https://doi.org/10.47183/mes.2024-26-4-114-122



## PROSPECTIVE DIRECTIONS IN HUMAN HEALTH MONITORING DURING LONG-TERM SPACEFLIGHTS

Viktor A. Ivanov[1]✉, Yaroslav D. Shansky[1], Kirill A. Prusakov[1], Julia A. Bespyatykh[1,2], Dmitry V. Basmanov[1]

[1] Lopukhin Federal Research and Clinical Center of Physical-Chemical Medicine, Moscow, Russia

[2] Mendeleev University of Chemical Technology of Russia, Moscow, Russia

**Introduction.** The increasing duration of spaceflights and the associated prolonged exposure of space crewmembers to unfavorable microgravity conditions necessitate the development of improved approaches to diagnosing the health status directly during the flight. This study is aimed at searching and selecting promising biological markers suitable for studying directly during spaceflights.

**Objective**. To review the current status of the abovementioned problem and to identify biochemical and molecular markers most promising for biomedical research in spaceflight conditions.

**Methods**. A literature review of methods currently used for monitoring the level of biological markers characterizing variations in the immune, excretory, reproductive, musculoskeletal, and blood coagulation systems caused by spaceflight conditions was carried out.

**Findings**. Data concerning biological markers used for monitoring the health status of space crewmembers were analyzed. The authors argue that protein markers reflecting bone tissue remodeling hold particular promise. The decrease in bone tissue density developed as a result of microgravity carries potential risks of traumatism, thus making screening diagnostics of the state of the musculoskeletal system a key focus of laboratory diagnostics. The conducted literature review suggests that P1NP and osteocalcin may serve as the most informative markers of new bone tissue formation, while collagen C-telopeptide, pyridine cross-links, and tartrate-resistant acid phosphatase may serve as markers of bone tissue lysis.

**Keywords:** aerospace medicine; bone remodeling; molecular markers; bone mineralization; micro-RNA; spaceflight; microgravity; thrombosis

**For citation:** Ivanov V.A., Shansky Y.D., Prusakov K.A., Bespyatykh J.A., Basmanov D.V. Prospective directions in human health monitoring during long-term spaceflights. *Extreme Medicine*. 2024;26(4):114–122. https://doi.org/10.47183/mes.2024-26-4-114-122

**Funding:** the study was performed within the framework of the state assignment “Amalthea-1”, R&D Reg. No. 124031500113-3.

**Potential conflict of interest:** the authors declare no conflict of interest.

✉ Viktor A. Ivanov Vanov.va@inbox.ru

**Received:** 7 July 2024 **Revised:** 7 Oct. 2024 **Accepted:** 9 Oct. 2024

## ПЕРСПЕКТИВНЫЕ НАПРАВЛЕНИЯ МОНИТОРИНГА СОСТОЯНИЯ ЗДОРОВЬЯ ЧЕЛОВЕКА В УСЛОВИЯХ ДЛИТЕЛЬНОГО КОСМИЧЕСКОГО ПОЛЕТА

В.А. Иванов[1]✉, Я.Д. Шанский[1], К.А. Прусаков[1], Ю.А. Беспятых[1,2], Д.В. Басманов[1]

[1] Федеральный научно-клинический центр физико-химической медицины им. академика Ю.М. Лопухина, Москва, Россия

[2] Российский химико-технологический университет имени Д.И. Менделеева, Москва, Россия

**Введение.** В связи с увеличением длительности космических полетов растет и продолжительность пребывания членов экипажа в неблагоприятных условиях микрогравитации, что требует разработки подходов, направленных на диагностику состояния здоровья непосредственно в процессе полета. Данное исследование направлено на поиск и выбор перспективных биологических маркеров, целесообразных для изучения в условиях космического полета.

**Цель.** Изучить современное состояние проблемы и определить биохимические и молекулярные маркеры, наиболее перспективные для направления медико-биологических исследований, выполняемых в условиях космического полета.

**Результаты.** Проведен анализ данных литературы, посвященных изучению методов контроля уровня биологических маркеров, характеризующих вызываемые условиями космического полета изменения иммунной, выделительной, репродуктивной систем, опорно-двигательного аппарата и системы свертывания крови.

**Выводы.** В настоящем обзоре рассмотрены данные, касающиеся биологических маркеров, позволяющих контролировать состояние здоровья космонавтов. По мнению коллектива авторов, наиболее перспективными являются белковые маркеры, отражающие перестройку костной ткани. Развивающееся в результате микрогравитации снижение плотности костной ткани потенциально несет риски травматизма, поэтому скрининговая диагностика состояния опорно-двигательной системы является актуальной проблемой лабораторной диагностики. Исходя из данных литературы, наиболее информативными маркерами образования новой костной ткани могут служить P1NP и остеокальцин, а ее лизиса — С-телопептид коллагена, пиридиновые сшивки и тартрат-резистентная кислая фосфатаза.

**Ключевые слова:** космическая медицина; ремоделирование кости; биологические маркеры; минерализация кости; космос; невесомость; тромбоз

**Для цитирования:** Иванов В.А., Шанский Я.Д., Прусаков К.А., Беспятых Ю.А., Басманов Д.В. Перспективные направления мониторинга состояния здоровья человека в условиях длительного космического полета. *Медицина экстремальных ситуаций*. 2024;26(4):114–122. https://doi.org/10.47183/mes.2024-26-4-114-122

**Финансирование:** публикация подготовлена в рамках государственного задания «Амальтея-1», номер государственного учета НИОКТР 124031500113-3

**Потенциальный конфликт интересов:** авторы заявляют об отсутствии конфликта интересов.

✉ Виктор Андреевич Иванов Vanov.va@inbox.ru

**Статья поступила:** 07.07.2024 **После доработки:** 07.10.2024 **Принята к публикации:** 09.10.2024

## INTRODUCTION

The growing diversity and amount of works performed by crewmembers during spaceflights, including long-term technical and biological experiments, increase the duration of their stay on the Earth's orbit. Astronauts encounter long-term unfavorable effects associated with gravity, such as hypodynamia, prolonged stay in a closed environment, increased noise, radiation, mental workloads, as well as restricted diets [1]. The continued quest of the mankind for longer-term manned missions will require even longer stays under the conditions hostile to the human body. Measures aimed at maintaining the physical and mental health of astronauts should be based on the knowledge of the behavior of human organs and systems under conditions of long-term spaceflights. The current level of technical development makes it possible to create laboratory instruments adapted for work in space conditions, permitting timely assessment of numerous parameters of body functions and life processes followed by correction, if necessary, the diet, physical load, and living conditions.

Various approaches can be applied to assess the functional state of the body. These include measurements, both invasive and noninvasive, undertaken during the flight and zero-G conditions, as well as collecting and storing samples for analysis after returning to the Earth. Conducting measurements directly in zero-G conditions makes it possible to avoid additional manipulations associated with preservation, storage, and delivery of biological samples, thus excluding the influence of storage and transportation conditions on target components in these biospheres.

Much attention has been paid to the development and application of noninvasive methods for monitoring astronauts' health status, such as Doppler blood cell composition detection [2], assessment of body fluid distribution [3], bicycle ergometry, etc. Unfortunately, such methods prove ineffective for evaluating minor changes in parameters and analyzing specific markers of the clinical status of the human body. The widespread approach involving collection of biological samples during the flight with their subsequent transportation to the Earth is inapplicable for many clinical and laboratory parameters due to the impossibility of their analysis after freezing and/or long-term storage of biospecimens. In addition, this approach allows analysis of changes in laboratory parameters only after a prolonged period of time, thus excluding the possibility of introducing the necessary adjustments to the experiment protocol along with monitoring and adjusting the health status of astronauts in real time [4].

Therefore, the search for biological markers that could reflect changes in human health during spaceflights represent a highly relevant research task. This will contribute to the development and improvement of valid diagnostic test systems for assessing the functional state of human health during spaceflights.

In this work, we aim to review prospective directions of modern biomedical research conducted in spaceflight conditions.

## STUDY RESULTS

### Current state of the problem

At present, a large number of studies focus on investigating astronauts' health status. However, most of them involve the stage of clinical and laboratory analysis after the astronauts return to the Earth. The results of such studies, although replenishing the knowledge base in the field of space medicine, are unsuitable for assessing the state of human health during the flight. The diagnostic value of such samples is minimal due to their inability to reflect the dynamics of changes in the state of astronauts' organs and systems at the moment of their stay in space. It should be noted that in zero-G conditions, the long-term storage and delivery of biological samples containing whole cells to the Earth conditions is hard to organize. In addition, a number of biological substrates, protein-containing ones in particular, should be analyzed directly during the flight due to the inadmissibility of their freezing.

The low gravity environment of space expeditions makes biological fluids change their behavior, thus changing the requirements for biosampling. Invasive biosubstrate collection using conventional techniques (syringe or open technique) becomes difficult. In space conditions, blood collection procedure is associated with venipuncture, which carries risks of low-dispersed aerosol formation in the air or development of infectious complications. Hence, the use of biological media available through minimally invasive or self-administered collection methods becomes relevant for analysis. Such biosubstrates include saliva, sweat prints, and urine samples. If blood components are to be tested, aliquot volumes should be minimized and the limitations of vacuum sampling systems should be considered.

In addition, preferences for a certain technique for introducing samples into the working area of the test system change. Thus, incubation of freely poured liquid when performing conventional enzyme-linked immunosorbent assay (ELISA) becomes unrealistic. Adhesive/capillary interactions of liquid with the solid phase keep under microgravity conditions, which currently allows the use of diagnostic systems in the form of test strips on the International Space Station (ISS). However, such test systems have a number of limitations, one of which is the semi-quantitative estimation of concentrations. It should be noted that the rapid development of microfluidic technologies and their successful application in terrestrial conditions makes it possible to assume that the adaptation of these technologies to microgravity conditions will facilitate the transition to the analysis of biological markers, including ELISA methods.

Currently, a number of physiological parameters, such as hemoglobin levels, blood glucose concentration, etc., are measured in space environments using immunochemiluminescence on test strips (Reflotron, F. Hoffmann-La Roche Ltd. / Roche Diagnostics GmbH, Germany) [5]. Other analytes and samples require transportation to terrestrial conditions followed by their analysis upon completion of the flight. Such samples include blood

serum, washes from the internal surfaces of the station and the surfaces of samplers, samples for microbiological studies, e.g., those collected as part of a Chromatomass-spectrum M experiment. Such studies are important for studying the continuously changing microbiome of the station [6], associated with the constant exchange of microflora in astronauts [7], but the urgency of obtaining such results is significantly lower than in the case of monitoring the health of the crew.

Recently, the results of a number of projects on studying astronauts' health using individual indicative markers of various states of the body systems in microgravity conditions have been published. Thus, as part of the Splanch experiment on the International Space Station (ISS), and earlier on the MIR space station, the levels of markers of cardiovascular damage, aspartate aminotransferase (AST), and alanine aminotransferase (ALT) were measured using a modified Reflotron-4 device. Hematological studies in spaceflight conditions were carried out on board the ISS, and earlier on the MIR station for 15 months during three MIR expeditions (15th, 16th, and 17th). These studies included an assessment of the blood cell composition, hematocrit, hemoglobin, reticulocytes, and the leukogram [8]. The analyses were carried out on smears of capillary blood using a Mikrovzor device, which combines a microscope with a television transmitter. In the Russian segment of the ISS, hematocrit was measured using a Hematocrit device. Kunz H, Quiriarte H et al. recorded that the hematocrit level was significantly elevated in the early stages of the flight, remaining unchanged throughout the entire stay in space, which is associated with a decrease in the volume of circulating blood and blood plasma under microgravity conditions. In the early post-flight period, a decrease in hematocrit below the pre-flight level was noted, indicating a loss of the cell pool [9]. Some authors (Mikhailov P.A. et al.) noted the development of functional erythropenia and an increase in the number of abnormal erythrocytes during orthostatic suspension in animal experiments [10].

## DISCUSSION OF RESULTS

### Influence of spaceflight conditions on the immune system

The need to elucidate the specifics of functioning of crewmembers' immune system is determined by harsh conditions, including confined spaces, hidden cavities, and reduced gravity, which contributes to the aerosol formation in the air. All this forms a favorable environment for the growth and transmission of pathogenic microorganisms, including herpes virus [11]. Paul AM, Mhatre SD et al. studied the leucogram and cytokine profile as an assessment of the cellular component [12–13]. They found a decrease in the number of eosinophils and a slight increase in the number of neutrophils when T-lymphocytes were passivated in vitro, which is probably due to a decrease in the expression of CD3 and IL-2 receptors on the T-lymphocytes surface [14]. Other studies revealed a decrease in the leukocytes number, in particular lymphocytes, monocytes [9], and leukocyte differentiation [15].

Microgravity promotes increased production of tumor necrosis factor and the development of immune cells apoptosis [16], which was confirmed by studies on cell lines. C.A. Savary et al. found that dendritic cells obtained by differentiation of donor $CD34^+$ progenitor cells in a rotating culture cell, modeling microgravity conditions, showed a decreased ability to phagocytose *Candida albicans* fungi and antigen presentation [17]. A number of studies have recorded a decrease in the cytotoxic function of natural killer cells against leukemia cells of the K562 line *in vitro* occurring in the early stages of spaceflights [18].

V.K. Ilyin et al. found that the levels of sIgA, IgM, and IgA immunoglobulins in saliva and gingival fluid decrease during a spaceflight. The noted shifts are highly likely to trigger a decrease in the protective function of saliva, thus contributing to the risk of infectious and inflammatory processes when the main paradontopathogenic strains of pathogens in the oral cavity are detected in the subjects [19].

The studies conducted by C.M. Ott showed that microgravity conditions and prolonged spaceflights stimulate the reactivation of latent herpesviruses, as evidenced by the increased frequency of human herpes virus type 1 detection in the saliva of astronauts and increased incidence of shingles. In addition, researchers diagnosed cytomegalovirus in the urine of 47% of Space Shuttle crewmembers [20].

A number of scientific studies have confirmed the formation of a general imbalance of the immune system in the conditions of spaceflights: about 46% of ISS crew members experienced these immune disorders [21]. A decrease in local immunity and functional activity of natural killer cells was registered, which led to reactivation of latent viruses, in particular, herpes virus. A number of publications (about 17% of reports) noted allergic reactions due to both a shift in the cytokine profile and other spaceflight factors (stress, space radiation) affecting the immune system status [21].

The National Aeronautics and Space Administration (NASA) has also identified changes in the immune response of astronauts during the Apollo (1975) and Skylab (Skylab-3 mission, 1973) missions. Serum samples collected during spaceflights were analyzed for miR-21 microRNAs, the expression of which increases approximately twofold during early T-cell activation. By quantitative polymerase chain reaction (PCR) tests of four biological samples, suppression of miR-21 expression under spaceflight conditions and suppression of expression of 85 genes was detected. In particular, the expression of early growth response protein 3 (EGR3), Fas-ligand of TNF superfamily (FASLG), protein family (BTG2), Spruti homolog 2 (SPRY2), and T-cell GTPase activator protein (TAGAP), whose regulation is carried out specifically by miR-21, was reduced. According to Hughes-Fulford M et al, the change in TAGAP expression can be functionally associated with the development of rheumatoid arthritis and multiple sclerosis, and a decrease in BTG2 gene expression reduces the cellular immune response [22].

### Influence of spaceflight conditions on the excretory system

According to the literature, the filtration function of kidneys in microgravity conditions is conventionally estimated by creatinine and urea levels, residual products of protein metabolism. Since creatinine is contained in muscle cells, given the relatively stable muscle mass, its level is not subject to significant fluctuations. Creatinine is excreted by the kidneys, which, in the absence of evidence of muscle injury, allows effective assessment of glomerular filtration rate [23]. Clinical urine analysis is a standard practice of clinical and laboratory diagnostics used to assess the excretory and filtering function of the kidneys and to evaluate the homeostasis of the body. In the practice of space medicine, this analysis is implemented using a Urolux urine analyzer included in the ISS onboard equipment, which uses 10-zone test strips. Measurements are carried out by the method of reflectance photometry. The parameters evaluated include urine specific gravity, acidity (pH), presence of leukocytes, nitrite, protein, glucose, ketone bodies, urobilinogen, bilirubin, and blood elements (erythrocytes, leukocytes) [24]. Currently, urine biochemical analysis is another routine comprehensive method for assessing the excretory system status, including under spaceflight conditions.

Keith Siew et al. reported a reversible renal tubular remodeling caused by adaptation to changes in the blood electrolyte composition and redistribution of body fluid due to cranial displacement. An increase in the secretion of calcium, phosphorus, and magnesium ions in the excreted urine was noted, which is presumably associated with bone resorption [25].

### Influence of spaceflight conditions on the blood coagulation system

Thrombosis in microgravity conditions is a relevant problem in modern astronautics. An ultrasound examination study conducted in 2019 found that six out of 11 ISS crewmembers had asymptomatic blood flow disorders in the head and neck vessels, with one of the astronauts having an occlusive thrombosis of the left internal jugular vein [26]. Thrombosis can be caused both a decrease in the velocity of blood flow through the vessels associated with hypodynamia and biochemical changes in the blood and endothelium. Changes in the blood protein composition affect the thickness and functional state of the vascular glycocalyx [27] and alter the rheological blood properties, increasing its viscosity, leading to an increased risk of thrombosis. In the early post-flight period, an increase in the parameters indicative of increased thrombosis potential, such as soluble fibrin monomer complexes (SFMCs) [28], factor XI, fibrinogen, fibrinopeptide A, plasminogen activator inhibitor serpin-3, etc., was observed [29].

Thrombosis formation may also be influenced by the planned use of oral contraceptives during flight to achieve medical amenorrhea [30], practiced for hygienic and water-saving reasons in female astronauts. According to published data, regular administration of a drug containing drospirenone leads to decrease in the plasma albumin level. In the absence of pharmacotherapy, the plasma protein composition and associated risks of thrombosis showed no sex differences [31]. However, the studied sample of astronauts is currently small, and its further increase during the development of space programs in the future may reveal prerequisites for the occurrence of multidirectional abnormalities in the hemostasis system.

To date, the increased risk of thrombosis caused by prolonged stay in microgravity has been associated with a number of factors that are difficult to correct, such as changes in blood circulation, blood cell composition, and activity of signaling molecules. In order to detect a pathological link in the coagulation system, determination of a large number of biomarkers is required. This task is associated with simultaneous processing of different types of biomaterials and a prolonged stage of sample preparation. Collection of different types of biomaterials under zero-G conditions is undesirable due to technical limitations and additional risks for astronauts' health. These limitations make it necessary to create test systems capable of analyzing a minimum number of integral indicators in the blood coagulation system, sufficient for monitoring the health of astronauts, with the prospect of being capable of determining the mechanisms of a particular pathology. In case of detection of thrombosis of large vessels, it is impossible to provide surgical assistance due to logistic constrains. The use of drug therapy is also problematic due to a limited set of pharmaceuticals and the development of possible complications that can aggravate the astronaut's condition.

Thus, the search for early biomarkers of the hemostasis system state and scientific substantiation of methods for diagnostics/correction and prevention of coagulopathies in space crewmembers is a promising direction of space medicine. However, the current list of biological markers required for complete characterization of coagulation processes is rather extensive, which makes their measurement in flight conditions highly difficult.

MicroRNAs, small non-coding RNA molecules (16–25 nucleotides) that perform regulatory functions in relation to a number of genes, may be potential informative markers of the blood coagulation system. In studies on rodents, as well as in the NASA Twins Study, microRNAs associated with the hemostasis system and more intensively expressed under spaceflight conditions were identified: miR-125, miR-16, and let-7a/7c [32–33]. These microRNAs are associated with the mechanisms of radiation damage in the vascular wall and, therefore, may be predictors of thrombosis. Changes in the level of miR-16, which has anti-inflammatory and antithrombotic effects, were observed in [32]. To date, there has been no precise information regarding the prognostic value of microRNA estimates in the human blood due to the lack of accumulated scientific data. This does not allow microRNA to be used as a biomarker of crew health control. Micro-RNA studies are associated with PCR, which is not yet feasible in zero gravity.

### Influence of spaceflight conditions on the reproductive system

The IMMUNO experiment conducted from 2012 to 2017 was aimed at monitoring the reproductive system of astronauts during long-term missions to the ISS. The experimental group included exclusively male individuals, which fact should be taken into account when interpreting the data. The levels of luteinizing hormone (LH) and follicle-stimulating hormones (FSH) affecting testosterone synthesis were measured by interstitial Leydig cells [34]. The levels of activin A, which is responsible for the regulation of FSH synthesis, regulating the immune response and the process of wound healing [35], as well as the antagonistic protein produced by Sertoli cells, inhibin B, which inhibits the synthesis of follicle-stimulating hormone [36], were determined.

The study was supplemented by estimating levels of antisperm immunoglobulins A, G, M (AS-IgA, AS-IgG, AS-IgM) and the sum of antisperm antibodies, as well as the total and free cortisol in saliva [37], testosterone, estradiol, and aldosterone [38]. It is worth noting that all studies were performed after the completion of the spaceflight by the ELISA method. It was found that long-term spaceflights lead to an increase in the estrogen level, which was associated with a decrease in the content of specific and nonspecific transport proteins in the body. At the same time, an increase in the concentration of stress hormone cortisol was noted. The study by I.A. Nichiporuk et al. [37], although failing to definitely establish the totality of factors leading to hormonal imbalance, noted that the changes in the reproductive system are reversible.

### Influence of spaceflight conditions on the musculoskeletal system

During a long-term spaceflight, all astronauts are subject to degradation of the musculoskeletal system, which is manifested in a decrease in muscle mass, bone mineralization, and reorganization of collagen in bones, tendons, and ligaments. Most of the large bones, experiencing constant load under the conditions of Earth gravity, are subjected to partial resorption in zero gravity. This leads to both reorganization of the micro- and macrostructure of bone tissue and to demineralization of most bones. The greatest danger is associated with damage to the skeleton of the lower limbs, lumbar vertebral bodies, pelvic bones, highly loaded bones of the skull base, and cervical vertebrae. Less critical is partial demineralization of thin spongy bones that carry less load. The only exception is the bones of the upper part of the skull, whose density increases in zero gravity due to the natural compensatory response to the changing load.

These conditions strongly affect the physical health of astronauts and their ability to perform their main tasks. Under spaceflight conditions, to assess the physical state of an individual, muscle volume measurements [39], dynamometry, myography [40], strain gauging, and bioimpedanceometry were performed. The latter are indirect methods that give no clear picture of the processes occurring in the body. In the pre-flight and early post-flight periods, examinations were performed using a noninvasive method of osteodensiometry [41]. It was found that the influence of microgravity is not limited to the development of osteopenia, being accompanied instead by redistribution of bone mineral density. The state of the muscular system and the effectiveness of training before spacewalking are evaluated by bicycle ergometry; the results are influenced by age, cardiovascular system, and fatigue. Moreover, data on previous measurements of the subject are required.

An optimal approach consists in the estimation of clinical molecular markers, conducted directly during the flight and allowing assessment of both the general state and its changes under the influence of loads and other external factors. Some markers include low molecular weight compounds and those excreted by the kidneys, which makes their estimation in urine accessible. Other markers are unable to pass the renal barrier and their estimation is possible only in blood.

Damage to the muscle system can be estimated by the level of creatine kinase, which was performed in the research [42]. However, this parameter is nonspecific with respect to the type of muscle tissue and can indicate damage to both skeletal muscle and myocardium.

Bone resorption is a relatively slow process carried out by osteoclasts. An activated osteoclast is fixed by specific integrin proteins to the bone matrix, triggering the synthesis of Cathepsin-K. The latter is an acidic protease capable of degrading type I structural collagen, which makes up more than 80% of the organic matter of bone. As a result of this process, large fragments of collagen containing large amounts of pyridine cross-links are released into the bloodstream. Osteoclasts synthesize matrix metalloproteases, whose work in the resorption focus result in the release of large fragments of collagen, consisting of two C-telopeptides of type I collagen, quickly excreted with urine, in the bloodstream. The third important component of bone resorption is the transmembrane transport of matrix degradation products into the cell by tartrate-resistant acid phosphatase.

In the process of resorption, calcium ions are released and their concentration in the blood increases, which may contribute to the development of uro- and nephrolithiasis. The study [43] showed an increased calcium content in morning urine and suggested using its determination to monitor the state of the bone system. However, the concentration of ionized calcium depends not only on the processes occurring in bone tissue, which significantly reduces the diagnostic value of its determination.

Thus, the control of collagen C-telopeptide, pyridine cross-links (pyridinoline and deoxypyridinoline) and tartrate-resistant acid phosphatase are convenient markers of bone resorption.

In parallel to bone resorption, bone tissue is also formed by osteoblasts. The resorption Howship's lacuna is filled by fibroblasts and osteoblasts synthesizing collagen of the first type, forming osteoid. In the process of collagen maturation, N-propeptide (P1NP, amino-terminal propeptide of procollagen type 1) determined in the blood becomes detached, which can be considered as a

marker of bone matrix formation. At the same time, a high mechanical strength of bone is provided by the mineral component, whose formation is affected by osteocalcin, a non-collagen protein, which promotes bone mineralization due to the stacking of oriented hydroxyapatite crystals [44]. The enzyme alkaline phosphatase, the exact function of which remains unknown, is involved in bone matrix formation. Considering that insufficient blood calcium levels may prevent the formation of new bone or provoke a decrease in mineralization, the level of ionized calcium in the blood should be taken into account when interpreting the results obtained [45].

Earlier studies showed a decrease in P1NP and bone alkaline phosphatase already by the eighth day of zero gravity. Simultaneously, such markers of bone resorption as pyridine cross-links and C-telopeptides of collagen type I in blood and urine increased [46].

The influence on bone remodeling processes extends beyond hypodynamia and orthostatic hypotension. The direction of the processes is regulated by the humoral system, including steroid hormones. Spaceflights are associated with a serious physical and psycho-emotional stress, which leads to the production of cortisol. Cortisol interferes with the formation of new bone tissue, shifting the equilibrium of bone remodeling toward impaired bone trophic and resorption [47].

In the context of bone tissue loss of its typical structure under spaceflight conditions, the participation of microRNAs in the regulation of de novo bone tissue formation is of interest. To test the hypothesis about changes in microRNA secretion by human osteoblasts under microgravity conditions, the expression of micro-RNAs in rat femur bone was studied. Thus, 14 microRNAs were identified that significantly decreased their expression under conditions of simulated zero gravity and 5 microRNAs whose expression significantly increased under these conditions. The main targets regulated by these microRNAs were genes of Wnt/β-catenin signaling pathway and estrogen-mediated cell cycle regulation [48]. It is assumed that the results obtained could indirectly indicate the state of bone tissue; however, such results are not available in the literature at the moment.

In 2014–2015, NASA carried out studies of urine compounds reflecting the state of the musculoskeletal system: urea, phosphorus and calcium, and creatinine. The results were presented as a ratio to creatinine as an indicator reflecting the glomerular filtration rate. A temporary decrease in urea/creatinine and phosphorus/creatinine ratios was noted, while an increase in calcium/creatinine ratio was observed [49].

A study of the urine proteome after a prolonged spaceflight showed the disappearance of the following peptides: tyrosine kinase receptor (IPI00296992); cytoskeletal keratin-1 (IPI00009865); G-protein coupled receptor from the C family (IPI00789902); inter-α (globulin) H4 inhibitor (IPI00944960); and SERPING1 gene protein (IPI00879931) [41, 50].

## CONCLUSION

The conducted review of published literature has allowed us to identify the most promising markers for assessing the status of human bone, blood coagulation, and immune systems under the conditions of zero gravity (microgravity).

The decreased density of bone tissue under the action of microgravity potentially carries risks of traumatism, e.g., during astronauts' return to the Earth. Therefore, monitoring the state of human bone tissue is a relevant problem of laboratory diagnostics. P1NP, bone alkaline phosphatase, and osteocalcin may serve as the most informative markers of bone tissue formation, along with its lysis — collagen C-telopeptide, pyridine cross-links (pyridinoline and deoxypyridinoline), and tartrate-resistant acid phosphatase. Micro-RNAs are also promising markers of the state of the musculoskeletal apparatus. However, their participation in the regulation of a number of body systems restricts identification of the processes occurring in bone tissue on the basis of individual micro-RNAs. In this regard, a multivariate analysis of a set of several microRNAs appears promising. The difficulty of PCR staging in zero gravity also requires its own technological solution.

The pathologies of the cardiovascular system, the risk of which increases in microgravity conditions, include a decrease in microcirculation and the development of thrombosis. According to the available literature data, the most informative markers of risk and dynamics of thrombotic complications may include soluble fibrin monomer complexes (SFMCs), factor XI, fibrinogen, fibrinopeptide A, and plasminogen activator inhibitor serpin-3. MicroRNA families, such as miR-125, miR-16, and let-7a/7c, are also promising molecular markers of cardiovascular complications.

This review has not considered markers of the state of central nervous system (CNS). Currently, there is a lack of definite molecular and biochemical markers to diagnose a specific neurologic disorder and to identify differences between healthy individuals and patients with CNS disorders. Additionally, CNS dysfunctions in humans are detected already at the stage of selecting candidates for flights.

Longer space exploration projects, including those associated with the prospect of manned interplanetary flights, impose stricter requirements on the control of astronauts' health condition. Although monitoring of the overall health status remains relevant, the limitations associated with zero gravity and space station conditions shift the focus to a gradual introduction of individual diagnostic markers. According to the authors, these include, e.g., the biological markers characterizing the process of bone tissue remodeling, characterized by bone resorption in the lower parts of the body, hyper-mineralization of skull bones and cervical spine. At the same time, analytical systems based on microfluidic technologies seem to be the most promising tool for monitoring these markers during spaceflights.

## References


1. Zwart S.R., Mulavara A.P., Williams T.J., George K., Smith S.M. The role of nutrition in space exploration: Implications for sensorimotor, cognition, behavior and the cerebral changes due to the exposure to radiation, altered gravity, and isolation/confinement hazards of spaceflight. *Neuroscience & Biobehavioral Reviews.* 2021;(127):307–31. https://doi.org/10.1016/j.neubiorev.2021.10.008
2. Kovaleva A.A., Pichulin V.S., Skedina M.A. Neinvazivnye metody issledovanija kletochnogo sostava krovi v uslovijah kosmicheskogo poleta. *TrudyMAI*. 2013;(65):12 (In Russ.). EDN: RBWGMV
3. Raketno-kosmicheskaja korporacija «Jenergija» imeni S.P. Koroljova. jeksperiment "profilaktika-2 [Electronic resource]. URL: https://www.energia.ru/ru/iss/researches/human/26.html (Available from: 29.05.2024) (In Russ.).
4. Tang O., Selvin E., Arends V., Saenger A. Short-Term Stability of Hematologic Parameters in Frozen Whole Blood. *The journal of applied laboratory medicine*. 2019;4(3):410–4. https://doi.org/10.1373/jalm.2018.028357
5. Jeksperiment "Gematologija" [Electronic resource]. URL: https://tsniimash.ru/science/scientific-experiments-onboard-the-is-rs/cnts/experiments/gematologiya/ (accessed: 30.05.2024) (In Russ.).
6. Checinska Sielaff A., Urbaniak C., Mohan G.B.M., Stepanov V.G., Tran Q., Wood J.M. et al. Characterization of the total and viable bacterial and fungal communities associated with the International Space Station surfaces. *Microbiome.* 2019;7(1):50. https://doi.org/10.1186/s40168-019-0666-x
7. Tozzo P., Delicati A., Caenazzo L. Skin Microbial Changes during Space Flights: A Systematic Review. *Life.* 2022;12(10):1498. https://doi.org/10.3390/life12101498
8. Polyakov V.V., Ivanova S.M., Noskov V.B., Labetskaya O.L., Yarlykova Yu.V., Karashtin V.V. et al. Hematological investigations in conditions of long-term space flights. *Aviakosm Ekolog Med.* 1998;2(32):9–18 (In Russ.). EDN: TOKWLD
9. Kunz H., Quiriarte H., Simpson R..J, Ploutz-Snyder R., McMonigal K., Sams Cet all. Alterations in hematologic indices during long-duration spaceflight. *BMC Hematol.* 2017;17(1):12. https://doi.org/10.1186/s12878-017-0083-y
10. Mikhailova P.A., Kropotova M.O., Shikulsky A.S., Nesterenko T.S., Grigoryev D.A., Gorbunov M.M. The change in the qualitative and quantitative composition of the blood in rats under the influence of weightlessness. *Forum molodyh uchenyh*. 2018;9(25):568–71 (In Russ.). EDN: RBWGMV
11. Sobisch L-Y., Rogowski K.M., Fuchs J., Schmieder W., Vaishampayan A., Oles P., et al (2019) Biofilm Forming Antibiotic Resistant Gram-Positive Pathogens Isolated From Surfaces on the International Space Station. *Frontiers in microbiology.* 2019;(10):543. https://doi.org/10.3389/fmicb.2019.00543
12. Paul A.M., Mhatre S.D., Cekanaviciute E., Schreurs, A-S., Tahimic C.G.T, Globus R..K et al. Neutrophil-to-Lymphocyte Ratio: A Biomarker to Monitor the Immune Status of Astronauts. *Frontiers in Immunology*. 2020;(11):564950. https://doi.org/10.3389/fimmu.2020.564950
13. Krieger S.S., Zwart S.R., Mehta S., Wu H., Simpson R.J., Smith S.M. et al. Alterations in Saliva and Plasma Cytokine Concentrations During Long-Duration Spaceflight. *Frontiers in Immunology*. 2021;(12):725748. https://doi.org/10.3389/fimmu.2021.725748
14. Akiyama T., Horie K., Hinoi E., Hiraiwa M., Kato A., Maekawa Y. et al. How does spaceflight affect the acquired immune system?. *NPJ Microgravity*. 2020;6(1):14. https://doi.org/10.1038/s41526-020-0104-1
15. Crucian B., Stowe R.P., Mehta S., Quiriarte H., Pierson D., Sams C. et all. Alterations in adaptive immunity persist during long-duration spaceflight. *NPJ Microgravity*. 2015;(1):15013. https://doi.org/10.1038/npjmgrav.2015.13
16. Bakos A., Varkonyi A., Minarovits J., Batkai L. Effect of simulated microgravity on human lymphocytes. *J Gravit Physiol.* 2001;8(1):69–70.
17. Bakos A., Varkonyi A., Minarovits .J, Batkai L. Characteristics of human dendritic cells generated in a microgravity analog culture system. *In Vitro Cell Dev Biol Anim.* 2001;37(4):216–22. https://doi.org/10.1007/BF02577532
18. Bigley A.B., Agha N.H., Baker F.L., Spielmann G., Kunz H.E., Mylabathula P..L et al. NK cell function is impaired during long-duration spaceflight. *J Appl Physiol*. 2019;126(4):842–53. https://doi.org/10.1152/japplphysiol.00761.2018
19. Ilyin V.K., Shumilina G.A., Solovieva Z.O., Nosovsky A.M., Kaminskaya E.V. Some characteristics of the oral cavity and teeth of cosmonauts on missions to the international space station. *Aerospace and Environmental Medicine.* 2016;50(6):25–30 (In Russ.). EDN: XHTNYN
20. Ott C. M, Oubre C., Wallace S., Mehta S., Pierson D., et al. Risk of Adverse Health Effects Due to HostMicroorganism Interac. Report Number JSC-CN-38050 Houston, 2016.
21. Crucian B., Babiak-Vazquez A., Johnston S., Pierson D.L., Ott C.M., Sams C. Incidence of clinical symptoms during long-duration orbital spaceflight. *Int J Gen Med.* 2016;(9):383–91. https://doi.org/10.2147/IJGM.S114188
22. Hughes-Fulford M., Chang T.T., Martinez E.M., Li C.F. Spaceflight alters expression of microRNA during T-cell activation. *The FASEB Journal*. 2015;29(12):4893–900. https://doi.org/10.1096/fj.15-277392
23. Kayukov I.G., Galkina O.V., Timshina E.I., Zubina I.M., Miheeva A.U., Berdichevsky G.M. Creatinin in the modern evaluation of the kidneys functional condition(Literature review and own data). *Nephrology (Saint-Petersburg)*. 2020;24(4):21–36 (In Russ.). https://doi.org/10.36485/1561-6274-2020-24-4-21-36
24. Noskin A. Bortovoe medicinskoe oborudovanie rossijskogo segmenta Mezhdunarodnoj kosmicheskoj stancii (RSMKS). *Ajerokosmicheskij kur'yer*. 2000;3:26–7 (In Russ.)
25. Siew K., Nestler .KA., Nelson C., D'Ambrosio V., Zhong C., Li Z., et al. Cosmic kidney disease: an integrated pan-omic, physiological and morphological study into spaceflight-induced renal dysfunction. *Nature communications*. 2024;15(1):4923. https://doi.org/10.1038/s41467-024-49212-1
26. White N.J., Wenthe A. Managing Hemostasis in Space. *Arterioscler Thromb Vasc Biol*. 2023;43(11):2079–87. https://doi.org/10.1161/ATVBAHA.123.318783
27. Aldecoa C., Llau J.V., Nuvials X., Artigas A. Role of albumin in the preservation of endothelial glycocalyx integrity and the microcirculation: a review. *Ann Intensive Care*. 2020;10(1):85. https://doi.org/10.1186/s13613-020-00697-1
28. Kuzichkin D.S., Kochergin A.YU. Influence of means of prevention of adverse effects of space flight on the plasma component of the system of regulation of the aggregate state of hu-

man blood. *Meditsina Truda I Promyshlennaya Ekologiya. Institut meditsiny truda RAMN*. 2020;60(11):818–20 (In Russ.). https://doi.org/10.31089/1026-9428-2020-60-11-818-820
29. Kashirina D.N., Percy A.J., Pastushkova L.K., Borchers C.H., Kireev K.S., Ivanisenko V.A., et al. The molecular mechanisms driving physiological changes after long duration space flights revealed by quantitative analysis of human blood proteins. *BMC Med Genomics.* 2019;12(S2):45. https://doi.org/10.1186/s12920-019-0490-y
30. Jain V., Wotring V.E. Medically induced amenorrhea in female astronauts. *NPJ Microgravity*. 2016;2(1):16008. https://doi.org/10.1038/npjmgrav.2016.8
31. Zwart S.R., Auñón-Chancellor S.M., Heer M., Melin M.M., Smith S.M. Albumin, oral contraceptives, and venous thromboembolism risk in astronauts. *Journal of applied physiology*. 2022;132(5):1232–9. https://doi.org/10.1152/japplphysiol.00024.2022
32. Malkani S, Chin C.R., Cekanaviciute E., Mortreux M., Okinula H., Tarbier M. et all. Circulating miRNA Spaceflight Signature Reveals Targets for Countermeasure Development. *Cell reports*. 2020;33(10):108448. https://doi.org/10.1016/j.celrep.2020.108448
33. Garrett-Bakelman .FE., Darshi M., Green S.J., Gur R.C., Lin L, Macias B.R., et al. The NASA Twins Study: A multidimensional analysis of a year-long human spaceflight. *Science.* 2019;364(6436):eaau8650. https://doi.org/10.1126/science.aau8650
34. Chauvigné F, Verdura S., Mazón M.J., Duncan N., Zanuy S., Gómez A. et all. Follicle-Stimulating Hormone and Luteinizing Hormone Mediate the Androgenic Pathway in Leydig Cells of an Evolutionary Advanced Teleost1. *Biol Reprod.* 2012;87(2). https://doi.org/10.1095/biolreprod.112.100784
35. Muttukrishna S., Farouk A., Sharma S., Evans L., Groome N., Ledger W. et all. Serum activin A and follistatin in disorders of spermatogenesis in men. *Eur J Endocrinol*. 2001;144(4):425–9. https://doi.org/10.1530/eje.0.1440425
36. Galimova E.F., Akhmadullina G.Kh., Bulygin K.V., Mochalov K.S., Galimov Sh.N. Inhibin b and activin a in the pathogenesis of idiopathic male infertility. *Kazan medical journal.* 2015;96(5):749–52 (In Russ.). https://doi.org/10.17750/KMJ2015-749
37. Nichiporuk I.A., Chistokhodova S.A. The effects of long term spaceflight on the male reproductive system and its relationship to body composition. *International Research Journal***.** 2023;1(127):76 (In Russ.) https://doi.org/10.23670/IRJ.2023.127.64
38. Nichiporuk I.A, Chistokhodova .SA, Vorontsov A.L. The effect of steroid hormones on physiological parameters and human body composition during short-term simulation of space flight factors. *International Research Journal*. 2022;12(126):70. https://doi.org/10.23670/IRJ.2022.126.35
39. Gopalakrishnan R, Genc K.O., Rice A.J., Lee S.M., Evans H.J., Maender C.C., et al. Muscle Volume, Strength, Endurance, and Exercise Loads During 6-Month Missions in Space. *Aviat Space Environ Med.* 2010;81(2):91–104. https://doi.org/10.3357/asem.2583.2010
40. Bensch .L, Nilsson T., Cowley A. Electroencephalography (EEG), electromyography (EMG) and eye-tracking for astronaut training and space exploration. 2022. *Preprint*. https://doi.org/10.48550/arXiv.2212.06139
41. Oganov V.S., Bakulin A.V., Novikov V.E., Murashko L.M., Kabitskaya O.E. Izmeneniya kostnoy tkani chelovekav kosmicheskom polete: o vozmozhnykh mekhanizmakh osteopenii. *Osteoporosis and Bone Diseases.* 2005; 8(2):2–7 (In Russ.) EDN: MTBJPN
42. Clarkson P.M., Kearns A.K., Rouzier P., Rubin R., Thompson P.D. Serum Creatine Kinase Levels and Renal Function Measures in Exertional Muscle Damage. *Medicine and science in sports and exercise.* 2006;38(4):623–7. https://doi.org/10.1249/01.mss.0000210192.49210.fc
43. Thamer S., Buckey J.C. First Void Urinary Calcium for Tracking Bone Loss and Kidney Stone Risk in Space. *Aerospace medicine and human performance*. 2022;93(7):546–50. https://doi.org/10.3357/AMHP.5979.2022
44. Caillot-Augusseau A., Vico L., Heer M., Voroviev D., Souberbielle J.C., Zitterman A. et al. Space Flight Is Associated with Rapid Decreases of Undercarboxylated Osteocalcin and Increases of Markers of Bone Resorption without Changes in Their Circadian Variation: Observations in Two Cosmonauts. *Clinical chemistry*. 2000;46(8):1136–43.
45. Kuchin R.V., Stogov M.V., Nenenko N.D., Maksimova T.A. Functional hypocalcemia as a probable trigger for bone mass growth in skiers. *Theory and practice of physical culture.* 2022;(9):57–9 (In Russ.). EDN: BEYONH
46. Caillot-Augusseau A, Lafage-Proust M.H., Soler C., Pernod .J, Dubois F., Alexandre C. Bone formation and resorption biological markers in cosmonauts during and after a 180-day space flight (Euromir 95). *Clinical chemistry.* 1998;44(3):578–85.
47. Korokin M.V., Soldatov V.O., Gudyrev O.S., Koklin I.S., Taran E.I., Mishenin M.O. The role of cortisol metabolism in the realization of pathogenetic links in the development of osteoporosis — the rationale for the search for new pharmacotherapeutic targets (review). *Research Results in Biomedicine.* 2022;8(4):457–73 (In Russ.). https://doi.org/10.18413/2658-6533-2022-8-4-0-5
48. Thomas N.J., Choi C.Y., Alwood J.S. Skeletal Micro-RNA Responses to Simulated Weightlessness [Electronic resource]. URL: https://ntrs.nasa.gov/citations/20160009015 (Available from: 28.05.2024). Document ID 20160009015.
49. Bilancio G., Cavallo .P, Lombardi C., Guarino E., Cozza V., Giordano F. et all. Urea and Minerals Monitoring in Space Missions by Spot Samples of Saliva and Urine. *Aerospace medicine and human performance.* 2019;90(1):43–7. https://doi.org/10.3357/AMHP.5200.2019
50. Pastushkova L.K., Valeeva O.A., Larina I.M., Dobrokhotov I.V., Kononikhin A.S., Nikolaev E.N., et al. *Changes of protein profile of human urine after long-term orbital flights.* 2013;156(2):201–4 (In Russ.). EDN: OWMSSD

**Authors' contributions.** All the authors confirm that they meet the ICMJE criteria for authorship. The most significant contributions were as follows. Viktor A. Ivanov, Yaroslav D. Shansky — the concept and design of the study, information collection, text writing; Kirill A. Prusakov — text editing; Julia A. Bespyatykh, Dmitry V. Basmanov — the concept and design of the study, text editing, general management.

**AUTHORS**

**Viktor A. Ivanov**
https://orcid.org/0000-0003-4766-1386
Ivanov@rcpcm.org

**Yaroslav D. Shansky**, Cand. Sci. (Biol.)
https://orcid.org/0000-0003-4672-2474
yar.shansky@rcpcm.org

**Kirill A. Prusakov**
https://orcid.org/0000-0002-7244-5741
kaprusakov@gmail.com

**Julia A. Bespyatykh**, Cand. Sci. (Biol.)
https://orcid.org/0000-0002-4408-503X
JuliaBes@rcpcm.org

**Dmitry V. Basmanov**
https://orcid.org/0000-0001-6620-7360
dmitry.basmanov@niifhm.ru