular structures was layered on the Percoll gradient, centrifuged for 15 min at 21000 g, after which the separation into 3 phases was observed; the lower layer of mitochondria was left and resuspended with the isolation medium. The next mitochondria washing procedure was carried out by centrifugation for 10 minutes at 15000 g at a temperature of 0 - 2°C. The prepared mitochondrial samples (protein concentration 4-6 g/l) were stored at -80°C in the isolation medium before their analysis. In the mitochondrial samples, using ELISA tests, we have determined concentrations of superoxide dismutase 2 (SOD 2) (pg/mg protein) (ABfrontier, Netherlands), 8-hydroxy 2′ deoxyguanosine (8-OHdG) (ng/mg protein) (Enzo Life Sciences, Switzerland); the total SOD activity (units/mg protein), the Mn SOD activity (units/mg protein) and the Cu-Zn SOD activity (units/mg protein) (Cusabio Biotech, China); the total SOD activity (units/mg protein), the Mn SOD activity (units/mg protein) and the Cu-Zn SOD activity (units/mg protein) (Cayman Chemical, USA); the protein concentration in mg/ml was identified with biuret method (Olvex Diagnosticum, Russia) employing automatic analyzer ChemWell (Awareness Technology INC, USA). Coefficient values SOD 2 activity/antigen have been computed.

The obtained statistics data have been processed with software Statistica 10.0.

The data were analyzed for the compliance of the features distribution with the normal distribution law
using the Shapiro-Wilk test (small sample size). The comparison of quantitative data in the above groups (independent samples) was performed by applying the Kruskal-Wallis test. The table data are presented in the M±m form, where M is the arithmetic mean, and m is the standard error of the mean; we have used the significance level p<0.05 (see Table 1 herein). The obtained statistics data have been processed in compliance with general applicable medical research recommendations.

Results

The results of our analysis of the obtained lipid metabolism and antioxidant protection indicators are given in Table 1 herein. It has been found that the only CHP effect recorded in the heart cell mitochondria, as compared with the data thereon in the intact animals, has been reflected in a statistically significant reduction in the SOD 2 level by 2.9 times, in the total SOD activity by 1.5 times (p<0.05) and in the Cu-Zn SOD activity by 2.7 times, respectively, while the coefficient value of SOD 2 activ./antig. has increased by 2.3 times.

The initial stage (week 1) of the standard growth of melanoma B16/F10 has produced a sharp decrease in the SOD 2 level in the heart cell mitochondria by 4.8 times, then later, since week 2, we have observed a gradual increase therein, when the difference against that found in the intact animals has been recorded to be not so substantial: it has decreased by 1.9 times (p<0.05) only, and upon expiration of 3 weeks the value in question has exceeded the intact animal level by 1.5 times (p<0.05). The total SOD activity within the entire period of the standard melanoma growth have been recorded to be below the intact group values by 1.8 times on average. The Mn SOD activity in week 1 has been recorded to be below the intact group values by 1.5 times (p<0.05). The total SOD activity within the entire period of the standard melanoma growth have been recorded to be below the intact group values by 1.8 times on average. 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to be 1,9 times lower. The standard growth of melanoma has produced no effect on the 8-OHdG amount in the heart mitochondria at all stages of our observation. The MDA level has remained stable in week one and two in the period of the standard melanoma growth, and it has demonstrated its tendency to increase by 1,32 times (p<0,05) as against the intact animal value.

With the growth of melanoma under the CNP conditions, another dynamics of the studied biochemical indicators in the heart cell mitochondria has been revealed. So, after week 1 of the tumor growth accompanied by CNP, the SOD 2 level has increased by 3,2 times, as against the values in the reference group, and remained high after 2 and 3 weeks exceeding the reference values by 4,3 and 3,6 times, respectively. Some changes in the total SOD activity have been recorded after 3 weeks of the melanoma growth against the CNP background: this indicator has demonstrated an increase by 1,7 times (p<0,05). A similar dynamics has been detected in case of the Mn SOD activity: we have observed an increase therein by 2 times after 3 weeks of the melanoma growth accompanied by CNP. The Cu-Zn activity measured after 1 week against the CNP background drops reaching practically undetectable values, then, 2 weeks later, it is recorded to be within the reference value range followed by its decline by 3 times upon expiration of 3 weeks. As to the values of coefficient SOD 2 activity/ antigen, throughout the entire period of the tumor growth against the CNP background, we have recorded low values thereof: after weeks 1-2 we have them recorded by 4,6 times and after week 3 by 1,8 times lower on average (p<0,05). The initial and logarithmic progression stages of the tumor growth against the CNP background (1 – 2 weeks) have been accompanied by an accumulation of products of free radical oxidation (FRO) in the heart cell mitochondria. So, after week 1, the 8-OHdG level has become 4 times higher, and upon expiration of week 2 it has been recorded to be 6,6 times higher. The MDA level measured after 1 week and 2 weeks has exceeded the reference values by 2 times on average. Upon expiration of 3 weeks of the tumor growth with CNP, both indicators have been found to be within the reference value range. The complete set of the presented data on the lipid metabolism and antioxidand protection system under the melanoma growth conditions against the CNP background in female mice demonstrates an aggravation of the dysfunction of mitochondria in the presence of comorbid pathology.

Discussion and conclusions

We have used in our research work the CHP model as comorbid pathology with the growing inoculated melanoma B16/F10 in mice [12]. The progression of melanoma against the CNP background in the examined mice cohort has been characterized by rapid formation of the tumor, the double-focused growth of the latter, early metastatizing and a decline in the animal life duration. In the female mice, under the growing tumor accompanied by CNP, metastases have been detected not only at their usual locations like the liver or the lungs, but also at their unusual destinations, namely, in the spleen and uterus. As a consequence, we may conclude that CNP as comorbid pathology used in our experimental study can cancel the genetically determined inhibition of a malignant tumor [12, 14].

In the framework of this experimental study, we have revealed that there is a high level of a secondary product of lipid peroxidation (LPO): it is MDA found in the heart cell mitochondria under the melanoma growth stimulated by CNP, in week one and two of the tumor formation, followed by a decrease in the level of this indicator after three weeks. It is known that MDA belongs to highly reactive molecules and is capable of damaging DNA due to production of exocyclic DNA adducts: most of them demonstrate a strong mutagenic effect. MDA is considered to be the highly mutagenic product formed in lipid peroxidation [15].

The capability of MDA to damage DNA has been evidenced in our experiment by an increase in the level of a biomarker of oxidative stress: it is biomarker 8-OHdG, which indicates DNA damage. Therewith, we have recorded a similar tendency of changes in the 8-OHdG and MDA contents in the heart cell mitochondria under the melanoma B16/F10 growth stimulated by CNP. So, we have established that there is an increase in the 8-OHdG content in the first two weeks of the tumor growth and a decrease therein after three weeks. It follows that the obtained evidence data on the LPO product content in the heart cell mitochondria under the melanoma growth stimulated by CNP can point to an immediate damaging effect produced by MDA on DNA.

A rise in the 8-OHdG level at the initial stages and a decline therein at the later stage of the stimulated tumor growth are in good agreement with the data obtained previously by other researchers, who note an increase in the 8-OHdG level at early stages of the malignant process and a decrease therein at its later stag-
Moreover, the authors of the research reports show that there is an increase in 8-OHdG available in oncology patients, who suffer from chronic diseases. A higher rate of production of reactive species of oxygen (ROS) leads to many modifications of the DNA nucleotide bases. 8-hydroxy 2’ deoxyguanosine (8-OHdG) is a product of oxidation of the guanine base, which shows the least oxidation potential as compared with other bases. Consequently, the guanine residues are more liable to attacks by free radicals. 8-OHdG attracts a great attention by researchers and is usually involved as a biomarker of oxidative stress indicating DNA damage. This damage of DNA (the 8-OHdG residues) induces a tranversion mutation by pairing with adenine or cytosine in the process of replication. The above type of mutation has been considered to be the major somatic mutation which appears in case of human cancer. As a consequence, the presence of 8-OHdG in the cells bears witness to the mutagenesis ability and increases potential risks of cancerogenesis.

To defend attacks produced by the free radical oxidation system, there is an antioxidant protection system in operation, and, according to our evidence data, within the first two weeks of the melanoma growth stimulated by CNP, weakening of the mitochondria protection takes place due to lowering of the Mn SOD activity and the total SOD activity, but by week three observed has been an activation of the ferments. When comparing the obtained data on the operation of the activators and inhibitors involved in oxidative stress, it can be seen that under weakening of the protection function of the antioxidant ferments we observe an immediate accumulation of the LPO products, while under growing of the antioxidant activity, on the contrary, we can identify a reduction in the LPO product concentration.

Previously, in our research works [17] we have demonstrated that the state of the antioxidant protection system (APS) in humans depends on the volume of the tumor node. Since CNP affects the melanoma progression that is manifested, among the other things, by a double-focused growth of the tumor [12], we cannot exclude the fact of making an effect by the tumor volume on the detected changes in FRO-APS in the heart cell mitochondria. In our experimental study, we have recorded a permanently stable level of SOD 2 within the entire period of the melanoma growth stimulated by CNP, and in this case the SOD 2 activity has been found to be not in conformity with the amount of this ferment in question. It is probable that within the first two weeks of the stimulated melanoma growth the SOD 2 active centers in the heart cell mitochondria have been disabled/closed that has reflected in the value of coefficient SOD activ./antigen.

We cannot state that under this variant of the tumor growth, disabling of the above active centers in week 1 and 2 has been performed by neutralizing of the reactive oxygen species, because we have recorded an increase in MDA and 8-OHdG content. It is possible that in this case closing of the SOD 2 active centers is linked with some conformational changes in the protein molecule of the ferment.

Therefore, to generalize the evidence data obtained from our research, we can arrive at conclusions as follows: 1. The revealed changes in the FRO-APS system in the heart cell mitochondria in female mice under the standard and CNP stimulated melanoma growth conditions point to a pronounced systemic character of the damaging effect of the tumor disease on the organism with comorbid pathology. 2. Concomitant chronic neurogenic pain is a contributor to a substantial re-setting of the oxygen-dependant systems in the cell mitochondria of the heart not affected by the tumor that develops stress and promotes further depletion of the heart energy resources under the growing tumor accompanied by CNP.

Statement on ethical issues
Research involving people and/or animals is in full compliance with current national and international ethical standards.

Conflict of interest
None declared.

Author contributions
The authors read the ICMJE criteria for authorship and approved the final manuscript.

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References


10. Kit OI, et al. Proessy samoorganizacii mitohondrij pri roste eksperimental’nyh opuholej v uslovi-
Insulin-like growth factors and their binding proteins in the heart in rats in experimental diabetes mellitus, growing Guerin’s carcinoma and under their combination

Elena M. Frantsiyants1, Valerija A. Bandovkina*, Irina V. Kaplieva1, Ekaterina I. Surikova1, Natalia D. Cheryarina1, Alla I. Shikhlyarova1, Irina V. Nesubina1, Yulia A. Pogorelova1, Lidija K. Treptaki1, Irina A. Goroshinskaya1, Inga M. Kotieva1, Maria I. Morozova1

1 National Medical Research Centre of Oncology, Russia, 344037, Rostov-on-Don, 14 liniya, 63, building 8
* Corresponding author:
email: valerryana@yandex.ru

Abstract
Diabetes mellitus is an additional risk factor for the development of heart diseases, cardiovascular dysfunction and malignant tumors. The aim of the study was to analyze levels of IGF and IGFBP in heart samples of animals with diabetes mellitus and/or growing Guerin’s carcinoma. The study included white outbred rats of both genders weighing 180-220 g. The rats of each gender were divided into groups of 8 animals: the intact group; test groups 1 (with diabetes) and 2 (with transplanted Guerin’s carcinoma); the main group (transplanted Guerin’s carcinoma growing in the presence of diabetes mellitus). Levels of IGF-I, IGF-II, IGFBP-1, and IGFBP-2 were measured by ELISA (Mediagnost, Germany) in heart homogenates in animals of all groups.

Results
The levels of IGF were not changed in female rats with diabetes and/or Guerin’s carcinoma; however, diabetes mellitus upregulated IGFBP1 and downregulated IGFBP2. The levels of IGF-I, IGF-II, IGFBP-1, and IGFBP-2 were measured by ELISA (Mediagnost, Germany) in heart homogenates in animals of all groups.

Conclusion
The study has demonstrated that the system of insulin-like growth factors and binding proteins in the heart has a gender specificity, which probably determines the pathogenetic mechanisms of diabetes-induced damage to the heart muscle. The metabolic characteristics typical of this endocrine disease dominate in the IGF-heart system over the changes caused by the growth of a malignant tumor, thereby determining the possible mechanisms for the stability or restoration of the heart function in various pathological conditions.

Keywords
Diabetes mellitus, Guerin’s carcinoma, Heart, IGF, IGFBP

Imprint

Introduction
One of the primary causes of death of the population in the modern world is cardiovascular diseases (CVD) including heart insufficiency, diabetes mellitus (DM) and malignant neoplasms [1]. As is shown by research studies, the above pathologies are often found in a combination. While DM cannot be recognized as an immediate cause of death, it is responsible for polyorganic dysfunction, which may include nephropathy, retinopathy, neuropathy, atherosclerosis, heart diseases and cardiovascular dysfunction [2]. The existing approaches to the proper interpretation of mutual relations between various comorbid states and the main pathogenic factor require complex methodological techniques from most advanced digital technology of cardiometry, which implies non-invasive assessments of hemodynamics, heart metabolism and heart energetics, to molecular markers of the heart performance, which offer a differentiated estimation of pathogenetic mechanisms based on models of the tumor growth [3-6]. In addition, available epidemiologic data support that there are interrelations between DM and malignant tumors [7].

An aggravation of oxidative stress and the metabolism dysfunction, which develop in DM, may lead to DNA damaging and diabetic cardiomyopathy, which may result in alterations of the myocardial tissue, metabolic disorders and abnormalities in the vegetative function of the heart [8]. Heart failure is found approximately in 20-30% of the diabetes mellitus pa-
tients that considerably increases risk of disease incidence and death [9]. Alterations in the heart muscle metabolism and insulin signal transduction are the key disorders connected with hyperglycemia as well as development and progression of cardiovascular complications [2, 10]. So, the risk of development of heart failure in DM increases both in males and females as against those patients who do not suffer from DM [1].

Some clinical studies focus on investigations devoted to diabetes and its causal connection with a neoplasm. There are some epidemiologic research works which demonstrate that the patients suffering from DM have a higher risk of various types of cancer [7]. The states like hyperglycemia, hyperinsulinemia and inflammation are considered to be of primary importance for progression of cancer in case of diabetes. Actually, diabetes mellitus and cancer share many common risk factors including ageing, hyperlipidemia, obesity and gender. Diabetes mellitus of both types I and II is interconnected with a higher risk of cancer progression [7].

Cancer can cause signs similar to those found in diabetes, which include a higher level of insulin and insulin-like growth factors, secretion of leptin/adiponectin and immune-related disorders. Moreover, malignant cells increase use of glucose to maintain proliferation [11]. The insulin-like growth factor (IGF) demonstrates its higher levels in patients with diabetes and can promote cancer progression due to its powerful mitogenic and anti-apoptotic activity [2,7,9,12]. Recent discoveries made in vitro and in vivo have shown that IGFBP can operate independent of IGF as independent growth modulators [13,14]. The IGF system has a pleiotropic effect on the organism: it participates in the tissue restoration due to anti-apoptotic, pro-angiogenic and fibrosis-modulating mechanisms that results in its cardioprotective and neuroprotective action at one end of the scale and in a risk of cancerogenesis at the other [2,15].

At present it becomes apparent that the growing incidence rate of diabetes mellitus on a global scale represents a major threat to public health care systems, since it is a severe comorbidity which may accompany and/or provoke diseases like cancer and cardiovascular insufficiency [1,2,10]. Much thought should be given to modifiable risk factors in the context of the diabetes mellitus epidemic observed nowadays throughout the world. As to the future medicine, the most prevalent should be a variant thereof which implies a prevention version adjusted to a human individual having a non-optimal health state, with the presence of a number of comorbid pathologies (prior to manifestation of a tumor disease). Some experimental studies have demonstrated an essential effect produced by concomitant chronic neurogenic pain on the malignant tumor growth [16]. For a better understanding of pathogenesis of the malignant tumor growth against the background of diabetes mellitus as a comorbid disease required are further experimental investigations in vivo [17]. The aim of our study was to investigate the IGF and IGFBP levels in the heart samples in animals suffering from diabetes mellitus and/or having growing Guerin’s carcinoma as well in the rodents with the combination thereof.

**Materials and methods**

Our study has been conducted with the use of white outbred rats of both gender, with an individual body mass of 180-200 g, which have been delivered by the Federal State Medical & Biological Institution “Research Center of Biomedical Technologies” (Branch Andreevka, Moscow Region) at the Federal Medical & Biological Agency and which have been kept under natural lighting conditions with no restrictions on their access to water and food. The research in animals was completed in accordance with the Directive 86/609/EEC on the Protection of Animals Used for Experimental and Other Scientific Purpose, as well as in accordance with the International Guiding Principles for Biomedical Research Involving Animals and Order No. 267 “Approval of the Rules of Laboratory Practice” dated June, 19, 2003 issued by the Ministry of Health of the Russian Federation. The experimental research record was approved by the Commission on Bioethics at the Federal State Budgetary Institution “National Medical Research Center of Oncology”, the Ministry of Health of the Russian Federation, Record No. 21/99 dated 01.09.2020.

The male and female animals have been assigned to groups, 8 animals in each, as follows: the group of the intact animals; test group 1 with rodents having diabetes mellitus and test group 2 of animals inoculated with Guerin’s carcinoma cells; the main group covering the animals with growing Guerin’s carcinoma against the background of diabetes mellitus. To reproduce experimental diabetes mellitus, the animals were given an intraperitoneal injection of Alloxan at a single dose of 150 mg/kg of an individual body mass.
Later, within the week, their blood glucose concentrations were measured. At the time of the inoculation with Guerin’s carcinoma cells, the animals in the main group have been recorded to have an average glucose concentration level of 25.4±1.2 mmol/l, while the reference intact animal group values have been recorded to be 5.2±0.3 mmol/l.

Each rat in the group with the independently growing tumor, upon expiration of 1 week of stable hyperglycemia, has received a subcutaneous injection of 0.5 ml suspension of the Guerin’s carcinoma cells in a 1:5 dilution with saline solution. Three days after the inoculation of the Guerin’s carcinoma cells suspension, a subcutaneous tumor became detectable. Upon expiration of the 10-day period, the animals were decapitated with the guillotine. In the heart homogenates in the animals of all groups using the ELISA method we have measured the amounts of IGF-I, IGF-II, IGFBP-1 and IGFBP-2 (Mediagnost, Germany).

The Statistica 10.0 software has been utilized for the purpose of statistical analysis of the obtained data. The data are presented in the M±m form, where M is the arithmetic mean. The data have been analyzed for their compliance with the normal distribution law using the Shapiro-Wilk test. Significance of differences between independent samplings has been assessed with the Mann-Whitney U test. In this case, p<0.05 has been taken as the level of statistical significance.

Results

It has been found that in the heart in the intact male rats the levels of IGF-I, IGF-II and IGFBP-1 are 1.6, 1.7 and 1.8 times lower (p<0.05) as compared with the respective data recorded in the intact female animals (see Table 1 and 2 herein).

Considering the influence made by diabetes on the heart in the female rats, we have recorded no changes in the amount of IGF-I and IGF-II, however we have established that there is a multidirectional change in the IGFBP-1 and IGFBP-2 levels (see Table 1 herein): the IGFBP-1 level has increased by 1.9 times as against the respective values in the intact animals, while the IGFBP-2 level has decreased by 1.5 times (p<0.05). Accordingly, the IGF-to-binding proteins ratio values IGF-I/IGFBP1 and IGF-I/IGFBP2 have decreased by 1.9 times, while IGF-II/IGFBP2 has increased by 1.4 times.

The growing Guerin’s carcinoma has induced a decrease in the IGFBP-1 level by 1.9 times only, while the other indicators remain within their normal value ranges. Accordingly, reported have been the 1.8-fold values of ratios IGF-I/IGFBP1 and IGF-II/IGFBP2 (p<0.05). As a result from the growing Guerin’s carcinoma against the background of diabetes mellitus in the heart in female rats, no changes in the IGF level have been detected, but the amount of IGFBP-1 has increased by 2.3 times, while the IGFBP-2 level has decreased by 1.9 times as against the respective indicators in the intact animals, in a similar manner as it is the case with the processes typical of independently developing diabetes mellitus.

It is intriguing that in the female rats with the growth of the malignant tumor against the background of endocrine comorbidity, namely, induced diabetes mellitus, the changes in the values in ratio IGH to their binding proteins have integrated both signs of the independent diabetes mellitus and the independent malignant tumor growth. So, the values of ratios IGF-I/IGFBP1 and IGF-II/IGFBP1 have decreased by more than 2 times as against the independent tumor growth process and the respective indicators in the intact animals, similarly to the processes found in independent diabetes mellitus, while the values of ratios IGF-I/IGFBP2 and IGF-II/IGFBP2 have increased by 1.9 and 1.8 times, respectively, in the same manner that is the case with the processes under the independently growing malignant tumor.

A completely different type of the response produced by the IGF axis factors to the independently developing diabetes mellitus and the Guerin’s carcinoma has been detected in male rats (see Table 2 herein). In the male rats with diabetes mellitus, in their heart samples, we have found a reduction in the levels of IGF-I and IGF-II as well as IGFBP1 and IGFBP-2 by 1.8 times on average as compared with the respective data recorded in the intact male animals. In this case, no changes in the ratios between IGF and the binding proteins have been revealed. The Guerin’s carcinoma growth has initiated the changes in the amounts of IGF-I, IGF-II and IGFBP2 in the same direction that is the case with those found in diabetes mellitus, except for the absence of changes in the IGFBP1 concentration; we have detected a decrease in the above amounts by 1.6, 1.3 and 1.5 times, respectively, as against the values reported for the intact animals. As a result, we have observed in the male rats with the independently growing Guerin’s carcinoma in the heart samples the reduced values of the IGF-I/IGFBP1 and IGF-II/IG-
**Table 1**

Concentration of insulin-like growth factors and their binding proteins (ng/g tissue), and ratios between the insulin-like growth factors and the binding proteins in the heart in female rats

<table>
<thead>
<tr>
<th></th>
<th>Intact animals</th>
<th>DM</th>
<th>Guerin's carcinoma</th>
<th>Main group</th>
</tr>
</thead>
<tbody>
<tr>
<td>IGF-1</td>
<td>1422.7±123.4</td>
<td>1377.45±128.3</td>
<td>1380.2±121.7</td>
<td>1497.0±140.3</td>
</tr>
<tr>
<td>IGF-2</td>
<td>215.8±18.7</td>
<td>212.8±20.1</td>
<td>215.4±16.4</td>
<td>213.5±20.5</td>
</tr>
<tr>
<td>IGFBP-1</td>
<td>11.5±9.5</td>
<td>21.4±1.71</td>
<td>10.0±0.92</td>
<td>22.8±2.11</td>
</tr>
<tr>
<td>IGFBP-2</td>
<td>251.4±24.3</td>
<td>167.5±15.41</td>
<td>131.1±12.21</td>
<td>135.8±11.71</td>
</tr>
<tr>
<td>IGF-1/IGFBP-1</td>
<td>123.7±11.7</td>
<td>64.4±6.21</td>
<td>138.0±11.92</td>
<td>65.7±5.91</td>
</tr>
<tr>
<td>IGF-1/IGFBP-2</td>
<td>5.7±0.54</td>
<td>5.5±0.45</td>
<td>10.5±0.712</td>
<td>11.0±0.812</td>
</tr>
<tr>
<td>IGF-2/IGFBP-1</td>
<td>18.8±1.5</td>
<td>9.9±0.871</td>
<td>21.5±1.52</td>
<td>9.4±0.761</td>
</tr>
<tr>
<td>IGF-2/IGFBP-2</td>
<td>0.9±0.06</td>
<td>0.3±0.111</td>
<td>1.6±0.141</td>
<td>1.6±0.151</td>
</tr>
</tbody>
</table>

Notes: statistically significant differences as against: 1 - the intact animal values; 2 - the animals with diabetes mellitus (p<0.05)

**Table 2**

Concentration of insulin-like growth factors and their binding proteins (ng/g tissue), and ratios between the insulin-like growth factors and the binding proteins in the heart in male rats

<table>
<thead>
<tr>
<th></th>
<th>Intact animals</th>
<th>DM</th>
<th>Guerin's carcinoma</th>
<th>Main group</th>
</tr>
</thead>
<tbody>
<tr>
<td>IGF-1</td>
<td>877.6±69.9</td>
<td>468.7±43.11</td>
<td>546.7±53.11</td>
<td>437.1±41.11</td>
</tr>
<tr>
<td>IGF-2</td>
<td>124.4±11.2</td>
<td>71.1±6.71</td>
<td>94.2±8.912</td>
<td>80.3±7.71</td>
</tr>
<tr>
<td>IGFBP-1</td>
<td>6.3±0.60</td>
<td>3.5±0.311</td>
<td>6.6±0.572</td>
<td>5.2±0.462</td>
</tr>
<tr>
<td>IGFBP-2</td>
<td>224.5±21.2</td>
<td>126.4±11.11</td>
<td>150.4±14.41</td>
<td>125.6±12.41</td>
</tr>
<tr>
<td>IGF-1/IGFBP-1</td>
<td>139.3±13.1</td>
<td>133.9±12.4</td>
<td>82.8±7.612</td>
<td>84.1±7.612</td>
</tr>
<tr>
<td>IGF-1/IGFBP-2</td>
<td>3.9±0.30</td>
<td>3.7±0.32</td>
<td>3.6±0.33</td>
<td>3.5±0.34</td>
</tr>
<tr>
<td>IGF-2/IGFBP-1</td>
<td>19.7±1.8</td>
<td>20.3±1.9</td>
<td>14.3±1.412</td>
<td>15.4±12.212</td>
</tr>
<tr>
<td>IGF-2/IGFBP-2</td>
<td>0.6±0.05</td>
<td>0.6±0.05</td>
<td>0.6±0.06</td>
<td>0.6±0.05</td>
</tr>
</tbody>
</table>

Notes: statistically significant differences as against: 1 - the intact animal values; 2 - the animals with diabetes mellitus (p<0.05)

FBP-1 ratios by 1.7 times and 1.4 times, respectively, as compared with those in the intact animals and in the DM rodents.

In the male rats with the growing Guerin's carcinoma against the background of diabetes mellitus no significant differences from the indicators in the heart in independently developing diabetes mellitus or the independently growing Guerin's carcinoma with respect to IGF-I, IGF-II and IGFBP-2 have been detected; the only exception is IGFBP1, the level of which has been found 1.5 times lower, as compared with that in diabetes mellitus, and 1.3 times lower as against that found under the growing Guerin's carcinoma. The values of the ratios between IGF and their binding proteins under the growing Guerin's carcinoma against the background of diabetes mellitus show no significant variances from the respective data recorded in the group with the independent growth of the tumor only, however the values of ratios IGF-I/IGFBP1 and IGF-II/IGFBP-1 have been reported to be 1.6 and 1.3 times lower, respectively.

**Discussion**

We have derived from our studies that the levels of IGF-I, IGF-II and IGFBP-1 recorded in the heart samples of the female rats are higher than those found in the male rodents. The peculiar features can be explained by differences in the level of their gender-specific steroids. It is well known that IGFs participate in the maintenance of the structure and performance of the heart, make influence on the lipid parameters and reduce coronary disease risk and rate of the aorta growth; in this case the effect produced by estradiol modifies this influence [2,7,15,18,19]. The CVD risk is known to considerably experience an increase in woman after menopause that bears witness to the protective role of estrogens [20]. We can find more and more experimental data confirming the fact that estrogens do much to shape the gender specificity found in the cardiac muscle contractility [21]. It is suggested that both diabetes mellitus and CVD have some common genetic and ecological factors, which are considered as contributors to development of the above diseases [1,2]. It is thought that diabetes is linked with the microcirculation dysfunction and heart insufficiency as well as fluctuations in the insulin and IGF1 levels [10,22].

In the course of our study we have revealed an interesting regularity: in the female rats neither diabetes mellitus nor the growing Guerin's carcinoma, including the combination of both diseases, has made any impact on IGF amounts, while in the male rats we have detected a decrease in the IGF amount in the heart samples as a response to induced DM, the growing Guerin's carcinoma and their combined action. As multi-fac-
tor studies of various biological markers have shown, the IGF/IGFBP system is most closely connected with the development of DM; the next contributors thereto should be hormones and pathways of their regulations. It is assumed that the involvement of the additional biomarkers of system-related pathway IGF/IGFBP, like IGF-1, IGFBP-1 and IGFBP-3 would be useful for an assessment of the contribution of this system to the DM and CVD risks [2,7]. It has been found that the overexpression of IGF-II accelerates the differentiation of parthenogenetic stem cells (PSC) into cardiomyocytes, but at the same time it inhibits their proliferation via signal transduction IGF-II / IGF1R that is evidence for the protective role of IGF [12,23]. The insulin-like IGF-1 signal transduction regulates contractility, metabolism, hypertrophy, autophagy, ageing and apoptosis in the heart. The IGF-1 deficiency is connected with a higher CVD risk [10,24]. On the other hand, thyroid hormones, insulin, IGF-1 and estrogens are usually linked with the physiological hypertrophy of the heart, however they are capable of counteracting a pathological response [25]. So, insulin and IGF-1 are powerful activators of PI3K/Akt1 in cardiomyocytes. It has been demonstrated that an increased production of the heart-associated IGF-1 in sport athletes is connected with the physiological hypertrophy found in the heart in them. It turns out that there is a relationship between the low levels of the heart-related IGF-1 and a higher risk of developing heart insufficiency [25]. IGF-I – Akt involved to produce a cardioprotective effect is capable of accelerating and enhancing the transcriptional re-programming of fibroblasts to the functional cardiomyocytes that is a potential approach to restore the cardiac function after damage of the myocardium [26].

In the female rats in the heart samples, as a response to induced DM, we have detected multidirectional changes in the amount in the binding proteins of type 1 and 2, while for the male rats reported is a decrease therein. The growing Guerin's carcinoma has initiated a reduction in the IGFBP2 concentration found in the heart, while the IGFBP1 level in the animals of both genders has remained unchanged. In the main group, where we have observed the growing Guerin's carcinoma against the DM background, the amount of the binding proteins in the heart in the female rats is in compliance with the pattern under diabetes mellitus, while in the male rodents the pattern is in correspondence with that recorded in the male rodents both in case with DM and the tumor growth.

Initially, the role of IGFBP was limited to their binding function to prolong the life time of circulating IGF in all tissues only. But at the present time, the concept has been changed: it turns out that most of IGFBP compete for the IGF activity at the level of the receptors and counteract the IGF function, while some of them (for example IGFBP2) enhance the IGF signal transduction [27].

IGFBPs coordinate and control biological activity of IGF by several methods: 1) transport of IGF in blood plasma and control of its diffusion / discharge from the vascular space; 2) prolongation of time of half-life and regulation of IGFs clearance; 3) provision for sites of specific binding of IGF in the extra- and pericellular space; and 4) modulation of interaction between IGF and their receptors. IGFBPs bind IGF-1 and IGF-II, but they do not bind insulin. IGFBP-1 binds IGF-1 and IGFBP-2 shows a higher binding affinity for IGF-II [28]. The IGFBP levels can fluctuate and are variable due to different physiological or pathological states that may include day cycle variations, physical loading, pregnancy, DM and ageing, among them hormones, which modulate the levels of some IGFBP in blood serum and other bio-fluids [29]. It is assumed that IGFBP are tumor growth retardants inhibiting the IGF action, including proliferation, survival, migration/invasion and stimulation of apoptosis [27]. However, on the contrary, other studies have demonstrated a pro-tumorigenic action of some IGFBP, among them promotion to cell survival and migration/invasion. Specifically, presumably IGFBP-2 is mainly cancerogenic for a number of the cancer types, including glioma, and its circulating levels are found in good correlation with aggressiveness and other well-defined tumor markers found in prostate, breast and ovarian cancer [14,29].

The IGFBP2 expression is regulated by the transcription factor induced by hypoxia (HIF-1α), the expression of which, in its turn, is controlled by O2 concentration in the cells [14]. In our research work, we have revealed that there is a decrease in the IGFBP2 level in the heart samples in the animals of both genders in all experimental groups (DM, the growing Guerin's carcinoma and the growing Guerin's carcinoma against the DM background). We have detected that an introduction of IGFBP-2 can prevent adipogenesis and show anti-diabetic properties [14]. This evidence confirms the pathogenetic significance of changes in the IGFBP2 amounts both in case of DM and the malignant tumor growth.
In general, the completed study shows that the system of the insulin-like growth factors and their binding proteins in the heart has its own gender-specific feature, which probably governs the pathogenic mechanisms of cardiac muscle damaging against the DM background. The metabolism specificity, typical of this sort of the endocrine disease, predominates in the IGF system in the heart over those changes, which are induced by the growing malignant tumor that may identify possible mechanisms to provide the required stable heart performance or to restore the latter under different pathology states.

Statement on ethical issues
Research involving people and/or animals is in full compliance with current national and international ethical standards.

Conflict of interest
None declared.

Author contributions
The authors read the ICMJE criteria for authorship and approved the final manuscript.

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The incidence rate of arterial hypertension and high normal blood pressure in students of Medical Faculty at the Kabardino-Balkarian State University

Inna F. Pshigotizheva1*, Danetta M. Gubzhokova2, Aksana M. Kardangusheva1, Liliya V. Elgarova1, Antonina F. Budnik1, Inna M. Dudarova1, Asiyat N. Malukhova1

1 Federal State Budgetary Educational Institution of Higher Education “Kabardino-Balkarian State University named after H.M. Berbekov”
Russia, 360000, Nalchik, Chernyshevskogo str., 173
2 Pirogov Russian National Research Medical University of the Ministry of Health of the Russian Federation
Russia, 117997, Moscow, Ostrovityanova str., 1

* Corresponding author:
email: pshigotizheva@gmail.com

Abstract
The aim is to assess the incidence rate of arterial hypertension (AH) and high normal arterial pressure (HNAP) in students of the Medical Faculty at the Kabardino-Balkarian State University as well as the relationship between arterial pressure (AP) and behavioral and biological risk factors (RF) of cardiovascular diseases (CVD) to substantiate the need for preventive measures.

Materials and methods
1087 students (267 males and 820 females) aged 18 years and older (average age 20.7 ± 4.6 years) have been examined with standard epidemiological methods.

Results
The incidence rate of AH among students was reported to be 5% (8.6% in males and 3.4% in females, p < 0.001), HNAP was recorded to reach 4.2% (9.4% in males and 2.6% in females, p < 0.001), respectively. The interrelations between AP and anthropometric indicators, gender, smoking and physical activity were identified.

Conclusion
The study revealed the incidence rate of hypertension and HNAP in the Medical Faculty students and the interrelation between AP and the behavioral and biological RF of CVD. Our results indicate the need to develop and implement programs for the prevention of AH among students that can be used to prepare the required preventive measures.

Keywords
Arterial pressure, Arterial hypertension, High normal arterial pressure, Students, Young individuals

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Introduction
The study on arterial hypertension (AH) is a topical challenge due to high incidence rate and the predicted growth of AH morbidity and associated diseases in the Russian Federation [1-3] including particularly the Kabardino-Balkarian Republic [4]. Elevated arterial pressure (AP) is the main factor in premature death and the cause approximately of 10 million death cases and more than 200 million cases of disability in the world [5-7]. Identified is a direct relationship between the AP level and the risk of developing cardiovascular diseases (CVD), starting with the systolic AP value of 110-115 mm Hg and the diastolic AP level of 70-75 mm Hg. The latter served as the basis for the creation of the concept of prehypertension in 1939 and the subsequent definition of the high normal arterial pressure (HNAP). This issue is pressing due to the wide prevalence of HNAP and the possibility of taking early preventive measures in individuals with HNAP [6,7].

Despite a large number of studies on hypertension, the epidemiology of hypertension and HNAP among young people remains poorly understood. A special position among young people is occupied by students of medical universities, whose education and training are associated with high academic performance loading and whose actual lifestyle does not contribute to the maintenance of their health. No doubt that the formation of risk factors (RF) begins in childhood, and the negative consequences of their impact on the body accumulate throughout the life span of an individual.
Some rare medical examinations in students show a wide range of data on the incidence rate of HNAP and AH in different regions [9-12]. It is found that AP levels in adolescents with hypertension demonstrate high stability during their transition to the adult category [8]. The study of the incidence of HNAP and AH is a necessary condition for planning preventive measures, and medical students are the optimal model for their implementation [13].

The aim herein is to assess the incidence rate of AH and HNAP in students of the Medical Faculty at the Kabardino-Balkarian State University and the relationship between AP and the behavioral and biological RF of CVD to substantiate the need for preventive measures.

Materials and methods

In 2011 and 2017, two parallel population studies of students of the Medical Faculty at KBSU were conducted. 1087 students (267 males and 820 females) have been examined. The average age of the examined patients was 20.7±4.6 years. The study record included a survey using a standard questionnaire to identify passport data and lifestyle indicators (education, work information, smoking adherence, alcohol consumption, the physical activity (PhA) level and dietary habits), measurement of AP, pulse rate, body length (BL) and body weight (BW), waist circumference. Tonometry was performed with a standardized sphygmomanometer, in sitting position, on the right arm, with an accuracy of 2 mmHg, in the morning, not earlier than 1 hour after physical education lessons or tests. An average value of the three measurements was used for analysis. The systolic AP and the diastolic AP values were recorded utilizing the corresponding Korotkoff sound (phase I) and the respective Korotkoff sound (phase Y). The AP categories were verified for AH and HNAP based on the systolic AP and/or diastolic AP values according to the recommendations issued by the Russian National Medical Society for Arterial Hypertension and the Russian Society of Cardiology (Fourth revision, 2010) [14]. In the present article, our analysis of the AP levels, based on the merged data file, is carried out to enhance the sampling validity. BW was measured with an accuracy of 0.1 kg, BL data were delivered with an accuracy of 0.5 cm. The Quetelet index (QI) was calculated as the ratio of BW (kg) to the square of BL (m²). Students who smoked at least one cigarette a week were classified as smokers.

Data processing was performed using the STATISTICA 6.0 software (StatSoft Inc, USA) [15]. The results of the analysis are presented as the mean and its standard deviation for continuous variables and as a percentage for categorical variables. P<0.05 was accepted as the critical level of significance to test statistical hypotheses. The estimation of the linear relationship (association) between the qualitative characteristics was carried out using a correlation analysis. The dependence of a quantitative normally distributed trait on the values of two or more quantitative and qualitative factors was studied using multivariate parametric analysis of variance.

Results and discussion

In the examined population of students, the average value of the systolic AP was recorded to be 113.9±10.6 mm Hg, and the average value of the diastolic AP reached 73.4±8.1 mm Hg, respectively. The values of systolic and diastolic AP in males were found higher than those in the females (see Table 1 herein).

The incidence rate of AH in the students was found to be 5%, and HNAP was recorded to be 4.2%. A comparative assessment of the incidence rate of AH and HNAP in the student population revealed some gender differences (see Figure 1 herein). The prevalence of elevated AP levels in the males was higher than that found in the females: AH was recorded in 8.6% for the males and 3.4% for the females (p<0.001), and HNAP was reported in 9.4% of the males and 2.6% of the females (p<0.001).

It should be noted that the students’ awareness of the presence of AH was found to be at the low level assessed reaching 31.4%. The majority of students (58.6%) with AH reported an increase in AP during the last year. Hypotensive therapy was performed only in 6.9% of the cases.

According to studies conducted in various cities of the Russian Federation, the incidence rate of AH among students varies widely: from 9.9% in Tomsk [9] to 30-40% in Tula [12]. The highest incidence rate of HNAP was reported upon an epidemiological study covered the student cohort in Vladivostok: 5% of females and 31.4% of males [11]. As to the medical students in Perm, the incidence rate of HNAP was recorded to reach 7% [10]. The prevalence of elevated AP levels among students indicates their vulnerability to the development of cardiovascular pathology and, therefore, the need for early diagnosis-making and
adequate correction of elevated AP to reduce cardiovascular morbidity in the population. It is of practical importance to distinguish the category of individuals with HNAP. The implementation of preventive measures in the group of individuals with HNAP will increase the awareness of young people of the respective risk factors responsible for AH, reduce the risk of cardiovascular complications, the rate of progression of AH or avoid and prevent its development.

Associations of AP levels in the examined students with some biological (gender, age, BL, BW, QI, pulse rate) and behavioral (smoking, alcohol consumption, sleep duration, PhA, level of education) parameters were investigated using the correlation and regression analysis. Direct weak and moderate correlation coefficients were established between the levels of the systolic and diastolic AP values on the one hand and the respective anthropometric indicators (BL, BW, QI (r=0.17÷0.36, p<0.0001)), gender (r=0.38, p<0.0001), smoking (r=0.18, p<0.0001 and r=0.15, p<0.0001), smoking in the past (r=0.11, p<0.001 and r=0.09, p<0.01), regular sports (r=0.08, p<0.01 and r=0.07, p<0.05), completed hours in sports exercises (r=0.20, p<0.01 and r=0.21, p<0.01), heart rate (r=0.10, p<0.001 and r=0.07, p<0.05) on the other hand. The measured systolic AP value also correlated with the sleep duration (r=0.12, p<0.01), the number of cigarettes smoked per day by heavy smokers (r=0.26, p<0.05). Our multiple regression analysis showed that the 21% variance in the systolic blood pressure can be explained by such variables as gender (B=4.99, p<0.0001), BL (B=0.12, p<0.05), QI (B=0.90, p<0.001), pulse rate (B=0.31, p<0.0001), number of minutes per day for walking to the place of study (B=0.04, p<0.05), and the sleep duration (B=0.89, p<0.001). For the level of diastolic AP (R2=0.20), gender (B=2.09, p<0.05), pulse rate (B=0.17, p<0.01), QI (B=0.46, p<0.05), BL (B=0.10, p<0.05) and the sleep duration (B=0.45, p<0.05) were significant. It is known that the effect made by several CVD risk factors on the body can lead to the development of hypertension and its complications [8, 13]. Therefore, it is urgent to elaborate new methods for the prevention of cardiovascular complications, which may develop at the early stages of AH, when complications are formed against the seemingly full health. The results obtained by us can be used in the preparation of preventive measures aimed at keeping-up and strengthening the health of students.

Statement on ethical issues

Research involving people and/or animals is in full compliance with current national and international ethical standards.

Conflict of interest

None declared.

Author contributions

The authors read the ICMJE criteria for authorship and approved the final manuscript.

References

Hemodynamic and adaptive correlates of transcranial magnetotherapy in patients with high-grade malignant brain gliomas in the early postsurgery period

Ivan A. Popov¹, Alla I. Shikhlyarova⁴, Galina V. Zhukova¹, Yulia Y. Arapova⁴, Elena M. Frantsiyants¹, Marina A. Engibaryan¹, Irina V. Kaplieva¹, Eduard E. Rostorguev⁴, Lyudmila Y. Rozenko¹, Dmitry Y. Iozefi¹, Marina A. Gusareva¹

¹ National Medical Research Centre of Oncology
Russia, 344037, Rostov-on-Don, 14 liniya, 63, building 8

Corresponding author:
email: shikhlyarova.a@mail.ru

Introduction

One of the most important achievements in recent times has been the development of an advanced research methodology, namely, cardiometry, which equips modern cardiology with new, mathematically verified, laws to connect the logical relationship between the phase structure and the electrical, biophysical and biochemical processes within a cardiac cycle [1]. The design and development of the cardiometric technology with its rapid quantitative assessment of multiple hemodynamic parameters and the level of functional coupling between the main cardiac cycle phases have determined the high topicality of this new approach for translational medicine in connection with the demand of the latter to monitor and prevent cardiovascular pathology under the cancer disease and aggressive methods of antitumor treatment used [2-4].

International statistics data show a steady increase in the number of diseases with malignant tumors of the central nervous system (CNS) [5,6]. A similar situation is observed in Russia, where the level of CNS oncopathology increased by 32.89% and reached 6.08 cases per 100,000 population. Among primary CNS tumors, glial neoplasms are reported in 35% of the cases [7]. Of particular concern are high-grade brain gliomas (HGG), among which glioblastomas account for up to 80% and which are characterized by the highest degree of malignancy, Grade IV as well as by the highest mortality rate [8,9]. HGGs are characterized by their aggressive growth, which is caused by increased proliferation of tumor cells, impaired regulation of apoptosis, and active angiogenesis [10-12]. Disorders in microcirculation are considered as a pathognomonic sign of changes in hemodynamics, especially against the background of radiation therapy [13]. A critical functional load on the heart during the tumor growth and at the stage of surgical treatment of HGG can initiate a pathological response in the complex system of the self-regulation of the cardiac phase cycles, since cardiomyocytes are limited by their proliferative and regenerative potential with a tendency to persistent cardiotoxicity [4].

Early pericardial damage can occur in the form of acute pericarditis, electrical instability of the myocardium, in the pathogenesis of which microcirculation disorders, including increased vascular permeability and vascular stasis, play a dominant role [4,13]. At
later stages, radiation cardiotoxicity manifests itself in the progression of atherosclerosis, fibrotic alterations, formation of valve defects, rhythm and conduction disorders, which are detected using various instrumental methods (daily heart rhythm monitoring, 35-lead electrocardiography, echocardiography).

The question arises whether it is needed to monitor the complex of quantitative cardiometric data in the early postsurgery period before radiation therapy with the help of appropriate software and devices, like the CARDIOCODE cardiac analyzer. This makes it possible to identify some markers of the CVS damage, which will help to timely suppress and prevent some critical points of rise in cardiotoxicity, intensify the required preparation before radiation therapy and improve the quality of life of patients with HGG. Among the means for the correction of systemic disorders, the use of transcranial magnetic therapy (TMT) is of particular interest, which has already proven itself favorably in the treatment of some post-stroke functional disorders, significantly affecting the quality of life of patients [14, 15], as well as in the rehabilitation of the early postsurgery period in patients after the removal of intramedullary tumors [16]. Besides, sufficient experience has been accumulated in the use of magnetotherapy in experimental and clinical oncology [17-21]. The aim of the study is to identify signs of cardiotoxicity based on a comprehensive cardiometric analysis of the cardiac performance in patients with glioblastomas of the brain at an early stage after surgical treatment with the use of accompanying transcranial magnetic therapy.

Materials and methods

The clinical part of our research work is based on the analysis of the primary records of 50 patients with HGG affecting functionally significant areas of the brain, who underwent surgical treatment at the Department of Neuro-Oncology at the Federal State Budgetary Institution "The National Medical Research Center of Oncology", Ministry of Health of the Russian Federation. All examination records have been prepared in accordance with the Helsinki Declaration (1964, 2013 edition). By the method of simple randomization, the patients were divided into the control and main groups, 25 human individuals in each. All patients underwent surgical treatment in the scope of bone-plastic craniotomy, cytoreductive surgery with the intraoperative use of Medtronic StealthStation S7 navigation, accompanied by fluorescence microscopy and neurophysiological monitoring.

1 day before the surgery, as well as on day 7 and day 15 after the cytoreductive removal of the neoplasm, all patients were examined according to an electrocardiogram (ECG), which was recorded with the use of a single-lead placed on the aortic area surface, for 30 seconds in a lying position, employing the CARDIOCODE cardiac analyzer (Taganrog, Russia). Heart hemodynamic parameters, energetic state of myocardium (concentrations of oxygen, lactate and phosphocreatine) have been calculated using the original Cardiocode software. The complete set of the heart hemodynamic parameters has been assessed according to the data on blood phase volumes computed noninvasively by substituting cardiac cycle phase durations into the G. Poyedintsev - O. Voronova equation of hemodynamics, as follows: SV – stroke volume of blood, (ml); PV1 – volume of blood entering the ventricle of the heart during early diastole, (ml); PV2 – volume of blood entering the heart ventricle during atrial systole, (ml); PV3 – volume of blood ejected by the ventricle in rapid ejection phase, (ml); PV4 – volume of blood ejected by the ventricle in slow ejection phase, (ml); PV5 – volume of blood pumped by the ascending aorta in systole, (ml) [1,22]. The metabolism changes have been assessed by the concentrations of oxygen, lactate and phosphocreatine in the myocardium, calculated indirectly with the use of the Cardiocode software. A parameter value within the range of 0.7…0.85, 0.6…0.65, 0.5…0.55 has been considered as the normal value for the aerobic process; a parameter within the range 3.0…7.0 has been taken as the norm for the anaerobic-glycolytic process, and a value 2.0…4.0 has been accepted as the norm for the anaerobic-alactate process, respectively [11].

After surgery, all patients received analgesic, antimicrobial, and anticoagulant therapy according to the applicable standards of treatment of neuro-oncological patients. The patients of the main group in the early postsurgery period were additionally subjected to transcranial magnetic therapy (TMT) applied to the brain. TMT covered 10 sessions and was performed daily as scheduled below: from postsurgery day 2 till day 6 day (5 sessions), and then every other day, three times a week, until the patients were discharged from the hospital. Each session of the electromagnetotherapy implied two exposures: the first exposure was pro-
vided in the morning using magnetotherapy device Gradient-4M to induce an ultra-low frequency magnetic field (ULF MF) focused on the hypothalamus region in an exponential intensity mode (B) from 3mT to 1mT in the frequency & exposure algorithm as follows: 0.3 Hz (t=5min) – 3 Hz (t=1min) – 9 Hz (t=1min); the second exposure was provided by a pulsed magnetic field (PMF) 3 hours after the first exposure with Neuro-MSD device (Neurosoft Co.) focused on the perifocal zone of the bed of the removed tumor. PMF with an intensity of 15mT was used with the frequency and time algorithm F=0.3 Hz (t=5 min), F=3 Hz (t=1min), F=9 Hz (t=1min), and t total=7 min. Identification of the general non-specific adaptation reactions (GNAR) type in the patients of both groups, determining the content of lymphocytes, eosinophils and monocytes according to the Schilling blood count, was carried out before surgery, on day 1, 7, 15 and 30 after the cytoreductive surgery. After that, the ratio of antistress and stress types of reactions (K=AC/S) was calculated as an integral synthetic indicator of the effectiveness of therapy, including the use of TMT [23].

Results

Our analysis of hemodynamic parameters before surgical treatment and in the early postsurgery period in the patients of the control and main groups show that in the preoperative period they are found within the normal range (see Tables 1 and 2 herein).

The amount of oxygen in the patients of the main group is 0.6 arbitrary units (arb.u.), and that in the control group is 0.55 arb.u. within the lower norm, with the norm not lower than 0.5 arb.u. (see Figure 2 herein). The amount of lactate in the patients of the main group is 6.4 arb.u. within the normal range, while in the patients of the control group noted is an accumulation of lactate reaching 9.64 arb.u. against the normal value of 3.0...7.0 arb.u. (please, refer to Figure 3 herein). The amount of phosphocreatine indicating the ATP storage, intended for immediate energy consumption, in the patients of the main group is 4.7 arb.u., while in the patients of the control group noted is an accumulation of lactate of 3.4 arb.u. as compared to the norm of 3.4...7.0 arb.u. (see Figure 4 herein). In the early postsurgery period, on day 7, in the patients of the main group, hemodynamic index PV1 became 18% lower than the normal one, and PV4 exceeded the norm by 22%. On postsurgery day 15, the PV1 index was recorded to be 22% lower than the respective normal parameter, and the PV4 index was found to be 10% higher than its normal value. These changes in the values do not exceed 30% of the norm that allows us to state that there are no pathological hemodynamic changes in

Table 1
Hemodynamic parameters before surgery and in the early postsurgery period in patients of the control group

<table>
<thead>
<tr>
<th>SV (ml) Stroke volume</th>
<th>MV (l) Minute volume</th>
<th>PV1 (ml) Blood volume, entering the left ventricle in the early diastole phase</th>
<th>PV2 (ml) Blood volume, entering the left ventricle in the atrial systole phase</th>
<th>PV3 (ml) Blood volume ejected by left ventricle in the phase of rapid ejection</th>
<th>PV4 (ml) Blood volume ejected by left ventricle in the phase of slow ejection</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before surgery</td>
<td></td>
<td>43.6±4.5</td>
<td>3.1±0.4</td>
<td>27.7±1.9</td>
<td>16.3±2.7</td>
</tr>
<tr>
<td>Postsurgery Day 7</td>
<td></td>
<td>44.9±3.8</td>
<td>3.3±0.3</td>
<td>27.6±2.6</td>
<td>17.3±1.7</td>
</tr>
<tr>
<td>Postsurgery Day 14</td>
<td></td>
<td>41.4±4.2</td>
<td>3.1±0.4</td>
<td>22.9±2.1*</td>
<td>18.7±3.4</td>
</tr>
</tbody>
</table>

Notes: *significant differences compared to the parameter before surgical treatment (p<0.05)

Table 2
Hemodynamic ECG parameters before surgical treatment and in the early postsurgery period in patients of the main group

<table>
<thead>
<tr>
<th>SV (ml) Stroke volume</th>
<th>MV (l) Minute volume</th>
<th>PV1 (ml) Blood volume, entering the left ventricle in the early diastole phase</th>
<th>PV2 (ml) Blood volume, entering the left ventricle in the atrial systole phase</th>
<th>PV3 (ml) Blood volume ejected by left ventricle in the phase of rapid ejection</th>
<th>PV4 (ml) Blood volume ejected by left ventricle in the phase of slow ejection</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before surgery</td>
<td></td>
<td>39.0±3.5</td>
<td>2.8±0.3</td>
<td>27.5±2.4</td>
<td>15.8±2.3</td>
</tr>
<tr>
<td>Postsurgery Day 7</td>
<td></td>
<td>39.9±2.7</td>
<td>3.2±0.3</td>
<td>22.5±2.2</td>
<td>17.5±2.1</td>
</tr>
<tr>
<td>Postsurgery Day 14</td>
<td></td>
<td>39.8±3.5</td>
<td>2.7±0.2</td>
<td>21.4±2.6</td>
<td>14.4±1.7</td>
</tr>
</tbody>
</table>

Notes: *no reliable differences compared to the parameter before surgical treatment are available.
In the patients of the control group on postsurgery day 7, the PV4 parameter is 37% higher than its normal value; on day 15, the PV1 parameter is 17% less than its normal level, and the PV4 parameter is already 42% higher than its normal value. These data may indicate an increase in the load on the left ventricle, its hyper trophy, possibly due to aortic insufficiency. The values of PV1 on day 15 and PV4 on day 7 significantly differ from their respective values recorded before the operation (p=0.04). Changes in the energy parameters of the myocardium are also markers of pathological processes. On day 7, the oxygen value of 0.5 arb.u. and the phosphocreatine concentration of 3.3 arb.u. are still found at the lower limit of the norm, but there is an accumulation of lactate reaching 17.1 arb.u. On day 15, the amount of oxygen decreases to 0.4 arb.u., which is below the permissible value, with the accumulation of lactate reaching 15.1 arb.u. and with phosphocreatine achieving 4.8 arb.u. (see Figure 2,3,4 herein). In other words, there is suppression of the anaerobic energy processes in the myocardium with the activation of the anaerobic-glycolytic and anaerobic-alactate processes. The high level of lactate bears witness to the fact that the muscles are overloaded, there is a lack of oxygen due to disorders in the blood supply by the coronary vessels, but at the same time, there is a retardation of a reduction in the phosphocreatine amount that may indicate that there is a reserve of energy for their performance. Thus, the oxygen deficiency in the myocardium is compensated by an increase in the lactate and creatine phosphate concentration.
Our comparative analysis of the results of statistical data processing with the Mann-Whitney U-test criterion shows that in the early postsurgery period, upon the TMT application, significant differences in energy indicators in the patients of the main group versus the control group patients are manifested. Thus, it has been found that in the patients of the main group, in their early postsurgery period, TMT has induced an increase in the oxygen levels on postsurgery day 7 and day 15 (p=0.04), a reduction in the lactate values on day 7 and day 15 (p=0.03) and a rise in the phosphocreatine amount on day 7 after surgery (p=0.05). These data are evidence for the corrective effect produced by TMT on the initial cardiometric markers of stress and the TMT favorable effect as against the decreased metabolism data found in the control group.

Since the systemic cardiotropic effect made by TMT is realized within the framework of the regulation of integral nonspecific adaptation reactions by the body, the actual GNAR types have been identified.

Our analysis of the adaptation reaction patterns in patients of both groups before surgery showed a similar picture of the identified types of GNAR: they were the reactions of training, calm and elevated activation, acute and chronic stress. The same wide range of the initial normal pattern types of the antistress reactions immediately after surgical treatment available in both groups sharply narrowed to the single-type pathological response, namely, the development of acute stress in 70-80% of the cases. Starting from the first day after the surgery, the incidence rate of the stress reactions decreased, and the stable normal pattern types of the antistress reactions developed, ranging from the reaction of training to elevated activation that was observed in especially rapid manner in the control group. However one month later, the patients of the control group demonstrated a decrease in their antistressor potential. On the contrary, in the main group, under the TMT influence, a stable increase in the antistress potential had a smooth linear profile and 1 month later reached the level, which was 90% higher than the control one. In that case, the reaction of calm activation as the most balanced physiological response dominated, while the incidence rate of stress demonstrated an increase in the control group.

The dynamics of the ratio of the antistress reactions to stress, reflected by an integral coefficient (K=AC/C), changed in both groups. It is important to note that in the main group, 1 month after surgical treatment, the highest K was observed, reaching 5.9 arb.u., which was 3 times higher than the integral indicator of the adaptation reaction in the patients in the control group (see Figure 4 herein).

**Conclusions**

The completed cardiometric examination in the early postsurgery period in the control group of the patients with HGG allowed us differentiating complex hemodynamic disorders in the cardiac performance within the possible shortest time. Starting from postsurgery day 2, manifested was a statistically significant increase in the load on the left ventricle and the associated imbalance of metabolic processes of the myocardial energy overload in the form of a decrease in the level of oxygen and phosphocreatine against the background of an increase in lactate, i.e., we observed suppression of the aerobic processes with the activation of anaerobic-glycolytic and anaerobic-lactate processes in the myocardium. This indicated the prognostic significance of the disorders in hemodynamics and the metabolic processes detected by the Cardiocode technology that was recorded within the framework of unstable antistress GNARs, which might contribute to the manifestation of more aggressive forms of cardiotoxicity at the stage of radiation therapy.

The use of TMT in the early postsurgery period in the patients of the main group prevented the development of pathological changes in their hemodynamics and the energy parameters of the myocardium. The best state of hemodynamics and the energy indicators of the heart in the patients of the main group was...
also evidenced by the results of our statistical analysis, which recorded the absence of the myocardium over-
tension and overloading. Starting from day 2 of the
postsurgery period, the completed TMT course pro-
duced primarily a systemic synchronizing effect on the
body. The programmed frequency and low-intensity
algorithm mode of TMT provided acting in concert
by the central regulation of the cardiac performance
and the metabolic processes, which contributed to
removal of the functional myocardial tension. The
cumulative nature of TMT was clearly manifested in
the increasing dynamics of a stable cardiotropic effect
within the framework of integral antistress reactions
as a necessary condition for improving the quality of
life of the HGG patients.

Final conclusions
1. The cardiometric analysis of the state of the car-
diovascular system performance (Cardiocode) in pa-
tients with high-grade glial brain tumors in the early
postsurgery period is an express examination method
allowing us (here and now) to identify a complete set
of objective biophysical, metabolic and adaptive mark-
ers of the functional tension, which are of important
prognostic value for assessing hemodynamics during
subsequent chemoradiotherapy.
2. The application of the Cardiocode analytical
software confirms the feasibility of using transcrani-
al magnetic therapy in patients with high-grade glial
brain tumors in the early postsurgery period by reliev-
ing the emerging signs of tension and myocardial dys-
function by producing a prolonged anti-stress effect
that improves quality of life in patients of this sort.

Acknowledgments
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Statement on ethical issues
Research involving people and/or animals is in full
compliance with current national and international
ethical standards.

Conflict of interest
None declared.

Author contributions
The authors read the ICMJE criteria for authorship and
approved the final manuscript.

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The state of intracardiac hemodynamics in the elderly people with arterial hypertension, taking into account age, gender differences and geographic climatic living conditions

Irina K. Tkhabisimova1*, Maria M. Tkhabisimova1, Rimma Kh. Medalieva1, Aslanbek V. Akhmetov1, Radima Kh. Zhemukhova1

1 Federal State Budgetary Educational Institution of Higher Education “Kabardino-Balkarian State University named after H.M. Berbekov”
Russia, 360000, Nalchik, Chernyshevskogo str., 173
* Corresponding author: email: yka@kbsu.ru

Abstract
We conducted a study of the state of intracardiac hemodynamics in elderly patients with hypertension in the Kabardino-Balkarian Republic, taking into account age, gender and geographic climatic differences.

Materials and methods
In the course of the study, an echocardiographic study was used according to the standard protocol with the Aloka ultrasound machine (Japan), which included M-modal, two-dimensional and Doppler modes.

Results
The study of the criteria of intracardiac hemodynamics in elderly and middle-aged men revealed that the parameters of the end-diastolic dimension (EDD) of the left ventricle and the mass of the left ventricular myocardium (LVMM) prevailed in elderly men compared with middle-aged men. When assessing the indicators of the transmural blood flow in elderly men, a tendency towards a decrease in the value of the E/A ratio was found that was a reflection of diastolic dysfunction, namely, a disorder in myocardial relaxation. In elderly women, large sizes of the aortic root diameter, left ventricular myocardial mass (LVMM) and the thickness of the interventricular septum (IVS) were detected compared with the middle-aged women, which might be associated with age-related changes, namely, a decrease in elasticity, an increase in the diameter and wall thickening. Our analysis of the data delivered by our echocardiographic studies revealed that elderly patients with hypertension were characterized not only by the presence of LVH, but also by various changes in the geometry of the left ventricular myocardium. In the concentric hypertrophy group, the values of the relative wall thickness (RWT) and the left ventricular myocardial mass (LVMM) were significantly higher than those identified in the eccentric hypertrophy group, where there were higher values of the EDD parameters. A correlation was found between the average blood pressure (according to the ABPM data) and the parameters of the left ventricular myocardium in elderly patients with hypertension.

Discussion
The study of the intracardiac hemodynamic parameters in elderly people (both men and women) has revealed a high prevalence of changes in the geometry of the left ventricle with a predominance of the concentric type of remodeling, which is more pronounced in elderly women.

Conclusion
Our analysis of the prevalence of hypertension in elderly patients, considering the geographic climatic conditions of their residence, has revealed that the incidence rate of hypertension is higher in those individuals, who live in the lowland zone than in those living in the mountainous regions.

Keywords
Daily monitoring of blood pressure, ABPM, Left ventricular remodeling, Left ventricular hypertrophy, Arterial hypertension, Elderly people, Intracardiac hemodynamics

Imprint

Introduction
Among the diseases of the circulatory system in the elderly, the most common case is arterial hypertension (AH), which significantly reduces the quality and life expectancy of this category of patients [1-3]. According
to the relevant data provided by many researchers, the incidence of diseases and deaths among elderly patients correlates with an abnormal increase in their blood pressure [4-6]. The topic of studying hemodynamic changes in patients of various ages with hypertension has been sufficiently covered by the reference literature, but the dependence of these changes on sex, gender differences and geographic climatic living conditions has not been sufficiently investigated [7-9]. In connection with the above, it seems relevant to study the daily blood pressure profile in elderly people with hypertension and identify the correlation between the parameters of 24-hour blood pressure monitoring (ABPM) and their indicators of intracardiac hemodynamics.

The aim of the study is to analyze the state of intracardiac hemodynamics in elderly patients with hypertension, who reside in the Kabardino-Balkarian Republic, taking into account their age, gender and the geographic climatic factors.

Materials and methods

The material for the study covered 150 elderly patients with hypertension, who underwent inpatient treatment at the Republican Gerontological Rehabilitation Center and the Republican Clinical Hospital. The research method applied by us involved echocardiography (EchoCG) according to the relevant standard protocol, including M-modal, two-dimensional and Doppler modes, with the Aloka SSD-1700 ultrasound machine (Japan) equipped with the 3,5 MG transducer [11,12]. Analyzed parameters were as follows: anteroposterior dimension of the left atrium (LA) in diastole, end systolic dimension (ESD) and end diastolic dimension (EDD) of the left ventricular cavity, thickness of the interventricular septum (IVST) and posterior wall (LVPWT) at the end of diastole, end systolic and diastolic volumes, ejection fraction (EF), left ventricular myocardial mass (LVMM) according to the formula by R.B. Devereux: LVMM = 0,8x (1.04x ((LVPWT + IVS + EDD) i-EDDi)) + 0,6.

Research results

When analyzing the EchoCG indicators in elderly patients, taking into account the influence of the geographic climatic zone of their residence, there were no significantly significant differences revealed (see Table 1 herein). In elderly patients with AH, living in the mountainous zone, the indicators of the aorta size, EDD and EF were recorded to be slightly higher com-

<table>
<thead>
<tr>
<th>Indicator</th>
<th>Plain residence n = 24</th>
<th>Foothill zone residence n = 39</th>
<th>Mountain zone residence n = 18</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ao, mm</td>
<td>31,1±1,5</td>
<td>31,3±2,2</td>
<td>33,5±1,8</td>
</tr>
<tr>
<td>EDD, mm</td>
<td>50,6±3,2</td>
<td>50,4±2,3</td>
<td>53,4±4,9</td>
</tr>
<tr>
<td>ESD, mm</td>
<td>34,1±4,8</td>
<td>32,7±4,6</td>
<td>33,8±5,3</td>
</tr>
<tr>
<td>EF, %</td>
<td>60,9±6,4</td>
<td>59,1±4,8</td>
<td>65,4±3,5</td>
</tr>
<tr>
<td>LVPW, mm</td>
<td>10,5±1,6</td>
<td>11,2±1,2</td>
<td>10,8±1,5</td>
</tr>
<tr>
<td>IVST, mm</td>
<td>11,9±1,7</td>
<td>11,8±2,1</td>
<td>12,3±1,3</td>
</tr>
</tbody>
</table>

Note: *significance criterion in relation to the mountain zone (p<0.05).

Abbreviations in Tables 1-4: Ao - aorta, ESD - end-systolic dimension, EDD - end-diastolic dimension, EF- ejection fraction, LVPWT - left ventricle posterior wall thickness, IVST - interventricular septum thickness.

Table 2

<table>
<thead>
<tr>
<th>Indicator</th>
<th>Groups of examined individuals</th>
<th>Men n=40</th>
<th>Women n=41</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ao, mm (aorta)</td>
<td></td>
<td>33,0±2,9</td>
<td>32,6±2,7</td>
<td>&gt;0,05</td>
</tr>
<tr>
<td>EDD, mm</td>
<td></td>
<td>52,3±5,9</td>
<td>49,9±6,5</td>
<td>&gt;0,05</td>
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<tr>
<td>ESD, mm</td>
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<td>34,1±6,3</td>
<td>33,6±5,5</td>
<td>&gt;0,05</td>
</tr>
<tr>
<td>EF %</td>
<td></td>
<td>65,1±9,1</td>
<td>60,9±7,7</td>
<td>&gt;0,05</td>
</tr>
<tr>
<td>LVPWT, mm</td>
<td></td>
<td>10,6±1,4</td>
<td>10,5±1,3</td>
<td>&gt;0,05</td>
</tr>
<tr>
<td>IVST, mm</td>
<td></td>
<td>12,5±1,3</td>
<td>12,3±1,5</td>
<td>&gt;0,05</td>
</tr>
<tr>
<td>MLVM (mass of left ventricular myocardium)</td>
<td></td>
<td>216,4±54,5</td>
<td>201,4±49,8</td>
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<tr>
<td>RWT (relative wall thickness)</td>
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<td>0,42±7,2</td>
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<tr>
<td>E, m/s</td>
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<tr>
<td>A, m/s</td>
<td></td>
<td>65,2±10,6</td>
<td>68,9±11,2</td>
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</table>

<table>
<thead>
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<th>Indicator</th>
<th>Groups of examined individuals</th>
<th>Elderly age n=40</th>
<th>Average age n=33</th>
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<td>Ao, mm</td>
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<td>33,0±2,9</td>
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<tr>
<td>EDD, mm</td>
<td></td>
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<td>ESD, mm</td>
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<td>31,4±4,2</td>
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<tr>
<td>EF %</td>
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<td>65,1±9,1</td>
<td>65,2±5,3</td>
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<tr>
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<td>12±1,4</td>
<td>&gt;0,05</td>
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<tr>
<td>MLVM</td>
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<td>180,4±36,7</td>
<td>&lt;0,05</td>
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<td>RWT</td>
<td></td>
<td>0,42±7,2</td>
<td>0,40±6,3</td>
<td>&gt;0,05</td>
</tr>
<tr>
<td>E, m/s</td>
<td></td>
<td>64,3±9,2</td>
<td>67,6±13,8</td>
<td>&gt;0,05</td>
</tr>
<tr>
<td>A, m/s</td>
<td></td>
<td>65,2±10,6</td>
<td>61,9±10,4</td>
<td>&gt;0,05</td>
</tr>
</tbody>
</table>
Table 4
Indices of intracardiac hemodynamics in middle-aged and elderly women with AH (X ± SD)

<table>
<thead>
<tr>
<th>Indicator</th>
<th>Groups of examined individuals</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>A o, mm</td>
<td>Elderly age n=41</td>
<td>32,6±2,7</td>
</tr>
<tr>
<td></td>
<td>Average age n=31</td>
<td>29,1±2,2</td>
</tr>
<tr>
<td>EDD, mm</td>
<td>Elderly age n=41</td>
<td>49,9±6,5</td>
</tr>
<tr>
<td></td>
<td>Average age n=31</td>
<td>47,1±2,9</td>
</tr>
<tr>
<td>ESD, mm</td>
<td>Elderly age n=41</td>
<td>33,6±5,5</td>
</tr>
<tr>
<td></td>
<td>Average age n=31</td>
<td>30,7±2,5</td>
</tr>
<tr>
<td>EF %</td>
<td>Elderly age n=41</td>
<td>60,9±7,7</td>
</tr>
<tr>
<td></td>
<td>Average age n=31</td>
<td>64,9±4,2</td>
</tr>
<tr>
<td>LVPWT, mm</td>
<td>Elderly age n=41</td>
<td>10,5±1,3</td>
</tr>
<tr>
<td></td>
<td>Average age n=31</td>
<td>10,1±1,1</td>
</tr>
<tr>
<td>IVST, mm</td>
<td>Elderly age n=41</td>
<td>12,3±1,5</td>
</tr>
<tr>
<td></td>
<td>Average age n=31</td>
<td>10,2±1,0</td>
</tr>
<tr>
<td>MLVM</td>
<td>Elderly age n=41</td>
<td>201,4±49,8</td>
</tr>
<tr>
<td></td>
<td>Average age n=31</td>
<td>166±51,8</td>
</tr>
<tr>
<td>RWT</td>
<td>Elderly age n=41</td>
<td>0,43±8,9</td>
</tr>
<tr>
<td></td>
<td>Average age n=31</td>
<td>0,41±9,8</td>
</tr>
<tr>
<td>E, m/s</td>
<td>Elderly age n=41</td>
<td>66,7±10,3</td>
</tr>
<tr>
<td></td>
<td>Average age n=31</td>
<td>74,2±11,4</td>
</tr>
<tr>
<td>A, m/s</td>
<td>Elderly age n=41</td>
<td>68,9±11,2</td>
</tr>
<tr>
<td></td>
<td>Average age n=31</td>
<td>59,3±9,9</td>
</tr>
</tbody>
</table>

Table 5
Variants of LV remodeling in elderly men and women with hypertension

<table>
<thead>
<tr>
<th>LV remodeling variant</th>
<th>Men</th>
<th>Women</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal LV geometry</td>
<td>35,6%</td>
<td>32,8%</td>
</tr>
<tr>
<td>Concentric remodeling</td>
<td>19,7%</td>
<td>20,6%</td>
</tr>
<tr>
<td>Concentric hypertrophy</td>
<td>23,8%</td>
<td>28,1%</td>
</tr>
<tr>
<td>Eccentric hypertrophy</td>
<td>20,9%</td>
<td>18,5%</td>
</tr>
</tbody>
</table>

Note: *p<0,05 compared with eccentric hypertrophy group

When comparing the transmirtal blood flow in elderly men, a tendency towards a decrease in the value of the E/A ratio was revealed that was associated with diastolic dysfunction: a disorder in the myocardial relaxation (see Table 3 herein).

In elderly women, larger values of the diameter of the aortic root, MLVM and the thickness IVS were found in comparison with the middle-aged women that might be associated with age-related changes, namely, a decrease in elasticity, an increase in the diameter and thickening of the walls (Table 4, p<0,05).

Our comparison of the parameters of the transmirtal blood flow in elderly women revealed lower peak blood flow rates in the phase of passive filling of the left ventricle and higher peak blood flow rates in the active filling phase compared to the middle-aged women, that might be attributed to the severity of the diastolic dysfunction in elderly patients with hypertension (p<0,05; see Table 4 herein).

In the study of the structural and functional state of the myocardium, depending on the type of remodeling in elderly patients with hypertension, a high prevalence of changes in the LV geometry was revealed. Our analysis of the obtained echocardiographic data showed that elderly patients with hypertension were characterized not only by the presence of LVH, but also by various changes in the geometry of the left ventricular myocardium (see Table 5 herein).

Among elderly men, 35,6% of them had the normal LV geometry, while among elderly women we recorded 32,8% of the normal LV geometry cases. The eccentric LV hypertrophy was detected in 20,9% of the elderly men and in 18,5% of the elderly women. The LV concentric hypertrophy and concentric remodeling were more common in the group of elderly women. Among women, the LV concentric hypertrophy was recorded in 28,1% of the cases, in the group of men it was reported to be detected in 23,8% of the individuals (see Table 5 herein).

Our analysis of remodeling variants in elderly patients with hypertension showed that the variant of the normal LV geometry was more common among the elderly men and in 18,5% of the elderly women. The LV concentric hypertrophy and concentric remodeling were more common in the group of elderly women. Among women, the LV concentric hypertrophy was recorded in 28,1% of the cases, in the group of men it was reported to be detected in 23,8% of the individuals (see Table 5 herein).

Our analysis of remodeling variants in elderly patients with hypertension showed that the variant of the normal LV geometry was more common among the elderly men, and among the women in the same age group with hypertension, the concentric variants of LV remodeling were more often found.

Discussion

Our analysis of the above mentioned structural and functional changes in the heart depending on the type
of LV remodeling showed statistically significant differences found in the groups with the concentric hypertrophy and eccentric hypertrophy. In the concentric hypertrophy group, the RWT and MLVM values were significantly higher than those detected in the eccentric hypertrophy group, where the EDD parameters were recorded to be higher (see Table 6 herein).

We conducted an analysis of the correlation dependence between the averaged values of the day- and night-time BP values and the indicators characterizing the structural and functional state of the heart. In the group of elderly men, a correlation was identified between the indicators of the "hypertensive load" SBP in the day-time and IVS (r = 0.5, p < 0.05), as well as between night-time DBP and IVS. A significant correlation was revealed between the indicators of the "hypertensive load" SBP during sleep with the LVPW value (r = 0.4, p < 0.05) as well as with the RWT value (r=0.3, p<0.05). A weaker correlation, which was treated as not significant, was identified between the day- and night-time values of the indicators. When analyzing the correlation between the MLVM values and the ABPM indicators, a significant correlation with the daily SBP values was found (r=0.3, p<0.05). In general, a combination of higher correlation coefficients between the MLVM values and the averaged SBP values and the weakening relationship of MLVM with similar DBP values was identified (see Table 7 herein).

When analyzing the correlation dependence in the group of women, a significant relationship was detected between the characteristics of ABPM (day-time SBP, night-time DBP) and the IVS indicators (r = 0.5; r = 0.3, p <0.05, respectively), between SBP during sleep and LVPWT (r = 0.4, p <0.05), between the day-time SBP and MLVM (r = 0.3, p <0.05), and between the sleep SBP and RWT (r = 0.3, p < 0.05; see Table 8 herein).

**Conclusion**

The given study of the intracardiac hemodynamic parameters in the elderly has revealed a high prevalence of changes in the geometry of the left ventricle with a predominance of the concentric type of remodeling, which is more pronounced in elderly women. When studying the prevalence of hypertension depending on gender, it has been found that in elderly men the incidence rate of hypertension is 49.8%, while that in women is recorded to be 54.3%. Some gender characteristics of blood pressure have been identified: among men, hypertension becomes widespread at their earlier age, while in women, the prevalence of hypertension can be detected in the sixth decade of their life span. Our analysis of the prevalence of hypertension in elderly patients, depending on the geographic climatic conditions of their residence, has shown that the incidence rate of hypertension is higher in those who reside in the plain zone as against those living in the mountainous regions. The incidence rate of hypertension in the elderly is 47.9% in the mountainous regions, while this parameter reaches 50.6% for the foothill area elderly and 57.8% in the elderly on the plain.

**Statement on ethical issues**

Research involving people and/or animals is in full compliance with current national and international ethical standards.
Conflict of interest
None declared.

Author contributions
The authors read the ICMJE criteria for authorship and approved the final manuscript.

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