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# МЕДИЦИНА ЭКСТРЕМАЛЬНЫХ СИТУАЦИЙ

НАУЧНО-ПРАКТИЧЕСКИЙ РЕЦЕНЗИРУЕМЫЙ ЖУРНАЛ ФМБА РОССИИ




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Bugaev-Makarovskiy NA, Ershov PV ✉, Volkova AG, Makarova AS, Keskinov AA

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Malignant neoplasms occupy a leading place among non-communicable diseases based on the number of patients and mortality rate. There are several fundamental approaches to cancer therapy, however, none of them are universal or show a high level of clinical response. Furthermore, all the approaches are characterized by a large number of adverse side effects. Today, immunotherapy used alone or in combination with other therapies is considered to be the most promising. Immunotherapy is usually the use of specific antibodies (immune checkpoint inhibitors) or special bioproducts, such as dendritic cells and artificially synthesized peptides, such as neoantigens. The review considers strategies for development of the dendritic cell- and neoantigen-based anticancer vaccines, the possibilities of their improvement and the efficacy of combining with other anticancer drugs. The summary of current trials of the dendritic cell- and neoantigen-based vaccines is provided along with a brief analysis of the basic strategies, achievements and challenges faced by the developers of such vaccines.

**Keywords:** dendritic cells, dendritic cell vaccine, neoantigens, neoantigen vaccines, anticancer therapy, targeted therapy, cell technology

**Author contribution:** all authors made equal contributions to the review preparation, writing, and editing.

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# ПОДХОДЫ К РАЗРАБОТКЕ ДЕНДРИТНОКЛЕТОЧНЫХ И НЕОАНТИГЕННЫХ ПРОТИВООПУХОЛЕВЫХ ВАКЦИН

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Центр стратегического планирования и управления медико-биологическими рисками здоровью Федерального медико-биологического агентства, Москва, Россия

Среди неинфекционных заболеваний одной из ведущих патологий по количеству пациентов и показателям летальности являются злокачественные новообразования (ЗНО). Для терапии ЗНО есть несколько принципиальных подходов, однако ни один из них не является универсальным и не обладает высоким уровнем клинического ответа. Кроме того, для всех подходов характерно большое количество нежелательных побочных явлений. Наиболее перспективным в настоящее время считают применение иммунотерапии — как самостоятельный подход либо в комбинировании с другими видами терапии. Иммунотерапия обычно представляет собой использование специфичных антител (ингибиторов иммунных контрольных точек) либо применение специальных биопродуктов, таких как дендритные клетки (ДК) и искусственно синтезированные пептиды, например, неоантигены (НА). В обзоре рассмотрены стратегии разработки противоопухолевых вакцин на основе ДК и НА, возможности их усовершенствования и эффективность комбинирования с другими противоопухолевыми препаратами. Представлена также сводка актуальных в настоящее время клинических испытаний ДК- и НА-вакцин с кратким анализом базовых стратегий, достижений и трудностей, с которыми сталкиваются разработчики данного вида вакцин.

**Ключевые слова:** дендритные клетки, дендритноклеточная вакцина, неоантигены, неоантигенные вакцины, терапия онкологических заболеваний, таргетная терапия, клеточные технологии

**Вклад авторов:** все авторы внесли равный вклад в создание, написание и корректировку данной обзорной статьи.

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Cancer is still one of major non-communicable cause of death in the adult population. According to the World Health Organization, cancer occupies the leading position based on mortality rate among people aged under 70 in 112 countries of the world [1]. The most common malignant neoplasms (MNs) by detection rate include breast cancer (BRCA), non-small cell and small cell lung cancer (NSCLC and SCLC, respectively), colorectal cancer (CRC), gastric cancer (GC), liver cancer (LC), prostate cancer (PC), cervical cancer (CC), thyroid cancer (TC), and bladder cancer (BLCA). Melanoma, various types of primary central nervous system cancers (neuroblastoma and glioblastoma) and oncohematological diseases can be considered as the most aggressive MNs. MNs with the highest mortality rate include lung cancer, CRC, LC, GC, BRCA, PC, CC, as well as esophageal cancer, pancreatic cancer, and leukemia [1]. MNs are found in people of various age and gender, different nationalities and professions. The important role in carcinogenesis is played by the genetic predisposition factors, harmful habits (such as tobacco smoking), and environmental factors(such as harsh industrial environment) that significantly increases the risk of MNs [2]. That is why early detection of MNs in the groups with occupational risks, adequate choice and implementation of timely anticancer therapy is important.

The main treatment for solid MNs (stages I–III) is surgical resection of the tumor with adjuvant and/or neoadjuvant therapy [3]. The combination therapy is often used: surgical treatment combined with radiation and chemoradiation therapy [3], as well as the combination with immunotherapy, for example, therapy with immune checkpoint inhibitors (ICIs) [3, 4]. In particular, in 2022 the U.S. Food and Drug Administration (FDA) has approved seven ICIs for the programmed cell death protein 1 (PD-1)/programmed cell death 1 ligand 1(PD-L1) pathway: pembrolizumab, nivolumab, durvalumab, atezolizumab, avelumab, cemiplimab, dostarlimab [4].

The other cancer immunotherapy option is represented by the use of the so-called dendritic cell vaccines (DC-vaccines) [5, 6]. It is believed that clinical efficacy of DC-vaccines is associated with targeting the populations of immunosuppressive

cells in the tumor microenvironment and subsequent immunogenic tumor cell death induction [7].

DCs are involved in antigen presentation, immune response regulation, inhibition of immunosuppressive T cells. DCs also can sensitize other effector cells of the innate antitumor immunity [5, 6]. Several DCs subpopulations are distinguished based on the origin and antigen receptors: myeloid DCs, lymphoid DCs, plasmacytoid DCs, Langerhans DCs, and monocyte-derived DCs [5, 6]. As a link of antitumor immune response, DCs are involved in recognition and presentation of the neoantigens, emerging de novo in the tumor cells, to the immunocompetent cells [5, 6]. It is rational to use this ability of the DCs loaded with tumor antigens ex vivo for further activation of the $CD4^+$ helper and $CD8^+$ cytotoxic T cells in order to determine the directions of the immune responses [8]. Today, only PROVENGE, the autologous cellular product, consisting of the antigen-presenting cells activated by the PA2024 recombinant chimeric protein, has been approved by FDA for treatment of PC based on the phase III clinical trial results () (NCT00779402).

Since the tumor neoantigens (NAs) stimulate specific antitumor immune response in the patient's body, the new personalized therapeutic approaches in the field of neoantigen vaccines (NA-vaccines) creation have been developed in recent years [9]. Neoantigens are highly specific for tumor cells. They can be divided into common ones, which are produced by the mutations in oncogenes and personalized ones (unique for the tumor found in a certain patient [10]. At least, two NA-based immunotherapy approaches are under activey development: peptide and RNA vaccines. Thus, peptide vaccines may contain the mixtures of synthetic peptides with adjuvants or the DCs loaded with peptides [11, 12].

The limitations of DC-vaccines are associated with time- and resource-consuming process of vaccine preparation. Sometimes this is the reason why the disease progression occurs, which reduces the clinical benefit of therapy. Furthermore, some patients might not survive to the end of the therapy course [5, 13]. The high cost of biological stimulators that are critical for correct DC differentiation and loading of DCs with antigens also prevents the timely production of vaccines and their introduction into clinical practice [5, 9, 13]. It is also pertinent to note that, despite the facts of achieving pathomorphological responses of tumors and stabilization of disease while administrating DC-vaccines, together with favourable pharmacological safety data, there is an objective problem of increasing the vaccine efficacy. This can be solved through various modifications of the existing vaccine compositions and combinations with other anticancer drugs [5, 6].

The aim of the review was to systematize the literature data in the approaches to the development of the DC- and NA-vaccines as candidate anticancer drugs in terms of optimizing methodological and some technological aspects of the drug development in order to overcome the abovementioned problem. The review also reports the features of interaction between the DC vaccines and human immune cells and the most advanced developments based on the data of preclinical and clinical trials (PCTs and CTs, respectively).

## Clinical trials of the DC- and NA-vaccines for treatment of MNs

As of December 2022, a total of 410 and 96 records of the clinical trials (CTs) of the DC- and NA-vaccines, respectively, were found in the ClinicalTrials database [14]. Among all CTs focused on DC-vaccines, 191 CTs (46.58%) were completed, 45 CTs (10.97%) were terminated, 24 CTs (5.85%) were withdrawn (suspended). Among a hundred of active CTs, 32 CTs (7.80%) were assigned the status "active, not recruiting", 57 CTs (13.90%) had the "recruiting" status, and 11 CTs (2.68%) had the "not recruiting" status. The status of another 50 CTs (12.20%) was "unknown".

Among the successfully completed CTs of anticancer DC-vaccines, a total of 29 CTs (86% — phase II, 14% — phase III). were analyzed Table 1 provides basic information about the CTs conducted (title, phase, status, disorder, group of patients, DC-vaccine dosing regimen, drug in combinations, etc.). The CTs focused on clinical assessment of safety, tolerability efficacy of the DC-vaccines used in treatment of various cancer types have been distributed as follows. The group of malignant neoplasms (stage III) includes two CTs of DC-vaccines only for treatment of PC. The other two CTs are focused on DC-vaccines in combination with dasatinib for treatment of metastatic melanoma (stage III) or glioma in individuals receiving temozolomide (TMZ). The group of MNs (stage II) includes ten CTs of DC-vaccines used alone and 15 CTs of DC-vaccines used in combination with other pharmacotherapeutics, most often combinations with interleukin 2 (IL2), TMZ or interferon-α (IFNα). Other MNs are distributed as follows: glioma (five CTs), melanoma (three CTs), sarcoma (three CTs), prostate cancer (three CTs), ovarian cancer (two CTs) and breast cancer (two CTs). It must be acknowledged that the vast majority of clinical trials are focused on assessing the combination of DC-vaccines and ICIs. Information about the active CTs phases II and III is provided in Tables 2 and 3, respectively.

The number of CTs registered in the ClinicalTrials database and devoted to and NA-vaccines was about four times lower than that of the DC-vaccines. Among 96 CTs, 11 CTs were completed, eight were terminated, three were suspended; there were 60 active CTs and 14 CTs with unknown status. By analogy with DC-vaccines, clinical assessment of NA-vaccines involved mostly individuals receiving ICIs, and the spectrum of MNs targeted by CTs was almost the same. The safety and anticancer efficacy of the NA-vaccine in individuals receiving pembrolizumab and nivolumab were confirmed in NCT03633110 (phase II) only. Among eight terminated CTs, three were terminated due to long development time, and the other five were terminated due to underinvestment.

Analysis of DC-vaccines CTs (phase I and II) details has helped reveal a number of issues in this field. First, a small number of individuals (usually not exceeding 20) enrolled is the main factor of the CTs' termination. Second, complications with interpretation data obtained on different anticancer treatment regimens in the same CT. Third, specific design of the CT that includes a single cohort of patients or the CT without randomization. Despite the fact of achieving the endpoints of safety and tolerability of the anticancer vaccine, a common trend of moderate efficacy of the DC-and NA-vaccines administrated alone should be noted. It defines the relevance of their combination with other pharmacotherapeutics. However, there are exceptions. For example, the DC-vaccine for intratumoral administration obtained in the presence of IFNα and granulocyte macrophage colony-stimulating factor (GM-CSF) showed high immune responses even in the absence of tumor-associated antigen. It ensured complete regression of follicular lymphoma in some individuals who received low doses of rituximab [15]. It is important to note that the combinations of DC-vaccines with targeted or immunotherapy drugs showed higher efficacy than the DC-vaccines administrated alone. The objective response rate (ORR) reached 50%, and the difference in progression-free survival (PFS) and/or overall survival (OS)

**Table 1.** The main results of the completed clinical trials of DC-vaccines

| Clinical trial (CT) title | Phase | Disorder | Number of groups | Dosing regimen | Drugs in combination | CT results | CT ID in ClinicalTrials.gov |
|---|---|---|---|---|---|---|---|
| Vaccine therapy in treating patients with metastatic prostate cancer that has not responded to hormone therapy | III | Prostate cancer | 2 | 127 subjects. Experimental group: 3 infusions of Sipuleucel-T with an interval of two weeks. Control group: DC-vaccine, no PA2024 activation | No | Median OS in the experimental group was 25.9 months vs 21.4 in the placebo group. The 8-fold increase in the stimulated T cell counts relative to the controls was achieved in response to the DC vaccine (16.9 vs 1.99; $p < 0.001$) | NCT00005947 |
| Provenge treatment and early cancer treatment (PROTECT) | III | Prostate cancer | 2 | 176 subjects. Experimental group: 3 infusions of Sipuleucel-T with an interval of two weeks. Control group: DC-vaccine, no PA2024 activation | No | No differences in quality of life between the experimental and control groups were revealed. The 50th percentile of the PSA levels exceeding 3 ng/mL was 15 vs 12 months in the experimental and control groups | NCT00779402 |
| Dendritic cell vaccines + dasatinib for metastatic melanoma | III | Metastatic melanoma | 2 | 15 subjects. Intradermal injections of the drug (dose of $1 \times 10^7$ cells) in the vicinity of the lymph nodes on days 1 and 15 of the cycle. Cohort A — DC preparation + dasatinib (starting on day 1 of the cycle), cohort B — DC preparation + dasatinib (starting on day 1 of the second cycle — after 5 weeks) | Dasatinib | Among 13 CT participants, specific response of the T cells to the vaccine administration was achieved in 6. Partial response was achieved in 4 cases, and the disease stabilization in two cases. The other 7 participants did not respond to vaccination (disease progression). Cohort A vs cohort B: ORR 66.7% vs.28.6%, OS 15.45 vs 3.47 months and progression-free survival (PFS) 7.87 vs 1.97 months | NCT01876212 |
| Study of a drug [DCVax®-L] to treat newly diagnosed GBM brain cancer (GBM) | III | Glioma | 2 | Control group (temozolomide + intradermal injections of DCVax-L). Experimental group (temozolomide + autologous PBMC (placebo). Injections (on weeks 0, 10, 20, 8, 16, 32, 48, 72, 96, and 120) | Temozolomide | The safety of use has been confirmed. The differences in the patients' survival between groups have not yet been revealed | NCT00045968 |
| A study of ICT-107 immunotherapy in glioblastoma multiforme (GBM) | II–III | Glioma | 2 | 124 subjects: 18–80 years. Group 1 (81) — therapy with autologous DCs, group 2 (43) — placebo | No | Median OS: DC-vaccine — 18 months, placebo — 16.7 months. Median PFS: DC-vaccine — 11.2 months, placebo — 9 months | NCT01280552 |
| Dendritic cell vaccine study (DC/PC3) for prostate cancer | II | Prostate cancer | 1 | 13 subjects. Subcutaneous injection of the DC-vaccine alone | No | Increased T cell proliferation in response to the DC-vaccine administration | NCT00345293 |
| Vaccine therapy in treating patients with stage I, stage II, or stage III non-small cell lung cancer | II | NSCLC | 1 | 32 subjects. Patients with histologically verified stage I-IIIB NSCLC. 16 intradermal injections, once a month | No | Assessment of immunogenicity: antigen-specific response to DC-vaccine is reported in 40%, non-specific response is reported in 40% | NCT00103116 |
| Ovarian cancer vaccine for patients in remission | II | Ovarian cancer | 3 | 63 subjects. 6–8 intradermal injections (forearm and thigh) (dose of $60 \times 10^6$ cells). Groups: control, randomization, no randomization | No | PFS 13 vs. 5 months and OS 42 vs 26 months in the cohorts DC-vaccine vs control, respectively | NCT01068509 |
| Safety and effectiveness of a vaccine for prostate cancer that uses each patients' own immune cell | II | Prostate cancer | 2 | 24 subjects. Subcutaneous injection of the vaccine. Cohort 1: placebo for 8 weeks, then DCs for more than 8 weeks. Cohort 2: DCs for more than 8 weeks | No | The DC-vaccine production method affected the efficiency of the T cell activation in response to the DC-vaccine administration | NCT00289341 |
| Vaccine therapy in treating patients with liver or lung metastases from colorectal cancer | II | CRC | 2 | 13 subjects. Cohort 1: Intradermal or subcutaneous injection of the DC-vaccine. Cohort 2: DC-vaccine + GM-CSF | No | There were little differences in the 2-year PFS between the cohorts (47% and 55%). There were no significant differences in the rate and intensity of the T cell immune responses between the cohorts | NCT00103142 |
| Ovarian cancer vaccine for patients who have progressed during the CAN-003 study (CAN-003X) | II | Ovarian cancer | 1 | 9 subjects. 3 doses of DCs were administered during 4 weeks, the other 3 doses during the subsequent 12 weeks, the remaining 6 doses during the subsequent 44 weeks | No | No data on efficacy available | NCT01617629 |
| Vaccine for patients with newly diagnosed or recurrent low-grade glioma | II | Glioma | 1 | 5 subjects. Administration of the drug on days 0, 14, 28 | No | No data on efficacy available | NCT01635283 |
| Therapy to treat Ewing's sarcoma, rhabdomyosarcoma or neuroblastoma | II | Sarcoma | 2 | 44 subjects. Cohort A — baseline: administration of the CD25 and 8H9 depleted autologous lymphocytes + DC vaccine. Cohort B — baseline + recombinant IL7 (administration on days 0, 14, 28, 42) | No | The immune responses associated with the use of IL7 were reported in 57% of patients. The median OS was 2.4 and 4.3 months in the cohorts A and B, respectively | NCT00923351 |
| A phase II feasibility study of adjuvant intra-nodal autologous dendritic cell vaccination for newly diagnosed glioblastoma multiforme | II | Glioma | 1 | 11 subjects. Three doses of the vaccine were injected into the neck lymph node with an interval of two weeks | Temozolomide, radiation therapy | The $CD4^+$ cell activation was correlated to the patients' survival rate. The median PFS was 9.5 (5–41) months | NCT00323115 |
| A pilot study of autologous t-cell transplantation with vaccine driven expansion of anti-tumor effectors after cytoreductive therapy in metastatic pediatric sarcomas | II | Sarcoma | 1 | 42 subjects. Intramuscular injections of the DC-vaccine in a dose of $1 \times 10^6$ cells every 6 weeks | Indinavir (oral), infusions of IL2, IL7 | The T cell responses were 60%, and the overall survival was two times higher in individuals who received DCs (73% vs 37%) | NCT00001566 |
| DC vaccine combined with IL-2 and IFNα-2a in treating patients with mRCC | II | Metastatic kidney cancer | 1 | 18 subjects. Induction therapy: Injections of the DC-vaccine into the lymph nodes — days 0 and 14 along with the IL2 (days 1–5 and 15–19) and interferon alpha (days 1, 3, 5, 15, 17, and 19) therapy. Adjuvant therapy: DC-vaccine (days 42, 70, and 98); IL2 — days 43–47, 71–75, and 99–103; IFNα (days 43, 45, 47, 71, 73, 75, 99, 101, and 103) | IL2, interferon alpha | Among 18 patients, the overall response was 50% with three complete responses. The counts of the circulating $CD4^+$ regulatory T cells were strongly correlated to the outcomes | NCT00085436 |
| Vaccine therapy, tretinoin, and cyclophosphamide in treating patients with metastatic lung cancer | II | Lung cancer | 1 | 24 subjects. Triple intradermal injection of the DC-vaccine every 14 days, the other three doses were injected once a month | Cyclophosphamide, tretinoin | The median OS was 8 months. The median PFS was 1.7 months. Among 14 patients, activation of the $CD8^+$ T cells associated with vaccination was achieved in 5 patients | NCT00601796 |
| Vaccine therapy plus interleukin-2 in treating patients with stage III or stage IV melanoma | II | Melanoma | 2 | 40 subjects. Cohort 1: DC-vaccine. Cohort 2: peptides injected in the form of emulsion with GM-CSF and the Montanide ISA-51 adjuvant. | IL2 | In the cohort 1 the T cell immune responses were reported in 11–13%, while in the cohort 2 these were reported in 42–80%. ORR was observed in 10% of patients in the cohorts | NCT00003222 |

**Table 1.** Продолжение

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| External beam radiation with intratumoral injection of dendritic cells as neo-adjuvant treatment for sarcoma | II | Sarcoma | 1 | 17 subjects. Intratumoral injections of three doses of the DC-vaccine ($10^7$ cells) during the course of radiation therapy. | Radiation therapy 50 Gy, 25 sessions | Survival of 67% of patients without systemic relapses within 2–8 years. In some cases, the immune response to the DC-vaccine administration was correlated to the clinical response | NCT00365872 |
| Vaccine therapy, trastuzumab, and vinorelbine in treating patients with locally recurrent or metastatic breast cancer | II | BRCA | 1 | 17 subjects. DCs + GM-CSF | Vinorelbine, trastuzumab | The increase in the share of the cytokine-producing $CD8^+$ cells by 36% | NCT00266110 |
| Dendritic cell (DC)-based vaccines loaded with allogeneic prostate cell lines in combination with androgen ablation in patients with prostate cancer | II | PC | 2 | Cohort A. 3 months – androgen blockade, then 3 months — combination of androgen blockade + DC-vaccine. Cohort B: 3 months — combination of androgen blockade + DC-vaccine, then 3 months — androgen blockade | Androgen blockade | No data on efficacy available | NCT00970203 |
| Dendritic cell/myeloma fusion vaccine for multiple myeloma | II | Multiple myeloma | 3 | 203 subjects. Subcutaneous injection of the DC-vaccine ($3 \times 10^6$ cells) in the upper third of the thigh on day 1 of each of 4 cycles of adjuvant therapy with lenalidomide | Lenalidomide, GM-CSF, melphalan | In the cohort with the корорте DC-vaccine + lenalidomide + GM-CSF (68 patients): 16% — complete response, 54% — partial response | NCT02728102 |
| DC migration study for newly-diagnosed GBM (ELEVATE) | II | Glioma | 3 | 64 subjects. Treatment course: 10 doses of the activated DC-vaccine ($2 \times 10^7$ cells) were injected intradermally in the inguinal area | Temozolomide, basiliximab | The increase in the patients' median OS 16.5 vs 23.8 months, DC-vaccine with adjuvant (diphtheria toxoid) vs. DC-vaccine with no adjuvant. There were no significant changes in the PFS | NCT02366728 |
| Study of gene modified immune cells in patients with advanced melanoma (F5) | II | Metastatic melanoma | 1 | 14 subjects. After the chemotherapy course the patients received intradermal injections of $1 \times 10^9$ transgenic cytolytic T cells and $1 \times 10^7$ DCs, as well as IL-2 500,000 IU/$m^2$ twice a day for 14 days | IL2 | No data on efficacy available | NCT00910650 |
| A vaccine (CDX-1401) with or without a biologic drug (CDX-301) for the treatment of patients with stage IIB-IV melanoma | II | Melanoma | 2 | 60 subjects. Experimental group: (CDX-301, CDX-1401, poly-ICLC). Control group: (CDX-1401, poly-ICLC) | Poly-ICLC, Flt3L, cytokine | In the experimental group stimulation of the immune response was reported in 53% of patients, while in the control group in was reported in 38% of patients. There were no significant changes in the time of recurrence (range 360–390 days) | NCT02129075 |
| Vaccine therapy and 1-MT in treating patients with metastatic breast cancer | I–II | Metastatic BRCA | 1 | 44 subjects. Intradermal injection of 6 doses of Ad.p53-DC on weeks 1, 3, 5 and 10, then every 3 weeks | 1-methyl-D-tryptophan | Among 21 patients receiving the DC-vaccine, 1 complete response, 7 partial responses, and 2 cases of the disease stabilization were reported | NCT01042535 |
| αDC1 vaccine + chemokine modulatory regimen (CKM) as adjuvant treatment of peritoneal surface malignancies | I–II | Mesothelioma | 1 | 64 subjects. The DC-vaccine was injected in the lymph node once during the cycle in a dose of $3 \times 10^6$ cells + intradermal injection of the same dose. | Celecoxib, INFa-2b, rintatolimod | Average time to progression — 16 months, OS — 52 months. The treatment-associated chemokine production was reported | NCT02151448 |
| Vaccination-dendritic cells with peptides for recurrent malignant gliomas | I–II | Glioma | 1 | 22 subjects. DC-vaccine treatment regimen: initial injection in the lymph nodes (week 1), booster phase 1 (week 13) + poly-ICLC, booster phase 2 (week 33) + poly-ICLC. | Poly-ICLC | OS: dose of DCs ($1 \times 10^7$ cells) + Poly-ICLC — (33 CI 14–37 months). Dose of DCs ($3 \times 10^7$ cells) + Poly-ICLC — (13 CI 6–37 months) | NCT00766753 |

was up to 100% depending on the treatment regimen. Thus, DC-vaccines in combinations with other therapy may have a more prominent anticancer effect ensuring higher OS.

The other trend found is — DC- and NA- vaccines are considered as a "last choice therapy" option. It may be the cause of their low efficacy in the CTs in a group of individuals with late-stage cancers. Alternatively, stimulation of the tumor-infiltrating immune cells and local immune responses has all the chance to demonstrate much better efficacy for treatment of early-stage cancers, when it is necessary to prevent metastasis.

## Optimization of some manufacture and application steps of biotherapeutic anticancer vaccines

*Options of accelerating, simplifying and cost-reducing of the DC-vaccines manufacturing*

*1. Options for accelerating the DC-vaccines manufacture process*

The use of nucleic acids to load the dendritic cells is the first approach to accelerating the DC-vaccine manufacture [9]. Synthesis of nucleic acids is a less time-consuming process than the synthesis of target peptides. Similarly, the nucleic acid purification procedure is less time-consuming than purification of the peptides or polypeptides. Nucleic acids, that are more stable than peptides, are adjuvants that can activate pro-inflammatory molecular pathways involving the Toll-like receptors (TLR) associated with activation of innate immunity [16].

The second approach involves modification of cultivating conditions of manufacturing cell strains. For example, the transfer of murine bone marrow progenitor cells into monolayers of murine OP9 stromal cells expressing the delta-like Notch 1 ligand (OP9-DL1) after three days of incubation with the FMS-like tyrosine kinase 3 ligand (FLT3L) led to the fact that the cells expressed the murine markers (CD103, CD24, DEC205 and CD8α) of myeloid DCs, the population that did not arise after incubation with FLT3L only. The transcriptional gene expression profile of such DCs was most similar to that of autologous DCs of the spleen. Meanwhile, the survival rate of laboratory animals increased, which could be due to enhanced lymphocyte migration to the tumor lesions [6]. The co-culture of human hematopoietic stem and progenitor cells and OP9-DL1 enabled a 20-fold increase in the yield of DCs of all types relative to conventional cell culture methods [17].

The third approach involves stimulation of the cell culture with various cytokines, such as GM-CSF [17, 18]. The transcriptional profiles of the DCs obtained were almost identical to that of primary DCs, while the cells themselves demonstrated normal cytokine responses to TLR agonists, including secretion of IL12, TNFα and IFNγ, and effectively induced the $CD4^+$ and $CD8^+$ T cell proliferation [17, 18].

The fourth approach was implemented by using the genetic editing technologies. Thus, viral transduction [19] and RNA interference methods [20] together with the CRISPR/CRISPR-Cas9 genome editing system [21] were used to generate the DC-vaccines. Pre-clinical trials showed that all methods were highly effective and could presumably be scaled to the DC-vaccines manufacture.

Another reported vector-free approach for acceleration of the DC-vaccine preparation is based on the Cell Squeeze® technology which involves forcing the target molecules through

**Table 2.** Open-label clinical trials of the DC- and NA-vaccine efficacy (active, not recruiting)

| Vaccine title | Vaccine composition | Phase | Disorder | Patient recruitment | Vaccine dosing regimen | Drugs in combination | CT ID in ClinicalTrials.gov |
|---|---|---|---|---|---|---|---|
| no | DCs + RNA | III | Uveal melanoma | 200 individuals, (18–75), M and F | Group A — 8 vaccine doses within 2 years, group B — control | no | NCT01983748 |
| ADCTA-SSI-G1 | DCs + tumor cells | III | Glioblastoma multiforme | 118 individuals (18–70), M and F | 10 doses: 2–4 × $10^7$ cells for the first dose (double dose) and 1–2 × $10^7$ cells for the doses 2–10, 3 vaccines twice a week | no | NCT04277221 |
| DEN-STEM | DCs + mRNA of cancer stem cells, surviving or hTERT | III | Glioblastoma | 60 individuals, (18–70), M and F | Intradermal injection of DCs, up to 6 cycles of temozolomide after 4 weeks | Adjuvant temozolomide | NCT03548571 |
| GIMI-IRB-19006 | DCs | II | Solid cancer types | 100 individuals, (18–80), M and F | No details available | no | NCT04085159 |
| CCRG12-001 | DCs | II | Acute myeloid leukemia | 130 individuals, (18+), M and F | Vaccination with DCs, combining with chemotherapy is possible (if earlier prescribed) | no | NCT01686334 |
| no | DCs | II | Acute myeloid leukemia | 75 individuals, (18+), M and F | No details available | no | NCT03059485 |
| ADCV01 | DCs | II | Glioblastoma | 24 individuals, (20–75), M and F | A total of 10 doses (1 mL/dose; 2 ± 0.5 × $10^7$ cells/dose) of ADCV01 will be administered to patients in the experimental group. ADCV01 will be injected in the axillary subcutaneous regional lymph nodes on both sides (half of the volume about 0.5 mL ADCV01) once a week for the first 4 doses; the next 2 procedures will be performed every two weeks. The last 4 procedures will be performed every 4 weeks | no | NCT04115761 |
| no | DCs with tumor lysate (with a concentration of 1x106 cells)/ or WT1 and MUC1 proteins (for patients with certain HLA type (HLA-A2)) + immature DCs (as a load with the carrier protein - keyhole limpet hemocyanin (KLH)) | II | Ovarian cancer | 36 individuals, (18+), F | Three injections in the inguinal area with an interval of two weeks (6 weeks) | no | NCT00703105 |
| DENDR1 | DCs + tumor lysate | II | Glioblastoma | 76 individuals, (18–70), M and F | 4 vaccines every second week (vaccines I, II, III, IV), another 2 vaccines monthly (vaccines V, VI) and the last vaccine (vaccines VII) 2 months after the sixth one. Injections I, V, VI and VII will deliver 10 million DCs + tumor lysate, while the other injections will deliver 5 million cells only | no | NCT04801147 |
| IRST153.04 | DCs + tumor homogenate | II | Metastatic CRC | 19 individuals, (19+), M and F | Each vaccine dose contains 1 × $10^7$ DCs + tumor homogenate. | no | NCT02919644 |
| IRST100.42 | DCs + tumor homogenate | II | Head and neck cancers, neuroendocrine tumors, soft tissue sarcoma | 51 individuals, (18+), M and F | 7–14 × $10^6$ DCs + tumor homogenate, delivered by intradermal injection (day 1) | no | NCT04166006 |
| HER2 DC1 | HER2-sensitized DCs | II | BRCA, HER2+ BRCA | 60 individuals, (18+), F | Ultrasound-guided intranodal injections, each dose containing 1.0–2.0 × $10^7$ cells will be injected in one left and one right inguinal lymph nodes | no | NCT03630809 |
| CSTI571ADE60 | DCs + peptides of bcr/abl, WT-1 + proteinase-3 | II | Chronic myeloid leukemia | 30 individuals, 18–80, M and F | Ten vaccinations within 26 with the use of the 10 × $10^6$ freshly thawed DCs, intradermal injections (1–2 mL) | no | NCT02543749 |
| IOR-IISML42037 | DCs | II | SCLC | 20 individuals, (18+), M and F | Intradermal injections (no more than 6 doses) on weeks 1, 3, 6, 9, 21, 33 | Atezolizumab, carboplatin | NCT04487756 |
| DC1 | DCs | II | BRCA (stages I–III), HER2+ BRCA | 110 individuals, (18+), F | Weekly intranodal injections between weeks 1 and 6 (the window between the vaccines 8–21). The booster vaccines will be administered with an interval of about 3 months on months 6, 9 and 12 (with an interval of +/– 1 month) | WOKVAC vaccine | NCT03384914 |
| MSDCV | DCs | II | Hepatocellular carcinoma | 600 individuals, (18–70), M and F | Once every 4 weeks during 0–20 weeks, about 5 × $10^7$ cells per dose, a total of 6 intravenous injections | Cyclophosphamide (Endoxan) | NCT04317248 |
| MC1685 | DCs | II | Lymphoma | 44 individuals, (18+), M and F | Therapy with DCs on days 2, 8 and 15 of the cycles 2 and 3, day 2 of the cycles 4 and 5 | Pembrolizumab, 13-valent pneumococcal conjugate vaccine | NCT03035331 |
| CA209-7R9 | DCs + NA | II | Hepatocellular carcinoma, CRC with liver metastasis | 60 individuals, (21+), M and F | 10 doses of vaccine will be administrated by intradermal route together with the nivolumab AT | Nivolumab (Opdivo) | NCT04912765 |
| IRST172.02 | DCs + tumor lysate/homogenate | II | Stages III–IV melanoma | 24 individuals, (18–70), M and F | Intradermal injections of the vaccine on weeks 1, 4, 6 and 8 during the induction phase and every four weeks during the maintenance phase, up to 14 vaccine doses (each dose is followed by administration of 3 MU of IL2 per day) | IFNα | NCT01973322 |
| CCRG13-002 | DCs + WT1 mRNA | II | Malignant pleural mesothelioma | 20 individuals, (18+), M and F | 4 intradermal injections of 8–10 × $10^6$ DCs + WT1 mRNA; on day 14 +/- 3 days after the start of each chemotherapy cycle | Platinum-based drugs/ pemetrexed | NCT02649829 |
| no | DCs + A2B5+ stem cells | II | Glioma, glioblastoma multiforme | 100 individuals, (18–70), M and F | 8–10 × $10^6$ DCs in 0.5 mL of phosphate buffer saline are administered by intradermal injection in the shoulder close to the posterior surface of the neck to facilitate the DC transfer into the neck lymph nodes | Temozolomide | NCT01567202 |
| MG-7-DC | DCs + MG-7 antigen | II | GC | 45 individuals, (18–80), M and F | Six intranodal injections of the DC vaccine will bedone on days 1, 8, 15, 21, 28, 35; 1–3 × $10^6$ cells | Sintilimab | NCT04567069 |
| CCRG14-001 | DCs + WT1 mRNA | II | Glioblastoma multiforme | 20 individuals, (18+), M and F | Weekly (+/- 1 day) injections of DCs + WT1 mRNA during 3 weeks | Temozolomide | NCT02649582 |
| GlioVax | DCs + tumor lysate | II | Glioblastoma | 136 individuals, (18+), M and F | Vaccination with DCs + tumor lysate (7x, 2–10 × $10^6$ DCs per intradermal injection, weekly on weeks 11–14, then on weeks 17, 21, 25) | Temozolomide | NCT03395587 |

**Table 2.** Продолжение

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| no | DCs + IL12 | II | Glioblastoma | 10 individuals, (18–75), M and F | Intradermal injection in the vicinity of the neck lymph node after surgery with subsequent radiation therapy (2 Gy/day for 30 days). | Temozolomide | NCT04388033 |
| pp65 DC | DCs + pp65-shLAMP mRNA + GM-CSF | II | Glioma, glioblastoma multiforme | 175 individuals, (18+), M and F | Intradermal injection on day 22–24 after the first course of temozolomide, then with an interval of 2 weeks. The doses 4–10 will be administered on day 22–24 of each cycle of temozolomide. Administration of the doses will be resumed until the total number reaches 10 or the disease progression/unacceptable toxicity is reported | Tetanus-diphtheria toxoid | NCT02465268 |
| PDC*lung01 | DCs + synthetic peptide (NY-ESO-1, MAGE-A3, MAGEA4, Multi-MAGE, SURVIVN, MUC1) or + peptide obtained from the Melan-A antigen | II | NSCLC | 64 individuals, (18+), M and F | In the cohorts A1 (low dose cohort) and A2 (high dose cohort), patients with NSCLC will be treated with low/high doses of PDC*lung01, administered by serial subcutaneous injections and then by intravenous route. In the cohorts B1 and B2, the first injection of PDC*lung01 will be started within 48 h after the first anti-PD-1 infusion. The fourth PDC*lung01 injection will be started within 48 h after the infusion of the second anti-PD-1 cycle | Alimta, Keytruda | NCT03970746 |
| no | Flt3L/CDX-301 + Poly-ICLC | II | Non-Hodgkin lymphoma, metastatic BRCA, squamous cell carcinoma of the head and neck | 56 individuals, (18+), M and F | Intravenous infusion of 200 mg of pembrolizumab (Keytruda) for 30 min, then DCs together with Flt3L | Keytruda, hiltonol | NCT03789097 |
| no | DCs + tumor lysate | II | Pediatric glioblastoma | 25 individuals, (3–21), M and F | 4 weekly intradermal injections of DCs + tumor lysate, with 3 subsequent monthly booster vaccines containing the tumor lysate and additional booster vaccines every three months | Cyclophosphamide (Endoxan), nivolumab, ipilimumab | NCT03879512 |
| Pro00082570 | DCs + CMV pp65-LAMP mRNA | II | Glioblastoma | 112 individuals, (18+), M and F | $2 \times 10^7$ DCs are administered by intradermal route in the inguinal area on both sides (the dose is split evenly between two sides of the inguinal region). The patients will receive a total of up to 10 doses of the DC-vaccine | Temozolomide, tetanus-diphtheria toxoid, varlilumab | NCT03688178 |
| no | DCs + WT1 mRNA | II | High grade glioma, diffuse intrinsic pontine glioma | 10 individuals, (1–17), M and F | 1) Induction immunotherapy: intradermal injection of DCs + WT1 mRNA, weekly (–1 day, +2 days) during 3 weeks, starting from week ≥ 1 after radiation therapy. 2) Induction immunotherapy: intradermal injection of DCs + WT1 mRNA, weekly (–1 day, +2 days) during 3 weeks, starting from week ≥ 4 after apheresis | Temozolomide | NCT04911621 |
| no | DCs +GSC-DCV | II | Glioblastoma | 40 individuals, (18–70), M and F | Every 3 weeks if there is no disease progression or unacceptable toxicity | Camrelizumab | NCT04888611 |
| GCO 13-1347 | Flt3L+Poly-ICLC | II | Low-grade B-cell lymphoma | 21 individuals (18+), M and F | Intratumor injections on days 1–5 and 8–11. Weekly intratumor injections of Poly-ICLC on weeks 2–8 | Hiltonol | NCT01976585 |

the membrane pores emerging due to temporary membrane integrity disruption [22]. It has been shown that this DC loading technique can be used *ex vivo* and it is suitable for transfer of various antigens to cytosol [23].

*2. Options for reducing the cost of the DC-vaccines manufacture process*

Among all available options for reducing the cost of DC-vaccines there are exosome preparations obtained from DCs (DEXs). DEXs are considered as more technologically feasible and less expensive compared to conventional DC-vaccine preparation. Both *in vitro* and *in vivo* studies have shown that DEXs can activate the $CD4^+$ and $CD8^+$ T cells and stimulate the effective antigen-specific responses of cytotoxic lymphocytes. However, the desired anticancer efficacy has not been achieved in several CTs, putting into question the prospects of DEXs application [24]. The DCs pretreatment with interferon — (IFNγ) resulting in the increased expression of *CD40*, *CD80*, *CD86* and *CD54* is an option to increase the DEX efficacy. However, this approach, well proven in PCTs [25], was less effective in the CT (phase II) [26].

*3. Options for simplifying the DC-vaccines manufacture process*

Preparation of the DC-vaccines based on primary DCs extracted from the patient's peripheral blood is much simpler than ex vivo DC preparation, with such limitation as the low DC content (less than 1%) in the monocyte fraction [27]. Low circulating DCs counts have been revealed in blood samples of patients with melanoma [28] and breast cancer [29], while abnormal DC differentiation is reported in the breast cancer and pancreatic cancer models [30]. Therefore, the effectiveness of DCs isolation from the peripheral blood of patients with these tumor types was minimal. Since the successful implementation of this approach has yet been demonstrated only *in vivo* in the murine model with xenotransplantation of B16/F10 and B16-Flt3L cells (melanoma) as well asMC38 cells (CRC) [31], the prospects of preparation the DC-vaccines (DCs type I) against the majority of tumors seem to be hardly feasible.

## Options of the anticancer vaccines application in combination therapy

*Growth factors*

The combinations of DC-vaccines and growth factors are designed to enhance the antigen-specific response. GM-CSF is most often used in combinations with DC-vaccines because it functions as a hematopoietic growth factor and immunomodulator. GM-CSF was also used as a low-toxic adjuvant during treatment with the DC- or NA-vaccines containing peptides [32]. Another approach based on the use of DC-vaccines and FLT3L has been reported. Thus, a significant increase in the generation of autologous DCs, including plasmacytoid DCs, has been revealed in the murine models in the presence of FLT3L. It is assumed that the increase in the mature DCs functional activity in the presence of FLT3L is mediated through the signaling pathways involving phosphoinositide 3-kinase (PI3K) and mTOR kinase [33].

*ICIs*

The combinations of ICIs and DC-vaccines lead to activation of T cells and NK cells, reduced immunosuppressive activity

**Table 3.** Open-label clinical trials of the DC-vaccine efficacy (recruiting)

| Vaccine name | Vaccine composition | CT phase | Disorder | Patients | Vaccine dosing regimen | Drugs in combination | CT ID in ClinicalTrials.gov |
|---|---|---|---|---|---|---|---|
| NL55823.000.15 | DCs + NA | III | Melanoma | 210 individuals, (18+), M and F | No more than 3 cycles, 3 intranodal DC injections ($3–8 \times 10^6$) per cycle. | no | NCT02993315 |
| DCP-001 | DCs | II | Acute myeloid leukemia | 20 individuals, (18+), M and F | Low dose — patients receiving 4 2-week $25 \times 10^6$ cells vaccines/vaccination with DCP-001 and 2 revaccinations with $10 \times 10^6$ cells/vaccination, High dose — patients receiving 4 2-week $50 \times 10^6$ cell vaccines/vaccination with DCP-001 and 2 revaccinations with $10 \times 10^6$ cells/vaccination | no | NCT03697707 |
| no | DCs | II | Acute myeloid leukemia | 63 individuals, (18+), M and F | 2–3 vaccine doses with an interval of 4 weeks | no | NCT01096602 |
| DC-005 | DCs + mRNA of tumor cells, survivin or hTERT | II | Prostate cancer | 30 individuals, (18–75), M | No details available | no | NCT01197625 |
| no | DCs + TARP peptide | II | Prostate cancer | 40 individuals in 2015 (actually 14 in 2020), (18+), M | $20 \times 10^6$ of viable cells/dose were administered intradermally on weeks 3, 6, 9, 12, 15 and 24 | no | NCT02362464 |
| no | DCs + total tumor RNA (ttRNA) | II | Medulloblastoma | 26 individuals, under 30 (children and adults), M and F | Intradermal injection of $1 \times 10^7$ cells every 2 weeks, a total of 3 doses | no | NCT01326104 |
| AV-GBM-1 | DCs + tumor-associated antigens (AV-GBM-1) | II | Glioblastoma | 55 individuals, (18–70), M and F | No details available | no | NCT03400917 |
| no | DCs + GM-CSF | II | Kidney cancer | 38 individuals, (18+), M and F | 3 vaccines with an interval of 3 weeks | no | NCT00458536 |
| no | DCs + NA | II | CRC | 25 individuals, (18–75), M and F | No details available | no | NCT01885702 |
| TLPLDC | DCs + yeast cell wall particles + tumor lysate | II | Melanoma | 184 individuals, (18–99), M and F | 6 flasks containing a single dose for intradermal injection x 3 every months with further booster injections after 6, 12 and 18 months in the same area of the lymph node drainage (preferably in the anterior thigh) | no | NCT02301611 |
| no | DCs + WT1 mRNA | II | Acute myeloid leukemia | 5 individuals, (18–70), M and F | 4 doses, once every 2 weeks | no | NCT03083054 |
| no | DCs + GM-CSF | II | Ovarian cancer, primary peritoneal cancer, fallopian tube cancer | 23 individuals, (18+), Ж | Subcutaneous injection once every 3 weeks | Imiquimod | NCT00799110 |
| no | DCs + NY-ESO-1 protein | II | MNs without clarification | 6 individuals, (16+), M and F | The patients can receive 3 additional doses of the peptide vaccine based on the NY-ESO-1 dendritic cells (157–165) after day 90 of therapy | Fludarabine phosphate, cyclophosphamide | NCT01697527 |
| no | DCs + tumor lysate | II | Gliomas, glioblastoma | 60 individuals, (18–70), M and F | Intradermal injection of DC-vaccine and tumor lysate (in all patients). Cohort 1 — optional application of the placebo cream, the vaccine is supplemented by saline, cohort 2 — the vaccine is supplemented by resiquimod, cohort 3 — the vaccine is supplemented by hiltonol | Resiquimod, hiltonol | NCT01204684 |
| Ad.p53-DC | DCs + p53 | II | SCLC | 14 individuals, (18+), M and F | 4 cycles of 21 days: the individuals will receive a p53-vaccine on days 1 and 15 of cycle 1, then once again on day 8 of cycle 2. Adjuvant immunotherapy started on day 1 of cycle 5: three additional doses of the p53-vaccine (every 4 weeks during 12 weeks) | Nivolumab, ipilimumab | NCT03406715 |
| no | DCs + CT-011 | II | Multiple myeloma | 35 individuals, (18+), M and F | The DC vaccination is performed 1–3 months after the autologous transplantation. Vaccination is performed with an interval of 6 weeks | CT-011 | NCT01067287 |
| no | DCs + cytokines | II | Breast cancer | 400 individuals, (18–75), M | 4 cycles of the DC-CIK treatment (annually) | Capecitabine | NCT02491697 |
| no | Exact formulation is not available | II | Prostate cancer | 19 individuals, (18+), M | Intradermal injection 6 times every 2 weeks, then 9 times every 4 weeks | Nivolumab | NCT03600350 |
| BVAC-C | Autologous B cells and monocytes trasfected with the HPV gene E6E7 | II | Cervical MNs | 32 individuals, (20+), F | Intravenous injections of BVAC-C on weeks 0, 4, 8, then on weeks 0, 4, 8, 12. After that in combination with topotecan on weeks 0, 4, 8, 12 | Topotecan | NCT02866006 |
| no | DCs + IL2 | II | Melanoma | 1230 individuals, (12+), M and F | $1 \times 10^7$ to 2.5x108 DCs with the MART-1 peptide administered intravenously for 20–30 min, about 4 h after the T cell administration | Fludarabine phosphate, cyclophosphamide, IL2 | NCT00338377 |
| no | DCs + tumor proteins | II | Melanoma (stage III–IV) | 7 individuals, (18+), M and F | The patients are administered mature DCs on day 1 or 2 of the course 2 or 3 after the low temperature exposure | Pembrolizumab | NCT03325101 |
| no | DCs + NY-ESO-1 and Melan-A/MART-1 peptides | II | Melanoma | 36 individuals, (18+), M and F | Intradermal administration of 100 μg/L of the peptide (NY-ESO-1 and Melan-A/MART-1) + 10 to $15 \times 10^6$ DCs per pertide antigen (NY-ESO-1 and Melan-A/MART-1) (no more than $50 \times 10^6$ cells in total) | Hiltonol, montanide | NCT02334735 |

of regulatory T cells [5, 34], and therefore to the increase in the DC- vaccine efficacy. In turn, the DC-mediated activation of NK cells and DC γδ T cells [35, 36] can increase the efficacy of ICIs. Synergistic antitumor effect of the combination of nivolumab and DC-vaccine was revealed in individuals with BRCA, myeloma, melanoma, lung cancer, lymphoma and glioblastoma [37]. In addition, the DC-vaccine was proven to be safe for patients; low number of side effects related to the use of nivolumab was reported [37].

*NK cells*

One more promising approach involves the combination of anti-cancer DC-vaccines and NK cell-based vaccines. NK cells present in the tumor microenvironment can produce a number of chemokines that positively affect the DC activity along with the FLT3L that enhances the autologous DC generation [38]. Furthermore, the activated NK cells can kill immature DCs and induce the adaptive immune response in the secondary

lymphoid organs. The mature DCs produce cytokines (mainly IL2, IL12, IL18) that stimulate production of IFNγ, TNFα or GM-CSF by the NK cells, thereby accelerating the DC maturation process [39].

**Modifications of DC- and NA-vaccines**

*DC-vaccines*

The contemporary trend in the development of anti-cancer vaccines is represented by the targeted approach based on the tumor-associated antigens (TAAs). These include overexpressed antigens, normal differentiation antigens and cancer stem cell antigens, as well as NAs. A peptide, chimeric protein, DNA or RNA can be the active ingredient of such vaccines [16].

One approach to modification of DC-vaccines involves the use of nanoparticles that are easily internalized by DCs through endocytosis and can be used as carriers of nucleic acids or peptides [32]. In this context, nanoparticles have some advantages: immunogenicity and the ability to be translocated through lymphatic vessels, if the particle size does not exceed 200 nm. The tumor antigens can be conjugated with nanoparticles by adsorption, encapsulation, chemical conjugation and self-assembly [32].

Another promising approach to modification of DC-vaccines involves genetic reprogramming of somatic cells by inducing the expression of key cell differentiation factors. The moDCs are more appropriate for this approach compared to other DCs. For example, the SmartDC technology enables reprogramming of autologous $CD14^+$ monocytes using the lentiviral vector that carries genes encoding GM-CSF, IL4 and TRP2 (dopachrom tautomerase). Transduction with the viral vector triggers differentiation of monocytes into the $TRP2^+$ moDCs.The SmartDC technology is simpler and less time-consuming compared to conventional DC-vaccine preparation [19].

*NA vaccines*

Developments of machine learning algorithms and neural networks allow for rather accurate identification of the patient's NAs and predicting the protein (peptide) structure [9]. Information about the predicted and tumor NAs is systemized in the specialized databases, such as dbPepNeo [40]. However, not all tumor NAs can be used to develop the NA-vaccines. Such parameters of NAs, as allogeneity, clonal distribution, abundance of the major histocompatibility complexes I and II (MHC-I, MHC-II), affinity of T cells for NAs, and the presence of driver mutation in the gene encoding NAs, have to be taken into account [41]. It is well known that the NA-vaccine efficacy results largely from the tumor mutational burden (TMB), i.e., the number of mutations per DNA fragment with the length of 1 million base pairs, but it can be limited due to low TMB values of some MNs. It should be remembered that TMB is considered to be a predictive biomarker for melanoma and NSCLC only [41, 42]. It was noted that the cultured DCs or DCs isolated from the patient's blood can be easily loaded with NAs using the routine procedures: electroporation or lentiviral transduction [43]. This contributes to the development of the mixed DC-NA-vaccines that have already shown their anticancer efficacy in the PCTs involving the models of PC, BRCA, NSCLC, CRC and Merkel cell carcinoma. Some of these vaccines are being studied in CTs (Table 2).

CONCLUSION

The DC- and NA-vaccines represent an intensively developed branch of the high-techbiotherapeutic anticancer drugs for the personalized application. Since certain technological aspects of the DC- and NA-vaccine preparation are characterized by considerable duration, high labor and resource intensity, optimization of preclinical developments aimed at accelerating,simplifying and cost reducing the DC-vaccine manufacture processis relevant. These developments will significantly increase the scale of the DC- and NA-vaccines applications in the future.

The approach directed totargeting the vaccines to cancer stem cells (CSCs) and their NAs seems to be ambitious and promising. According to a number of studies, tumors with aggressive phenotypes can contain large populations of CSCs that determine high proliferative potential and the disease progression [44]. However, a more detailed investigation of the CSC molecular genetic profile and the spectrum of the CSC specific biomarkers is needed to improve this approach.

Since the DC- and NA-vaccines have proved to be effective against a number of similarmalignant neoplasms, clinical assessment of the mixed (combined) NA-DC-vaccines should be considered as a promising area, however, the results of such CTs have not yet been published.

According to the analysis of the completed and active CTs, the combinations of DC-vaccines and ICIs currently demonstrate the highest anticancer efficacy along with acceptable safety and tolerability in patients with solid malignant neoplasms.

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## HEALTH RISK ASSESSMENT PROBLEMS IN THE SETTING OF CHEMICAL POLLUTION OF THE ENVIRONMENT

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Existing approaches to health risk assessment focus, primarily, on the comparative priority of pollutants and their sources in the environment. But these approaches cannot be used to predict real changes in the mortality or morbidity rates of the population living in a given territory, and therefore cannot be used to develop health-prevention measures aimed at preserving or restoring human health. In this regard, in this study it is proposed to use the concept of mitigation (in this context, actions aimed at reducing environmental pollution) and the concept of adaptation (actions aimed at reducing the vulnerability of populations to environmental pollution). The existing risk assessments can be used to develop mitigation measures, but are not much instrumental in development of adaptation measures, which need to concentrate on early diagnosis and prevention of diseases caused by environmental pollution, as well as on the development of rehabilitation measures. It has been noted that hygiene and epidemiological research has not paid enough attention to the differences between these areas of public chemical and radiation safety. Yet, better targeting when assessing the risk will help to more effectively design interventions to manage these risks.

**Keywords:** Environmental pollution, health risks, chemical safety

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## ПРОБЛЕМЫ ОЦЕНКИ РИСКА ЗДОРОВЬЮ ПРИ ХИМИЧЕСКОМ ЗАГРЯЗНЕНИИ ОКРУЖАЮЩЕЙ СРЕДЫ

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Подходы к оценке риска здоровью позволяют характеризовать прежде всего сравнительную приоритетность загрязняющих веществ и источников их поступления в окружающую среду, но не могут быть использованы для предсказания реальных изменений уровней смертности или заболеваемости населения, проживающего на конкретной территории, а следовательно, и для разработки медико-профилактических мероприятий, направленных на сохранение или восстановление его здоровья. В обзоре предложено при анализе влияния загрязнения окружающей среды на здоровье населения использовать такие понятия, как митигация (в данном контексте это действия, направленные на снижение загрязнения окружающей среды) и адаптация (действия, направленные на снижение уязвимости населения при загрязнении окружающей среды). Существующие оценки риска эффективно могут быть использованы для разработки мероприятий по митигации, но не позволяют разрабатывать мероприятия по адаптации, основные направления которых — ранняя диагностика и профилактика заболеваний, обусловленных загрязнением окружающей среды, а также разработка реабилитационных мероприятий. Отмечено, что в исследованиях в области гигиены и эпидемиологии не уделяется достаточного внимания различиям между этими направлениями обеспечения химической и радиационной безопасности населения. Вместе с тем более точное определение целей при оценке риска поможет более эффективно разрабатывать мероприятия по управлению этими рисками.

**Ключевые слова:** загрязнение окружающей среды, риски здоровью, обеспечение химической безопасности

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Chemical and biological risk monitoring is one of the priorities of the state policy in the field of chemical and biological security [1]. It is also important to justify and implement medical and preventive measures for persons exposed to adverse effects of hazardous chemical and biological factors in potentially hazardous chemical and biological facilities and territories, and in areas impacted by these adverse factors.

Currently, human health risk assessment is understood as a quantitative measure of the probability of the development of adverse effects on human health or the health of future generations resulting from exposure to environmental factors [2, 3]. Traditionally, the analysis of chemical and radiation risks distinguishes between carcinogenic and non-carcinogenic effects.

In assessing the risk of developing non-carcinogenic effects, it is generally assumed that there is a threshold value (reference level of exposure) for radiation dose or concentration of a chemical below which exposure does not markedly increase health risk to sensitive populations. A risk below $1 \times 10^{-5}$ is acceptable [2–4]. Exceeding the reference level increases the probability of developing adverse effects. However, it is not possible to estimate this probability, so the characteristics of the degree of adverse effect using threshold doses and concentrations are called hazard quotient and indices, which emphasises their difference from the traditional concept of risk as a quantitative assessment of the probability of a harmful effect developing [2]. These indicators are calculated as follows. A hazard quotient is calculated for a specific pollutant in a component of the environment (soil, ambient air, water, etc.) as the ratio of the averaged dose of that substance ingested by a human body to the corresponding threshold value. The

hazard index is defined as the sum of the hazard quotients of all contaminants acting simultaneously. It is assumed that if the hazard quotient of a substance is less than 1, then if it is ingested daily over a lifetime, the probability of a person developing adverse effects is negligible. The values of the quotients and hazard indices are due to the interaction mechanisms of the substances concerned and the living organism. The threshold concentration is the minimum concentration that causes an adverse effect in at least one organ or system that is referred to as critical for such exposure. Threshold concentrations are usually determined in experiments on small animals (rats, mice). The human threshold concentrations are recalculated using appropriate reserve factors due to the significantly lower metabolic rate in human body and the uncertainty associated with extrapolating data from animal stidues that are taxonomically distant from humans (different order within the Mammals [2]. Hazard indicators describe a substance that has the potential to contaminate the environment. They are not related to the duration or other characteristics of exposure (such as climatic), nor do they depend on whether the substance has been acting on any living organism [2]. In contrast, risk is the result of exposure to a pollutant under certain conditions, characterized by the duration of exposure and the condition of the organism exposed to it.

Under the influence of some environmental pollutants, both chemical and radiation, biological effects have been revealed (first of all, damage to the genetic apparatus), the probability of the appearance of which is proportional to the influencing dose, while the severity of the manifestation does not depend on it. This is apart from non-carcinogenic effects for which appropriate concentration and dose thresholds can be established. Because such damage contributes to the development of cancer, these effects are called carcinogenic effects. To quantify the frequency of such stochastic effects, the hypothesis of a linear non-threshold dependence of the probability of developing negative effects on the exposure dose has been adopted. This hypothesis is based on extrapolating the effects of high exposure doses to much lower doses [2, 3]. For a chemical substance that is carcinogenic and can induce direct damage to the genome (genotoxic carcinogen), the main parameter in assessing carcinogenic risk is the carcinogenic potential of the substance. It represents the degree of increase in carcinogenic risk as a function of increasing the exposure dose of a substance (slope of a straight line constructed by linear extrapolation for several points characterizing the dependence of carcinogenic risk on the exposure dose and obtained under experimental conditions). In the case of radiation contamination, coefficients $5.6 \times 10^{-2}$ $Sv^{-1}$ [5] or $5.5 \times 10^{-2}$ $Sv^{-1}$ [6] are used to assess the dose-response relationship for carcinogenic risk.

When analyzing the carcinogenic and non-carcinogenic effects of chemical factors, first of all, the state of the so-called critical organs and systems is analyzed, the critical organs and systems are the organs and systems that are most sensitive to the investigated conditions according to experimental studies and in which specific negative changes occur that entail specific effects [3, 7].

In recent decades, however, numerous studies have shown that both chemical contamination and prolonged low-dose radiation exposure induce the development of oxidative stress and inflammation in the human body, the main target organs being the blood vessels. This points to the limited informative value of using the concept of "critical organs" [8–10].

As noted by many researchers, traditional approaches to risk assessment and analysis, including the use of the hazard index concept, are most valuable for the comparative characterisation of environmental exposures in different areas or over different time periods; they are also useful for comparing the effectiveness of environmental measures [2–4]. Using such risk assessments researchers are able to obtain quantitative characteristics of possible damage, compare the potential consequences of exposure to pollutants, identify priority sources of danger, and rank residential areas by the degree of influence of the factors in question [4, 11]. However, it should be noted that such approaches cannot be used to predict real changes in the mortality or morbidity of the population living in a particular area [3]: indeed, they do not take into account the factors that characterize the vulnerability of the population to the effects of pollution, such as the proportion of children and the proportion of the elderly, the degree of unfavorable natural and climatic conditions and the standard of living of the population. As these factors can significantly influence morbidity and mortality rates from some common causes [12–15], such approaches cannot be used to develop medical and preventive measures aimed at maintaining or restoring the health of the population living or working in the contaminated area.

It is therefore constructive, when analyzing the impact of environmental pollution on public health, to use such concepts that are widely used in the analysis of the negative impact of climate change, such as mitigation and adaptation [16]. Mitigation, in this context, refers to actions aimed at reducing environmental pollution, and adaptation refers to actions aimed at reducing the vulnerability of population to environmental pollution, inevitable at this technological age. It should also be noted that existing risk assessments, which primarily characterize the comparative priority of particular pollutants and sources of their release into the environment, can be effectively used to develop mitigation measures. Yet, they are not so useful for the development of adaptation measures, which focus primarily on early diagnosis and prevention of the main diseases caused by environmental pollution, and on development of rehabilitation and recovery measures, including those that curb negative changes in the body at the inception stage.

Despite the large number of studies in the fields of hygiene and epidemiology, the scientific literature does not pay enough attention to the differences between these significantly different areas of ensuring chemical and radiation safety of the population: there are only a few publications. One way of developing risk assessment methodology should be to link existing health risk assessments with the results of epidemiological studies [17].

In epidemiological studies aimed at analyzing the impact of environmental pollution on health, such concepts as attributable risk, relative risk, additional population risk, and additional share of population risk are traditionally used. Relative risk is the ratio of morbidity (mortality) indicators of persons exposed to and not exposed to a polluting factor, and attributive (additional) risk is the difference between the corresponding morbidity (mortality) indicators.

An analysis of publications on health risk assessments due to environmental pollution reveals significant problems that lead to an underestimation of the actual risk to public health. This may come from the emphasis on cancer, diseases of the respiratory and digestive organs, skin, eye, etc. [17, 18] although the findings of numerous modern studies indicate that environmental pollution has the greatest impact on morbidity and mortality from circulatory diseases [8–10, 19, 20]. Among such studies it is necessary to single out those that show that workers of chemically hazardous facilities have an earlier development and wider spread of circulatory system

diseases of atherogenic nature [19, 20]. In addition, this group of workers is at increased risk of hepatobiliary damage [21] and of developing various forms of immune-mediated pathology [22]. In this regard, many authors point out that in order to strengthen the monitoring of the health of workers at particularly hazardous chemical production facilities, it is necessary to expand the range of diagnostic tests both at the time of hiring and in subsequent dynamic monitoring [20-23].

Another problem contributing to the underestimation of actual risk is the presence of modulating factors, such as natural-climatic and socio-economic factors, which significantly affect the vulnerability of populations to the effects of pollution [12, 24]. It is well known that cold climates remain the cause of elevated concentrations of pollutants, as many of the toxicants carried by warm air currents from low- and mid-latitude regions are deposited when they collide with cold Arctic air masses. In permafrost conditions, self-purification processes of natural objects are significantly slowed down, mobility of soil solutions and circulation of surface water are limited, the rate of physical and chemical reactions and the intensity of biological (microbial) degradation and assimilation of pollutants are reduced. The synergistic effects of cold and air pollution accelerate human disease and ageing in high latitudes, affecting the circulatory system to the greatest extent. The climatic features of the polar latitudes (low ambient temperature and strong winds) induce an increase in thermogenesis and, as a result, an increase in the concentration of reactive oxygen species and other free radicals, and also cause adaptive changes in the respiratory system, which indirectly contribute to an increase in the negative impact of air pollution [13–15]. Since with moderate cooling, pulmonary ventilation significantly increases, for gases absorbed in the respiratory tract (for example, sulfur dioxide, hydrogen fluoride, etc.), this leads to an increase in the absorbed dose, and the lengthening of the inhalation phase when breathing cold air further contributes to an increase in the settling of suspended particles [13–15]. In addition, during cooling, the functional activity of the adrenal glands and the level of their blood supply increase, which, apparently, causes the accumulation of toxic substances in them with the simultaneous action of cooling and pollutants. [25].

The additional influence of socioeconomic conditions on the risk of major non-communicable diseases and the increased vulnerability to the negative impact of environmental pollution in populations with low socioeconomic status has been shown in many studies [12, 26].

In this regard, it seems appropriate to develop approaches to the integral assessment of the impact of all exposure factors (chemical, physical, natural-climatic, socio-economic). This kind of exposure risk for non-communicable diseases does not determine the risk for a particular individual, like the SCORE scale [27], but aims to identify areas whose populations have an increased risk of developing certain non-communicable diseases. In these areas, in addition to measures aimed at mitigating environmental pollution, actions are needed to increase the adaptation of the population by reducing its vulnerability to the impact of negative factors. This implies, on the one hand, additional medical and preventive measures aimed at the early detection of markers of the development of relevant non-communicable diseases, and, on the other hand, clarification of which particular exposure factors may have a dominant influence. The purpose of this is to narrow down the pool of people who need additional medical and prophylactic measures.

As we see, despite the sufficiently long period of research and the existence of a plethora of whitepapers written using different approaches, the task of assessing the chemical and radiation risks to public health remains relevant, taking into account all the main factors involved. The solution of this problem requires a joint effort of specialists in various fields: hygienists, toxicologists, radiologists, cardiologists, as well as physicists, mathematicians, biologists, and geographers.

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# ROLE OF RADIOLOGY TECHNIQUES AND HYBRID PET-MRI TECHNIQUE IN THE DIAGNOSIS OF PHARMACORESISTANT EPILEPSY

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The surgical treatment outcome in patients with pharmacoresistant epilepsy directly depends on the epileptic focus localization accuracy. Conventional diagnostic algorithms for patients with epilepsy involve starting with video EEG monitoring and magnetic resonance imaging. It is not possible to localize epileptogenic foci with the use of these techniques in a large segment of patients with pharmacoresistant epilepsy, or the test results are discordant. The review provides the analysis of literature data on the current possibilities of SPECT, PET and new hybrid PET-MRI technique when used for preoperative planning in patients with refractory epilepsy.

**Keywords:** epilepsy, MRI, SPECT, PET, PET-MRI

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# РОЛЬ РАДИОЛОГИЧЕСКИХ МЕТОДИК И ГИБРИДНОГО МЕТОДА ПЭТ-МРТ В ДИАГНОСТИКЕ ФАРМАКОРЕЗИСТЕНТНОЙ ЭПИЛЕПСИИ

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Результаты хирургического лечения пациентов с фармакорезистентной эпилепсией напрямую зависят от точности диагностики локализации эпилептогенного очага. Диагностический алгоритм при эпилепсии традиционно начинается с видео-ЭЭГ мониторинга и магнитно-резонансной томографии. У существенной части пациентов с фармакорезистентной эпилепсией с помощью этих методик не удается локализовать эпилептогенный очаг либо результаты этих исследований не согласуются между собой. В обзоре представлен анализ данных литературы о современных возможностях методов ОФЭКТ, ПЭТ и новой гибридной методики ПЭТ-МРТ в предоперационном планировании пациентов с фармакорезистентной эпилепсией.

**Ключевые слова:** эпилепсия, МРТ, ОФЭКТ, ПЭТ, ПЭТ-МРТ

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Epilepsy is a disorder characterized by recurrent seizures caused by dysfunction of brain structures of different etiology and localization. The prevalence of epilepsy is 4–5 cases per 1000 population [1]. Despite the use of a wide variety of antiepileptic drugs, up to one quarter of patients (9–26%) remain resistant to antiepileptic therapy [2, 3].

Such invasive techniques, as resection surgery, endoscopic disconnection surgery, embolization, laser ablation, and stereotactic radiosurgery (Gamma Knife), are used for treatment of pharmacoresistant epilepsy. The effectiveness of surgical treatment is as high as 55–80%, it is directly dependent on the diagnosis accuracy [4, 5]. The goals of epilepsy surgery are to control seizures by resecting epileptogenic tissues and to minimize neuropsychological impairments and other neurological disorders by preserving important areas of the brain [6]. Accurate localization of epileptogenic zones and mapping of those against functionally significant cortical areas form an integral part of preoperative assessment of patients with pharmacoresistant epilepsy performed when planning surgery [7, 8]. According to multiple researchers, patients with epilepsy showing concordant EEG and MRI results recover fully after surgery in 30–90% of cases [9–11].

Conventional diagnostic algorithms for patients with epilepsy involve starting with video electroencephalography (EEG) monitoring, the noninvasive and rather accessible method. The use of scalp electrodes may be insufficient for accurate localization of foci in patients with the deep seated foci and rapidly propagating seizures [12].

MRI is the modality of choice for morphological confirmation of functional alterations detected by EEG that allows one to identify a whole range of abnormalities capable of causing epileptic seizures, such as tumors, vascular malformations, focal cortical dysplasia, mesial temporal sclerosis, brain matter alterations caused by injury or infarction, cortical migration disorders, and other conditions (Fig. 1).

It should be noted that it is not possible to accurately localize epileptogenic foci using MRI and EEG in 20–50% of patients with pharmacoresistant epilepsy, or the MRI results are inconsistent with the data of video EEG monitoring and clinical features. In such cases, intracranial EEG may be used

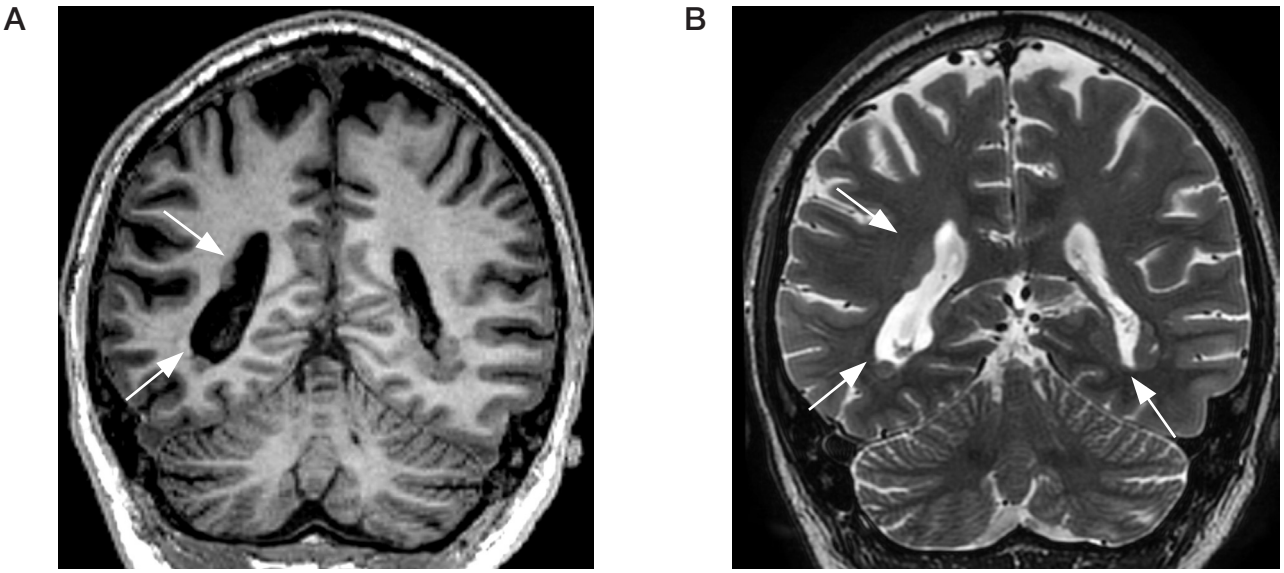


**Fig. 1.** Brain MRI, oblique coronal scan, T1- (**A**) and T2-weighted images (**B**). Multiple areas of subependymal nodular grey matter heterotopia (*arrows*)

to clarify the epileptogenic focus localization [9, 10, 13], additional information may be also obtained by using functional MRI, MR spectroscopy or radionuclide diagnostics, SPECT and PET [3, 14]. The MR imaging-based morphometry (the method for quantitative assessment of volumes of various brain regions) may be used as a supporting techniques to clarify the epileptogenic focus localization and detect changes in the volumes of brain regions that are not directly related to the epileptogenic focus and are sometimes located in the contralateral hemisphere, which can be considered as a prognostic factor of the surgical treatment outcome [15].

Functional MRI can also be used for localization of the primary motor and sensory cortical areas, Wernicke's and Broca's areas, as well as the arcuate fasciculus, relative to the intended resection area in order to estimate the risk of neurologic deficit [16–18] (Fig. 2).

### Single photon emission computed tomography

The functional radionuclide diagnosis method, single photon emission computed tomography (SPECT), is widely used in assessing cerebral perfusion [19, 20]. Cerebral perfusion and metabolism are closely related in most physiological and pathological processes; cerebral blood flow (CBF) is often correlated with the neuronal activity. As compared with $^{18}$F-FDG PET used for assessment of the brain, SPECT that has lower spatial and higher temporal resolution makes it possible to detect a primary excitation focus. In the ictal phase, the increase in neuronal activity results in the increased metabolism and perfusion (CBF).

The regional cerebral blood flow measured using SPECT is considered to be an indirect marker of neuronal activity. Subtraction of ictal SPECT image from interictal image with subsequent co-registration to MRI using $^{99m}$Tc-hexamethyl propylene amine oxime (HMPAO) or 99mTc-ethylene cysteine dimer (ECD) in parallel with EEG monitoring is the most sensitive and specific technique [21]. The technique involving sequential $^{99m}$Tc-HMPAO SPECT and CT scanning within the same study is a more commonly used method.

Computational techniques enable estimation of differences between the SPECT perfusion indicators acquired in the ictal

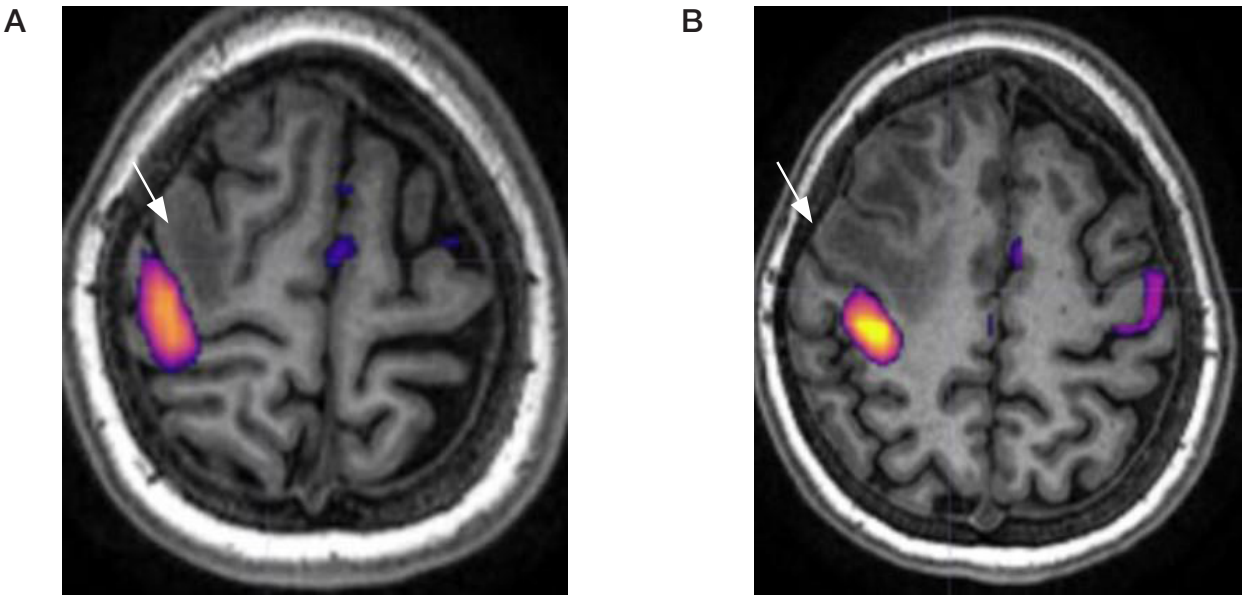


**Fig. 2.** Functional MRI. Motor cortex mapping in patient with refractory epilepsy (**A**, **B**). Distribution of the areas showing activation when moving the left fingers is marked by the arrowhead, the epileptogenic zone is marked by the *arrow*

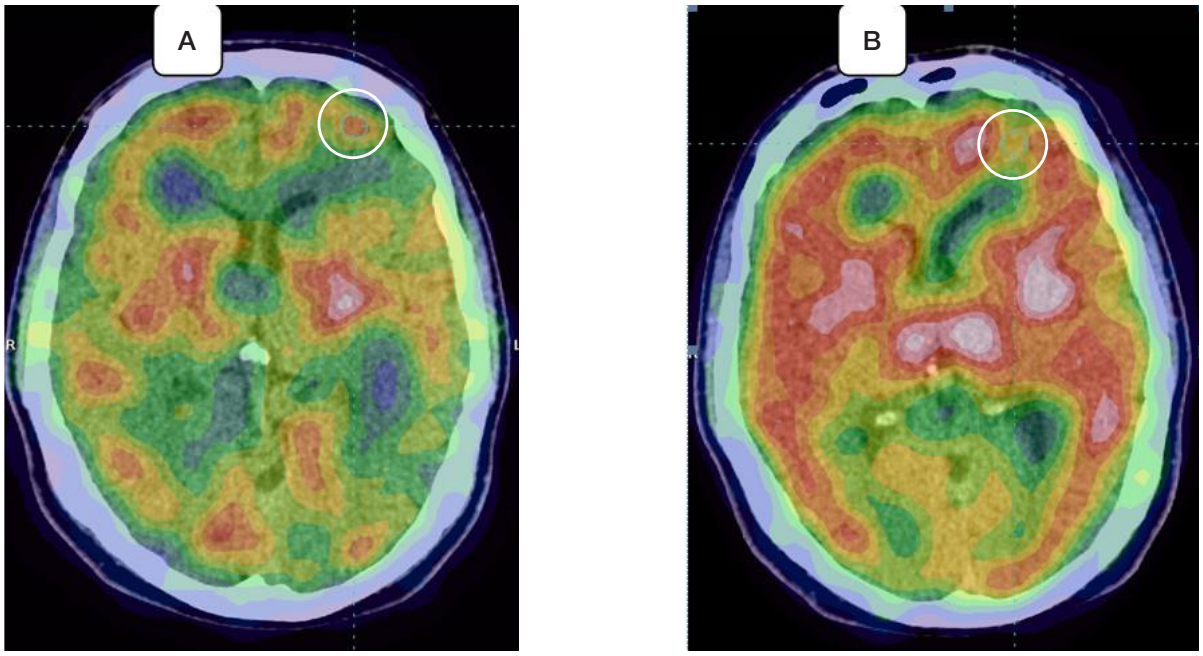


**Fig. 3.** SPECT perfusion maps (RPA $^{99m}$Tc-Teoxim) of the patient with refractory epilepsy performed in ictal (**A**) and interictal (**B**) phases. The annular area in the left middle frontal gyrus markes the zone of hyperperfusion in the images obtained during the ictal phase that corresponds to the hypoperfusion zone in the images obtained during the interictal phase

and interictal phases, and the results obtained are further co-registered to CT or MRI. Thus, SPECT allows one to clarify the presence of blood perfusion changes in the affected brain regions identified by neuroimaging or to reveal MR-negative regions showing abnormal perfusion [22–24] (Fig. 3).

Xenon-133 ($^{133}$Xe), the diffusing capacity of which allows one to measure cerebral blood flow (CBF) in mm/min per 100 g of tissue, is considered to be a reference radiopharmaceutical for estimation of perfusion. At the same time, $^{133}$Xe has a number of disadvantages, such as rapid clearance, short scan time and lower spatial resolution, that is why its clinical use is limited. In the past, $^{123}$I-amines were used along with $^{133}$Xe, however, the capability of redistibution imposes the same restrictions as the method involving the use of 133Xe. The above radioactive tracers now have mostly historical value.

Today, the most widely used radioactive tracers for perfusion imaging are technetium labeled compounds, the $^{99m}$Tc-examethylpropyleneamine oxime ($^{99m}$Tc-HMPAO) and the technetium ethyl cysteinate dimer ($^{99m}$Tc-ECD). Redistibution is not typical for these radiopharmaceuticals, and their primary distribution is proportional to volumetric blood flow at the time of injection regardless of the blood flow fluctuations. This enables performing scanning within hours after the injection [22].

The recommended scan time for optimal signal-to-noise ratio is 30–90 min for $^{99m}$Tc-HMPAO and 30–60 min for $^{99m}$Tc-ECD. $^{99m}$Tc-HMPAO and $^{99m}$Tc-ECD have similar pharmacokinetic parameters and show comparable sensitivity and specificity when used for estimation of cerebral perfusion and localization of epileptogenic foci. The above radioactive tracers show differences in the mehanisms of absorption and dosimetry, as well as different patterns of distribution in brain matter: $^{99m}$Tc-HMPAO is accumulated mainly in the cerebellum, while increased accumulation in primary visual cortex is typical for $^{99m}$Tc-ECD. The features of the $^{99m}$Tc-ECD pharmacokinetics are more selective fixation in brain tissues and more rapid urinary excretion allowing for administration of higher doses resulting in the higher quality of images compared to that obtained using $^{99m}$Tc-HMPAO. According to some reports, $^{99m}$Tc-ECD shows slightly higher sensitivity and specificity than $^{99m}$Tc-HMPAO [23]. That is why it is advantageous to use the same radiopharmaceutical and the same scanning parameters when monitoring the dynamics [22]. The patient is recommended to stay in a stable environment, in a quiet, dimly-lit room at the time of the radioactive tracer injection and for several minutes before and after the injection to mitigate the impact of external stimuli on brain activation. When the patient is in a brightly lit room with his/her eyes open, the increased uptake of radiopharmaceutical in the visual cortex can be observed [23]. When performing interictal assessment, EEG monitoring is started 2 h before the injection and terminated not earlier than 15 min after the injection in order to avoid possible effects of epileptic seizure on the blood perfusion parameters: ictal hyperperfusion followed by hypoperfusion capable of affecting the entire cerebral hemisphere and even of contralateral spreading. Furthermore, the data of EEG monitoring are used to estimate bioelectric activity of the brain at the time of injection [20, 22]. When performing ictal assessment, the most accurate epileptogenic focus localization can be achieved when the radiopharmaceutical is injected no later than 20 s after the epileptic seizure. Assessment performed with administration of radiopharmaceutical after 45 s or later results in multiple ambiguous or even false results [25–27].

Ictal and interictal scans are visually assessed in accordance with the published guidelines [23, 28], in addition, quantitative indices are measured using ROI (region of interest), atlases of normal $^{99m}$Tc-HMPAO and $^{99m}$Tc-ECD distribution based on the data of healthy volunteers, voxel-based morphometry [23, 28]. After that image subtraction is performed: the areas of hyperperfusion revealed in interictal images are subtracted from ictal images.

The combined SPECT/MRI protocol (SISCOM, subtraction single photon emission CT co-registered to MRI) that implies overlaying the subtraction SPECT images on MRI scans, thereby allowing one to specify anatomic localization of alterations detected by SPECT and improve accuracy of the scan interpretation, is of special interest [29]. According to meta-analysis performed in 2016, co-registration of subtraction

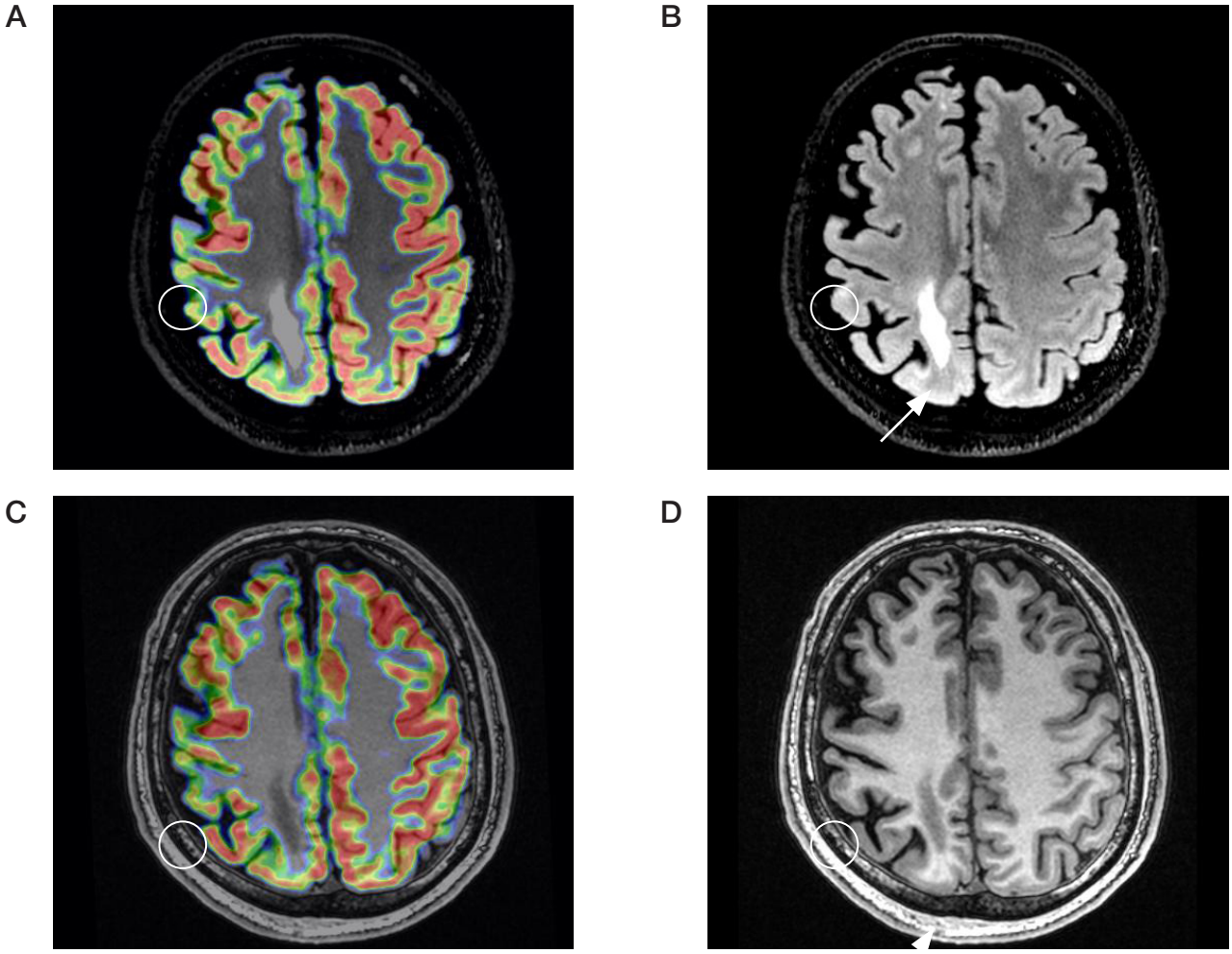


**Fig. 4.** $^{18}$F-FDG-PET-MRI of the brain in patient with pharmacoresistant epilepsy and unsatisfactory outcome of epilepsy surgical treatment. **A**, **C**: area of hypometabolism in the dorsolateral cortex of the right frontal lobe (*circle*). Brain MRI: axial FLAIR (**B**), IR-FSPGR (**D**). Focal cortical thickening with FLAIR hyperintensity in the area of hypometabolism (*circle*). Postsurgical structural brain changes in the paramedian area of the right parietal lobe (*arrow*)

SPECT to MRI ensures positive prognostic value (PPV) of 56% when the data obtained using these techniques are concordant [30]. In addition to the diagnosis of MR-negative epileptogenic foci, the technique has found use in planning of partial resection of epileptogenic foci in patients with large zones of structural alterations. This can make it possible to achieve control over seizures. In addition, the technique can be used when planning re-surgery in patients with unacceptable results of previous surgical treatment [30, 31].

The literature data on comparison of the SPECT and [$^{18}$F]-FDG diagnostic capabilities vary considerably depending on the study design, patient enrollment, and the expertise of medical personnel. According to retrospective studies performed in 2013, the SPECT sensitivity that reached 87% [32, 33] significantly exceeded the PET sensitivity (56%). Such high SPECT sensitivity values may be due to shorter time lag between the seizure and the radioactive tracer injection. The data of SPECT and PET can provide additional information and complement each other within the framework of multimodality approach to preoperative examination of patients with pharmacoresistant epilepsy [34].

### Positron emission tomography

Another radionuclide brain imaging method, positron emission tomography (PET), is used to provide visualization and quantification of various cellular and biological processes, such as blood perfusion and metabolism, synthesis of proteins and DNA, receptor expression, etc. The detection feature of PET scanners is that images are formed entirely by the pairs of oppositely directed gamma rays, which results in higher spatial resolution compared to SPECT.

Standard preparation of a patient for PET study is conventionally performed in accordance with the European guidelines issued in 2021 [35]. Video EEG monitoring (10–20 electrode placement system) is performed within 2 h before intravenous administration of the radioactive tracer and throughout the period of the radiopharmaceutical uptake (at least 20 min) in order to ensure there are no seizures during the procedure. The patient should stay in a quiet dark room, in a comfortable position for at least 15 min before the radiopharmaceutical administration. The patient should be informed about the importance of maintaining a relaxed resting state before the procedure: to lay still with the eyes closed, not to talk, read or listen to music. If there is a need to use medical sedation, sedatives should be used as late as possible: at least 20 min after administration of radioactive tracer, several minutes before scanning. In case of static image acquisition procedure, scanning is started between 30 and 60 min after the radiopharmaceutical administration [36]. It is recommended to use the same scanning protocol, including the same scanning start time after administration of radiopharmaceutical when performing the follow-up assessment. When there is a need to level up the patient's motion artifacts or to perform quantitative

analysis, the dynamic scanning can be carried out within the specified time interval; the optimal dynamic scanning start time is 60–90 min after the radiopharmaceutical injection [35].

There are a lot of radioactive tracers, however, $^{18}$F-fluorodeoxyglucose is most commonly used in clinical practice to identify epileptogenic foci. The use of $^{18}$F-fluorodeoxyglucose makes it possible to indirectly evaluate metabolism based on the quantitative changes in the glucose uptake. A rather long half-life (110 min) is one of the biggest advantages of $^{18}$F-FDG. Interictal PET is performed. This is due to the fact that sufficient $^{18}$F-FDG concentration in the brain is achieved 30–40 min after the injection, it reflects the summational metabolism of this period. Such a long radiopharmaceutical accumulation time makes it impossible to estimate transient neural processes, such as epileptic seizure that usually lasts one or few minutes [36].

Epileptogenic foci in patients with both temporal and extratemporal lobe epilepsy are associated with the areas of decreased glucose metabolism, which are usually larger that the foci themselves. The zone of 18F-FDG hypometabolism is likely to include the areas of initiation and propagation of excitation, that is why PET enables lateralization and only approximate localization of epileptogenic zones. However, it may be difficult to accurately define the area of the epileptic seizure origin using PET only, especially when the focus is small [37, 38].

In 10–43% of cases, additional hypometabolic zones, that are most often localized in the ipsilateral frontal lobe and are probably induced by the seizure, may be found in patients with epilepsy. Furthermore, the regional hypometabolism may be caused by a number of other factors not directly related to epilepsy: co-occurring structural alterations resulting from infarction, injury, infection, and neurodegenerative diseases, such as Alzheimer's disease. When interpreting the PET images, it should be borne in mind that reduced sensitivity of epileptogenic foci to $^{18}$F-FDG-PET can be observed in patients with focal-onset seizures prone to secondary generalization. That is why PET data interpretation should involve comparison with clinical data and EEG results [39, 40].

The $^{18}$F-FDG-PET results may be important for prediction of the disease course. Thus, identification of hypometabolic zones limited by the epileptogenic zone was associated with better surgical outcomes in patients with temporal lobe epilepsy, while no such correlation was found in patients with extratemporal lobe epilepsy [41, 42]. A mismatch between the hypometabolic zone localization according to PET, the epileptogenic zone revealed using EEG, and the clinical features may be a predictor of adverse surgical outcome. The combined analysis of PET and MRI data makes it possible to identify 81–95% of patients with beneficial surgical outcomes when the data obtained using these techniques are concordant [43]. The reasons for conflicting results have not been reliably established, however, there is a hypothesis that these discrepancies are due to the rapid spread of epileptic activity or the long history of epilepsy [43, 44]. The results of studies focused on assing sensitivity and specificity of positron emission tomography applied during preoperative planning in patients with pharmacoresistant epilepsy vary considerably. According to the meta-analysis conducted in 2007, positive prognostic value (PPV) reaches 72–89% depending on the study design [45].

*PET. Antagonist of central GABA receptors*

$^{11}$C-flumazenil (FMZ) is a specific reversible antagonist of the GABA receptor complex central benzodiazepine fragment. One of the papers reports reduced cortical $^{11}$C-FMZ binding in the epileptogenic foci of 85% of patients [46]. The zones with reduced cortical binding located at a distance from the epileptogenic focus have been also revealed in 55% of patients, which could complicate the diagnosis. The other studies show lower frequency of the areas of $^{11}$C-FMZ accumulation by epileptogenic zones compared to hypometabolic areas based on the $^{18}$F-FDG-PET data. This has a potential of more accurate epileptogenic foci localization [47, 48]. According to the meta-analysis conducted in 2021, the $^{11}$C-FMZ-PET sensitivity and specificity were 62% and 72%, these were comparable with the sensitivity and specificity of $^{18}$F-FDG-PET (66 and 71%, respectively). Moreover, higher sensitivity of $^{11}$C-FMZ in the subgroup of patients over 30 years of age having a long history of extratemporal lobe epilepsy was reported [48]. The results of the quantitative analysis involving the data of $^{18}$F-FMZ-PET and $^{18}$F-FDG-PET were consistent with the invasive EEG data in 86% and 71% of cases, respectively [49]. This allows us to consider these two techniques as a noninvasive diagnostic alternative.

*PET. TSPO receptor ligands*

Despite the fact that the first studies involving TSPO ligands, the neuroinflammation biomarkers, were conducted more than 20 years ago, new radioactive tracers have not yet found wide application in clinical practice. The results of a number of studies [50–52] confirm the increased accumulation of TSPO ligands in the epileptogenic substrate, which could be indicative of microglial activation and suggest the role of inflammation in the pathogenesis of epilepsy. A diffuse increase in the TSPO ligand uptake in brain matter, except for the cerebellum, within a week after status epilepticus has been reported in one of the studies. The authors suggest that duffuse accumulation of radiopharmaceutical may be a reflection of the “epileptic network”. After that the buildup of TSPO ligands was localized in the brain areas where epileptogenic foci were typically localized, amid continued low-intensity diffuse accumulation [53]. The correlation of TSPO expression with the surgical outcomes, drug resistance and behavioral disorders revealed in a number of experimental studies is of scientific interest and requires further investigation. TSPO ligands have potential as medications for assessment of the efficiency of new therapeutic strategies, as well as for prediction of the disease course and detection of drug resistance [54].

*PET. Markers of metabolism and distribution of serotonin receptors*

The results of pathomorphological assessment indicative of the increased serotonin synthesis in the epileptogenic cortex, including in patients with focal cortical dysplasia [55], necessitated the search for markers enabling its qualitative and quantitative assessment.α-$^{11}$C-methyl-l-tryptophan (AMT), capable of tracing tryptophan metabolism through the serotonin and kynurenine pathway and detecting epipeptogenic foci in the interictal phase even in patients with tuberous sclerosis and cortical malformations, mostly IIB type, including those with no hypometabolic areas according to $^{18}$F-FDG-PET, is one such marker. The technique sensitivity is about 70%, and specificity is close to 100% [56]. These studies take on special importance in the diagnosis of the cortical tubers' epileptogenicity. Tuberous sclerosis is characterized by the presence of multifocal hypometabolic areas that significantly limits the use of $^{18}$F-FDG for estimation of each area epileptogenicity, although such estimation might

have result in more selective surgical interventions, increased effectiveness of surgery and reduced surgical risks. According to the study conducted in 2013, the $^{11}$C-AMT data were consistent with the data of ictal video EEG monitoring in 68 patients with type 1 and 2 tuberous sclerosis out of 95, and the more accurate localization of epileptogenic foci was achieved in 28 of patients. Moreover, the use of $^{11}$C-AMT-PET made it possible to identify epileptogenic foci in 10 patients with no distinct epileptic foci according to the data of ictal EEG out of 17 [57]. According to other sources, sensitivity of the technique in the group of 12 patients with tuberous sclerosis was 17%, despite high specificity reaching 100%. This made it possible to identify epileptogenic foci in 17% of patients [58]. Little prior experience of using α-$^{11}$C-methyl-l-tryptophan (AMT) PET is among other things due to short $^{11}$C half-life (about 20 min) and the consequent need to use cyclotron for AMT synthesis. That is why the subject of scientific inquiry is the search for ligands with longer half-life, including those based on [$^{18}$F], the half-life of which is 110 min. One of such ligands used in scientific research, $^{18}$F-fluorobenzamidoethylpiperazine ($^{18}$F-MPPF), is used to assess the distribution of 5-hydroxytryptamine-1A (5-HT1A) serotonin receptors allowing one to identify the areas of the synaptic serotonin release reduction due to astroglial activation [59]. According to one of the studies conducted in 2018, the 18F-MPPF accumulation was consistent with both 18F-FDG data and severity of epileptic seizures and behavioral disorders [60].

*PET. Opioid receptor ligands*

The following radioactive tracers are used to assess binding to various types of opioid receptors: $^{11}$C-carfentanil ($^{11}$C-CFN), the μ opioid receptor agonist; $^{11}$C-N1'-methylnaltrindole (11C-MeNTI) that binds to — receptors; $^{11}$C-diprenorphine (11C-DPN), the nonselective opioid receptor agonist. As far back as in 1988–1991, there were reports about the changes in binding of μ and — opioid receptors in the temporal cortex ipsilateral to the epileptogenic focus during the postictal phase [61–63]. Lower frequency of areas showing the increased binding to opioid receptors compared to the areas with the decreased $^{18}$F-FDG uptake was noted. Later, in 2007, a hypothesis was put forward on the anticonvulsant effect of opioid peptides and the role of endogenous opioid system in the seizure control [64]. In 2023, the correlation of $^{11}$C-CFN binding to the μ opioid receptors with higher anxiety levels and depression was revealed in the group of patients with temporal love epilepsy. The authors assume that desensitization and inhibition of opioid receptors caused by seizures may represent a potential mechanism underlying the development of mood disorders often associated with epilepsy [65].

**PET/MRI**

The positron emission tomography and magnetic resonance imaging (PET/MRI) combined modality is a new hybrid technique that enables succesive or simultaneous assessment of structural and metabolic alterations in the brain within a single scan [66] (Fig. 4). This hybrid, painless, minimally invasive technique shows high reproducibility, allows one to reduce the radiation dose, requires no careful and lengthy preparation for the study, and has a small number of contraindications and probable side effects [67]. The technique can be used for mapping of epileptogenic zones in patients with temporal lobe epilepsy [68–70] or epileptic zones located outside the temporal lobes [71]. PET/MRI has a number of advantages when used to assess patients with pharmacoresistant epilepsy. Among other things, the technique has a potential of increasing identification rate of focal cortical dysplasia (FCD) that is in some cases not easy to detect by MRI, although surgical treatment of such malformations can completely stop seizures or significantly reduce the severity of epilepsy. Assessment of the hybrid PET/MRI technique efficiency has shown better diagnostic outcomes and more accurate identification of epileptogenic foci (including in patients with refractory epilepsy) compared to the separate use of PET or MRI, and compared to PET-CT [71, 72].

Scientific studies published in the last few years used mathematical models (statistical and correlation analysis), allowing one to clarify the indications for PET/MRI aimed at noninvasive localization of epileptogenic zones, to confirm this hypothesis. According to the results of these studies, PET/MRI can be recommended to patients having discordant results of MRI and EEG along with no distinct epileptogenic foci, as well as to patients with multiple affected areas. The correlation analysis results have shown that PET/MRI can confirm the MR-positive epileptogenic foci and allows one to distinguish patients having indications for surgical treatment from inoperable patients. The post-processing techniques, such as calculation of the asymmetry index using the brain atlases and statistical parametric mapping (SPM) analysis, can provide quantitative confirmation of visual PET/MRI scan assessment along with additional information required for the treatment tactics selection [73]. The method for automated analysis of the PET data interhemispheric metabolic asymmetry performed after the automated anatomical symmetrization co-registered to MRI (the PASCOM protocol) is independent from the PET atlases of healthy volunteers, less limited, it enables more accurate lateralization and localization of the epileptogenic zone [74].

Simultaneous fMRI-EEG acquisition represents an alternative noninvasive epileptogenic zone imaging method. According to the studies piblished in 2012, positive prognostic value of detecting the epileptiform activity and the BOLD response changes within 2 cm from the epileptogenic zone was as high as 78%, while negative prognostic value was as high as 81% [74–77]. The technique involving simultaneous fMRI-EEG acquisition also makes it possible to discern changes in the BOLD response at the seizure onset, during the propagation of excitation, and in the pre-ictal phase [78].

CONCLUSION

Essential steps of preoperative assessment of patients with pharmacoresistant epilepsy are represented by video EEG monitoring (for ictal EEG recording and analysis of epileptiform patterns by neurophysiologist) and MRI (for detection of morphological changes in the brain). The surgical treatment outcome is directly dependent on the accuracy of the epileptogenic substrate localization by the diagnostic technique. At the same time, the MRI and video EEG monitoring results are not always concordant, in some cases MRI and EEG are insufficient for both accurate localization of epileptogenic foci and distinguishing between operable and inoperable epileptogenic foci.

Despite the significant number of papers, the data of which show benefits of using single photon emission and positron emission tomohraphy, as well as hybrid PET/MRI modality, when selecting candidates for surgery, the optimal plan for preoperative assessment of patients with pharmacoresistant epilepsy remains the subject of scientific debate. The algorithm

for selection of the radioisotope technique or the combination of such techniques in patients with various disorders resulting in pharmacoresistant epilepsy requires further investigation.

Currently, the SPECT/CT technique enables quantitative estimation of the differences in blood perfusion changes observed in the affected brain regions in the inctal and interictal phases with subsequent coregistration to MRI for clarification of the epileptogenic nature of the detected structural changes in the brain or localization of MR-negative regions showing abnormal blood perfusion.

PET is used maily in the interictal phase, that is why a less thorough preparation compared to SPECT/CT is required. The technique enables lateralization and localization of the area of the excitation initiation and propagation. The combined analysis of PET, MRI and EEG data may be effective in evaluating the epileptogenic zone resectability and predicting the surgical outcome. Further studies of the ligands binding to the translocator protein (TSPO) receptors, opiod receptors and serotonin metabolism markers are of great scientific interest. The use of such ligands has a potential of improving the epileptogenic substrate detection specificity and the surgical treatment selectivity, especially in patients with focal cortical dysplasia and tuberous sclerosis, and can change perceptions about the pathogenetic mechanism of epilepsy and mood disorders often co-existing with epilepsy.

The hybrid PET/MRI technique, including involving the use of the most accessible 18F-FDG molecule, has a potential of significant contribution to the development of the algorithm of preoperative assessment of patients with pharmacoresistant epilepsy and will make it possible to clarify the presence of epileptogenic zones in patients with dubious MRI results, small foci or ambiguous metabolic patterns. Introduction of correlation analysis into clinical practice is one promising direction of the method development, the obtained quantitative data can be useful in determining the possibility of surgical treatment in patients with pharmacoresistant epilepsy.

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# METABOLIC SYNDROME: RISKS IN YOUTH SPORTS

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Metabolic syndrome is one of the pre-nosological conditions that implies strain on several systems of the body and disruption of all types of metabolism. The key components of the syndrome are visceral obesity, peripheral tissue insulin resistance, arterial hypertension and non-alcoholic fatty liver disease. There is a number of diseases associated with the syndrome, which makes diagnosing its preclinical manifestations important. Overweight and obesity only continue spreading; moreover, these conditions are registered in people of increasingly younger age. Metabolic syndrome in childhood increases the risk of cardiovascular disease in adulthood. Top tier athletes are no exception. Some sports and playing roles promote body weight growth. A young athlete may have specific constitutional features, and, without proper control, motivating such athletes to grow muscles means they also grow fat. The recommendation is to pay special attention to children under the age of 11 that play rugby, American football as line men, in heavy weight categories. Application of the latest diagnostic criteria with their actualization on a regular basis, as well as search for additional markers and parameters identifiable in laboratory settings, would ensure adjustment of the athlete's condition in a timely manner.

**Keywords:** metabolic syndrome, insulin resistance, obesity, arterial hypertension, underage athletes, elite sports, top tier athletes

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# МЕТАБОЛИЧЕСКИЙ СИНДРОМ: РИСКИ В ДЕТСКО-ЮНОШЕСКОМ СПОРТЕ

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Метаболический синдром — одно из донозологических состояний, при котором происходят напряжение сразу нескольких систем организма и нарушения во всех видах обмена. Его главные составляющие — висцеральное ожирение, инсулинорезистентность периферических тканей, артериальная гипертензия и неалкогольная жировая болезнь печени. Синдром ассоциирован с риском развития ряда заболеваний, поэтому важно диагностировать его доклинические проявления. Число людей, страдающих избыточным весом и ожирением, только увеличивается, более того, эти состояния активно молодеют. Наличие метаболического синдрома в детском возрасте увеличивает риск развития сердечно-сосудистых заболеваний во взрослом. Высококвалифицированные спортсмены не исключение. Ряд спортивных дисциплин и игровых амплуа способствуют увеличению массы тела. У молодых спортсменов могут быть конституциональные особенности, и мотивирование их к наращиванию мышечной массы без должного контроля приводит к тому, что у них вырастает и объем жировой ткани. Рекомендуется обратить особое внимание на детей в возрасте до 11 лет, занимающихся регби, американским футболом на позициях лайнменов, выступающих в тяжелых весовых категориях. Следование последним диагностическим критериям и регулярное их уточнение, поиск дополнительных маркеров и лабораторных показателей позволят не упустить время и скорректировать состояние спортсмена.

**Ключевые слова:** метаболический синдром, инсулинорезистентность, ожирение, артериальная гипертензия, несовершеннолетние спортсмены, спорт высших достижений, высококвалифицированные спортсмены

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In the context of sports medicine, metabolic syndrome (MS) is a rather new factor considered. Regular physical activity were believed to largely safeguard athletes from the development of MS. However, the significant and often over-the-top physical strain associated with professional sports is a risk factor for inflammatory processes and oxidative stress, which, in turn, causes endothelial dysfunction and affects the vascular tone regulation [1, 2].

At the same time, in some sports, high results require excessive weight and even obesity (body mass index > 30). Such sports include martial arts (heavy weight category in sumo, judo, sambo, Greco-Roman wrestling), rugby, American football, weightlifting, strongman competition, powerlifting, bobsleigh [2]. These facts, along with the new scientific data obtained in the recent years, dictate the need to revise the previously practiced approaches.

## Metabolic syndrome: definition and prevalence

How do experts interpret metabolic syndrome today? Clinical Guidelines of 2013 by the Ministry of Health of the Russian Federation suggest the following definition: "MS is

characterized by growth of the visceral fat mass, decrease of the insulin sensitivity in peripheral tissues and hyperinsulinemia, which trigger disorders of carbohydrate, lipid, purine metabolism and arterial hypertension (AH) [3].

Some medical professionals consider the spread of MS to constitute a new pandemic; its prevalence exceeds that of diabetes mellitus. According to the WHO Fact Sheet, from 1975 to 2016, the number of obese people worldwide has more than tripled. The prevalence of MS in the population varies from 10 to 30%; in Russia, the figure is from 20 to 35%. Currently, 1.9 billion adults over 18 are overweight, with more than 650 million of them obese. This is 13% of the world's adult population [3–6].

Along with obesity, in adults and adolescents over 16, MS manifests as insulin resistance, dyslipidemia and arterial hypertension [3, 7, 8].

Previously, obesity was considered to be specific to high-income countries, but nowadays it grows increasingly prevalent in low- and middle-income countries. In 2016, about 41 million children under the age of 5 were overweight or obese, while among those older than 5 the conditions were registered in 340 million children and adolescents [3, 9]. Another study states that at least 10–15% of children and adolescents are overweight [6]. In the 0 through 14 years age group, 350 children out of 100,000 in Russia had their first obesity diagnosis, and in the 15 through 17 years age group this figure was almost twice as large almost doubled (708 cases per 100,000 children) [10].

There is an array of diseases associated with obesity, with cardiovascular diseases (CVDs) and diabetes mellitus being the most common thereof. The complications of obesity also include dyslipidemia, non-alcoholic fatty liver disease (NAFLD), reproductive disorders and dysfunctions of the reproductive system, disorders of the musculoskeletal system, obstructive sleep apnea syndrome etc. [7]. Currently, medical professionals and researchers have no univocal view whether these conditions are a complication of obesity or concomitant diseases that, progressing, exacerbate obesity. Nevertheless, many studies have shown that overweight and obesity in children and adolescents are the risk factors for metabolic syndrome, diabetes mellitus and cardiovascular disease later in life. In this connection, early detection and prevention of the metabolic syndrome in children and adolescents is an urgent public health problem [8].

In its work providing comprehensive recommendations for cardiovascular diseases and reduction of risk thereof in children and adolescents, a group of experts has underscored the importance of preventing the development of risk factors (primary prevention) and cardiovascular disease in the future [11].

The recently published data from the study of prevalence of obesity in the population of the Russian Federation (main age groups, duration 1995 through 2019) has shown that on the level of the country's federal districts and constituent entities 15% of children aged 0–14 years are obese, and in the group of 15 through 17 years of age the figure is 7%. At the regional level, the largest proportion of obese children in the 0-14 years age group is in the Kaluga region (37%), Jewish Autonomous Region and Republic of Tyva (35%), and in the 15–17 years age group — Jewish Autonomous Region (14%), Republic of Tatarstan (13%) and Perm region (12%). The incidence of obesity in children aged 0 through 14 years has increased 4 times, from 367.6 per 100,000 population in 1995 to 1417.1 in 2019. Since 2002, the obesity indicator among adolescents aged 15–17 years has grown almost sixfold, which is a matter of great concern; in 2019, it has rapidly increased from 865.1 to 3411.7 per 100,000 population [5].

In 2007, the European regional office of the WHO developed the Childhood Obesity Surveillance Initiative (COSI), which aims to identify the causes of overweight and develop and implement dietary and physical activity guidelines for children of the school age. COSI is one of the largest population-based studies of overweight and obesity in this age group; it includes over 300,000 children from 38 European countries. Its Moscow part took place in 2017–2018 and included 2166 7-year-old children; the study revealed that 27% of boys and 22% of girls were overweight and 10% and 6%, respectively, — obese [12]. These data are consistent with the global trends that indicate both the growing prevalence of these conditions and their increasing registration in younger age groups [6].

Some authors state that the true prevalence of overweight and obesity in children is greater than what is officially reported [13]. They have also shown that childhood obesity is concomitant with poor bone metabolism and imbalanced bone formation and bone resorption processes. An examination of children in the Sverdlovsk region (Russia) has revealed that the prevalence of obesity as registered based on the preventive screenings is significantly higher than what is recorded in the official statistical reports that draw upon the data on people seeking medical assistance willingly. In particular, the prevalence of obesity in children of the 0–14 years age group in the Sverdlovsk region was 18.4% higher than generally in Russia, and for the 15–17 years age group this figure was 9.7% [14].

**Diagnostic criteria**

In 2007, the International Diabetes Federation (IDF) adopted new age-specific criteria for abdominal obesity and metabolic syndrome in children and adolescents. The criteria dictate diagnosing abdominal (visceral) obesity in the 6–15 years age group if the waist circumference (WC) is equal to or greater than the 90$^{th}$ percentile of the percentile distribution of this indicator. Metabolic syndrome is not diagnosed in the 6–9 years age group, but an obese (visceral obesity) patient with an aggravated family history of MS, type 2 diabetes mellitus, CVD (including hypertension) and/or obesity should be additionally examined and followed-up routinely [15].

In the 10–15 years age group, MS may be diagnosed if, in addition to abdominal obesity, at least two of the following criteria are met: TG ≥ 1.7 mmol/L, HDL < 1.03 mmol/L, BP ≥ 130/85 mm Hg, fasting glucose ≥ 5.6 mmol/L (or type 2 diabetes; Table 1). From 16 years on, the diagnosis of MS is established based on the same criteria as for adults [16].

Along with anthropometric parameters and physiological ranges of blood pressure, puberty significantly alters the fat distribution patterns, which is accompanied by the drop of the adiponectin level and insulin sensitivity by about 30% and additional growth of secretion of this hormone. Such transformations, most pronounced in the pubertal period of development, make interpretation of the laboratory-measured indicators of adolescents a complex matter. This complexity is one of the reasons why medical professionals cannot reach the consensus about what threshold values of certain parameters should be considered signs of MS criteria, especially in the view of differing diagnostic significance and contribution of each of the components [15].

In 2009, experts from the International Diabetes Federation (IDF), the National Heart, Lung and Blood Institute (NHLBI), the American Heart Association (AHA ), the World Heart Federation (WHF), the International Atherosclerosis Society (IAS), and the International Association for the Study of Obesity (IASO)

**Table 1.** Metabolic syndrome criteria for children and adolescents

| Age group (years) | Obesity (waist circumference) | Triglyceride level | HDL cholesterol level | Blood pressure (BP) | Glucose level or diagnosed type 2 diabetes |
|---|---|---|---|---|---|
| 6–9 | ≥ 90th percentile | Metabolic syndrome is not diagnosed, but family history of MS, type 2 diabetes, dyslipidemia, CVD, hypertension, and/or obesity call for additional examinations | | | |
| 10–15 | ≥ 90th percentile (adult criterion if lower) | ≥ 1.7 mmol/L (≥ 150 mg/dL) | < 1.03 mmol/L (< 40 mg/dL) | Systolic ≥ 130 mmHg or diastolic ≥ 85 mmHg | ≥ 5.6 mmol/L (100 mg/dL) (or diagnosed type 2 diabetes). If ≥ 5.6 mmol/L, an oral glucose tolerance test is recommended |
| 16 and older | Application of the criteria developed by the IDF for adults | | | | |

have developed unified criteria for diagnosing MS in adults and adolescents aged 16 and over [16]. These criteria factor in the scientific data accumulated to the moment. The consensus prescribes diagnosing the condition in case at least three of the below-mentioned criteria (Table 2) are met.

The Russian Metabolic Syndrome Clinical Guidelines developed in 2013 note that there are practically no prognostic data substantiating the benefits of various MS diagnosing criteria [3]. In this connection, it is obviously necessary to harmonize and adjust the existing diagnostic criteria for the conditions of the Russian Federation: factor in the ethnic and genetic differences of the Russian population, national nutritional characteristics, lifestyle and economic background in the state. The suggested key diagnostic criterion is the central (abdominal) type of obesity registered at > 80 cm WS in women and > 94 cm WS in men, with a number of additional criteria; the MS diagnosis is considered reliable when the patient exhibits signs of the key criterion and two additional criteria:

– blood pressure level > 140 and 90 mmHg or pharmaceutical treatment of hypertension;

– increased triglyceride levels (≥ 1.7 mmol/L);

– decreased HDL cholesterol levels (<1.0 mmol/L in men; <1.2 mmol/L in women);

– impaired glucose tolerance (IGT) — elevated plasma glucose level 2 hours after loading 75 g of anhydrous glucose with OGTT ≥ 7.8 and < 11.1 mmol/L, provided that the fasting plasma glucose is below 7.0 mmol/L;

– impaired fasting glycemia (IFG) — elevated fasting plasma glucose level ≥ 6.1 and < 7.0 mmol/L, provided that plasma glucose after 2 hours with OGTT is below 7.8 mmol/L;

– combined IFG/IGT — elevated fasting plasma glucose ≥ 6.1 and < 7.0 mmol/L in combination with plasma glucose after 2 hours with OGTT ≥ 7.8 and < 11.1 mmol/L [3].

Abdominal, or visceral obesity should be considered a canonical MS symptom. It has been shown that WC correlates more strongly with visceral adipose tissue than body mass index (BMI). Here, it is important to take into account a fact well-known in sports medicine: BMI depends, interalia, on the type of sport, the athlete's muscle mass and some other factors, which reduces its informativeness as an objective indicator.

A study that included 1037 boys and 950 girls (mean age — 11 years) undertook a stepwise multiple regression analysis of such variables as total cholesterol, triglycerides, high and low density lipoproteins and blood pressure and established that WS is the most significant predictor, regardless of gender [17]. Another study employed dual-energy x-ray absorptiometry (DXA) and showed that it is the WC that allows clear and accurate (87% accuracy for girls and up to 90% for boys) identification of children with low and high body fat mass [18].

In adults, WC is widely used as a diagnostic criterion to judge distribution of fat in the abdominal region, but in children this parameter can be influenced by growth and sexual development, which reduces the accuracy of the visceral adipose tissue assessment. Ethnicity also plays an important role in this context [19].

In 2020, a study that involved 113,453 normal weight children from eight countries (Bulgaria, China, Iran, Korea, Malaysia, Poland, Seychelles and Switzerland) aged 6 through 18 established reference values for the WC percentiles. The researchers have also confirmed that the 90th WC percentile can be used to predict cardiovascular risk in children of normal weight [20]. In 2021, same WC threshold values were recommended in the international consensus made by the experts in the pediatric metabolic fatty liver disease [21].

Metabolic fatty liver is closely associated with obesity, insulin resistance, dyslipidemia and other metabolic constituents of MS; it is often considered the "liver constituent" thereof. Hypertriglyceridemia and MS were shown to be independent factors associated with the development of non-alcoholic steatohepatitis, and hypertriglyceridemia is known to often manifest in top tier athletes, as it supports the body during intense training sessions. In addition to the accumulation of excessive amounts of triglycerides in the liver, induction of oxidative stress plays an important role in the non-alcoholic fatty liver disease (NAFLD) pathogenesis. This kind of stress often emerges against the background of extreme sports-related body overload. According to DIREG 2, a large-scale study, the frequency of NAFLD in Russia in 2007 was 27%, and in 2014 it rose up to 37.1%, which makes this condition the most common liver disease [22].

According to a number of authors, prevalence of NAFLD grows in parallel with the growth of prevalence of obesity

**Table 2.** MS criteria in adults and adolescents over 16 years of age [16]

| MS criteria | Indicators |
|---|---|
| Abdominal obesity | Critical value exceeded<br>WC based on ethnicity |
| Triglycerides | > 1.7 mmol/L |
| HDL cholesterol<br>male<br>female | <br>< 1.0 mmol/L<br>< 1.3 mmol/L |
| Arterial pressure | ≥ 130 / ≥ 85 mmHg |
| Fasting glucose | ≥ 5.6 mmol/L |

and MS [23]. An objective assessment is a complex issue, the complexity thereof backed by the limited individual value of the routine liver pathology laboratory diagnostics methods (determination of bilirubin, ALT, AST, g-GT, albumin, ferritin, complete blood count and INR), which are common for outpatient practice. For example, twofold increase of the ALT and AST levels is registered only in 30% of NAFLD patients, and the indicators correlate with the severity of the disease quite weakly. Therefore, this study cannot help establish the prognosis of metabolic steatohepatitis [24]. It is advisable to factor in the influence sports-associated loads have on the ranges of values of indicators measured with laboratory tests. National studies that involved large samples have shown that in sportsmen, reference ranges of the majority of the listed biochemical markers differ significantly from those peculiar to the regular people. Gender differences, and especially age differences, are also of great importance [25].

An analysis of systematic reviews available on PubMed, Scopus and Web of Science yielded 36 prospective studies (5,802,226 patients) that state the association of NAFLD with a moderate increase in the risk of fatal and non-fatal cardiovascular events [26].

Discussing the concept of MS as a complex of conditions predisposing to cardio-metabolic risk factors and the possibility of diagnosing the syndrome in pediatric practice, it is necessary to recognize the fuzziness of the criteria currently applied to children. This fuzziness is the reason behind the wide variation of the assessments of MS prevalence. Thus, in the papers published in 2014–2019 the prevalence of MS was assessed within the range from 0.3 to 26.4%, which, to a certain, is the result of the variety and reliability of the diagnostic criteria used [27].

It is also important to remember that a child, as he/she grows and develops, may meet the applicable criteria at one time and not meet them at another time, and it is not clear whether the recorded changes represent an improvement or deterioration in his/her health status. Nevertheless, there is conclusive evidence that childhood MS and childhood overweight are associated with a greater than 2.4-fold risk of developing MS in adulthood, and the possible future condition can be predicted from the age of 5 years [28]. A certain conformation of this statement can be found in a study that demonstrated the relationship between childhood MS and cardiovascular diseases in adults 25 years later [29].

As an individual component of MS, arterial hypertension has a number of clinical peculiarities in its course. The said peculiarities include frequently observed development of refractory hypertension, early damage to target organs, such as the development of left ventricular hypertrophy that quickly leads to myocardial dysfunction, renal hyperfiltration, decreased elasticity of the aorta and arteries. According to the ABPM data, hypertension patients with metabolic disorders, compared to hypertensive patients without such, are diagnosed with more pronounced disturbances in the blood pressure's circadian rhythm, higher rates of pressure load at night and increased variability [3].

According to some researchers, screening of children and adolescents for overweight and obesity should be complemented with screening for high blood pressure [30]. It is fairly well known that BP levels in children and adolescents are closely associated with age, sex, and body length. However, the wide variety of anthropometric data seen even within one age group, as well as gender differences, substantiate the need to use special centile tables based on the results of the relevant population (national) studies [31].

A 2016 study that involved not overweight children and adolescents aged 6–17 years from seven countries (China, India, Iran, Korea, Poland, Tunisia and USA) established the international reference BP percentiles depending on sex, age and height. These international reference BP values were taken as criteria for comparison of prevalence of elevated BP in children and adolescents [32]. In the same 2016, Russian cardiologists prepared clinical guidelines for arterial hypertension diagnosing and treatment in children and adolescents, which suggest using centile tables as criteria for assessing blood pressure values and factor in age, gender and height [33].

In September 2022 there was published a consensus prepared by a number of European associations that covered hypertension in children and adolescents. This paper recommended measuring blood pressure and interpreting the results taking into account the centile values for age, sex and height, and provided the criteria for assessing modifiable risk factors, including overweight, obesity, dyslipoproteinemia, hyperglycemia, and insufficient physical activity [34]. However, none of the above international and domestic consensus documents grades children's BP values factoring in their sports activities, although many millions of children and adolescents go in for sports worldwide. Moreover, various studies have convincingly shown the significant impact of excessive (professional) sports load on systemic blood pressure and the subsequent high risk of violations of its regulation, which can ultimately cause arterial hypertension and other cardiovascular events.

To a certain extent, the expediency of BP control and early detection of AH as an early marker of MS is confirmed by the data on pathophysiological role of insulin resistance and hyperinsulinemia in the development of endothelial dysfunction brought by an imbalance in the synthesis of vasodilators and vasoconstrictors, with subsequent development of hypertension [35, 36].

Many different markers synthesized in the body adipose tissue correlate with metabolic and cardiovascular risk factors; in particular, such are pro-inflammatory cytokines (TNFα, IL6) and adipokines (eg, adiponectin, leptin, chemerin). Measuring the level of of most of these adipokines is not yet part of routine laboratory examinations, however, a number of researchers suggest testing for adipokines and inflammatory markers in the context of basic examination of obese children and adolescents to assess the risk of cardio-metabolic diseases.

Only a few works describe the relationship between age, gender, specifics of pubertal development and the level of adiponectin in childhood. For example, the level of adiponectin in children was found to be higher than in adults, but during puberty it goes down significantly. It has also been shown that in children, adiponectin is negatively correlated with the body fat percentage, and significant weight loss during treatment of obesity is associated with the growing level of adiponectin and improving insulin resistance. This correlates with the results of studies in adults that describe the close relationship between adiponectin and body fat and insulin resistance [37].

A number of papers highlight the high percentage of detection of metabolic disorders and overweight, up to obesity, in young American football players. It should be noted that the desire to gain more muscle mass for a certain role on the field, which is typical for professional players, is not considered to be a significant risk factor, since young athletes, college students, do not have a clear role and change it during the season. Still, according to the surveys involving national samples, overweight and obesity are the conditions registered in them 45% and 42% more often, respectively [2, 38].

Compared to the general population, young rugby players (aged 9–14) in France, Europe grow overweight and obese more often. The conducted balance studies confirmed it is

the high mass of fat, not muscle, that is the primary cause of overweight in young athletes [39].

Overweight and obesity pose more risks for the health of young athletes than only the risk of MS. Several meta-analyses and original studies provide evidence that these conditions make young athletes more prone to trauma. Development of inflammatory reactions to injury is an important aspect, as proven by the high values of a number of cytokines (TNFα, IL1, IL6) and such markers as CRP and fibrinogen, which correlate with a high risk of obesity. The role of inflammatory reactions and endothelial dysfunctions, which, as mentioned above, are peculiar to MS, disallows excluding formation of a certain vicious circle brought by injuries in overweight athletes, this circle ultimately undermining both the athlete's health and his/her sports performance [6, 30, 40].

Discussion of the pathophysiological processes that condition development of MS necessitates recalling the hypothesis of chronic stress. Studies of different years allow a high degree of certainty in considering the physiological role nonspecific chronic stress reaction plays in energy support of specific adaptive components. One of such studies suggests differentiating between energy-tropic and trophotropic stages of chronic stress, with further subdivision of the stages into phases: intense adaptation, relative compensation, and decompensation [41]. The energy-tropic stage, diagnosed, for example, in children with intrauterine malnutrition, as well as in older children and adults, often transforms into the trophotropic stage, which is accompanied by the development of obesity, diabetes mellitus, CVD and especially often — arterial hypertension. According to many researches, the latter group of diseases constitutes the metabolic syndrome. Thus, this hypothesis suggests interpreting MS as a trophotropic stage of chronic stress [42, 43]. It should be noted that young athletes undergo significant pubertal transformations that make them especially vulnerable to the negative impact of stress factors. However, the results of studies investigating the relationship between MS, chronic stress and sports loads have not yet been published.

## CONCLUSION

The problem of MS is obviously relevant to the sports medicine; further targeted research is needed to develop diagnostic criteria for this condition and methodological approaches to its management tactics applicable to children and adolescents, such criteria and approaches factoring in not only age and gender differences but also the specifics of the practiced sport through the lens of pubertal development. The results of such research would allow adjusting the MS diagnostic algorithm proposed in the literature to the characteristics of children and adolescents involved in sports, with the appropriate standard and reference values of the parameters that, as a rule, are looked into as part of in-depth medical examinations. Practical implementation of this algorithm can fundamentally improve the quality of early diagnosis and treatment and prevention programs developed for this syndrome.

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# PARTICULARITIES OF CURATION OF ATHLETES WITH PROTRACTED COURSE OF COVID-19

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This paper reviews publications covering the delayed clinical and functional manifestations of SARS-CoV2 among athletes competing at national and international levels; we describe the prevalence of multiorgan failure associated with protracted COVID as registered in sportsmen and people not going in for sports. The review reports the results of a retrospective analysis of data yielded from clinical, instrumental and laboratory tests undertaken by the Russian national team athletes that had COVID-19. We highlight the most informative indicators that reflect the condition of sportsmen with protracted coronavirus infection course, define the approaches making resumption of active training  safe and compile the list of the most significant criteria supporting admission to such training and competitions. Lastly, the paper presents the parameters subject to inclusion in the prognostic model (binary logistic regression) describing the dynamics of residual multiorgan failure in athletes, including minors, who have had COVID-19 or viral pneumonia of a different etiology.

**Keywords:** elite sports, elite athletes, SARS-CoV2 coronavirus infection, post-Covid syndrome, cardiovascular system, myocarditis in sports, multisystem inflammatory syndrome, binary logistic regression

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# ОСОБЕННОСТИ КУРАЦИИ СПОРТСМЕНОВ ПРИ ЗАТЯЖНЫХ ВАРИАНТАХ ТЕЧЕНИЯ COVID-19

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Представлен обзор публикаций, посвященных отсроченным клиническим и функциональным проявлениям коронавирусной инфекции SARS-CoV2 в контингенте спортсменов национального и международного уровней; дана характеристика распространенности полиорганных поражений при затяжном течении коронавирусной инфекции среди спортсменов, а также лиц, не занимающихся спортом. Описаны результаты ретроспективного анализа данных клинико-инструментального и лабораторного тестирования спортсменов сборной команды России, перенесших COVID-19. Выделены наиболее информативные показатели, отражающие состояние спортсменов с пролонгированным течением коронавирусной инфекции; определены подходы к безопасному возобновлению спортивной деятельности, сформирован перечень наиболее значимых критериев допуска к тренировочному и соревновательному процессу. Определены параметры, подлежащие включению в прогностическую модель (бинарную логистическую регрессию) динамики резидуальных полиорганных нарушений у спортсменов, в том числе несовершеннолетних, перенесших коронавирусную инфекцию или вирусную пневмонию иной этиологии.

**Ключевые слова:** спорт высших достижений, элитные спортсмены,  коронавирусная инфекция SARS-CoV2, постковидный синдром, кардиоваскулярная система, миокардит в спорте, мультисистемный воспалительный синдром, бинарная логистическая регрессия

**Финансирование:** статья подготовлена в рамках прикладной научно-исследовательской работы «Изучение влияния новой коронавирусной инфекции COVID-19 на здоровье и функциональное состояние высококвалифицированных спортсменов и разработка методических рекомендаций по обследованию спортсменов, включая несовершеннолетних, по допуску спортсменов к тренировкам и соревнованиям после перенесенного заболевания COVID-19, по восстановлению их здоровья и функционального состояния» (шифр «COVID-22»),  выполняемой ФГБУ ФНКЦСМ ФМБА России по государственному контракту №107.003.22.14 от 25 июля 2022 г.

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The effect of COVID-19 reverberates through all aspects of our lives, including sports, a socially significant area. In addition to the risks associated with the severity of acute symptoms and the possibility of complications, SARS-CoV2 often entails longterm consequences that may hinder professional activities of recovering patients, including athletes competing at national and international levels, or elite and sub-elite athletes, in the terminology of the IOC. Mitigation of these risks requires complex (multidirectional) measures taken not only during the acute course of the disease [1], but also generally when there is a risk of an epidemic and when the patient is followed-up after recovery and resumes training. Currently, seeking to develop an effective tool preventing severe delayed effects of SARS-CoV2, researchers continue searching for early markers of both latent and persistent damage to various functional systems. These efforts are especially significant in the field of sports and, above all, for young athletes. First of all, it is not fully understood how high-intensity physical exertion affects the course of coronavirus and other viral infections, which disallows identification of the risk group in the cohort of seemingly healthy individuals with high functional state characteristics and effective adaptive and compensatory mechanisms. In addition, there are no well-described concrete options of prognosis of COVID course and outcomes in athletes, and there is no specific data on the most probable risks of complications depending on age, gender and type of sports activity, on the severity and nature of the clinical course of the disease. All these aspects add urgency to the investigation of coronavirus infection's effect on the health of athletes and search for predictors of complications (including their delayed varieties) manifesting, first of all, in the cardiovascular system.

## COVID-19 course variants

The WHO defines COVID-19 as a disease manifesting as a severe acute respiratory syndrome caused by the SARS-CoV2 coronavirus. The infection caused by the new virus was first reported on December 31, 2019, and on March 11, 2020 the WHO announced the pandemic [2].

Initially, COVID-19 was considered an acute infection with full resolution of manifestations (mildly and and moderately severe) within 2-3 weeks. However, over time, there appeared a growing body of evidence confirming symptoms persisting for a longer time, up to 6 months or even longer.

On October 30, 2020, National Institute for Health and Care Excellence (NICE) suggested differentiating between following forms of COVID-19:

1) acute COVID-19, with subjective and objective manifestations lasting up to 4 weeks;

2) ongoing symptomatic COVID-19, lasting from 4 to 12 weeks;

3) post-COVID syndrome [3].

*Definition of post-COVID syndrome*

Initially, the term "post COVID-19" was introduced to describe the condition that typically develops in people with suspected or confirmed SARS-CoV-2 3 months after the onset of the disease, with symptoms that cannot be attributed to another disease lasting at least 2 months [3]. The ICD-10 code U09.9 for post COVID-19 condition was legitimized in September 2020; in ICD-11, it is RA02, currently used to describe only unspecified conditions. The respective complex of symptoms complex may manifest after acute form of COVID-19 or persist post recovery. The most common symptoms are fatigue, shortness of breath, cognitive dysfunction etc.; as a rule, they complicate daily activities and may change over time or recur [4].

## Prevalence of multiple organ symptoms of the post COVID-19 syndrome

According to a statistical review and meta-analysis of 60 studies that jointly included 257,348 people, male patients tend to suffer the post COVID-19 syndrome more often than female, and the symptoms may recur in patterns [5]. Table 1 presents the predominant attributes of the syndrome.

## Cardiac manifestations of SARS-CoV-2 infection and post COVID-19 syndrome

The current and expected state of the cardiovascular system (CVS) largely conditions achievements in sports, cyclic and competitive in particular; CVS is one of the most significant, but at the same time vulnerable systems ensuring general and special physical performance, which means that effectiveness of the CVS is one of the most important factors shaping athletic performance [6], when the former is reduced, the latter is limited, with ensuing prospect of further professional growth, including the risks of premature termination of sports career or development of catastrophic cardiac events, i.e. sudden cardiac death, or SCD. A past coronavirus infection in the background may complicate differential diagnosis of adaptive remodeling, identification of signs of stress cardiopathy and post-inflammatory myocardial damage [7, 8].

Our preliminary observations (data from the Ogarev University Medical Institute) show that hearts of almost 40% of athletes who had COVID-19 have underwent persistent changes, both morphometric (heart size) and functional (left ventricular myocardial contractility), as registered with echocardiography (Figure 1).

In the contingent of athletes we observed, the signs of SVR maladaptation registered in the controlled exertion tests (decreased tolerance to controlled physical load, hemodynamic shifts, electrical instability of the myocardium) were virtually consistent but significantly less stable.

The laboratory indicators that are diagnostically significant in the cases of myocardium inflammation and stress-induced SVR changes also varied significantly in their intensity and stability, as shown by the biochemical markers of myocardial damage. Compared to their less trained fellows, professional athletes with signs of maladaptive SVR remodeling more often had higher levels of cardiac fraction of creatine phosphokinase and troponin I: 6.3% vs 27%, respectively ($p < 0.05$). In addition, 65% of the observed athletes had the cortisol levels going up during onset of COVID-19, with their subsequent return to the reference values in 55% of them. In 80% and 58% of the athletes, we registered pronounced COVID-19-induced changes in the levels of CPK and LDH (recovered subsequently in 65% and 60% of them).

The mentioned markers can reflect not only the load on the mechanisms supporting the current state of myocardial function, but also predict the vector of subsequent changes with a certain degree of probability (factoring in the dynamics recorded in a month or more after verification of the disease).

Manifestations of the current long-term variants of COVID-19 and post COVID-19 syndrome vary (intra-group differences) and often have a wavy pattern, which translates into a wide range of manifestations and their associations, as well as heterogeneity of dynamics. It should be clarified that the data on the prevalence of prolonged Covid-19 is currently

**Table 1.** The frequency of occurrence of signs of post COVID-19 syndrome (according to Alkodaymi et al.)

| Observation period / Sign | 3–6 months | 6–9 months |
|---|---|---|
| General and pulmonary manifestations | | |
| Fatigue | 32% (*n* = 7268, 25 studies) | 36% (*n* = 8191, 19 studies) |
| Shortness of breath | 25% (*n* = 8132, 28 studies) | – |
| Cough | 15 % (*n* = 7539, 22 studies) | – |
| Alopecia | 9% (*n* = 478, 4 studies) | 10% (*n* = 4276, 5 studies) |
| Neuropsychic manifestations | | |
| Sleep disorders | 24% (*n* = 4369, 8 studies) | 29% (*n* = 242000, 12 studies) |
| Anxiety | 21% (*n* = 4324, 7 studies) | 23% (*n* = 240756, 7 studies) |
| Depression | 14% (*n* = 4099, 5 studies) | 23 % (*n* = 4377, 6 studies) |
| Clouded sensorium | 22% (*n* = 466, 5 studies) | 22 % (*n* = 854, 4 studies) |
| Cognitive disorders | 14% (*n* = 670, 6 studies) | 15% (*n* = 1987, 5 studies) |
| Headache | 12% (*n* = 5699, 12 studies) | 14% (*n* = 7170, 13 studies) |
| Anosmia | 9% (*n* = 5400, 16 studies) | 15 % (*n* = 6596, 17 studies) |
| Ageusia | 8% (*n* = 5127, 13 studies) | 13% (*n* = 6505, 16 studies) |
| Cardiovascular manifestations | | |
| Exercise intolerance | 19% (*n* = 5203, 6 studies) | 45% (*n* = 850, 5 studies) |
| Rapid heartbeat | 14 % (*n* = 5401, 8 studies) | 14% (*n* = 4735, 7 studies) |
| Chest pain | 11 % (*n* = 5758, 15 studies) | 12% (*n* = 4318, 10 studies) |
| Musculoskeletal system disorders | | |
| Joint pain | 14% (*n* = 4829, 8 studies) | 23% (*n* = 5288, 8 studies) |
| Myalgia | 12% (*n* = 5453, 10 studies) | 19 % (*n* = 3490, 9 studies) |
| Gastrointestinal tract disorders | | |
| Diarrhea | 10% (*n* = 4908, 7 studies) | 5% (*n* = 3318, 8 studies) |
| Nausea | 8% (*n* = 480, 3 studies) | 4% (*n* = 3419, 8 studies) |

insufficient, but, judging by a number of studies, more than 144 million people live with this condition in the world, and most of them suffer from symptoms that generally affect the quality of life and functional status negatively [9].

The echocardiographic evidence from our study correlates, to a certain extent, with the previously registered COVID-19 consequences, including autonomic dysregulation associated with aortic rigidity, as well as ventriculo-aortic insufficiency and left ventricular dysfunction, all seen even 6 months after discharge from the hospital. These disorders may be caused by prolongation of the infection [10].

The published data also suggest that vaccination of people with post COVID-19 syndrome can prevent progression of persistent symptoms. In some cases, specific immunoprophylaxis can even completely eliminate the symptoms, with the probable explanation therefor being the cumulative protective effect of multiple vaccinations in the context of prevention of recurring viral infections. In UK case-control study that involved over 4,000 patients, two doses of the vaccine reduced the prevalence and severity of persistent cardiac manifestations of COVID-19 (compared with the unvaccinated) and often made the course asymptomatic [11].

The TriNetX retrospective cohort study undertaken in the US gave similar but more significant results. The researchers examined over 81 million electronic medical records seeking to evaluate the delayed complications of COVID-19, i.e. those identified 6 months after confirmed SARS-CoV-2 infection, and, in particular, uncover the probable effect the number of vaccinations (1 vs 2) has thereon. A single dose of vaccine was associated with a lower subsequent risk of admission to the intensive care unit, intubation or lung ventilation, hypoxemia, oxygen demand, respiratory failure, hypercoagulopathy, venous thromboembolism, seizures, psychotic disorders and hair loss; for most of such outcomes, the risk was even lower for the revaccinated, who, possibly, have the advantage of a less severe course and smaller chance of re-infection [12]. Another study was conducted in France; it involved 910 patients already diagnosed with long COVID-19 and found that vaccination translated into a milder post COVID-19 syndrome and improved quality of life 2 months after administration of the vaccine. In effect, vaccination doubled the frequency of remission of the persisting symptoms [13]. However, such studies need to be continued.

There is also a certain shortage of publications documenting aggravation of cardiovascular dysfunction in patients with post COVID-19 syndrome that developed against the background of chronic diseases of the CVS. To a large extent, the reason behind this situation lies in the insufficiency of understanding of how SARS-CoV-2 itself and the acute pathological processes it initiates affect functioning of the CVS and the progression of its chronic disorders.

In case of COVID-19, most medical interventions are trials designed to compensate and prevent aggravation of acute clinical manifestations and to prove the efficacy of drug therapy. In the field of cardiology, research efforts were mainly aimed at finding cellular mechanisms driving the said manifestations and, accordingly, at developing sound diagnostic and therapeutic strategies. The respective studies relied on the activities of multicenter/national research groups (e.g., the European Society of Cardiology), which prepared a scientific statement on COVID-19-related cardiovascular complications, covering myocardium and pericardium diseases. Then, the focus shifted to research projects dedicated to promotion of active lifestyle, which

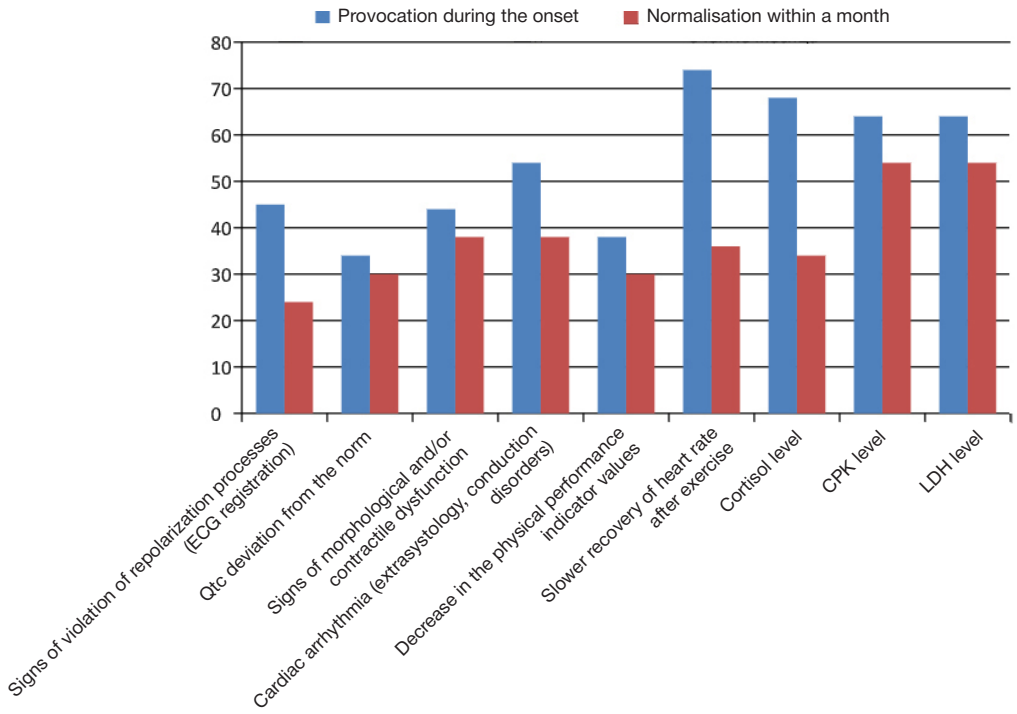


**Fig**. Dynamics of the CVS clinical and laboratory markers in athletes who had coronavirus infection

allows realization of the preventive aspects of measures taken to counter chronic pathology. In view of the duration of the COVID-19 pandemic and the probabilistic risks of other global shocks caused by viral invasion, it is necessary to widely implement national protocols aimed not only at elimination of the immediate threats, but also at creation of long-term behavioral determinants that reduce the prevalence of CVD [14]. In our opinion, predictive models based on the mathematical analysis of valid indicators reflecting the state of essential systems of the body can play a significant role in increasing the effectiveness of this activity of the world community.

### Manifestations (reflected in instrumental and laboratory tests) confirming a SARS-CoV-2 and post COVID-19 syndrome diagnosis

In some COVID-19 patients, cardiac symptoms (e.g., chest pain, shortness of breath, fatigue and palpitations) persist for several months after the disease, but there is also another scenario: gradual, prolonged manifestation of signs of damage to the organs, including myocardium, that were detected only with instruments and laboratory testing [15–19].

One of the main problems is the development of myocarditis, since its course can be asymptomatic or subclinical but it still remains arrhythmogenic, which translates into a pronounced risk of sudden cardiac death [20, 21]. Moreover, physical exertion during the acute phase of myocarditis can aggravate myocardial damage and trigger progression to fatal arrhythmias [20–23]. In this connection, there have been developed the Return to Play recommendations that suggest the routine of resuming sports activities after coronavirus infection [24, 25].

In addition, it is acknowledged necessary to differentially diagnose inflammatory myocardial damage and its sports remodeling [20, 22].

A prospective multicenter observational study that involved over 19000 US athletes included a cohort of SARS-CoV-2-positive patients ($n$ = 3018) that had their CVS examined. ECG revealed changes in 0.7% (21/2999) of them, EchoCG — in 0.9% (24/2556) [26].

A systematic review analyzed data from 16 studies, which involved 890 COVID-19 convalescents; 14% of them were diagnosed with myocarditis, and late gadolinium enhancement (LGE), a contrast technique for MRI, allowed detecting myocardial fibrosis in 20.5% of the examined [22]. Among the sample, 35.5% were athletes, and 17.1% had documented MRI-registered changes, including myocarditis in 2.5% of cases, while among people not going in for sports the results were 62.5% and 23.9%, respectively [27].

Another, more representative review presents an analysis of articles that cover data on 3131 athletes who had COVID-19. In the studies, from 0 ti 15% of the athletes were diagnosed with myocarditis, pericardial effusion was the diagnosis in 0–58% of cases and MRI later contrast enhancement allowed detecting changes in 0-46% of the participants [28]. Obviously, the significant differences result from the different inclusion criteria (e.g., timing of examinations considering the time of confirmation of the infection status), lack of a control group and various results assessment methods, although MRI is the most sensitive and specific non-invasive method for diagnosing myocarditis in any category of persons, including those who had COVID-19. The diagnostic value of the method is believed to grow 1–3 months after the disease [29].

**Table 2.** The degree of risk of COVID-19-induced CVS damage when the changes are detected with instruments and laboratory tests

| Sign risk | Reduced performance | High CPK level | Rhythm and conduction disturbances | Increased cortisol level | Heart rate recovery time extension against load |
|---|---|---|---|---|---|
| Relative, cu | 3.75 | 2.34 | 3.77 | 2.42 | 3.86 |
| Absolute, cu | 0.6 | 0.22 | 0.38 | 0.35 | 0.58 |

MRI-detected changes — focal myocardial fibrosis, both post-myocarditis and post-ischemic, — are quite common among elite athletes (24–38% of cases); these manifestations are not associated with coronary pathology, and it is necessary to monitor the dynamics as they can trigger life-threatening arrhythmias [30-32].

A small study [33] that involved 26 athletes presents, as we believe, gives data closest to the real values. The athletes had COVID-19 and underwent cardiac MRI thereafter: 4 (15.4%) of them showed signs of myocarditis, 8 (30.8%) had late contrast enhancement without increased T2, which indicates previous damage/fibrosis of the myocardium. Overall, among the recovering athletes, MRI signs of myocarditis in combination with other criteria allowing establishing the respective diagnosis, were registered in 0 — 2.3–7.6% of cases, with other signs — pericardial effusion, myocardial contractility disorder, abnormal movements of walls or septum — detected more often [34–38]. It was established that myocarditis is more common in male athletes and in persons under 21 years of age with COVID-19-associated multisystem inflammatory syndrome (MSIS) [26, 35, 36, 39–43].

Daily monitoring with a Holter monitor uncovered supraventricular and ventricular extrasystoles in athletes ($n$ = 90) who had asymptomatic or mild COVID-19 (53.3% and 52.2% of cases, respectively) [44].

The level of troponin, one of the biochemical markers of myocardial damage, exceeded the upper limit of the reference range in 0.9% (24/2719) of athletes, but a clear connection between biomarker levels and changes detected with EchoCG and MRI was not reported. There is a number of other studies [26, 28, 33, 34, 45] that also mention no such connection, which once again confirms the problematic nature of differential diagnosis of post COVID-19 changes of the myocardium and its transformation caused by sports loads.

Thus, there are very diverse data on myocardial changes in athletes after COVID-19. Young athletes, in general, tend to run a lower risk of cardiovascular complications; some athletes may have no clinical symptoms of involvement of heart in the pathological process [27, 28]. Most authors agree that subjective manifestations (complaints of weakness, fatigue, poor performance) are not typical for recovering athletes. Patients with increased troponin levels, ECG abnormalities that suggest myocarditis (diffuse inversion of the T wave, ST segment elevation without reciprocal depression, expansion of the QRS complex), and/or echocardiographic anomalies typical of myocarditis (ventricular wall movement anomalies, decreased myocardial contractility, pericardial effusion, ventricular dilation, abnormal ventricular tension) are referred to a cardiologist for consultation and, with respective indications, MRI scanning [34]. In the context of detection of myocardial electrical instability and arrhythmias, Holter monitor is more significant diagnostically than standard ECG; EchoCG in combination with biomarkers (troponin I/T) provides the maximum amount of information relevant to the diagnosis of myocardial damage in athletes that had COVID-19.

Thromboembolic complications are registered in 27–31% of COVID-19 patients [46]. Factoring in the risk of coagulopathies, some cases require determining the parameters of clotting (D-dimer, international normalized ratio (INR), activated partial thromboplastin time (APTT), prothrombin time) [24, 45]. Before the COVID-19 pandemic, D-dimer level above 400 ng/ml was registered in 7.9% of rugby players, and the median was 231 ng/ml (215; 270); coronavirus infection doubled the number of such cases: D-dimer above 400 ng/ml is seen in 17.3% of cases, and the median has grown to 270 ng/ml (215; 318) [47]. At the same time, it should be remembered that sports injuries inherent in rugby can also drive the D-dimer level up [47].

A variety of psychological and autonomic disorders, the symptoms of asthenia, are also typical manifestations of the post COVID-19 syndrome [48, 49].

Athletes may experience post-exercise weakness resembling chronic fatigue or fibromyalgia [50]. Some sportsmen complain of shortness of breath and palpitations, including when upright, which requires checking for the postural tachycardia syndrome [51].

It should be noted that organizational approaches to diagnostic search in the sample of athletes with cardiac symptoms differ from the those practiced generally, which are sufficient for verification of the post COVID-19 syndrome. The objective characterization of the type and severity of clinical manifestations is the decisive component of the diagnosing, and patients with suspected cardiovascular system damage mandatorily undergo the following tests:

1) basic laboratory testing (full blood count, basic metabolic panel, troponin T, C-reactive protein tests);
2) ECG;
3) EchoCG;
4) Holter monitoring;
5) comprehensive assessment of the state of respiratory system; checks for orthostatic hypotension and postural tachycardia are also recommended [34].

A consultation with a cardiologist is recommended to patients (including recovering patients) that:

1) have abnormal results of cardiological testing;
2) have a documented pathology of the CVS with newly emerged or worsening symptoms;
3) have confirmed COVID-19-induced cardiac complications;
4) have cardiac and/or respiratory symptoms that cannot be explained in any other way [34].

## Methodological aspects of safe return to professional sports

Currently, there are various clinical recommendations regulating resumption of physical activity after a coronavirus infection. All the adopted protocols stratify athletes in accordance with the course of the disease. For example, for asymptomatic COVID-19 the recommendation is to refrain from physical exertion for at least 2 weeks, the monitoring method of choice is ECG at rest, values beyond the normal range therein is a reason for EchoCG and exercise tests as a minimum [52, 53].

A similar, that is, rather conservative, but at the same time more multimodal approach to assessment of the possibility of resumption of training activities is being developed by the American College of Cardiology [34, 52, 54]. This approach includes the so-called "triad of tests": 12-lead ECG, EchoCG, cardiac troponin level measurement (by a highly sensitive method). However, almost in all cases it is the exercise testing that is crucial for the decision to resume or refrain from training activity; this is the position of the cardiological expert communities of the West as consolidated in 2015 [56].

The efficacy of protocols that include MRI requires a separate discussion: it enables verification of damage to the myocardium and pericardium associated with COVID-19 [33, 36, 37, 39], but the analysis of data from the American Professional cohort ($n$ = 789) and the multicenter register of cardiac outcomes in athletes ($n$ = 3018) demonstrated low detection of myocarditis in athletes (0.6–0.7%) [26, 40]. One of the subsequent studies aimed at summarizing the data on the diagnostic significance of MRI screening among sportsmen

showed the that myocarditis occurred in 2.3% of cases [35]. Experts of the European Association of Preventive Cardiology (EAPC) recognized troponin level growth as an indication for a heart MRI with contrast [23]; for athletes, admission to a hospital or documented heart damage should call for expansion of the diagnostic methods with primarily biochemical tests (brain natriuretic peptide and its terminal fragment) [56].

In other words, professional communities have agreed on the need for triad screening regardless of the nature of symptoms. In addition, they deemed expedient to monitor athletes under the age of 21 after a coronavirus infection; observation lasting up to 8 weeks can enable timely diagnosis of MSIS [55].

In general, in case of myocarditis it is necessary to follow the previously adopted recommendations, since currently there is no evidence that myocarditis associated with COVID-19 clinically and pathophysiologically differs significantly from its other forms [47]. Resumption of training is recommended after 3-6 months, with the function of the left ventricular and levels of cardiac biomarkers back to normal, no clinical manifestations, Holter monitoring and ECG-registered disorders in the context of exercise tests [23, 56].

**The approaches to building a predictive mathematical model based on the valid predictors of multi-organ lesions peculiar to long COVID-19**

Forming the approaches to predicting the levels of risks associated with coronavirus infection, we used methods of multidimensional statistical analysis, binary logistic regression in particular. Logistic regression is a type of multiple regression enabling analysis of the relationship between several independent variables (also called regressors or predictors) and a dependent variable. Binary logistic regression is used when the dependent variable is binary (that is, it can take only two values).

The necessary calculations were carried out as part of the retrospective analysis of medical records of 59 National team athletes that had COVID-19; they underwent in-depth medical examinations seeking readmission to sports activities six months after resumption of the training process. The characteristics meeting the requirements for inclusion in the mathematical model and thus most promising were: exercise tests aimed at registration of performance deterioration, heart rate recovery time extension, episodes of increased levels of CPK and cortisol (Table 2).

Such factors as reduced physical performance and extended post-exercise recovery period corresponded to a higher level of absolute risk. The possible explanation for the slower dynamics of restoration of performance during the recovery period lies in the resistance of disorders of recovery processes associated with the viral invasion (figure 1).

CONCLUSION

The published papers report a wide range of frequency of occurrence of COVID-19-induced CVS damage in the recovering athletes, with those under the age of 25 running a lower risk of cardiac symptoms associated with coronavirus than their older counterparts.

Most athletes that recovered from asymptomatic or mild COVID-19 had the biomarkers of myocardial damage, as well as indicators of ECG and EchoCG within the reference values range. If the athlete has complaints of cardiac profile and/or suffered the diseases in moderate and severe forms, the decision on resuming sports activities can be made only based on the data of cardiological examination, including, as a minimum, ECG, EchoCG and troponin level measurement.

The following diagnostic criteria applied to gauge the post-COVID-19 damage to the myocardium are the most informative: diffuse inversion of the T wave, ST segment elevation without its reciprocal depression, expansion of the QRS complex, new non-physiological ECG patterns; among the indicators registered with EchoCG — anomalies of the left ventricular wall movement, decreased myocardial contractility, ventricular dilation, abnormal ventricular tension, pericardial effusion, new disorders and aggravation of the existing ones.

The inclusion of these indicators in a mathematical model — logistic regression — opens up prospects for predicting the risks of post COVID-19 syndrome and multi-organ lesions.

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# SIBLING POSITION AS A CONDITION FOR THE FORMATION OF SOME FORMAL AND DYNAMIC FEATURES OF ATHLETES

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An athlete's performance is a major issue of the elite sports. The current studies are focused on various factors of success, however, the effect of sibling position on the athlete's personality formation is poorly understood. The study was aimed to assess the correlation of the formal and dynamic features with the resource potential in athletes who played badminton based on their sibling position using the structured interviews; Questionnaire of Formal and Dynamic Properties of Personality by V.M. Rusalov; Perinatal Experience – Resource Potential test by N.P. Kovalenko; correlation analysis. A total of 40 athletes were enrolled (20 boys and 20 girls; average age 14.5 year). In the "eldest or only child" subsample, the athletes' communicative plasticity negatively correlated with the parameters of sensory and physiological systems ($r$ = –0.50), as well as stress tolerance ($r$ = –0.60). Correlations between the stress tolerance and psychomotor ergicity ($r$ = 0.63), etc., were revealed. The athletes of the "second or later-born child" subsample had higher psychomotor plasticity and a larger resource potential ($p < 0.05$). The basic perinatal matrix is integrated into the formal and dynamic structure of the athlete's personality. Thus, athletes of the "second or later-born child" subsample have a higher resource potential. Athletes of the "eldest or only child" subsample show lower psychomotor plasticity, however, they are more tenacious in fulfilling their goals. The areas of concern for the sports psychologist who works with athletes are highlighted in accordance with the results.

**Keywords:** athletes, sibling position, basic perinatal matrices, resource potential, communicative emotionality, communicative plasticity

**Author contribution:** Bogun TV — data acquisition, study concept and design, manuscript writing, literature review, data interpretation; Rakitina OV — literature review, planning the empirical phase of the study, analysis and interpretation of the results, editing; Gornov SV — editing, approval of the final version of the article, general management.

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# СИБЛИНГОВАЯ ПОЗИЦИЯ КАК УСЛОВИЕ ФОРМИРОВАНИЯ НЕКОТОРЫХ ФОРМАЛЬНО-ДИНАМИЧЕСКИХ ОСОБЕННОСТЕЙ СПОРТСМЕНОВ

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В спорте высших достижений проблема результативности спортсмена — одна из ключевых. В современных исследованиях уделяют внимание различным факторам успешности, но остается неизученным влияние сиблинговой позиции на формирование личности спортсмена. Целью работы было изучить взаимосвязь формально-динамических свойств и ресурсного потенциала спортсменов, занимающихся бадминтоном, в зависимости от их сиблинговой позиции с использованием структурированного интервью; «Опросника формально-динамических свойств индивидуальности» В. М. Русалова; теста «Перинатальный опыт — ресурсный потенциал» Н. П. Коваленко; корреляционного анализа. В исследовании участвовало 40 спортсменов (20 юношей и 20 девушек; средний возраст — 14,5 лет). В подвыборке спортсменов «старший или единственный ребенок» коммуникативная пластичность отрицательно коррелирует с показателями сенсорных и физиологических систем ($r$ = –0,50) и стрессоустойчивостью ($r$ = –0,60). Выявлены корреляции между стрессоустойчивостью и психомоторной эргичностью ($r$ = 0,63) и др. Спортсмены подвыборки «второй и последующие дети» обладают большей психомоторной пластичностью и большим ресурсным потенциалом ($p < 0,05$). Базовая перинатальная матрица интегрирована в формально-динамическую структуру личности спортсмена. Таким образом, спортсмены подвыборки «второй и последующие дети» обладают более высоким ресурсным потенциалом. Спортсмены подвыборки «старший или единственный ребенок» менее психомоторно пластичны, но более упорны в достижении целей. В соответствии с результатами обозначены направления работы спортивного психолога со спортсменами.

**Ключевые слова:** спортсмены, сиблинговая позиция, базовые перинатальные матрицы, ресурсный потенциал, эмоциональность коммуникативная, пластичность коммуникативная

**Вклад авторов:** Т. В. Богун — сбор материала, дизайн и концепция исследования, написание статьи, обзор литературы и интерпретация данных; О. В. Ракитина — анализ литературы, планирование эмпирического этапа исследования, анализ и интерпретация результатов, редактирование; С. В. Горнов — редактирование, утверждение окончательного варианта статьи, общее руководство.

**Соблюдение этических стандартов:** исследование проведено в соответствии с методическими рекомендациями «Организация и проведение психофизиологических обследований спортсменов сборных команд России в рамках углубленных медицинских осмотров», утверждено Ученым советом ФГБУ «Федеральный научно-клинический центр спортивной медицины и реабилитации ФМБА России» (протокол № 4 от 3 июня 2016 г.), а также в соответствии с методическими рекомендациями «Оценка психологического состояния высококвалифицированных спортсменов при проведении УМО», утверждено Ученым советом ФГБУ «Федеральный научно-клинический центр спортивной медицины и реабилитации Федерального медико-биологического агентства» (протокол № 16 от 29 марта 2018 г.); все участники подписали добровольное информированное согласие на участие в исследовании.

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Athletic performance is a major issue of sports psychology. The current studies are focused on such factors of success in elite sports, as the athlete's age, morphological features of his/her body [1, 2], features of birth [3], influence of the family, personality traits, societal attitudes [4], etc.

However, among various papers on elite sports, no reports taking into account the impact of sibling position on the athlete's personality formation in general and specifically on his/her athletic performance have been found. At the same time, there are papers not related to sports psychology that are focused on the rather thorough investigation of the family relationships taking into account the sibling positions of children and adults during the study of personality. Thus, in perinatal psychology, extensive theoretical and empirical experience of studying the impact of the features of birth on the individual's mental and physical development has been accumulated. The papers by the leading experts in perinatal psychology [5–10] provide the review of various areas and directions of perinatal psychology, which include not only such conventional areas, as perinatal medicine (fetal diagnosis and therapy) [8]; adaptability theory; concept of instincts and psychosomatic disorders [7]; Jungian analysis [9]; but also a rather new area, the concept of perinatal matrices [9].

Perinatal matrix is an information unit of the personal unconscious associated with birth experience [9]. Perinatal experience is manifested in three patterns of experiences, basic perinatal matrices (BPM), which correspond to four clinical stages of birth [5]. The birth is first and foremost overcoming the barriers. Successful birth outcome reinforces the motor patterns of birth as a certain scheme which leads to success, including, as we assume, success in sports.

Thus, birth experience represents the moment when intrinsic behavioral acts are formed, which are related to mobilization of the individual's internal resources directed towards overcoming the external and internal conflicts and realization of activity program, including that of sports activity [3].

According to the BPM concept, the resource potential of personality is an important construct. The resource potential is a combination of the subject's qualities or a special integral quality that performs the function of the subject's "realization", i.e. determines and ensures the effectiveness of implementing all kinds of activity in certain objective conditions [11].

The concept of sibling position (siblings or sibs are brothers and sisters born in the same family) includes formal characteristics of the individual's circumstances of birth: birth order, gender, and spacing between births. The first idea that the child's personality depends on his position among brothers and sisters belongs to A. Adler. He discussed this matter within the framework of the area of individual psychology he had developed and claimed that birth order was an important factor of personality development that determined the individual's personality characteristics in childhood and adulthood [12]. The hypothesis of the study is as follows. It is assumed that formal and dynamic features, as well as the resource potential of athletes playing badminton significantly correlate with their sibling positions: the athletes being the second or later-born children have more prominent formal and dynamic features and a higher resource potential compared to athletes being the eldest or only children.

The study was aimed to assess the correlation of formal and dynamic features and the resource potential of athletes who played badminton with their sibling positions. The objectives were as follows: 1) to identify the specifics of formal and dynamic features of athletes playing badminton taking into account their sibling positions; 2) to identify the features of the resource potential of athletes with different sibling positions; 3) to identify the points of corrective interventions aimed at improving the resource potential and athletic performance during the period of competitions for the sports psychologist.

## METHODS

### Subjects

The study was performed in the Training Center, Kratovo urban locality (Moscow Region), where the badminton training session for athletes (juniors) was carried out.

The study involved 40 athletes, among them 20 boys and 20 girls aged 13–15 (average age 14.5 years). The subjects were candidates for master of sports realizing themselves in elite sports. The athletes were divided into two groups based on the birth order: the first group included the "eldest and only children", and the second one included the "second and later-born children" (according to the order of birth in the family). Inclusion criteria: first and second grade athletes, candidates for master of sports. Exclusion criteria: masters of sports, twins.

Theoretical and methodological approach. Our study was based on the idiodynamic research paradigm by V.M. Rusalov allowing one to assess the formal and dynamic features of the athlete's personality resulting from consolidation of innate biological programs operating under the logic of the "body" or the individual's general biological constitution [13, 14]. Our study of the sibling positions was based on the review and assessment reports of the research on sibling relationships [12, 15, 16].

### Methods

*Structured interview*

We conducted structured interviews with the athletes enrolled in the study, during which we consistently collected the data on the athlete's sibling position in the family.

*Questionnaire of Formal and Dynamic Properties of Personality (QFDPP) by V.M. Rusalov [17]*

This test is used for the diagnosis of the "objective-practical" (psychomotor and intellectual spheres) and "communicative" aspects. Such spheres of personality, as emotional, psychomotor, intellectual, and communicative speres, are assessed.

The emotional sphere is represented by sensitivity, impulsivity, strength and dynamics of emotions. The psychomotor (motor) sphere is represented by general activity, i.e. the pace, rate, rhythm and overall number of movements. The intellectual sphere is represented by intellectual capabilities and the drive for activity related to mental strain. The communicative sphere is represented by the need for communication, desire to make acquaintances, sociability.

We also assessed the characteristics of personality that manifested in these spheres: ergicity (individual's endurance, both physical and mental), plasticity (the ability of mind to adapt to new conditions), rate (pace of responses, behavior, and mental processes), and emotionality (sensibility, impulsivity, sensitivity to emotional influences and possible discrepancies with the initial ideas).

*Perinatal Experience — Resource Potential test (N.P. Kovalenko)*

This method is based on the system for identification of the memory units related to perinatal experiences. Perinatal

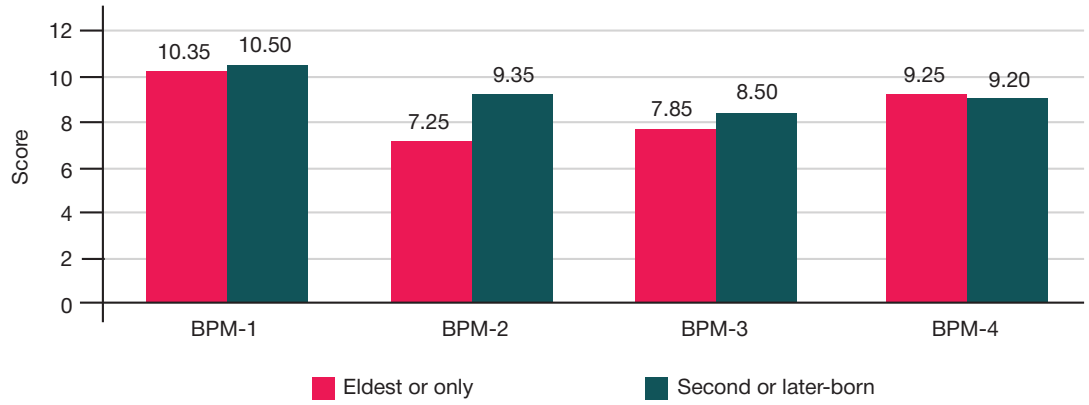


**Fig. 1.** Comparison of the features of the resource potential between athletes with different sibling positions (based on the Mann–Whitney *U* test) according to the Perinatal Experience – Resource Potential method

psychology has shown that perinatal experience has a significant influence on the formation of basic psychophysiological programs of the subconscious [9]. This means that such experiences affect behavioral patterns, strategies of self-realization and development. Perinatal trauma can be pushed deep down into the subconscious or compensated during life, but despite this such trauma can affect the individual's general mental health and resource potential [9]. The method allows one to assess these early experiences based on four matrices (BPM).

High values obtained for the first matrix (BPM 1) indicate maturation of sensory and physiological systems, accumulation of vital energy and good immunity, the ability to relax and enjoy life. Low values are indicative of problems in this sphere.

The values obtained for the second matrix (BPM 2) help to estimate the athlete's mobilization and stress tolerance, his/her ability to overcome difficulties and make quick decisions. Low values are indicative of bad experience, feeling helpless, desperate and guilty, when it is necessary to be active; the subject can be through "brainstorm" or feel scared.

The third matrix (BPM 3) allows one to estimate vital power, experience of struggle for survival or freedom, confidence in achieving the goal, activation of the leadership instinct. Low values are indicative of hesitant behavior, inability to fight, emergence of difficulties when trying to overcome the obstacles; the emergence of the attitude "someone else will do things for me" is possible.

The fourth matrix (BPM 4) allows one (in case of positive experience) to obtain information about the individual's quest for self-realization, his/her adaptive capacity, shaped basic trust in the world, activation of the leadership and survival instincts. When the earlier experiences are bad, the results for this matrix are indicative of vulnerability, distrust of the world, and feeling lonely.

*Using the Mann–Whitney U test*

Significance of differences between groups was determined using the Mann–Whitney *U* test.

*Statistical analysis*

The Spearman's rank correlation coefficient was used to assess significant correlations between the studied phenomena. Statistical processing of the results was performed using the STATISTICA ver. 10.0 software package (StatSoft; USA).

## RESULTS

The study has made it possible to identify the specifics of the athletes' resource potential and formal and dynamic features taking into account the athletes' sibling positions.

One of the objectives of the study was to determine the specifics of formal and dynamic features of personality in athletes playing badminton based on their sibling positions ("eldest or only child" or "second or later-born child").

The analysis of values obtained for BPM 2 related to activation and dynamization of the processes related to ensuring sports activity together with the use of the Mann–Whitney *U* test has made it possible to reveal significant differences in the athletes' resource potential between groups (Fig. 1; Table 1).

Athletes of the "second or later-born child" group show significantly higher values (score 9.35) for BPM 2 compared to the athletes of the "eldest or only child" group (score 7.25). Athletes of the "second or later-born child" group have a 2.1% higher stress tolerance, ability of mobilization; it is easier for them to go through hardships, they can make decisions faster than athletes of the "eldest or only child" group.

The athletes' formal and dynamic features have been estimated using the QFDPP questionnaire (by V.M. Rusalov), the results are provided in Fig. 2.

The use of the Mann–Whitney U test (Table 1) has made it possible to reveal significant differences in psychomotor plasticity (flexibility of switching from one type of physical activity to another, propensity for various forms of motor activity) between groups.

**Table 1.** Significant differences in the indicators of formal and dynamic features of the athletes with different sibling positions (based on the Mann–Whitney *U* test)

| Indicators of formal and dynamic features | Average values for BPM | | Median (lower quartile; upper quartile) | | | | Significance level |
|---|---|---|---|---|---|---|---|
| | Eldest or only child | Second or later-born child | Eldest or only child | Second or later-born child | *U* | *Z* | *p* |
| BPM-2 | 7,25 | 9,35 | 7 (5,5; 9) | 9 (8; 11) | 114 | 2,35 | 0,018533 |
| PP | 33,85 | 36,1 | 34 (31; 36,5) | 37,5 (31; 40) | 129 | 1,91 | 0,048523 |

**Note:** * — differences at the significance level of $p < 0.05$; BPM 2 — the second basic perinatal matrix; PP — psychomotor plasticity.

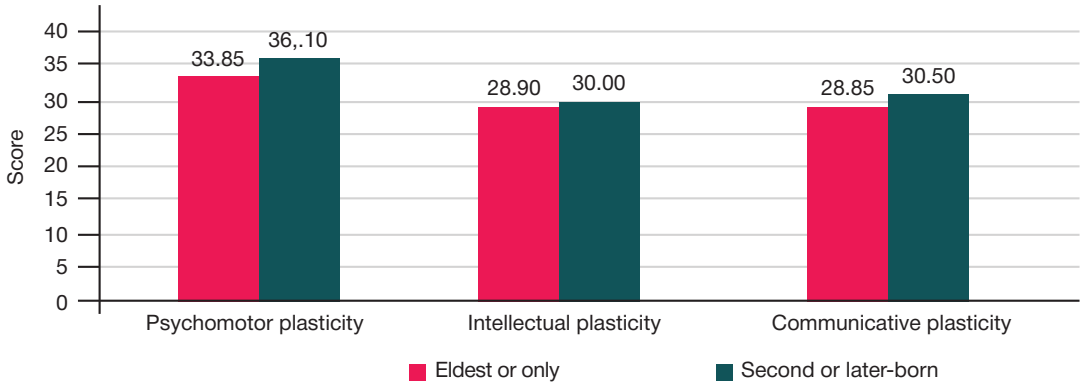


**Fig. 2.** Comparison of the formal and dynamic features of athletes with different sibling positions (based on the Mann–Whitney *U* test) according to the QFDPP questionnaire

Athletes of the "second or later-born child" group are characterized by the significantly higher psychomotor plasticity (score 36.1) compared to the athletes of the "eldest or only child" group (score 33.85) (Table 1). The athletes, who were the second or later-born children in their families, were 2.25% more active and dynamic, they showed easier and more successful overcoming of obstacles, and had the better shaped processes of activation and dynamization of livelihood. The athletes, who were the first or only children in their families, showed lower plasticity; they had to invest more energy to get results.

It has been found that the athletes, who are the second or later-born children in their families, are characterized by not only higher dynamism and activity (being their strengths in sports), but also by lower emotional stability. This makes them more vulnerable compared to the athletes, who are the first or only children in their families, and have higher emotional stability and tolerance due to lower plasticity. The patterns determined should be taken into account when working with athletes in order to improve their athletic performance during the competition cycle.

The correlations of the athletes' formal and dynamic features and the resource potential with various sibling positions are provided in Table 2.

No significant correlation between the resource potential and formal and dynamic characteristics was revealed in the "eldest or only child" group. This suggests that these psychological substructures of personality are rather isolated from each other in athletes of this category (Table 2).

A number of significant negative correlations were revealed in the "second or later-born child" group of athletes: between the indicators of maturation of the sensory and physiological systems (BPM 1) and communicative plasticity ($r$ = –0.50); between the indicators of stress tolerance, the ability to go through hardships, and communicative plasticity ($r$ = –0.60).

In the same group ("eldest or only child"), positive correlations between the indicators of stress tolerance, the ability to go through hardships (BPM 2), psychomotor ergicity ($r$ = 0.63), and psychomotor rate ($r$ = 0.61) were revealed, i.e. athletes of this group were characterized by normal muscle tone and normal motor activity, moderate desire for physical stress and medium muscle performance, as well as by the medium rate of motor operations. The upper limit of the medium values of psychomotor ergicity and medium values of psychomotor rate were combined with medium scores for BPM 2, i.e. with such constructs, as activation and dynamization of the assurance processes, stress tolerance, the ability to go through hardships, the ability to make quick decisions. A less significant positive correlation ($r$ = 0.44) between the athletes' intellectual plasticity and their ability to go through hardships (stress tolerance) was also revealed.

Positive correlations between the indicators of vitality (BPM 3) and intellectual rate ($r$ = 0.53), as well as between the indicators of vitality and intellectual plasticity ($r$ = 0.47) were revealed in this group.

Significant correlations for each of four matrices were revealed in the "second or later-born child" group, which

**Table 2.** Matrices of intercorrelations among the athletes' formal and dynamic features and their resource potential (based on the Spearman's rank correlation coefficients; according to the QFDPP method)

| Formal and dynamic characteristics of athletes | Sibling position: "elder or only child" | | | | Sibling position: "second or later-born child" | | | |
|---|---|---|---|---|---|---|---|---|
| | BPM-1 | BPM-2 | BPM-3 | BPM-4 | BPM-1 | BPM-2 | BPM-3 | BPM-4 |
| PER | 0.42 | 0.63 | 0.26 | 0.02 | 0.35 | 0.49 | 0.25 | 0.08 |
| IER | –0.02 | –0.02 | –0.14 | 0.21 | 0.17 | –0.05 | 0.58 | 0.05 |
| IP | 0.13 | 0.44 | 0.47 | 0.13 | 0.08 | 0.28 | 0.23 | 0 |
| CP | –0.50 | –0.60 | –0.31 | –0.05 | –0.27 | –0.29 | –0.46 | –0.32 |
| PR | 0.28 | 0.61 | 0.34 | –0.12 | 0.28 | 0.21 | 0.31 | 0.06 |
| IR | 0.34 | 0.39 | 0.53 | 0.18 | 0.3 | 0.2 | 0.32 | 0.15 |
| ME | –0.22 | –0.28 | 0.05 | 0.23 | –0.29 | –0.34 | –0.16 | –0.49 |
| IE | –0.28 | –0.21 | –0.17 | 0.07 | –0.44 | –0.47 | –0.39 | –0.20 |
| CE | –0.33 | –0.24 | –0.02 | 0.04 | –0.34 | –0.49 | –0.72 | –0.25 |

**Note:** $n$ = 20; BPM 1 — the first basic perinatal matrix; BPM 2 — the second basic perinatal matrix; BPM 3 — the third basic perinatal matrix; BPM 4 — the fourth basic perinatal matrix; PER — psychomotor ergicity; IER — intellectual ergicity; IP — intellectual plasticity; CP — communicative plasticity; PR — psychomotor rate; IR — intellectual rate; ME — motor emotionality; IE — intellectual emotionality; CE — communicative emotionality. Gray — correlations at the significance level of $p < 0.05$; light gray — correlations at the significance level of $p < 0.01$; dark gray — correlations at the significance level of $p < 0.001$.

suggested the high degree of the matrix parameter integration into the formal and dynamic structure of the athlete's personality (Table 2). Thus, a negative correlation between the indicators of maturation of the sensory and physiological systems (BPM 1) and intellectual emotionality ($r$ = –0.44) was revealed.

The less significant negative correlations between the indicators of stress tolerance and intellectual emotionality ($r$ = – 0.47), as well as communicative emotionality ($r$ = –0.49) were also found in this group. Athletes of this group are characterized by medium severity of emotional distress in case of failures in work and in the situations that require mental exertion; medium flexibility of switching from one type of physical activity to another; moderate propensity for various forms of motor activity.

In the "second or later-born child" group there was a positive correlation ($r$ = 0.49) between the indicators of stress tolerance and psychomotor ergicity. The upper limit of the medium values of psychomotor ergicity (PER) was combined with the mostly high scores obtained for BPM 2, i.e. with such constructs, as activation and dynamization of the assurance processes, stress tolerance and the ability to go through hardships, as well as the ability to make quick decisions. Furthermore, there was a strong negative correlation ($r$ = –0.72) between the indicators of vitality (BPM 3) and communicative emotionality (CE). The lower limit of the medium CE values was combined with the mostly high scores obtained for BPM 3, i.e. with such constructs, as confidence in achieving the goal, activation of the leadership instinct, and the desire for freedom. Athletes of this group showed a negative correlation ($r$ = –0.46) between the indicators of vitality (BPM 3) and communicative plasticity. Moreover, in these athletes the indicators of vitality positively correlated ($r$ = 0.58) with the indicators of intellectual ergicity.

## DISCUSSION

Our findings are consistent with the results obtained by other researchers [9, 13, 14, 18], however, it should be emphasized that the authors of the above papers never conducted sibling studies in terms of elite sports. The results of our study make it possible to identify the points of psychocorrectional interventions when working with athletes of the selected categories.

Thus, when working with athletes, who are the first or only children in their families, it is reasonable to focus on improving stress tolerance, cultivate the ability to mobilize, teach the athletes to learn and use various coping mechanisms when experiencing hardships, and to learn the methods of quick decision making. Furthermore, the key directions are psychocorrectional exercises on switching attention from one type of motor activity to another, as well as relaxation activities aimed at improving the recovery processes in athletes.

It is important to teach the athletes, who are the second or later-born children in their families, the methods for self-regulation of functional state and the methods for self-organization of educational, training and competitive activities, as well as to improve the athletes' volitional qualities, emotional stability and tolerance [19].

Furthermore, it is important for the sports psychologist working with the athletes enrolled (who are engaged in elite sports) to take into account a rather broad age range of the sports team when selecting and using the methods for working.

The development of the guidelines on working with athletes that take into account the athletes' sibling positions will allow the sports psychologist to organize psychological support of educational, training and competitive activities to make it even more differentiated and effective.

## CONCLUSIONS

We have revealed significant differences in the resource potential of athletes playing badminton that result from their sibling positions: the athletes, who are the second or later-born children, have a significantly higher resource potential than the athletes, who are the first or only children in their families, having medium resource potential. The athletes of the "first or only child" group are characterized by significantly lower psychomotor plasticity compared to the athletes of the "second or later-born child" group; they overcome this conditional limitation due to tenacity and constant drive for results. It has been found that the athletes, who are the first or only children in their families, achieve high results due to intense and hard training, in contrast to the athletes of the second group. Strong correlations between the indicators of communicative emotionality and confidence in achieving the goal, as well as between the leadership potential and the desire for freedom have been revealed in the "second or later-born child" group. Thus, the research problems have been solved, and the goal has been accomplished. The research hypothesis has been confirmed. Psychologists are recommended to use the study results for correction of the athletes' educational and training activities, since this can improve the results of competitions. The long-term objectives of further research are as follows: 1) to identify, describe and explain the mechanisms underlying the patterns we have revealed; 2) to study the sibling position as a factor of athletic performance during training and competitions in elite sports.

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# THE RELATIONSHIP BETWEEN THE VARIANTS OF IMMUNE RESPONSE AND THE CORTISOL AND ADRENALINE LEVELS ASSOCIATED WITH COOLING

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The development of adaptive response to cold exposure is associated with the increased synthesis of the adrenal hormones involved in regulation of the immunocompetent cells' functional and metabolic activity. Even people residing permanently in the North show different variants of response to cold. The study was aimed to determine the relationship between the baseline cortisol and adrenaline levels, as well as the changes in their concentrations associated with the adaptive immune response to whole body cooling. A total of 173 individuals were assessed before and after the short-term whole body cooling. White blood cell differential, cortisol, adrenaline and ferritin levels, and the presence of glycogen in lymphocytes were determined in peripheral blood. Three variants of response were defined: 1) the relatively low baseline levels of cortisol and adrenaline together with no increase in these levels after the cold exposure have no significant effect on the lymphocyte migration activity; 2) predominant activation of the sympathetic–adrenal–medullary axis is associated with lymphocyte mobilization into the bloodstream along with the decrease in their glycolytic activity; 3) the higher baseline levels of cortisol and further increase in its concentration until it reaches the upper limit of the normal range following cooling are associated with intensification of glycolisis in lymphocytes and the increase of lymphocyte migration to the tissues.

**Keywords:** cooling, adrenaline, cortisol, lymphocyte, adaptation

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**Compliance with ethical standards:** the study was approved by the Ethics Committee of the N. Laverov Federal Center for Integrated Arctic Research, the Ural branch of RAS (protocol № 4 of 7 December 2016, protocol № 6 of 14 February 2022) and conducted in accordance with the principles of the Declaration of Helsinki (1975, rev. 2013).

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# ВЗАИМОСВЯЗЬ ВАРИАНТОВ ИММУННОГО РЕАГИРОВАНИЯ С УРОВНЕМ КОРТИЗОЛА И АДРЕНАЛИНА ПРИ ОХЛАЖДЕНИИ

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Формирование адаптивной реакции в ответ на холодовое воздействие связано с повышением синтеза гормонов надпочечников, регулирующих функциональную и метаболическую активность иммунокомпетентных клеток. Варианты реагирования на холод могут значительно различаться даже у людей, длительное время проживающих на северных территориях. Целью работы было определить взаимосвязь фонового уровня кортизола и адреналина, а также изменения их концентрации при формировании адаптивной иммунной реакции в ответ на общее охлаждение. Обследовали 173 человека до и после кратковременного общего охлаждения. В периферической крови определены лейкограмма, уровень кортизола, адреналина и ферритина, наличие в лимфоцитах гликогена. Установлены три варианта реагирования: 1) относительно низкая фоновая концентрация кортизола и адреналина, без повышения их уровня после холодового воздействия не оказывает значимого влияния на миграционную активность лимфоцитов; 2) преимущественная активизация симпатико-адреналово-медуллярной оси связана с мобилизацией лимфоцитов в кровоток, при снижении их гликолитической активности; 3) более высокий фоновый уровень кортизола и дальнейшее повышение его концентрации до верхней границы нормы после охлаждения связаны с активизацией гликолиза в лимфоцитах и усилением их миграции в ткани.

**Ключевые слова:** охлаждение, адреналин, кортизол, лимфоцит, адаптация

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Interaction between the immune and nervous systems ensures the development of adaptive responses to living conditions. There are neurotransmitter receptors on the membranes of immunocompetent cells, through which neurotransmitters can affect the cells' functional and metabolic activity. However, T cells can control both synthesis and degradation of neurotransmitters [1, 2]. The cold exposure leads to the changes in the levels of hormones involved in instant and long-term adaptation. The development of adaptive physiological processes involves mainly two systems: the sympathetic–

adrenal–medullary and hypothalamic-pituitary-adrenal axes. Low blood levels of adrenaline, the hormone of the sympathetic–adrenal–medullary axis, are reported in the normal state, while the exposure to stressor results in the significant increase in adrenaline concentration that intensifies catabolic processes. Catecholamines control a number of leukocyte functions, such as proliferation and differentiation, mitogenic responses, lytic activity of natural killers and cytokine production. High doses of catecholamines cause abnormal platelet aggregation [3–6]. Cortisol, the steroid hormone of the hypothalamic-pituitary-adrenal axis, can elevate blood glucose levels and suppress the immune system, thereby inducing apoptosis in pro-inflammatory T cells, it also can suppress antibody production by B cells and reduce migration of neutrophils during inflammation [7]. Cortisol reduces glycogen synthesis and contributes to the fat, protein and carbohydrate metabolism via gluconeogenesis [8]. Gluconeogenesis makes it possible to respond quickly to the changes in the need for ATP. Intensification of lipolysis in the adipose tissues under exposure to cortisol facilitates the release of glycerol and free fatty acids that are also involved in β-oxidation and are used as a source of energy by other cells. The effects of cortisol exposure result mostly from the duration of exposure and the dose. Activation of catabolic mechanisms aimed at meeting the body's energy needs is justified in case of short-term stress, while chronic exposure to the stressor and the long-term immunosuppressive effects of high cortisol doses can result in the body's adaptation reserve depletion. Even people residing permanently in the North show different degrees of adaptation to specific environmental conditions, including cold exposure. This is largely due to baseline levels of the neuro-immuno-endocrine system activation and the spare capacity enabling adequate responses to external stimuli. The study was aimed to determine the relationship between the baseline levels of cortisol and adrenaline, as well as the changes in their concentrations associated with the adaptive immune response to whole body cooling.

## METHODS

The changes in immunological, hematological and biochemical parameters of peripheral venous and capillary blood were studied in 173 generally healthy people. Inclusion criteria: no acute disorders at the time of examination; non-use of cold exposure training; age 20–60 years. Blood was collected twice by skilled medical specialists: 1) before staying in the USHZ-25N cooling chamber (Xiron-Kholod; Russia) at –25 °C for 5 min and 2) immediately after staying in the chamber. Blood serum and plasma were separated by centrifugation, the samples were freezed once at a temperature of –20 °C. Assessment was performed in the morning (8 to 10 am), strictly in the fasting state. Differential blood count was determined using the XS-1000i hematological analyzer (Sysmex; Japan). Enzyme immunoassay was used to determine the levels of adrenaline with the test kit (IBL, Hamburg; Germany), as well as the levels of cortisol (DBC; Canada) and ferritin (ORGENTEC Diagnostika GmbH; Germany). The results were assessed using the Multiskan FC microplate photometer (Thermo Scientific; Finland). The levels of glycogen in lymphocytes were determined by the cytochemical method (Abris+; Russia) involving calculating the percentage of positively stained cells with the Biomed 4 LED microscope (Biomed; Russia). The study results were processed with the Statistica 6.0 software package (StatSoft; USA). The data were described using the median (Me) and 25–75$^{th}$ percentiles. The nonparametric Mann–Whitney U test was used to determine the significance of differences. When testing the statistical hypotheses, the significance level ($p$) was considered to be 0.05.

## RESULTS

We compared the data in the groups depending on the changes in peripheral blood lymphocyte counts after the short-term whole body cooling. In group 1 ($n$ = 52) the lymphocyte counts decreased by 1.5–2 times (from 2.1 (1.77; 2.44) × $10^9$ to 1.69 (0.95; 2.16) × $10^9$ c/L ($p$ < 0.001)), in group 2 ($n$ = 42) these increased from 1.49 (1.26; 1.74) × $10^9$ to 2.22 (1.48; 2.61) × $10^9$ c/L ($p$ < 0.01), in group 3 ($n$ = 79) no significant differences were revealed (1.88 (1.46; 2.17) × $10^9$ and 1.82 (1.46; 2.56) × $10^9$ c/L). The development of adaptive response associated with the decrease in the circulating lymphocyte counts (group 1) took place against the background of relatively high cortisol levels (317.4 (283.5; 732.2) mmol/L), the levels of which increased to reach the upper limit of normal range, i.e. 606.3 (281.0; 963.2) mmol/L, after the whole body cooling in this group. When peripheral blood lymphocyte counts increased in response to whole body cooling (group 2) or no significant differences were revealed (group 3), the baseline cortisol concentration was actually 2 times lower and did not change after the cold exposure (Fig. 1).

Glucocorticoids that are the main effector molecules of the hypothalamic-pituitary-adrenal axis ensure mobilization of energy substrates into the bloodstream. Cortisol can alter bioenergetic cell fuctions via transactivation or transrepression of target nuclear and mitochondrial genes, as well as activation of cytoplasmic signaling pathways, thereby affecting mitochondrial activity. ATP production largely depends on the cellular oxygen consumption via the oxidative phosphorylation system (OXPHOS) located in the inner mitochondrial membrane [9–11]. It is well known that the short-term exposure to cortisol induces mitochondrial biogenesis and enzyme activity of some OXPHOS subunits, causing higher mitochondrial activity, since the long-term exposure to high doses of cortisol results in the decrease in mitochondrial activity, OXPHOS dysfunction, increased production of reactive oxygen species, and structural abnormalities [12]. Thus, higher baseline cortisol levels and their further increase in response to cold exposure can be associated with the decrease in functional activity of mitochondria observed in case of glycolytic activity intensification. On the one hand, intensification of glycolysis is necessary for reprogramming of lymphocytes, their rapid activation. On the other hand, according to the model of mitochondrial allostatic load, mitochondrial function can determine the limits of the human ability to adapt to external stressors, whereby the higher mitochondrial content and function are associated with the increased adaptability and biological stability potential, while the decreased mitochondrial function limits the ability to adapt and opens the door to stress-related disorders [13]. We have found that the adaptive response to cooling, accompanied by the decrease in circulating lymphocyte counts against the background of long-term exposure to higher cortisol concentrations, is associated with intensification of glycolysis and the decrease in the glycogen content of lymphocytes from 4.01 to 2.83% ($p$ < 0.01), which is essential for effective ATP production in case of immunocompetent cell activation.

Adrenaline is an effector mediator of the sympathetic–adrenal–medullary axis. There no differences in the baseline adrenaline levels between three groups: in group 1 the level was 30.7 (25.7; 43.8) ng/mL, in group 2 it was 30.0 (29.14; 44.6) ng/mL, and in group 3 it was 32.5 (26.38; 39.0) ng/mL. The significant increase in adrenaline concentrations was

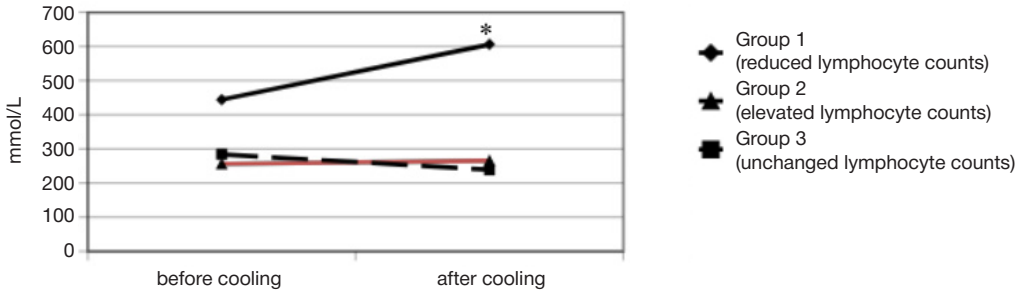


**Fig. 1**. Changes in cortisol levels after the whole body cooling; * — $p < 0.01$

revealed after the whole body cooling in the subjects, for whom the increased lymphocyte counts were reported (group 2) (Fig. 2). No significant changes in the concentrations of this catecholamine were revealed in groups 1 and 3.

Adrenaline causes the increase in succinate dehydrogenase activity and inhibits α-ketoglutarate dehydrogenase activity in mitochondria of peripheral blood lymphocytes [14], reduces oxidative phosphorylation in mitochondria, and causes mitochondrial dysfunction [15, 16]. The elevated adrenaline levels found in group 2 are associated with no changes in the glycogen content of lymphocytes (3.6% before cooling, 4.8% after cooling, respectively). This is probably due to the adrenaline ability to increase the ADP phosphorylation time by more than two times, thereby significantly reducing the ATP synthesis rate.

The stress-induced alterations due to adrenaline and cortisol exposure can affect the iron metabolism. The levels of ferritin are considered as an indirect criterion of adequate iron levels. On the one hand, these represent the iron depot, and on the other hand these are considered as a marker of acute inflammation, while the increase in blood ferritin levels can be associated with inflammatory tissue destruction. Iron ensures effective adaptation to cold, maintains energy balance and thermogenesis [17]. Assessment of the correlations of the adrenaline, cortisol and ferritin levels associated with various types of response to whole body cooling has shown that baseline ferritin levels in groups 1 and 2 are almost the same, while in group 3 lower ferritin levels are reported that significantly increase after the cold exposure while still within normal range (Fig. 3).

## DISCUSSION

Adaptation to low temperatures is ensured by interaction between the immune and endocrine systems. Exposure to external factors affects the body in different ways depending on its baseline state and the ability to respond, i.e. the reserve capacity to form instant and long-term adaptive responses. The exposure to stressor results in the adrenal gland activation, secretion of adrenaline and cortisol capable of changing the number of immunocompetent cells in the bloodstream. However, the research data are controversial. There are data supporting both stimulation and immunosuppressive effects, and the mechanism of such interaction is poorly understood [18–21]. Furthermore, enough iron stored is a factor that is important for realization of adaptive responses to hypothermia. Ferritin, that represents the iron depot and regulates the iron metabolism, controls the integrity and function of mitochondria in the cells, thereby ensuring the energy and thermal homeostasis [22]. Our findings show that the increase in the baseline activity of the hypothalamic–pituitary–adrenal axis in response to the whole body cooling results in intensification of glycolysis and the decrease in the circulating lymphocyte counts that may be due to migration of these. At the same time, prolonged exposure to high concentrations of cortisol can be considered as the chronic stress that has an effect similar to that of the long-term antigenic stimulation and can contribute to production of lymphocytes showing signs of replicative aging [23]. The increase in the circulating lymphocyte counts after the cold exposure associated with the sympathetic–adrenal–medullary axis activation can be mediated by the enchanced release of cells from the depot or the change in the ratio of the marginated and circulating lymphocyte pools caused by vasoconstriction. No changes in lymphocyte counts in individuals subjected to whole body cooling are associated with no significant changes in the concentrations of cortisol and adrenaline, but with significant changes in ferritin levels. Cooling contributes to the ferritin heavy chain degradation, thereby causing ferroptosis, release of free iron ions, induction of reactive oxygen species accumulation, and inhibition of the glutathione- glutathione peroxidase 4 (GPX4) pathway [24, 25].

## CONCLUSIONS

It has been found that the variant of response to whole body cooling is correlated mainly with activation of the sympathetic–adrenal or hypothalamic-pituitary-adrenal axis. Low baseline levels of cortisol and adrenaline along with no changes in their concentrations after the whole body cooling are not associated with intensification of glycolysis in lymphocytes. On the one hand, this suggests more stable condition of the body and no stress

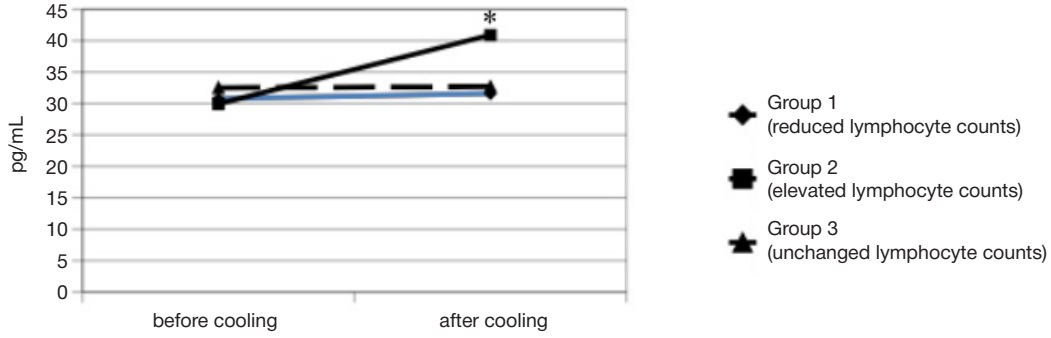


**Fig. 2**. Changes in adrenaline levels after the whole body cooling; * — $p < 0.01$

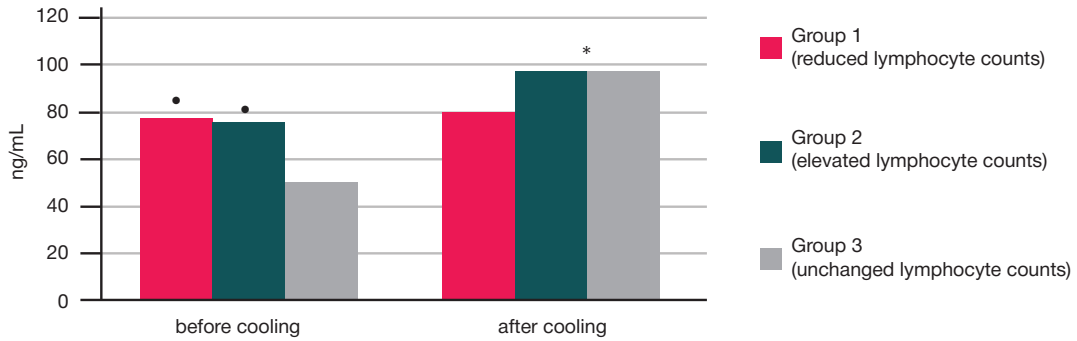


**Fig. 3**. Ferritin concentrations before and after the whole body cooling. * — $p_3 < 0.01$ (significance of differences of 0.01 in the volunteers of group 3 before and after the whole body cooling); • — $p_{1-3,\ 2-3} < 0.01$ (significance of differences of 0.01 between the volunteers of groups 1 and 3 and groups 2 and 3, respectively, before the whole body cooling)

caused by such cold exposure. On the other hand, elevated ferritin levels can be indicative of inflammation. The effects of cortisol and adrenaline are associated with the changes in the circulating cell counts and metabolic activity of these cells. The higher baseline levels of cortisol cause intensification of glycolysis together with the decrease in glycogen content and therefore further intense ATP production that is essential for activation of immunocompetent cells. In this context the decrease in circulating lymphocyte counts is observed that may be due to rearrangement of functionally active cells in the tissue aimed at providing the effective immune defense. Possibly due to decelerating the rate of ADP phosphorylation, adrenaline does not allow such a rapid (within 5 min) increase in glycolytic activity of lymphocytes. The increase in adrenaline concentration in response to the cold exposure is associated with elevation of circulating lymphocyte counts, since the duration of exposure eliminates the possibility of lymphopoiesis. This fact can be explained by rearrangement of cells from the marginated to the circulating layer due to vasoconstrictor effects of adrenaline or the release of cells from the depot. The data obtained add to the knowledge about the role of the neuro-immuno-endocrine regulation in the development of individual cold sensitivity.

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# ASSESSMENT OF THE VITAMIN D, CALCIUM AND PHOSPHORUS SUFFICIENCY IN INDIVIDUALS DEPLOYED IN ARCTIC

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Inadequate ultraviolet insolation is one of the the key prerequisites for the pathogenesis of body's vitamin D insufficiency in the North. The study was aimed to assess the body's vitamin D, calcium and phosphorus sufficiency in the contract servicemen deployed in Arctic. The contract servicemen deployed on the Cape Chelyuskin and Dixon Island were surveyed ($n$ = 51). The serum levels of 25(OH)D, the intermediate of the vitamin D conversion, along with the ionized calcium, total calcium, and inorganic phosphorus levels, were determined in June. Three degrees of the vitamin D sufficiency were revealed in the military, who had been deployed in Arctic for 5.9 ± 0.4 years: deficiency (in 29.4%), insufficiency (in 52.9%), and optimal levels (in 17.7%). However, the optimal levels revealed were close to the lower limit of normal range. Low ionized calcium levels were found in 29.4% of blood samples (15.5 ± 0.6 ng/mL). A total of 70.6% of samples that were within normal range were close to the lower limit of normal range based on $Q_{25}$ (1.16 mmol/L) and were within the lower half of normal range (1.15–1.35 ng/mL) based on $Q_{75}$ (1.22 mmol/L). The measured total calcium and inorganic phosphorus levels were close to the lower limits of reference ranges (2.29 ± 0.009 and 0.83 ± 0.006 mmol/L, respectively). In general, the reduced ionized calcium levels associated with vitamin D insufficiency were revealed, which were indicative of impaired calcium metabolism. The vitamin D deficiency results from the total calcium and inorganic phosphorus concentrations that are close to lower limits of reference ranges. Further negative changes in the body's vitamin D, phosphorus and calcium sufficiency should be expected during polar night. The study actualizes the year-round replenishement of the vitamin D and mineral deficiency in the military.

**Keywords:** Arctic, contract servicemen, vitamin D, total calcium, ionized calcium, inorganic phosphorus

**Author contribution:** Rakhmanov RS — developing the study concept and design, manuscript writing; Bogomolova ES — editing, approval of the final version of the article; Narutdinov DA — primary data acquisition; Razgulin SA — literature review; Bakhmudov GG — statistical processing of the results; Zaitsev LL — participation in statistical data processing and data interpretation.

**Compliance with ethical standards:** the study was approved by the Ethics Committee of the Privolzhsky Research Medical University (protocol № 4 of 14 March 2022), it was carried out in accordance with the ethical principles stipulated in the Declaration of Helsinki of the World Medical Association; the informed consent was submitted by all study participants.

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# ОЦЕНКА ОБЕСПЕЧЕННОСТИ ВИТАМИНОМ D, КАЛЬЦИЕМ И ФОСФОРОМ ЛИЦ, ПРОХОДЯЩИХ СЛУЖБУ В УСЛОВИЯХ АРКТИКИ

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Неадекватная ультрафиолетовая инсоляция является одним из ключевых условий в патогенезе развития D-витаминной недостаточности организма на Севере. Целью работы было оценить насыщенность организма витамином D, кальцием и фосфором военнослужащих, проходящих службу по контракту в Арктике. В исследовании участвовали военнослужащие, проходящие службу по контракту, работающие на мысе Челюскин и острове Диксон ($n$ = 51). В июле определяли содержание в сыворотке крови 25-ОН — промежуточного продукта превращения витамина D, уровень кальция ионизированного и общего, фосфора неорганического. В летний период года у военнослужащих, работающих в Арктике 5,9 ± 0,4 года, выявлено три уровня обеспеченности витамином D: дефицит (у 29,4%), недостаточность (у 52,9%) и оптимальный, но в нижней зоне границы нормы, уровень (у 17,7%). Низкое содержание ионизированного кальция определено в 29,4% проб крови (15,5 ± 0,6 нг/мл). В 70,6% проб, входящих в границы нормы, по $Q_{25}$ были близки к нижней границе нормы (1,16 ммоль/л), по $Q_{75}$ (1,22 ммоль/л) — в нижней половине зоны нормы (1,15–1,35 нг/мл). Общий кальций и фосфор неорганический выявлены на уровне нижней зоны референтных границ (соответственно 2,29 ± 0,009 и 0,83 ± 0,006 ммоль/л). В целом на фоне недостаточной насыщенности организма витамином D выявлено снижение содержания ионизированного кальция, что свидетельствует о нарушении кальциевого обмена. Его дефицит обусловлен концентрацией и общего кальция и неорганического фосфора, находящихся в нижних зонах референтных значений. В период полярной ночи следует ожидать более негативные изменения D-витаминной и фосфорно-кальциевой насыщенности организма. Исследование актуализирует проведение в течение всего года восполнение дефицита D и минеральных веществ у военнослужащих.

**Ключевые слова:** Арктика, военнослужащие по контракту, витамин D, кальций общий, кальций ионизированный, фосфор неорганический.

**Вклад авторов:** Р. С. Рахманов — разработка дизайна и концепции исследования, написание статьи; Е. С. Богомолова — редактирование, утверждение окончательного варианта статьи; Д. А. Нарутдинов — сбор первичного материала; С. А. Разгулин — обзор литературы; Г. Г. Бахмудов — статистическая обработка результатов; Л. Л. Зайцев — участие в статистической обработке материала и интерпретации данных.

**Соблюдение этических стандартов:** исследование одобрено этическим комитетом ФГБОУ ВО «ПИМУ» Минздрава России (протокол № 4 от 14 марта 2022 г.), проведено с соблюдением этических норм Хельсинкской декларации Всемирной медицинской ассоциации; все участники исследования подписали добровольное информированное согласие.

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Arctic is a region with extreme environmental conditions that adversely affect body's functional state by reducing its reserve capacity, complicate the daily routine and implementation of the people's professional activity [1]. This climate zone is characterized by inadequate ultraviolet insolation being the key prerequisite for the pathogenesis of body's vitamin D insufficiency [2–6].

**Table 1.** Serum levels of 25(OH)D and minerals in individuals of the study group (abs.)

| № | Studied parameter, reference range | M ± *m* | Me | $Q_{25}$–$Q_{75}$ |
|---|---|---|---|---|
| 1 | 25(OH)D, 30–100 ng/mL | 24,1 ± 0,9 | 24 | 17,9–28,7 |
| 2 | Ionized calcium, 1.15–1.35 mmol/L | 1,2 ± 0,005 | 1,18 | 1,14–1,2 |
| 3 | Total calcium, 2.02–2.6 mmol/L | 2,2 ± 0,009 | 2.2 | 2,14–2,24 |
| 4 | Inorganic phosphorus, 0.7–1.8 mmol/L | 0,8 ± 0,006 | 0,83 | 0,79–0,85 |

Vitamin D serves a number of important functions in the human body; the receptors susceptible to the effects of this vitamin are found in many cells of the body. The vitamin affects both innate and adaptive immunity; it has been found that the vitamin plays a certain role in regulation of neurohormonal effects on the brain development, maintaining cognitive function, memory, and behavior associated with mental disorders [7, 8]. It has been shown that low vitamin D levels are associated with the increased risk of a number of cancer types and infectious diseases, cardiovascular disorders, diabetes mellitus of both types, tuberculosis, bronchial asthma, reproductive dysfunction, mental disorders, complications of pregnancy [9–11]. The vitamin D deficiency exacerbates the severity of autoimmune disorders [12, 13] and affects the incidence of infectious and inflammatory diseases [14–19]. A correlation between the vitamin D deficiency and the increase in hospital admissions of elderly people has been determined [20]. Vitamin D plays an important role in the mechanisms of oxidative stress and damage to tissues and cells of the body [21, 22]. On the one hand, the vitamin D system is regulated by epigenetic mechanisms, and on the other hand it is involved in regulation of epigenetic events [23].

It is well-known that the vitamin D sufficiency is closely related to the calcium and phosphate metabolism [24–26].

The study was aimed to assess the vitamin D, calcium and phosphorus sufficiency in the contract servicemen deployed in Arctic.

## METHODS

The study that was carried out in summer (July) involved males ($n$ = 51), the contract servicemen who were deployed in the Arctic zone of Russia, on the Cape Chelyuskin and Dixon Island.

The age of individuals in the study group was 35.3 ± 0.6 years. The total enlistment period of the individuals in the study group was 12.8 ± 0.76 years, the servicemen had been doing their duty in Arctic for 5.9 ± 0.36 years. The median value was 6 years; the interquartile ranges were 4–7 years. The servicemen were engaged in professional activities in shifts: they worked one day on and one or two days off. On the working days the time spent in an open area was 3–7 h. On the days off, except those in summer, the time spent in an open area was minimal due to weather conditions.

Meals were provided by the canteens at the military units in accordance with the food ration № 1 taking into account additional food items distributed in the regions of the Far North according to regulatory documents.

The body's vitamin D sufficiency was judged by the levels of 25(OH)D, the intermediate of the vitamin D conversion, in blood samples. Identification was performed by tandem mass spectrometry using the AB SCIEX QTRAP 5500 mass spectrometer (SCIEX; Germany). The body's vitamin D sufficiency was distinguisged based on the 25(OH)D levels: severe deficiency (5–10 ng/mL), deficiency (10–20 ng/mL), insufficiency (20–30 ng/mL), optimal levels (30–100 ng/mL) [24, 27].

The ionized calcium and total calcium levels were determined. The ionized calcium represented a metabolically active form (free calcium); the total calcium represented a biologically inactive form, it was linked to proteins and other molecules. The ionized calcium levels were assessed by ion-selective potentiometry using the AVL9180 electrolyte analyzer. The total calcium and inorganic phosphorus levels were determined using the helium–neon laser operating in a fully automatic mode in the AU5800 hematology analyzer (Abbott; USA).

The reference ranges were 2.02–2.6 mmol/L for the total serum calcium concentration, 1.15–1.35 mmol/L for the ionized calcium, and 0.7–1.8 mmol/L for inorganic phosphorus [28].

Statistical processing of primary data was performed using the Statistica 6.1 software package (StatSoft; USA). The mean and standard error of the mean (M ± $m$), median values and quartile deviations ($Q_{25}$–$Q_{75}$) were calculated. Primary data were tested for normality using the Kolmogorov–Smirnov test, significance of differences for parametric samples was calculated using the Student's $t$-test for the probability of $p < 0.05$.

## RESULTS

The 25(OH)D levels in the study group were within the range estimated as “close to optimal” (Table 1). However, the $Q_{25}$ value showed that there were individuals, whose levels of this vitamin corresponded to deficiency.

The average ionized and total calcium levels and average inorganic phosphorus levels were within reference ranges.

When assessing the 25(OH)D levels based on individual data, three cohorts of subjects were distinguished showing different body levels of 25(OH)D (Table 2). The bulk of the group showed 25(OH)D deficiency of insufficient 25(OH)D levels. The levels of this vitamin in the cohort with deficiency were significantly lower (by 1.6 times; $p$ = 0.001) than in the cohort where the 25(OH)D levels were estimated as insufficient, and 2.2 lower than in the cohort with optimal levels ($p$ = 0.0001). The 25(OH)D levels in the cohort 2 were 1.3 times lower than in the group 3 ($p$ = 0.001).

**Table 2.** Characteristics of the study group, 25(OH)D levels

| № | Estimated levels | Abs., M ± *m*, ng/mL | Me | $Q_{25}$–$Q_{75}$ | Share of the group, % |
|---|---|---|---|---|---|
| 1 | Severe deficiency | – | – | – | 0 |
| 2 | Deficiency | 15,5 ± 0,6 | 16,2 | 14,0–16,7 | 29,4 |
| 3 | Insufficiency | 25,4 ± 0,6 | 25,25 | 23,3–28,6 | 52,9 |
| 4 | Optimal levels | 34,1 ± 0,8 | 34,8 | 31,7–35,25 | 17,7 |

**Table 3.** Characteristics of the study group, ionized calcium levels

| № | Estimated levels | Abs., M ± *m* mmol/L | Me | $Q_{25}$–$Q_{75}$ | Share of the group, % |
|---|---|---|---|---|---|
| 1 | Low levels | 1,12 ± 0,003 | 1,125 | 1,12–1,14 | 29,4 |
| 2 | Normal levels | 1,195 ± 0,005 | 1,2 | 1,16–1,22 | 70,6 |

The average ionized calcium level based on $Q_{25}$ was 1.14 mmol/L, i.e. it was beyond the lower limit of normal range (1.15–1.35 mmol/L). The average value of $Q_{75}$ (1.2 mmol/L) showed that this value was just above the median (1.18 mmol/L). As for individual data, almost one third of the study group showed low levels of this mineral; these were significantly (by 6.7%) lower ($p$ = 0.001) than in the cohort with normal levels (Table 3). In individuals with normal levels of ionized calcium, the $Q_{25}$ value exceeded the lower limit of normal range just by 0.01.

The individual total calcium levels varied between 2.1 and 2.27 mmol/L. The median value was 2.2 mmol/L, and the interquartile range ($Q_{25}$–$Q_{75}$) was 2.14–2.24 mmol/L. Furthermore, individual blood levels of this mineral in subjects of the study group were close to the lower limit of reference range.

Inorganic phosphorus was within normal range. Individual levels varied between 0.77–0.9 mmol/L, the median value was 0.83 ng/mL, and the interquartile range ($Q_{25}$–$Q_{75}$) was 0.79–0.85 ng/mL. Blood levels of this mineral in subjects of the study group were also close to the lower limit of reference range.

Thus, the reduced levels of ionized calcium associated with the body's vitamin D insufficiency were revealed, which were indicative of the calcium metabolism disorder [28]. Deficiency of this vitamin resulted in low total calcium and inorganic phosphorus concentrations that were close to the lower limits of reference ranges.

## DISCUSSION

The values of vitamin D insufficiency in the population vary significantly depending on the country, gender, and season [29, 30]. Vitamin D deficiency and insufficiency is common in the Russian Federation [24].

Ultraviolet light plays an important role in regulation of the body's vitamin D sufficiency. Sufficient sunlight exposure of the skin sufrace can ensure 80% of the vitamin D synthesis; not only the number of sunny days, but also the intensity of the exposed body surface UVB irradiation is important [2].

Artic is a zone of severe ultraviolet light deficit resulting from the changes in the sun's altitude height above the horizon. Even in summer the conditions for absorption of natural UV radiation are minimal due to low sun's altitude height and considerable losses on foggy and cloudy days (the number of such days reaches 75–90%) [1].

It is well known that the body's vitamin D levels depend on a number of factors, which include the season. For example, in St. Petersburg the vitamin D concentrations measured in summer were 1.75 times higher than that measured in winter. The seasonal improvement in the body's vitamin sufficiency was observed in 61.4% of the surveyed individuals in Samara versus sufficient vitamin levels found only in 23.4% in winter. In autumn, winter, and spring the body does not synthesize enough vitamin D [3–6, 31].

Despite the fact that our study was conducted in summer, the majority of the organized group members had vitamin D insufficiency or deficiency. The optimal levels were found only in one sixth of the surveyed individuals, however, the average levels were close to the lower limit of optimal range. The lowest value of interquartile range ($Q_{25}$) was close to the upper limit of the range estimated as deficiency (31.75 ng/mL).

Our findings are consistent with the data provided by other researchers. Thus, 29% of the adult population of Arkhangelsk (subarctic region) have vitamin D deficiency in spring and summer, and 41% have vitamin D insufficiency, while students show vitamin D deficiency and insufficiency in 40 and 32% of cases, respectively (another 8% have severe deficiency) [5].

Vitamin D and its metabolites are an important component of the endocrine system that regulates the body's calcium homeostasis [32, 33]. The vitamin D active form is an key regulator of the calcium and phosphate homeostasis: it is involved in maintaining the calcium and phosphate homeostasis, bone tissue mineralization and remodeling [23–26].

Calcium contained in bones provides structure and strength to the skeleton, while calcium present in extracellular fluid and cytosol is essential for maintaining numerous biochemical processes [26].

During the study the reduced blood levels of ionized calcium were found in almost 30.0% of the surveyed individuals, while in the others the ionized calcium levels were close to the lower limit of reference range. This means that it's physiological function, i.e. involvement in blood coagulation as a cofactor, maintaining the optimal levels of ions for bone mineralization, involvement in stabilization of plasma membranes via binding of phospholipids in the lipid bilayer, and involvement in regulation of the membrane permeability to sodium, was impaired. The increase in membrane permeability to sodium reduces activity of all excitable tissues [26].

The bound calcium and inorganic phosphorus were within normal ranges; the values of these parameters were close to the lower limits of normal ranges in all the surveyed individuals.

The findings suggest that more considerable changes in the body's vitamin D and phosphorus and calcium sufficiency would take place during the polar night.

Thus, the negative shifts in the balance of the vitamin and minerals pose health risks to the military deployed in Arctic for a long time. The year-round preventive measures should be applied fill the deficit in the body's levels of vitamin D and minerals.

## CONCLUSIONS

In summer, 29.4% of the military, who had been deployed in Arctic for 5.9 ± 0.4 years, had vitamin D deficiency, 52.9% had vitamin D insufficiency, and 17.7% had optimal vitamin levels. Low ionized calcium levels were found in 29.4% of cases. The assessment results of 70.59% of samples that were within normal ranges were at the lower limit of normal range based on $Q_{25}$. The total calcium and inorganic phosphorus levels appeared to be close to the lower limits of normal ranges.

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# NEUROPHYSIOLOGICAL METHOD FOR STUDYING CHANGES IN THE BRAIN'S DEFAULT MODE NETWORK ACTIVITY

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Curiosity about the activity of neural networks in the human brain results from the search for definition of human self-consciousness as an identifier of human personality. Today, the RS-fMRI technology occupies a leading position among methods used to study this problem. The widespread use of the technology is limited by certain drawbacks. Starting from 2010, there is a growing interest in the possibility of using neurophysiological methods for the diagnosis of the brain's default mode network (DMN) state based on the analysis of EEG microstates. The study was aimed to demonstrate the possibility of recording the activity of brain networks both at rest and under exposure to the stimulus evoking a known response. A total of 42 people underwent assessment in the relaxed wakefulness state with the eyes closed that involved extraction of certain EEG microstate sequences and the EEG inverse problem solution. The data obtained were tested for adequacy via comparison with the results obtained by the preset stimulation of auditory and language function. The conclusion was made about the possibility of assesing the brain's DMN's activity by combining the analysis of EEG microstates with the EEG inverse problem solution. The proposed technology can be used in both scientific research and clinical practice in the form of new techniques and systems allowing one to determine alterations in neuropsychological processes.

**Keywords:** thinking, technology, default mode network (DMN), neuropsychological processes, neurophysiology, EEG microstates, hybrid research methods, EEG inverse problem solution

**Compliance with ethical standards:** the study was approved by the Ethics Committee of the National Research Nuclear University "MEPhI" (protocol № 09-01/23 of 09 January 2023), it was conducted in accordance with the principles of the Declaration of Helsinki issued in 1964 and its subsequent revisions.

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# НЕЙРОФИЗИОЛОГИЧЕСКИЙ МЕТОД ИССЛЕДОВАНИЯ ИЗМЕНЕНИЯ АКТИВНОСТИ СЕТИ ПАССИВНОЙ РАБОТЫ ГОЛОВНОГО МОЗГА

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Интерес к активности нейронных сетей покоя человеческого мозга обусловлен поиском определения человеческого самосознания как идентификатора человеческой личности. В настоящее время в изучении данной проблемы лидирующие позиции занимает технология фМРТ покоя. Определенные недостатки ограничивают ее широкое применение. С 2010 г. все больший интерес вызывает возможность применения нейрофизиологических методов диагностики состояния сетей пассивной работы мозга на основании анализа ЭЭГ-микросостояний. Целью исследования было продемонстрировать возможность регистрации поведения сетей головного мозга как в состоянии пассивной работы, так и в ответ на раздражитель, вызывающий заранее известный ответ. Обследовано 42 человека в состоянии пассивного расслабленного бодрствования с выделением отдельных последовательностей ЭЭГ-микросостояний и решением обратной задачи ЭЭГ-исследования. Проверку адекватности полученных данных проводили путем сравнения с результатами, получаемыми при заданной стимуляции слухоречевой функции. Сделан вывод о возможности исследования активности дефолтных сетей головного мозга с помощью комбинирования анализа ЭЭГ-микросостояний с решением обратной ЭЭГ-задачи. Предлагаемая технология может найти применение как в научных исследованиях, так и в клинической практике в виде новых технологий и приборов, позволяющих определять изменения нейропсихологических процессов.

**Ключевые слова:** мышление, технология, сеть работы мозга, нейропсихологические процессы, нейрофизиология, ЭЭГ-микросостояния, гибридные методы исследования, обратная ЭЭГ-задача.

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Curiosity about the activity of neural networks in the human brain results from the search for definition of human self-consciousness. As far back as 1637, R. Descartes made the allegation "Je pense, donc je suis" (I think therefore I am) in his treatise "Discours de la méthode" [1]. He defined human self-consciousness as a key factor inherent only in human being. However, it is generally accepted that organization of thinking is individual in its essence. Social influence may create some similarity of views and systems of cultural perception, however, the thinking process in each individual person appears to be individual and unique when the issue is considered from the perspective of any specific case.

The human brain never loses its activity, even in the resting state. The brain processes the accumulated information that is stored in the memory and makes the goal-directed decisions based on this information. According to the modern literature [2], these processes are strongly associated with the activity of the brain's default mode networks (DMN), the complex neuronal structures showing their activity beyond implementation of the effector neural functions. The mode of activity in such networks depends on numerous factors, such as intensity of recall, analysis of the current situation, planning of processes, etc., however the study of brain activity in different groups of people during transmission of information revealed some

similarities of the results [3]. The studies made it possible to detect the characteristic changes in the pathological process intensity resulting from the effects of spontaneous discharges in patients with idiopathic generalized epilepsy and people with autism [4–6]. Alterations of activity in these brain networks associated with various subtypes of depression have been demonstrated [7, 8]. These papers support the idea that the brain function is implemented via activity of separate neural networks or autonomous parts of the common brain network, that are capable of exerting activity not only when the brain is engaged, but also during the periods of relaxed wakefulness, as the components directly related to individual characteristics of the person's thinking.

It is believed that the main DMN of the brain consists of the posterior medial cortex, medial prefrontal cortex, and the temporoparietal junction, thereby integrating the areas responsible for estimation of information and the regions involved in the information analysis [9]. This construct is considered as a kind of “inherent” system showing high activity at rest and decreased activity when engaged in executing experimental tasks. Some researchers compare this phenomenon with the “mind-wandering” associated with activity of the tertiary cortical areas responsible for analysis of the past and modeling of the future [10–12]. Mind-wandering shows up in the form of spontaneous imagery. Furthermore, is has been suggested that the common brain network is divided into networks of the “internal” and “external” systems [13], similar to the functional concept of the “internal” and “external” information processing pathways [14]. However, these assumptions require a more detailed objective confirmation involving further research with the use of more accessible methods than the currently available RS-fMRI technique.

The first objective studies of the brain's DMN activity were conducted using neuroradiological methods, specifically positron emission tomography and single photon emission computed tomography (PET/SPECT). However, such an approach requires administration of radioisotopes to humans that makes it possible to conduct only one-off studies. Today, the RS-fMRI is a leading diagnostic method. This is a more safe method that has been put into practice after the study conducted by Biswal et al. that was focused on assessing the functions of motor cortex and independent sources of spontaneous activity in the brain [15]. Human brain exerts functional activity even at rest. The main postulate of the resting state fMRI (RS-fMRI) is represented by the differences in blood oxygen levels (BOLD) enabling the detection of active areas in the nervous tissue based on the increased oxygen consumption. To date, inclusion of multiple mathematical methods of data analysis in the method has made it possible not only to identify certain areas showing spontaneous brain activity at rest, but also to acquire information about their interplay by developing the concept of integration of brain networks into a single structure, the connectome [16]. Despite all the benefits of the RS-fMRI [17], significant delays occurring during investigation do not allow one to record rapid processes, since the BOLD signal, that is based on transformation of oxyhemoglobin into carboxyhemoglobin and is associated with alterations of the nervous tissue activity, is generated within 2 s at an average. The technique requires using additional equipment and specific software (related to the system for suppression of noise of the equipment running), it also has low temporal resolution that makes it difficult to record rapid changes in brain activity [18–20].

In 2010, the idea of using EEG, the method to record activity of the brain's DMN by assessing certain brain rhythms, was proposed and implemented [21]. However, the study results that reflected the data of frequency analysis made it impossible to judge the possibility of the technique widespread introduction into the diagnostic process.

According to the PubMed database, at least 300 papers on using the frequency analysis of EEG signals for assessment of the brain's DMN had been published over the next 11 years. All the papers describe two common problems: difficulties in matching the results with the RS-fMRI data and low spatial resolution associated with specific pattern of biopotential distribution over the scalp surface [22, 23]. As a result, despite the occasionally emerging interest, neurophysiological methods, even those implemented on the basis of advanced EEG systems, are seldom used to study the structure and functions of distinct brain networks.

However, a specific way to record the activity of distinct groups of neurons by assessing the EEG signal was proposed by D. Lehmann as early as in 1998: after monitoring certain variants of the compound scalp potential he concluded that the compound electrical activity of the brain could be represented by a sequence of bioelectric patterns that were fixed in time and had a duration of about 40–120 ms, within which the distributed pools of neurons showed synchronous activity and generated stable scalp spatial potential topographies, referred to as EEG microstates [24, 25]. It was concluded that the duration of individual microstate could be interpreted as a reflection of preservation and stability of function of the neuronal assembly the microstate was based on, and the recording frequency could be interpreted as activity of certain neural generators during implementation of the studied brain function. However, recording of isolated EEG microstates still not allowed to define brain structures (networks) that were the sources of activity and the extent to which this process was specific for thinking.

In 1994–1997, R.D. Pascual-Marqui proposed the system for the EEG inverse problem solution based on the technique of matching the dipole localization to the layered head model, called low resolution brain electromagnetic tomography (LORETA).

Conclusion about the localization (1998) was made based on the statistical parametric mapping applied to the LORETA scans with high temporal resolution. Starting from 1999, the technique was enhanced by quantitative neuroanatomy based on the templates provided by the Brain Imaging Centre of the Montreal Neurological Institute (MNI). The combination of these solutions brought LORETA to the level of conventional functional imaging methods, such as PET and fMRI [26].

In 2008, the team of R. Grech assessed various techniques of dipole source localization and concluded that sLORETA provides the best solution among the most widely used officially presented and registered EEG post-processing and analysis tools for localization of sources that exert rhythmic activity considering both localization errors and false sources. In 2014, simultaneous fMRI-EEG studies showed that the LORETA technique used to determine EEG power for the alpha, beta, delta and theta-bands in the region of interest revealed a strong link between the spontaneous BOLD - fluctuations in the brain's DMN and various EEG rhythms [27]. This suggests that certain extracted neural nerwork is characterized by specific “electrophysiological signature” created by the combination of brain rhythm serving different putative functions [28].

Thus, today, neural networks of the brain can be studied not only by the conventional RS-fMRI method, but also by combining the methods for extraction of cetrain EEG microstates with the EEG inverse problem solution. Such a combination allows one to study their activity with the delay lower than that

of neuroradiological methods. Furthermore, spatial resolution of the combination is enough to enable combining with the results obtained by neuroradioimaging methods that are widely used nowadays.

The study was aimed to demonstrate the possibility of recording activity of the brain's DMN by neurophysiological methods and demonstrate adequacy of the data obtained by the example of changes in the studied characteristics in response to the external stimulus evoking a known response. Null hypothesis of the experiment: if no expected changes in bioelectric activity are recorded after applying specific load, the results obtained when studying the relaxed wakefullness state with the eyes closed do not represent activity in the brain's DMN.

## METHODS

The study involved the results of EEG tests of 42 volunteers. Inclusion criteria: no diagnosed neurological or mental disorders as stated in the informed consent. Exclusion criteria: the subject is not a native Russian speaker; left-handedness (even the forced right-handedness); the use of psychoactive substances. The age of subjects in the surveyed group varied between 19–40 years, the average age was 32.37 ± 8 years. All the subjects were right-handed (this was confirmed by using the previously proposed technique [29]) Russian-speakers.

EEG fragments showing background activity were compared in the real time mode in the state of relaxed wakefulness with the eyes closed as previously reported [30]. A 52 channel bioamplifier approved for use in medical institutions with the sampling rate of the analog-to-digital conversion of 500 Hz (Medical Computer Systems; Zelenograd, Russia) and the fragment of recording, when the subject listened to a short story in his/her native (Russian) language (3 min), selected from the full EEG record were used.

EEG was recorded using monopolar montage of electrodes with an average reference electrode. This ensured that the potential differences were equal to the baseline scalp potential at each electrode.

The listening test was selected as a test load due to the possibility of executing the test in the state most close to the baseline state of relaxed wakefulness, which enabled assessment of functional test isolated effects on brain activity. Another reason was a relative knowledge of the human auditory perception system and spatial localization of its major components. The subjects had no preliminary acquaintance with the text to listen to, as well as no additional motivation.

All the tests were performed in the dark and quiet room, however, it was decided not to use a completely soundproofed room due to impossibility to exclude the noise signals of the equipment running and the likelihood of the subject's covert psychogenic response to the isolated room. The latter could cause more severe distortion than the interfering tones and noise with the known characteristics that evoked rhythmic activity over the cortical areas 37 and 47.

The common technological stages of the experiment were as follows. Primary testing of the subject in the relaxed wakefulness state with the eyes closed was performed. The judgement of achieving the relaxed wakefulness state was made based on the stabilization of occipital alpha activity and formation of the clearly defined differences between zones. After the relaxed wakefulness state was achieved, a long epoch was recorded for 3–5 min and saved in a separate data file. The patient's state was assessed after data acquisition. If the differences between zones observed in the EEG recording persisted, the functional load was applied that involved listening for 3–5 min (using the audio recording of speech in native language).

The results of EEG tests were saved in a separate file of the European Data Format (*.edf), allowing for further analysis using the sLORETA software package for EEG signal processing.

Artifacts of the recording were suppressed by the independent component analysis allowing one to separate native EEG data from oscillatory phenomena of a different nature, such as muscle tension, electrooculogram, ECG, etc. After the artifacts were removed from all the selected epochs of the recording, individual stable EEG microstates were calculated by K-means clustering using the software package (sLORETA; Switzerland). It is believed that the minimum number of active neural networks that is enough to describe the activity observed in the relaxed wakefulness state is four [31, 32]. That is why we used a theoretical model consisting of eight contingent neural networks with the twice higher level of base number discretization in accordance with the basic provisions of the Nyquist-Shannon-Kotelnikov sampling theorem to confirm the data obtained and prove the possibility of the brain networks' response to the functional stimulus [33].

Then distinct microstates reflecting the activity of certain groups of neurons [34], that had been extracted from the recording, were subjected to the EEG inverse problem solution in accordance with the previously reported algorithm [35] allowing one to define the main aggregate source of cortical rhythmic activity within Brodmann area 1 (implemented in the sLORETA software package) according to the spatial coordinates stated in the MNI digital MRI atlas [36]. The results obtained were represented as diagrams reflecting the quantitative representation of the EEG microstate recording in accordance with the localization of certain cortical areas.

Thus, the general research model involved extraction of at least eight most frequently repeated sequences associated with the activity of certain cortical macronetworks, that were later spatially localized by using the algorithm for the EEG inverse problem solution, from the general EEG recording. Such an approach allowed us to both define the variants of brain activity alterations and their spatial connection with distinct anatomical structures.

Statistical data processing was performed using the SPSS Statistics 23.0 software package (IBM; USA) in accordance with the guidelines [37]. The null hypothesis was rejected at $p < 0.05$ by using the chi-squared test.

## RESULTS

Testing primary data for consistency using the Cronbach's alpha test showed that the Cronbach's alpha was 0.89, which was typical for high internal consistency of the primary sample and enabled further calculations.

Monitoring of bioelectric activity in the relaxed wakefulness state showed that the largest number of rhythmic phenomena occurred in the occipital regions (Brodmann areas 17, 18, 19). This reflected rhythmic activity of cortical structures observed in the relaxed wakefulness state, which was well-known to the specialists. Such activity was represented by alpha rhythm produced by visual cortical areas in the “standby” or “rest (idle)” mode [38–39].

The rhythmic activity recorded over the cortical auditory and language areas was confined to the areas 22, 37 and 40, which are principally responsible for perception of musical tones rather than language components that are characterized by the involvement of the Wernicke's area tertiary cortical areas (areas

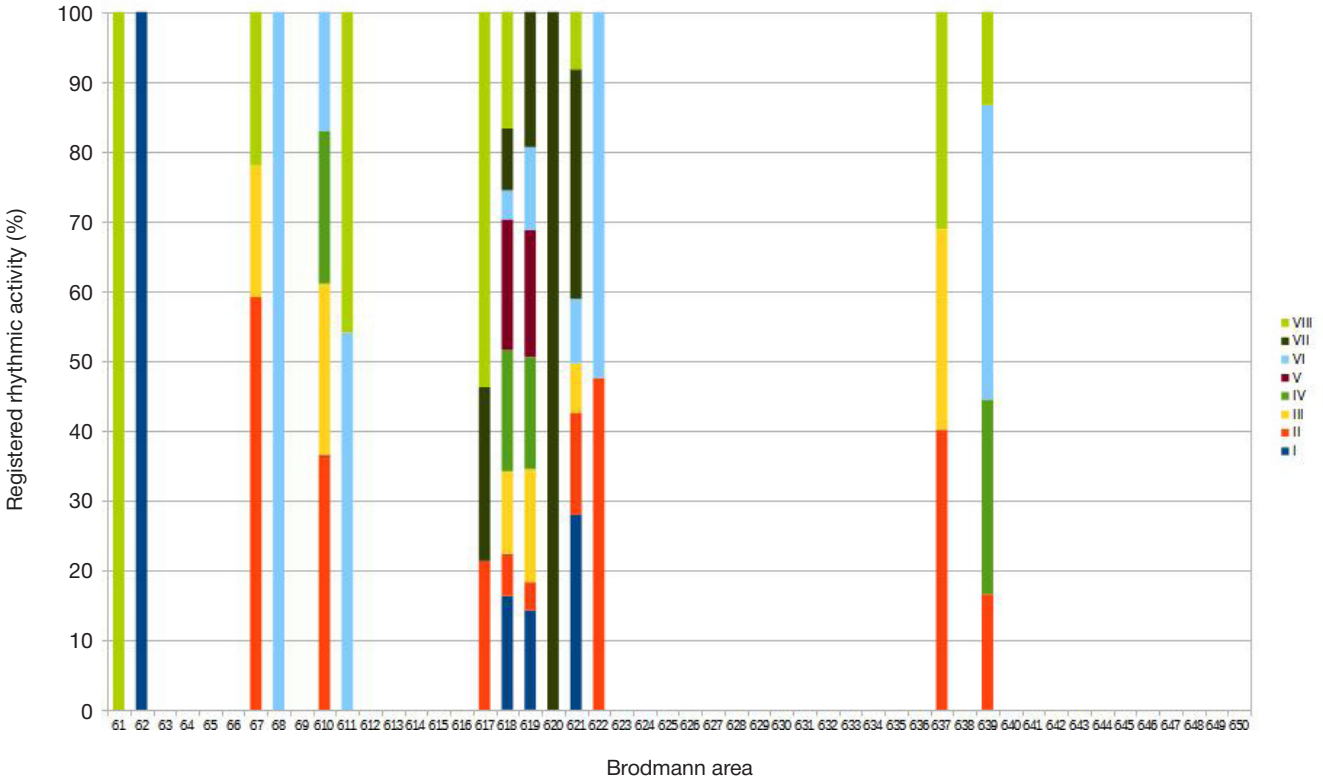


**Fig. 1.** Characteristics of rhythmic activity of individual EEG microstates (classes I–VIII) recorded over individual Brodmann areas in the relaxed wakefulness state (percentage)

39 and 40). The involvement of sensorimotor cortical areas (1 and 2) was also reported (only within a single microstate) together with rhythmic activity over the supplementary motor area (area 6), tertiary cortical areas of the area 7 and prefrontal cortical area (area 10) that charactesized external information processing (Fig. 1).

When executing the functional test that involved listening we expected to detect the activity over the cortical areas related to realization of speech function: areas 37–47 (responsible for musical perception and production of music), area 22 (responsible for perception of tones and noise), areas 39, 40 (constitute tertiary cortical areas of the Wernicke's area), and areas 44, 45 (constitute teriary Broca's area).

Individual analysis of each and every case revealed various individual sequences of the involvement of certain cortical areas that formed the unique profile of each subject. It was also noted that the sequences not necessarily included the well-known speech areas.

In fact, several variants of the response represented by sequences showing involvement of noise perception (area 22) together with area 39, the combination of rhythmic activity over the areas 37 and 47 responsible for musical perception and production of music, conventional involvement of the auditory and language areas 39, 40, 44, and 45, and the variants not invoving the areas related to auditory and language perception were recorded.

However, the group comparison revealed the phenomenon of ordering the ordered structure of EEG microstates compared to the relaxed wakefulness state with no load applied and the changes in characteristics of rhythmic activity exerted by the cortical structures (Fig. 2).

Thus, despite the persisting predominance of rhythmic activity recorded from the areas 17, 18, and 19 that reflected the subjects' staying with their eyes closed, activity of the cortical areas responsible for information processing was concentrated in the cortical areas 6, 7, and 10. Furthermore, in addition to activity recorded from the areas 22, 37, and 40, the emergence of rhythmic activity over the areas 39, 44, 45, 46, and 47 associated with the structures constituting the lateral stream of the sensory system involved in speech (Wernicke's, Broca's areas) was detected in patients during listening.

Statistical comparison using the nonparametric chi-squared test made it possible to define a satisfactory significance level for the data obtained (< 0.001), thereby allowing us to reject the null-hypothesis of the study.

## DISCUSSION

Currently, the methods of assessing the brain's DMN reported in the majority of scientific papers are implemented using the direct results of EEG frequency analysis [21–23]. Matching the data obtained using EEG and the resting state fMRI is one of the challenges faced by the researchers, since the technique for extraction of the DMN activity during fMRI involves representation of the functionally active areas of cerebral structures as the zones showing alterations in BOLD signal, while EEG analysis detects rhythmic activity, that does not represent a physiological equivalent of excitation, in the area of interest [35–39].

According to our findings, the simplest solution of this problem may be recording the prolonged sequence of phenomena of the transition from excitation to the state of generating rhythmic activity within the prolonged epoch of the recording integrated into a single EEG microstate.

In this study we used a proposition that the recording of distinct EEG microstates reflects the activity of the finite number of neural networks, that is why applying the technique of the EEG inverse problem solution to each extracted EEG microstate makes it possible to determine spatial distribution of certain brain structures involved in realization of total brain

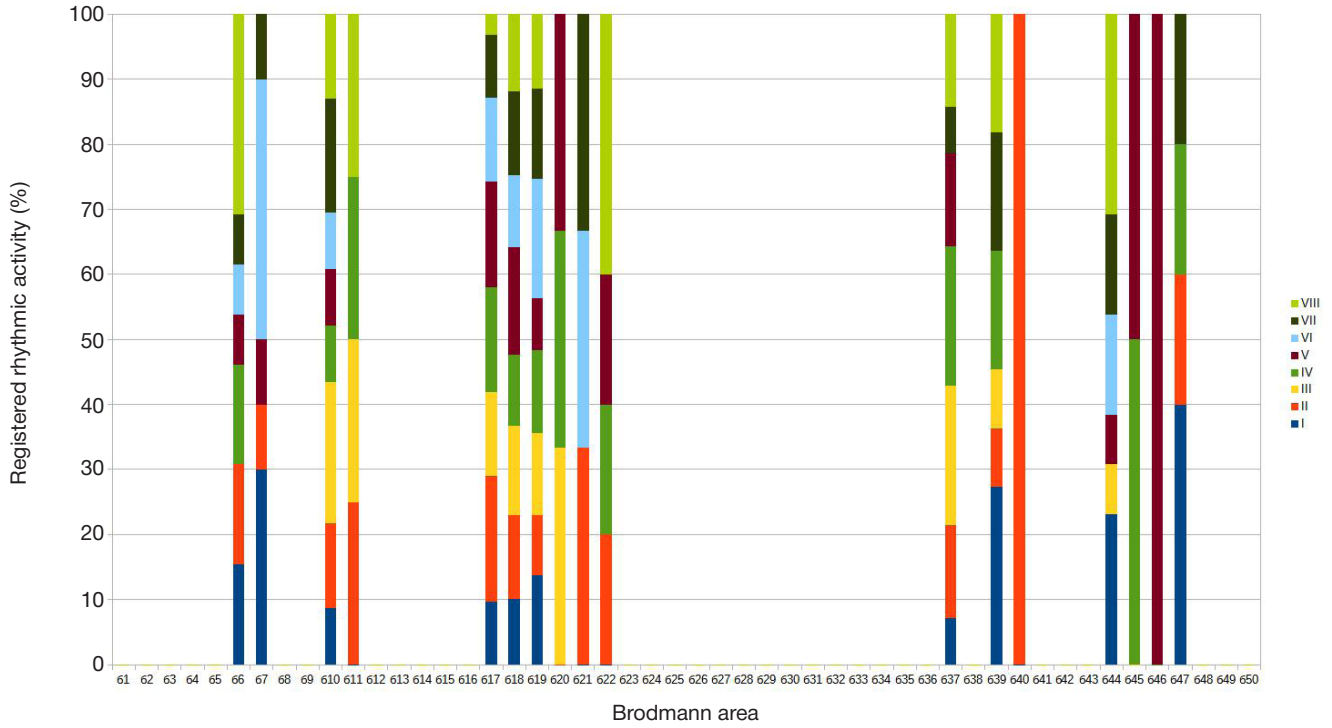


**Fig. 2.** Changes in the characteristics of rhythmic activity of individual EEG microstates (classes I–VIII) recorded over individual Brodmann areas during the functional test (percentage; comparison with the results of background recording; $p < 0.001$, chi-squared test)

activity at rest with higher spatial resolution than the use of frequency analysis only.

In particular, it was shown that the activity of cortical structures, including sensorimotor and visual cortex, was determined in the state of relaxed wakefulness, which, in our opinion, could be characteristic of brain activity related to analysis and processing of information the human deals with at rest [10, 13].

At the same time, inclusion of the functional stimulus that resulted in the significantly changed activity and the expected involvement of the tertiary auditory areas allowed us to interpret the data obtained by analysis of the relaxed wakefulness state as a true manifestation of activity exerted by the brain's resting state networks.

Thus, introduction of the model of EEG microstates shaped the native EEG signal pre-processing, thereby allowing us to extract the signal components produced by certain neural structures. The combination of the model with solving the inverse problem implemented in the processing software packages that are available for researchers makes it possible to propose a simple and more cost-effective technique to determine alterations in neuropsychological processes.

## CONCLUSIONS

The study demonstrates the possibility of performing EEG analysis of the brain's DMN and opens up the prospect of performing affordable and objective studies of cognitive processes to obtain the results with high temporal resolution, since the process of the brain's bioelectric activity recording is not limited to slow biochemical reactions. Such an approach will make it possible to assess spatial localization of the brain structures responsible for implementation of distinct cognitive functions and to determine the dynamics and sequence of their involvement in the cognitive process. Such systems can be developed using advanced equipment, including free open source tools. This makes it possible to propose a new approach to extensive development and the use of EEG technology in both scientific research and practical studies. Active introduction of the systems will allow us to define new prospects in studying human thinking and, perhaps, to propose new cognitive control systems to be used under ordinary conditions and in the environment associated with increased psychoemotional stress.

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## IMMUNE RESPONSES ASSOCIATED WITH HODGKIN LYMPHOMA

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HL is characterized by significantly enlarged lymph nodes and the presence of rare Hodgkin and Reed-Sternberg cells. Pathogenesis is not fully understood. The increase in the disease risk can be associated with immunosuppression, HIV, parenchymal organ transplantation, autoimmune disorders, etc. The possibility of differentiating pathogenetic and protective immune responses associated with this disease will help understand the causes of the disease and the treatment prognosis. The study was aimed to determine the features of immune responses in HL depending on the disease duration and the circulating lymphocyte counts. A total of 134 patients with HL were assessed. The cytogram and phagocytosis were assessed in blood smears stained by the Wright-Giemsa procedure. The expression of lymphocyte markers in lymphocytes was determined using the indirect immunoperoxidase technique and flow cytometry. Serum levels of cytokines, immunoglobulins, autoantibodies and circulating immune complexes were assessed by enzyme immunoassay. Comparative analysis of the immune responses depending on peripheral blood leukocyte counts is provided. It has been found that prolonged HL course is associated with the decrease in the functionally active T cell counts, progressive neutropenia and monocytopenia, along with the increased activity of the reaginic reactions and autosensitization. In individuals with lymphocytopenia, mainly small lymphocytes die, the 3-fold decrease in the counts of such lymphocytes is observed; lymphocytopenia is associated with the deficiency of circulating T cells, both mature and immature, the concentrations of which decrease by 2.5–3 times, while B cell counts show no dramatic changes. The disease progression is associated with reduction of the lymphocyte homeostasis control by granulocytes and monocytes, along with progressive neutropenia and monocytopenia.

**Keywords:** Hodgkin disease, autosensitization, lymphopenia, lymphocytosis, reagins, antibody-dependent cytotoxicity

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**Compliance with ethical standards:** the study was approved by the Ethics Committee of the Laverov Federal Center for Integrated Arctic Research, the Ural branch of RAS (protocol № 4 of 7 December 2016, protocol No. 6 of 14 February 2022) and conducted in accordance with the principles of the Declaration of Helsinki (1975, rev. 2013).

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## ИММУННЫЕ РЕАКЦИИ ПРИ ЛИМФОМЕ ХОДЖКИНА

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ЛХ характеризуется значительным увеличением лимфатических узлов и наличием редких клеток Ходжкина и Штернберга–Рид. Патогенез до конца не изучен. Риск развития может увеличиваться при иммуносупрессии, у пациентов с ВИЧ, трансплантацией паренхиматозных органов, аутоиммунными состояниями и т. д. Возможность дифференцировать патогенетические и защитные иммунные реакции при этой болезни позволит помочь разобраться в причинах заболевания и прогнозах лечения. Цель работы — определить особенности иммунных реакций при ЛХ в зависимости от длительности болезни и уровня циркулирующих лимфоцитов. Обследовано 134 пациента с ЛХ. Цитограмму и фагоцитоз изучали в мазках, окрашенных по Романовскому–Гимзе. На лимфоцитах методами непрямой иммунопероксидазной реакции и проточной цитометрии определяли экспрессию маркеров лимфоцитов. В сыворотке крови методом ИФА определяли содержание цитокинов, иммуноглобулинов, аутоантител и циркулирующих иммунных комплексов. Проведен сравнительный анализ иммунных реакций в зависимости от содержания лимфоцитов в периферической крови. Установлено, что длительное течение ЛХ сопряжено со снижением числа функционально активных Т-лимфоцитов, нарастанием нейтропении и моноцитопении, на фоне повышения активности реагиновых реакций и аутосенсибилизации. При лимфопении погибают преимущественно малые лимфоциты, их концентрация снижается в 3 раза; лимфопения ассоциирована с дефицитом циркулирующих Т-лимфоцитов и касается как зрелых, так и незрелых Т-клеток, концентрации которых в крови падают в 2,5–3 раза, при этом содержание В-лимфоцитов не претерпевает резких изменений. Прогрессирование болезни сопровождается сокращением резервов регуляции лимфоцитарного гомеостаза со стороны гранулоцитов и моноцитов с нарастанием нейтропении и моноцитопении.

**Ключевые слова:** лимфома Ходжкина, аутосенсибилизация, лимфопения, лимфоцитоз, реагины, антителозависимая цитотоксичность

**Финансирование:** работа выполнена в рамках программы фундаментальных научных исследований по теме лаборатории экологической иммунологии Института физиологии природных адаптаций ФГБУН ФИЦКИА УрО РАН № гос. регистрации 122011300377-5.

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Lymphogranulomatosis (Hodgkin lymphoma, HL) is lymphoproliferative disease, the etiology of which is still being debated. First of all, the issue of the origin of cells affected by this disease is addressed. In fact, the lymph node is transformed into granuloma, in which the clusters of lymphoid, reticular, plasma cells, granulocyte neutrophils and eosinophil granulocytes are unevenly distributed. Mononuclear Hodgkin cells (cells with a strongly basophilic cytoplasm and large nucleoli that actively divide) are the main diagnostic feature. The Hodgkin cell is transformed into the multinucleate Reed–Sternberg cell, having cytoplasm with fewer basophils and low division rate, via endomitosis [1]. Such cells actively interact with their microenvironment, create optimal conditions for growth due to the cell proliferation autocrine and paracrine mediators, apoptosis inhibition and suppression of cytotoxic cells [2]. The Reed–Sternberg cells lose most of their B-cell identity, including B cell receptors, and do not undergo programmed cell death. It is well known that immunosuppression (HIV, infections caused by Epstein–Barr virus, autoimmune diseases, etc.) significantly increase the risk of lymphoma [3]. Infections caused by such viruses, as respiratory syncytial viruses, adenoviruses, parainfluenza viruses, coxsackievirus and echoviruses, herpes viruses and many other viruses, have a cytopathic effect, i.e. cause degenerative changes in the cellular structures affecting future cell proliferation, and have an anti-apoptotic effect. These infections equally affect cells of different origin having no lymphoid elements. Studying the features of the immune system function in individuals with proliferative disorders is necessary to understand the body's immune defenses against this disorder.

## METHODS

A total of 134 patients with the final diagnosis of HL, who contacted the Biocor Medical Center (Arkhangelsk, Russia), were studied. The immune status and blood counts of patients were assessed during remission. Inclusion criteria: patients of both genders; duration of the disease of 2–18 years; duration of remission of 5 months to 2.5 years. The diagnosis was confirmed by histological examination: the Hodgkin cells were found in all cases, and the Reed-Sternberg cells were found in 78 patients (58.21%). When making a diagnosis, the mixed cell lymph node involvement was detected in 98 patients (73.13%), while in other cases nodular sclerosis prevailed. Localization of the lymph nodes involved was as follows: mostly axillary (125 cases; 93.28%), mediastinal (65 cases; 48.51%) or inguinal (52 cases; 38.81%); the cases of submandibular lymph node involvement were rare (21 patients, 15.67%). The cytogram and phagocytosis were assessed in blood smears stained by the Wright-Giemsa procedure, the numbers per 100 cells were counted. The expression of lymphocyte markers in lymphocytes was determined using the indirect immunoperoxidase technique and flow cytometry (Epics XL; USA). Serum levels of cytokines IL1β, IL2, IL4, IL10, IFNγ, TNFα (Bender MedSystems; Austria), anti-dsDNA, IgG, IgA, IgM (ORGenTec Diagnostika; Germany), IgE (Monobind; USA), circulating immune complexes were assessed by enzyme immunoassay using the Multiskan FSC system (ThermoFisher Scientific Inc., Finland) and the Evolis automated analyzer (Bio-Rad; USA). The study results were processed using the Statistica 6 software package (StatSoft; USA). The Shapiro–Wilk test was used to test the statistical hypothesis of differences between the values. The data obtained were described using the mean values and standards deviations, as well as the rate of elevated concentrations. Significant differences between groups were identified using the parametric Student's t-test for independent samples and the nonparametric Mann–Whitney U test. The differences were considered significant when p did not exceed 0.05.

## RESULTS

Comparative analysis of the mean indicators of immune status depending from the lymphocyte counts in peripheral venous blood was conducted (Table 1).

The increase in lymphocyte counts is accompanied by activation of the granulocyte neutrophils and monocytes. Activation of neutropils is observed in 62% of HL cases with normal or elevated lymphocyte counts in peripheral venous blood. The increase in neutrophil counts is associated with the left shift; the stab neutrophil counts are markedly higher in patients having no lymphocytopenia. It is well known that activation of the neutrophilic leukocyte proliferation is accompanied by the increase in CD10 expression associated with the increased cell's ability to respond to numerous inflammatory peptides [4–8]. In cases of lymphocytopenia, there is no response of granulocyte neutrophils. The increase in neutrophil counts in HL patients with normal or elevated lymphocyte counts is associated with the increase in monocyte counts, signs of monocyte proliferation activation, and elevated promonocyte counts. Hence, HL in patients with normal blood lymphocyte counts was associated with neutrophilia and monocytosis, while patients with severe lymphocytopenia had a 4 times lower rate of elevated monocyte and neutrophil counts. Such a response is quite similar to the body's response to glucocorticoids. Glucocorticoids, that have long been used for treatment of neutropenia [9–12], affect migration of progenitor cells, granulocyte-monocyte progenitors in blood flow from bone marrow with the 2–4-fold increase in the number of colony forming cells. Glucocorticoids stimulate production of colony-stimulating factors by monocytes, the release of mature granulocytes from the bone marrow into the blood, granulocyte rearrangement in bloodstream and migration [13–16]. The following question remains open: what is observed in HL patients with lymphocytopenia, no response of glucocorticoids or no significant effect of hormones on all the above processes? It was found that the disease duration was a significant factor that affected lymphocytopenia associated with HL: no lymphocytopenia was observed in almost all patients (61 individuals; 89.71%) with the disease duration of up to two years (68 patients); in contrast, the decrease in absolute lymphocytes counts was found in 46 cases (88.46%) with the disease duration of four years or more (52 patients). Lymphocytopenia developing in patients with the long-term disease results in the increasing circulating mature T cell ($CD3^+$) deficiency and is correlated to the decrease in the levels of circulating neutrophils and monocytes ($p < 0.001$).

Neutropenia ($< 2 \times 10^9$ c/L) was found in 19 cases (31.14%), and monocytopenia ($< 0.2 \times 10^9$ c/L) was revealed in 6 patients (9.84%). Given the fact that granulocyte neutrophils and monocytes secrete a wide variety of interleukins capable of changing the cells' receptor activity together with the cell adhesive and migratory abilities, chemotaxis, as well as the activating, colony-stimulating and mitogenic products, it can be assumed that the changes in their counts are the criteria of poor disease outcome. Neutrophils are not only important effector cells, but also regulate and shape the lymphocyte responses. Neutrophils are actively engaged in shaping the paracrine cytokine profile, since these cells, secreting all known cytokines, are involved in apoptosis, contribute to antibody-dependent cellular cytotoxicity and form extracellular traps

**Table 1.** Содержание лейкоцитов в венозной периферической крови больных при ЛХ (M ± *m*)

| Studied parameters | Lymphocytosis (*n* = 73) | Lymphocytopenia (*n* = 61) |
|---|---|---|
| Leukocytes, $10^9$ c/L | 11.54 ± 0.29 | 5.86 ± 0.28 |
| Neutrophils, % | 49.05 ± 0.19 | 36.69 ± 0.21 |
| Neutrophils, $10^9$ c/L | 5.66 ± 0.19 | 2.15 ± 0.21*** |
| Neutrophilia rate, number/% | 62 / 84.93 | 8 / 13.11 |
| Neutropenia rate, number/% | 1 / 1.37 | 27 / 44.26 |
| Monocytes, $10^9$ c/L | 1.98 ± 0.06 | 0.67 ± 0.05*** |
| Monocytosis rate, number/% | 59 / 80.82 | 12 /19.67 |
| Lymphocytes, % | 31.46 ± 0.18 | 36.69 ± 0.21 |
| Lymphocytes, $10^9$ c/L | 3.63 ± 0.15 | 1.24 ± 0.06*** |
| Lymphocyte subset panel: | | |
| small, $10^9$ c/L | 1.93 ± 0.12 | 0.92 ± 0.04*** |
| medium, $10^9$ c/L | 1.51 ± 0.05 | 0.29 ± 0.01*** |
| large, $10^9$ c/L | 0.19 ± 0.03 | 0.15 ± 0.02 |
| Mature $CD3^+$ T cells, $10^9$ c/L | 1.66 ± 0.09 | 0.55 ± 0.09*** |
| Immmature T cells, $10^9$ c/L | 1.39 ± 0.07 | 0.06 ± 0.01*** |
| $CD3^+$ deficiency, number/% | 22 / 30.14 | 61 / 100 |
| B cells, $CD19–20^+$, % | 15.55 ± 0.32 | 50.81 ± 0.19*** |
| B cells, CD19–20+, $10^9$ c/L | 0.57 ± 0.06 | 0.63 ± 0.05 |
| Elevated $CD19^+$, number/% | 62 / 84.93 | 53 / 86.88 |
| $CD16^+$, $10^9$ c/L | 0.43 ± 0.06 | 021 ± 0.04** |
| $CD10^+$, $10^9$ c/L | 0.47 ± 0.04 | 0.34 ± 0.05* |
| $CD71^+$, $10^9$ c/L | 0.63 ± 0.05 | 0.31 ± 0.06** |
| $CD25^+$, $10^9$ c/L | 0.51 ± 0.04 | 0.42 ± 0.03* |
| $HLADRII^+$, $10^9$ c/L | 0.55 ± 0.05 | 0.47 ± 0.07 |
| $CD8^+$, $10^9$ c/L | 0.76 ± 0.05 | 0.21 ± 0.06*** |
| $CD4^+$, $10^9$ c/L | 0.52 ± 0.02 | 0.58 ± 0.06 |
| $CD95^+$, $10^9$ c/L | 0.45 ± 0.05 | 0.28 ± 0.05** |
| Cortisol, nmol/L | 218.32 ± 10.34 | 129.48 ± 9.67** |
| Norepinephrine, nmol/L (lying down) | 1.59 ± 0.27 | 9.22 ± 0.51** |

**Note:** *** — $p < 0.001$; ** — $p < 0.01$; * — $p < 0.05$.

[17–22]. The activated neutrophils influenced by IFNγ express high-affinity CD64 (FcγRI) that determines antibody-dependent cytotoxicity [23–29]. It can be assumed that the decrease in the neutrophil response activity indicates depletion of the the lymphoproliferative process reserve capacity, lymphocyte migration and recirculation control. The same pattern underlies the monocyte response. Like neutrophilic leukocytes, monocytes shape the cytokine profile, serete colony-stimulating factors, enable phagocytosis and antibody-dependent cytotoxicity [30–37]. Perhaps, the effects of monocytes are more prolonged, since the tissue-resident monocyte counts are several times higher than the tissue-resident neutrophil counts, and lifespan of tissue-resident monocytes can be several years. Thus, the neutrophil and monocyte activation responses associated with HL can compensate for functional impairment of lymphocytes and perform regulatory functions of maintaining the portion of lymphocytes that retains the capability of performing functional duties.

When there are relatively high neutrophil and monocyte counts, some favorable features of the leukocyte cellular compositions are revealed: on average, normal mature T cell counts, while $CD3^+$ deficiency is found in individuals with lymphocytopenia, and the $CD3^+$ deficiency detection rate is 2.5 times higher; predominance of cell-mediated cytotoxicity over the helper effects of T cells; finally, the relative large pool of lymphocytes capable of further differentiation. Small lymphocytes that belong to inactive cells carrying genetic information hold the largest share of the patients' circulating lymphocytes. It is believed that small (usually dark-colored, "naked") lymphocytes sized 6–8 μm account for 10–15% of the whole lymphocyte population circulating in the venous blood of generally healthy people [38–41]. The significant increase in the blood small lymphocyte counts (up to 53.17 and 74.19% of total lymphocyte counts) in patients with the diagnosis of Hodgkin disease suggests activation of lymphocyte recirculation in individuals with this disorder. It is well known that lymphocyte recirculation gives lymphocytes access to all parts of the body, enables cell–cell interaction and interaction of lymphoid masses with the lymph nodes, as well as preservation of adaptive counts of lymphocytes circulating in blood.

The most striking differences revealed were represented by mature $CD3^+$ T cell counts against severe deficiency of such cells in individuals with lymphocytopenia. Insufficient amounts of mature circulating T cells were revealed in all patients with lymphocytopenia, while in individuals with no lymphocytopenia the deficiency of mature T cells was revealed only in 30% of cases. However, in fact this significant difference had almost no effect on the activated T cell counts and helper T cell counts. By contrast, individuals with lymphocytopenia had 3 times lower concentrations of cytotoxic T cells. It should be borne in mind

**Table 2.** Levels of cytokines, immunoglobulins and autoantibodies in peripheral venous blood of patients with LH (M ± *m*)

| Studied parameters | Lymphocytosis (*n* = 73) | Lymphocytopenia (*n* = 61) |
|---|---|---|
| IL1β, pg/mL | 4.37 ± 0.06 | 1.57 ± 0.05 *** |
| IL2, pg/mL | 8.34 ± 0.15 | 6.32 ± 0.19 ** |
| IL4, pg/mL | 15.29 ± 0.22 | 12.36 ± 0.23 |
| IL10, pg/mL | 1.57 ± 0.07 | 1.79 ± 0.05 |
| IFNγ, pg/mL | 21.58 ± 0.12 | 10.94 ± 0.09 ** |
| TNFα, pg/mL | 24.43 ± 0.23 | 11.67 ± 0.13 ** |
| IgM, g/L | 1.62 ± 0.19 | 1.98 ± 0.22 |
| IgG, g/L | 21.34 ± 0.59 | 19.85 ± 0.41 * |
| IgA, g/L | 0.92 ± 0.08 | 1.52 ± 0.11 *** |
| IgE, IU/mL | 108.13 ± 2.35 | 194.45 ± 3.89 *** |
| Elevated IgE rate, number/% | 25 / 34.25 | 49 / 80.32 |
| Anti-dsDNA, U/mL | 73.29 ± 1.51 | 116.52 ± 3.58*** |
| Elevated anti-dsDNA rate, number/% | 32 / 50.68 | 48 / 96.72 |
| Elevated CICs (circulating immune complexes), number/% | 51 / 69.86 | 55 / 78.68 |

**Note:** *** — $p < 0.001$; ** — $p < 0.01$; * — $p < 0.05$.

that a sharp decrease in total lymphocyte counts is due to not only cytotoxic T cells, but also to the equal extent lymphocytes having no CD3 on the membrane, and the share of these cells is relatively large (38.57%). Hence, the disease progression and long disease duration are associated with the decrease in total lymphocyte counts, mostly due to $CD3^+CD8^+$ cytotoxic T cells and unidentified lymphocytes. The almost 2-fold decrease in the counts of lymphocytes labeled for apoptosis indirectly indicates that the levels of lymphoproliferative activity in patients with lymphocytopenia are significantly lower. Indeed, HL is associated with reduced lymphocyte functional activity: lower levels of blast transformation induced by phytohemagglutinin (22.56 ± 1.13 и 43.24 ± 1.89%, respectively); individuals with lymphocytopenia have lower counts of activated T cells, especially cells having IL2 receptors. The proliferating part of lymphocytes ($CD10^+$) is probably represented mostly by B cells: both types are more abundant in individuals with lymphocytopenia; patients with lymphocytopenia have higher concentrations of autoantibodies against dsDNA and reagins (IgE), as well as T cells having FcIg $CD23^+$ receptor. B cell counts were significantly higher than normal in 85–87% of cases against the background of moderate increase in total lymphocyte counts and lymphocytopenia; patients of both groups had almost the same absolute B cell counts (Table 2).

The decrease in effector cell activity also affects phagocytes. Among the surveyed individuals with normal lymphocyte counts and lymphocytopenia, phagocytic deficiency was found in 65 (89.04%) and 60 (98.36%) patients, respectively. However, the most significant differences were related to phagocytic activity of monocytes: the percentage of actively phagocytic monocytes was very low, in individuals with normal lymphocyte counts is was 21.35 ± 1.33%, while in individuals with lymphocytopenia the phagocytic cell counts were 9.57 ± 0.27%. Reduced functional activity of the effector cells, especially monocytes, is probably one of the key factors having a pathogenetic significance for the HL development. High concentrations of circulating immune complexes in 69 and 78% of surveyed patients confirm failure of the phagocytic ability of macrophages in this situation. Perhaps, autoantibodies are of some importance, the secretion of which is obviously enhanced in affected individuals, of IgE synthesis intensification. It is well known that synthesis of this class of antibodies (reagins) is associated with low doses of antigen and the duration of antigen exposure. Reagins, having the highest sensitivity, recognize even the conformational antigen alterations and realize one of the most strong and effective antibody-dependent cytotoxicity responses.

## DISCUSSION

HL is characterized by alterations in the lymph node structure and formation of inflammatory tumor microenvironment due to crosstalk between the Hodgkin Reed-Sternberg cells and the immune infiltrate [42, 43]. The study results have made it possible to develop various HL treatment options, including targeted small molecule therapy, treatment with antibody-drug conjugates and checkpoint inhibitors [44, 45]. However, there is not enough information about the functional activity of immunocompetent cells associated with the impact of inflammatory microenvironment depending on the disease duration. We have found that HL is associated with the immune response involving activation of cell-mediated and antibody-dependent responses of isolation and clearance of the pathogen in the lymphocyte or of the transformed lymphocyte itself. Perhaps, all the options, including the backup and rather risky protective mechanisms, such as intensification of the synthesis of autoantibodies and immunoglobulins of specific class (IgE) enabling the increased responses, are used in this case. It is likely that nodular sclerosis in the lymph nodes also represents the form of pathogen isolation that is considered to be enforced in this situation. The intense or long-term struggle may be followed by the phase of the immune defense reserve reduction and the period of lymphocyte depletion. The latter is characterized by mature T cell deficiency with all the consequences. There is a question about the Hodgkin cells and the ensuing Reed-Sternberg cells. The presence of immunoglobulins, receptors of the Fc portion of immunoglobulin (FcIgR), immune complexes on the membranes of these cells, and, most importantly, the phagocytic ability of these cells testify in favor of their macrophagal origin. This issue is still being debated. However, if we assume that initiation of immune response by phagocytic and presenting cells predetermines its further intensity, we can accept all the difficulties related to pathogen isolation in case the pathogen is preserved and even reproduced in the phagocyte. Anyway, our findings show that monocytes of the surveyed patients are characterized by extremely low activity;

accumulation of extremely high concentrations of circulating immune complexes actually utilized only by monocytes is reported. This view is also supported by the fact of reduced blood $CD16^+$ mononuclear cell counts in case of the disease progression in individuals with lymphocytopenia. It is well known that expression of the gene encoding the CD16 protein is not associated with the phagocytosis rate and is inherent to the cells actively secreting cytokines. Thus, lymphocytopenia observed in affected individuals actually represents a phase of the immune homeostasis maintenance reserve depletion due to impaired function of phagocytes and cells showing antibody-dependent cytotoxicity. This phase of depletion results from imbalance of the catecholamine and cortisol responses. However, in contrast to cortisol, catecholamine levels do not decrease, even when stress becomes habitual [46–48]. From this perspective, lymphocytopenia associated with HL is likely to be a criterion of adverse outcome. Such lymphocytopenia is associated with both the decrease in activated T cell counts and the deficiency of differentiated immunocompetent cells.

## CONCLUSIONS

The major events in lymphoid organs of individuals with HL are represented by the lymphocyte death. Perhaps, lymphocytes die due to severe antibody-dependent cytotoxicity involving granulocyte neutrophils, and later eosinophils and macrophages. Antibodies that ensure such responses belong mainly to IgA and IgE. This response is supported by IL1β and TNFα; it is well known that TNFα induces the IL1β and IL5 synthesis, thereby preventing apoptosis [49]. Predominantly small lymphocytes die, their concentrations decrease by 3 times in individuals with lymphocytopenia; lymphocytopenia is associated with the circulating T cell deficiency and involves both mature and immature T cells, the 2.5–3-fold decrease in blood counts of which is observed in lymphocytopenia, however, there are no dramatic changes in B cell counts. Increasing lymphocytopenia, i.e. the disease progression, is accompanied by reduction of the lymphocyte homeostasis control reserve by granulocytes and monocytes with increasing neutropenia and monocytopenia.

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# ASSESSMENT OF THE EFFECT OF CHRONIC EXPOSURE ON PREMATURE AGING OF HUMAN T-LYMPHOCYTES BASED ON UNSTABLE CHROMOSOME ABERRATIONS

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For more than 60 years, residents of the villages on the Techa River have been chronically exposed to combined radiation, receiving a wide range of doses. Red bone marrow (RBM) is the critical system in the exposure conditions. This study aimed to assess the effect of chronic exposure on premature aging of T-lymphocytes based on the frequency of unstable chromosome aberrations; the subjects were the residents of the Southern Urals that have been chronically exposed to radiation. The increased frequency of occurrence of dicentrics and rings in T-cells of the exposed persons was the marker of cellular aging, with the associated doses to the red bone marrow (RBM dose) at 0.5–2.5 Gy. The participants (RBM donors), both exposed and non-exposed, were divided into three age subgroups: 40–59 years old, 60–69 years old, 70–79 years old. The differences in the RBM dose among the exposed individuals were insignificant. In the exposed group, unstable chromosome aberrations (UCA) were recorded significantly more often than in the control group ($p$ = 0.04). The age group of 40–59 years was the one where the exposed donors had significantly more frequently occurring chromosome aberrations compared to the non-exposed participants. There were no such differences registered in other age groups. The age-associated increase of the amount of chromosome aberrations was registered in the non-exposed group only. Chronic exposure to radiation indirectly promotes premature aging of T-lymphocytes: 1) in the long term, the exposed individuals had UCA significantly more often; 2) compared to the control group, the 40–59 years age subgroup of the exposed group had increased cytogenetic index. In the context of this study, the number of dicentrics and rings was not registered as increasing in the older age subgroups of exposed individuals, which may be due to the specifics of the donor inclusion criteria, which, for the elderly, may favor radioresistant individuals.

**Keywords:** unstable chromosome aberrations, dicentrics, rings, Techa River, aging of T-lymphocytes, chronic radiation exposure, Southern Urals

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# ОЦЕНКА ВЛИЯНИЯ ХРОНИЧЕСКОГО ОБЛУЧЕНИЯ НА ПРЕЖДЕВРЕМЕННОЕ СТАРЕНИЕ Т-ЛИМФОЦИТОВ ЧЕЛОВЕКА НА ОСНОВЕ НЕСТАБИЛЬНЫХ ХРОМОСОМНЫХ АБЕРРАЦИЙ

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Более 60 лет жители прибрежных сел реки Теча подвержены хроническому сочетанному облучению в широком диапазоне доз. Критический орган при облучении — красный костный мозг (ККМ). Целью работы было оценить влияние хронического облучения жителей Южного Урала на преждевременное старение Т-лимфоцитов на основе частоты нестабильных обменных аберраций хромосом. Маркером клеточного старения была повышенная частота дицентриков и колец в Т-клетках облученных лиц (дозы на красный костный мозг — 0,5–2,5 Гр). Сформированы три возрастные подгруппы (40–59 лет, 60–69 лет, 70–79 лет) среди облученных и необлученных лиц. Подгруппы облученных лиц по дозам на ККМ достоверно не различались. Нестабильные хромосомные аберрации (НХА) в клетках облученных лиц отмечены достоверно чаще, чем в группе сравнения ($p$ = 0,04). Достоверно повышенную частоту хромосомных аберраций выявили у облученных доноров в возрасте 40–59 лет при сравнении с необлученными донорами такого же возраста. В двух других возрастных периодах различий нет. Только у необлученных доноров выявили возрастную динамику увеличения хромосомных аберраций. Хроническое облучение оказывает опосредованное влияние на преждевременное старение Т-лимфоцитов: 1) достоверно повышена частота НХА у облученных лиц в отдаленные сроки; 2) выявлено увеличение цитогенетического показателя у облученных лиц в возрасте 40–59 лет по сравнению с лицами, не подвергавшимися аварийному облучению. Отсутствие динамики увеличения дицентриков и колец в старших возрастных группах у облученных лиц может быть обусловлено особенностью критериев для включения доноров в цитогенетическое исследование. В старшем возрасте критерии могут способствовать включению лиц с радиорезистентными характеристиками.

**Ключевые слова:** нестабильные хромосомные аберрации, дицентрики, кольца, река Теча, старение Т-лимфоцитов, хроническое радиационное воздействие, Южный Урал

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Over 70 years ago, several accidents happened at the Mayak Production Association facility; as a result of these accidents, more than 100,000 residents of the Southern Urals were chronically exposed radiation. For several decades now, specialists of the Urals Research Center for Radiation Medicine have been monitoring health of the exposed people, studying the effects of ionizing radiation on the organs and tissues of the body and providing medical and psychological assistance to the affected and their offspring. Residents of the Techa riverside villages were exposed to combined radiation: internally, the sources of radionuclides were the food and water consumed (mainly $^{89,90}$Sr), and externally there was the γ radiation from the water of the river into which the Mayak PA discharged the liquid radioactive waste. It is important to note here that strontium, being similar to calcium, replaces it in bone tissue and bombards bone marrow cells with β particles, affecting hematopoiesis and immunity of the exposed person. This thesis is confirmed by the results of the published immunological, clinical, cytogenetic, and epidemiological studies that involved population of the Techa riverside villages [1].

In the recent decades, a cohort of residents of the Techa riverside villages has naturally stepped into the late phase of life, which allows starting investigation of the effect chronic radiation exposure has on human aging. Mechanisms of aging is research problem currently tackled by scientists and medical professionals around the world. The goal is to preserve the quality of life of an elderly person and, considering the increasing life expectancy in the developed countries, enable the older generation to fully participate in the life of the society [2].

In addition to studying the natural mechanisms of body aging at all system levels (cellular, tissue, organ, etc.), it is important to investigate the influence of adverse factors that can accelerate the processes leading to aging or trigger its mechanisms earlier, leading to what can be called premature (unnatural) aging of the studied systems or the body as a whole [3]. Hence, studying the effect ionizing radiation has on premature aging of biological systems is an urgent scientific problem, since radiation is one of the factors peculiar to the modern civilization.

Exchange-type chromosome aberrations (dicentric and circular chromosomes) are a generally accepted marker of cell aging; they are characteristic for the processes associated with instability of the human genome [4, 5]. The exchange between different chromosomes is the result of breaks of the DNA's two strands (less often — one strand) and the subsequent incorrect repair thereof. On average, a cell sees about 8.8 breaks of two strands and 55,000 breaks of one strand of the DNA in a day [2]. The efficiency of repair of such breaks and/or elimination of cells with unrepaired DNA damage decreases with age, consequently, the frequency of occurrence of chromosome aberrations increases.

In biodosimetry studies, the increased frequency of occurrence of dicentrics and rings in cells is perceived as a marker of exposure to ionizing radiation. The dose-effect dependencies for X-ray, gamma and other types of radiation have been studied well; this background enables assessing the radiation dose received by a person based on the frequency of unstable chromosome aberrations (UCA) if several months have passed since the exposure. In the long term, the increased frequency of UCA is a biomarker of exposure to ionizing radiation [6].

For over 40 years, the phytohemagglutinin-stimulated peripheral blood T-lymphocytes sampled from the exposed residents of the Southern Urals have been studied in the context of a cytogenetic investigation [7, 8]. Over the entire follow-up period, the chronically exposed persons had significantly elevated levels of UCA compared to the individuals that did not suffer such fallout of the accidents. However, the analysis of the dynamics of the UCA occurrence has shown a two-fold decrease of the frequency thereof, 25 and 50 years since the beginning of the exposure [9], which can be explained by the elimination of cells with unstable aberrations during mitosis due to violation of chromosome segregation into daughter cells. The presence of unstable rearrangements indicates that the cell with an aberration has entered its first mitosis *in vitro* or is a daughter of a precursor cell. It was shown that in the second mitosis, cells with dicentrics occurred 50% less often, but in the third mitosis, despite the 70% drop in the occurrence frequency, such cells were still present [10]. Dicentrics with closely spaced two centromeres had a noteworthy higher chance of surviving the cell cycle.

Numerous radiobiological studies have shown that the frequency of exchange-type chromosome aberrations correlates with age of the person. There were no dicentric chromosomes found in the phytohemagglutinin-stimulated T cells sampled from the newborns, but such did appear and became more common with age [11]. The UCA were obviously growing more frequent in groups of people divided by age with 10-year increments [12]. Thus, an increased frequency of UCA is a marker of exposure to ionizing radiation and cellular aging [13]. Based on the above, it can be assumed that exposure to ionizing radiation leads to the formation of additional chromosome aberrations in cells. Therefore, a greater frequency of chromosomal damage in exposed individuals compared to the same-age non-exposed people will indicate premature aging of cells under the action of radiation.

This study aimed to investigate the effect of ionizing radiation on premature aging of human peripheral blood T-lymphocytes based on the frequency of UCA with red bone marrow as the critical system affected by chronic exposure.

## METHODS

### Study Design

This work was based on the results of cytogenetic studies of samples taken from 800 exposed individuals (RBM doses from 0.001 Gy to 4.1 Gy, age from 40 to 89 years) and 100 non-exposed people; the tasks were to form age subgroups among the exposed and the unexposed, analyze the frequency of chromosome aberrations in each age subgroup and intercompare the indicators, assess the age-related dynamics of chromosome aberrations, and, based on the results, make a conclusion about the effect of chronic exposure, as suffered by the residents of the Southern Urals, on premature aging of T-cells in the long term.

### Characteristics of the donors

The exposure of residents of the Techa riverside villages to combined sources of radiation began in the 1950s, when Mayak Production Association facility discharged radioactive wastes into the river system. The external sources of γ-rays were bottom sediments and floodplain soils contaminated with radionuclides. The internal sources of β-rays were radionuclides that entered the body with river water and locally produced food. The internal exposure doses were estimated based on the measured content of $^{89,90}$Sr radionuclides in the body; external exposure doses were calculated based on the duration and frequency of presence of the participants (by age subgroups) in contaminated areas. The study relied on

the red bone marrow (RBM) exposure doses calculated at the biophysics laboratory of the Urals Research Center for Radiation Medicine using the TRDS-2016 system [14].

The cytogenetic study that involves local residents chronically exposed to radiation continues currently; the cytogenetic database receives regular updates. The inclusion criteria for the study are: residence in the Southern Urals (exposed and non-exposed); no history of autoimmune, oncological, chronic inflammatory diseases in the acute phase; for an exposed person — calculated cumulative exposure dose factoring in internal (β-rays) and external (γ-rays) sources [14]. People who underwent X-ray examination less than 6 months before blood sampling were excluded from the study. Same criteria applied to the control group gathered from the residents of uncontaminated areas.

Calculated exposure doses are significantly uncertain (30–60%), therefore, it was decided to include donors with RBM dose from 0.5 to 2.5 Gy. The age of the participants was 40 through 79 years. Thus, ultimately, the exposed group included 343 people (138 male and 205 female), and the control group 83 people. Three age subgroups were formed within each group, spanning ages 40 through 59 years, 60 through 69 years, 70 through 79 years. A fundamentally important fact is that the RBM doses did not differ significantly throughout the age subgroups. Table 1 shows the distribution of participants (donors) into age subgroups.

Thirty-eight of the exposed individuals underwent cytogenetic examination several times. After distribution of the participants into age subgroups, we found that we could follow the dynamics of indicators of only 15 people in different subgroups, and the remaining 23 participants that previously had been examined several times belonged to the same subgroup. Seven people in different age subgroups had zero chromosome aberrations; we did not include them in the analysis of the dynamics of indicators. Thus, we can show the dynamics of the UCA occurrence frequency for 8 people only, and since they were in different age subgroups, Table 2 disregards specific age and cites "early" and "late" age periods.

### Preparation and analysis of the slides with metaphase chromosomes

We made cytogenetic preparations of the phytohemagglutinin-stimulated peripheral blood T-lymphocytes following the protocol adopted by the Laboratory of Radiation Genetics of the Urals Research Center for Radiation Medicine. This protocol includes four successive stages: cell cultivation to the metaphase condition (duration — 52 hours, colcemid administered 3 hours before the end, ultimate concentration of 0.1 mg/mL); hypotonic treatment of metaphase cells (1 hour before fixation); fixation of metaphase spreads (freshly prepared fixative: 3 parts of ethanol and 1 part of glacial acetic acid); preparation of the slides with metaphase chromosomes. Metaphase chromosomes were stained with 2% Giemsa solution for 10 minutes, then the stain was washed off and the slides were dried at room temperature [7, 8].

We used AxioImager A2, Z2 microscopes to analyze the preparations (no karyotyping): took 46-chromosome cells with 1–2 overlaps, marked dicentric and circular chromosomes and acentric rings. The number of cells analyzed from each participant ranged from 100 to 500.

### Statistical methods

The obtained results were processed using the variation statistics methods: we calculated the median and the 25$^{th}$ and 75$^{th}$ percentiles, as well as the mean per 100 cells (if the percentile values were equal to 0, they were not put into the data table). Kolmogorov-Smirnov test enabled verification of normalcy of distribution of the indicators. Since the data we collected tended to distribute abnormally, we used the nonparametric Mann–Whitney test to compare values in the groups. The $\chi^2$ test allowed assessing dynamics of the individual indicators of exchange-type aberrations. STATISTICA 10.0 software package (StatSoft Inc.; USA) was used to statistically process the data obtained.

## RESULTS

Comparing the indicator data gathered from all exposed and all non-exposed individuals, we registered a significant excess of cells with metabolic UCA in the exposed individuals ($p = 0.04$). However, no linear correlation dependence of the studied parameters on the RBM dose was established in the group of exposed persons (all age subgroups) ($R = 0.125$; $p = 0.005$).

Table 1 presents the results of the study of dependence of the chromosomal aberrations occurrence frequency in different age subgroups.

It should be noted that exchange-type UCAs are rare events. In all subgroups (no exceptions), the median was zero, therefore, Table 1 shows the mean value. Absence of the range of 25$^{th}$ and 75$^{th}$ percentiles also reflects zero values. In the control group, the studied indicator grows with age (0, 0.18 and 0.30 per 100 cells). In both "60–69 years old" and "70*p*79 years old" age subgroups of the non-exposed group the frequency of chromosome aberrations was significantly higher than in the "40–59 years old" subgroup ($p^1 = 0.06$, $p^2 = 0.02$). There were more exchange-type events registered in the "70–79 years old" age subgroup, but the differences with the "60–69 years old" subgroup did not reach significance.

As for the frequency of occurrence of chromosome aberrations in the subgroups of the exposed group, the studied indicator did not grow with age. On the contrary, the respective values were the same in all three age subgroups ($p^1 = 0.69$, $p^2 = 0.37$), which constitutes another proof of the lack of linear correlation between frequency of chromosome aberrations and age ($R = 0.002$, $p = 0.76$).

Comparison of the frequencies of occurrence of exchange-type aberrations between the age subgroups of unexposed and exposed persons revealed a significant increase of the indicator's value in the "40–59 years old" subgroup of the exposed group ($p = 0.038$). In the exposed individuals aged 60–69 years, we detected cells with chromosome aberrations more often, but the differences were not significant. As for the oldest donors, there were no differences in cytogenetic parameters registered between the exposed and non-exposed groups.

Table 2 presents the analysis of dynamics of individual indicators reflecting frequency of occurrence of unstable exchanges in 8 exposed individuals.

The frequency of occurrence of chromosome aberrations has grown with age in 5 out of 8 examined participants, but the differences were not significant. However, it is noteworthy that the median frequency of UCA increased with age from 0.375 in the "early" group to 0.775 in the "late" group, while the RBM dose did not change significantly, (second figure after the decimal point).

## DISCUSSION

Investigation of the human aging mechanisms is a scientific problem made urgent by the change in the world's age balance:

**Table 1.** Frequency (%) of unstable chromosomal aberrations in the examined groups (median, 25 and 75%)

| Age subgroups | Control group median / mean 25–75% | | Exposed individuals RBM dose 0.5–2.5 Gy median / mean 25–75% | |
|---|---|---|---|---|
| | *n* M : F | exchanges, % | *n* M : F | exchanges, % |
| 40–59 y.o. | 17<br>5 : 12 | 0 / 0 | 55<br>23 : 32 | 0 / 0.23<br>$p = 0.038$ |
| 60–69 y.o. | 44<br>14 : 30 | 0 / 0.18<br>$p^1 = 0.06$ | 191<br>77 : 114 | 0 / 0.25<br>0–0.2<br>$p = 0.427$<br>$p^1 = 0.69$ |
| 70–79 y.o. | 22<br>8 : 14 | 0 / 0.30<br>0–0.625<br>$p^2 = 0.02$ | 97<br>38 : 59 | 0 / 0.24<br>0–0.225<br>$p = 0.973$<br>$p^2 = 0.37$ |

**Note:** *p* — statistical differences in indicators between similar age subgroups of the exposed and control groups; $p^1$ — statistical differences in indicators between age subgroups "40–59 years old" and "60–69 years old"; $p^2$ — statistical differences in indicators between age subgroups "40–59 years old" and "70–79 years old"; M — male; F — female.

the number of elderly people is growing, by 2050, there will be about 1.6 billion people over 65 [15]. Understanding the basics of the body's aging program, inter alia, rests on research looking into the patterns of aging with the environmental factors accounted for. Such research requires a reliable set of tools: markers, methods, approaches, which eventually form a methodology for the respective studies. A living organism is a multilevel system, therefore, the processes and effects of aging are studied at several levels: subcellular, cellular, levels of tissue, organ, organism [16].

We chose the cytogenetic method that allows assessing the state of chromosomal DNA to study the possible effect of ionizing radiation on premature aging of human cells. Our work focused on the unstable exchange-type chromosome aberrations in the phytohemagglutinin-stimulated peripheral blood T-lymphocytes. The choice of this object was not accidental: in addition to γ beams, precursors of T-cells were also exposed with osteotropic $^{89,90}$Sr radionuclides in the bone marrow. Currently, the youngest exposed individuals are 60 years old. It is known that after the age of 25, human thymus begins to involute, but there is a high probability of retaining UCA in cells that have not entered mitosis in the body or cells that survived 1–2 mitoses with aberrations. Circulating lymphocytes have been and are still exposed to the internal sources of radiation [17].

In the group of non-exposed participants, we detected an age-driven increase of the frequency of occurrence of cells with unstable metabolic aberrations. Moreover, this indicator was significantly higher in both the "60–69 years old" and "70–79 years old" age subgroups than in the "40–59 years old" subgroup. The regularities we discovered are consistent with the data from previously published papers, which have also noted the dependence of the amount of dicentric and ring chromosomes on age [11, 12]. However, we registered no such dependencies when analyzing the data describing chromosome preparations made from samples collected in the exposed group. In the three age subgroups, we have not detected the expected age-dependent increase in the frequency of chromosome aberrations. All indicators (Table 1) were similar and maximum for this study.

We revealed the influence of chronic exposure on the frequency of chromosome aberrations only in "40–59 years old" subgroup. Contrary to the expectations, the oldest participants (70–79 years old) from the exposed group had the indicators at the same level as that calculated for non-exposed donors. Thus, we registered that chronic exposure affects premature cellular aging in the residents of the Techa riverside villages aged 40–59 years only. Examining older exposed individuals, we found them to have similar indicator values as those from the control subgroups of the same age.

There is an explanation to the data that, at the first glance, looks "contradictory": the cytogenetic study inclusion criteria may bias the selection and fill the exposed group with the most radioresistant donors. For example, to participate, a person should have had a medical history without oncological, autoimmune diseases, diabetes mellitus. Such restrictions allow excluding the effect diagnostic and therapeutic measures may have on the frequency of occurrence of the studied cytogenetic indicators. Given that the above diseases more often begin to manifest in older ages, it is quite possible that the exposed radiosensitive individuals had the effects of irradiation triggering disorders in them earlier in life and, consequently, met the study exclusion criteria. There is a study that provides an indirect confirmation of this assumption: there, researchers noted that individuals with an increased frequency of chromosome aberrations were more likely to develop cancer in the future

**Table 2.** Dynamics of frequency (%) of unstable exchange-type chromosome aberrations in the same exposed individuals examined in different age subgroups

| Number of the person | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | Total. Median 25–75% |
|---|---|---|---|---|---|---|---|---|---|
| Early indicator | 0 | 1 | 0.98 | 0.75 | 0 | 0 | 0.85 | 0.8 | 0.375<br>0–0.9 |
| Late indicator | 0.6 | 0 | 0 | 1 | 0.25 | 0.5 | 0 | 1.5 | 0.775<br>0–0.9<br>$p = 0.8$ |

**Note:** *p* — statistical differences in the final indicators as registered between subgroups.

[18]. Taking into account all of the above, we can assume that while the younger groups participating in the cytogenetic research were comprised of people with a diverse genetic potential in terms of response to exposure to ionizing radiation, the older groups (above 60 years of age) included the so-called "radioresistant" individuals.

Despite some controversy in the results of the analysis, having conducted this study, we can conclude that chronic exposure did have an indirect effect on the premature aging of T-lymphocytes. The confirmations are, firstly, the significantly increased frequency of UCA registered in the exposed individuals in the long term, and secondly, the significantly more frequent occurrence of UCA in the exposed individuals aged 40–59 years compared to that found in their peers from the non-exposed group.

Thus, this article attempted to present the design of a cytogenetic study aimed at assessment of the effect of *in vivo* chronic ionizing radiation on the premature aging of human T-lymphocytes, as well as the subsequent analysis of the results of the study with its participants divided into three age subgroups. Ultimately, we cannot discount the possible effect of the inclusion criteria that may fill the exposed group with the most radioresistant individuals, which eventually may lead to "contradictory" results. This is a debatable topic; the investigation should be continued with a larger sample (both groups) and a greater number of the analyzed metaphase spreads.

## CONCLUSIONS

Relying on the cytogenetic index, we registered the effect of chronic exposure on premature aging of T-cells in the residents of Southern Urals that were 40–59 years old at the time of the examination.

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# THERAPEUTIC EFFICACY OF N-CHOLINOLITIC DRUG *N,N*-DIETHYL-5,5-DIPHENYL-2-PENTYNYLAMINE IN MODELS OF NON-CARDIOGENIC PULMONARY EDEMA

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The existing non-cardiogenic pulmonary edema (NCPE) treatment methods are not sufficiently effective. *N,N*-Diethyl-5,5-diphenyl-2-pentynylamine hydrochloride (DDPA), the N-cholinolitic drug, is of interest as a potential remedy for treatment of toxic pulmonary edema (TPE). The study was aimed to determine therapeutic efficacy of the drug in animal TPE models. TPE in white rats was induced through intraperitoneal thiourea injection or nitrogen dioxide inhalation. Treatment of animals involved inhalation of the DDPA aqueous solution. The efficacy was estimated based on the animals' survival rate and lung gravimetry data. The results were assessed based on descriptive statistics using the Student's *t*-test. In the model of thiourea-induced NCPE, the drug administered after the toxic exposure increased the animals' survival rate and significantly decreased lung hydration levels (149% vs. 262.5% in non-treated animals). In the model of nitrogen dioxide-induced NCPE, the drug significantly increased the rats' survival rate within the period between 0 and 5 h, however, the differences became non-significant within 24 h. The treated animals had 15–20% lower respiratory rate and pulmonary coefficients than non-treated animals 5 h after the $NO_2$ exposure. The use of DDPA improved the survival rate and overall health in both TPE models, however, the thiourea-based model showed better treatment outcomes compared to the $NO_2$–based model. Such differences can be explained by the deeper and more disruptive nature of the lung tissue injury caused by nitrogen dioxide compared to that caused by thiourea. Thus, the use of DDPA in individuals with injuries induced by pulmonotoxic chemicals may be promising at the prehospital stage.

**Keywords:** toxic pulmonary edema, model, *N,N*-diethyl-5,5-diphenyl-2-pentynylamine hydrochloride, inhalation

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**Author contribution:** Melikhova MV — assessment of the animals' physiological state, data processing and interpretation; Krasnov KA — modeling of toxic pulmonary edema in animals, prepress manuscript preparation; Rozhko MA — drug administration through inhalation and monitoring of external respiration parameters in animals; Lapina NV — general management; Krasnova AA — lung gravimetry in animals.

**Compliance with ethical standards:** all the procedures involving model animals were performed in accordance with the principles of Good Laboratory Practice and the Directive 2010/63/EU of the European Parliament and (2010) on the protection of animals used for scientific purposes.

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# ЛЕЧЕБНАЯ ЭФФЕКТИВНОСТЬ Н-ХОЛИНОЛИТИКА *N,N*-ДИЭТИЛ-5,5-ДИФЕНИЛ-2-ПЕНТИНИЛАМИНА НА МОДЕЛЯХ НЕКАРДИОГЕННОГО ОТЕКА ЛЕГКИХ

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Существующие методы лечения некардиогенного отека легких (НКОЛ) недостаточно эффективны. Препарат *N,N*-диэтил-5,5-дифенил-2-пентиниламина гидрохлорид (ДДПА) из группы н-холинолитиков представляет интерес как потенциальное средство для лечения токсического отека легких (ТОЛ). Целью исследования было определить его лечебную эффективность на моделях ТОЛ у животных. ТОЛ вызывали у белых крыс внутрибрюшинным введением тиомочевины или ингаляцией диоксида азота. Лечение животных проводили путем ингаляции водного раствора ДДПА. Эффективность оценивали по выживаемости и гравиметрическим параметрам легких у животных. Результаты оценивали на основе описательной статистики, используя критерий Стьюдента. На модели НКОЛ, вызванного тиомочевиной, препарат, введенный после отравления, повышал выживаемость животных и статистически значимо снижал степень гидратации легких (149% против 262,5% у нелеченых животных). На модели НКОЛ, вызванном диоксидом азота, препарат значимо повышал выживаемость крыс в период от 0 до 5 ч, однако в течение 24 ч различие становилось недостоверным. Через 5 ч после $NO_2$-затравки у леченных животных частота дыхания и легочные коэффициенты были ниже на 15–20%, чем у животных без лечения. Применение ДДПА увеличивало выживаемость и улучшало общее состояние животных на обеих моделях ТОЛ, но на тиомочевинной модели результаты лечения были лучше, чем на $NO_2$-модели ТОЛ. Это различие можно объяснить более глубоким и более деструктивным характером повреждений легочной ткани, вызываемых двуокисью азота, по сравнению с тиомочевиной. Таким образом, применение ДДПА может быть перспективно на догоспитальном этапе при поражении пульмонотоксикантами.

**Ключевые слова:** токсический отек легких, модель, *N,N*-диэтил-5,5-дифенил-2-пентиниламин, ингаляция

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**Вклад авторов:** М. В. Мелихова — оценка физиологического состояния у животных, обработка и интерпретация данных; К. А. Краснов — моделирование токсического отека легких у животных, подготовка материалов к печати; М. А. Рожко — ингаляционное введение препарата и контроль показателей внешнего дыхания у животных; Н. В. Лапина — общее руководство; А. А. Краснова — определение гравиметрических коэффициентов легких у животных.

**Соблюдение этических стандартов:** все процедуры с модельными животными были проведены в соответствии с Правилам лабораторной практики и директивой Европейского парламента и Совета Европейского союза 2010/63/ЕС (2010 г.) о защите животных, используемых для научных целей.

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Non-cardiogenic pulmonary edema (NCPE) is a life-threatening condition defined primarily as acute lung injury syndrome and its most severe form, acute respiratory distress syndrome [1], as well as severe bilateral pneumonia. These conditions are characterized by rapid progression of hypoxemia and hypoxia resistant to oxygen therapy [2, 3]. NCPE may result from poisoning with pulmonotoxic chemicals (often associated with fires, chemical accidents and other extreme situations) or from viral or bacterial pneumonia [3, 4], septic or hemorrhagic shock, multisystem organ failure, and other causes [5]. The mechanisms underlying these processes are different: toxic pulmonary edema (TPE) results from primary blood–air barrier damage [4], and cardiogenic edema results from the blood pressure increase in pulmonary circulation by more than 30 mmHg.

Pulmonotoxic effects are exerted by the substances capable of damaging alveolar membranes of the lung, such as phosgene, chlorine, dimethyl sulfate, nitrogen oxides and sulfur oxides, ammonia, acids, thiourea, and other volatile toxic agents [6, 7]. Inhalation of vapors or aerosols of some toxic agents (dichloroethane, trichloroethylene, etc.) results in mixed pulmonary edema showing similarities to both toxic and cardiogenic pulmonary edema [8]. The common mechanisms of the blood–air barrier damage also underlie the development of pulmonary edema caused by viral and bacterial infections [5].

The main focus of the ARDS therapy is to ensure adequate gas exchange by selecting the respiratory support conditions [9]. NCPE therapy involves the use of hyperbaric oxygen therapy [10] and other respiratory support methods [9, 11], as well as some categories of drugs: ganglionic blockers (such as pentamine), narcotic analgesics (morphine), cardiac glycosides (strophanthin, digoxin), diuretics (lasix), corticosteroids (prednisolone), antifoaming agents [12, 13], antihypoxants [14]. Sedatives, such as ketamine, central α-2 agonists, benzodiazepines, etc., have been proposed as pharmacological agents for treatment of TPE [15].

The approaches to TPE treatment continue to be developed. Thus, recently the aquaporin-5 blockers [16], innovation technologies involving the use of perfluorocarbon liquids [17], recombinant heat shock proteins (including those combined with pulmonary surfactant) attract attention in this context [18, 19].

However, no uniquely effective agents and methods for prevention and treatment of TPE caused by pulmonotoxic chemicals have been developed so far [20, 9]. Mortality among affected individuals with TPE reaches 60% even in hospital settings, and the vast majority of this number die within three days after the exposure to toxic chemical [5, 6].

Thus, the development of new, more effective anti-edematous drugs is a pressing issue of modern medicine.

Among potential remedies for treatment of NCPE, *N*,*N*-diethyl-5,5-diphenyl-2-pentynylamine hydrochloride (DDPA) synthesized in the Golikov Research Clinical Center of Toxicology of FMBA of Russia, is of interest. Pharmaceutical substance has been registered and is produced by the Federal State Unitary Enterprise Research and Production Center "Farmzashita" of FMBA of Russia (№ P N002888/01 of 6 June 2008). The structural formula of the active ingredient is provided in Fig.

Pharmaco-toxicological assessment of DDPA has been reported earlier [21]. DDPA is an active N-cholinolitic agent, it shows pronounced antispasmodic and anti-inflammatory activity, local anesthetic effect, improves blood circulation, and exerts antioxidant and membrane stabilizing effects. According to the available data [21], DDPA has a beneficial effect on the inhalation injury caused by pulmonotoxic chemicals and irritants, allowing one to expect its efficacy against TPE. This is also suggested by the previously published data [22] showing the DDPA therapeutic effect in the model of non-cardiogenic pulmonary edema.

The study was aimed to determine the therapeutic efficacy of DDPA in the animal models of toxic pulmonary edema.

## METHODS

The *N*,*N*-diethyl-5,5-diphenyl-2-pentynylamine hydrochloride (DDPA) pharmaceutical substance was provided by the Federal State Unitary Enterprise Research and Production Center "Farmzashita" of FMBA of Russia (99.7% purity). The study involved the use of the “chemically pure” and “pure for analysis” reagents.

The experiments involved male white outbred rats (age three months, body weight 200–220 g) obtained from the Rappolovo animal nursery (Leningrad region, Rappolovo, Russia; veterinary certificate № 15806716021). Animals were kept under standard conditions with the 12 h light/dark cycle and free access to water and food. Rats were selected for the experiment by randomization.

### Pulmonary edema modeling and experimental design

Two methods were used to create the rat models of acute NCPE. In the first case, pulmonary edema was induced by intraperitoneal thiourea injection, and in the second case it was induced by the nitrogen dioxide inhalation [5].

*Method to induce NCPE through intraperitoneal thiourea injection*

The rats received intraperitoneal injections of the 10% thiourea aqueous solution in a dose of 100 mg/kg. Immediately after that the animals were put in the 100 L inhalation chamber and kept in the atmosphere containing carbon monoxide at a concentration of 1500 mg/m$^3$ for 30 min to enhance pulmonary hypoxia. The concentration of CO in the chamber was monitored using the automatic gas analyzer. The experiment involved 80 male outbred rats divided into two groups: the control group (no treatment) and the group treated with DDPA. An hour later 20 animals were randomly selected in each group; macroscopic features of the lungs, pulmonary coefficient (PC), lung dry weight (LDW) measured as a percentage, lung hydration status (LHS) measured as a percentage were assessed in these animals. The rest of 40 animals were used to estimate survival rate. The results are provided in Table 1.

*Method to induce NCPE through $NO_2$ inhalation*

During the experiment cages with animals were put in the 100 L inhalation chamber, nitrogen dioxide was supplied from the cylinder with the air-gas mixture. A total of 10 experimental

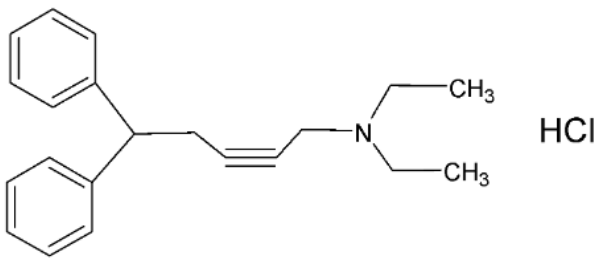


**Fig.** Chemical structure of DDPA

**Table 1.** The effects of DDPA on the thiourea-induced NCPE manifestations (M ± *m*; *n* = 20)

| Experimental group | Number of animals dead/total | Lung gravimetry of animals withdrawn from the experiment | | |
|---|---|---|---|---|
| | | CP | LDW, % | LHS, % |
| DDPA | 0/20* | 10,7 ± 1,2* | 15,0 ± 0,9 | 149,0 ± 30,5* |
| Control | 6/20* | 14,2 ± 1,4* | 13,7 ± 0,7 | 262,5 ± 35,0* |

**Note:** * — significant difference from control ($p < 0.05$)

rats were distributed into four cages, 2–3 animals per cage. $NO_2$ concentration in the chamber was monitored using the MONOLIT gas analyzer (Nord-West Engineering; Russia). At the same time, the concentrations of $CO_2$ (with the PKU-4/1-MK-S gas analyzer, Russia) and $O_2$ (with the PKG-4-K-N-1-M gas analyzer, Russia) in the chamber were monitored and maintained within normal limits. The rats were kept in the inhalation chamber for 18 min after the specified $NO_2$ concentration was established, the total inhalation time was 20 min. $NO_2$ concentration fluctuations in the chamber did not exceed 5% of the specified value.

Preliminary tests were used to determine the nitrogen dioxide toxic concentrations: LC20/20min = 180 mg/m$^3$, LC50/20min = 210 mg/m$^3$, and LC80/20min = 225 mg/m$^3$. These experiments involved 40 animals.

After selection of toxic concentrations, the experiments focused on assessing the DDPA therapeutic effects in TPE were conducted. In the first experiment involving 60 animals, we assessed the effects of DDPA on the survival rate of rats exposed to three nitrogen dioxide concentrations (Table 2). The second experiment involving 72 animals was focused on assessing the external respiration parameters and gravimetric pulmonary coefficients in survivors after exposure to the nitrogen dioxide concentration LC50/20min = 210 mg/m$^3$ (Tables 3, 4).

*Drug administration and assessment of treatment outcome*

The animals were divided into two groups immediately after the end of the NCPE induction procedure. Animals of the first group were administered 1% aqueous solution of DDPA via inhalation, and animals of the second group (controls) were administered pure water via the same route. The DDPA solution inhalation time was 2 min. The second DDPA solution inhalation was applied an hour later. The experiments involved the use of the OMRON NE-C28 PLUS nebulizer (China) with the nebulizing rate of 0.5 mL/min. The average generated aerosol particles dispersion was 1.5 μm, and the aerosol particle size distribution ranged between 0.2 and 10 μm.

The treatment outcomes were assessed relative to the control group. The animals' survival rate, overall health condition, clinical manifestations of poisoning, macroscopic features of lung injury, and gravimetric pulmonary coefficients were assessed in accordance with the common diagnostic criteria of pulmonary edema in laboratory animals [23]. The rats' external respiration parameters were measured using the M150P system (BIOPAC Systems; USA): respiratory rate per minute (RR); inspiratory capacity in mL (IC). The external respiration parameters and lung gravimetry were assessed before the exposure (baseline), 2 and 5 h after the exposure. Gravimetry was performed only in survivors withdrawn from the experiment by decapitation in groups of 12 animals per group.

The following gravimetry parameters were used to assess the NCPE severity: pulmonary coefficient (PC), lung dry weight (LDW) and lung hydration status (LHS) calculated according to formulas 1–3.

$$PC = \frac{m_1}{m_2} \cdot 1000 \quad (1), \text{ where}$$

$m_1$ — weight of the experimental animal's fresh lungs (g);
$m_2$ — body weight of the experimental animal (g).

$$LHS = \frac{m_1}{m_3} \cdot 100\ \% \quad (2), \text{ where}$$

$m_1$ — weight of the experimental animal's fresh lungs (g);
$m_3$ — weight of the experimental animal's dried lungs (g).

$$LDW = \frac{m_4}{m_1} \cdot 100\ \% \quad (3), \text{ where}$$

$m_1$ — weight of the experimental animal's fresh lungs (g);
$m_4$ — weight of the intact animals' fresh lungs (g).

The weight of the dried lungs (m$^3$) was determined by drying the animal's fresh lungs in the drying chamber at 100 °C until the constant weight was achieved.

## Statistical data processing

Statistical processing of the results performed using descriptive statistics involved calculation of the mean and standard error of the mean determined based on the standard deviation. The data obtained were tested for normality using the Shapiro–Wilk test. When proving the normal distribution of data in the experimental

**Table 2.** The effects of DDPA on the survival rate of rats with NCPE induced by $NO_2$ inhalation (M ± *m*; *n* = 10)

| $NO_2$ concentration, mg/m$^3$ | Experimental groups | | | |
|---|---|---|---|---|
| | Control (no treatment). number of dead animals/total | | DDPA, number of dead animals/total | |
| | Over 5 h | Over 24 h | Over 5 h | Over 24 h |
| 180 ± 10 | 2/10 | 6/10 | 0/10 | 4/10 |
| 210 ± 10 | 5/10* | 9/10 | 0/10* | 8/10 |
| 225 ± 10 | 8/10 | 10/10 | 6/10 | 10/10 |

**Note:** * — significant difference from control ($p < 0.05$).

**Table 3.** The effects of DDPA on the external respiration parameters in rats with NCPE induced by $NO_2$ inhalation (LC50/20min = 210 mg/m$^3$) (M ± *m*; *n* = 12)

| Experimental group | Time after $NO_2$ exposure, h | | | | | |
|---|---|---|---|---|---|---|
| | 0 (Baseline) | | 2 | | 5 | |
| | RR breaths/min | IC, mL | RR breaths/min | IC, mL | RR breaths/min | IC, mL |
| DDPA, NCPE | 139 ± 6<br>100% | 3,0 ± 0,2<br>100% | 178 ± 9*<br>128% | 2,1 ± 0,2*<br>70% | 197 ± 7*<br>142% | 2,0 ± 0,2*<br>67% |
| Control (water for injection), NCPE | 137 ± 6<br>100% | 3,1 ± 0,2<br>100% | 196 ± 10*<br>143% | 2,1 ± 0,2*<br>68% | 241 ± 15*<br>176% | 1,6 ± 0,1*<br>52% |
| Intact rats | 140 ± 5<br>100% | 3,0 ± 0,2<br>100% | 138 ± 5<br>99% | 3,0 ± 0,2<br>100% | 139 ± 5<br>99% | 3,1 ± 0,2<br>103% |

**Note:** * — significance of differences between the baseline values and the values obtained within 2 and 5 h ($p < 0.05$)

groups and the equality of variance, the parametric Student's *t*-test implemented in the StatPro application was used. The differences were considered significant at $p < 0.05$ [24, 25].

## RESULTS

Table 1 shows the treatment outcomes of NCPE induced by intraperitoneal thiourea injection in rats.

After inducing NCPE, the animals were put in the cage where they were kept to follow-up. Hypodynamia and dyspnea were observed in all animals 40–60 min after administration of thiourea. Later the control animals' condition continued to deteriorate progressively: accessory muscle breathing, adynamia, cyanosis, and disheveled fur showed up. Eventually, some animals of the control group died; death occurred within 1.5–2.0 h in conditions of the acute respiratory failure progression. After that no animals died during the follow-up period.

The condition of animals, treated with 1% DDPA solution immediately after the inhalation, stabilized, and no respiratory system-related worsening occurred. Motor activity and grooming were reported in rats.

Assessment of macroscopic features of the lungs in the animals that were withdrawn from the experiment revealed enlarged lungs, and burgundy color liquid discharge was observed in the section of parenchyma. The dark-red exudate in the amount of up to 5 mL was detected in the thoracic cavity. In the group of animals treated with DDPA, pale-yellow pleural effusion with no hemorrhagic component was observed.

The results obtained when studying the efficacy of nitrogen dioxide-induced NCPE treatment are provided in Tables 2–4.

Table 4 presents gravimetric pulmonary coefficients reported in animals at different times (2 h and 5 h) after poisoning compared to that of intact animals.

### Clinical manifestations of TPE in animals after exposure to nitrogen dioxide

Hypodynamia and dyspnea were observed in animals after the exposure in the chamber containing $NO_2$. After that the animals' condition worsened: accessory muscle breathing, adynamia, cyanosis, and disheveled fur showed up. The animals died as a result of progressive acute respiratory failure. Autopsy showed that the dead animals' lungs were filled with foamy fluid. Animals died in both groups, subjected and not subjected to treatment, however, the condition of animals that received DDPA inhalations stabilized, in contrast to the control group.

## DISCUSSION

The treatment outcomes of NCPE caused by intraperitoneal thiourea administration that are provided in Table 1 indicate the decrease in the pulmonary edema severity. No fatal cases along with significant decrease in gravimetric pulmonary coefficients are observed in the group of animals receiving DDPA inhalations. Assessment of pulmonary weight indices in animals shows that administration of the drug slows down the buildup of fluid in the lungs [24], thereby reducing the pulmonary edema severity.

According to the treatment outcomes of NCPE caused by $NO_2$ inhalation (Table 2), treatment with DDPA reduces mortality rate relative to controls. The DDPA beneficial effects were more obvious in animals intoxicated with medium

**Table 4.** The effects of DDPA on gravimetric pulmonary coefficients of rats with NCPE induced by $NO_2$ inhalation (LC50/20min = 210 mg/m$^3$) (M ± *m*; *n* = 12)

| Parameters | Time after $NO_2$ exposure, h | |
|---|---|---|
| | 2 | 5 |
| | Treatment with DDPA | |
| PC | 15,4 ± 1,3* | 17,8 ± 1,4* |
| LHS (%) | 257,6 ± 8,7* | 275,4 ± 7,8* |
| LDW (%) | 15,9 ± 1,4 | 15,4 ± 1,3 |
| | Control (water for injection) | |
| PC | 20,3 ± 1,3* | 25,6 ± 1,4* |
| LHS (%) | 289,8 ± 9,8* | 295,6 ± 8,9* |
| LDW (%) | 13,5 ± 1,4* | 13,1 ± 1,5* |
| | Intact animals | |
| PC | 7,4 ± 0,5 | 7,4 ± 0,5 |
| LHS (%) | 100 | 100 |
| LDW (%) | 18,6 ± 0,4 | 18,6 ± 0,4 |

**Note:** * — significance of differences between intact and experimental groups ($p < 0.05$)

concentration of LC50/20min = 210 mg/m$^3$ within the period of 2–5 h after exposure. Furthermore, DDPA increased the rats' life expectancy: significant differences from controls were revealed in animals intoxicated with low and medium $NO_2$ concentrations (LC50/20min = 210 mg/m$^3$). Treatment with DDPA results in the decrease in respiratory rate (RR) and the increase in inspiratory capacity (IC) relative to the control group within 5 h. Gravimetric pulmonary coefficients (Table 4) suggest inhibition of the buildup of fluid in the lungs of animals receiving DDPA. Significant differences from controls have been obtained for pulmonary coefficient (PC), other parameters demonstrate a trend of declining lung hydration status.

Thus, the findings demonstrate the efficacy of DDPA therapy in the TPE models used. The issue of the mechanism underlying realization of the DDPA therapeutic effect remains open. As the available data on the DDPA pharmacology might suggest, its effect is probably of combined nature. Due to its antispasmodic activity, DDPA can reduce blood pressure in pulmonary circulation. Presumably, this in combination with membrane stabilizing and antioxidant activity reduces lung hydration.

It should be noted that the treatment outcomes of TPE induced by intraperitoneal administration of thiourea are better than that of edema caused by $NO_2$ inhalation. Such differences can be explained by the fact that nitrogen dioxide causes a more deep irreversible lung tissue injury than thiourea. Thiourea is rapidly excreted from the body, it does not form stable adducts, and its damaging effects start to wear off almost immediately after the decrease in its blood levels. In contrast, nitrogen dioxide reacts with lung tissues, producing toxic metabolites and inducing destructive processes throughout the long period after poisoning. That is why toxic pulmonary edema caused by $NO_2$ is extremely difficult to treat.

## CONCLUSIONS

The study has shown that inhalation of DDPA solution has a therapeutic effect in two models of toxic pulmonary edema. The efficiency of treatment with DDPA is higher in the model of TPE caused by intraperitoneal tiourea administration than in the model of $NO_2$-induced TPE. Extrapolation of the data obtained to humans suggests that the use of DDPA in individuals with injuries induced by pulmonotoxic chemicals may be promising at the prehospital stage and during transfer to the hospital for treatment.

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# EFFECT OF SODIUM BICARBONATE ON THE DEVELOPMENT OF GASTRIC STASIS IN THE RAT MODEL OF MYELOABLATIVE CHEMOTHERAPY WITH CYCLOPHOSPHAMIDE

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Myeloablative cytostatic therapy is often associated with gastrointestinal (GI) stasis that is a component of pathogenesis of the bacterial overgrowth syndrome, endotoxicosis, systemic inflammation, sepsis, emetic syndrome. The study was aimed to test the hypothesis that sodium bicarbonate ($NaHCO_3$), the alkalinizing agent administrated by gavage in the rat model of myeloablative cytostatic therapy with cyclophosphamide (CP), would have a protective effect against GI stasis. We assessed the effects of intragastric $NaHCO_3$ administrations on the development of GI stasis, acute chemotherapy-induced mucositis of the small intestine, and urinary excretion of indican using 140 Wistar rats with the body weight of 161–190 g as a model of myeloablative cytostatic therapy with the intravenously injected CP. The CP administration in a dose of 390 mg/kg resulted in dystrophic changes in the small intestinal mucosa, the development of GI stasis with predominant gastric stasis within the first 24 h, and the increase in excretion of indican. Intragastric administration of $NaHCO_3$ in a dose equivalent to 350 mL of the 4% $NaHCO_3$ solution in humans to rats 30 min before and immediately after the CP administration prevented acute chemotherapy-induced mucositis of the small intestine and alleviated the symptoms of gastric stasis and excessive growth of the indole-producing gastrointestinal microbiota. The reported approach to emergency drug prevention of the myeloablative cytostatic drug therapy gastrointestinal complications holds promise for testing of the use of CP and other alkylating drugs as cytostatic agents.

**Keywords:** cyclophosphamide, myeloablative cytostatic therapy, rat model, acute cytostatic mucositis, gastric stasis, indican, sodium bicarbonate

**Author contribution:** Vakunenkova OA — experimental study; Ivnitsky JuJu — rationale, developing the experimental model, data interpretation and discussion; Gaykova ON — morphometry data interpretation; Kozlov AA — morphometry studies; Schäfer TV — experimental procedure, data processing and visualization, developing the experimental model. All authors contributed to discussion, manuscript writing and editing.

**Compliance with ethical standards:** the study was carried out in accordance with the principles of bioethics, approved by the European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes.

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# ВЛИЯНИЕ ГИДРОКАРБОНАТА НАТРИЯ НА ФОРМИРОВАНИЕ ГАСТРОСТАЗА У КРЫС ПРИ МОДЕЛИРОВАНИИ МИЕЛОАБЛЯЦИОННОЙ ХИМИОТЕРАПИИ ЦИКЛОФОСФАНОМ

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При миелоабляционной цитостатической терапии нередко возникает желудочно-кишечный стаз (ЖКС) — звено патогенеза синдрома избыточного бактериального роста, эндотоксикоза, системного воспаления, сепсиса, эметического синдрома. Целью исследования было проверить гипотезу о том, что ощелачивающий агент гидрокарбонат натрия ($NaHCO_3$), вводимый в желудок при моделировании на крысах миелоабляционной цитостатической терапии циклофосфаном (ЦФ), проявит профилактическую активность в отношении ЖКС. Изучали влияние вводимого в желудок $NaHCO_3$ на формирование желудочно-кишечного стаза, острого цитостатического мукозита тонкой кишки и экскрецию индикана с мочой при моделировании на 140 крысах линии Вистар массой тела 161–190 г миелоабляционной цитостатической терапии внутривенным введением ЦФ. Введение ЦФ в дозе 390 мг/кг вело к дистрофическим изменениям в слизистой оболочке тонкой кишки, развитию в течение ближайших суток ЖКС с преобладанием гастростаза и повышению экскреции индикана. Введение за 30 мин до и тотчас после ЦФ в желудок крыс $NaHCO_3$ в дозе, эквивалентной 350 мл его 4%-го раствора для человека, предупреждало формирование острого цитостатического мукозита тонкой кишки, смягчало проявления гастростаза и избыточного роста индол-продуцирующей желудочно-кишечной микрофлоры. Представленный подход к экстренной медикаментозной профилактике желудочно-кишечных осложнений миелоабляционной цитостатической фармакотерапии перспективен для апробации при использовании в качестве цитостатического агента не только ЦФ, но и других медикаментозных средств алкилирующего действия.

**Ключевые слова:** циклофосфан, миелоабляционная цитостатическая терапия, крысы, острый цитостатический мукозит, гастростаз, индикан, гидрокарбонат натрия

**Вклад авторов:** О. А. Вакуненкова — выполнение экспериментальной части работы; Ю. Ю. Ивницкий — научный замысел, разработка экспериментальной модели, интерпретация и обсуждение результатов; О. Н. Гайкова — трактовка результатов морфологических исследований; А. А. Козлов — морфометрические исследования; Т. В. Шефер — экспериментальная часть, обработка и визуализация данных, разработка экспериментальной модели. Все авторы участвовали в обсуждении результатов, подготовке и редактировании рукописи статьи.

**Соблюдение этических стандартов:** исследование проведено с соблюдением правил биоэтики, утвержденных Европейской конвенцией о защите позвоночных животных, используемых для экспериментальных и других целей.

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The previously reported study revealed retention of the X-ray shadow of barium sulfate administrated into the rat's stomach that was associated with acute cyclophosphamide toxicity [1] and was typical for gastrointestinal (GI) stasis. This potentially fatal complication often occurs during the patient's preparation for hematopoietic stem cell transplantation, myeloablative cytostatic conditioning [2]; in some recipients, the X-ray gastric shadow spreads to large parts of both abdominal and thoracic cavities [3]. The GI stasis clinical significance results from its negative impact on the chemotherapy outcome. GI stasis hampers the patients' feeding, makes it pointless to prescribe oral medications; intestinal bacterial overgrowth that is associated with GI stasis results in realization of quorum sensing [4], enhanced production of toxic substances by bacteria, as well as in endotoxemia and endotoxicosis. GI stasis contributes to the intestinal barrier damage associated with the Gram-negative bacterial lipopolysaccharide release into the bloodstream, systemic inflammation [5] and sepsis [6]. GI stasis is involved in the pathogenesis of delayed chemotherapy-induced vomiting; however, prescribing antiemetics [7, 8], prokinetics or antispasmodics [7] to such patients does not resolve the acute chemotherapy-induced gastrointestinal mucositis, a defensive response to the first stage of which is believed to be represented by GI stasis [9]. The use of enteroprotectors, i.e. medications capable of preventing acute gastrointestinal mucositis, seems to be a more promising approach to prevention of GI stasis associated with myeloablative conditioning. When using cytostatic therapy with cyclophosphamide (CP), enteroprotective effect can hypothetically be achieved by alkalinization of the chyme moving through the gastrointestinal tract. With increasing pH of biological media, spontaneous hydrolysis of the active CP metabolite produced in the liver is slowed down, accumulation of more toxic metabolites, acrolein and phosphoramide mustard, is inhibited [10], and toxicity of aldehyde dehydrogenase, the key enzyme responsible for the CP detoxification, the optimal pH value of which is within the alkaline range, is increased [11]. Higher normal pH values of the cytoplasm relative to the tumor cells contribute to the CP selective anticancer activity [12]. Acidification of the chyme by intragastric administration of weak acid solutions [13] or lactulose [14] increased the severity of acute cyclophosphamide toxicity in rats. Intragastric CP administration resulted in more severe GI stasis than intraperitoneal injection of the same dose, which could be partly due to more intense CP toxification in the acidic environment [1]. The study was aimed to test the hypothesis that $NaHCO_3$, the alkalinizing agent administrated by gavage in the rat model of myeloablative cytostatic therapy with cyclophosphamide, would have a protective effect against GI stasis.

## METHODS

The study involved 140 male Wistar rats (161–190 g) obtained from the Rappolovo laboratory animal nursery. The animals were treated in accordance with the Principles of Good Laboratory Practice, stated in the Order № 708n of the Ministry of Health of the Russian Federation of 1 August 2010. The standard rat diet and ad libitum water access were provided. The animals were randomized into experimental groups. To deprive the rats of food, they were placed in the slatter floor cages (to avoid coprophagy and consumption of the bedding components) with access to water only for a specified time. Myeloablative cytostatic therapy was modeled by a single lateral tail vein injection of the freshly prepared aqueous solution of Endoxan (Baxter Oncology GmbH; Germany) in the amount of 10 mL/kg in a dose of 390 mg/kg (≈ 1.7 $LD_{99/30\ day}$), which was equivalent to the daily dosage for humans of 60 mg/kg used in the myeloablative conditioning regimens [15]. Laparotomy and organ harvesting were performed under the mask halothane anaesthesia. The GI stasis severity was assessed based on the relative weight of chyme in the stomach and caecum calculated as a difference between the weight of the organ filled with chyme and the empty organ (*gaster*, *caecum*) in grams relative to the body weight in kilograms.

During the first phase of the study we assessed the dynamics of the GI stasis development after the myeloablative conditioning. For that the animals were distributed into seven groups, among which the first one was represented by intact rats ($n$ = 10) having unlimited access to food, another tree groups were deprived of food 4, 24 or 48 h after the CP administration, and the three remaining groups were deprived of food within the same time frame but did not receive CP ($n$ = 10 in each group). All animals were subjected to laparotomy 72 h after the CP administration to assess the GI stasis severity.

During the second phase of the study we assessed the effects of $NaHCO_3$ on the GI stasis severity and the growth rate of gastrointestinal microbiota. For that the rats deprived of food between 24 and 72 h after the CP administration were used. The animals were distributed into five groups, among which the first one was represented by intact rats ($n$ = 10), and all other groups were represented by the rats administered with CP ($n$ = 10 in each group). Rats of the second groups were administered CP only; the 4% $NaHCO_3$ solution (pH = 8.34) in the amount of 15 mL/kg was administrated by gavage to rats in the third group 30 min before the CP administration. In the fourth group, administration of $NaHCO_3$ 30 min before CP was supplemented by the repeated administration of $NaHCO_3$ in the same dose after the CP injection; in the fifth group, $NaHCO_3$ was administrated by gavage four times: 30 min before, immediately after, 60 and 120 min after the CP administration. All the rats were placed in metabolic cages for urine collection 48 h after the CP injection; 50 μL of the 10% trichloroacetic acid solution per chamber were added to the urinal chambers as a preservative. The GI stasis severity was assessed 72 h after the CP administration. To assess selectivity of the $NaHCO_3$ protective effect, the relative weight of the spleen was measured along with the relative weight of the gaster and caecum chyme as a measure of chemotherapy-induced damage to the hemopoietic system. Urinary excretion of indican was used as a measure of the gastrointestinal microbiota growth rate [16]. The volume of urine sampled within 24 h was measured, and indican, an intestinal endotoxicosis indicator [17], the urinary excretion of which was measured in micrograms per kilogram of body weight per hour, was quantified.

During the third phase of the study we assessed morphological changes in the small intestine associated with the myeloablative cytostatic therapy modeling by the above method, as well as the effects of double $NaHCO_3$ administration into the stomach (30 min before and immediately after CP) on these changes. The 10 cm long small intestine sections (*duodenum* proximal of *pylorus*; *jejunum* 10 cm distal of *flexura duodenojejunalis*; *ileum* proximal of *caecum*) were fixed in 10% formalin and embedded in paraffin. The annular slices were stained with hematoxylin and eosin and then examined with the 3DHISTECH Pannoramic MIDI scanning digital microscope (Carl Zeiss AG; Germany). We enumerated intestinal villi and measured their length in 58–73 slices of each organ obtained from three animals; the results were processed using the Case Viewer application (3DHISTECH Ltd.; Hungary).

The results were presented as the mean and standard error of the mean (M ± *m*). The effects of the injected substances on the quantitative parameters were estimated by analysis of variance. When the resulting models were significant, the intergroup comparison of mean values was performed using the Tukey's honestly significant difference test [18]. Correlations between traits were represented as the Spearman's rank correlation coefficients ($r_s$). The α-value of 0.05 was considered to be a critical significance level.

## RESULTS

Three days after the CP administration to rats deprived of food within 48 h before laparotomy, the dilated stomach that was filled with chyme occupied most of the abdominal cavity; it seemed to be empty in intact animals. The increase in the volume of the caecum associated with the CP administration was lower (Fig. 1). Food consumed within the first 24 h after the CP injection stayed in the stomach over the next 48 h. This resulted in the 7–13 fold gastric chyme relative weight increase. Excessive accumulation of chyme in the caecum was represented as a trend ($p$ = 0.075 for the animals deprived of food for 4 h; Fig. 2). The body weight of animals administered with CP measured on the day of laparotomy made up a smaller share of body weight measured prior to exposure, than in controls: 78.9 ± 0.8% vs. 86.2 ± 0.5% ($p < 0.05$).

Administration of $NaHCO_3$ into the stomach 30 min before and immediately after the CP administration prevented GI stasis: the gastric chyme relative weight was on average 2.6 times lower, than in unprotected animals, however, it was still three times higher than in intact rats. The decrease in the relative weight of the caecal chyme was represented as a trend ($p$ = 0.084). The four-time administration of $NaHCO_3$ had no benefit over the double administration, and a single preventive $NaHCO_3$ administration was ineffective. The $NaHCO_3$ administration had little effect on the CP-induced hypotrophy of the spleen (Fig. 3).

Urinary excretion of indican after the exposure to CP only was on average 1.9 times higher than in intact rats; the double intragastric $NaHCO_3$ administration resulted in the 1.4-fold increase, which manifested as a trend only ($p$ = 0.067; Fig. 4). In the rats administered with CP, this indicator showed a strong negative correlation ($r$ = –0.77; $p < 0.01$) with the body weight measured on a day of laparotomy as a percentage of body weight measured before the exposure. Excretion of indican in rats administered with CP only positively correlated with the relative weight of the caecal chyme ($r$ = 0.66; $p < 0.05$); when $NaHCO_3$ was administered together with CP, the correlation was weak ($r$ = 0.15).

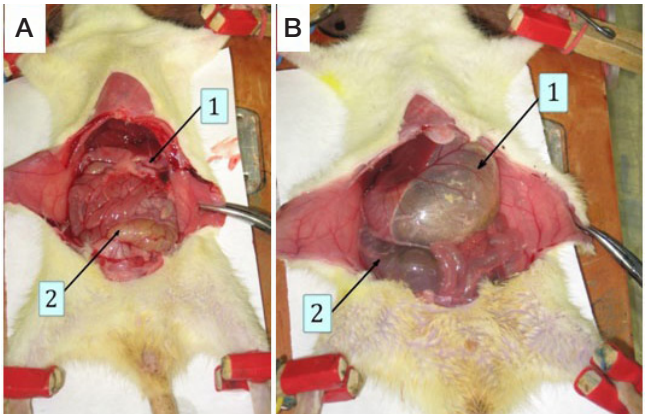


**Fig. 1.** Abdominal organs of the rats deprived of food 48 h before laparotomy: intact (**A**); 72 h after intravenous injection of cyclophosphamide in a dose of 390 mg/kg (**B**). *Arrows* show: 1 — stomach; 2 — caecum

Three days after the CP administration, the changes (congestion, inflammation, atrophy) were observed in the small intestine, the severity of which increased in the direction from the duodenum to the ileum. The average length of intestinal villi was reduced, and a downward trend in the number of villi was observed in the annular slices of the organ. Atrophy of villi was found in the ileum. No such alterations were found in rats administered with $NaHCO_3$ in addition to CP (Table; Fig. 5).

## DISCUSSION

Modelling myeloablative cytostatic therapy in rats was associated with deep inhibition of the gastrointestinal (GI) tract propulsive function with predominance of gastric stasis developing during the first hours after the CP administration. The gastric transit time exceeded three days. It is 10–48 min in healthy people [19], that is why it can be assumed that gastric stasis persists in the recipients of hematopoietic stem cells for much of the myeloablative conditioning course lasting 3–5 days [15]. It is possible that gastric stasis is involved in general health deterioration during myeloablative cytostatic therapy, as indicated by the fact that the animals lose about a

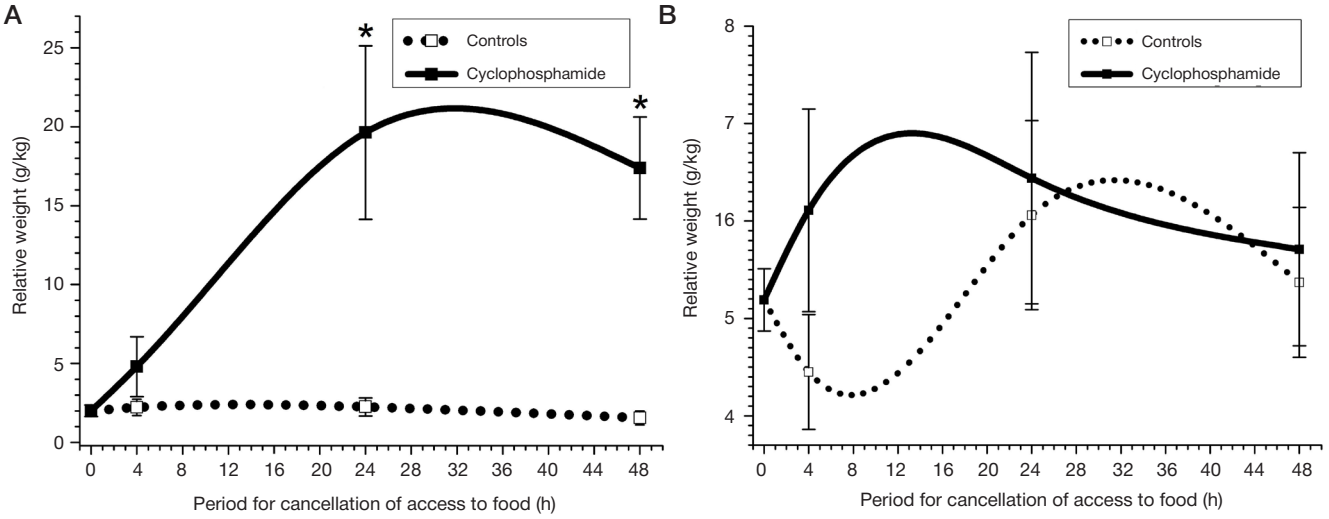


**Fig. 2.** Relative weight of the gastric (**A**) and caecal (**B**) chyme in rats 72 h after intravenous injection of cyclophosphamide in a dose of 390 mg/kg (M ± *m*; *n* = 10) depending on the time of access to food after the exposure. Controls — animals not administered with cyclophosphamide. At the beginning of the horizontal axis — values of the group of rats not administered with cyclophosphamide and having unlimited access to food. * — significant differences from controls ($p < 0.05$)

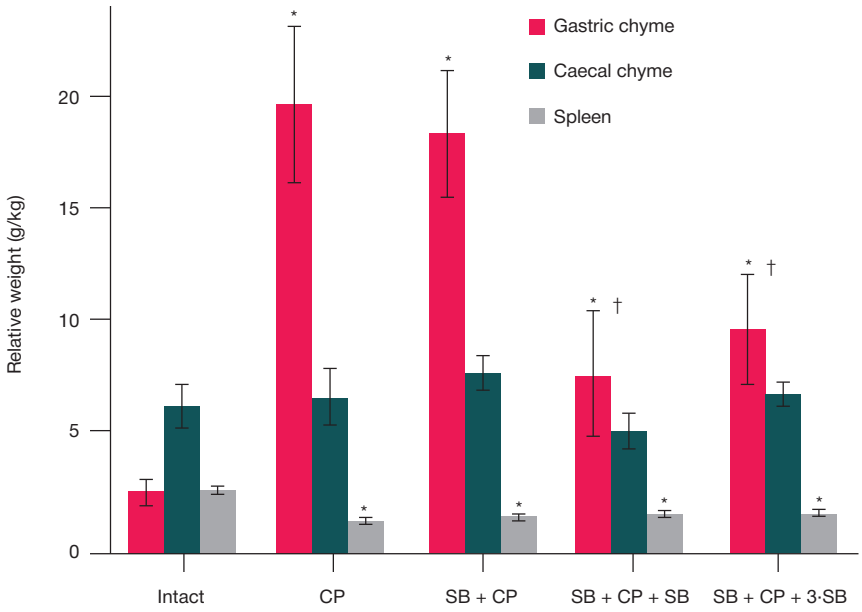


**Fig. 3.** Relative weight of the gastric chyme, caecal chyme and the spleen in rats 72 h after intravenous injection of cyclophosphamide in a dose of 390 mg/kg (M ± *m*; *n* = 10). "Intact" — rats not administered with drugs; "CP" — rats administered with cyclophosphamide only; "SB + CP" — rats that received intragastric administration of the 4% sodium bicarbonate solution 30 before the cyclophosphamide administration; "SB + CP + SB" — rats that received intragastric administration of the 4% sodium bicarbonate solution 30 before and immediately after the cyclophosphamide administration; "SB + CP + SB"— rats that received intragastric administration of the 4% sodium bicarbonate solution 30 before, immediately after, 1 and 2 h after the cyclophosphamide administration. All the animals were deprived of food 24 h after the cyclophosphamide administration. Significant differences ($p < 0.05$): * — from the intact group; † — from the "CP" and "$NaHCO_3$ + CP" groups

quarter of their initial body weight within three days after the CP administration.

Intragastric administration of two doses of $NaHCO_3$ to a total dose, which was equivalent to 350 mL of the 4% solution in humans, made it possible to a significant extent, although not completely, preserve the propulsive function of the stomach. $NaHCO_3$ was most effective during the period that did not exceed the CP $T_{½}$ after intravenous administration to rats, i.e. 0.5 h [20]. Consequently, inhibition of the CP toxification predominated in the mechanism underlying the $NaHCO_3$ protective effect. Because of the weakly alkaline nature of the $NaHCO_3$ solution and the salt's capability of being absorbed by the GI tract mucosa, the protective effect involved inhibition of the acrolein and phosphoramide mustard production in epithelial cells of the stomach and/or small intestine. This is also indicated by the $NaHCO_3$ inability to prevent the CP-induced hypotrophy of the spleen that can be explained by the buffering properties of blood not allowing one to ensure the comparable increase in pH of cells of the stem or proliferative pool of the hematopoietic system. Thus, the $NaHCO_3$ protective effect of the GI tract was selective, which was conductive to its testing in disorders requiring myeloablative cytostatic therapy.

The pH values that are optimal for the enzymes responsible for DNA repair are within the range close to neutral (6.5–7.5), that is why acidosis, that results from the shift from oxidative to glycolytic phosphorylation and comes along with mitochondrial damage caused by mustard agents [21], can violate DNA repair. Acidosis also leads to another effect that contributes to enterocytopenia: the expression of pro-apoptotic proteins that activate caspases [22]. This determines the possibility of the $NaHCO_3$ protective effects not only in acute poisoning with CP, but in poisoning with other alkylating cytostatic agents; the hypothesis needs further testing.

The CP-induced gastric stasis was associated with the increase in urinary excretion of indican (the indoxyl sulfate potassium salt), the end product of the liver metabolism of indole (oxidation to indoxyl and its sulfonation), the main source of which in experimental rats was represented by the reaction catalyzed by the gut microbiota tryptophanase. This indicates the increase in the weight and/or metabolic activity of intestinal microbiota, that produces indole but does not metabolize it, in GI stasis; in this case, a more intense production of both indole and another toxic product of the tryptophanase reaction, ammonia, in the GI tract is inevitable. The CP-induced increase in excretion of indican was lower that the increase in the relative weight of the gastric chyme, which was probably due to lower resorption capacity of the stomach compared to the intestine. When $NaHCO_3$ was administered, the differences in excretion of indican between the intact animals and the rats administered with CP were minor, which characterized the endotoxemia severity in the latter.

The myeloablative cytostatic therapy modeling was associated with damage to the small intestine. The severity of damage increased distally, as determined by the fact that the abundance of bacteria in the ileal chyme exceeded that in the duodenal chyme by four orders of magnitude [23]. This is consistent with the hypothesis that gastric stasis represents the

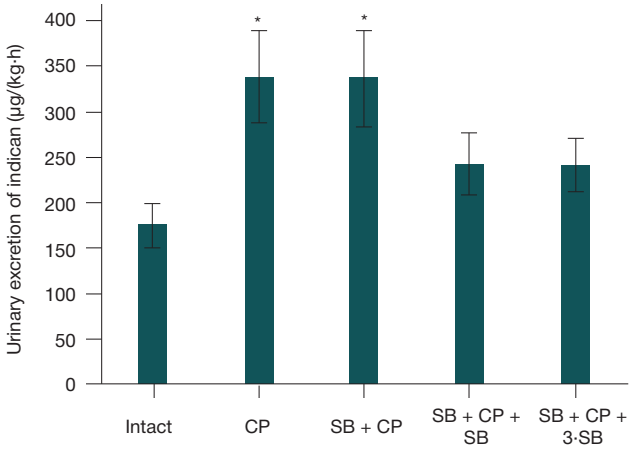


**Fig. 4.** Urinary excretion of indican in rats 72 h after intravenous injection of cyclophosphamide in a dose of 390 mg/kg (M ± *m*; *n* = 10). The symbols are the same as in Fig. 3

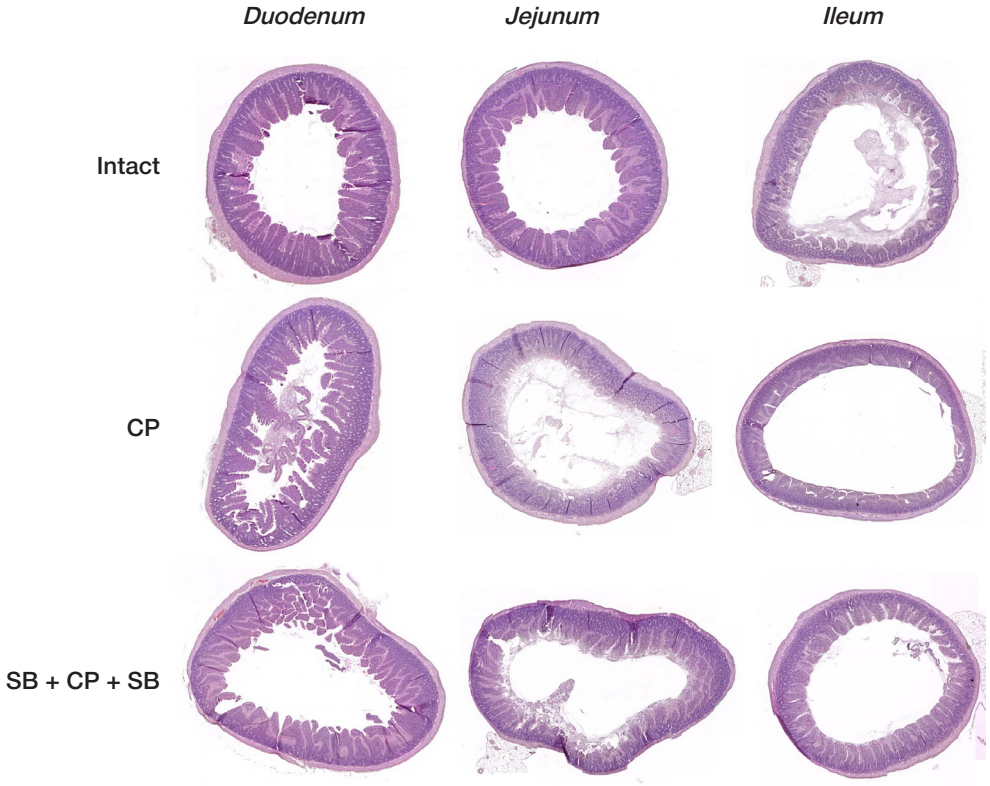


**Fig. 5.** Annular slices of the duodenum, jejunum, and ileum of the rats 72 h after intravenous injection of cyclophosphamide in a dose of 390 mg/kg

body's protective response, the biological meaning of which is to prevent injury of the small intestine damaged by the cytostatic agent, since the small intestine is a GI tract segment that is most sensitive to cytostatic agents [24]. The use of $NaHCO_3$ prevented dystrophic changes in all parts of the small intestine. As for duodenum and proximal jejunum, such an effect can be explained by local alkalinization, however, the $NaHCO_3$ protective effect on the ileum requires further investigation. This is due to the fact that the time it takes for the $NaHCO_3$ solution to reach it (at least 3 h according to preliminary data acquired by monitoring methylene blue administration in the rat stomach) far exceeds $T_{½}$ of CP administered to rats by intravenous injection [20].

The problem of acute chemotherapy-induced gastrointestinal mucositis treatment is far from being resolved. Antioxidants, anti-inflammatory agents and inhibitors of apoptosis are considered as possible therapeutic agents [25]. However, delivery of these agents to the GI tract segment most sensitive to cytostatic agents, the small intestine, is only achieved when there is no gastric stasis. That is why drug treatment should be preceded by the use of medications for emergency prevention of gastric stasis. The findings indicate potential benefits from early prescription of oral alkalinizing agents, such as sodium bicarbonate, for this purpose.

## CONCLUSIONS

1) Single intravenous injection of cyclophosphamide in a dose equivalent to that used for myeloablative conditioning to rats results in dystrophic changes in the small intestinal mucosa, the

**Table.** Morphological signs of acute chemotherapy-induced gastrointestinal mucositis in rats 72 h after intravenous injection of cyclophosphamide in a dose of 390 mg/kg

| Experimental group | Average number of villi, M ± *m* | Average villus length, M ± *m*, мкм | Main qualitative trait |
|---|---|---|---|
| *Duodenum* | | | |
| Intact | 39.0 ± 2.9 | 366 ± 8 | No |
| CP | 34.5 ± 3.7 | 294 ± 9* | Hyperemia |
| $NaHCO_3$ + CP + $NaHCO_3$ | 40.1 ± 2.0 | 355 ± 10† | No |
| *Jejunum* | | | |
| Intact | 31.5 ± 2.4 | 305 ± 7 | No |
| CP | 27.0 ± 3.6 | 208 ± 8* | Inflammation |
| $NaHCO_3$ + CP + $NaHCO_3$ | 35.0 ± 3.9 | 292 ± 5† | No |
| *Ileum* | | | |
| Intact | 36.2 ± 2.5 | 230 ± 6 | No |
| CP | No villi | | Atrophy |
| $NaHCO_3$ + CP + $NaHCO_3$ | 43.0 ± 2.5 | 239 ± 5 | No |

**Note:** "Intact" — rats that received no drug treatment; "CP" — rats that received cyclophosphamide only; "$NaHCO_3$ + CP + $NaHCO_3$" — intragastric administration by oral gavage of 4% sodium bicarbonate solution 10 min before and immediately after the cyclophosphamide administration. Significant differences ($p < 0.05$): * — from the intact group; † — from the "CP" group.

development of gastrointestinal stasis with predominant gastric stasis within 24 h, and the excess growth of the indole-producing gastrointestinal microbiota. 2) Intragastric administration of sodium bicarbonate in a dose equivalent to 350 mL of 4% sodium bicarbonate solution in humans to rats 30 min before and immediately after the cyclophosphamide administration to a considerable extent prevents acute chemotherapy-induced gastrointestinal mucositis, gastric stasis, and the excess growth of gastrointestinal microbiota. 3) Early oral administration of sodium bicarbonate represents a promising approach to prevention of gastric stasis when performing myeloablative chemotherapy with cyclophosphamide; the approach enables further oral administration of drugs for treatment of acute chemotherapy-induced mucositis of the small intestine to patients. 4) Testing of the above approach to prevention of gastric stasis associated with myeloablative cytostatic therapy with cyclophosphamide and other alkylating cytostatic drugs is of interest.

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# ASSESSING THE POSSIBILITY OF INTERACTIONS OF VARIOUS METALS WITH ALPHA-2-MACROGLOBULIN AND OTHER HUMAN BLOOD PROTEINS *IN VITRO*

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Homeostasis of metals plays an important role in functioning of the body. Not only the concentrations of toxic and essential metals in bodily fluids, but also their ability of interaction with proteins and enzymes defining the enzyme activity, are important. The study was aimed to compare the possibilities of binding interactions between various metal ions and human serum proteins. Chemical reactions between the immobilized metal ions ($Cu^{2+}$, $Zn^{2+}$, $Mn^{2+}$, $Ca^{2+}$, $Fe^{3+}$, $Mg^{2+}$, $Hg^{+}$, $Cd^{2+}$, $Pb^{2+}$, $Cr^{3+}$, $Co^{2+}$, $Ag^{+}$, $Bi^{2+}$, $Ba^{2+}$, $Sr^{2+}$) and the serum proteins or highly purified blood metalloprotein (alpha-2-macroglobulin, α2M) were assessed by the crossed immunoelectrophoresis with *in situ* adsorption in the second dimension. It has been shown that $Hg^{+}$, $Cu^{2+}$, $Zn^{2+}$, $Cd^{2+}$ ions more actively interact with metalloproteins (particularly, with α2M) and many other human blood proteins in *in vitro* reactions than other ions. We have demonstrated that α2M interacts not only with $Zn^{2+}$ and $Cd^{2+}$ ions, as earlier reported, but also with $Ca^{2+}$, $Mg^{2+}$, $Fe^{3+}$, $Mn^{2+}$, $Pb^{2+}$, $Sr^{2}$, $Ag^{+}$. Interaction of a number of metal ions, including highly toxic ones, with blood proteins that are not metalloproteins has been revealed. The findings confirm the fundamental possibility of the metal ion imbalance active involvement in metabolic disorders via effects on the body's regulatory and transport proteins, which requires further investigation

**Keywords:** metal ions, metalloproteins, alpha-2-macroglobulin, immunoelectrophoresis, intoxication

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**Compliance with ethical standards:** the study was carried out in accordance with the principles stated in the Declaration of Helsinki of the World Medical Association.

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# ИЗУЧЕНИЕ ВОЗМОЖНОСТИ ВЗАИМОДЕЙСТВИЯ РАЗЛИЧНЫХ МЕТАЛЛОВ С АЛЬФА-2-МАКРОГЛОБУЛИНОМ И ДРУГИМИ БЕЛКАМИ КРОВИ ЧЕЛОВЕКА *IN VITRO*

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Гомеостаз металлов играет важную роль в жизнедеятельности организма. При этом имеет значение не только концентрация токсичных и эссенциальных металлов в биологических жидкостях, но и их способность взаимодействовать с белками и ферментами, определяющая активность последних. Целью работы было сравнить возможности связывания различных ионов металлов с белками сыворотки крови человека. Изучение реакций иммобилизованных ионов металлов ($Cu^{2+}$, $Zn^{2+}$, $Mn^{2+}$, $Ca^{2+}$, $Fe^{3+}$, $Mg^{2+}$, $Hg^{+}$, $Cd^{2+}$, $Pb^{2+}$, $Cr^{3+}$, $Co^{2+}$, $Ag^{+}$, $Bi^{2+}$, $Ba^{2+}$, $Sr^{2+}$) с белками крови, а также с высокоочищенным металлопротеином крови (альфа-2-макроглобулин, а2-МГ) проводили методом перекрестного иммуноэлектрофореза с адсорбцией *in situ* во втором направлении. Показано, что в реакциях *in vitro* ионы $Hg^{+}$, $Cu^{2+}$, $Zn^{2+}$, $Cd^{2+}$ активнее других взаимодействуют с металлопротеинами (в частности с а2-МГ) и со многими другими белками крови человека. Продемонстрировано, что а2-МГ взаимодействует не только с ионами $Zn^{2+}$ и $Cd^{2+}$, как описано ранее, но и с $Ca^{2+}$, $Mg^{2+}$, $Fe^{3+}$, $Mn^{2+}$, $Pb^{2+}$, $Sr^{2}$, $Ag^{+}$. Выявлено взаимодействие ряда ионов металлов, в том числе высокотоксичных, с белками крови, не являющимися металлопротеинами. Результаты подтверждают принципиальную возможность активного участия дисбаланса ионов металлов в обменных нарушениях через воздействие на регуляторные и транспортные белки организма, что требует дальнейшего изучения.

**Ключевые слова:** ионы металлов, металлопротеины, альфа-2-макроглобулин, иммуноэлектрофорез, интоксикация

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Metal ions play an important role in metabolic pathways of living systems, from electron transfer and biocatalytic reactions to shaping the tertiary structure of metalloproteins that determines their biological activity. The disturbed essential metal homeostasis is associated with functional impairment and severe disorders. It is well-known that the non-physiological concentrations of Fe, Mn, Cu, Zn cause manifestations of neurotoxicity, while the excess levels of Zn and Cu trigger the toxicity-induced damage to the kidney, liver, cardiovascular system, gastrointestinal tract, and inhibit the function of the immune and central nervous systems [1]. Substantial amounts of metals are found in plaques, Lewy bodies, and cytoplasmic inclusions of the cells of individuals with neurodegenerative diseases (Alzheimer's disease, Parkinson's disease, etc.) and amyotrophic lateral sclerosis [2]. Furthermore, Zn ions provide antiatherogenic properties [3]. Deficiency of essential

metals also has an adverse effect on the body. In particular, Zn ions are essential for realization of the enzyme (alcohol dehydrogenase, alkaline phosphatase, carbonic anhydrase, leucine aminopeptidase and superoxide dismutase) function, and the Zn deficiency that triggers dermatoses, anorexia, and growth retardation, is observed in individuals with slow wound healing and impaired reproductive function [1]. Metals with variable valence may exert both positive and negative effects: Mn ions promote generation of the hydroxyl radical and at the same time are involved in the development of atherosclerosis as cofactors of antioxidant enzymes [3].

When assessing their effects on the body, it is extremely important to take into account reactions of metals, including metals that form part of organic compounds, enzymes and proteins, with each other. In particular, highly toxic Pb, Hg, Cd cause severe intoxication when ingested [4], and organoselenium compounds demonstrate antioxidant and antitoxic activity in individual poisoned with salts of heavy metals [5]. In general, about one third of serum proteins contain ions of metals [2]. Of greatest interest is the study of metalloproteins performing regulatory and transport functions that are capable of exerting indirect effects on the number of organs and systems of the body due to changes in ionic composition. In particular, human alpha-2-macroglobulin (α2M) contains four Zn ions. The levels of this protein are rather high (2–3 g/L), and its functions are diverse: inhibition of the broad spectrum of proteases; transportation and regulation of the synthesis of cytokines, hormones and growth factors; regulation of apoptosis, synaptogenesis, neuron growth, and proliferation; regulation of dopamine concentrations in dopaminergic neurons and synthesis of choline acetyl transferase [6].

When studying the role of metal ions in physiological processes, it is important to consider the distribution of those in tissues and bodily fluids. In particular, Ag, Ca, Cu, In, Li, Na, Se, Si, Sr ions are found mostly in human blood plasma, while Fe, K, Mn, Ni, V, Zn are found in blood cells. The distribution of heavy metals shifts from cell to blood plasma with the increase in ionic raduis, and vice versa the distribution of alkali metals shifts to cells [7]. Therefore, it is advantageous to use the inductively coupled plasma mass spectrometry (ICP-MS) allowing one to identify a broad spectrum of metals, including minor concentrations, to assess the whole body levels of metals. However, other methods should be used to study interactions with proteins: crystallography, nuclear magnetic resonance, electron paramagnetic resonance, fluorescence-based methods, spectrometry and surface plasmon resonance [2]. The vast majority of the above methods involve partial or complete protein denaturation and can hardly be used for analysis of mixtures. This makes it more difficult to obtain objective scientific data on the processes that take place in the living organism. The less sensitive but more gentle methods with good potential of comparative visualization of the results, such as variants of crossed immunoelectrophoresis, seem to be promising.

The study was aimed to compare the possibilities of binding interactions between various metal ions and human serum proteins.

## METHODS

The study involved drainage blood serum obtained from 40 generally healthy donors of both genders aged 20–40 in order to identify all possible interactions.

The highly purified alpha-2-macroglobulin (α2M) preparations were obtained from blood plasma by using the combination of fractional precipitation with PEG 6000, anion-exchange chromatography, and zinc-chelate chromatography [8].

Polyclonal rabbit antisera against all human blood proteins and human α2M were obtained by intradermal immunization of two groups of rabbits (with blood serum and highly purified α2M preparation, respectively).

The possibility of binding interactions between human serum proteins and metal ions was assessed using the crossed immunoelectrophoresis with *in situ* adsorption in the second dimension. Immunoelectrophoresis was run in the horizontal agarose gel slabs on the glass plates. For that type 1 agarose (Sigma; USA) solution in the 1% Tris Tricine buffer (pH 8.6) was used [9]. The round wells were cut out in the 1 mm thick gel layer formed, to which 5 μL of blood serum or α2M preparation per well were introduced. Electrophoresis in the first dimension was run for 1 h at 200 V. Then gel was cut into 10 mm wide strips that were moved to the edges of glass plates. Free space was poured with 1% agarose sol.

When the gel was formed, we cut out a 0.5 cm wide pouch approximately 2 mm from the border with the gel used for electrophoresis in the first dimension. The pouch freed from agarose was filled with gel with essential, conditionally essential, and toxic metals the salts consisted of, immobilized onto sorbent (Table 1).

To make gel, the sample of iminodiacetic acid agarose (IDA) (Sigma; USA) was placed on a chromatography column and washed successively with ten volumes of the following preparations: 1) 0.05 M ethylenediaminetetraacetic acid disodium salt; 2) double distilled water; 3) 0.05 M aqueous solution of the metal to be tested; 4) double distilled water; 5) Tris Tricine buffer, pH 8.6.

After filling the pouch with gel with immobilized metal, the agarose gel above the pouch was cut at 2 mm away from the pouch edge, and the vacant space was filled with 1% agarose sol containing 5% of appropriate antiserum. Immunoelectrophoresis in the second dimension was run for 18 h at 100 V. At the end of electrophoresis the gel plates were washed for 24 h with the 0.1 M NaCl solution, dried, and stained with the Coomassie Brilliant Blue (R-250) dye.

## RESULTS

According to the data obtained by studying the samples of drainage blood serum collected from healthy donors (Fig. 1), blood proteins bound to both essential and toxic metals contained in intermediate gel with appropriate decrease in the precipitate area in an electropherogram.

In particular, active binding of many serum proteins was observed in the presence of not only $Zn^{2+}$,$Cu^{2+}$ and $Cd^{2+}$ ions, but also $Hg^{+}$ in intermediate gel.

Moderate binding to serum proteins was found in the electropherograms, in which intermediate gel contained not only $Sr^{2+}$ and $Pb^{2+}$, but also $Ba^{2+}$. Moreover, active binding of $Sr^{2+}$ to serum glycoproteins was observed.

Weak affinity of certain serum proteins not only to $Fe^{3+}$ and $Mn^{2+}$ ions, but also to $Ag^{+}$ was reported. Weak binding interactions between certain proteins and $Ca^{2+}$ and $Bi^{2+}$ ions was shown.

Low binding of $Mg^{2+}$ ions to γ-globulins was revealed. Weak interactions between certain proteins and $Co^{2+}$ ions were observed. Assessment of interactions with $Cr^{3+}$ ions showed almost no reactions.

Assessment of the possibility of binding interactions between the highly purified α2M molecules and the metal ions (Fig. 2) showed that this metalloprotein which was also a

**Table 1.** List of substances used for immobilization of metals

| № | Sample | № | Sample | № | Sample | № | Sample |
|---|---|---|---|---|---|---|---|
| 1 | control (no metals) | 2 | $CuSO_4$ | 3 | $CdSO_4 \times 3\ H_2O$ | 4 | $AgNO_3$ |
| 5 | $Zn(CH_3COO)_2 \times 2\ H_2O$ | 6 | $FeCl_3 \times 6\ H_2O$ | 7 | $Pb(CH_3COO)_2$ | 8 | $Bi(NO_3) \times 5\ H_2O$ |
| 9 | $MnCl_2 \times 4\ H_2O$ | 10 | $CoCl_2 \times 6\ H_2O$ | 11 | $Sr(NO_3)_2$ | 12 | $Ba(CH_3COO)_2$ |
| 13 | $CrCl_3$ | 14 | $CaCl_2 \times 6\ H_2O$ | 15 | $MgCl_2 \times 6\ H_2O$ | 16 | $Hg(NO_3) \times 1/2\ H_2O$ |

glycoprotein actively interacted not only with $Zn^{2+}$ contained in intermediate gel, but also with $Cd^{2+}$ ions (peak height reduction by more than 50% of the baseline was reported). When the intermediate gel contained not only $Fe^{3+}$, $Mn^{2+}$, $Ca^{2+}$, $Mg^{2+}$ ions, but also $Pb^{2+}$, $Sr2^{+}$, and $Ag^{+}$, the peak height was 50–60% of the peak height observed in the electropherogram of the reference sample.

When the immobilized $Cu^{2+}$, $Hg^{+}$, $Ba^{2+}$ ions were included, the peak height reduction observed in the electropherogram was 40% or more, which was indicative of rather active interaction between these metals and the α2M molecule *in vitro*.

Inclusion of $Cr^{3+}$ and $Co^{2+}$ ions in intermediate gel resulted in the lowest binding to α2M, and the peak height reduction did not exceed 10% compared to the reference sample.

Semi-quantitative data on the intensity of the serum proteins' and α2M binding to the metal ions contained in intermediate gel are provided in Table 2.

## DISCUSSION

The study has shown that serum proteins are capable of binding to immobilized metals, even if they are not metalloproteins.

The fundamental possibility of protein binding without forming the metal chelate bonds is well-known, however, only binding to essential metals was earlier identified by using the combination of gel filtration chromatography and the inductively coupled plasma atomic emission spectroscopy (ICP-AES): two proteins interacted with zinc (alpha-2-macroglobulin and albumin), two proteins bound to iron (ferritin and transferrin), and four proteins bound to copper (ceruloplasmin, albumin, factor V, transcuprein) [10]. According to the results obtained by other authors, the use of the combination of affinity chromatography (immobilized metal contained in the sorbent) and LC-MS-MS showed that complement component 3 (C3), α2M, certain albumin isoforms, apolipoproteins, ceruloplasmin, serotransferrin, keratin, γ-globulins could interact not only with essential $Cu^{2+}$, $Zn^{2+}$, but also with the conditionally essential $Cd^{2+}$, $Pb^{2+}$ [1, 11].

According to our findings, the spectrum of blood proteins capable of interacting with metal ions is even broader, it includes interactions with macroelements ($Ca^{2+}$, $Mg^{2+}$) and toxic microelements ($Ag^{+}$, $Hg^{+}$, $Ba^{2+}$, $Bi^{2+}$).

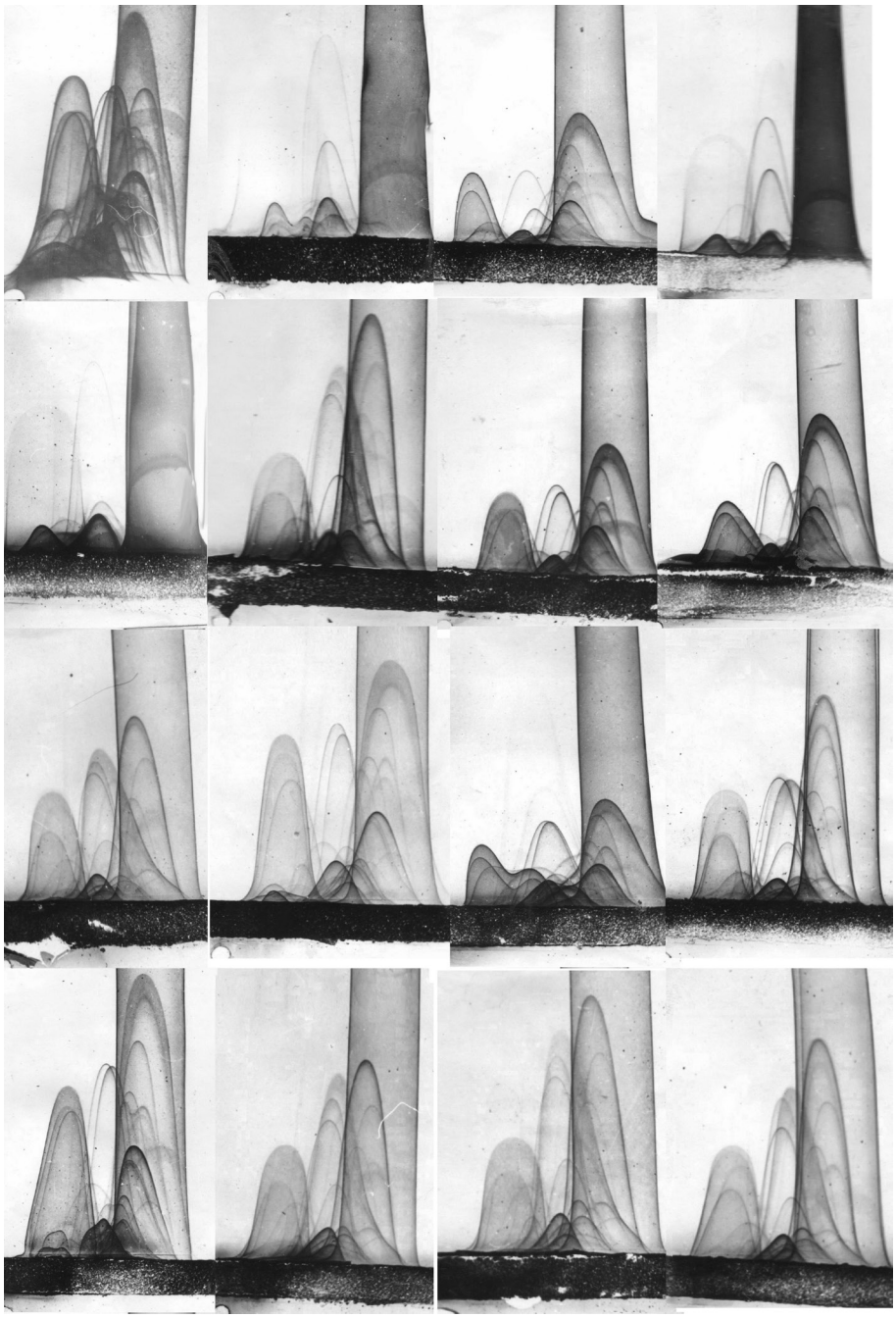
Certainly, the results of *in vitro* study need to be further confirmed by *in vivo* study, however, it can be assumed that metal toxicity may be also realized through competitive interactions with essential metals contained in proteins, as well as through formation of the metal–metal bonds that are not very strong but are capable of negatively affecting the protein conformation structure and its affinity to receptors and ligands, as is the case with competitive replacement of essential microelement. The underlying patterns and biological effects of such reactions in acute and chronic metal toxicity require further investigation.

According to the findings, it is not only the ions of essential microelements ($Cu^{2+}$, $Zn^{2+}$) that show high affinity (at the known level of assumptions, considering the identified interactions and the currently known properties) to the wide variety of serum proteins, as has been previously reported after studying the protein fraction of human γ-globulins and opposite effects of copper and zinc [11] together with $Cd^{2+}$ attributable to conditionally essential metals, but also the $Hg^{+}$ ions. It is well-known that many cadmium compounds are toxic. The thiol groups (–SH) of cysteines that are found in proteins are the most important targets for $Cd^{2+}$: cadmium can suppress the activity of many mitochondrial enzymes [12]. It can be assumed that when there are competitive or other interactions between cadmium ions and regulatory or transport metalloproteins or enzymes containing $Zn^{2+}$ and especially the less reactive $Fe^{3+}$ $Cu^{2+}$, $Mg^{2+}$ ions, these can significantly change properties of proteins, inhibit their original functions and even show new properties (such as immunogenicity). For good reason, among other things, administration of albumin, also a zinc-containing metalloprotein, is recommended when treating cadmium poisoning.

Mercury toxicity is well known. Among other things, mercury competes for metal (zinc, copper and other) binding

**Table 2.** Binding affinity of human serum proteins to the studied metal ions contained in intermediate gel during immunoelectrophoresis

| Object | Binding affinity | Ions of macroelements and essential microelements | Ions of conditionally essential microelements | Ions of toxic microelements |
|---|---|---|---|---|
| All serum proteins | +++ (high) | $Cu^{2+}$, $Zn^{2+}$ | $Cd^{2+}$ | $Hg^{+}$ |
| | ++ (moderate) | | $Pb^{2+}$, $Sr^{2+}$ | $Ba^{2+}$ |
| | + (weak) | $Ca^{2+}$, $Mn^{2+}$, $Fe^{3+}$ | | $Ag^{+}$, $Bi^{2+}$ |
| | +/– (low) | $Mg^{2+}$, $Cr^{3+}$, $Co^{2+}$ | | |
| Alpha-2-macroglobulin | +++ (≥ 50%) | $Zn^{2+}$ | $Cd^{2+}$ | |
| | ++ (≥ 40%) | $Ca^{2+}$, $Mg^{2+}$, $Fe^{3+}$, $Mn^{2+}$ | $Pb^{2+}$, $Sr^{2+}$ | $Ag^{+}$, |
| | + (≥ 30%) | $Cu^{2+}$ | | $Hg^{+}$, $Ba^{2+}$, $Bi^{2+}$ |
| | +/– (< 10%) | $Cr^{3+}$, $Co^{2+}$ | | |



| | | | |
|---|---|---|---|
| Control | $Cu^{2+}$ | $Cd^{2+}$ | $Ag^{+}$ |
| $Zn^{2+}$ | $Fe^{3+}$ | $Pb^{2+}$ | $Bi^{2+}$ |
| $Mn^{2+}$ | $Co^{2+}$ | $Sr^{2+}$ | $Ba^{2+}$ |
| $Cr^{3+}$ | $Ca^{2+}$ | $Mg^{2+}$ | $Hg^{+}$ |

**Fig. 1.** Interaction of immobilized metal ions with serum proteins. The reduced height and area of the peak are indicative of interaction between protein and metal contained in intermediate gel. The layout of metals in the samples is in table

sites in metalloproteins, thereby suppressing their activity, and demonstrates active high affinity interactions with the thiol, carboxylic, and other enzymes (ATPase, cholinesterase, alkaline phosphatase, glutathione peroxidase, glutamine synthetase, etc.) [5, 13]. Our findings show that mercury ions also can interact with other proteins. Biological effects of such interactions require further investigation.

The rather high reactivity of barium and lead ions relative to various human serum proteins also attracts attention. Despite the fact that lead is considered to be a conditionally essential microelement, most of lead compounds (especially the water-soluble ones) are toxic. It is well-known that the mechanism underlying toxic effects of lead is associated with inhibition of the thiol enzymes, interaction with the carboxyl and phosphate groups of biopolymers, and inactivation of esterases [14]. Our findings have clearly demonstrated the presence of such interactions with human blood proteins.

Despite the fact that copper is an essential microelement, and copper excess and deprivation have the extremely adverse effects on the body, pathogenesis of various neurodegenerative

diseases is associated with disturbed copper homeostasis [4]. It has been previously shown that $Cu^{2+}$ possesses properties of the redox-active metal that realizes its high oxidation potential in biological systems. The $Cu^{2+}$ chelation can cause partial breakdown of the side amino acid radicals, sugars, and sialic acids on the surface of macromolecule, as well as destructuring of the surface layer spatial arrangement, including the antigen determinants [11]. The identified broad spectrum of proteins that interact with copper ions substantiates the need for further identification aimed at clarifying their possible role in pathogenesis of various disorders.

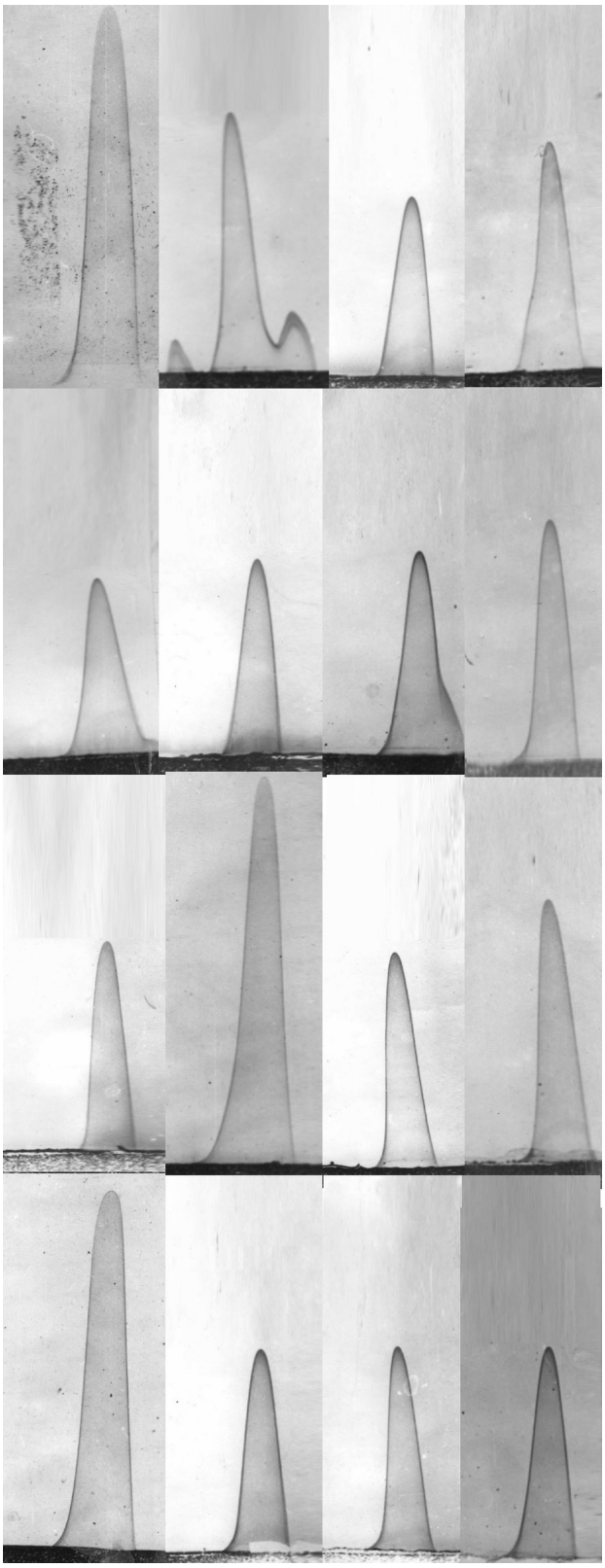
α2M was selected as a "model" protein for comparative assessment of the possibility of binding to metal ions. The study involved the use of protein in its native state obtained by gentle preparative low-pressure chromatography methods. This brought the results obtained closer to real processes taking place in the human body.

The observed interactions between α2M and $Zn^{2+}$ and $Cd^{2+}$ ions were the most intense. This result matches those on the α2M, $Cd^{2+}$, $Zn^{2+}$ bonding and to a lesser extent with those on the α2M, $Ni^{2+}$, and $Pb^{2+}$ bonding obtained earlier by other methods [1]. It is well-known that zinc is a structure-forming component of α2M, each of four α2M subunits contains one zinc ion. It has been previously shown that cadmium, that interacts with zinc contained in α2M, breaks the chelating bond between half-molecules, and the protein that is split into two parts loses most of its regulatory functions. Since α2M is involved in regulation of the cytokine profile, lipid metabolism, inhibition of the broad spectrum of proteases, signal transduction in the nervous system, inflammatory and autoimmune responses of the body [6], cadmium toxicity may result in massive failure of regulatory processes involving this protein, and the presence of such interaction, in turn, explains some mechanisms underlying cadmium toxicity.

Furthermore, zinc metabolism disorders play a key role in aging. Some authors recommend to use zinc as a dietary supplement to increase life expectancy [15]. It is obvious that cadmium that competes for binding to α2M has the exact opposite effect. In contrast to zinc ions contained in proteins, excess concentrations of free zinc in blood exert neurotoxic effects [4]. This can be one of the components of accelerated aging associated with chronic metal toxicity and one of the components of the senile dementia pathogenesis.

The identified interaction between α2M and lead is also important. It has been previously shown that lead can interact with active centers of a number of enzymes (ATPase, glucose-6-phosphate dehydrogenase, alkaline phosphatase, etc.) [16]. It is known that α2M is a universal proteinase inhibitor, however, in this case the direct effects of $Pb^{2+}$ on this enzyme inhibitor are observed instead of indirect ones. It can be assumed that circulation of the lead complexes with α2M along with the lead phosphates and albuminates takes place in lead toxicity [14]. Considering the lead hepatotoxicity, adverse effects on α2M come from two sources: death of the α2M-producing hepatocytes and inhibition of the α2M function via interaction with lead ions.

The identified ability of α2M to interact with manganese ions also can adversely affect the functions of this regulatory and transport protein. It is known that excess manganese disrupts catalytic activity of enzymes, and the reduced form ($Mn^{3+}$) contributes to oxidative stress [17]. In this case the adverse effects of this essential microelement on α2M can be realized via two mechanisms: change in functions of protein itself due to competing interaction involving zinc replacement and damage to the α2M molecule caused by superoxide radicals. It is



| | | | |
|---|---|---|---|
| Control | $Cu^{2+}$ | $Cd^{2+}$ | $Ag^{+}$ |
| $Zn^{2+}$ | $Fe^{3+}$ | $Pb^{2+}$ | $Bi^{2+}$ |
| $Mn^{2+}$ | $Co^{2+}$ | $Sr^{2+}$ | $Ba^{2+}$ |
| $Cr^{3+}$ | $Ca^{2+}$ | $Mg^{2+}$ | $Hg^{+}$ |

**Fig. 2.** Interaction of immobilized metal ions with alpha-2-macroglobulin from blood serum. The reduced height and area of the peak are indicative of interaction between protein and metal contained in intermediate gel. The layout of metals in the samples is in table

known that the "oxidized" form of α2M shows impaired ability of utilization (reduced receptor affinity) [6] and becomes potentially immunogenic due to altered conformation.

The previously described increased inhibition of the activated protein C (APC) in blood that involves α2M and occurs when exposed to ions of divalent metals (Zn, Mn, Cu) is indirect evidence of the influence of microelement imbalance on the physiological and pathological processes involving regulatory and transport proteins [18]. According to the findings, this phenomenon may be caused by the direct effects of essential microelements on α2M and its functions in the body.

The identified α2M ability of active interaction with macroelements (magnesium, calcium, iron) requires further investigation of the impact of such bonding on physiological and pathological processes.

The fact that most of clinical manifestations of acute metal toxicity (albeit less severe) are found in many conditionally healthy residents of large industrial cities attracts attention. These manifestations include conduction disorders and impaired limb sensitivity, frequent headaches and chronic fatigue, increase in the number of individuals with cognitive impairment and signs of early onset dementia, impaired liver function, etc. The findings suggest that the identified interactions of toxic and essential metals with the regulatory and transport proteins may affect the development of functional disorders and pathological processes.

## CONCLUSIONS

Most of human serum proteins, including those that are not metalloproteins, interact with metal ions in the *in vitro* experiment. High intensity of protein interaction with the conditionally essential cadmium and toxic mercury suggests that pathogenetic mechanisms of intoxication with these metals may be realized via blood protein structural and functional impairment. The identified *in vitro* α2M metalloprotein interaction with the conditionally essential and toxic metals may take the form of the competing metal–metal interactions and adversely affect the structure and functions of this regulatory and transport protein *in vivo*. The mechanisms underlying interaction and reversibility of protein binding to metals *in vivo* require further investigation.

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# MINI-INVASIVE TRANSMITRAL MYECTOMY AND MITRAL VALVE REPLACEMENT IN OBSTRUCTIVE HYPERTROPHIC CARDIOMYOPATHY CASE

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Primary hypertrophic cardiomyopathy is an isolated genetic heart disease characterized by thickening of the myocardium in the absence of an apparent hemodynamic cause. There are two patterns of the obstruction: static, with a muscle band narrowing the outflow tract of the left ventricle, and dynamic, which implies elongation of the anterior mitral valve leaflet. The key to correct treatment of the condition is understanding of the mechanism behind the obstruction. Myectomy is the gold standard of invasive treatment of obstructive hypertrophic cardiomyopathy; it aims to remove the static component of the obstruction. Another common adidition is the mitral valve surgery, aimed at elimination of the obstruction's dynamic component. This article presents a successful mini-invasive transmitral myectomy and mitral valve replacement in a case of obstructive hypertrophic cardiomyopathy with a damaged mitral valve.

**Keywords:** minimally invasive surgery, obstructive hypertrophic cardiomyopathy, mitral valve replacement

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**Author contribution:** Zemlyannikov ID, Nguyen HN — literature analysis, text authoring; Tsaregorodtsev AV — clinical case analysis, literature collection and analysis, text authoring; Ferzalieva ZR, Drozhdina AA — text editing.

**Compliance with the ethical standards:** the patient signed the form of voluntary informed consent for surgical treatment.

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# ТРАНСМИТРАЛЬНАЯ МИОЭКТОМИЯ И ПРОТЕЗИРОВАНИЕ МИТРАЛЬНОГО КЛАПАНА ИЗ МИНИДОСТУПА ПРИ ОБСТРУКТИВНОЙ ГИПЕРТРОФИЧЕСКОЙ КАРДИОМИОПАТИИ

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Гипертрофическая кардиомиопатия (первичная) — это изолированное генетическое заболевание сердца, выражающееся в утолщении миокарда без явной гемодинамической причины. Существует два механизма обструкции: статический — мышечный валик, стенозирующий выносящий тракт левого желудочка, и динамический — удлиненная передняя створка митрального клапана. Понимание механизма обструкции является ключом к правильному лечению. Золотым стандартом инвазивного лечения обструктивной гипертрофической кардиомиопатии является миоэктомия, устраняющая статический компонент обструкции. Ее дополняют вмешательствами на митральном клапане, которые помогают устранить динамический компонент обструкции. В статье представлен клинический случай успешной трансмитральной миоэктомии и протезирования митрального клапана из мини-торакотомии при обструктивной гипертрофической кардиомиопатии с поражением митрального клапана.

**Ключевые слова:** миниинвазивная хирургия, обструктивная гипертрофическая кардиомиопатия, протезирование митрального клапана

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**Соблюдение этических стандартов:** от пациента получено добровольное информированное согласие на оперативное лечение.

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Primary hypertrophic cardiomyopathy is an isolated genetic heart disease characterized by thickening of the myocardium in the absence of an apparent hemodynamic cause. There are over 1400 mutations in more than 11 genes encoding the cardiac sarcomere proteins that can cause the disorder [1]. In some nosologies, like the Noonan syndrome, the MELAS syndrome, the Sengers syndrome etc., heart damage is secondary.

The degree of obstruction of the left ventricular outflow tract (LVOT) defines the severity of hypertrophic cardiomyopathy. There are two patterns of the obstruction, one involving a muscle bundle narrowing the LVOT and another is the elongation of the anterior mitral valve (MV) leaflet. The key to correct treatment of the condition is understanding of the mechanism behind the obstruction. Although myectomy is the gold standard of invasive treatment of obstructive hypertrophic cardiomyopathy (OHC), MV surgery, which aims at elimination of the obstruction's dynamic component, should also be considered.

It is very important to make the myectomy sufficiently complete. The classical transaortic access, however, imposes limitations on manipulations deeper in the left ventricle. Moreover, even following all the rules of myectomy, there is still a risk of damage of the tracts. Transmitral access, on the other hand, involves partial amputation of the MV's anterior leaflet (which gives direct access to the interventricular septum (IVS)), followed by myectomy, reduction of the posterior leaflet's height (if it is more than 20 mm), and remodeling annuloplasty

with a support ring. The operation is finished with restoring the integrity of the MV's anterior leaflet by suturing in an oval autopericardium patch.

However, the question of advisability of the above-described plastic reconstruction of MV is still an open one. If the patient suffers from a pronounced SAM syndrome, there is a risk of recurrence of the dynamic obstruction.

This sort of intervention can be minimally invasive, but this approach to the matter has not yet been properly developed. In Russia, the share of surgeries through mini-access in isolated pathologies is very low, and the number of combined interventions is single-digit. Only the successful technique application cases are described in the published papers, which prevents accumulation of objective data thereon. However, it is possible that the negative consequences linked to mini-invasive access are mainly associated with the complexity of the incision itself and not with the procedures made through such access. In addition to the obvious advantages, minimally invasive procedures deliver results comparable to those attained through the classical access. This is convincing point in favor of the promise of development of the short-scar incision cardiac surgery.

This article presents a successful transmitral myectomy and mitral valve replacement through a mini access in a case of hypertrophic obstructive cardiomyopathy (HOCM) with a damaged mitral valve.

## Case description

A 65-year-old female patient was admitted to the Department of Cardiac Surgery of the I.V. Davydovsky City Clinical Hospital. Case history: for 15 years she has been suffering from shortness of breath and dizziness during physical exertion (climbing the stairs from one floor to the next one). Since December 2020, even minimal exertion, like climbing 2 or 3 steps, brought shortness of breath. The described condition was the reason for an outpatient examination. Echocardiography (EchoCG; June 16) revealed the EF to be at 70% and the IVS asymmetrically hypertrophied (up to 17 mm in the basal regions). Other findings included obstruction of the LVOT (HPmax in LVOT > 100 mm Hg), violation of myocardial relaxation, retraction of the MV's posterior leaflet in the left atrial (LA) up to 10 mm, severe mitral insufficiency (4th degree, type II by Carpentier classification). Transesophageal echocardiography (TEE; June 16) showed a significant deflection of the posterior leaflet in the area of P3 and P2 (caused by chordal detachment), several regurgitant jets, vena contracta — 0.45 $cm^2$, SAM. Coronary angiography revealed no lesions. The main diagnosis: severe mitral insufficiency caused by the detachment of chords of MV's posterior leaflet. Obstructive hypertrophic cardiomyopathy (asymmetric, IVS / LVOT > 1.6/1; HP > 100 mm Hg). Complications: chronic heart failure 2A (CHF 2A), FC 2 according to NYHA.

The patient underwent mitral valve replacement with a mechanical prosthesis and transmitral myoectomy through a short-scar thoracotomy with pharmaceutical and cold blood cardioplegia and cardiopulmonary bypass (femoral vein-femoral artery) (surgeon O.Yu. Pidanov). MV reconstruction was not undertaken because mini access makes anterior leaflet reduction and neochord reconstruction difficult, and there is a high risk of development of the SAM syndrome: the obstruction was not only static but also dynamic. This patient had a subaortic obstruction that could not be remedied after MV replacement, which is an indication for myectomy, the golden standard treatment for HOCM.

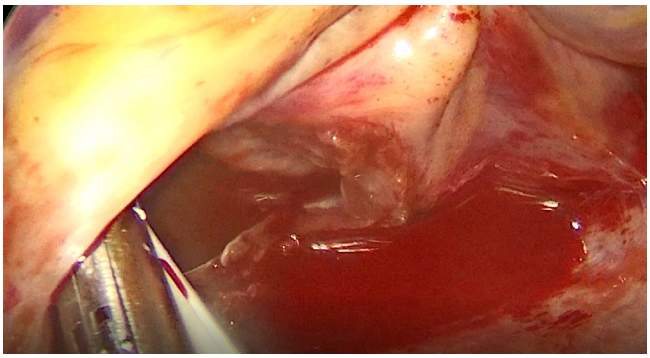

**Fig. 1.** Left atticotomy behind the interatrial sulcus

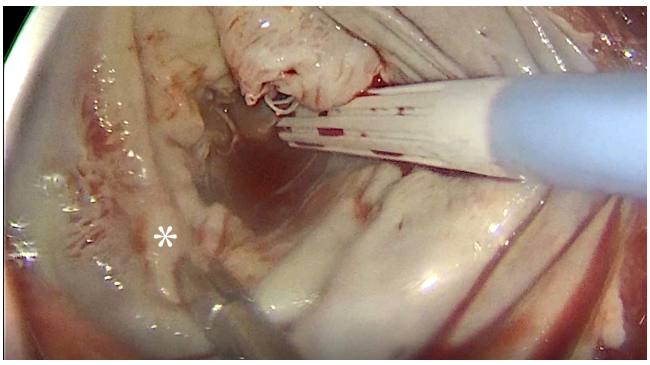

**Fig. 2.** Intraoperative view of the MV from the LA side. * — excessive mobility of the posterior leaflet in the P3 and P2 regions due to detachment of the chords

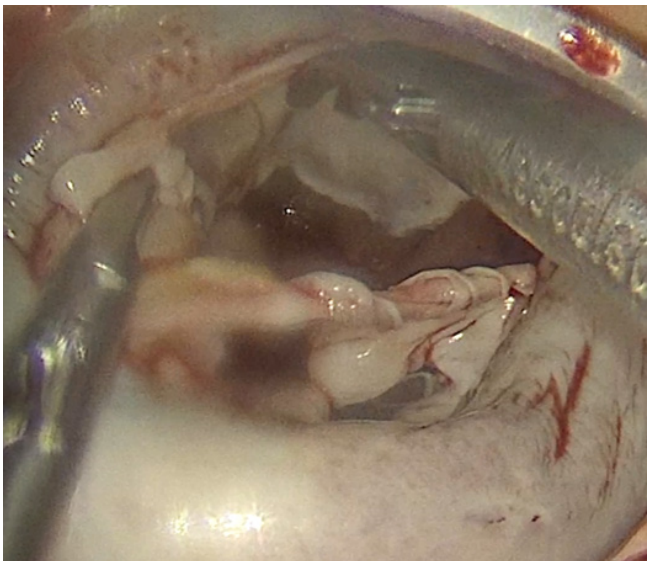

**Fig. 3.** MV excision, exposure of the LVOT obturation

Once the aorta was clamped, we placed a root aortic cannula to enable antegrade crystalloid cardioplegia (2000 ml). Carbonization was also part of the process. The MV was accessed from the left atrium through an incision behind the interatrial sulcus (Fig. 1).

To expose the MV and to move further into the left ventricle, an atrial retractor was placed. Intraoperative view of the MV: detachment of the chord in the P2 segment, severe insufficiency (Fig. 2).

The muscle bundle obturating the LVOT becomes visible as the MV is excised (Fig. 3).

Myectomy began immediately below the MV annulus (navigation lines indicate excision volume) (Fig. 4) and continued to the base of the papillary muscles (Fig. 5). The

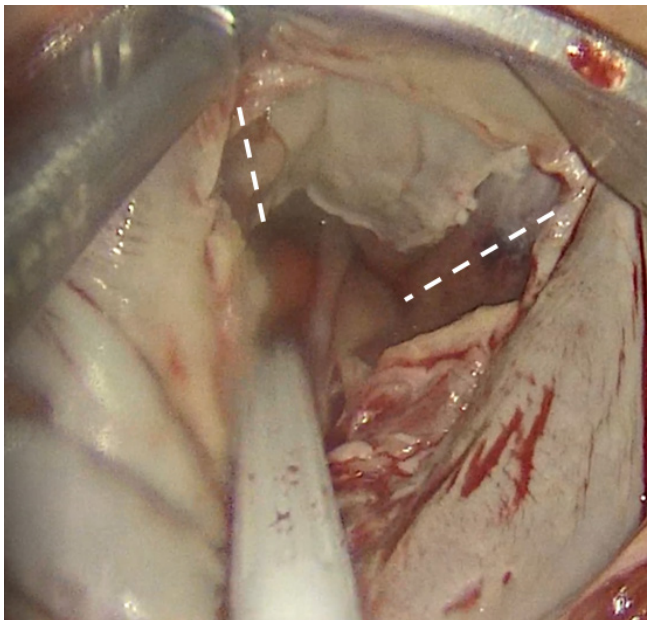

**Fig. 4.** Muscle bundle obtruding the LVOT. Navigation lines indicate the extent of the excision

excised fragment of the muscle measured 3 × 3 × 0.7 cm.

Excision of the MV with partial excision of the subvalvular structures was a myectomy (Fig. 6).

Final view after myectomy: excised MV and muscle bundle; LV cavity became larger, especially near the LVOT; the papillary muscles were intact (Fig. 7).

A mechanical MV was placed with 17 single matrass sutures (Fig. 8).

The function of the prosthesis was reviewed and assessed. The atriotomy was closed with a double-row suture; after careful deaeration, the aortic clamp was removed. Cardiac activity restored without external assistance, HR = 85. Bypass was stopped with stable hemodynamics. Decannulation was performed from the femoral vein and artery, wound in the femoral region was closed with layered sutures. The pleural cavity was drained with a silicone drain tube, the thoracotomy wound was closed layered sutured. The patient was on bypass for 123 minutes; the aorta was clamped for 68 minutes. The patient was transported to the cardiac intensive care unit.

The result of the operation: HPmax in LVOT — 8 mm Hg, mean pressure gradient in the MV prosthesis — 7 mm Hg, sinus rhythm, no signs of AV-, SA-blockades. The patient was discharged on the 20th day after resolution of the post-surgery complications: right-sided hemopneumothorax, 1st degree endobronchitis (Lemoine classification).

## Clinical case discussion

In cardiac surgery, minimally invasive techniques do have obvious advantages over the classical surgical access patterns: they reduce blood loss, pain, the likelihood of infectious complications, and shorten the rehabilitation period [2]. Few surgeons have mastered minimally invasive techniques; there are no standards regulating the respective training, which means the end result largely depends on the surgeon and may vary in different clinics. In addition, far from all clinical cases are described in the published papers: not all surgeons are ready to report their failures and mistakes, while everyone wants to talk about successful complex operations. Nevertheless, there is evidence that the results of surgeries done through a short-scar access are quite comparable with the results of operations

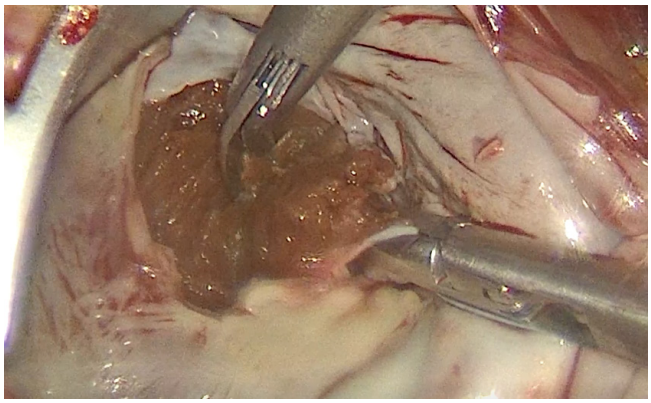

**Fig. 5.** The process of excision of the swelling muscle

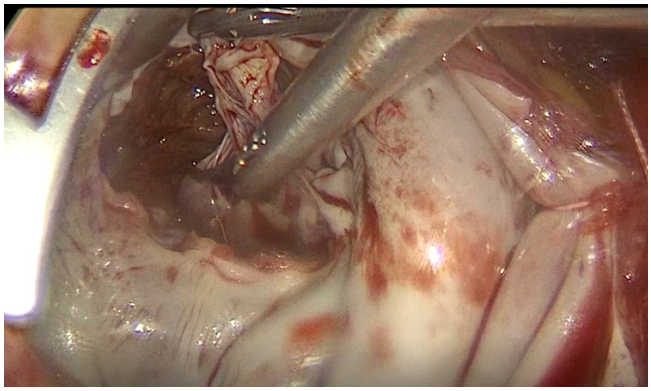

**Fig. 6.** Final stage of the process of excision of the MV and some of the subvalvular structures

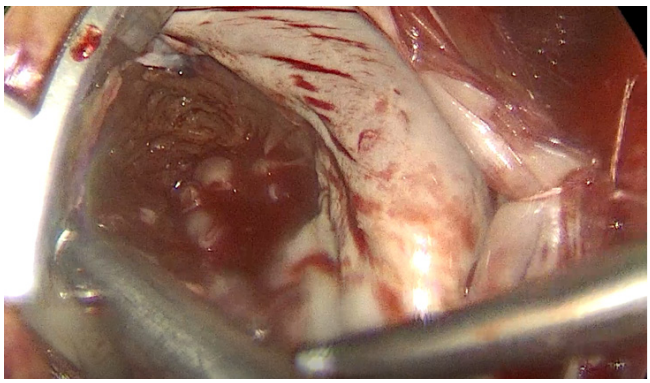

**Fig. 7.** LV after MV excision and myectomy (final view)

performed through a classic sternotomy [3]. Mini access probably can replace sternotomy, but only in the context of certain interventions. While the procedure is crucial for the patient, convenience of surgical access has to be a priority.

Myectomy can be transaortic, transapical, transventral, transmitral. Transaortic septal myectomy gives excellent long-term results [4] and is a classic solution for HOCM, but the decision to apply it should be based on rational reasons. The respective access is an optimal one when the target is an subaortic muscular stenosis, but if the obstruction is slightly lower, it can make the surgeon's work more difficult. There are improved myectomy techniques for such cases. The flaw with transaortic access is the ease with which surgeons can damage the conducting pathways passing at the central fibrous body's projection site (between right coronary and non-coronary cusps). Transmitral access is a good choice for myectomy at the midventricular level of the IVS [5]; the risk of damage to the pathways is lower with this approach. In the

described clinical case, we opted for the transmitral access, since it allows operating on the MV simultaneously.

If MV can be repaired, LV is exposed by cutting off the MV's anterior leaflet in case of a transmitral access. At the end of the myectomy, the valvotomy is sutured (if necessary, covered with patch), and the patient's valve can be saved. This technique has been used in and described in detail [6]. The method was repeated with video-assisted short-scar thoracotomy, with patients suffering from diffuse obstructive HOCM and SAM induced moderate mitral regurgitation.[7]

Preservation of the native MV and elimination of the obstruction are the main goals in the treatment of patients with HOCM. The frequency of MV replacement in the HOCM expert surgery centers is less than 5% [8], and in the Cardiology Republican Research and Practice Center (Minsk, Republic of Belarus) it is 0% (160 cases over the past 5 years). By itself, MV replacement for HOCM patients, compared with its repair or isolated myoseptectomy, is associated with worse immediate and long-term results; this is considered a disabling intervention, an opinion shared by Russian and foreign authors [9, 10]. Detachment of the chords in the MV's P2 segment (as in the case described) can be successfully corrected surgically in more than 95% of cases. For this patient, we decided to replace the MV taking into account her age and the unreasonable risk of repeated surgical intervention. In addition, there are no convincing data on the efficacy of reconstruction done through a mini access.

Thus, the surgical tactics, its expediency and conformity to the world experience in managing such patients are substantiated.

## CONCLUSION

The described clinical case demonstrates the possibility of correcting HOCM with MV lesion through a right side short-scar thoracotomy.

There are specific indications for MV replacement in such patients: obvious inexpediency of native valve repair or significant mitral regurgitation not associated with the HOCM, which renders the repair impossible.

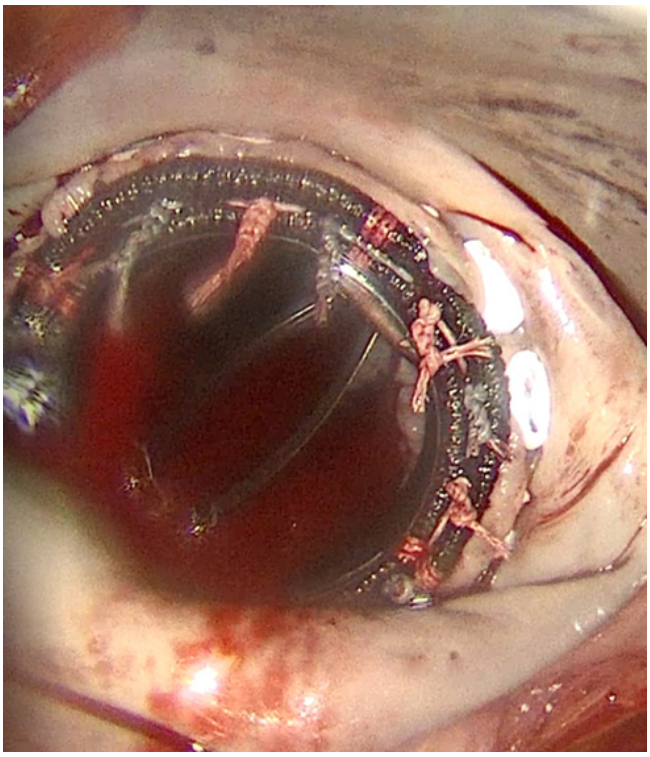

**Fig. 8.** MV prosthesis

On one hand, minimally invasive approaches in surgery still remain underutilized due to the technical complexity of the respective procedures and little experience accumulated in this area so far. On the other hand, minimally invasive techniques ensure results comparable to those achieved with open heart surgery. There is an obvious need for further development in this direction, especially considering the general trend in surgery towards minimal invasiveness.

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