RUSS-AGE: developed research protocol for the creation of Russian biological age calculators

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Abstract
Background. Life expectancy is increasing around the globe. However, chronological age is not the best indicator of health. For a more accurate assessment of body condition throughout life, in general, and aging, in particular, and identify potential points of geroprotective intervention, a specialized tool is needed. A tool that could prove beneficial is a biological age calculator, utilizing a range of biomarkers to analyze the degree of functional preservation of the body.

Aim. Many existing biological age calculators are limited by a small number of parameters to analyze and sensitivity to use in a specific population. Large-scale studies to create a mathematical model for calculating biological age based on the Russian population have not previously been carried out. In 2022, the RUSS-AGE study was launched to create biochemical, cognitive and microbiotic calculators of biological age and determine possible points of geroprotective interventions.

Materials and methods. The study intends to enroll at least 3,500 participants and analyze more than a hundred biomarkers using laboratory tests, questionnaires, neurocognitive and functional testing, and collection of anthropometric and physical indicators.
Results. Currently, the recruitment of participants is supported by a government grant under the Priority 2030 program. By November 2023, 510 participants had been enrolled in the study.
Conclusion. Further statistical processing of the information received and the development of prototypes of biological age calculators are planned.

Keywords: biological age; biological age calculator; biomarkers; gerosciences; aging research.

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INTRODUCTION

Over recent decades, global life expectancy for individuals over seventy has increased significantly [1]. While healthy life expectancy has also risen, the health status of older populations varies considerably between individuals, posing challenges for both healthcare systems and the economy. Chronological age is a poor proxy for health status [2]. Consequently, there is a pressing need for tools that enable a more precise analysis of aging processes at the individual level and identify potential intervention points prior to disease onset.

The international scientific community has long sought to address this challenge, investigating both the mechanisms of aging [3,4] and relevant biomarkers [2], as well as potential geroprotective interventions [5,6,7]. A significant body of knowledge has been accumulated, and the critical task now is to translate these findings into clinical practice.

Biological age calculators, or "aging clocks," are promising tools for assessing an individual's biological age — the functional integrity of their tissues and organs. These are computational formulas that utilize various aging biomarkers as variables. Depending on the inputs, calculators can estimate biological age based on DNA methylation (epigenetic clocks like DNAmAge, PhenoAge, GrimAge), gene expression data (transcriptomic clocks like Aging AI), omics complexes (proteomic, metabolomic clocks), circadian rhythms, routine blood tests,

The most advanced aging clocks are machine learning models that integrate multiple parameters reflecting aging across different tissues into a single composite biological age score. However, existing calculators have limitations. Some, like DNA methylation-based models, are not easily applicable in routine clinical practice. Others that use more clinically accessible parameters (e.g., biochemical and hematological markers) are sensitive to the specific population used to derive their reference values.

Therefore, tools developed using data from other populations cannot be confidently applied in Russia, or at least would entail significant calculation error. Assessing the biological age of Russians requires a tool built on data from the Russian population itself, accounting for its genetic makeup, multi-ethnic specifics, and lifestyle. No prior studies have described such a mathematical model for the Russian population.

In 2022, the Institute of Aging at the Pirogov Russian Gerontology Research and Clinical Centre initiated the multicenter cross-sectional RUSS-AGE study. Its aim is to develop biological age calculators based on Russian population data and identify potential points for geroprotective

interventions. Our objective is to create biochemical, microbiotic, and cognitive calculators. To achieve this, we have selected over a hundred aging biomarkers to be collected via biosampling, questionnaires, and testing from a nationally representative age-stratified sample. This article describes the biomarker selection criteria, the developed study protocol, and its current status.

PARTICIPANT SELECTION AND ENROLLMENT

The study plans to enroll at least 3,500 participants residing in the Russian Federation, with a target gender ratio of 1:1. Key inclusion criteria are age ≥18 years and provision of signed informed consent. While the lower age limit is set at 18, there is no upper age limit. Participants will be divided into 5-year age cohorts, with a target of at least 200 participants per cohort.

Exclusion criteria (see Appendix) are designed to focus on individuals without significant sensory or cognitive deficits, life-threatening or other acute health conditions, uncorrectable diseases, and other specific health issues. Assessment against exclusion criteria does not involve preliminary diagnostics by the Organizer. Instead, potential participants self-assess their health status against the listed exclusion criteria after being informed.

Prior to signing consent, volunteers are informed about the study's aims, procedures (including questionnaires, examination, and biosampling), and can ask questions. Participants may withdraw at any time and revoke consent for biobanking. Data collected prior to withdrawal may still be used for statistical analysis. Participants may also be excluded if any exclusion criterion is identified during study procedures, with reasons documented in the source documentation and individual registration card (IRC).

LOCAL ETHICS COMMITTEE AND DATA CONFIDENTIALITY

Strict confidentiality of personal data is maintained. Data collected is limited to what is necessary for the study objectives and includes: full name, date and place of birth, residence address, personal phone number, optional relative's phone number, nationality, marital status, and number of children. This list may be expanded via a protocol amendment.

To protect privacy, each participant is assigned a unique identification code that encrypts their information in the electronic database. The electronic IRC does not display names, addresses, contact details, or medical record numbers.

Access to collected data is restricted. Direct access is granted only to investigating physicians communicating with participants. Organizations processing biomaterials receive the minimum necessary personal data. For statistical processing, all data is anonymized. Personal data on physical media (paper questionnaires) are stored securely in the Organizer's archive.

All personnel with access to study information sign non-disclosure agreements. Personal data will not be disclosed to third parties except as required by law or authorized audits.

DATA ENTRY AND DATABASE STORAGE

A software-hardware complex with a client-server architecture and web interface is used for data collection, storage, and processing. Data is stored in a relational database (MS SQL Server) under a full mirroring regime on two geographically separate servers in Moscow.

Each system user (investigating physician) is assigned a specific role and unique login credentials. User roles determine read/write access permissions.

Data entry occurs via a graphical web interface. The system performs automated two-stage validation: 1) checking for required fields and data type conformity; 2) applying additional automated rules for specific fields. All data entries and modifications are logged in an audit trail, recording date, time, user login, and old/new values. Data for statistical analysis is exported in MS Excel format.

PARTICIPANT RECRUITMENT METHODS

Volunteers are recruited through information dissemination at the Russian Gerontology Research and Clinical Centre (RGNCC), via media, and at events involving RGNCC staff, particularly from the Laboratory of Aging Biomarkers.

Some participants are invited from databases of previous RGNCC studies. Those who consented to re-contact are phoned, informed about RUSS-AGE, and screened for exclusion criteria. Eligible individuals are then invited for an interview and biosampling.

Large companies and organizations like Moscow Longevity Centers also assist in recruitment through mailings, oral presentations, and flyers. Data of individuals interested in participating are centrally collected in the Laboratory of Aging Biomarkers database.

RUSS AGE STUDY PROCEDURES

Participation involves seven mandatory stages (see flowchart "RUSS-AGE Study Stages"):
1) Screening against exclusion criteria; 2) Signing informed consent; 3) Completing questionnaires; 4) Anthropometric assessment by a physician; 5) Functional tests; 6) Neurocognitive testing by a physician; 7) Biosampling (blood, urine, gut microbiota).

Typically, all stages require one meeting with the physician and one separate visit for biosampling. For some participants (e.g., centenarians), procedures may be split across different days.

Questionnaires
The questionnaire stage is standardized across age cohorts and includes both study-specific and internationally validated instruments.

Study-specific questionnaires assess: chronic disease risk factors; medical history (including past/current diseases, COVID-19 history); medication/supplement use; emotional state; nutritional status.

Internationally validated questionnaires include: SF-12 Quality of Life [8,9]; Hospital Anxiety and Depression Scale (HADS) [10]; Insomnia Severity Index (ISI) [11]. Participants aged ≥65 are assessed for frailty using the "AGE IS NOT AN ISSUE" questionnaire [12] and functional status using the Barthel Index [12].

Neurocognitive Testing
All participants undergo neurocognitive testing: Symbol Digit Modalities Test (SDMT) [13] and Trail Making Tests (TMT-A/B) [14,15]. Additional tests are age-dependent: Stroop Test [16] (ages 18-39); Montreal Cognitive Assessment (MoCA) [17,18] (ages 40-89); Mini-Mental State Examination (MMSE) [19] (age ≥90).

Functional Tests and Anthropometric Data
During the visit, the investigating physician measures: height, weight, BMI; waist, arm, calf circumference; blood pressure, heart rate; handgrip strength (dynamometry); Short Physical Performance Battery (SPPB) [12] (age ≥65). For younger cohorts, balance (semi-tandem stand) and chair rise tests are performed.

Biomaterials
Participants provide blood, urine, and stool samples. Blood (55 mL) is collected aseptically into 14 tubes (with/without preservatives). Most samples are centrifuged (3000 rpm, 10 min). Aliquots of whole blood, plasma, and serum are sent for lab analysis within 4 hours or transported at +4–8°C. Remaining aliquots are stored in the Organizer's biobank at ≤ -75°C. Urine is collected in sterile containers, aliquoted, and biobanked. Stool is collected in sterile tubes with a preservative stabilizing the microbiome at room temperature for several weeks prior to lab analysis.

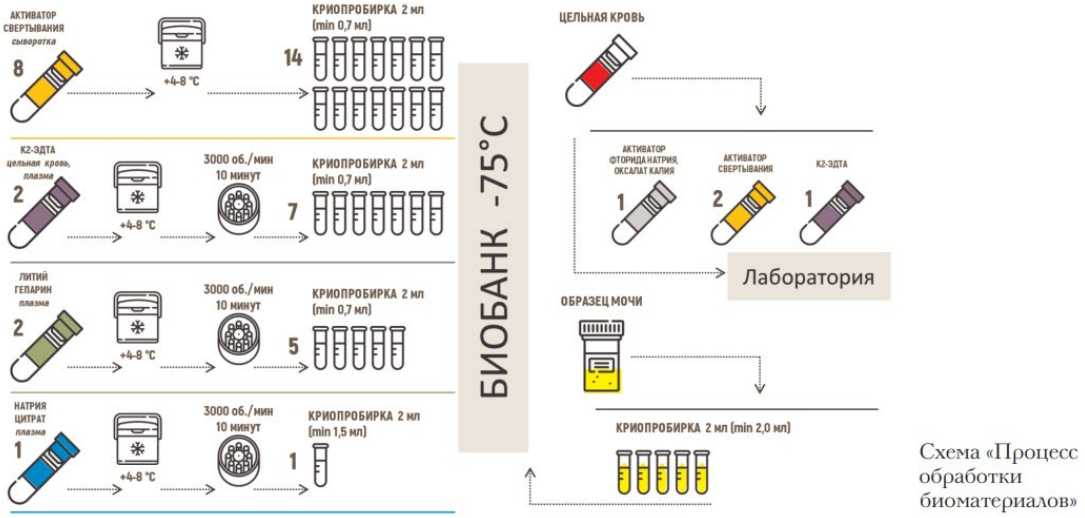

Схема «Процесс обработки биоматериалов»

SELECTION OF BIOMARKERS FOR ANALYSIS

RUSS AGE plans to analyze over 100 biomarkers related to aging mechanisms [20], including markers of mitochondrial dysfunction, oxidative stress, chronic sterile inflammation, telomere shortening, and others.

Selection criteria required: ≥3 independent clinical studies per biomarker (published 2015 or later); and a meta-analysis (published 2018 or later) of ≥3 RCTs with a total sample ≥300.

Literature search (PubMed) identified parameters for analysis encompassing: complete blood count; serum biochemistry; carbohydrate/lipid metabolism; adipokines; hormonal/vitamin status; inflammation markers; mitochondrial dysfunction/cellular senescence markers; extracellular matrix markers; markers of cellular barrier integrity/endothelial dysfunction; immunosenescence indicators; quantitative amino acid analysis; leukocyte telomere length; and select urinary parameters. Data from questionnaires and tests also serve as biomarkers.

The protocol also allows for optional sub-studies with participant consent, e.g., whole-genome DNA sequencing, fecal microbiota RNA sequencing, and optical coherence tomography (OCT) of the eye.

CURRENT STUDY STATUS

Recruitment began in December 2022 and is supported by a state grant under the Priority 2030 program. By November 2023, 510 participants had been enrolled (376 women, 134 men). Recruitment for age cohorts 25-69 is complete. Enrollment continues for ages 18-24 and ≥70. All enrolled participants have completed questionnaires, testing, and biosampling.

STUDY LIMITATIONS

The primary limitation is the current cross-sectional design. However, upon completion, the collected data may permit a follow-up longitudinal study in a subset of participants. Another limitation is the reliance on self-reported data for assessing exclusion criteria. Furthermore, recruitment is currently geographically biased toward Moscow and the Moscow region, potentially limiting ethnic and socioeconomic representativeness given Russia's multi-ethnic composition. Recruitment from other regions depends on future funding.

Concentration in a metropolis may also bias the sample toward middle-class participants. As socioeconomic status influences aging processes and biomarker levels [21,22,23], this may limit the generalizability of findings to the broader national population.

Finally, the study's ambitious scope is constrained by financial, human, and temporal resources. The project, conducted under the seven-year Priority 2030 program, currently has grant support for recruiting 600 participants.

CONCLUSIONS

Recruitment for RUSS-AGE will continue until 2029. We have successfully established procedures for participant enrollment, communication, data/biosample collection, transport, storage, and processing.

Preliminary statistical analysis is planned to obtain general characteristics of laboratory, anthropometric, functional, and questionnaire data. Based on this, initial prototypes of biochemical, microbiotic, and cognitive biological age calculators will be developed. Beyond standard statistics, we plan to employ artificial intelligence to create a machine learning-based "aging clock" model integrating all three assessment domains (biochemical, microbiotic, cognitive), aiming for a more objective biological age estimate than existing calculators.

Prototypes are planned by the end of 2024. Following study completion in 2030, a translational phase is envisaged to test and implement the developed tools in clinical practice.

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APPENDIX

Checklist of Inclusion and Exclusion Criteria for Potential Participants

Inclusion Criteria
• Age ≥18 years.
• Consent to participate in the study.

Exclusion Criteria
• Pregnancy or breastfeeding.
• Refusal to participate or provide informed consent.
• Significant cognitive/sensory impairments or mental disorders hindering communication, as judged by the investigator.
• Acute illness/condition, exacerbation of a chronic disease, or surgical intervention within the last month prior to inclusion.
• Active cancer or anticancer therapy completed less than three years prior to inclusion.
• History/medical records indicating infectious diseases (Hepatitis C, Hepatitis B including HBsAg carriage, HIV).
• Severe chronic non-communicable diseases:
• Life-threatening arrhythmias (severe, uncontrolled by medication);
• Chronic heart failure NYHA Class III-IV;
• Ejection fraction <40%;
• Coronary artery disease CCS Class 3-4;
• Chronic kidney disease Stages 3b-5;
• Type 1 diabetes mellitus;
• Type 2 diabetes mellitus with terminal-stage complications;
• Systemic connective tissue diseases;
• Chronic obstructive pulmonary disease with respiratory failure ≥ Grade 1 (dyspnea on walking 150-300 m or climbing one flight of stairs);
• Asthma requiring glucocorticosteroid therapy;
• Osteoarthritis Functional Class IV;
• BMI ≥40 kg/m²;
• Documented history of myocardial infarction or stroke.
• Any other factors deemed by the investigator to preclude participation.

Additional Criteria for Participants Providing Stool Samples:
• Systemic antibiotic use for ≥3 days within 3 months prior to the study.
• Any invasive procedures on the large intestine within the last 3 weeks prior to the study.