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Brief Title: Volatilome and Single-Lead Electrocardiogram Optimize Ischemic Heart Disease Diagnosis Using Machine Learning Models

Official Title: Biomarkers of the Exhaled Breath and Single-Lead Electrocardiography in the Diagnosis of Myocardial Ischemia

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Study Protocol

1. Relevance of the topic.

The relevance of the proposed scientific problem is beyond doubt. Cardiovascular disease (CVD) is the leading cause of mortality and morbidity in the 21st century, despite modern progress in therapeutic strategies and technologies [1]. Unfortunately, every second person worldwide dies from cardiovascular event. According to the World Health Organization data published in 2021, it is known that in 2019, 17.9 million people died from CVD, which is 32% of all deaths worldwide. Cardiovascular diseases (CVD) are the leading cause of death in the Russian Federation and account for about 54 % of all cases. The leading position among these diseases is occupied by ischemic heart disease, the annual mortality from ischemic heart disease is 38% [2,3].

For several decades, there have been active attempts to develop reliable diagnostic methods for coronary heart disease applicable to mass population screening. It is known that the diagnostic accuracy of the most accessible method for detecting signs of myocardial ischemia - the stress ECG test, does not exceed 70%. In this regard, the search for various biomarkers that increase the accuracy of stress testing is a practically significant and urgent task. There is a growing interest in studying the composition of exhaled air (volatile organic compound concentrations). Exhaled air analysis using mass spectrometry (MAS) in stable coronary heart disease (CHD), as well as in the diagnosis of CHD, has not been studied and requires further development. In addition, predicting changes in lipidome and inflammasome in correlation with exhaled air (EA) analysis has also not been studied and can determine the pathogenetic basis for the development of CHD.

We analyzed various databases (PubMed, Embase, Cochrane library, China National Knowledge Infrastructure), found a limited number of works aimed at studying volatile organic compounds in exhaled air in patients with coronary heart disease. There are many descriptive works on the methodology for performing and analyzing volatilome, a limited number of works devoted to the search for cardiometabolic substances in exhaled air.

The attention to the MSM BB method is also justified by the fact that the test does not require expensive consumables and is carried out within one minute, which allows this method to be used in addition to the load test, without changing the test protocol significantly.

Also, to improve the diagnosis of coronary heart disease, a single-channel electrocardiogram is used with pulse wave assessment and the ability to predict the risk of cardiovascular events using artificial intelligence algorithms.

2. Novelty of the proposed topic based on literary sources and patent documentation.

For the first time in the world, use real time method of analysis of the composition of the exhaled breath using time-of-flight mass spectrometer-proton transfer reaction- mass spectrometer-1000. There is no study reporting changes in the analysis of exhaled air using this method in patients with ischemic heart disease, confirmed by stress induced myocardial perfusion defect.

For the first time in the world, single-channel ECG with pulse wave function analysis using machine learning models will be performed to predict a positive test result for ischemic heart disease.

3. **Objective and tasks of the planned research.**

Goal:

1. To increase the diagnostic accuracy of functional tests with physical activity under the control of a 12-channel ECG with parameters for analyzing the molecular composition of exhaled air, single-channel ECG data with pulse wave function.

Tasks:

1. Assessing the diagnostic accuracy of the stress electrocardiography in the diagnosis of ischemic heart disease (AUC, Sensitivity, Specificity, Sensitivity, NPV, and PPV)

2. Analyze the volatile organic compounds of the exhaled breath in individuals with stress-induced myocardial perfusion defect on stress computed tomography myocardial perfusion imaging (CTP) with vasodilation test (adenosine triphosphate) and compare them with individuals without stress-induced myocardial perfusion defect after a physical stress test, and compare them with rest results as independent variables. Machine learning model will be used to assess the diagnostic accuracy of the exhaled breath in the diagnosis of ischemic heart disease

3. Analyze the parameters of the single-lead electrocardiogram with pulse wave function in individuals with stress-induced myocardial perfusion defect on stress computed tomography myocardial perfusion imaging (CTP) with vasodilation test and compare them with individuals without stress-induced myocardial perfusion defect as an independent variable. Machine learning model will be used to assess the diagnostic accuracy of the single-lead ECG with pulse wave function in the diagnosis of ischemic heart disease.

4. Analyzing the taken blood samples for total cholesterol, TG (mmol/L), LDL (mmol/L), LDL (mmol/L), HDL (mmol/L), and VLDL (mmol/L) in individuals with stress-induced myocardial perfusion defect on stress computed tomography myocardial perfusion imaging (CTP) with vasodilation test and comparing them with individuals without stress-induced myocardial perfusion defect as independent variables.

5. Analyzing the taken blood samples for Apolipoprotein B (g/L) in individuals with stress-induced myocardial perfusion defect on stress computed tomography myocardial perfusion imaging (CTP) with vasodilation test and comparing them with individuals without stress-induced myocardial perfusion defect as independent variables.

6. Analyzing the taken blood samples for lipoprotein (a) (mg/L) and c-RP (mg/L) in individuals with stress-induced myocardial perfusion defect on stress computed tomography myocardial perfusion imaging (CTP) with vasodilation test and comparing them with individuals without stress-induced myocardial perfusion defect as independent variables.\

7. Analyzing the taken blood samples for IL- 6 (pg/mL) in individuals with stress-induced myocardial perfusion defect on stress computed tomography myocardial perfusion imaging (CTP) with vasodilation test and comparing them with individuals without stress-induced myocardial perfusion defect as independent variables.

4. Research design.

A prospective, non-randomized, minimally invasive, cohort case-control study includes patients (men and women) aged ≥ 40 years. Participants will be recruited from 01.11.2023 to 10.06.2024 at the University Clinical Hospital No. 1 of Sechenov University, Moscow.

Once enrolled in the study, participants will be divided into two groups based on the results of a stress computed tomography myocardial perfusion (CTP) imaging with vasodilation test using the adenosine triphosphate:

- The first group is planned to include 31 people with myocardial perfusion defect on the stress computed tomography myocardial perfusion Imaging (by using contrast enhanced multi-slice spiral computed tomography (CE-MSCT) using adenosine triphosphate (ATP)).
- The second group is planned to include 49 people without myocardial perfusion defect on the stress computed tomography myocardial perfusion imaging (by using contrast enhanced multi-slice spiral computed tomography (CE-MSCT) using adenosine triphosphate (ATP)).

All patients are planned to undergo the following examinations:

1. Exhale into proton transfer reaction- time of flight-mass spectrometry-1000 for one minute using the PTR-TOF-MS-1000 device (Patients/control group come to the hospital (ate two hours before the test to avoid hypoglycemia during the stress test)/did not brush their teeth with toothpaste).

To prepare the participant for the time-of-flight mass spectrometry with proton transfer ionization method, the participant enters the office, sits on a chair in front of the device, the patient is informed that he/she needs to clasp the breathing tube with his/her lips, breathe through the nose and exhale through the mouth into the tube, which is connected to the PTR-TOF-MS-1000 device (IONICON PTR-TOF-MS - Trace VOC Analyzer, Eduard-Bodem-Gasse 3, 6020 Innsbruck, Austria (Europe)), for 1 minute.

2. After the procedure of exhaled air analysis in PTR TOF-1000, patients undergo a bicycle ergometry study, according to the Bruce protocol or the modified Bruce protocol). Then, immediately after stopping the load test, patients undergo a repeated study of exhaled air by the method of time-of-flight mass spectrometer with ionization by the proton transfer method for 1 minute, and after 3 minutes a repeated analysis of exhaled air is carried out, also for 1 minute. The obtained exhaled breath data will be analyzed using machine learning models.

3. On the morning of study enrollment, a single 10 mL venous blood sample will be collected via peripheral venipuncture using vacuum biochemical tubes from all participants. The samples will undergo centrifugation at 2000 rpm for 20 minutes to separate plasma, after which the plasma fraction was aliquoted into seven sterile tubes using calibrated pipetting systems. Each tube will be labeled with the participant's unique registration number and sample collection date, then sealed within individual bags displaying duplicated identification data externally. Samples were cryopreserved at -82 to -84°C in ultra-low temperature freezers for long-term storage. Following complete blood collection across the cohort, frozen plasma specimens will be transferred to the laboratory for quantification of total cholesterol (TC), low-density lipoprotein (LDL), very low-density lipoprotein (VLDL), high-density lipoprotein (HDL), triglycerides (TG), lipoprotein(a), apolipoprotein B (ApoB), C-reactive protein (CRP), and interleukin-6 (IL-6).

4. Before and immediately after the exercise test, all patients are scheduled to have a single-channel ECG and pulse wave recorded using a portable single-channel recorder (Cardio-Qvark) (Russia, Moscow). Single lead ECG with pulse wave parameters will be analyzed using machine learning models.

5. All participants will undergo CTP with vasodilation test.

5. The object of the study and the planned number of observations, with justification for the selection of patients.

The object of the study is 80 human.

Inclusion Criteria:

1. Age ≥40 years;
2. Absence of acute exacerbations of psychiatric disorders or cognitive impairments that would preclude study participation;
3. Provision of written informed consent for study participation, blood sample collection, and anonymous publication of research results;
4. Pre-test probability of ischemic heart disease between 1% and 33%.

Non-inclusion criteria:

1. Pregnancy and breastfeeding;
2. Diabetes mellitus;
3. Presence of acute myocardial ischemia (acute coronary syndrome or myocardial infarction within the preceding 48 hours) or a history of myocardial infarction;
4. Active infectious or non-infectious inflammatory diseases in the acute/exacerbation phase;

5. Connective tissue diseases (regardless of disease activity);

6. Respiratory disorders (e.g., bronchial asthma, chronic bronchitis, cystic fibrosis, or other conditions associated with significant respiratory dysfunction);

7. Acute pulmonary thromboembolism involving the pulmonary artery or its branches;

8. Aortic dissection;

9. Hemodynamically significant decompensated cardiac valvular defects;

10. Active malignancy;

11. Decompensated chronic heart failure (NYHA class III-IV) or acute heart failure;

12. Neurological disorders (e.g., Parkinson's disease, multiple sclerosis, acute psychosis, Guillain-Barré syndrome);

13. Cardiac arrhythmias or conduction abnormalities contraindicating stress testing;

14. Musculoskeletal disorders precluding exercise testing (e.g., bicycle ergometry);

15. Allergy to radiocontrast agents and/or adenosine triphosphate (ATP);

16. Chronic kidney disease with an estimated glomerular filtration rate (eGFR) <30 mL/min/1.73 m² (CKD-EPI formula);

17. Severe hepatic insufficiency and/or Child-Pugh class B or C liver cirrhosis.

Exclusion Criteria:

1. Poor recording quality of single-channel electrocardiogram (ECG) and/or plethysmography data;

2. Failure to complete the stress test due to reasons unrelated to cardiac conditions;

3. Voluntary withdrawal of consent to continue participation in the study;

4. Post-enrollment development of conditions or identification of pathologies listed in the exclusion criteria.

6. **Evaluated outcomes (primary endpoints).**

The results in the primary are subjective and subject to the objectivity of the study.

1. Assessing the diagnostic accuracy of the stress electrocardiography in the diagnosis of ischemic heart disease (AUC, Sensitivity, Specificity, Sensitivity, NPV, and PPV)

2. Analyze the volatile organic compounds of the exhaled breath in individuals with stress-induced myocardial perfusion defect on stress computed tomography myocardial perfusion imaging (CTP) with vasodilation test (adenosine triphosphate) and compare them with individuals without stress-induced myocardial perfusion defect after a physical stress test, and compare them with rest results as independent variables. Machine learning model will be used to assess the diagnostic accuracy of the exhaled breath in the diagnosis of ischemic heart disease

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7. **Main stages of the research.**

1. Search and recruitment of patients to participate in the study in the departments of the Clinical Center of Sechenov University.
2. Conducting instrumental examinations (analysis of exhaled air (time-of-flight mass spectrometer with proton transfer ionization and glow discharge emission spectrometry), sphygmometry, single-channel ECG recording, bicycle ergometry, CT of coronary arteries with contrast) .
3. Preparation of the obtained material for subsequent statistical processing. Statistical analysis of the obtained data.
4. Writing publications

8. **Methods of the planned study (method of statistical processing of results).**

The study plans to use statistical methods, including descriptive statistics, student test, chi-square test, ANOVA test and Pearson correlation test. Statistical processing of the obtained data materials will be carried out using the Statistica 12 program (StatSoft, Inc. (2014). STATISTICA (data analysis software system), version 12. www.statsoft.com).

9. **Expected research result.**

To create an optimal multifactorial model that determines the optimal examination of ischemic heart disease patients at rest and at peak physical exertion test. Additionally, to improve the diagnostic accuracy of the standard stress ECG test in detecting coronary insufficiency.

10. **Calendar deadlines for completing the work. The work is supposed to be completed in accordance with the individual plan that was approved and agreed upon.**

1. October 2023 – June 2024 (Patient recruitment)
2. June 2024 – September 2024 (Statistical analysis of results)
3. September 2024 – June 2025 (Interpretation of statistical results, writing publications)
4. June 2025 – September 2025 (Dissertation writing)

11. References

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