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Dear colleagues,

Yury I. Grinshtein

Igor S. Yavelov

We would like to bring to your attention the issue of the Russian Journal of Cardiology, dedicated to lipid metabolism disorders, atherosclerosis and related cardiovascular diseases. This is one of the most actively developing areas of modern cardiology, as evidenced by the presented publications.

In two detailed works by Alieva A. S. et al. and Ezhova M. V. et al., performed under the guidance of chief cardiologists of the Russian Ministry of Health Shlyakhto E. V. and Boytsov S. A., experience of successful functioning of lipid centers in Russia and prospects for developing novel models optimizing the healthcare for patients with lipid metabolism disorders at the population level are discussed, including diagnosis and treatment of familial hypercholesterolemia.

The section Original Articles describes fundamental issues of atherosclerosis, stable coronary artery disease (CAD), and heart failure (HF). In the study by Zhatkina M. V. et al., prognostic value of various biomarkers in non-invasive CAD diagnosis is being studied. Osyaev N. Yu. et al. showed specifics of calcification and angiogenesis in atherosclerotic plaques of extracranial arteries, which specifies their instability and risk of stroke. The paper by Nadzhafov R. N. describes the relationship between vascular age and atherosclerosis-related cardiovascular diseases.

Two works are devoted to relevant issues of antithrombotic therapy. Komarov A. L. et al. presented the results of observational register of long-term antithrombotic therapy REGATTA-1. The authors identified predictors of upper gastrointestinal bleeding and optimized the bleeding risk score in patients with CAD receiving long-term antiplatelet therapy, which is important for clinical practice. In the article by Goncharov M. D. et al., the molecular and metabolic characteristics of changes in the platelet sensitivity to antiplatelet therapy in patients with CAD are discussed.

Kazantsev A. N. et al. analyzed the outcomes of carotid endarterectomy (CE) in the acute phase of ischemic stroke (IS), obtained within a multicenter study.

We draw the readers' attention to works devoted to HF. Using modeling, Drapkina O. M. et al. estimated HF-related socio-economic impact n Russia. Soldatova A. M. et al. offer a comprehensive model of personalized selection of patients with HF for cardiac resynchronization therapy. In the article by Lyasnikova E. A. et al., modification of the algorithm for diagnosing heart failure with mid-range ejection fraction and focusing on personalized echocardiographic data, taking into account obesity and indexing threshold values of natriuretic peptides in patients with a body mass index $\geqslant$30 kg/m^2, are discussed. Osokina A. V. et al. discuss the role of fibrosis biomarkers (PICP, PIIINP, galectin-3) in subacute period of myocardial infarction with preserved ejection fraction for predicting and detecting postinfarction diastolic dysfunction.

In the section Clinical Findings and Pharmacotherapy, personalized approaches to trimetazidine prescription in CAD and the role of physiotherapy in treating uncontrolled hypertension are discussed. Attention is drawn to reviews devoted to C-reactive protein and decision-making system in lipid metabolism disorders.

We are confident that these materials will be useful to a wide range of medical practitioners, scientists and public health professionals.

Yuri I. Grinstein, Doctor of Medical Science, Professor
Igor S. Yavelov, Doctor of Medical Science

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BEST PRACTICES IN THE ORGANIZATION OF CARDIOLOGICAL CARE
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Implemented models and perspectives of managing lipid metabolism disorders. Concept of rare lipid disease centers

Alieva A. S.[1], Reutova O. V.[1], Pavlyuk E. I.[1], Duplyakov D. V.[2,9], Khripun A. V.[3], Efimova I. P.[1], Guryanova Yu. A.[10], Timoshchenko E. S.[4], Nekrasov A. A.[4], Namitokov A. M.[5,7], Zafiraki V. K.[6,7], Kosmacheva E. D.[5,6], Korneva V. A.[8], Vezikova N. N.[8], Skopets I. S.[8], Zvartau N. E.[1], Shlyakhto E. V.[1]

Despite the advances in lipidology over the past decade, the control of dyslipidemia at the population level in Russia, as in a number of European countries, remains unsatisfactory. The need for novel organizational approaches to solving the problem at the regional and federal levels is obvious. This publication provides an overview of the implemented projects and the successful practical experience of lipid centers in Russia, as well as the prospects for the development of novel models that will optimize the care provision for patients with lipid metabolism disorders at the population level.

Keywords: dyslipidemias, population-based strategy, lipid centers.

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Dyslipidemia is one of the key modifiable risk factors, delayed detection of which leads to an increase in cardiovascular morbidity and mortality. Despite the evidence of key role of lipid metabolism disorders in the development of atherosclerotic cardiovascular diseases (CVD) and the common position of European and Russian guidelines on the need to lower the low-density lipoprotein (LDL) cholesterol levels, the study results demonstrate insufficiently effective control of dyslipidemia at the population level. Thus, the DA VINCI study, which assessed the effectiveness of both primary and secondary CVD prevention in more than 18 European countries, demonstrated a significant gap between the current clinical guidelines (2019) and actual clinical practice — on average, only 33% of patients achieved target LDL values. Among patients with very high cardiovascular risk (CVR) while receiving lipid-lowering therapy, only 17% and 22% of patients achieved LDL values of <1,4 mmol/L within primary and secondary prevention, respectively [1].

There are following possible problems leading to inadequate control of dyslipidemia: 1) inaccurate stratification of CVR, mainly within primary prevention; 2) no continuity in the management of patients with lipid metabolism disorders; 3) low adherence of patients to lipid-lowering therapy; 4) limited availability of apheresis and subsidized pharmaceutical provision programs for the three main lines of lipid-lowering drugs, especially within primary prevention.

Patients with lipid metabolism disorders represent an extremely heterogeneous group, for the management of which competent risk stratification is required. Over the past decades, serious advances have been made in the treatment of patients with high and very high CVR due to the introduction of high-tech medical care and secondary prevention measures. Nevertheless, according to Rose's prevention paradox, at the population level, most cardiovascular events occur in patients with moderate and low risk. In this regard, working with these cohorts of patients has the greatest potential for reaching long-term targets. All this emphasizes the need to improve the system for managing lipid metabolism disorders with the development of criteria for referral to lipid centers.

Management of care for patients with familial hypercholesterolemia

Within the primary prevention, the greatest difficulty is the identification of patients with a heterozygous familial hypercholesterolemia (FH). Despite the progress achieved in genetic testing in addition to novel treatment technologies, the prevalence of detection and adequate management of patients with FH remains low, especially given the prevalence of heterozygous FH (1:200-1:250) in the population [2]. The development of nationwide clinical and genetic screening programs contributes to early detection and adequate management through assessment of cascade screening data, lifestyle changes, and timely pharmacological intervention. So, created in 1994 in the Netherlands, and in 2009 in Italy, the National Screening Program for FH have demonstrated effectiveness in CVR management in this category of patients [3, 4].

Since 2015, the project of the European Atherosclerosis Society (EAS) EAS FH Studies Collaboration has been successfully implementing, the purpose of which was to identify and treat patients with FH [5]. The Russian National Atherosclerosis Society participates in this initiative, as a part of which the register RENESSANS was maintained. The maintenance of such a register made it possible to draw attention to this problem and significantly increase the proportion of patients with FH. Currently, the EAS registry numbers >62 thousand patients from 62 countries, among which 41 thousand patients are persons with a definite or probable FH according to clinical and/or genetic criteria. Up-to-date information on the register is available on EAS website: https://www.eas-society.org/.

One of the key issues concerning patients with FH in Russia is the possibility of providing expensive classes of lipid-lowering drugs and apheresis. It should be noted that most of them receive therapy as part of the primary cardiovascular prevention. In this regard, the Almazov National Medical Research Center, with the support of Russian Society of Cardiology (RSC), initiated a register creation for patients with suspected FH (Dutch Lipid Clinic Network score 6 or more) [6], within which genetic testing is provided. This register, covering a number of Russian regions, is considered as a possible tool for choosing patients for subsidized pharmaceutical provision with lipid-lowering therapy, including based on positive genetic testing results.

Practical aspects of managing care for patients with lipid metabolism disorders. EAS and RSC project — Lipid Clinics Network

EAS initiated the **Lipid Clinics Network project**, the aim of which was to introduce common European standards for the diagnosis and treatment of lipid metabolism disorders, as well as to ensure the continuity of managing these patients at the local, regional and federal levels. In accordance with this, a possible organization chart of lipid service has been developed and a mechanism of bidirectional interaction at the regional and local levels has been worked out (Figure 1). Federal and regional centers carry out clinical, research and educational functions with a different competency.

Figure 1. Organization chart of the lipid service.
Abbreviation: HTHC — high-tech healthcare.

There are following criteria for referral to regional and federal lipid centers:

• severe hypercholesterolemia (total cholesterol level >8,0 mmol/L and/or LDL >5,0 mmol/L, and/or triglycerides >10 mmol/L), requiring determination of optimal patient management (indications for high-dose and/or combination therapy with lipid-lowering drugs, including monoclonal antibodies);

• cardiovascular events/CVDs under the age of 50, including revascularization due to atherosclerosis (timely, aggressive secondary prevention is required);

• difficulties in selecting lipid-lowering therapy (intolerance, side effects, concomitant diseases).

At the same time, it is necessary to differentiate the groups of patients needing consultation in lipid centers to optimize diagnosis and treatment, and the dynamic follow-up groups, which, due to the severity of disease course, require observation within a specialized center. Such separation of patients will provide the timely in-depth examination without an unreasonable increase in the number of lipid centers.

To introduce European standards into actual clinical practice, together with key European atherosclerosis experts, work is performed to develop an algorithm to create a decision support system that will be used for assessing the risk and determining management tactics in accordance with the latest guidelines on lipid metabolism disorders.

It is necessary to conduct specialized training events for therapists, cardiologists, endocrinologists and dermatologists, as well as active telemedicine consultations in difficult cases of lipid metabolism disorders, including with the involvement of international experts. So, in February 2020, on the basis of Almazov National Medical Research Center, the first online consultation with professor A. L. Katapano was held. Three participating regions brought up difficult clinical cases for discussion, during which a council of experts adjusted the management tactics for 6 patients with severe combined genetic dyslipidemias.

Currently, a mechanism of bidirectional interaction the Almazov National Medical Research Center with regional lipid centers in the Rostov and Samara Oblasts, the Chuvash Republic and Nizhny Novgorod has been worked out with provision of genetic testing for patients with suspected severe hereditary lipid metabolism disorders with increased LDL levels and hypertriglyceridemia, hyperlipoproteinemia (a).

Successful experience of regional lipid centers

Republic of Karelia

In order to identify patients with severe dyslipidemia and the selection of personalized lipid-lowering therapy in the Republic of Karelia, a Lipid Center was created on the basis of Laboratory of Clinical Epidemiology of Petrozavodsk State University Medical Institute.

There are following tasks of the lipid center:

• Provision of qualified consultative, diagnostic, therapeutic and prophylactic care for outpatients with severe lipid metabolism disorders and/or early aggressive atherosclerosis, and/or intolerance to lipid-lowering drugs;

• Standardization of treatment and diagnostic approaches in the management of cardiovascular patients with atherosclerosis and patients with high and very high risk of their development, including the introduction of novel methods of examination and treatment into practice;

• Conducting research and educational events dedicated to diagnostics, effective methods of primary and secondary prevention and invasive treatment of dyslipidemia;

• Development of guidelines on creating education programs for patients with dyslipidemia and their relatives;

• Determination of indications for high-tech treatment methods (proprotein convertase subtilisin/kexin type 9 (PCSK9) inhibitors), follow-up of patients receiving this therapy;

• Selection of patients with severe dyslipidemia for referral to specialized federal lipid centers for consulting on treatment and rehabilitation.

The following indications for referring patients to a consultation in Lipid Center have been determined:

• Severe dyslipidemia before starting lipid-lowering therapy (total cholesterol >7,8 mmol, LDL >4,9 mmol/L) after ruling out secondary causes of hyperlipidemia;

• Severe hypertriglyceridemia (>10 mmol/L), resistant to therapy;

• Tendons xanthomatosis at any age, arcus senilis in persons under 45;

• Early onset of coronary artery disease (CAD) or stroke (up to 40 years);

• Patients resistant to statin therapy, with statin intolerance, and those who did not achieve the target lipid profile levels with standard lipid-lowering therapy.

Funding of subsidized pharmaceutical provision for patients in need of PCSK9 inhibitor therapy is carried out within the departmental special-purpose program approved by the order of the Ministry of Health of the Republic of Karelia dated December 24, 2015 № 2504 "Prevention of disability of the population of the Republic of Karelia". The program is reviewed annually — new subprograms are added and the drug list of is updated.

Through the subprograms "Cardiac rehabilitation" and "Individual measures aimed at providing people with drugs for life threatening chronic diseases (conditions)", PCSK9 inhibitors are purchased for patients with FH and aggressive atherosclerosis.

During the Lipid Center's work, a register of FH patients was created (n=277; mean age, 48 years). The prevalence of FH in Karelia was determined (1:300). Patients are genotyped and new mutations determine the FH development have been identified. In addition, selection and management of patients receiving PSCK9 inhibitor therapy is underway. Currently, 31 patients (men, 61%; age, 39-74) receive treatment with drugs of this group: 15 people — alirocumab, 16 people — evolocumab. A protocol for patient management using PCSK9 inhibitors was developed; reminders for primary care physicians and for patients receiving these drugs were created.

Krasnodar Krai

In the Krasnodar Krai, two lipid offices have been created — on the basis of Research Institute (RI) of Regional Clinical Hospital (RCH) № 1 and RCH № 2, supporting with the Therapy Department № 1 of the Kuban State Medical University.

The lipid office on the basis of RI-RCH № 1 began to work in March 2017, where patients with suspected FH, as well as patients with very high CVR, are referred for consultative and diagnostic assistance.

In 2019, an office for patients with lipid metabolism disorders began working on the basis of RCH № 2. The office functions as a subdivision of outpatient cardiology department of the ambulatory clinic for specialized course outpatient treatment of RCH № 2. Organized in 1989 as an experimental project, for more than 30 years, this polyclinic provides high-quality outpatient care for patients in the Krasnodar city and Krasnodar Krai, working in conjunction with a diagnostic center. Its peculiarity is the possibility of conducting not only one-time consultations, but also performing diagnostic and therapeutic interventions within repeated visits of patients, during which a diagnostic search and selection/adjustment of therapy is carried out.

Since both hospitals are the main medical institutions of the Krasnodar Krai, patients in lipid offices receive comprehensive laboratory examination and vascular screening — from non-invasive techniques (ultrasound, exercise stress tests) to computed tomography angiography and coronary angiography. If necessary, hospitalization in specialized departments for surgery is possible. The organization of lipid rooms based on hospitals with a powerful diagnostic potential and the availability of X-ray endovascular diagnostics is the best solution.

Indications for referral to lipid office are:

• Total cholesterol >8 mmol/L;

• LDL cholesterol >4,9 mmol/L;

• Lipoprotein (a) >30 mg/dL;

• Triglycerides >10 mmol/L;

• Failure to achieve target LDL-C levels using highest tolerated doses of statins;

• Combination of atherosclerotic CVD with diabetes, as well as the rapid progression of atherosclerosis, despite therapy with highest tolerated doses of lipid-lowering drugs.

Participation in the all-Russian register allows to create a database of patients with FH, which makes it easier to enroll patients in regional and federal programs of subsidized pharmaceutical provision (PCSK9 inhibitors). Another function of these offices is to select patients with severe hypercholesterolemia to participate in international clinical trials with promising lipid-lowering molecules. Specialists of lipid offices are also employees of the Therapy Department of the Kuban State Medical University and carry out educational activities among residents and cardiologists of the Krasnodar Krai.

Nizhny Novgorod Oblast

Taking into account the great urgency of cardiovascular morbidity, in March 2019, a regional lipid center was created on the basis of Nizhny Novgorod City Clinical Hospital № 5, according to the order of the Ministry of Health of Nizhny Novgorod Oblast № 315-131/19P/od dated March 1, 2019.

There are following indications for referral to the lipid center: hyperlipidemia (total cholesterol >7,5 mmol/L, or LDL >4,9 mmol/L, or triglycerides >10 mmol/L), requiring a high-dose and/or combination therapy with lipid-lowering drugs; early CVDs, including revascularization (<55 years of age), requiring aggressive secondary prevention; suspected intolerance to lipid-lowering therapy due to side effects or its insufficient effectiveness; patients of difficult clinical situations to consider a lipid-lowering therapy.

Patients are referred to the lipid center with above indications from outpatient clinics: from primary prevention offices, from general practitioners, cardiologists, endocrinologists. Also, the patients are referred to the lipid center by internal selection (counseling service of city cardiology dispensary specialists, as well as among patients discharged from the primary vascular centers and cardiology departments of Nizhny Novgorod CCH № 5, who, if indicated, are referred by hospital-based physician) and self-referral. Today, the referral ways to the lipid center are now distributed as follows: internal selection — 78,1%, external referral — 15,1%, self-referral — 6,8%.

Due to the small proportion of patients referred by external medical centers, one of the priority areas of the lipid center today is the widespread dissemination of information on its functioning and specifics of referring patients with lipid metabolism disorders. During 2020, 372 patients were consulted (small number of consultations are associated with the COVID-19 pandemic and related restrictions). More than 40 patients with FH and 1 patient with severe familial hypertriglyceridemia are followed up. Currently, 73 patients with LDL >4,9 mmol/L are regularly followed up in the regional lipid center, of which 38 (52,1%) receive PCSK9 inhibitor therapy. The main funding sources to receive PCSK9 inhibitors are compulsory medical insurance funds (resource of diagnostic related groups) (n=20; 52,6%) and federal benefits (n=18; 47,4%).

All patients have prior CVD, including CAD (86,3%) and/or peripheral arterial atherosclerotic diseases (45,2%). In more than half of the patients, CAD developed at a young age (women, <60; men, 55). Most patients had prior recurrent cardiovascular events and a history of revascularization. As a result, all followed up patients have very high or extremely high CVR (63,1% and 36,9%, respectively). At the same time, in the subgroup of patients receiving PCSK9 inhibitors, the proportion of individuals with extremely high is naturally higher and amounts to 55,3%.

A significant proportion of patients in the lipid center have a hereditary predisposition to lipid metabolism disorders. Sixteen (21,9%) patients had relatives with a certain FH. More than one third of patients (35,6%) had a family history of CVD. According to the Dutch Lipid Clinic Network (DLCN), the FH was established in 26 (35,6%) patients, probable — 15 (20,5%), possible — 14 (19,1%) patients. As a result, 55 patients out of 73 (75,3%), with varying degrees of probability, could have FH. The limited genetic testing potential makes it difficult to make an accurate diagnosis and makes it difficult to predict treatment strategy. Treatment of patients with PCSK9 inhibitors showed high efficiency in achieving target LDL levels (although it was used in the most severe patients, among whom more than half had extremely high CVR). During treatment, the LDL decreased to ⩽1,0 mmol/l in 23 (60,5%) patients, ⩽1,4 mmol/l in 27 (71,1%), ⩽1,8 mmol/l in 33 (86,8%).

Perspectives

The creation of a network of lipid centers will make it possible to effectively implement the strategy for managing lipid metabolism disorders both in high-risk groups and at the population level. The introduction of uniform standards for managing dyslipidemia will ensure a high-quality care for each patient, and the network structure will ensure the availability of consultations by leading specialists in difficult cases. The ideology of this project implies a further expansion of the structure and its openness; all data on joining the project is available on the RSC website (available at: https://scardio.ru/proekty/sovmestnye_proekty_s_amdzhen/proekt_evropeyskogo_obschestva_po_aterosklerozu__lipid_clinics_network/).

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References

1. Ray KK, Molemans B, Schoonen WM, et al., 2020. EU-wide cross-sectional observational study of lipid-modifying therapy use in secondary and primary care: the DA VINCI study. European Journal of Preventive Cardiology. 2020;zwaa047. doi:10.1093/eurjpc/zwaa047.
2. Yezhov MV, Bliznyuk SA, Tmoyan NA, et al. Register of patients with familial hypercholesterolemia and patients of very high cardiovascular risk with lipid-lowering therapy underperformance (RENESSANS). Russian Journal of Cardiology. 2019;24(5):7-13. (In Russ.) doi:10.15829/1560-4071-2019-5-7-13.
3. Besseling J, Reitsma JB, Gaudet D, et al. Selection of individuals for genetic testing for familial hypercholesterolaemia: development and external validation of a prediction model for the presence of a mutation causing familial hypercholesterolaemia. Eur Heart J. 2017;38(8):565-73. doi:10.1093/eurheartj/ehw135.
4. Averna M, Cefalù AB, Casula M, et al. LIPIGEN Group. Familial hypercholesterolemia: The Italian Atherosclerosis Society Network (LIPIGEN). Atheroscler Suppl. 2017;29:11-6. doi:10.1016/j.atherosclerosissup.2017.07.001.
5. Overview of the current status of familial hypercholesterolaemia care in over 60 countries — The EAS Familial Hypercholesterolaemia Studies Collaboration (FHSC). Atherosclerosis. 2018;277:234-55. doi:10.1016/j.atherosclerosis.2018.08.051.
6. Goldberg AC, Hopkins PN, Toth PP, et al. National Lipid Association Expert Panel on Familial Hypercholesterolemia. Familial hypercholesterolemia: screening, diagnosis and management of pediatric and adultpatients: clinical guidance from the National Lipid Association Expert Panel on Familial Hypercholesterolemia. J Clin Lipidol. 2011;5(3 Suppl):S1-8. doi:10.1016/j.jacl.2011.04.003.

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Organization of lipid centers in the Russian Federation — new potential

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Project of the Russian National Atherosclerosis Society

In 2016, Guidelines on the medical care organization to the patients with hereditary atherogenic lipid disorders in the regions of Russia were published, which described and presented the principles of routing patients with hereditary dyslipidemia and the organization of medical care for them within the current regulatory documents. In December 2018, the Russian Ministry of Health approved clinical guidelines for the diagnosis and treatment of familial hypercholesterolemia. Thus, persons with a severe hereditary dyslipidemia were able to get free medication with expensive lipid-lowering drugs and receive apheresis. Following the European ones, the Russian guidelines on the management of lipid metabolism disorders were updated: lower target low density lipoprotein cholesterol levels were adopted. In the Russian population, there is a high prevalence of hypercholesterolemia, including familial monogenic and polygenic types. Therefore, timely detection and routing to a lipid center or an office to a specialist (cardiologist, lipidologist), adequate and modern prescription of lipid-lowering therapy will make an important contribution not only to secondary, but also to primary prevention of atherosclerotic cardiovascular complications.

Keywords: lipid center, familial hypercholesterolemia, dyslipidemia, atherosclerosis, prevention.

Relationships and Activities: none.

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In 2016, "Methodical recommendations on the organization of medical care for patients with hereditary atherogenic disorders of lipid metabolism in the subjects of the Russian Federation" were published, which described the principles of routing and managing patients with hereditary dyslipidemia, the qualification requirements of a physician, payment methods within the current regulatory documents [1]. Over the past 5 years, certain successes have been achieved in the treatment of patients with dyslipidemia. In December 2018, the Russian Ministry of Health approved clinical guidelines on familial hypercholesterolemia (FH) [2]: persons with severe hereditary dyslipidemia can be included in the drug benefit program receiving expensive lipid-lowering drugs and apheresis. Following the European ones [3], the Russian guidelines on management of lipid metabolism disorders [4] were updated, and with them new, lower, target levels of low density lipoprotein cholesterol (LDL-C) were adopted. In the Russian population, there is a high prevalence of hypercholesterolemia, including familial monogenic and polygenic types [5-7]. Therefore, timely detection, correct routing to a lipid center or an office to cardiologist or lipidologist, adequate prescription of lipid-lowering therapy will significantly contribute not only to the secondary, but also to the primary prevention of atherosclerotic cardiovascular events (CVE).

Lipid centers and a program for lipid metabolism disorders' treatment through specialized structures, including lipid centers, is an extremely important part of a high-risk prevention strategy implementation. As part of the adult outpatient screening in Russia in accordance with the orders of the Russian Ministry of Health № 124n dated March 3, 2019 and № 173n dated March 29, 2019, persons with a total cholesterol (TC) >8 mmol/l should be followed up, while hereditary dyslipidemia should be excluded or confirmed and the target LDL-C level should be achieved [2, 8, 9]. Thus, now there are new tasks for lipid centers — not only the identification of persons with lipid metabolism disorders, including with hetero- and homozygous FH, and initiation and maintenance of statin therapy, but also their high-dose use, the introduction of novel lipid-lowering medication classes, including in combination with statins, as well as lipoprotein apheresis, allowing to achieve target LDL-C levels in most patients.

Creation of a lipid clinic network

The European Atherosclerosis Society Familial Hypercholesterolaemia Studies Collaboration (EAS-FHSC) initiative can be used as a platform for developing a network of lipid centers in Europe, including the Russian Federation. The FHSC is a pan-European database with over 62500 FH patients from various clinical centers, including 8500 children. The initiative involved 68 countries, including the Russian Federation [10]. Joint work is underway with decision-making structures such as the World Heart Federation and the World Health Organization. In Europe, much attention is paid to hypercholesterolemia, including its family forms. EAS-FHSC believes that the global burden of FH can be reduced by creating effective interaction between countries on all continents. It is necessary to create an international standardized register of patients with FH and a unified digital platform for data exchange, harmonization and analysis. All interested researchers should be able to access this data. Politicians should keep abreast of what is happening and support research. The FHSC plans to provide insights and recommendations to patients and patient organizations. After conducting training programs, it is planned to evaluate their effectiveness and create conditions for the exchange of advanced developments with each other.

Lipid clinic network creates a platform for developing unified European standards on dyslipidemia. Data from various sources are analyzed and remote virtual consultations are carried out. Each participating center has access to the EAS-FHSC network, which makes it possible to discuss the results and analyze clinical cases. Individual consultations are possible.

Organization of lipid centers in Russia

The development of a network of lipid centers in Russia is extremely relevant due to high cardiovascular mortality and insufficient effectiveness of lipid-lowering therapy, especially in refractory dyslipidemias. Severe lipid metabolism disorders are often diagnosed at late stages. In fact, there are no system for their preventive detection and counseling service for statin intolerance. The regularity and effectiveness of statin therapy is poor. According to the RECORD-3 register study in 2015, before hospitalization for acute coronary syndrome, only 19% of patients took statins, and 34% — after rehospitalization [11]. These are extremely low values, although they have slightly improved in comparison with previous RECORD registers [12, 13]. Only the rate of in-hospital statin therapy was at high level — 89,6% [11].

The second major problem is the failure of Russian patients with very high cardiovascular risk (CVR) to achieve target LDL-C levels. According to the DYSIS study, this parameter is only 12% [14].

According to the ESSE-RF study, a pronounced increase in LDL-C level >4,9 mmol/l is present in 7,7% of Russian adult population [6]. At the same time, with the same LDL-C level, the presence of FH-related mutations increases the CVE risk

several times [15]. Monogenic hypercholesterolemia can also differ in CVRSo, carriers of low density lipoprotein receptor (*LDLR*) gene mutations have the highest CVR, while carriers of apolipoprotein B-100 (*ApoB*) gene mutations — an intermediate, compared with the general population [16].

In Russia, the prevalence of heterozygous FH is 1:173 (95% confidence interval: 1:208-1:145) [2]. When recalculated, it turns out that in Russia there are more than 840 thousand patients with FH, not counting patients with other hereditary atherogenic dyslipidemias. In order to cover this entire contingent, a system for the identification and routing of patients with FH and other atherogenic dyslipidemias is needed, in which the participation of healthcare facilities at all levels is expected, with the obligatory involvement of both medical institutions conducting outpatients screening or providing cardiac care, and specialized lipid centers.

On December 11, 2014, the expert council on FH developed proposals on creating a lipid center network within the all-Russian project, to determine their structure and function, and on September 21, 2015, the expert council of Russian National Atherosclerosis Society approved the creation of lipid centers. Then, a working group and pilot regions have been identified, and the organization of interaction with authorities has begun. In 2016, guidelines were published on health care organization for patients with hereditary atherogenic lipid metabolism disorders in Russian regions, as well as for persons with severe lipid metabolism disorders without a confirmed hereditary disorder and for those with statin intolerance [1]. Over the past years, a lot of work has been done and significant changes have occurred, but many points of these guidelines are still relevant now. At the same time, after 5 years, the procedural framework of lipid centers' operation should be updated.

To date, ~20 lipid centers have been organized and operate, which is extremely small, taking into account the current statistics on lipid metabolism dispensers in Russia.

Role of the Russian register

The tasks of lipid centers include the analysis and adjustment of routine prescription of lipid-lowering therapy, especially in high and very high-risk groups, maintaining local registers of patients with severe lipid metabolism disorders, evaluating the effectiveness of lipid-lowering therapy optimization, cascade screening, entering data into the all-Russian register (RENESSANS).

The RENESSANS register was initiated to obtain consolidated information on the clinical characteristics, approaches to diagnosis and treatment of patients with severe lipid metabolism disorders, many of whom have a high or very high risk of CVE. Its purpose is to take into account and correctly manage patients not only with FH, but also with refractoriness to lipid-lowering therapy, including its intolerance. This project has a multicenter observational non-interventional design. Data is collected continuously: medical centers included in the register constantly enter information on patients into an original electronic system, where the data is converted into a depersonalized table. Centralized data upload and analysis is performed twice a year. First- and second-degree relatives are included in the cascade screening for FH.

The majority of patients are women (60%). The mean age is 54 years (mean age at diagnosis, 45 years). To date, there are more than 1700 people in the register, 10 of whom suffer from homozygous FH. More than 600 patients are classified as very high CVR. Most (>1100) patients with FH receive statin agents. Proprotein convertase subtilisin/kexin type 9 inhibitors (PCSK9i) are taken by only 50 people so far [17]. Unfavorable outcomes are associated with male sex (relative risk increases by 2 times), coronary artery disease (7 times), a burdened history (2 times), hypertension (3 times), lipoprotein(a) level (3 times). According to the register data, the target LDL-C level can be achieved in 2% of patients using treatment, which is 10 times more than at 1 visit to the lipid center [17].

Difficulties and problems of lipid centers operation in 2016-2020

Lipid centers (offices) can be created as structural or functional units based on medical facilities of various profiles. A lipid center can be either a separate structural unit with its own position and staff, or a functional specialized medical appointment of a cardiologist or a general physician who studied a program in lipidology (36 hours) within the continuing medical education. It is the last option that seems to be optimal, since it does not require additional approvals with the Federal Compulsory Medical Insurance Fund, and all consultative and therapeutic activities provided by a lipidologist are carried out within agreed outpatient tariff rates.

An example of the effective work of lipid center as a functional unit is a specialized medical appointment of a cardiologist-lipidologist, deployed in September 2016 based on cardiology outpatient clinic of the L. S. Barbarash Kemerovo Regional Clinical Cardiology Dispensary. Initially, researchers from the Research Institute for Complex Issues of Cardiovascular Diseases worked as lipidologists of this center, which made it possible to quickly and efficiently translate advanced evidence-based approaches to prescribing lipid-lowering therapy in high and very high-risk patients into actual clinical

practice. Currently, the regional register of patients with identified severe lipid metabolism disorders numbers >190 patients. For 2021, the territorial compulsory health insurance fund in Kuzbass agreed to receive 12-month treatment it PCSK9 inhibitors for 10 patients followed up in a lipid center.

Lipid centers should also provide organizational and methodological assistance to practical healthcare, help patients in obtaining regional and federal benefits when prescribing expensive therapy. With the participation of lipid centers, combination and expensive lipid-lowering therapy should become more accessible. The issue of the availability of free molecular genetic diagnostics in hereditary lipid metabolism disorders has also ripened, possibly within the compulsory health insurance system.

The study of lipid centers profile in Russia showed that most of them are regional or municipal. The main source of funding for them is the compulsory health insurance system, which ideally should provide, if necessary, expensive drugs, such as PCSK9 inhibitors.

Difficulties were found in the follow-up of patients: conducting vascular investigations and consultations of doctors of other specialties, insufficient awareness of doctors and patients, low adherence of patients to follow-up visits to lipid center, limitation of providing effective but expensive drugs. Additional legal and research support are needed, as well as new guidelines.

The 2020 clinical guidelines on chronic coronary syndromes [18], as well as non-ST [19] and ST [20] segment elevation acute coronary syndrome, state that if the target LDL-C level cannot be achieved with highest tolerated dose of statins in combination with ezetimibe, or in patients with intolerance to HMG-CoA reductase inhibitors, it is recommended to prescribe one of the PCSK9 inhibitors to prevent CVEs [2-4, 18-20]. If during therapy with HMG-CoA reductase inhibitors at maximum tolerated doses, the LDL-C level remains significantly increased (>2,5 mmol/L), adding PCSK9 inhibitors without prior ezetimibe use should be considered [19, 20]. The principles of using all three classes of lipid-lowering drugs are harmonized with European guidelines.

The central contingent of lipid center is high and very high-risk patients, including patients requiring additional therapy to control LDL-C. These include patients with atherosclerotic diseases in combination with diabetes, FH, with multiple vascular involvement, rapid progression of atherosclerosis (2 or more vascular events within 2 years) and who have not reached the target LDL_C values. One of the options for adjusting CVR is combination lipid-lowering therapy, including PCSK9 inhibitors [2-4], which reduce the CVE risk and improve the prognosis of patients [21]. Adherence to therapy is increased by confirming the hereditary nature of lipid metabolism disorders using genetic tests.

The lipid center determines the indications for high-tech treatment methods, including PCSK9 inhibitors, and, which is very important, manage the follow-up of these patients. A special protocol has been developed taking into account the monitoring of effectiveness and safety of treatment. Check lists for primary care physicians and patients have also been developed.

Children are the most important cohort for the primary prevention of lipid metabolism disorders. An increase in LDL-C is manifested from the very birth, but in Russia there is no system for its detection. The difficulty lies in the fact that most children (with the exception of children with homozygous FH) do not have such clinical manifestations of hypercholesterolemia as xanthomas, xanthelasmas, and arcus senilis. In addition, the study of cholesterol profile is not included in the list of investigations within screening follow-up of children. Another obstacle to correcting the lipid profile in children is resistance from pediatricians and parents. Most physicians prefer delaying the initiation of lipid-lowering therapy until the age of 18. This problem can be partially solved by lipid centers. It is necessary to introduce universal screening in children aged 7 to 11 years, or to carry out selective screening. Adults should refer children and grandchildren to lipid center or medical facilities with lipid office. Physicians and adult cardiologists should refer children and grandchildren of index patients to pediatric outpatient clinics and pediatric lipid centers. Universal screening is the examination of all children in population. In European countries, it is carried out in Slovenia in newborns and children aged 5 years and is confirmed in almost half of the cases by DNA testing [22]. To detect high cholesterol levels, the German Society of Pediatrics and Adolescent Medicine recommends testing for all children aged 5 years [23]. In the United States, universal screening of children is carried out at the age of 9-11 years, because selective screening, which conducted previously, was insufficiently effective [24]. Thus, the optimal age for screening is considered to be 9-11 years, since hormonal changes during puberty in children can reduce the LDL-C level. Currently, the introduction of universal screening in Russia is being discussed. Children with suspected heterozygous FH can be screened from the age of 5 [25]. In case of suspected homozygous FH, screening is carried out as early as possible [26], for example, during immunization at the age of 1-2 years.

New day-patient treatment options

An important milestone in providing health care to patients with high and very high CVR with hyperlipidemia is the possibility of day-patient treatment for lipoprotein apheresis (from 2018) or therapy with PCSK9 inhibitors (from 2021).

In November 2017, the Russian Ministry of Health and the Federal Compulsory Medical Insurance Fund for the first time included the diagnosis-related group "Treatment of hereditary atherogenic lipid metabolism disorders using apheresis in patients with ineffectiveness of basic therapy" in the Methodological Guidelines on methods of paying for medical care at the expense of compulsory medical insurance within day-patient cardiology treatment.

In 2019-2020, this diagnosis-related group was included in the tariff agreements of at least 28 Russian regions (Altai, Krasnodar, Krasnoyarsk, Arkhangelsk, Voronezh, Volgograd, Vologda, Ivanovo, Irkutsk, Kemerovo, Omsk, Penza, Rostov, Samara, Saratov, Sverdlovsk, Tver, Tomsk, Tula, Primorsky Krai, Jewish Autonomous Oblast, the republics of Kalmykia, Crimea, Mari El, Mordovia, the Kabardino-Balkarian Republic, the cities of St. Petersburg and Sevastopol). At the same time, treatment was organized only in St. Petersburg (once every 2 weeks) and Samara (once a month).

There is a precedent when a patient from Moscow has been traveling to St. Petersburg twice a month for more than one and a half years for lipoprotein apheresis. Payment is made in accordance with the Federal Compulsory Medical Insurance Fund Order dated May 8, 2009 № 97. That is, there is possibility of treating patients from other nearby regions.

According to payment methods for health care at the expense of compulsory medical insurance funds for 2021, the current diagnosis-related group ds36.004 "Treatment with the use of biopharmaceuticals and selective immunosuppressive agents" included diagnoses of pure hypercholesterolemia (E78.0) and mixed hyperlipidemia (E78.2). Hospitals with following profiles can treat patients with these methods: therapy, cardiology, cardiac surgery, or endocrinology. An important advantage of this diagnosis-related group is the patient's stay in the hospital for only 1 day (actually, several hours), which is sufficient for subcutaneous administration of PCSK9 inhibitor. The cost of a completed case in most regions and Federal medical facilities is sufficient to cover medicines and other costs of institutions. The conditions for paying for hospitalizations for this diagnosis-related group are the presence of drug in Essential Medicines List and the corresponding indication according to product instruction and clinical guidelines. These conditions are met in 2021, since PCSK9 inhibitors are included in Essential Medicines List and in the Clinical guidelines on "Diagnostics and correction of lipid metabolism disorders in order to prevent and treat of atherosclerosis, VII revision" [4] and "Familial hypercholesterolemia" [2].

New opportunities for Federal healthcare organizations appear in 2021 in connection with the order of Russian Ministry of Health dated December 23, 2020 № 1363n.

Improving patient routing

The main challenges today are late diagnosis of lipid metabolism disorders (in case of FH, this is of decisive importance and can shorten a full-quality life to 20 years), inadequate lipid-lowering therapy (patient inaction, prescribing inadequately low statin doses, insufficient combination therapy use), and as a consequence — failure to achieve target LDL-C values and an increase in CVE risk. It is necessary to oblige all laboratories to send a patient to lipid centers or offices if total cholesterol >8,0 mmol/L is detected. The importance of cholesterol monitoring must be equated with blood glucose monitoring.

Criteria for referring a patient to a lipid center/office:

To rule out/confirm hereditary dyslipidemia, at least one criterion must be met:

1. Serum (plasma) total cholesterol >10 mmol/L and/or serum (plasma) LDL-C >8,5 mmol/L and/or serum (plasma) triglycerides >11 mmol/L.
2. Serum (plasma) total cholesterol >8,0 mmol/L and/or serum (plasma) LDL-C >5,0 mmol/L and/or triglyceride level >5,0 mmol/L and/or lipoprotein (a) level >50 mg/dl in combination with a positive family history of early (up to 55 years in men, up to 60 years in women) atherosclerotic cardiovascular disease.
3. Ineffectiveness (lowering LDL-C by less than 30%) of drug lipid-lowering therapy in highest tolerated doses for at least 3 months, including due to intolerance.
4. Prior early (up to 40 years) atherosclerotic cardiovascular disease.
5. All first-degree relatives (parents, children, siblings) of a patient with hereditary dyslipidemia.

Conclusion

There are following necessary steps to solve the problem of early detection and treatment of patients with hereditary dyslipidemias: creation of system for routing patients with severe dyslipidemia, where all levels of healthcare should be involved, with the obligatory involvement of both facilities conducting screening or providing cardiac care and specialized lipid centers; introduction of molecular genetic testing in persons with severe lipid profile

disorders into the healthcare system; creation of a cascade screening system; handling a problem of supply with a modern effective drugs; introduction of lipoprotein apheresis methods for treating patients with insufficient effectiveness of drug therapy; increase of patient medical adherence.

Each Russian region, based on its material-and-technical, staffing capabilities, the prevailing regional characteristics of healthcare system and medication provision, can create an optimal routing system for patients with dyslipidemia, create lipid offices or centers as a structural unit in medical organizations providing cardiology and therapeutic care.

Relationships and Activities: none.

References

1. Sokolov AA, Alexandrova OYu, Kashtalap VV, et al. Methodical recommendations on the organization of medical care for patients with hereditary atherogenic disorders of lipid metabolism in the subjects of the Russian Federation. The Journal of Atherosclerosis and Dyslipidemias. 2016;4:14-20. (In Russ.)
2. Ezhov MV, Bazhan SS, Ershova AI, et al. Clinical guidelines for familial hypercholesterolemia. The Journal of Atherosclerosis and Dyslipidemias. 2019;1:5-43. (In Russ.)
3. Mach F, Baigent C, Catapano AL, et al.; ESC Scientific Document Group. 2019 ESC/EAS Guidelines for the management of dysli pidaemias: li pid modification to reduce cardiovascular risk. Eur Heart J. 2020;41(1):111-88. doi:10.1093/eurheartj/ehz455.
4. Kukharchuk VV, Ezhov MV, Sergienko IV, et al. Diagnostics and correction of lipid metabolism disorders in order to prevent and treat atherosclerosis. Russian recommendations VII revision, 2020. (In Russ.) doi:10.34687/2219-8202.JAD.2020.01.0001.
5. Meshkov AN, Ershova AI, Shalnova SA, et al. Cross-sectional Study to Estimate the Prevalence of Familial Hypercholesterolemia in Selected Regions of the Russian Federation: Relevance, Design of the Study and Initial Characteristics of the Participants. Rational Pharmacotherapy in Cardiology. 2020;16(1):24-32. (In Russ.) doi:10.20996/1819-6446-2020-02-17.
6. Meshkov AN, Ershova AI, Deev AD, et al. Distribution of lipid profile values in economically active men and women in Russian Federation results of the ESSE-RF study for the years 2012-2014. Cardiovascular Therapy and Prevention. 2017;16(4):62-7. (In Russ.) doi:10.15829/1728-8800-2017-4-62-67.
7. Ershova AI, Meshkov AN, Bazhan SS, et al. The prevalence of familial hypercholesterolemia in the West Siberian region of the Russian Federation: A substudy of the ESSE-RF. PLoSOne. 2017;12(7):e0181148. doi:10.1371/journal.pone.0181148.
8. Order of the Ministry of Health of the Russian Federation № 869n of October 26, 2017 "On Approval of the Procedure for Conducting Medical examinations of certain groups of the Adult Population". (In Russ.)
9. Order of the Ministry of Health of the Russian Federation of 29.03.2019 N 173n "On approval of the procedure for conducting dispensary monitoring of adults" (Registered with the Ministry of Justice of the Russian Federation on 25.04.2019 N 54513). (In Russ.)
10. EAS Familial Hypercholesterolaemia Studies Collaboration; EAS Familial Hypercholesterolaemia Studies Collaboration (FHSC) Investigators. Overview of the current status of familial hypercholesterolaemia care in over 60 countries — The EAS Familial Hypercholesterolaemia Studies Collaboration (FHSC). Atherosclerosis. 2018;277:234-55. doi:10.1016/j.atherosclerosis.2018.08.051.
11. Ehrlich AD, Barbarash OL, Kashtalap VV, Graziansky NA, on behalf of all participants of the RECORD-3 registery. Compliance with clinical practice guidelines in non ST-segment elevation acute coronary syndrome: association between outcomes and predictors of "poor" management (RECORD-3 registery data). Complex problems of cardiovascular diseases. 2016;5(2):75-82. (In Russ.)
12. Ehrlich AD, Graziansky NA, Participants of the RECORD Registry. The RECORD Registry. Treatment of Patients With Acute Coronary Syndromes in Hospitals With and Without Possibilities to Perform Invasive Coronary Procedures. Kardiologiia. 2010;7:8-14. (In Russ.)
13. Ehrlich AD, Kharchenko MS, Barbarash OL, et al. Adherence to Guidelines on Management of Acute Coronary Syndrome in Russian Hospitals and Outcomes of Hospitalization (Data From the RECORD-2 Registry). Kardiologiia. 2013;1:14-22. (In Russ.)
14. Oganov RG, Kukharchuk VV, Arutyunov GP, et al. Persistent dyslipidemia in statin-treated patients: Russian real-world clinical practice data (Russian part of the DYSIS Study). Cardiovascular Therapy and Prevention. 2012;11(4):70-8. (In Russ.) doi:10.15829/1728-8800-2012-4-70-78.
15. Sturm AC, Knowles JW, Gidding SS, et al.; Convened by the Familial Hypercholesterolemia Foundation. Clinical Genetic Testing for Familial Hypercholesterolemia: JACC Scientific Expert Panel. J Am Coll Cardiol. 2018;72(6):662-80. doi:10.1016/j.jacc.2018.05.044.
16. Page MM, Bell DA, Watts GF. Widening the spectrum of genetic testing in familial hypercholesterolaemia: Will it translate into better patient and population outcomes? Clin Genet. 2020;97(4):543-55. doi:10.1111/cge.13685.
17. Chubykina UV, Ezhov MV, Ershova AI, et al. Cardiovascular events and effectiveness of hypolipidemic therapy in patients with familial hypercholesterolemia and patients of very high cardiovascular risk: 3-year follow-up of the RENAISSANCE Registry. Kardiologicheskij Vestnik. 2020;3:27-36. (In Russ.)
18. Russian Society of Cardiology (RSC). 2020 Clinical practice guidelines for Stable coronary artery disease. Russian Journal of Cardiology. 2020;25(11):4076. (In Russ.)
19. Barbarash OL, Duplyakov DV, Zateischikov DA, et al. 2020 Clinical practice guidelines for Acute coronary syndrome without ST segment elevation. Russian Journal of Cardiology. 2021;26(4):4449. (In Russ.) doi:10.15829/1560-4071-2021-4449.
20. Russian Society of Cardiology. 2020 Clinical practice guidelines for Acute ST-segment elevation myocardial infarction. Russian Journal of Cardiology. 2020;25(11):4103. (In Russ.) doi:10.15829/29/1560-4071-2020-4103.
21. Zhuravleva MV, Prokofiev AB, Shikh EV, et al. PCSK9 Inhibitors in the Management of Patients with Atherosclerotic Cardiovascular Diseases: Guidelines and Reimbursement Issues. Rational Pharmacotherapy in Cardiology. 2020;16(4):644-53. (In Russ.) doi:10.20996/1819-6446-2020-08-21.
22. Kusters DM, de Beaufort C, Widhalm K, et al. Paediatric screening for hypercholesterolaemia in Europe. Arch Dis Child. 2012;97:272-6. doi:10.1136/archdischild-2011-300081.
23. Ramaswamia U, Humphriesb SE, Priestley-Barnhamc L, et al. Current management of children and young people with heterozygous familial hypercholesterolaemia — HEART UK statement of care. Atherosclerosis. 2019;290:1-8. doi:10.1016/j.atherosclerosis.2019.09.005.
24. La Bresh KA, Lazorick S, Ariza AJ, et al. Implementation of the NHLBI Integrated Guidelines for Cardiovascular Health and Risk Reduction in Children and Adolescents: Rationale and Study Design for Young Hearts, Strong Starts, a Cluster Randomized Trial Targeting Body Mass Index, Blood Pressure, and Tobacco. Contemp Clin Trials. 2014;37(1):98-105. doi:10.1016/j.cct.2013.11.011.
25. Wiegman A, Gidding S, Watts G, et al. Familial hypercholesterolaemia in children and adolescents: gaining decades of life by optimizing. Eur Heart J. 2015;36:2425-37. doi:10.1093/eurheartj/ehv157.
26. Sjouke B, Kusters DM, Kindt I, et al. Homozygous autosomal dominant hypercholesterolaemia in the Netherlands: prevalence, genotype-phenotype relationship, and clinical outcome. Eur Heart J. 2015;36(9):560-5. doi:10.1093/eurheartj/ehu058.

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Molecular and metabolic characteristics of changes in the platelet sensitivity to antiplatelet therapy in patients with coronary artery disease before and after coronary artery bypass grafting

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Aim. To study the production of reactive oxygen species (ROS) by platelets in patients with coronary artery disease (CAD) before and after coronary artery bypass grafting (CABG), depending on their sensitivity to acetylsalicylic acid (ASA) as a part of ASA monotherapy and dual antiplatelet therapy (DAPT) (ASA+clopidogrel).

Material and methods. The study included 104 patients with CAD (ASA monotherapy, 64 patients; DAPT, 40 patients). From day 1 after CABG, they took 100 mg a day of enteric-coated ASA. In the DAPT group, clopidogrel was prescribed for 2-3 days after CABG. All measurements were performed before surgery, on the 1st day and days 8-10 after surgery. Control group consisted of 36 healthy donors. Resistance to ASA was determined at a level of optical platelet aggregation with arachidonic acid ≥20% at least at one observation point. The spontaneous and ADP-induced chemiluminescence (CL) of platelets with luminol and lucigenin was assessed according to the following parameters: time to maximum intensity (Tmax), maximum intensity (Imax), area (S) under the CL curve, and the ratio of ADP-induced CL S to spontaneous CL S.

Results. Throughout the study, 71 patients with CAD were sensitive to ASA (sASA) (ASA monotherapy, 46 patients; DAPT, 25 patients), three patients — resistant (rASA) (ASA monotherapy, 1; DAPT, 2). Sensitivity of other 30 patients (ASA monotherapy, 17; DAPT, 13) changed in different follow-up periods. Compared to the control group, sASA patients had increased values of platelet CL parameters throughout the study, while in the rASA group (ASA monotherapy), Tmax was higher before CABG, and in the rASA group (ASA therapy+clopidogrel), Imax and S were higher on the first day after CABG, while Imax — on days 8-10 after CABG. Compared to sASA, the values of S and Imax before CABG, Imax after CABG, as well as Imax and S on the days 8-10 after CABG in rASA (ASA monotherapy) were significantly lower, while in rASA (ASA therapy+clopidogrel), only the Tmax values were lower on the 8-10 days after CABG.

Conclusion. In patients with CAD, depending on the sensitivity to ASA and antiplatelet therapy after CABG, the metabolic activity of platelets in terms of ROS production differs. In sASA patients, ROS synthesis is higher than in healthy individuals, while, in rASA patients (ASA monotherapy), platelets produce ROS levels lower than in sASA. CABG surgery and the addition of clopidogrel to ASA therapy leads to increased ROS production in rASA patients in the postoperative period.

Keywords: coronary artery disease, clopidogrel, acetylsalicylic acid, resistance, reactive oxygen species, platelet.

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Cardiovascular disease is a leader among all causes of death, affecting the population decline, including working-age persons, which is a socially significant problem that requires close attention and search for solutions.

In severe hemodynamically significant coronary involvement, one of the options for restoring blood flow is coronary artery bypass grafting (CABG). After CABG, patients are prescribed antiplatelet therapy with acetylsalicylic acid (ASA) or clopidogrel to maintain patency of venous and arterial shunts. Despite the effectiveness of such antiplatelet treatment, in some patients there is a reduced sensitivity to both ASA and clopidogrel, which increases the risk of shunt thrombosis. Among the many reasons for platelet resistance to ASA, one of the main ones is the insufficient suppression of cyclooxygenase-1 (COX-1), which is involved in thromboxane A2 synthesis, which causes platelet aggregation. Resistance to clopidogrel is due to genetic polymorphisms of adenosine diphosphate (ADP) receptors and enzymes involved in medication metabolism, low adherence to therapy, and insufficient absorption.

Ultimately, the sensitivity of platelets is specified by their functional activity and activity of metabolic processes providing energy and plastic substrates during the life of these cells. With the harmonious work of all internal mechanisms, the receptors on platelet membrane are assembled, the active substances are synthesized and packed into granules, as well as the platelets are provided with energy resources. Here, an important role is played by reactive oxygen species (ROS), which can participate as signaling molecules, as well as activate platelets and their receptors [1]. ROS synthesis is associated with function of plasma membrane NADPH oxidase, myeloperoxidase, superoxide dismutase, and the inner mitochondrial membrane electron transport chain. In addition, the production of superoxide anion radical and hydroxyl radical is possible through receptor-mediated signaling pathways, as well as in the metabolism of arachidonic acid with the participation of COX-1 inside platelets, especially with collagen stimulation [2]. The resulting primary and secondary ROS can initiate further cascade of synthesis of other ROS. Since the ASA point of application is COX-1, it is likely that platelet susceptibility to it depends on the metabolism of arachidonic acid, which can be judged, in particular, by the production of ROS using chemiluminescence (CL) assay. To detect primary ROS, lucigenin, which does not penetrate into the cell, can be used, while for secondary ROS — luminol (passes through the cell membrane).

In the literature, there are studies revealing the variability in platelet sensitivity due to various reasons (cardiopulmonary bypass, renal dysfunction, selective non-steroidal anti-inflammatory drug therapy in the postoperative period, reduced bioavailability of enteric-coated ASA, inflammatory response) [3, 4].

Therefore, it seems interesting and extremely important to study the effect of CABG surgery on platelet sensitivity to ASA from the standpoint of their metabolic activity in relation to ROS production. At the same time, it is also necessary to assess the platelet production of ROS in patients resistant and sensitive to ASA, both with ASA monotherapy and with ASA+clopidogrel therapy after CABG.

Thus, the aim was to study the production of ROS by platelets in patients with coronary artery disease (CAD) before and after CABG, depending on their sensitivity to ASA as a part of ASA monotherapy and dual antiplatelet therapy (DAPT) (ASA+clopidogrel).

Material and methods

The study included 104 patients (79 men and 25 women) aged 35 to 76 years (mean age, 61±5,5 years) with Canadian Cardiovascular Society (CCS) class II-IV angina. Sixty-four patients took ASA monotherapy and 40 patients — DAPT (ASA+clopidogrel). All patients underwent CABG. Coronary artery atherosclerosis was confirmed by coronary angiography. The exclusion criteria were chronic kidney disease (glomerular filtration rate <60 ml/min/1,73 m^2), liver failure (exceeding the hepatic transaminase normal range by 3 or more times), gastric and/or duodenal ulcer, intolerance to ASA and clopidogrel. The characteristics of patients by clinical and laboratory characteristics are presented in Table 1.

Patients took the following drug groups before CABG: β-blockers — 91,9%, angiotensin-converting enzyme inhibitors — 90,8%, angiotensinogen II receptor blockers — 5%, calcium channel blockers — 16,9%, diuretics — 42,4%, aldosterone antagonists — 40,6%, statins — 100%. Before CABG, for 5 days, patients stopped receiving antiplatelet drugs, and from the first day after surgery they were prescribed with enteric-coated ASA 100 mg a day, while in the group of patients using DAPT, clopidogrel was prescribed by 2-3 days after CABG. Measurements throughout the study for each patient with CAD were carried out three times (before surgery, on day 1 immediately after surgery, and 8-10 days after surgery). This study design makes it possible to establish the effect of CABG and ASA intake on the studied parameters. The control group consisted of 36 healthy donors matched for sex and age.

All patients signed written informed consent. The study was carried out in accordance with the

Table 1

Patient characteristics

Characteristics	All patients	sASA	rASA	p
Sex, female/male, n (%)	25 (24%)/79 (76%)	14 (19,7%)/57 (80,3%)	11 (33,3%)/22 (66,7%)	0,131
Age (years), Me (C_{25}-C_{75})	63 (56-65)	62 (57-65)	62 (55-66)	0,319
Smoking (current status), %	39,7	42,5	29,6	0,227
Total cholesterol, mmol/l	4,28 (3,69-5,58)	4,50 (3,79-5,66)	3,93 (3,56-5,49)	0,132
Leukocytes, 10^9/l	7,57 (6,44-8,60)	7,60 (6,70-8,70)	7,10 (6,10-8,38)	0,774
Platelets, 10^9/l	228,0 (203,0-276,0)	222,0 (201,0-267,5)	229,0 (214,5-283,5)	1,0
Erythrocytes, 10^{12}/l	5,00 (4,7-5,3)	5,0 (4,7-5,3)	4,9 (4,7-5,2)	0,386
Hemoglobin, g/l	141,0 (133,0-152,3)	141,0 (133,5-154,5)	141,0 (132,0-147,0)	0,878
Creatinine, mmol/l	109,0 (97,8-119,0)	109,0 (98,5-118,5)	112,0 (97,0-119,0)	0,688
Class II angina, %	49,6	50,5	48,0	0,578
Class III angina, %	39,3	37,4	48,0	0,508
Diabetes, %	27,3	23,4	40,7	0,075
Prior myocardial infarction, %	62,8	62,8	62,9	0,986
Obesity, %	32,2	35,1	48,2	0,077

Note: data represent medians and interquartile range (Me (C_{25}-C_{75})). $p<0,05$ between the indices of patients with sASA and rASA (Mann-Whitney U-test). The χ^2 test was used to compare categorical variables, unless the expected frequencies in the contingency tables were less than 5, in which case Fisher's exact test was used.

Abbreviations: rASA — acetylsalicylic acid-resistant patients, sASA — acetylsalicylic acid-sensitive patients.

Helsinki Declaration and Rules for Good Clinical Practice in Russia approved by Order of the Russian Ministry of Health dated June 19, 2003 № 266. The study protocol was approved by the Ethics Committee of V. F. Voino-Yasenetsky Krasnoyarsk State Medical University.

The study used blood stabilized with 3,2% sodium citrate in a 9:1 ratio. To determine the resistance of platelets to ASA, platelet-rich (blood centrifugation at 140 g for 10 min, at 24° C) and platelet-poor plasma (blood centrifugation at 1500 g for 15 min, temperature 24° C) were obtained. Determination of ASA resistance was carried out in 500 µl of platelet-rich plasma relative to platelet-poor plasma on a Chronolog 490 aggregometer (USA) with the addition of 5 µl of arachidonic acid (0,5 mM) (CHRONO-PAR, USA). If the measurement was carried out before ASA therapy initiation, then platelet aggregation with arachidonic acid was determined after their incubation with 10 µl ASA (3,36 mM, purity ⩾99,0%, A5376 Sigma Aldrich) *in vitro* for 3 min at 37° C to predict aspirin resistance. ASA resistance was determined at a level of platelet aggregation with arachidonic acid ⩾20% at least at one observation point: on antiplatelet therapy on days 8-10 after CABG or during incubation of the patient's plasma with ASA *in vitro* before the start of surgery and ASA treatment.

To determine the CL activity of platelets, platelet-rich plasma, after standing the blood in a thermostat for 30 min, was obtained by centrifuging stabilized blood at 140 g for 10 min at 24° C. The supernatant was collected and transferred into a plastic tube, adjusted with buffer № 1 (90 mM NaCl, 5 mM KCl, 36 mM sodium citrate, 10 mM EDTA, pH 762) to 2-fold dilution. The resulting mixture was centrifuged at 400 g for 15 min at 24° C. The pellet was resuspended in buffer № 1 (5 ml) and recentrifuged at 400 g for 1 min. The resulting supernatant was centrifuged again at 400 g for 15 min. The supernatant was carefully discarded; 10 ml of buffer № 2 (0,13 M NaCl, 0,02 M Tris-HCl buffer, 0,03 M EDTA, 0,015 M glucose, pH 7,4) was added to the sediment and centrifuged for 50 sec at 140 g. Evaluation of the number and purity of isolated platelets was carried out on a Sysmex XE-5000 hematology analyzer (Sysmex Inc., USA). The purity of isolated platelets was 98-100%. For the study, platelets were used in the amount of 2×10^7 cells per sample. The reaction mixture also included 50 µl of lucigenin or luminol at a concentration of 50 µg/ml, 50 µl of 0,1 M ADP (for assessing induced ROS synthesis) and 250 µl (for assessing spontaneous ROS synthesis) or 200 µl (for assessing ADP-induced ROS synthesis) of buffer (0,13 M NaCl, 0,02 M Tris-HCl buffer, 0,03 M EDTA, 0,015 M glucose, pH 7,4). Evaluation of spontaneous and ADP-induced CL was carried out for 90 min on a 36-channel biochemiluminescence analyzer BLM-3607 (OOO MedBioTech, Russia) [5].

Table 2

CL activity of platelets with lucigenin in patients with CAD before CABG (Me (C_{25}-C_{75}))

Parameters	Control (n=36)	sASA (n=71)	rASA (ASA monotherapy) (n=17)	rASA (ASA+clopidogrel) (n=13)
Spontaneous CL				
Tmax, sec	219 (82-719)	813 (222-2841) p_1=0,027	611 (339-1908)	212 (70-560)
Imax, CU × 10^3	0,074 (0,06-0,086)	0,12 (0,09-0,50) p_1=0,006	0,061 (0,047-0,086) p_2=0,006	0,088 (0,07-0,096)
S, CU × sec × 10^6	0,225 (0,171-0,266)	0,30 (0,18-0,79)	0,136 (0,12-0,321)	0,227 (0,162-0,233)
Induced CL				
Tmax, sec	319 (73-1393)	1036 (445-3745) p_1=0,004	764 (509-1979)	636 (287-1201)
Imax, CU × 10^3	0,076 (0,062-0,082)	0,13 (0,08-0,43) p_1=0,013	0,071 (0,058-0,124)	0,1 (0,075-0,132)
S, CU × sec × 10^6	0,241 (0,161-0,295)	0,41 (0,25-1,11)	0,234 (0,18-0,344)	0,27 (0,202-0,312)
S_{ind}/S_{spont}	1,03 (0,96-1,24)	1,06 (0,89-1,28)	1,13 (1,01-1,21)	0,98 (0,93-1,65)

Note: p_1 — statistical significance of differences in comparison with control group; p_2 — statistical significance of differences in comparison with sASA patients with CAD.

Abbreviations: ASA — acetylsalicylic acid, CAD — coronary artery disease, CU — conventional units, rASA — acetylsalicylic acid-resistant patients, sASA — acetylsalicylic acid-sensitive patients, CL — chemiluminescence, Tmax — time to maximum chemiluminescence intensity, Imax — maximum chemiluminescence intensity, S — area under chemiluminescence curves.

The following characteristics were determined: time to maximum plasma concentration (Tmax), maximum intensity (Imax), as well as the area under the CL curve (S). The enhancement of ADP-induced CL was assessed by the ratio of area under the induced CL curve of area under the spontaneous CL curve (S_{ind}/S_{spont}).

The sample description was made by calculating the median (Me) and interquartile range in the form of 25 and 75 percentiles (C_{25} and C_{75}). The statistical significance of differences between the independent samples was assessed by the nonparametric Mann-Whitney U test. Differences were considered significant at $p<0,05$. Statistical analysis was performed using the Statistica 8.0 software package (StatSoft Inc., USA).

Results

Out of 104 patients with CAD, 71 (68,3%) were sensitive to ASA (sASA) throughout the study (46 patients, ASA monotherapy; 25 patients, ASA+clopidogrel). The remaining 33 (31,7%) patients with CAD showed resistance to ASA (rASA) at least at one follow-up point, which is consistent with other studies where resistance to ASA ranged from 5 to 50% [6-8]. Only 3 (9%) patients of all rASA patients with CAD were resistant during the entire observation period (1 patient, ASA monotherapy; 2 patents, ASA+clopidogrel therapy), and the sensitivity of remaining 30 rASA patients with CAD (17 patients, ASA monotherapy; 13 patents, ASA+clopidogrel therapy) changed in different follow-up periods, which is of interest for studying this phenomenon.

Study results showed that in sASA patients with CAD, many platelet CL parameters is significantly higher than in the control group. Before CABG, these are CL with lucigenin (Tmax and Imax in the spontaneous and ADP-induced test) and luminol (Imax in the spontaneous and ADP-induced test and S in the ADP-induced test). On the first day after CABG surgery, these are CL with lucigenin (Tmax and Imax in the spontaneous test) and luminol (Imax and S in the spontaneous test, Tmax and Imax in the ADP-induced test). On days 8-10 after CABG surgery, these are CL with lucigenin (Tmax and Imax in the spontaneous test, Imax and S in the ADP-induced test) and luminol (Imax and S in the spontaneous test, Tmax, Imax and S in the ADP-induced test) (Tables 2-7).

In the rASA patients with CAD (ASA monotherapy), compared with the control group, only the Tmax in the ADP-induced test with luminol before CABG was higher (Table 5). In the rASA group (ASA+clopidogrel), compared with the control group, the following CL indices were higher: Imax in the spontaneous test with lucigenin, Imax in the ADP-induced test and S in the spontaneous test with luminol (in the first days after CABG), as well as Imax in spontaneous and ADP-induced test with

Table 3

CL activity of platelets with lucigenin in patients with CAD on the first day after CABG (Me (C_{25}-C_{75}))

Parameters	Control (n=36)	sASA (n=71)	rASA (ASA monotherapy) (n=17)	rASA (ASA+clopidogrel) (n=13)
Spontaneous CL				
Tmax, sec	219 (82-719)	494 (255-2043) p_1=0,008	408 (95-710)	212 (89-382)
Imax, CU × 10^3	0,074 (0,06-0,086)	0,1 (0,08-0,30) p_1=0,036	0,065 (0,05-0,077) p_2=0,006	0,097 (0,086-0,117) p_1=0,049
S, CU × sec × 10^6	0,225 (0,171-0,266)	0,35 (0,21-0,56)	0,182 (0,11-0,245)	0,36 (0,22-0,398)
Induced CL				
Tmax, sec	319 (73-1393)	458 (177-985)	509 (66-799)	282 (178-2519)
Imax, CU × 10^3	0,076 (0,062-0,082)	0,1 (0,08-0,45)	0,058 (0,054-0079) p_2=0,006	0,09 (0,072-0,097)
S, CU × sec × 10^6	0,241 (0,161-0,295)	0,32 (0,21-0,67)	0,176 (0,162-0,218)	0,314 (0,156-0,354)
S_{ind}/S_{spont}	1,03 (0,96-1,24)	1,03 (0,97-1,17)	0,92 (0,81-1,02)	0,89 (0,79-0,99) p_2=0,006

Note: p_1 — statistical significance of differences in comparison with control group; p_2 — statistical significance of differences in comparison with sASA patients with CAD.

Abbreviations: ASA — acetylsalicylic acid, CAD — coronary artery disease, CU — conventional units, rASA — acetylsalicylic acid-resistant patients, sASA — acetylsalicylic acid-sensitive patients, CL — chemiluminescence, Tmax — time to maximum chemiluminescence intensity, Imax — maximum chemiluminescence intensity, S — area under chemiluminescence curves.

Table 4

CL activity of platelets with lucigenin in patients with CAD on days 8-10 after CABG (Me (C_{25}-C_{75}))

Parameters	Control (n=36)	sASA (n=71)	rASA (ASA monotherapy) (n=17)	rASA (ASA+clopidogrel) (n=13)
Spontaneous CL				
Tmax, sec	219 (82-719)	611 (185-2198) p_1=0,015	212 (89-637)	124 (0-410) p_2=0,006
Imax, CU × 10^3	0,074 (0,06-0,086)	0,11 (0,09-0,34) p_1=0,002	0,064 (0,057-0,101)	0,085 (0,078-0,109)
S, CU × sec × 10^6	0,225 (0,171-0,266)	0,32 (0,20-0,85)	0,172 (0,158-0,199)	0,31 (0,244-0,329)
Induced CL				
Tmax, sec	319 (73-1393)	1625 (161-3099)	574 (171-819)	301 (53-1141)
Imax, CU × 10^3	0,076 (0,062-0,082)	0,12 (0,09-0,53) p_1=0,001	0,058 (0,051-0,097) p_2=0,006	0,085 (0,078-0,112)
S, CU × sec × 10^6	0,241 (0,161-0,295)	0,35 (0,28-1,47) p_1=0,037	0,13 (0,076-0,314) p_2=0,006	0,244 (0,145-0,315)
S_{ind}/S_{spont}	1,03 (0,96-1,24)	1,10 (0,88-1,95)	1,02 (0,44-1,36)	0,92 (0,51-1,1)

Note: p_1 — statistical significance of differences in comparison with control group; p_2 — statistical significance of differences in comparison with sASA patients with CAD.

Abbreviations: ASA — acetylsalicylic acid, CAD — coronary artery disease, CU — conventional units, rASA — acetylsalicylic acid-resistant patients, sASA — acetylsalicylic acid-sensitive patients, CL — chemiluminescence, Tmax — time to maximum chemiluminescence intensity, Imax — maximum chemiluminescence intensity, S — area under chemiluminescence curves.

luminol (8-10 days after CABG surgery) (Tables 3, 6, 7).

In the rASA patients with CAD (ASA monotherapy) compared with the sASA ones, the S values in the ADP-induced test with luminol and Imax in the spontaneous test with lucigenin before CABG were significantly lower (Tables 2, 5). The same trend was observed on the first day after CABG for Imax in the spontaneous and ADP-induced test with lucigenin (Table 3), as well as on days 8-10

Table 5

CL activity of platelets with luminol in patients with CAD before CABG (Me (C_{25}-C_{75}))

Parameters	Control (n=36)	sASA (n=71)	rASA (ASA monotherapy) (n=17)	rASA (ASA+clopidogrel) (n=13)
Spontaneous CL				
Tmax, sec	141 (0-680)	229,5 (40,2-1833,5)	255 (85-1655)	95 (71-319)
Imax, CU × 10^3	0,077 (0,057-0,085)	0,12 (0,08-0,55) p_1=0,01	0,06 (0,048-0,14)	0,081 (0,079-0,339)
S, CU × sec × 10^6	0,226 (0,12-0,285)	0,29 (0,2-0,95)	0,141 (0,09-0,26)	0,29 (0,21-0,35)
Induced CL				
Tmax, sec	95 (0-598)	454,5 (0-1800)	710 (234-2197) p_1=0,024	776 (61-1426)
Imax, CU × 10^3	0,07 (0,059-0,081)	0,113 (0,08-0,5) p_1=0,008	0,06 (0,056-0,098)	0,1 (0,072-0,434)
S, CU × sec × 10^6	0,222 (0,151-0,251)	0,3 (0,22-0,88) p_1=0,041	0,148 (0,132-0,277) p_2=0,006	0,313 (0,181-0,367) p_3=0,025
S_{ind}/S_{spont}	1 (0,88-1,1)	0,98 (0,75-1,13)	1,09 (1,01-1,19)	1,02 (0,67-1,11)

Note: p_1 — statistical significance of differences in comparison with control group; p_2 — statistical significance of differences in comparison with sASA patients with CAD; p_3 — statistical significance of differences in comparison with rASA patients with CAD (ASA monotherapy).

Abbreviations: ASA — acetylsalicylic acid, CAD — coronary artery disease, CU — conventional units, rASA — acetylsalicylic acid-resistant patients, sASA — acetylsalicylic acid-sensitive patients, CL — chemiluminescence, Tmax — time to maximum chemiluminescence intensity, Imax — maximum chemiluminescence intensity, S — area under chemiluminescence curves.

Table 6

CL activity of platelets with luminol in patients with CAD on the first day after CABG (Me (C_{25}-C_{75}))

Parameters	Control (n=36)	sASA (n=71)	rASA (ASA monotherapy) (n=17)	rASA (ASA+clopidogrel) (n=13)
Spontaneous CL				
Tmax, sec	141 (0-680)	141 (0-1107)	159 (90-2189)	141 (51-976)
Imax, CU × 10^3	0,077 (0,057-0,085)	0,15 (0,086-0,46) p_1=0,012	0,072 (0,049-0,088)	0,093 (0,084-0,185)
S, CU × sec × 10^6	0,226 (0,12-0,285)	0,33 (0,22-0,86) p_1=0,043	0,21 (0,084-0,328)	0,34 (0,299-0,444) p_1=0,015
Induced CL				
Tmax, sec	95 (0-598)	198 (0-1529) p_1=0,046	89 (0-1514)	71 (0-266)
Imax, CU × 10^3	0,07 (0,059-0,081)	0,130 (0,09-0,45) p_1=0,011	0,077 (0,053-0,103)	0,083 (0,078-0,293) p_1=0,024
S, CU × sec × 10^6	0,222 (0,151-0,251)	0,33 (0,17-1,05)	0,16 (0,011-0,234)	0,326 (0,155-0,36)
S_{ind}/S_{spont}	1 (0,88-1,1)	0,94 (0,83-1,1)	0,96 (0,84-1,17)	0,99 (0,81-1,19)

Note: p_1 — statistical significance of differences in comparison with control group.

Abbreviations: ASA — acetylsalicylic acid, CAD — coronary artery disease, CU — conventional units, rASA — acetylsalicylic acid-resistant patients, sASA — acetylsalicylic acid-sensitive patients, CL — chemiluminescence, Tmax — time to maximum chemiluminescence intensity, Imax — maximum chemiluminescence intensity, S — area under chemiluminescence curves.

after CABG surgery for Imax in the ADP-induced test with luminol and lucigenin and S in the ADP-induced test with lucigenin (Tables 4, 7).

In rASA patients with CAD (ASA+clopidogrel) compared with sASA ones, the S_{ind}/S_{pont} values in lucigenin test on the first day after CABG surgery (Table 3), as well as Tmax in the ADP-induced test with luminol and Tmax in the spontaneous test with lucigenin on days 8-10 after surgery, CABG were significantly lower (Tables 4, 7).

Significant differences between rASA patients with CAD receiving ASA monotherapy and ASA+clo-

Table 7

CL activity of platelets with luminol in patients with coronary artery disease on days 8-10 after CABG (Me (C_{25}-C_{75}))

Parameters	Control (n=36)	sASA (n=71)	rASA (ASA monotherapy) (n=17)	rASA (ASA+clopidogrel) (n=13)
Spontaneous CL				
Tmax, sec	141 (0-680)	264 (0-1164)	382 (53-1134)	71 (0-660)
Imax, CU × 10^3	0,077 (0,057-0,085)	0,14 (0,09-1,253) p_1=0,001	0,06 (0,057-0,167)	0,134 (0,086-0,149) p_1=0,035
S, CU × sec × 10^6	0,226 (0,12-0,285)	0,4 (0,3-2,35) p_1<0,001	0,19 (0,171-0,416)	0,304 (0,172-0,358)
Induced CL				
Tmax, sec	95 (0-598)	492 (84,5-1876,5) p_1=0,01	355 (139-2211)	35,5 (0-279) p_2=0,006
Imax, CU × 10^3	0,07 (0,059-0,081)	0,18 (0,09-1,57) p_1<0,001	0,062 (0,052-0,12) p_2=0,006	0,117 (0,081-0,133) p_1=0,041
S, CU × sec × 10^6	0,222 (0,151-0,251)	0,41 (0,22-2,27) p_1=0,038	0,18 (0,126-0,409)	0,247 (0,131-0,33)
S_{ind}/S_{spont}	1 (0,88-1,1)	0,9 (0,62-1,5)	0,91 (0,57-1,03)	0,83 (0,6-1,14)

Note: p_1 — statistical significance of differences in comparison with control group; p_2 — statistical significance of differences in comparison with sASA patients with CAD.

Abbreviations: ASA — acetylsalicylic acid, CAD — coronary artery disease, CU — conventional units, rASA — acetylsalicylic acid-resistant patients, sASA — acetylsalicylic acid-sensitive patients, CL — chemiluminescence, Tmax — time to maximum chemiluminescence intensity, Imax — maximum chemiluminescence intensity, S — area under chemiluminescence curves.

pidogrel therapy were found only for S CL in the ADP-induced test with luminol before CABG (Table 5).

Discussion

The variability of platelet sensitivity to ASA depends on various factors, but many mechanisms of this phenomenon are still not fully understood. Platelets, as direct participants in not only hemostatic, but also immune and inflammatory processes, interact with a large number of cells and active molecules. At the same time, intercellular contacts of platelets with leukocytes are most pronounced in a chronic nonspecific inflammatory process, which, along with lipid disorders, underlies atherosclerosis. This can lead to a different response of platelets to ASA, depending on microenvironment. The increased basic and induced metabolic activity of platelets, manifested in high rates of production of primary and secondary ROS in sASA patients with CAD throughout the study, indicates a cell activeness and their potential upon additional stimulation. At the same time, both CABG surgery and antiplatelet therapy in some cases did not deplete internal resources of platelets, and the persisting high activity of platelets could initiate rethrombosis [9].

It is noteworthy that in the rASA patients with CAD (ASA monotherapy), the level and intensity of platelet ROS production does not differ from the control group. Only before CABG the time for reaching the maximum intensity of induced ROS production is increased, which indicates a slowdown in their production with additional stimulation. In the rASA patients with CAD (ASA+clopidogrel), a different picture is observed. After CABG surgery and while taking ASA and clopidogrel, platelets produce increased primary and secondary ROS relative to control values, which indicates the influence of these factors on platelet metabolism in this category of patients with CAD. Probably, residual platelet reactivity is due to stimulation of cells by cardiopulmonary bypass circuit, leukocytes, and production of new platelets 8-10 days after CABG [10]. Moreover, DATT does not reduce the production of ROS.

We have previously shown that in rASA patients with CAD (regardless of therapy), the level and intensity of ROS production by platelets is lower than in sASA ones [11]. The sensitivity is associated with increased metabolic activity of platelets for ROS synthesis, including for arachidonic acid (with COX-1 participation), which platelets can also receive from neutrophils during intercellular interaction [12]. In this study, this picture remains expressed in rASA patients with CAD (ASA monotherapy), while in the group of rASA patients with CAD (ASA+clopidogrel), only the time of ROS production was reduced and only by 8-10

days after CABG, which may be associated with the effect of clopidogrel on platelet receptors for ADP. In addition, platelets of sASA and rASA (ASA+clopidogrel) produce more secondary ROS upon stimulation before CABG surgery than r ASA patients with CAD (ASA monotherapy). These data indicate that the CL activity of platelets in rASA patients with CAD (ASA+clopidogrel) is at the level of sASA patients, i.e. using DAPT, it is possible to overcome the resistance to ASA.

Higher rates of ROS production in sASA patients with CAD and rASA (ASA+clopidogrel) may indicate that the NADPH oxidase activity in the platelets of these patients is higher than in rASA ones (ASA monotherapy). Thanks to DAPT, 2 pathways of platelet activation are blocked at once, but this can be compensated due to their increased contact with leukocytes both immediately after the operation and with taking antiplatelet drugs through the transfer of active substances through microvesicles, but not through the receptor-mediated leukocyte-platelet complex formation, since the number of such aggregates is significantly reduced along with the pro-inflammatory functions of platelets on clopidogrel therapy in patients with CAD [13]. Because of this, the amount of arachidonic acid, may increase, during the metabolism of which ROS is produced. And since in this group of patients with CAD, COX-1 resistance to ASA is observed, then high substrate levels contribute to increased formation of intermediate ROS products, and the main antiplatelet effect is provided not by ASA, but by clopidogrel. Purinergic receptors for ADP are also present on leukocyte cells. They are involved in many processes, including in leukocyte adhesion to endothelial cells during inflammation and atherosclerosis, therefore, they are also blocked by clopidogrel, which leads to disruption of intercellular bridges. In addition, leukocyte-platelet complexes are capable of producing ROS themselves, and the resulting nitric oxide can reduce platelet aggregation activity, thereby preventing recurrent thrombosis [14]. Therefore, in the group of rASA patients with CAD on DAPT, clinical outcomes may be more favorable due to clopidogrel presence. The addition of clopidogrel to ASA does not affect the very phenomenon of aspirin resistance, but it has an anti-inflammatory and additional antiplatelet function. And the probable disruption of intercellular contacts in rASA patients with CAD (ASA monotherapy) levels the antiplatelet effect of ASA due to the insufficient arachidonic acid amount in platelets, which causes a reduced sensitivity of such patients to ASA [15].

Conclusion

Thus, we obtained data indicating a difference in the metabolic activity of platelets in ROS production in CAD patients, depending on both the sensitivity to ASA and the options for antiplatelet therapy after CABG (ASA or ASA+clopidogrel). In sASA patients with CAD, ROS production is higher than in healthy individuals in the pre and postoperative period. In rASA patients with CAD treated with ASA monotherapy, platelets produce ROS levels lower than in sASA patients with CAD. However, CABG and the addition of clopidogrel to ASA therapy leads to increased ROS production in rASA patients in the postoperative period, which equates this group of patients with sASA patients in terms of ROS synthesis. These results may indicate a complete disruption of the intercellular interactions of platelets and leukocytes in rASA patients with CAD (ASA monotherapy) and partial (only at the receptor level) in patients with CAD (ASA+clopidogrel) with intact transport of substances using microvesicles, which is the result of blocking receptors by clopidogrel on both platelets and leukocytes.

Sensitivity to ASA may depend not only on COX-1 activity, but also on the presence and metabolic characteristics of enzyme substrate, on the activity of other cells that can interact with platelets, which, accordingly, depends on the environment (medication therapy, invasive interventions, comorbidities, etc.). Further study of these metabolic processes may lead to the discovery of additional mechanisms of aspirin resistance and ways to overcome this phenomenon.

Relationships and Activities. The study was financially supported by the Russian Foundation for Basic Research, the Government of the Krasnoyarsk Krai, the Krasnoyarsk Regional Science Foundation within the research project № 18-415-243003 “Personification of antiplatelet therapy in patients with coronary artery disease (CAD), depending on the level of P-selectin gene expression, the activity of intercellular interaction and inflammation”.

References

1. Morel A, Bijak M, Miller E, et al. Relationship between the Increased Haemostatic Properties of Blood Platelets and Oxidative Stress Level in Multiple Sclerosis Patients with the Secondary Progressive Stage. Oxidative Medicine and Cellular Longevity. 2015;2015:240918. doi:10.1155/2015/240918.
2. Delaney MK, Kim K, Estevez B, et al. Differential Roles of the NADPH-Oxidase 1 and 2 in Platelet Activation and Thrombosis. Arteriosclerosis Thrombosis and Vascular Biology. 2016;36(5):846-54. doi:10.1161/ATVBAHA.116.307308.
3. Yassin AS, Abubakar H, Mishra T, et al. Aspirin Resistance: Cardiovascular Risk Game Changer. American Journal of Therapeutics. 2019;26(5):593-9. doi:10.1097/MJT.0000000000000780.
4. Grinshtein YuI, Kosinova AA, Grinshtein IYu, et al. Clinical and laboratory characteristics of patients with ischemic heart disease resistant to acetylsalicylic acid in perioperative period of coronary artery bypass graft: results of an open prospective study. Rational Pharmacotherapy in Cardiology. 2016;12(3):265-71. (In Russ.) doi:10.20996/1819-6446-2016-12-3-265-271.
5. Savchenko AA, Goncharov MD, Grinsthein YuI, et al. Chemiluminescent Analysis of Reactive Oxygen Species Synthesis by Platelets from Patients with Coronary Heart Disease. Bulletin of Experimental Biology and Medicine. 2020;169(4):535-8. doi:10.1007/s10517-020-04924-4.
6. Jastrzebska M, Marcinowska Z, Oledzki S, et al. Variable gender-dependent platelet responses to combined antiplatelet therapy in patients with stable coronary-artery disease. Journal of Physiology and Pharmacology. 2018;69(4):594-605. doi:10.26402/jpp.2018.4.10.
7. Klasic A, Lakusic N, Gaspar L, Kruzliak P. The monitoring of antiaggregation effect of acetylsalicylic acid therapy by measuring serum thromboxane B2 in patients with coronary artery bypass grafting. Blood Coagulation and Fibrinolysis. 2016;27(4):370-3. doi:10.1097/MBC.0000000000000438.
8. Hartinger J, Novotny R, Bilkova J, et al. Role of Dipyrone in the High On-Treatment Platelet Reactivity amongst Acetylsalicylic Acid-Treated Patients Undergoing Peripheral Artery Revascularisation. Medical Principles and Practice. 2018;27(4):356-61. doi:10.1159/000489970.
9. Ivert T, Dalén M, Ander C, et al. Platelet function one and three months after coronary bypass surgery in relation to once or twice daily dosing of acetylsalicylic acid. Thrombosis Research. 2017;149:64-9. doi:10.1016/j.thromres.2016.11.018.
10. Bauer A, Hausmann H, Schaarschmidt J, et al. Shed-blood-separation and cell-saver: an integral part of MiECC? Shed-blood-separation and its influence on the perioperative inflammatory response during coronary revascularization with minimal invasive extracorporeal circulation systems a randomized controlled trial. Perfusion. 2018;33(2):136-47. doi:10.1177/0267659117728195.
11. Grinshtein YuI, Savchenko AA, Kosinova AA, et al. Resistance to Acetylsalicylic Acid in Patients with Coronary Heart Disease Is the Result of Metabolic Activity of Platelets. Pharmaceuticals. 2020;13(8):178. doi:10.3390/ph13080178.
12. Serebryanaya NB, Shanin SN, Fomicheva EE, et al. Blood platelets as activators and regulators of inflammatory and immune reactions. Part 2. Thrombocytes as participants of immune reactions. Medical Immunology. 2019;21(1):9-20. (In Russ.) doi:10.15789/1563-0625-2019-1-9-20.
13. Wang X, Deng H, Li T, et al. Clopidogrel reduces lipopolysaccharide-induced inflammation and neutrophil-platelet aggregates in an experimental endotoxemic model. Journal of Biochemical and Molecular Toxicology. 2019;33(4):e22279. doi:10.1002/jbt.22279.
14. Zang Y, Popat KC, Reynolds MM. Nitric oxide-mediated fibrinogen deposition prevents platelet adhesion and activation. Biointerphases. 2018;7;13(6):06E403. doi:10.1116/1.5042752.
15. Goncharov MD, Grinshtein YuI, Savchenko AA, et al. Chemiluminescent activity and platelet aggregation in chronic coronary heart disease during the acetylsalicylic acid therapy before and after coronary bypass surgery. Pathological Physiology and Experimental Therapy. 2021;65(1):42-51. (In Russ.) doi:10.25557/0031-2991.2021.01.42-51.

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Regularities of plaque stabilization in various scenarios of neointimal calcification and vascularization

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Aim. To study the relationships between phenotypes of extracranial arteries' plaques (stable/unstable), their calcification and its causes, in particular, vascularization.

Material and methods. The study included 88 patients: patients (n=44) with ischemic stroke and those (n=44) with chronic brain ischemia. In all subjects, the parameters of systemic mineral homeostasis were assessed (total and ionized calcium, phosphate, total protein, albumin, and calcification propensity). Atherosclerotic plaques have been obtained during carotid endarterectomy, fixed in formalin, postfixed in 1% osmium tetroxide, stained in 2% osmium tetroxide, dehydrated in ascending ethanol series and acetone, stained with 2% alcoholic uranyl acetate and embedded into epoxy resin with its further polymerization. Epoxy resin blocks were grinded, polished, counterstained with Reynolds' lead citrate and sputter coated with carbon. Sample visualization was performed employing backscattered scanning electron microscopy. Number and area of calcium deposits and neointimal vessels were quantified using ImageJ. Statistical analysis was carried out using Mann-Whitney U-test and Spearman's rank correlation coefficient

Results. It was found that area of neointimal calcification, but not number of calcium deposits, was associated with the stable plaque phenotype. The stabilizing effect of calcification was manifested in retarding stenosis associated with plaque rupture and stroke. Calcification extent directly correlated with total and local plaque vascularization, which have been associated with unstable and stable plaque phenotype, respectively. In addition, plaque calcification negatively correlated with total protein and albumin, thereby reflecting the impaired systemic mineral homeostasis.

Conclusion. Atherosclerotic plaque calcification and active local vascularization reduce stenosis extent and stabilize plaque. In contrast, total plaque calcification contributes to the atherosclerosis progression and promotes major acute cardiovascular events.

Keywords: atherosclerosis, ischemic stroke, neointima, calcification, stenosis.

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Extracranial artery (ECA) atherosclerosis, as it progresses, inevitably leads to hemodynamic disorders and insufficient cerebral blood supply [1]. In particular, plaque rupture in ECAs leads to an acute discrepancy between the blood delivery and oxygen demand (ischemic stroke), while permanent ECA stenosis is manifested by chronic cerebral ischemia (CCI). Thus, plaques can be subdivided into unstable (causing stroke) and stable (causing CCI) [2]. Despite the fundamental differences in the clinical phenotype of stable and unstable ECA atherosclerosis, the pathophysiological factors of plaque instability and especially the mechanisms of their regulation remain largely unknown. At the same time, predicting cardiovascular outcome in patients with multifocal atherosclerosis requires a clear understanding of regulating balance between plaque rupture and stabilization.

Despite the fact that the role of plaque calcification in its stabilization has been studied well, the reasons for development and progression of neointimal calcification, as well as the causal relationships between calcification and other determinants of unstable plaque phenotype, have not been adequately studied [3]. It is unclear why a number of plaques remain uncalcified until the cardiovascular event, and what factors are leading in calcification development. It also remains unclear which types of neointimal calcification stabilize the plaque and which, on the contrary, contribute to its rupture.

The study of this problem is complicated by the fact that sample preparation of tissues with extra skeletal mineralization for histology is extremely difficult due to impaired tissue integrity due to significant differences in the density of calcium deposits and surrounding tissues. As a result, the analysis of the interaction of calcification with other pathological processes occurring in the neointima becomes almost impossible. Our group previously developed an original method for preparing calcified plaques for electron microscopy, which consists in staining formalin-fixed tissues with osmium tetroxide and uranyl acetate, further embedding dehydrated tissues in epoxy resin, grinding and polishing polymerized epoxy blocks, followed by backscattered-electron imaging [4-6]. This method allows one to preserve the integrity of calcified plaques and investigate their colocalization with neointimal vessels (*vasa plaquorum*) [7].

The aim was to study the relationships between the phenotypes of ECA plaques (stable/unstable), their calcification, and the causes of calcification. Ultimately, this made it possible to identify the calcification types characteristic of stable and unstable plaques, as well as the pathomorphological determinants of calcifying and non-calcifying phenotype in ECAs.

Material and methods

The study included 88 patients hospitalized in Neurosurgery department of the L. S. Barbarash Kuzbass Clinical Cardiology Dispensary with ECA stenosis verified by ultrasound (44 patients with stroke and 44 patients with CCI). Plaques were considered unstable in stroke and stable in CCI. The study was carried out in accordance with Good Clinical Practice and Declaration of Helsinki. The study protocol was approved by the local ethics committee. All patients signed written informed consent. Cerebrovascular diseases (CCI and ischemic stroke), as well as concomitant diseases (hypertension, heart failure, chronic obstructive pulmonary disease, asthma, chronic kidney disease, diabetes, overweight and obesity) were diagnosed and treated according to current clinical guidelines and standards developed by European Society of Cardiology, Global Initiative for Chronic Obstructive Lung Disease, Global Initiative for Asthma, Kidney Disease: Improving Global Outcomes, American Diabetes Association and European Association for the Study of Obesity). Glomerular filtration rate was estimated according to the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation. Left ventricular ejection fraction was assessed using echocardiography (Sonos 2500 ultrasound system, Hewlett Packard). The percentage of ECA stenosis in patients with cerebrovascular disease was assessed using color Doppler ultrasound (Vivid 7 Dimension Ultrasound System, General Electric Healthcare). Data on age, sex, smoking status and pharmacological history were collected at admission time.

In all patients participating in the study, upon hospital admission, the parameters of systemic mineral homeostasis (concentration of total and ionized blood calcium, phosphorus, total protein and albumin) were determined on a biochemical analyzer (Konelab 60i, Thermo Scientific). All patients underwent carotid endarterectomy, during which a number of them (21 patients with stroke and 27 patients with CCI) received ACB for further ultrastructural examination. After 24-hour fixation in formalin (B06-003, BioVitrum), each biomaterial was postfixed with 1% osmium tetroxide (19110, Electron Microscopy Sciences) in 0,1 M phosphate buffer solution for 12 h, then stained with 2% osmium tetroxide in bi-distilled water for 48 h. Then the samples were dehydrated through a series of ethanol solution in ascending concentration (50, 60, 70, 80, and 95%, all in two changes, each change for 15 min), stained with 2% uranyl acetate

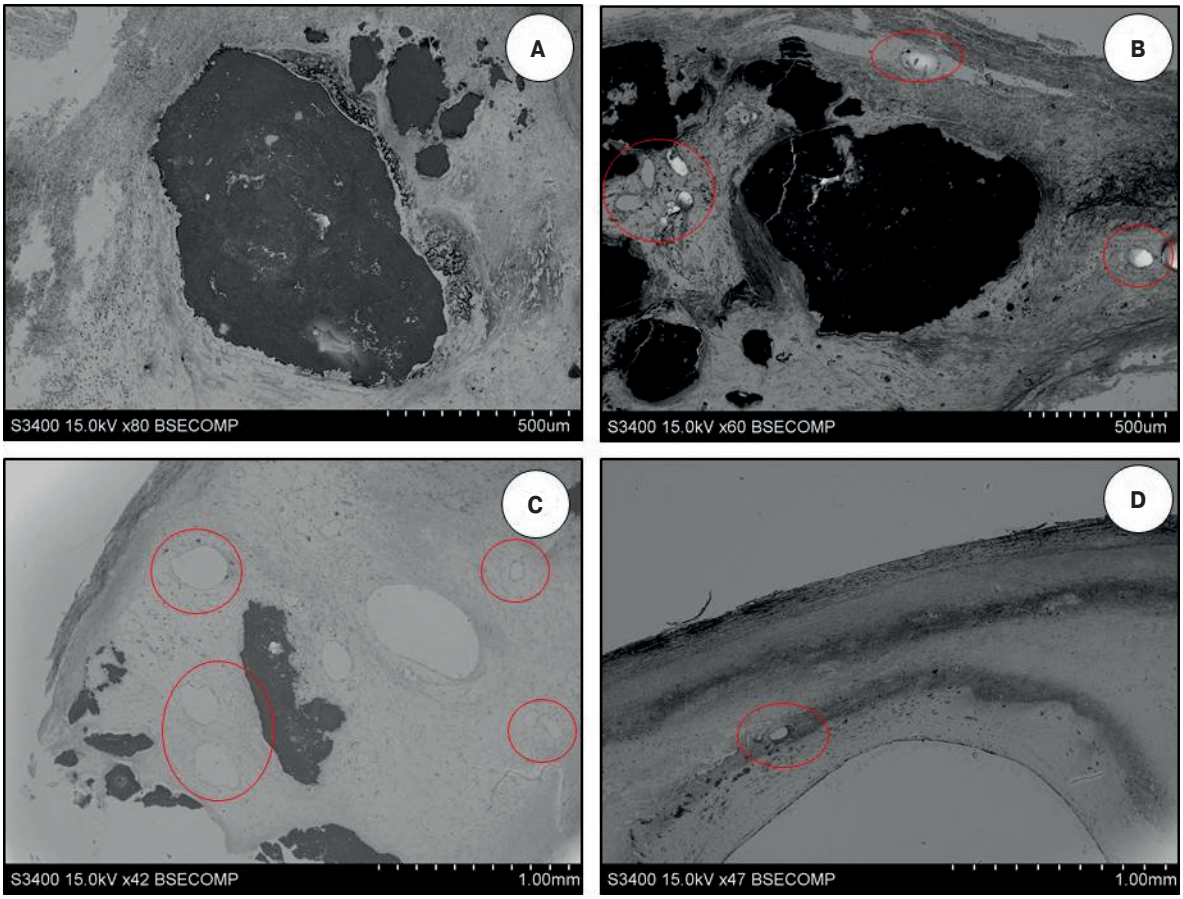

Figure 1. Interposition of neointimal vessels and calcifications in plaques. **A.** No blood vessels around calcifications; **B.** Large number of vessels around the calcification; **C.** Large number of vessels both around the calcification and in the plaque; **D.** No calcium, in the presence of newly formed vessels. New neointimal vessels are marked in red.

(22400-2, Electron Microscopy Sciences) in 95% ethanol (5 h), dehydrated with 99m7% isopropanol (06-002, BioVitrum) for 5 h and acetone (150495, LenReaktiv) for 1 h, impregnated with acetone mixture and epoxy resin Epon (14120, Electron Microscopy Sciences) in a ratio of 1:1 (6 h), after which it was transferred into new epoxy resin portion (for 24 h) and then polymerized in FixiForm tools (40300085, Struers) at 60° C. After that, the samples in epoxy blocks were grinded and polished on the TegraPol-11 system (Struers). Contrasting with lead citrate (17810, Electron Microscopy Sciences) was performed according to Reynolds methods for 7 min by applying the solution to polished sample surface, followed by washing it with bi-distilled water. Then, epoxy carbon blocks were sprayed onto the polished surface using a vacuum coater (EM ACE200, Leica). The structure of samples was visualized by backscattered electron microscopy using a S-3400N (Hitachi) electron microscope in the BSECOMP mode at an accelerating voltage of 10 kV.

The number and area of neointimal calcifications and vessels were analyzed both over the entire plaque and immediately around calcifications (one per plaque). Area of neointimal calcifications and vessels were estimated using the ImageJ software (National Institutes of Health). Statistical processing and graphical presentation of the results were performed using the GraphPad Prism 7 software (GraphPad Software). Due to the insufficient distribution of sample size for assessing the distribution normality, the data were described by nonparametric criteria (median and interquartile range), while intergroup comparison was performed using the Mann-Whitney U test. Correlation analysis was performed using the Spearman's rank correlation coefficient. Differences were considered significant at $p \leqslant 0,05$.

Results

At the initial phase, when studying the plaque ultrastructure by scanning electron microscopy, the main interposition types of neointimal calcifications and vessels were established (Figure 1). This made it possible to further reveal the features of plaque calcification and vascularization in different phenotypes. First of all, the hypothesis about the stabilizing effect

Table 1

Sex and age characteristics, comorbidities and pharmacological history of the included subjects

Patient group/studied cofactor	Patients with chronic cerebral ischemia	Patients with ischemic stroke	P
Sex and age characteristics			
Male gender	26/44 (59,09%)	31/44 (70,46%)	0,37
Age	67,0 (61,0-73,7)	64,50 (59,25-70,0)	0,07
Comorbidities or pathological conditions			
Hypertension	41/43 (95,3%)	39/41 (95,1%)	0,64
Heart failure	36/43 (83,7%)	37/41 (90,2%)	0,57
Chronic obstructive pulmonary disease or asthma	3/43 (7,0%)	7/41 (17,1%)	0,27
Smoking	2/43 (4,6%)	6/41 (14,6%)	0,24
Chronic kidney disease	4/43 (9,3%)	4/41 (9,8%)	0,76
Diabetes	10/43 (23,2%)	13/41 (31,7%)	0,53
Overweight	25/43 (58,1%)	20/41 (48,8%)	0,52
Obesity	5/43 (11,6%)	9/41 (22,0%)	0,33
Quantitative parameters			
Body mass index, kg/m^2	27,6 (24,2-32,0)	26,3 (24,6-32,8)	0,88
Glomerular filtration rate, ml/min/1,73 m^2	73,0 (60,0-82,0)	77,0 (66,0-91,5)	0,13
Left ventricular ejection fraction, %	64,0 (60,5-65,5)	65,0 (64,0-67,0)	0,10
Percentage of extracranial artery stenosis	75,0 (70,0-83,5)	86,0 (75,5-95,0)	0,01
Medical history before hospital admission			
Antiplatelet agents	25/43 (58,1%)	15/41 (36,6%)	0,08
Beta-blockers	16/43 (37,2%)	13/41 (31,7%)	0,76
Angiotensin-converting enzyme inhibitors	7/43 (16,3%)	3/41 (7,3%)	0,35
Statins	29/43 (67,4%)	14/41 (34,1%)	0,01
Nitrates	0/43 (0,0%)	0/41 (0,0%)	0,91
Angiotensin II receptor blockers	16/43 (37,2%)	5/41 (12,2%)	0,02
Aldosterone antagonists	2/43 (4,7%)	1/41 (2,4%)	0,96
Calcium channel blockers	14/43 (32,5%)	5/41 (12,2%)	0,05
Diuretics	0/43 (0,0%)	1/41 (2,4%)	0,97
Anticoagulants	5/43 (11,6%)	1/41 (2,4%)	0,23

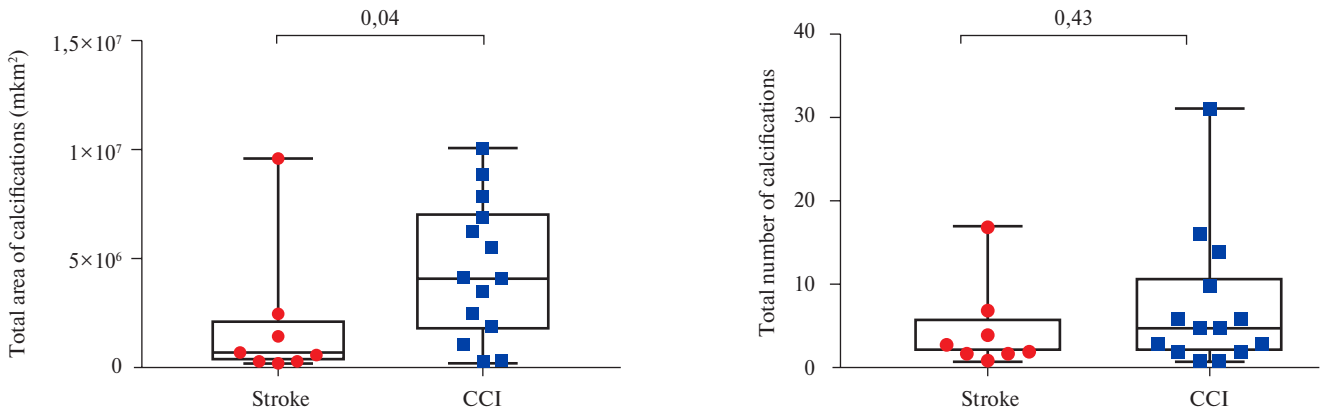

Figure 2. Total area of calcifications (left) and total number of calcifications (right) in the plaque of patients with stroke and CCI. Mann-Whitney test. P values are shown above the graphs.
Abbreviation: CCI — chronic cerebral ischemia.

Table 2

Correlation matrix for assessing the relationship between plaque volume, its blood supply and calcification

Spearman's rank correlation coefficient (r; P)							
	Stenosis percentage	Neointimal vessels number	Neointimal vascular area	Neointimal vessels number around Ca	Neointimal vascular area around Ca	Total Ca area	Number of calcifications
Stenosis percentage		0,41; 0,13	0,33; 0,23	-0,08; 0,83	-0,21; 0,54	-0,41; 0,18	-0,27; 0,39
Neointimal vessels number	0,41; 0,13		0,94; 0,0001	0,70; 0,0005	0,65; 0,001	0,53; 0,02	0,58; 0,007
Neointimal vascular area	0,33; 0,23	0,94; 0,0001		0,70; 0,0004	0,68; 0,001	0,58; 0,008	0,60; 0,006
Neointimal vessels number around Ca	-0,08; 0,83	0,70; 0,0005	0,70; 0,0004		0,96; 0,0001	0,63; 0,003	0,54; 0,01
Neointimal vascular area around Ca	-0,21; 0,54	0,65; 0,001	0,68; 0,001	0,96; 0,0001		0,56; 0,01	0,52; 0,02
Total Ca area	-0,41; 0,18	0,53; 0,02	0,58; 0,008	0,63; 0,003	0,56; 0,01		0,69; 0,0004
Number of calcifications	-0,27; 0,39	0,58; 0,007	0,60; 0,006	0,54; 0,01	0,52; 0,02	0,69; 0,0004	

Note: significant correlations are highlighted.
Abbreviation: Ca — representative calcification.

of calcification was tested, given the high prevalence and severity of comorbidities in patients (Table 1). In some works, it was shown that calcification in general helps to stabilize plaque, protecting it from rupture, however, the predominance of microcalcifications over macrocalcifications leads to the opposite effect [8]. Electron microscopy revealed that an increase in the total area, but not in calcifications number (Figure 2), has a stabilizing effect on plaques in patients with ECA atherosclerosis, which confirms this data [9-11]. Thus, it can be concluded that severe calcification prevents plaque rupture and specifies its stable phenotype.

However, the mechanism of plaque stabilization by calcification remains unclear. Quantitative image analysis found that the total area (but not the total number) of calcifications negatively correlated with vascular stenosis degree (r=-0,41) (Table 2), which was higher in unstable plaques (stroke) than in stable (CCI) (Figure 3). It should be noted that the stenosis degree also positively correlated (r=0,41) (Table 2) with the number of neointimal vessels, which reflects the volume of plaque blood delivery and, according to the literature, also contributes to its growth and rupture [12]. One may conclude that the morphological substrate of calcification stabilizing effect is the containment of plaque from growing into the vessel lumen, which sooner or later will lead to neointimal instability and fibrous cap rupture.

Further, the task was set to investigate neointimal calcification progression. The number and area of

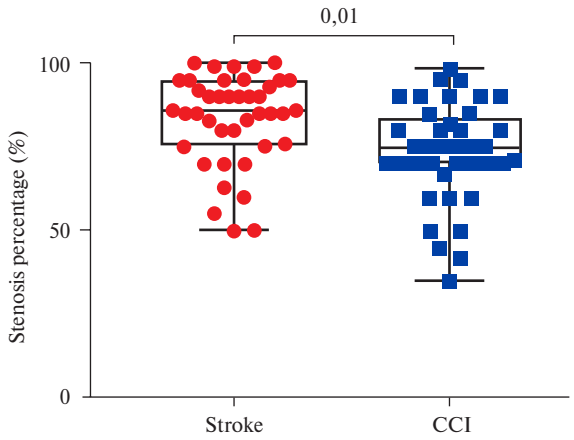

Figure 3. Stenosis percentage in patients with stroke and CCI. Mann-Whitney test. P value is shown above the graph.
Abbreviation: CCI — chronic cerebral ischemia.

calcifications, regardless of its phenotype, correlated with the total blood supply to neointima (r=0,53-0,60) (Table 2) and with the direct blood supply near the representative calcification (r=0,52-0,63) (Table 2). The total number and area of neointimal vessels strongly correlated with each other (r=0,94) (Table 2) and with the number and area of vessels around calcifications (r=0,65-0,70) (Table 2), which confirms the validity of using both of these measures to assess the plaque blood delivery. Based on the above, one may assume that the general and local (near the calcification) plaque blood circulation causes the active calcification progression (Figure 4).

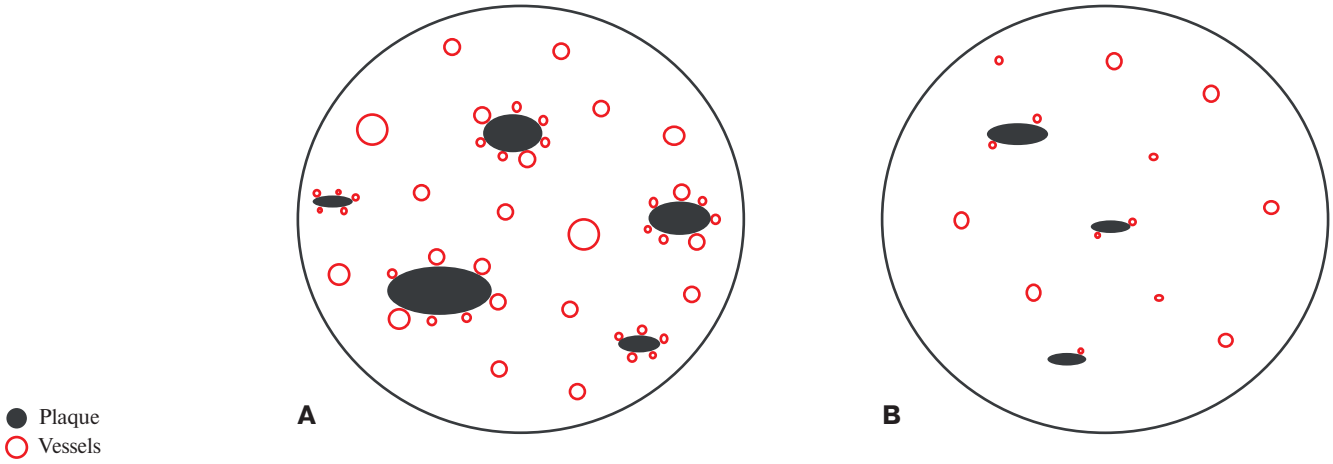

Figure 4. Arrangement of neointimal vessels in the plaque. **A** — calcifying phenotype, **B** — non-calcifying phenotype.

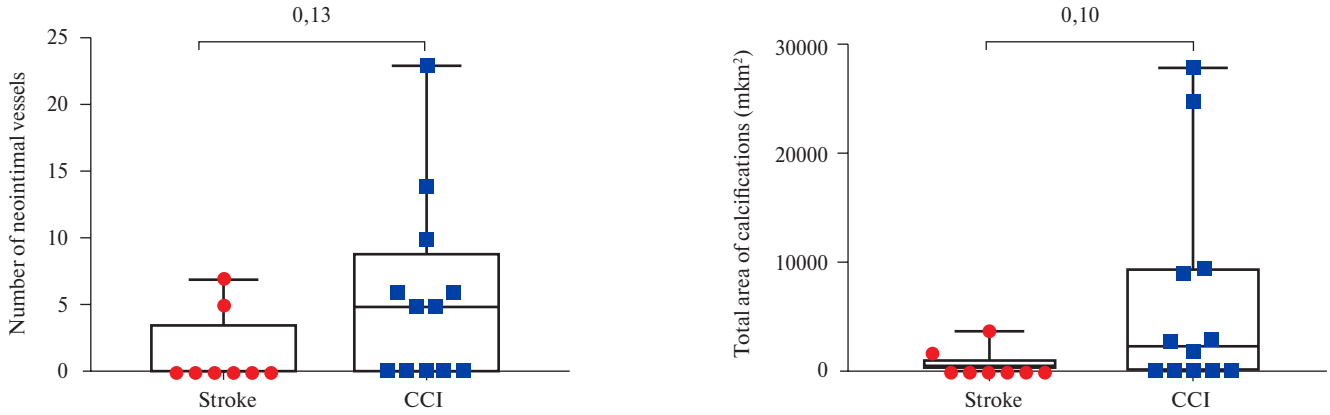

Figure 5. Total number of neointimal vessels around the representative calcification (left) and the total area of neointimal vessels around the representative calcification (right). Mann-Whitney test. P values are shown above the graphs.
Abbreviation: CCI — chronic cerebral ischemia.

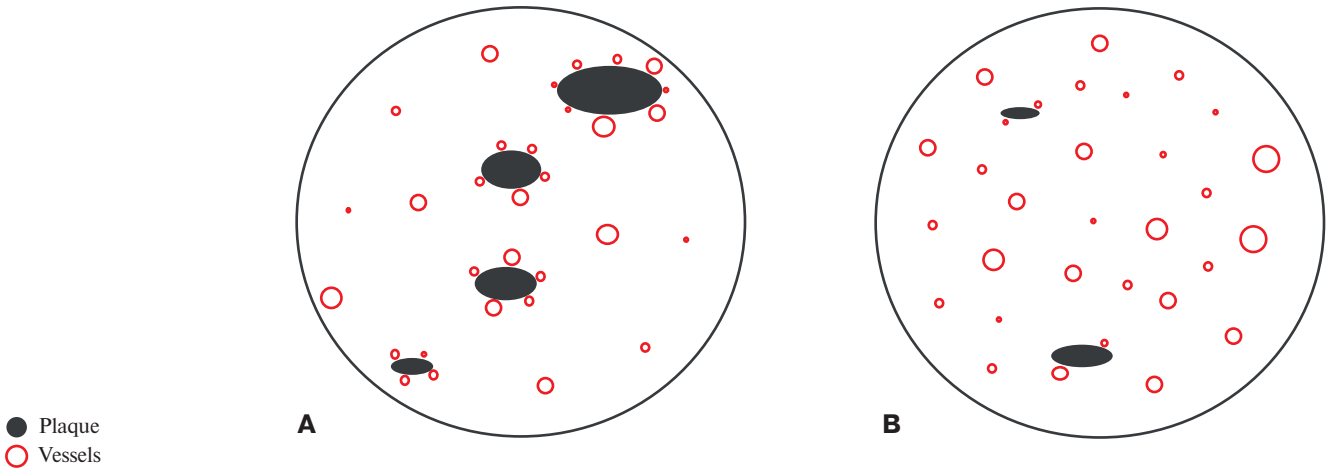

Figure 6. Arrangement of neointimal vessels in the plaque. **A.** Stable phenotype. **B.** Unstable phenotype.

Thus, the following paradox arises: the calcification contributing to plaque stabilization progresses due to vascularization, which, however, is associated with an unstable plaque phenotype. To explain this paradox, we studied the association of the extent of plaque general and local blood supply with its stability/instability. It was found that active blood delivery near calcification is associated with a stable plaque phenotype (Figure 5), in contrast to the general one, which is associated with plaque growth

leading to fibrous cap rupture. Therefore, satisfactory local blood supply to neointimal calcifications contributes to its enlargement and plaque stability, while poor general blood supply contributes to plaque instability (Figure 6).

Finally, it was investigated whether plaque calcification was associated with above mineral homeostasis parameters. Both the total area and the number of plaque calcifications negatively correlated with levels of total protein (-0,37 and -0,38, respectively) and albumin (-0,34 and -0,40, respectively), reflecting the neointimal calcification during depletion of calcium ion depot. At the same time, the total calcification area also negatively correlated with phosphorus level (-0,48), and the number of calcifications — with the total calcium level (-0,38), which may indicate a partial transition of these ions from serum to ectopic calcifications (plaques).

Discussion

It has been proven that neointimal calcification is a long-term, complex and multifactorial process that plays one of the key roles in atherogenesis, while certain calcification phenotypes are a predictor of plaque progression [13]. A in-depth study of the determinants of stable and unstable atherosclerotic phenotypes made it possible to reveal the factors contributing to plaque stabilization. According to the literature, an increase in the total calcification area contributed to stabilizing effect [9-11, 14]. This was revealed in this study as well. Therefore, one may assume that the calcification severity determines the stable plaque phenotype and prevents its rupture. The stabilizing effect of calcification is manifested in a decrease in stenosis degree by restraining plaque from growing into the vessel lumen.

New neointimal vessels saturate the plaque with oxygen and nutrients necessary for its metabolism, but at the same time they also contribute to plaque proliferation, thereby increasing the stenosis degree. Some authors showed that active blood supply to the carotid plaque aggravates the clinical prognosis and leads to an increased risk of ischemic stroke [12]. As the plaque grows, the ECA stenosis degree increases. With a decrease in the vessel lumen, the transmural pressure in new neointimal vessels increases, which destabilizes the hemodynamic response of the plaque to blood pressure changes and leads to its even greater expansion during systole [12]. According to literature data, the network of newly formed vessels occupies up to 14% of neointimal tissues [12]. These vessels are characterized by frequent intraplaque hemorrhage and an increased pressure gradient in the neointimal tissues due to a lumen decrease and transverse pulsating movements of the neointimal vessels [12].

A detailed study of neointimal blood delivery made it possible to establish that its vascularization contributes not only to plaque growth, but also to calcification progression. When studying the role of vascularization in plaque calcification, attention should be paid to location of intraplaque vessels. The active general neointimal blood supply is the most characteristic of the unstable phenotype (stroke). At the same time, active local blood supply promotes the calcification growth and, consequently, the plaque stabilization and the development of a stable phenotype (CCI).

At a certain stage of plaque development, neointimal cells die due to impaired homeostasis and a lack of oxygen and nutrients with a critical extracellular matrix thickening. Plaque phagocytes cease to efficiently process dead cells [15]. The starting point for calcification onset can be the deposition of microscopic calcium granules on the remains of dead cells and necrotic nucleus of plaques. Other important aspects of calcification are osteogenic differentiation of vascular smooth muscle cells and high activity of phosphate-generating enzymes, in particular, alkaline phosphatase [14]. It can be assumed that the proliferation of neointimal vessels in the area of newly formed calcification contributes to constant delivery of mineral ions and bioactive substances necessary for calcification.

Conclusion

An in-depth study of the relationships between the ECA plaque phenotypes revealed that the stable phenotype (CCI) is characterized by neointimal calcification (contributing to a decrease in stenosis degree) and active local blood supply directly in the area of macrocalcification. The active general neointimal blood supply contributes to a non-calcifying unstable phenotype (stroke), which contributes to the rapid plaque proliferation into the vessel lumen, the progression of atherosclerosis and development of adverse events. Neointimal blood supply specifics significantly effect the calcification processes, and various types of neointimal calcification make a significant contribution to plaque phenotype formation.

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References

1. Każmierski P, Pająk M, Kruś-Hadała J, et al. Screening test for extracranial carotid lesions' detection in patients of an outpatient vascular clinic. Pol Przegl Chir. 2019;91(5):5-11. doi:10.5604/01.3001.0013.4520.
2. Ragino Yul, Volkov AM, Chernyavskiy AM. Stages of atherosclerotic plaque development and unstable plaque types: pathophysiologic and histologic characteristics. Russian Journal of Cardiology. 2013;18(5):88-95. (In Russ.)
3. Nicoll R, Henein M. Arterial calcification: A new perspective? Int J Cardiol. 2017;228:11-22. doi:10.1016/j.ijcard.2016.11.099.
4. Mukhamadiyarov RA, Kutikhin AG. Structure of calcificates in human carotid artery atherosclerotic plaques by means of backscattered scanning electron microscopy. Atherosclerosis. 2020;16(2):5-15. (In Russ.)
5. Mukhamadiyarov RA, Kutikhin AG. Backscattered scanning electron microscopy approach to assess microvessels in health and disease. Bulletin of Experimental Biology and Medicine. 2020;169(4):514-20. (In Russ.)
6. Mukhamadiyarov RA, Kutikhin AG. Histology and histopathology of blood vessels: backscattered scanning electron microscopy approach. Fundamental and Clinical Medicine. 2019;4(1):6-14. (In Russ.)
7. Galis ZS, Lessner SM. Will the real plaque vasculature please stand up? Why we need to distinguish the vasa plaquorum from the vasa vasorum. Trends Cardiovasc Med. 2009;19(3):87-94. doi:10.1016/j.tcm.2009.06.001.
8. Shioi A, Ikari Y. Plaque Calcification During Atherosclerosis Progression and Regression. J Atheroscler Thromb. 2018;25(4):294-303. doi:10.5551/jat.RV17020.
9. Jinnouchi H, Sato Y, Sakamoto A, et al. Calcium deposition within coronary atherosclerotic lesion: Implications for plaque stability. Atherosclerosis. 2020;14:S0021-9150(20)30291-4. doi:10.1016/j.atherosclerosis.2020.05.017.
10. Otsuka F, Sakakura K, Yahagi K, et al. Has our understanding of calcification in human coronary atherosclerosis progressed? Arterioscler Thromb Vasc Biol. 2014;34(4):724-36. doi:10.1161/ATVBAHA.113.302642.
11. Karlöf E, Seime T, Dias N, et al. Correlation of computed tomography with carotid plaque transcriptomes associates calcification with lesion-stabilization. Atherosclerosis. 2019;288:175-85. doi:10.1016/j.atherosclerosis.2019.05.005.
12. Xu C, Yuan C, Stutzman E, et al. Quest for the Vulnerable Atheroma: Carotid Stenosis and Diametric Strain — A Feasibility Study. Ultrasound Med Biol. 2016;42(3):699-716. doi:10.1016/j.ultrasmedbio.2015.11.002.
13. Yahagi K, Kolodgie FD, Lutter C, et al. Pathology of Human Coronary and Carotid Artery Atherosclerosis and Vascular Calcification in Diabetes Mellitus. Arterioscler Thromb Vasc Biol. 2017;37(2):191-204. doi:10.1161/ATVBAHA.116.306256.
14. Akers EJ, Nicholls SJ, Di Bartolo BA. Plaque Calcification: Do Lipoproteins Have a Role? Arterioscler Thromb Vasc Biol. 2019;39(10):1902-10. doi:10.1161/ATVBAHA.119.311574.
15. Bentzon JF, Otsuka F, Virmani R, et al. Mechanisms of plaque formation and rupture. Circ Res. 2014;114(12):1852-66. doi:10.1161/CIRCRESAHA.114.302721.

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Biochemical markers of coronary atherosclerosis: building models and assessing their prognostic value regarding the lesion severity

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Aim. To assess the individual and complex prognostic value of various blood biochemical parameters (biomarkers) in the non-invasive diagnosis of coronary artery (CA) atherosclerosis.

Material and methods. The study included 216 patients (men, 115; women, 101) aged 24 to 87 years (mean age, 61,5±10,7 years), who underwent indicated coronary angiography. All patients underwent a biochemical blood tests to determine the parameters of lipid, carbohydrate and nitrogen metabolism, the hemostatic system, inflammatory markers, as well as the creatinine level as an indicator of renal function.

Results. Analysis revealed biomarkers, the deviations in the level of which contribute to the diagnosis and determination of the coronary involvement. These biomarkers include glucose, creatinine, C-reactive protein, and adiponectin. Using these biochemical parameters, a multivariate model (MVM) was constructed, which was significant for the diagnosis of coronary atherosclerosis and determination of its severity. With the help of ROC-analysis, the cutoff point of MVM of 2 was found. MVM >2 with a sensitivity of 72% indicate CA atherosclerosis of any severity, as well as with a specificity of 62,5%, it can be ruled out. Using MVM data and a cutoff point of 2, a binary logistic regression model was built, according to which, with a MVM >2, the odds for detecting CA atherosclerosis of any degree is 2,1 times higher (95% confidence interval (CI), 1,2-3,8; p=0,010), severe CA — 4,7 times (95% CI, 1,9-12,0; p=0,001) compared with individuals with MVM ≤2, who have 2,8 times (95% CI, 1,4-4,9; p=0,002) a higher chance of detecting intact CAs.

Conclusion. Thus, the total MVM score of 0-2 indicates the absence of coronary atherosclerosis, while 3-4 points –CA atherosclerosis of any severity.

Keywords: atherosclerosis, biochemical parameters, biochemical models, coronary arteries, risk factors.

Relationships and Activities: none.

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Coronary artery (CA) atherosclerosis is the pathological basis of ischemic heart disease, the prevalence and mortality from complications of which remain high both in Russia and throughout the world [1]. One of the reasons for this is the late disease establishment. Therefore, the search for early-stage markers of CA atherosclerosis remains an urgent area of modern cardiology.

Impaired function of some human body systems (lipid-transport, carbohydrate, hemostasis systems), chronic inflammation and related changes in blood parameters are a stimulus for an active search and study of early-stage biomarkers of atherogenesis. It has long been noted that the analysis of blood biochemical parameters as criteria reflecting various pathophysiological pathways of atherogenesis can improve the prediction of cardiovascular risk (CVR) [2].

Numerous studies considered various blood parameters, which make it possible to assess the relationship between changes in their blood content both with atherosclerosis in general and specifically with CA atherosclerosis [3, 4]. For a long time, lipid metabolism indicators were considered the main markers of atherosclerosis [5]. However, atherosclerotic cardiovascular events also develop with normal lipid profile [6]. Therefore, the researchers' attention is now attracted by other biochemical blood parameters, reflecting the relationship of their deviations from the norm. Moreover, due to the widespread use of statins and other lipid-lowering drug classes, the interpretation of lipid metabolism without an effect on lipid-transport system is difficult.

It should be noted that the contribution of most blood biomarkers to atherosclerosis prediction was studied separately for each indicator. At the same time, there is evidence that the use of their combination can increase the prognostic value and improve CVR stratification [7, 8]. The literature presents several studies combining imaging and circulating markers or involving the use of circulating, genetic and/or imaging markers (Framingham Heart Study, Malmö Diet and Cancer Study, MORGAN, Cardiovascular Health Study) [8-10]. Previously, we analyzed mathematical models including various combinations of blood biochemical parameters with each other, as well as with imaging plaque characteristics obtained by carotid duplex scanning and risk factors (RFs) of cardiovascular disease (CVD) [11]. Based on the study of a wide range of blood biochemical parameters and imaging markers, analysis of their various combinations, a novel indicator was developed, called an integrative biomarker (i-BIO), which reflects the total contribution of a complex of biochemical, clinical and instrumental markers to presence and severity of CA atherosclerosis. Following blood parameters were included in i-BIO: triglycerides (TG), glucose, fibrinogen (FG), high sensitivity C-reactive protein (hsCRP), and adiponectin. This set of biomarkers made it possible to diagnose severe CA atherosclerosis, however, the detection of subclinical atherosclerosis was less effective [11].

The aim of this study was to assess the individual and complex prognostic value of various blood biomarkers in the non-invasive diagnosis of CA atherosclerosis.

Material and methods

We analyzed a cohort of patients admitted to the National Medical Research Center for Therapy and Preventive Medicine in the period from 2016 to 2019, who, according to indications, underwent diagnostic coronary angiography (CAG). The study was carried out in accordance with the Declaration of Helsinki. The study protocol was approved by the Ethics Committee (№ 09-05/19). All patients signed informed consent for participation in the study, personal data processing, collection and biobanking of blood.

There were following inclusion criteria: patients aged >18 years who signed an informed consent for inclusion in the study, collection and biobanking of blood.

Exclusion criteria were as follows: acute clinical complication of atherosclerosis within prior 6 months; any acute inflammatory disease; stage ⩾III chronic kidney disease (glomerular filtration rate (GFR) <60 ml/min/1,73 m^2); decompensated type 1 and 2 diabetes (fasting blood glucose >11 mmol/l); left ventricular ejection fraction <40%; cancer; blood and immune system diseases, pregnancy or lactation.

All patients underwent CAG by the Judkins technique (1967) [12] using radial or transfemoral access on angiographic x-ray systems Philips Integris Allura and General Electric Innova 4100. For the quantitative assessment of stenosis, the computer program of General Electric Innova 4100 system was used.

The patients were admitted to the hospital for CAG for various indications, such as:

— Sternum or left-sided chest pain (presumably coronary origin); impossibility, contraindications or refusal of a patient to perform stress tests and CA multislice computed tomography (MSCT);

— Positive or questionable results of exercise stress tests (treadmill or stress echocardiography) or CA stenosis >50% according to MSCT;

— Abnormalities revealed by conventional electrocardiography (ECG) or 24-hour Holter monitoring (presumably ischemic origin) and the impossibility, contraindications or refusal of a patient to perform stress tests and CA MSCT;

— Patients who underwent inpatient treatment at the National Medical Research Center for Therapy and Preventive Medicine, who meet the inclusion criteria and agreed to participate in clinical testing, the protocol of which implies CAG;

— Professional activity peculiarities (professions with an increased risk for other people).

All patients underwent a biochemical blood tests. Determination of blood parameters was carried out in serum or plasma obtained by standard methods from venous blood taken after 12-hour fasting. The standardization and quality control was carried out in accordance with the requirements of the "Federal system for external quality control of clinical laboratory procedures".

The concentration of total cholesterol (TC), TG and high-density lipoprotein cholesterol (HDL-C) was determined by the enzymatic assay using Abbott reagents on an Architect C8000 Clinical Chemistry Analyzer (Abbot Diagnostics, USA). The concentration of low-density lipoprotein cholesterol (LDL-C) was estimated using the Friedewald equation at a TG level ⩽4,5 mmol/l: LDL-C = TC - (HDL-C+TG/2,2) mmol/l. The cholesterol concentration not included in HDL (non-HDL cholesterol) was calculated as the difference: TC - HDL-C. The level of lipoprotein(a) (Lp(a)) was determined on a Sapphire-400 analyzer (Japan) using enzyme kits. The concentration of the main proteins LDL and HDL (apolipoproteins (apo) B and apo AI) was determined on the same analyzer using DiaSys diagnostic kits. When assessing the lipid metabolism parameters, the normal ranges adopted in the 2019 ESC/EAS Guidelines for the management of dyslipidaemias were used [13].

The serum glucose concentration was determined by the glucose oxidase assay on an Architect C 8000 analyzer using Abbott reagents, and the insulin level was determined by Chemiluminescent immunoassay on an Architect i2000SR analyzer. An elevated glucose level was considered ⩾6,1 mmol/L, insulin — ⩾14,0 μU/ml [14].

The levels of adiponectin and leptin were determined using an enzyme-linked immunosorbent assay: adiponectin (BioVendor, Czech Republic), leptin (Diagnostic Biochem Canada Inc., Canada). Reduced adiponectin level was considered <8,0 μg/ml [11], which coincided with the data obtained in this study (ROC-analysis); an elevated leptin level was considered ⩾18 ng/ml, which corresponds to the median distribution of presented cohort for this indicator.

The CRP level was determined by a high-sensitivity turbidimetric immunoassay based on the interaction of test sample CRP with specific anti-CRP antibodies using an Architect C8000 analyzer. Levels of hsCRP ⩾1,0 mg/L were considered elevated [15].

The plasma FG level was determined by the Claus method. The measurements were carried out on an ACL Elite automatic coagulometer (USA) with Hemosil reagents (USA). An elevated level was considered the upper quartile for presented cohort >4,0 g/L.

To determine the serum content of stable nitric oxide metabolites (nitrates and nitrites (NO_X)), at the first stage, the blood serum was deproteinized by centrifugation. The NO_X concentration was determined spectrophotometrically using a Multiskan MCC/340 system (LabSystems, Finland) [16]. NO_X <36 μmol/L was considered reduced, which corresponds to the cut-off point found using the ROC analysis in our cohort of patients.

Creatinine levels were determined using enzyme-linked immunosorbent assay. The measurements were carried out on an Architect C 8000 analyzer. The range of values from 70 to 110 μmol/L is taken as a normal creatinine level according to international standards. In our study, according to the cut-off point found by ROC analysis, the creatinine level ⩾73 μmol/L was considered elevated.

Statistical analysis. Statistical analysis of results was carried out using the statistical software packages Statistica v.10 and SPSS v.20. Depending on the type of distribution of continuous variables, the arithmetic mean with standard deviation (total cholesterol, LDL-C, HDL-C, non-HDL cholesterol, apo AI, apo B, FG, glucose, creatinine) or the median and interquartile range, indicating maximum and minimum values (TG, Lp(a), NO_X, hsCRP, insulin, adiponectin, leptin) was estimated. To evaluate the cut-off points of continuous variables, ROC analysis was used with the creation of curves to determine the sensitivity and specificity of the test. The threshold level was determined by a combination of sensitivity and specificity values at curve intersection, giving 100% in total. A binary logistic regression was used to estimate odds ratios and 95% confidence interval (CI). Differences were considered significant at $p<0,05$.

Results

The study included 216 patients: 115 men and 101 women aged 24 to 87 years (mean age, 61,5±10,7 years), who were selected in accordance with the inclusion criteria in one of the following groups:

Group 1 (n=73) — asymptomatic patients without CA atherosclerosis (intact CA);

Group 2 (n=71) — asymptomatic patients with subclinical CA atherosclerosis (CA stenosis ⩽50%);

Group 3 (n=72) — symptomatic patients with severe CA atherosclerosis (stenosis in two or more CAs with hemodynamically relevant involvement of

Table 1

Biochemical blood parameters depending on CA involvement degree

Biochemical parameters	Group		
	Group 1 (intact CA) (n=73)	Group 2 (subclinical CA atherosclerosis) (n=71)	Group 3 (severe atherosclerosis of CA) (n=72)
Lipid metabolism parameters			
Total cholesterol, mmol/l	4,5±1,15	4,7±1,00	3,9±0,98[b,c]
LDL-C, mmol/l	2,6±0,98	2,8±0,91	2,2±0,84[b,c]
HDL-C, mmol/l: Men Women	 1,0±0,22 1,3±0,33	 1,2±0,33 1,3±0,28	 1,0±0,33[c] 1,1±0,21[b,c]
Non-HDL cholesterol, mmol/l	3,3±1,07	3,4±0,9	2,8±0,92[b,c]
TG, mmol/l	1,4 [1,00; 1,99]; (0,11-4,85)	1,3 [0,84; 1,74]; (0,38-3,62)	1,4 [1,1; 1,75]; (0,60-5,72)
Lp(a), mg/dl	15,9 [7,31; 36,8]; (0,9-229,5)	16,9 [6,82; 33,89]; (0,9-241,27)	15,2 [5,84; 54,21]; (1,46-169)
Apo AI, mg/dl	157±32,12	159±26,82	136±25,9[b,c]
Apo B, mg/dl	86±23,61	92±23,73	84±22,43
Hemostatic parameters			
Fg, g/l	4,6±1,42	4,5±0,87	5,2±1,42[b,c]
Inflammatory markers			
hsCRP, mg/l	2,3 [0,88; 5,37]; (0,14-186,2)	2,2 [1,38; 3,62]; (0,28-29,8)	4,5 [2,14; 10,05][b,c]; (0,52-136,69)
Carbohydrate metabolism markers			
Glucose, mmol/l	5,9±1,37	6,3±1,47	6,9±2,0[b,c]
Insulin, μU/L	8,9 [6,07; 12,45]; (1,30-73,80)	10,0 [6,9; 15,05]; (1,00- 81,00)	11,0 [8,20; 17,65]; (4,60-50,60)
Metabolism parameters of visceral adipose tissue			
Adiponectin, mg/ml	7,7 [5,97; 10,55]; (4,19-23,20)	8,8 [7,02; 11,65]; (1,10-56,30)	6,8 [4,73; 10,40][b,c]; (2,13-22,70)
Leptin, ng/ml	19,4 [5,8; 57,3]; (0,00-182,0)	27,2 [4,98; 73,2]; (0,33-225,0)	10,5 [4,0; 37,0]; (0,75-139,0)
End product of creatine phosphate reaction			
Creatinine, μmol/l	75,4±22,68	77,7±17,22	93,0±32,98[b,c]
NO metabolites			
NO_x, μmol/l	41,8 [42,04; 60,00]; (12,64-222,68)	30,0 [24,46; 42,06][a]; (13,91-132,12)	30,5 [25,18; 43,93][b,c]; (15,60-115,72)

Note: p<0,05: [a] — between 1 and 2 groups.; [b] — between 1 and 3 groups.; [c] — between 2 and 3 groups. The interquartile range and minimum and maximum values are indicated in square brackets and parentheses, respectively.

Abbreviations: apo — apolipoproteins, hsCRP — high-sensitivity C-reactive protein, CA — coronary arteries, Lp(a) — lipoprotein(a), LDL — low density lipoproteins, HDL — high density lipoproteins, TG — triglycerides, FG — fibrinogen, NO_x — nitric oxide metabolites.

one and/or several vessels). Hemodynamically relevant stenoses were considered as follows [17]:

- stenosis >50% in any CA with imaging data confirming myocardial ischemia in the area of corresponded affected vessel;
- stenosis >90% in any CA even without ischemia confirmation by imaging methods.

Table 1 shows the serum biochemical parameters in groups with different CA involvement.

Analysis presented in Table 1 showed that in the group with severe CA atherosclerosis (group 3), the lipid profile was less atherogenic compared to patients with intact CA (group 1) and subclinical coronary artery disease (group 2), which, most likely, is due to statin therapy. Indeed, 93% of group 3 patients took statins, 50,7% — group 2, and 26% — group 1. At the same time, HDL-C cholesterol and apo AI levels in women with severe CA atherosclerosis were lower than in other patients, and in men — lower than in patients with subclinical CA involvement. The levels of FG and hsCRP, as inflammation indicators, were higher in patients with severe coronary atherosclerosis in comparison with patients without hemodynamically significant CA atherosclerosis (group 1 and 2).

Glucose and insulin values, reflecting the carbohydrate metabolism activity, was higher in patients with any CA atherosclerosis (group 2 and 3), compared with those without coronary atherosclerosis (group 1). Among visceral adipose tissue

Table 2

Univariate and multivariate logistic regression models for determining coronary atherosclerosis of any severity (groups 2 and 3)

Biochemical parameters	Univariate analysis		Multivariate analysis	
	OR (95%; CI)	p	OR (95%; CI)	p
Glucose, mmol/l	2,44 (1,34-4,47)	0,004	2,17 (1,15-4,11)	0,017
Creatinine, μmol/l	2,37 (1,33-4,22)	0,003	2,45 (1,35-4,52)	0,004
hsCRP, mg/l	2,80 (1,36-5,76)	0,005	2,83 (1,37-6,41)	0,008
Adiponectin, μg/ml	0,71 (0,40-1,25)	0,232	0,51 (0,27-0,96)	0,037

Note: glucose, mmol/L ⩾6,1 (1), <6,1 (0); creatinine, μmol/l ⩾73 (1), <73 (0); hsCRP, mg/l ⩾1 (1), <1 (0); adiponectin, μg/ml <8 (1), ⩾8 (0).
Abbreviations: CI — confidence interval, hsCRP — high-sensitivity C-reactive protein, OR — odds ratio.

Table 3

Univariate and multivariate logistic regression models for determining severe coronary atherosclerosis (group 3)

Biochemical parameters	Univariate analysis		Multivariate analysis	
	OR (95%; CI)	p	OR (95%; CI)	p
Glucose, mmol/l	2,87 (1,60-5,15)	0,000	2,41 (1,31-4,43)	0,005
Creatinine, μmol/l	3,03 (1,63-5,62)	0,000	2,79 (1,47-5,33)	0,002
hsCRP, mg/l	3,83 (1,42-10,39)	0,008	3,48 (1,24-9,77)	0,018
Adiponectin, μg/ml	1,61 (0,91-2,85)	0,103	1,33 (0,72-2,45)	0,368

Note: glucose, mmol/L ⩾6,1 (1), <6,1 (0); creatinine, μmol/l ⩾73 (1), <73 (0); hsCRP, mg/l ⩾1 (1), <1 (0); adiponectin, μg/ml <8 (1), ⩾8 (0).
Abbreviations: CI — confidence interval, hsCRP — high-sensitivity C-reactive protein, OR — odds ratio.

metabolism parameters, adiponectin and leptin were considered. The highest leptin values were obtained in group 2, and the lowest — in group 3. Creatinine levels increased from group 1 to 3. Lower NO_X values were obtained in patients with subclinical CA atherosclerosis, and higher values — in those without CA atherosclerosis compared with the group of severe CA atherosclerosis.

To determine the contribution of blood parameters to the likelihood of CA atherosclerosis and its severity, logistic regression was used; at the same time, significant differences between the groups in the analyzed blood parameters were taken into account in univariate and multivariate models (Table 1).

Univariate analysis with the inclusion of above biochemical parameters revealed that an independent contribution to the diagnosis of severe coronary atherosclerosis (group 3) is made by an increased levels of HDL-C >1 mmol/L in men (p=0,006) and >1,2 mmol/L in women (p=0,002), non-HDL cholesterol >2,2 mmol/L (p<0,001), glucose ⩾6,1 mmol/L (p=0,000), hsCRP ⩾1 mg/L (p=0,008) and creatinine ⩾73 μmol/L (p<0,001). When diagnosing CA atherosclerosis of any severity, the following parameters maintain its value in univariate models: glucose ⩾6,1 mmol/L (p=0,004), hsCRP ⩾1 mg/L (p=0,005), creatinine ⩾73 μmol/L (p=0,003), and NO_X <36 μmol/L (p=0,001). However, multivariate models that take into account various combinations of lipid metabolism parameters, as well as their combinations with other biochemical indicators, turned out to be insignificant neither for detecting CA atherosclerosis, nor for determining its severity (p>0,05).

At the same time, models including glucose, creatinine, hsCRP, and adiponectin turned out to be significant. According to univariate analysis (Table 2), which determines the independent contribution of each parameter to the assessment of presence and severity of CA atherosclerosis, all included indicators, except for adiponectin, were significant.

The multivariate model, including the above parameters (Table 3), turned out to be significant both for the detection of atherosclerosis of any severity (group 1 and 2) (p<0,001), and the detection of severe coronary involvement (group 3) ($p=10^{-4}$).

Given the multifactorial etiology of atherosclerosis, the close conjugation of biomarkers with each other and their mutually potentiating effect, as well as the results of univariate and multivariate analysis, we have formed a multivariate biochemical model (MVM) that allows non-invasive diagnosis of CA atherosclerosis. The following biomarkers were included in the MVM: glucose, creatinine, hsCRP,

Table 4
MVM scoring of biochemical deviations

Biochemical parameters	Score
Glucose, mmol/l	⩾6,1 mmol/l — 1 <6,1 mmol/l — 0
Creatinine, μmol/l	⩾73 μmol/l — 1 <73 μmol/l — 0
hsCRP, mg/l	⩾1 mg/l — 1 <73 mg/l — 0
Adiponectin, μg/ml	<8 μg/ml — 1 ⩾8 μg/ml — 0

Abbreviation: hsCRP — high-sensitivity C-reactive protein.

Table 5
Determination of CA atherosclerosis severity according to MVM

MVM, total score	Group
0-2	Intact coronary arteries (no CA atherosclerosis)
3-4	CA atherosclerosis of any severity (plaques in CA ⩾20%)

Abbreviation: CA — coronary arteries.

adiponectin. The deviation from the norm of one biochemical parameter was estimated at 1 point. Thus, the total MVM can range from 0 to 4 points (Table 4).

The ROC analysis was used to assess the MVM significance for diagnosis of CA atherosclerosis; a cut-off point of 2 was found. MVM >2 points with a sensitivity of 72% indicated the CA atherosclerosis of any severity, and with a specificity of 62,5%, it was ruled out. Using the MVM data and a cutoff point of 2, a binary logistic regression model was built, according to which, with a MVM >2, the likelihood of detecting any CA atherosclerosis increases by 2,1 times (95% CI, 1,2-3,8; p=0,010), severe atherosclerosis — by 4,7 times (95% CI, 1,9-12,0; p=0,001). MVM ⩽2 points increased the likelihood of detecting intact CAs by 2,8 times (95% CI, 1,4-4,9; p=0,002). The interpretation of the results depending on total MVM score is presented in Table 5.

Discussion

The study of biomarkers of key pathophysiological mechanisms of atherogenesis is a promising direction, since their action is carried out at the molecular-cellular level and covers all stages of atherosclerosis development.

We considered markers of lipid and carbohydrate metabolism, hemostasis system, inflammatory mediators, metabolic products of visceral adipose tissue, and the end product of creatine-phosphate reaction.

The search for atherosclerosis markers, lipid metabolism parameters are traditionally studied. Despite the fact that about half of cardiovascular events occur in patients with normal lipid profile, dyslipidemia continues to occupy the leading place among CVD RFs, and its correction is the primary task of treating patients with high and very high CVR [13]. In this regard, we analyzed the main lipid profile parameters. It turned out that in the examined cohort of patients, a less atherogenic lipid profile was observed in patients with severe CA atherosclerosis (group 3), which is probably due to statin therapy. The created univariate and multivariate regression models, including various lipid metabolism indicators, as well as their combination with other biomarkers, was insignificant (p>0,05).

The analysis of relationship between disorders of insulin-dependent glucose uptake by cells showed that glucose >6,1 mmol/L and adiponectin <8 μg/ml reliably indicate CA atherosclerosis in patients. Our results are consistent with the literature data, which show that patients with atherosclerosis are significantly more likely to have metabolic disorders [18-20].

Taking into account the pathogenetic aspects of atherosclerosis associated with chronic inflammation, we examined such indicators as FG and hsCRP. These biomarkers were not chosen due to evidence-based medicine and practical significance [21-23]. According to research data, an increased FG level is significant diagnostic and prognostic marker of atherosclerosis, in which a number of pronounced hemostatic disorders are associated with complications and higher cardiovascular mortality [24, 25]. According to literature data, the level of hsCRP is independently associated with atherosclerosis severity and stenosis area. However, the rationale of using hsCRP to detect subclinical atherosclerosis is not fully clear [23]. A meta-analysis of 22 studies showed that hsCRP concentration ⩾3,0 mg/L was associated with a 60% probability with coronary artery disease development. At the same time, for cardiovascular risk stratification, hsCRP ⩾1,0 mg/l is considered as high [24]. The study results showed a significant relationship between FG and hsCRP not only with the presence, but also with the severity of CA atherosclerosis.

Chronic kidney disease is also associated with an increased CVD risk independently of other RFs. Decreased GFR increases the cardiovascular death risk. According to the European clinical guidelines on cardiovascular disease prevention in clinical practice [25], in the presence of chronic kidney disease with

GFR <30 ml/min, a patient is classified with very high risk, <60 ml/min — a high risk. Since the creatinine level is used in calculating GFR, we analyzed its possible associations with CA atherosclerosis and its severity, both in isolation and in combination with other biomarkers. It turned out that the level of creatinine is positively associated not only with the presence, but also with CA atherosclerosis severity.

The feasibility of assessing NO_X level to assess the endothelial NO-producing activity has been confirmed in studies both in laboratory animals and in humans [26, 27]. Literature data show that NO_X is associated not only with cardiovascular mortality, but also with all-cause mortality [28]. There is evidence that NO_X is associated with atherosclerosis [29]. In our study, when analyzing the blood concentration of NO_X, we found a cut-off point of 36 μM/L and revealed a negative correlation of this biomarker with the presence and severity of CA atherosclerosis. However, given the small sample and the complexity of NO_X determination, this indicator was not included in multivariate regression models. This result requires further study in a larger cohort.

Based on this analysis of blood biomarkers, an MVM was developed, which makes it possible to verify coronary atherosclerosis. However, the potential of this model for verifying the atherosclerosis severity are limited. This is most likely due to the fact that detected deviations are associated with the atherosclerosis initiation, and during its progression they do not change significantly.

Conclusion

Thus, blood biochemical parameters were selected for diagnostic scale, which makes it possible to verify patients with and without CA atherosclerosis. The quantitative determination of glucose, creatinine, hsCRP and adiponectin levels included in MVM is quite accessible, which allows it to be widely used in practical health care. According to the obtained MVM score, patients can be reclassified according to CA atherosclerosis likelihood and, if necessary, send them to additional studies to confirm the diagnosis and determine atherosclerosis severity.

Relationships and Activities: none.

References

1. Andreev EM. Mortality in Moscow and other megacities of the world: similarities and differences. Demographic review. 2016;3(3):39-79. (In Russ.)
2. Vasan RS. Biomarkers of cardiovascular disease: molecular basis and practical considerations. Circulation. 2006;113:2335-62. doi:10.1161/CIRCULATIONAHA.104.482570.
3. Huang Y, Gulshan K, Nguyen T, Wu Y. Biomarkers of Cardiovascular Disease. Dis Markers. 2017;2017:8208609. doi:10.1155/2017/8208609.
4. Lyngbakken MN, Myhre MR, Røsjø H. Novel biomarkers of cardiovascular disease: Applications in clinical practice. Crit Rev Clin Lab Sci. 2019;56(1):33-60. doi:10.1080/10408363.2018.1525335.
5. Preiss D, Sattar N. Lipids, lipid modifying agents and cardiovascular risk: a review of the evidence. Clin Endocrinol. 2009;70:815-28. doi:10.1111/j.1365-2265.2008.03490.
6. Oravec S, Dukat A, Gavornik P, et al. Atherogenic normolipidemia — a new phenomenon in the lipoprotein profile of clinically healthy subjects. Neuro Endocrinol Lett. 2011;V32(3):317-21.
7. Subirana I, Fitó M, Diaz O, et al. Prediction of coronary disease incidence by biomarkers of inflammation, oxidation, and metabolism. Sci Rep. 2018;8(1):3191. doi:10.1038/s41598-018-21482-y.
8. Wang TJ, Gona P, Larson MG, et al. Multiple biomarkers for the prediction of first major cardiovascular events and death. N Engl J Med. 2006;355:2631-9. doi:10.1056/NEJMoa055373.
9. Melander O, Newton-Cheh C, Almgren P, et al. Novel and conventional biomarkers for prediction of incident cardiovascular events in the community. JAMA. 2009;302:49-57. doi:10.1001/jama.2009.943.
10. Blankenberg S, Zeller T, Saarela O, et al. Contribution of 30 biomarkers to 10-year cardiovascular risk estimation in 2 population cohorts: the MONICA, risk, genetics, archiving, and monograph (MORGAM) biomarker project. Circulation. 2010;121:2388-97. doi:10.1161/CIRCULATIONAHA.109.901413.
11. Metelskaya VA, Gavrilova NE, Yarovaya EA, et al. An integrative biomarker: opportunities for non-invasive diagnostics of coronary atherosclerosis. Russ J Cardiol. 2017;(6):132-8. (In Russ.) doi:10.15829/1560-4071-2017-6-132-138.
12. Merculov EV, Mironov VM, Samko AN. Coronary angiography, ventriculography, bypass angiography in graphics and diagrams. M.: Media Medika 2011;100. (In Russ.)
13. 2019 ESC/EAS Guidelines for the management of dyslipidaemias: lipid modification to reduce cardiovascular risk. Eur Heart J. 2020;41:111-88. doi:10.1093/eurheartj/ehz455.
14. 2019 ESC Guidelines on diabetes, pre-diabetes, and cardiovascular diseases developed in collaboration with the EASD. Russian Journal of Cardiology. 2020;25(4):3839. (In Russ.) doi:10.15829/1560-4071-2020-3839.
15. Genkel VV, Kuznetsova AS, Salashenko AO, et al. Increased level of highly sensitive C-reactive protein as a marker of multifocal atherosclerosis in patients with cardiovascular diseases. Medical Board. 2019;(16):86-93. (In Russ.) doi:10.21518/2079-701X-2019-16-86-93.
16. Metelskaya VA, Gumanova NG. Screening-method for determining the level of nitrogen oxide metabolites in blood serum. Clinical laboratory diagnostics. 2005;6:15-8. (In Russ.)
17. 2019 ESC Guidelines for the diagnosis and management of chronic coronary syndromes. Russian Journal of Cardiology. 2020;25(2):3757. (In Russ.) doi:10.1093/eurheartj/ehz425.
18. Shuvaev IP, Asymbekova EU, Buziashvili Yul. Features of coronary heart disease in metabolic syndrome. Creative cardiology. 2017;11(1):20-30. (In Russ.) doi:10.15275/kreatkard.2017.01.03.
19. Lakka HM, Laaksonen DE, Lakka TA, et al. The metabolic syndrome and total and cardiovascular disease mortality in middle-aged men. JAMA. 2002;4;288(21):2709-16. doi:10.1001/jama.288.21.2709.
20. American Diabetes Association. Standards of medical care in diabetes. Diabetes Care. 2004;27:15-35. doi:10.2337/dc20-S002.
21. Danesh J, Lewington S, Thompson SG, et al. Plasma fibrinogen level and the risk of major cardiovascular diseases and nonvascular mortality: an individual participant meta-analysis. JAMA. 2005;294(14):1799-809. doi:10.1001/jama.294.14.1799.
22. Ridker PM, Buring JE, Cook NR, Rifai N. C-reactive protein, the metabolic syndrome, and risk of incident cardiovascular events: an 8-year follow-up of 14719 initially healthy American women. Circulation. 2003;107(3):391-7. doi:10.1161/01.cir.0000055014.62083.05.

23. Ridker PM, Hennekens CH, Buring JE, Rifai N. C-reactive protein and other markers of inflammation in the prediction of cardiovascular disease in women. N Engl J Med. 2000;342:836-43. doi:10.1056/NEJM200003233421202.
24. Cheng JM, Oemrawsingh RM, Garcia-Garcia HM, et al. Relation of C-reactive protein to coronary plaque characteristics on grayscale, radiofrequency intravascular ultrasound, and cardiovascular outcome in patients with acute coronary syndrome or stable angina pectoris (from the ATHEROREMO-IVUS study). Am J Cardiol. 2014;114(10):1497-503. doi:10.1016/j.amjcard.2014.08.013.
25. 2016 Europen guidelines on cardiovascular disease prevention in clinical practice. Russ J Cardiol. 2017;(6):7-85. (In Russ.) doi:10.15829/1560-4071-2017-6-7-85.
26. Granger DL, Anstey NM, Miller WC, Weinberg JB. Measuring nitric oxide production in human clinical studies. Methods enzymol. 1999;301:49-61. doi:10.1016/s0076-6879(99)01068-x.
27. Bachetti T, Comini L, Pasini E, et al. ACE-inhibition with quinapril modulates the nitric oxide pathway in normotensive rats. J Mol Cell Cardiol. 2001;33:395-403. doi:10.1006/jmcc.2000.1311.
28. Gumanova NG, Deev AD, Zhang W, et al. Serum nitrite and nitrate levels, NOx, can predict cardiovascular mortality in the elderly in a 3-year follow-up study. Biofactors. 2016;43(1):82-9. doi:10.1002/biof.1321.
29. Naseem KM. The role of nitric oxide in cardiovascular diseases. Mol Aspects Med. 2005;26(1-2):33-65. doi:10.1016/j.mam.2004.09.003.

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Risk factors and outcomes of gastrointestinal bleeding in patients with stable coronary artery disease: data from the observational registry of long-term antithrombotic therapy REGATTA-1

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Aim. To optimize the upper gastrointestinal bleeding (UGIB) risk scale in patients with chronic coronary artery disease (CAD) receiving long-term antiplatelet therapy.

Material and methods. The UGIB risk scale was developed based on the prospective REGistry of long-term AnTithrombotic TherApy-1 REGATTA-1 (ClinicalTrials.gov Identifier: NCT04347200). The registry includes 934 patients with stable CAD (men, 78,6%; median age, 61±10,7 years), 76% of whom were included after elective percutaneous coronary interventions and received dual antiplatelet therapy for 6-12 months. After a UGIB episode, patients were prescribed proton pump inhibitors. The 2015 European Society of Cardiology (ESC) scale was used for assessing the UGIB risk. In addition, we evaluated the ultrasound data on atherosclerotic burden (abdominal aorta and peripheral arteries).

Results. The median follow-up was 2,5 years [1,1-14,7 years]. The incidence of UGIB was 1,9 cases per 100 patient/years. Recurrent UGIB episodes and thrombosis was recorded in 13,7% and 31,4%, respectively. Based on the results of a multivariate logistic regression, a novel scale for assessing the UGIB risk (REGATTA) has been developed. In accordance with the odds ratio, points were assigned for each independent risk factor (RF): age ≥80 years — 3 points, prior gastric erosion, peptic ulcer disease or UGIB — 3 points for each RF, anticoagulation therapy — 4 points, non-steroidal anti-inflammatory drug therapy — 2 points. The atherosclerotic burden (peripheral atherosclerosis and/or abdominal aortic aneurysm; 2 points) and heart failure (in most cases after a myocardial infarction; 2 points) were marked as a new independent predictor. The cutoff value (≥4 points) was determined, reflecting the high UGIB risk (sensitivity, 80,4%; specificity, 84,5%). The REGATTA scale was more powerful than the traditional 2015 ESC scale: AUC of 0,88, (95% confidence interval, 0,86-0,9) vs AUC of 0,79, (95% confidence interval, 0,76-0,82) (p=0,04).

Conclusion. The identified UGIB predictors (atherosclerotic burden and heart failure) and the developed REGATTA scale made it possible to improve the prognosis and prevention of UGIB in patients with stable CAD receiving long-term antiplatelet therapy.

Keywords: coronary artery disease, antiplatelet therapy, gastrointestinal bleeding, percutaneous coronary intervention, proton pump inhibitors, peripheral atherosclerosis.

Trial ID: ClinicalTrials NCT04347200.

Relationships and Activities: none.

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Upper gastrointestinal bleeding from the tract (UGIB) is one of the most common complications of antithrombotic therapy associated with high mortality [1]. Adequate score for assessing the bleeding risk in patients with coronary artery disease (CAD) would make it possible to select candidates for primary UGIB prevention (primarily by using proton pump inhibitors (PPIs)), to plan active monitoring of such patients (complete blood count, fecal occult blood test, esophagogastroduodenoscopy (EGD)). In addition, the high risk of UGIB may be the basis for a more careful choice of antithrombotic therapy.

There are two approaches to UGIB risk stratification in patients with CAD. The first one predicts the likelihood of any bleeding and takes into account general risk factors (RF) characterizing the severity of patient's condition as a whole (PRECISE-DAPT [2] and ARC-HBR [3] scores). The second approach [4] by the European Society of Gastrointestinal Endoscopy (2015), which was recommended for patients with CAD by the European Society of Cardiology (ESC), involves taking into account age and medications (steroids, non-steroidal anti-inflammatory drugs (NSAIDs), anticoagulants), as well as local RFs characterizing the mucous membrane. We assume that combining various RFs into a single model can enhance the prognostic value of known scores and allow careful predicting UGIB.

The aim was to optimize the UGIB risk scale in patients with chronic CAD receiving long-term antiplatelet therapy.

Material and methods

To create a UGIB risk scale and assess its predictive value, a cohort of patients with stable CAD was used, included in the single-center prospective register of long-term antithrombotic therapy REGATA [5], created on the basis of the National Medical Research Center of Cardiology (Figure 1). Within this study, patients included in the period from 2003 to 2019 was analyzed.

The study was performed in accordance with standards of Good Clinical Practice and Declaration of Helsinki. The study protocol was approved by the local ethics committee. All patients signed written informed consent.

Inclusion and exclusion criteria have been detailed in our previous publications [5]. Briefly, the study included patients with stable CAD (most of them after elective percutaneous coronary interventions (PCI)) who have no contraindications to standard antithrombotic therapy.

Study progress. In addition to the standard examination, screening for concomitant peripheral atherosclerosis was performed, including ankle-brachial index (ABI) test, Doppler ultrasound of extracranial arteries, abdominal aorta and its branches (and/or contrast-enhanced abdominal multislice computed tomography if indicated). In the presence of intermittent claudication and/or a decrease in ABI <0,9, lower limp artery ultrasound was performed. To define the "peripheral atherosclerosis", generally accepted criteria were used [4]. Attention was also paid to prior gastric erosions and ulcer, verified by EGD.

The scale recommended by ESC in 2015 was chosen for assessing the UGIB risk [4]. For all patients, the UGIB risk was stratified according to this scale, which, however, had a number of limitations. Thus, *H. pylori* infection was assessed by the decision of an attending physician. Due to insufficient data, this parameter was not included in further statistical processing. For the same reason, the presence of gastroesophageal reflux disease was not taken into account. Also, for a large number of patients, no reliable information was obtained regarding the alcohol consumption. Therefore, this symptom was also not analyzed.

The planned duration of prospective follow-up was more than 2 years. Follow-up visits were carried out 6 and 12 months after inclusion, then — every 12 months. If necessary, unscheduled visits were carried out due to CAD progression. Telephone contacts were made every 3 months.

End points. The primary endpoint was overt UGIB (verified by EGD or typical symptoms — melena, vomiting blood, or a combination thereof). Bleeding counts met BARC class 2-5.

The following adverse events were also recorded: death (with an indication of cause), myocardial infarction, unstable angina requiring hospitalization, ischemic stroke, transient ischemic attack, peripheral arterial thrombosis.

Medication therapy. All patients received standard treatment according to the current Russian Society of Cardiology and ESC guidelines on CAD and myocardial revascularization [4]. All patients were prescribed with aspirin 75-100 mg a day. After PCI, patients also received clopidogrel for 6-12 months. The adherence to treatment with antiplatelet agents, statins and other drugs that affect the cardiovascular prognosis was monitored. At the discretion of an attending physician, preventive therapy with PPIs was prescribed, appointment of which was assessed using medical records. Patients who underwent UGIB after being included in the registry were additionally observed by a gastroenterologist. Mandatory prescription of PPIs and assessing adherence to treatment was envisaged. If indicated, additional EGD and eradication therapy for *H. pylori* were performed.

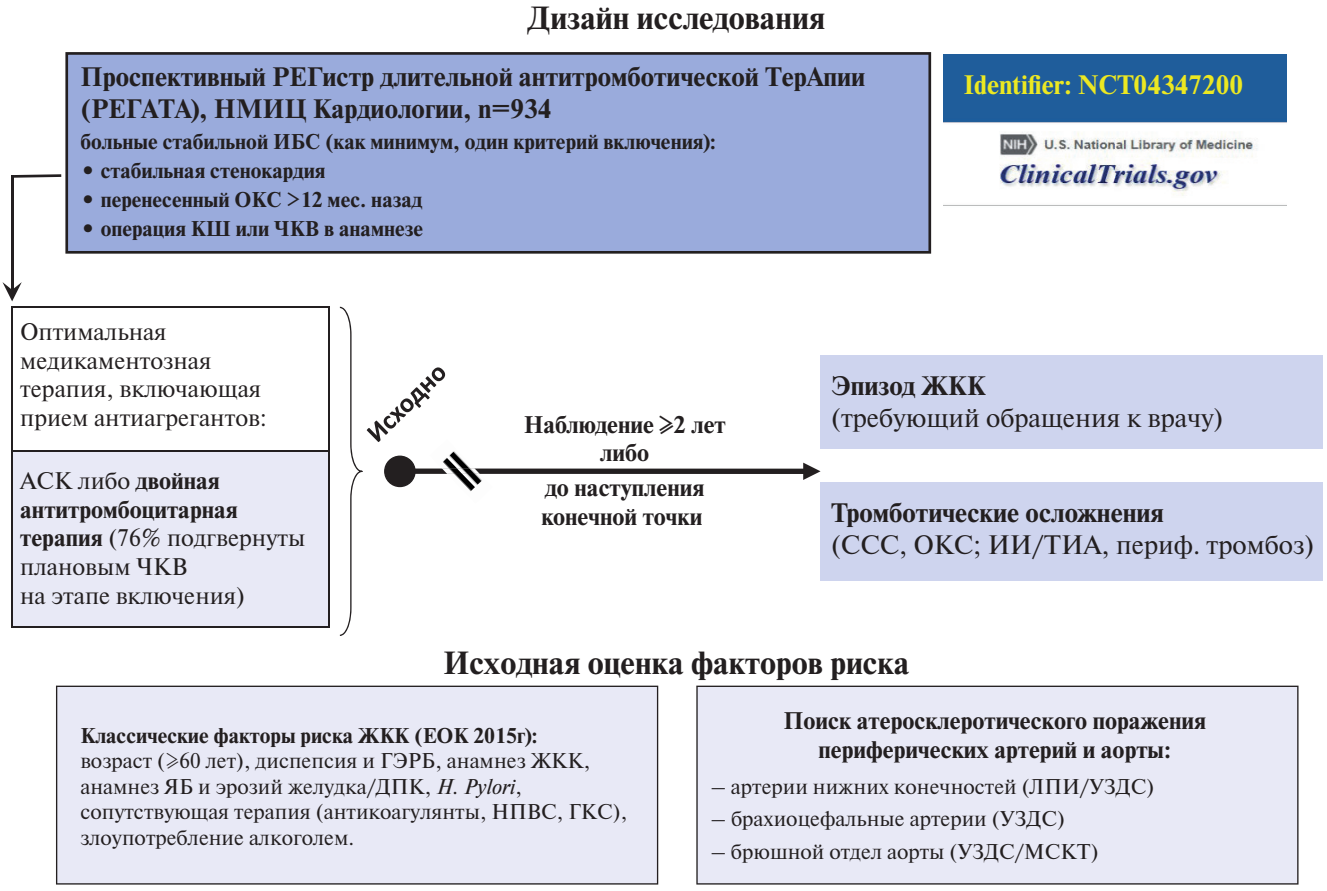


Figure 1. Study design.
Abbreviations: ASA — acetylsalicylic acid, GCSs — glucocorticosteroids, GERD — gastroesophageal reflux disease, ESC — European Society of Cardiology, UGIB — upper gastrointestinal bleeding, CAD — coronary artery disease, ABI — ankle-brachial index, MSCT — multislice computed tomography, NSAIDs — non-steroidal anti-inflammatory drugs, ACS — acute coronary syndrome, CVD — cardiovascular death, TIA — transient ischemic attack, PCI — percutaneous coronary intervention.

Statistical processing and risk scale development. Statistical processing was carried out using the Statistica 10.0, SPSS 20 and MedCalc 18.11 software packages. Univariate and multivariate regression was used to identify potential RFs of UGIB. Parameters with a significant odds ratio (OR) (95% confidence interval (CI) of which did not include one) were selected by multiple logistic regression model. Based on the OR values obtained in multiple logistic regression, the identified independent predictors of UGIB were assigned scores characterizing the contribution of each factor to the total bleeding risk. Taking into account the relatively small sample size and follow-up period, the characteristics of patients (for example, initially, patients receiving anticoagulants, NSAIDs, steroids were practically not included), number of assigned scores for RFs that are rare in our sample was adjusted in accordance with literature data covering more representative cohorts.

To assess the predictive value of scale, we used the ROC analysis with determining area under the curve (ROC curve). As a cutoff point for score determining the high UGIB risk, the value with closest sensitivity and specificity was chosen.

To assess the adequacy of cut-off point, the incidence of UGIB for subgroups of patients allocated in accordance with score was compared with the incidence of UGIB, which we theoretically calculated, which was considered as high. The calculation was based on the following data:

1) Academic Research Consortium experts proposed a cut-off point of 4% per year as a high-risk criterion for all major bleeding (BARC 3-5) after PCI [3];

2) among all such bleedings, the proportion of UGIB is ~60% [6],

3) among all gastrointestinal bleeding, more than half (64,4%) falls on the upper gastrointestinal tract [7].

Thus, the cutoff line of high risk for major UGIB is 4% × 0,6 × 0,64 = 1,536%.

This calculation takes into account only major bleeding. It is known that 3 cases of major gastrointestinal bleeding (BARC 3-5) account for 1 additional BARC 2 bleeding [8]. Due to the above, the cutoff point of high risk for major and clinically

Table 1

Prevalence of gastrointestinal bleeding RFs in patients with stable CAD, depending on bleeding within the follow-up period

Total, n (%)	All patients, n=934	Patients with gastrointestinal bleeding within follow-up, n=51	Patients without gastrointestinal bleeding within follow-up, n=883	P_{1-2}
Sex				
Male	723 (78,6%)	42 (78,1%)	681 (77,9%)	0,386
Female	211 (21,4%)	9 (21,9%)	202 (22,5%)	
Age				
<70 years	762 (81,6%)	32 (58,8%)	730 (82,7%)	<0,001
70-79 years	152 (16,3%)	12 (23,5%)	140 (15,9%)	0,149
⩾80 years	20 (2,1%)	7 (13,7%)	13 (1,5%)	<0,001
Clinical risk factors:				
• BMI >30 kg/m^2	544 (58,2%)	36 (70,6%)	375 (57,5%)	0,073
• Diabetes	190 (20,3%)	11 (21,6%)	179 (20,3%)	0,823
• Stage ⩾3a chronic kidney disease	97 (10,4%)	12 (23,5%)	85 (9,6%)	0,002
• Prior MI	538 (57,6%)	29 (56,9%)	509 (57,6%)	0,701
• PCI within 12 months prior to inclusion	707 (75,7%)	25 (49,0%)	682 (60,2%)	<0,001
• HF*	73 (7,8%)	17 (33,3%)	56 (6,3%)	<0,001
• Prior ischemic stroke + TIA	72 (7,7%)	5 (9,8%)	67 (7,6%)	0,564
• Peripheral arterial atherosclerosis	176 (18,8%)	18 (35,3%)	158 (17,9%)	0,012
• Abdominal aortic aneurysm	22 (2,4%)	10 (19,6%)	12 (1,4%)	<0,001
Condition of the upper gastrointestinal tract:				
• Prior mucosal erosion	238 (25,5%)	29 (56,9%)	209 (23,7%)	<0,001
• Prior mucosal ulcer	164 (17,6%)	23 (45,1%)	141 (16,0%)	<0,001
• Erosion exacerbation after PCI	138 (14,8%)	11 (21,6%)	127 (14,4%)	0,162
• Prior gastrointestinal bleeding	6 (0,64%)	6 (11,8%)	0	<0,001
Drug therapy:				
• NSAIDs	4 (0,43%)	4 (7,8%)	0	<0,001
• Anticoagulation (dual or triple therapy)	48 (5,1%)	12 (23,5%)	36 (4,1%)	<0,001
• PPIs	264 (28,3%)	51 (100%)	213 (54,1%)	<0,001

Note: * — in 90% of patients with HF developed after MI.

Abbreviations: MI — myocardial infarction, BMI — body mass index, PPIs — proton pump inhibitors, NSAIDs — non-steroidal anti-inflammatory drugs, TIA — transient ischemic attack, HF — heart failure, PCI — percutaneous coronary intervention.

relevant UGIB was increased by a third and amounted to 2,06%. Thus, >2 events per 100 people per year was chosen as a criterion for the high UGIB risk (BARC 2-5).

Comparison of the predictive value of developed scale with 2015 ESC scale [4] was carried out by comparing the corresponding areas under the ROC curves.

Differences were considered significant at p<0,05.

Results

Initial characteristics of patients. In total, the study included 934 patients with stable CAD (median age, 61 years [53-68 years]; men, 78,6%) (Table 1). At the enrollment, 687 patients (76%) underwent elective PCI, and therefore received dual antiplatelet therapy (DAPT) for 6-12 months. We have described in detail the clinical and demographic characteristics of patients, classical UGIB RFs, the prevalence of peripheral atherosclerosis and abdominal aortic aneurysm in the studied cohort previously [5].

Upper gastrointestinal bleeding. The median follow-up was 2,5 years [1,1-14,7 years]. Major and clinically relevant UGIB (BARC ⩾2) was recorded with a frequency of 1,9 cases per 100 people per year (Figure 2). It should be emphasized that most of UGIB occurred in the first 2 months from treatment initiation (median duration before UGIB development was 71 days [13-212]). Most of the bleeding (62,8%) was verified by EGD; the rest were diagnosed retrospectively by typical clinical picture.

Table 2

Gastrointestinal bleeding risk assessment scale REGATA: RFs and their relative contribution to gastrointestinal bleeding risk

Risk factors	Score	OR [95% CI]	p
Age:			
— 70-79 years old	1*	1,2 [0,5-2,9]	0,616
— ⩾80 years old	3	4,8 [1,2-18,7]	0,024
Abdominal aortic aneurysm and/or peripheral atherosclerosis	2	3,4 [1,7-6,9]	0,0005
Heart failure	2	4,4 [1,9-10,3]	0,0007
Prior gastric/duodenal erosion	3	5,6 [2,7-11,3]	0,000002
Prior gastric/duodenal ulcer	3	4,9 [2,4-10,1]	0,00002
Prior gastrointestinal bleeding	3*	2,2 [1,5-35,7]	<0,0001
NSAIDs	3	8,16 [3,4-124,7]	<0,0001
Anticoagulant therapy (including in combination with antiplatelet agents)	4	164,8 [15,5-1755,6]	0,00002

Notes: * — assigned score is corrected in accordance with literature data. Risk factors that are absent in the 2015 ESC scale are marked in color.

Abbreviations: CI — confidence interval, NSAIDs — non-steroidal anti-inflammatory drugs, OR — odds ratio.

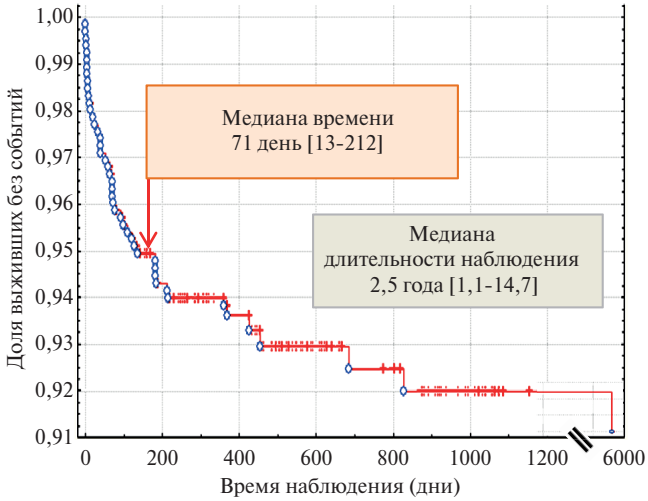
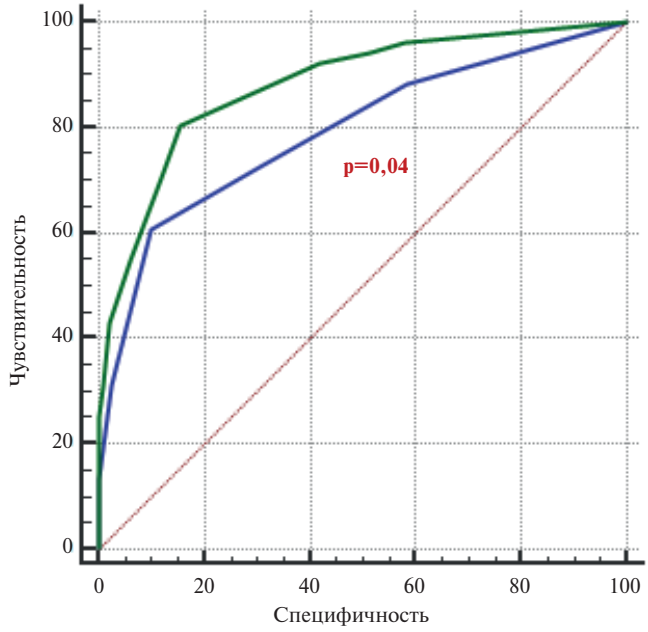


Figure 2. Cumulative incidence of upper gastrointestinal bleeding in patients with stable CAD (Kaplan-Meier curve).

All patients with further gastrointestinal bleeding have information in their medical records about PPI appointment, but adherence to therapy is not known. After an episode of bleeding, the patients were followed up by a gastroenterologist. A set of measures was carried out aimed at the secondary gastrointestinal bleeding prevention, including long-term PPI therapy and correction of modifiable RFs (withdrawal of NSAIDs, alcohol, eradication of *H. pylori* if indicated, etc.). Against the background of such events, the incidence of recurrent gastrointestinal bleeding was only 13,7%.

To identify gastrointestinal bleeding predictors, in addition to conventional RFs, other indicators were analyzed, reflecting renal function, atherothrombosis, prior vascular events and heart failure



	Отрезное значение	Чувствительность	Специфичность
Шкала ЕОК 2015г	⩾2 баллов	72,6%	69,3%
Шкала Регата	⩾4 баллов	80,4%	84,5%

Figure 3. Comparison of the predictive value of gastrointestinal bleeding risk scales REGATA and 2015 ESC using ROC analysis.
Abbreviations: CI — Confidence Interval, ESC — European Society of Cardiology.

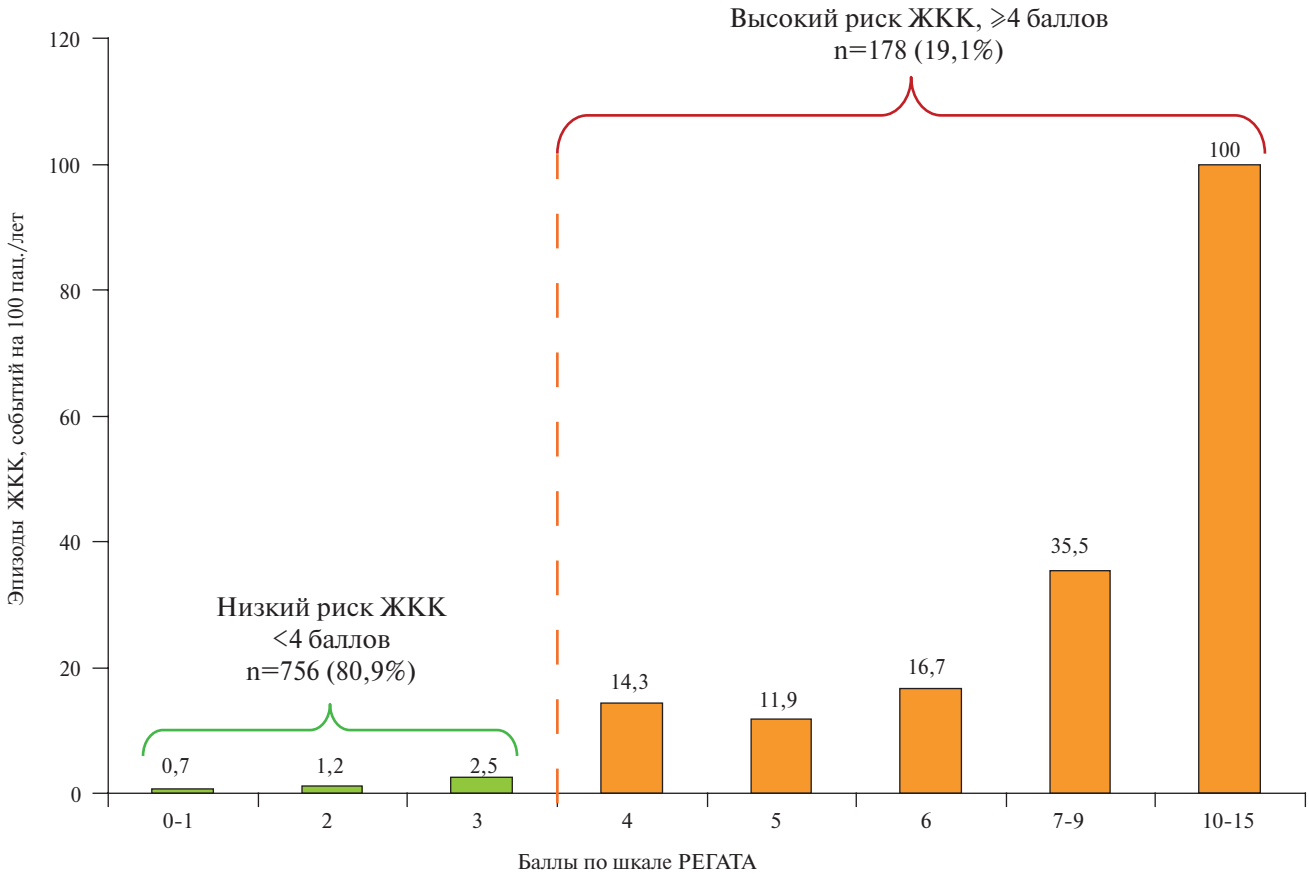


Figure 4. Gastrointestinal bleeding rate depending on score on the REGATA scale.
Abbreviation: UGIB — upper gastrointestinal bleeding.

as the final stage of cardiovascular continuum (Table 1). We have identified a symptom that most fully reflects the atherosclerosis extent — presence of peripheral atherosclerosis and/or abdominal aortic aneurysm. Age as one of the leading RFs of gastrointestinal bleeding was analyzed by groups (<70 years old, 70-79 years old, and ⩾80 years old); the criteria for dividing patients into groups by age were borrowed from large-scale observational studies carried out in 2017-2019 [1, 9]). Since dyspepsia as a term does not have clear criteria, and EGD confirmation of gastroesophageal reflux disease in actual clinical practice is often difficult, these RFs were not taken into account in further analysis.

According to multivariate analysis (Table 2), the already known factors were independent UGIB predictors in our cohort of patients with stable CAD: history of peptic ulcer disease or upper gastrointestinal erosions, age over 80 years, prior gastrointestinal bleeding, as well as NSAID and anticoagulant therapy. We also found that the risk of gastrointestinal bleeding increased in patients with advanced atherosclerosis (peripheral atherosclerosis and/or abdominal aortic aneurysm), as well as in patients with heart failure.

Risk stratification scale for UGIB in patients with stable CAD REGATA. Based on the results obtained, we have developed a novel risk stratification scale for UGIB in patients with stable CAD — REGATA (Table 2). Based on the OR values obtained by multivariate analysis, each of the RFs we found was assigned a certain score: factors with OR <1,5 were assigned 1 point, 1,5⩽ OR <4,5 — 2 points, 4,5⩽ OR <10 — 3 points, ⩾10 — 4 points. For signs that are rare in these patients (prior gastrointestinal bleeding), as well as for the age of 70-79 years, the score was adjusted in accordance with literature data (first of all, we relied on similar indicators in the 2015 ESC scale [5]).

We compared the predictive value of REGATA and 2015 ESC scales (Figure 3). It was found that the area under the curve (ROC AUC) for REGATA scale was larger than in 2015 ESC scale. The ROC AUC was 0,88 (95% CI, 0,86-0,9) and 0,79 (95% CI, 0,76-0,82), respectively (p=0,04). For REGATA scale, the optimal cut-off point, which determines the high bleeding risk, was a score of 4. With such a cut-off point, the sensitivity and specificity were 80,4% and 84,5%, respectively.

All patients were divided into 8 groups in accordance with score on the REGATA scale. For

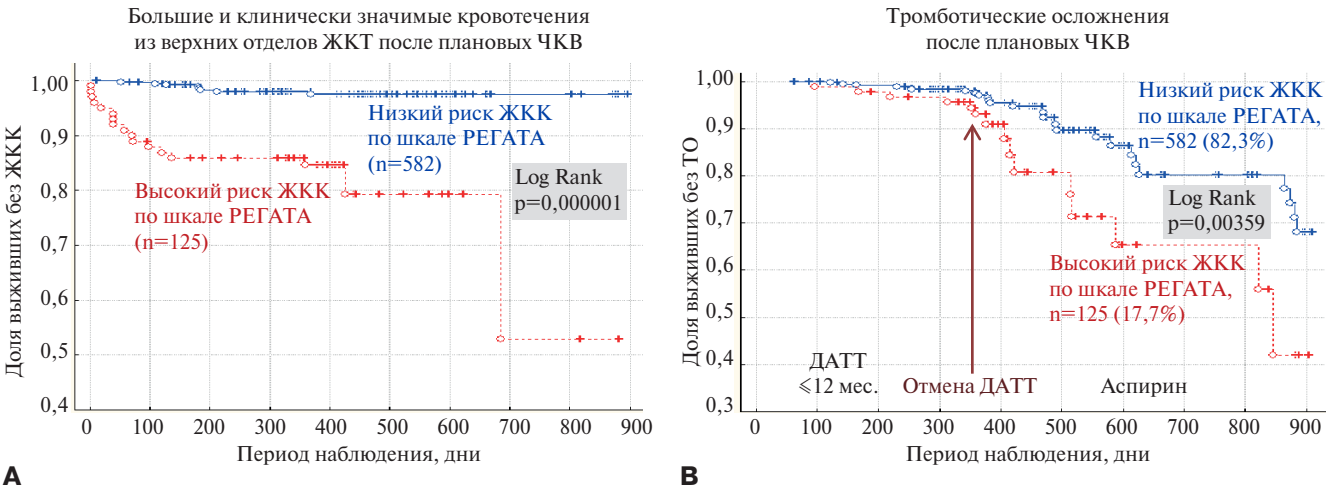


Figure 5. Survival without upper gastrointestinal bleeding (**A**) and thrombotic events (**B**) in the high and low risk groups according to the REGATA scale (Kaplan-Meier curves).
Abbreviations: DAPT — dual antiplatelet therapy, UGIB — bleeding from the upper gastrointestinal tract, TE — thrombotic events, PCI — percutaneous coronary intervention.

each group, the incidence of gastrointestinal bleeding was estimated (Figure 4). The incidence of gastrointestinal bleeding in patients with score of 3 (highest score in the low-risk category) was 2,5 cases per 100 patients per year. This indicator was very close to the theoretically calculated value of 2% per year, which is an additional criterion for the high predictive value of the scale we developed. In 19,1% of patients, the risk of gastrointestinal bleeding was defined as high on the REGATA scale.

Logistic regression showed that the incidence of gastrointestinal bleeding in the subgroup of patients with high REGATA risk (⩾4) was significantly higher than in patients with low risk (1-3): 23% vs 1,3% (p=0,000001).

Predictive value of the REGATA scale in patients after elective PCI. Three quarters of patients at the inclusion stage received DAPT after elective PCI. We analyzed the outcomes in this subgroup separately.

The REGATA scale demonstrated good predictive value of gastrointestinal bleeding in this subgroup of patients as well (Figure 5 A). In addition, patients with a high gastrointestinal bleeding risk were also characterized by a high risk of thrombotic events (TEs) (Figure 5 B). At the same time, the incidence of TEs for the entire follow-up period was higher than gastrointestinal bleeding. It is important that the divergence of survival curves without TEs began at the start of second year after PCI — after the planned cancellation of DAPT.

Prognosis of CAD patients who underwent gastrointestinal bleeding during prospective follow-up. The analysis of outcomes in patients after gastrointestinal bleeding showed that the incidence of recurrent bleeding in this subgroup was 13,7%, while the incidence of TEs in them was twice as high — 31,4%.

The likely reason for the increase in TEs after gastrointestinal bleeding was the complete cancellation or decrease in the intensity of antiplatelet therapy. Among 51 patients who underwent gastrointestinal bleeding, 7 died in the next week, 5 died within a year from causes not related to thrombosis and bleeding, 39 patients survived after the first bleeding episode. Antiplatelet therapy was resumed in full by 30 people, and 9 patients reduced or completely canceled antiplatelet agents. It should be emphasized that among 9 people who reduced or canceled antiplatelet drugs, the frequency of thrombotic events subsequently was almost 2 times higher — 55,6% vs 26,7% in those who continued antiplatelet therapy. However, the small sample size did not allow us to assess the reliability of revealed differences.

Discussion

Gastrointestinal bleeding occupies a leading position in pattern of hemorrhagic events in patients receiving antiplatelet drugs. The prognosis of patients with various manifestations of atherothrombosis after such bleeding is considered unfavorable: 1-year mortality after any clinically relevant episode of gastrointestinal bleeding is 20-25% [1, 5].

The existing approach to assessing the likelihood of gastrointestinal bleeding is based solely on the opinion of experts who have combined the generally recognized RFs [4] into a common scale that has not undergone any validation. The high risk of gastrointestinal bleeding, determined in accordance with this scale, is a formal indication for PPI ap-

pointment. Nevertheless, it has not yet been possible to convincingly demonstrate the feasibility of this method of prevention. All of the above determines the need for well-organized observational studies and registers aimed both at assessing the predictive value of this scale and at finding new RFs. In our work, we chose just such an approach, which made it possible to integrate already known and new factors into a unified prognostic model.

The stratification was changed in relation to one of the key RFs, which is age. According to our data, the previously accepted division of patients into groups over and under 65 years of age is not optimal: a significant increase in gastrointestinal bleeding risk was noted in older people (Table 2), which was logically reflected in REGATA scale. A similar change in risk stratification with an emphasis on older age groups has been proposed in recent years by other authors [1, 6].

There were certain limitations that did not allow adequately validating the gastrointestinal bleeding risk scale proposed by the ESC in 2015 in relation to patients from the REGATA register. Our cohort of patients was characterized by a relatively low rate of using NSAIDs and anticoagulants. In addition, the register did not include persons who required corticosteroid therapy. It was also difficult to qualify the alcohol abuse. Routine screening for reflux disease and *H. pylori* infection has not been performed.

Another problem was recording the dyspepsia symptoms. There are no clear criteria for dyspepsia, which determines the well-known subjectivity when calculating gastrointestinal bleeding risk score. We, in turn, drew attention to another, more objective criterion associated with certain dyspepsia symptoms, namely, with gastrointestinal erosions. It is known that such a lesion is characteristic of *H. pylori* infection [10], which logically explains the significance of detected RF in relation to bleeding.

The presence of erosion may indicate impaired reparative processes, including due to gastrointestinal mucosa ischemia. In this regard, it seems logical to emphasize the importance of the discovered RFs characterizing the atherothrombosis burden, namely, the involvement of peripheral arteries and abdominal aorta, as well as heart failure, which developed in most cases after MI (Table 1). The relationship between widespread atherosclerotic lesions and the development of gastrointestinal bleeding has been demonstrated by other authors [11]. For obvious reasons, no work analyzed the state of arteries directly supplying the upper gastrointestinal tract. Nevertheless, given the systemic nature of atherothrombotic process, it is logical to assume ischemia in this vascular system, both due to anatomical flow obstruction and due to distal embolism by damaged plaques and thrombi, the source of which is most likely the abdominal aorta.

Taking into account novel RFs, reflecting the prevalence and severity of atherothrombotic process, allowed us to improve the prognosis of gastrointestinal bleeding. The sensitivity and specificity of REGATA scale developed by us was significantly higher than the ESC risk score.

Thrombosis and bleeding RFs are closely related [12]. One of these common RFs was atherothrombosis burden, which determines the need for long-term therapy, optimally including a second antithrombotic agent in addition to aspirin [13].

We have previously shown [5] that the outcomes of patients after gastrointestinal bleeding are determined by the high TE rate, while the incidence of recurrent bleeding was significantly less. An analysis of a cohort of patients undergoing elective PCI and receiving initial therapy with two antiplatelet agents (Figure 5) demonstrated an increase in TE rate in the group with a high risk of gastrointestinal bleeding, which corresponded to the timing of DAPT withdrawal. Thus, our results suggest that net clinical benefit in these patients is determined by the risk of thrombosis rather than bleeding. The data obtained in large-scale registers [12] also indicate that in most patients with many RFs of TEs, the risk of cardiovascular events significantly exceeds the likelihood of bleeding. From all of the above, an important practical conclusion follows that the bleeding risk is not a reason for routine withdrawal of antithrombotic therapy, which is prescribed due to high risk of ischemic events.

This provision may apply to the most vulnerable category of patients — those with prior gastrointestinal bleeding. There is reason to believe that treatment (at least one antiplatelet agent) is preferable to maintain, if there is adequate endoscopic hemostasis [14]. Our research has also demonstrated the benefits of this approach. Taking into account all the possible limitations associated with the small number of groups, the lack of data on factors influencing the choice of antithrombotic drugs by physicians, etc., it should be stated that TE rate is lower in the case of resuming the previous antiplatelet therapy a week after gastrointestinal bleeding.

The safety of antithrombotic therapy can be maintained only if there is an active search and correction of all modifiable factors that increase the bleeding risk, as well as preventive administration of PPIs. Compliance with these rules allowed us to ensure low recurrence rate in patients with bleeding. The specifics of observational study did not allow us to carry out the same measures in all patients included in registry.

An active search for a potential source of bleeding could be discussed as an additional preventive measure. We showed that the largest number of gastrointestinal bleeding developed in the coming months from the initiation/intensification of treatment. Antithrombotic therapy is a kind of stress test that reveals the silent initial pathology of the gastrointestinal mucosa [15]. Thus, it is prudent to provide for endoscopic screening or, at least, fecal occult blood test, if there are any concerns about the safety of prescribed therapy.

Conclusion

As a result, we discovered novel predictors and developed a scale for assessing the gastrointestinal bleeding risk in patients with CAD receiving long-term antiplatelet therapy. The relationship with gastrointestinal bleeding was demonstrated for RFs characterizing the atherothrombosis burden, namely, peripheral artery and abdominal aorta involvement, as well as heart failure due to myocardial infarction. A high risk of gastrointestinal bleeding should not be a routine reason for refusal from antithrombotic therapy. The proposed modification is designed to facilitate the implementation of measures (PPI prescription, EGD screening, *H. pylori* eradication) aimed at preventing gastrointestinal bleeding in patients with various clinical manifestations of atherothrombosis.

Relationships and Activities: none.

References

1. Sehested TSG, Carlson N, Hansen PW, et al. Reduced risk of gastrointestinal bleeding associated with proton pump inhibitor therapy in patients treated with dual antiplatelet therapy after myocardial infarction. European Heart Journal. 2019;40(24):1963-70. doi:10.1093/eurheartj/ehz104.
2. Costa F, van Klaveren D, James S, et al. PRECISE-DAPT Study Investigators. Derivation and validation of the predicting bleeding complications in patients undergoing stent implantation and subsequent dual antiplatelet therapy (PRECISE-DAPT) score: a pooled analysis of individual-patient datasets from clinical trials. Lancet. 2017;389:1025-34. doi:10.1016/S0140-6736(17)30397-5.
3. Urban P, Mehran R, Colleran R, et al. Defining high bleeding risk in patients undergoing percutaneous coronary intervention: a consensus document from the Academic Research Consortium for High Bleeding Risk. Eur Heart J. 2019;40(31):2632-53. doi:10.1093/eurheartj/ehz372.
4. Roffi M, Patrono C, Collet J-P, et al. 2015 ESC Guidelines for the management of acute coronary syndromes in patients presenting without persistent ST-segment elevation: Task Force for the Management of Acute Coronary Syndromes in Patients Presenting without Persistent ST-Segment Elevation of the European Society of Cardiology (ESC). European Heart Journal. 2016;37(3):267-315. doi:10.1093/eurheartj/ehv320.
5. Shakhmatova OO, Komarov AL, Korobkova VV, et al. Upper gastrointestinal bleeding in patients with stable coronary artery disease (registry of antithrombotic therapy "REGATTA" results). Terapevticheskii arkhiv. 2020;92(9):30-8. (In Russ.) doi:10.26442/00403660.2020.09.000699.
6. Généreux P, Giustino G, Witzenbichler B, et al. Incidence, Predictors, and Impact of Post-Discharge Bleeding After Percutaneous Coronary Intervention. J Am Coll Cardiol. 2015;66(9):1036-45. doi:10.1016/j.jacc.2015.06.1323.
7. Shaukat A, Waheed S, Alexander E, et al. Etiology of gastrointestinal bleeding in patients on dual antiplatelet therapy. J Dig Dis. 2018;19:66-73. doi:10.1111/1751-2980.12575.
8. Koskinas KC, Räber L, Zanchin T, et al. Clinical impact of gastrointestinal bleeding in patients undergoing percutaneous coronary interventions. Circ Cardiovasc Interv. 2015;8(5):e002053. doi:10.1161/CIRCINTERVENTIONS.114.002053.
9. Jensen BES, Hansen JM, Larsen KS, et al. Randomized clinical trial: the impact of gastrointestinal gastrointestinal risk factor screening and prophylactic proton pump inhibitor therapy in patients receiving dual antiplatelet therapy. Eur J Gastroenterol Hepatol. 2017;29(10):1118-25. doi:10.1097/meg.0000000000000934.
10. Crowe SE. Helicobacter pylori Infection. N Engl J Med. 2019;380(12):1158-65. doi:10.1056/NEJMcp1710945.
11. Jolobe OMP. The Association of Peptic Ulcer Disease and Abdominal Aortic Aneurysm. The American Journal of Medicine. 2019;132(9):e724. doi:10.1016/j.amjmed.2019.03.047.
12. Bhatt DL, Steg PG, Ohman EM, et al. International prevalence, recognition, and treatment of cardiovascular risk factors in outpatients with atherothrombosis. JAMA. 2006;295(2):180-9. doi:10.1001/jama.295.2.180.
13. Knuuti J, Wijns W, Saraste A, et al. 2019 ESC Guidelines for the diagnosis and management of chronic coronary syndromes. The Task Force for the diagnosis and management of chronic coronary syndromes of the European Society of Cardiology (ESC). European Heart Journal. 2020;41(3):407-77. doi:10.1093/eurheartj/ehz425.
14. Sung JJ, Lau JY, Ching JY, et al. Continuation of low-dose aspirin therapy in peptic ulcer bleeding: a randomized trial. Ann Intern Med. 2010;152(1):1-9. doi:10.7326/0003-4819-152-1-201001050-00179.
15. Clemens A, Strack A, Noack H, et al. Anticoagulant-related gastrointestinal bleeding–could this facilitate early detection of benign or malignant gastrointestinal lesions? Annals of Medicine. 2014;46:672-8. doi:10.3109/07853890.2014.952327.

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LITERATURE REVIEW
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Clinical decision support system for lipid metabolism disorders: relevance and potential

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Current guidelines for the management of patients with dyslipidemia are well known and easily accessible. Despite this, according to research data based on actual clinical practice, selection of optimal tactics for managing patients with dyslipidemia often causes difficulties and leads to a failure to achieve the target levels. Tools such as clinical decision support system (CDSS) can help clinicians follow current clinical guidelines, taking into account the diversity of phenotypic profiles and side effects. This review highlights the effectiveness of CDSS implementation in medical practice as a means for making decisions in managing patients with dyslipidemia, as well as presents the algorithm for CDSS for lipid metabolism disorders created by specialists of the Almazov National Medical Research Center and the University of Milan.

Keywords: cardiovascular risk, prevention, dyslipidemia, statins, digital technologies, decision support systems.

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Failure to reach target low-density lipoprotein cholesterol levels is a stumbling block in cardiovascular prevention

At the heart of cardiovascular prevention is the control of modifiable cardiovascular risk factors (CVR) and the determination of an individual approach in patient management. Today, modification of risk factors (RFs) is actively used in the group of people with high and very high CVR. However, at the population level, the majority of cardiovascular deaths occur in groups with low and moderate CVR, because they are much more numerous (Rose's paradox). Dyslipidemia is one of the key modifiable cardiovascular RFs. It is known that a decrease in low-density lipoprotein (LDL) level for every 1 mmol/L leads to a decrease in risk of all-cause mortality by 10%, cardiovascular mortality — by 20%, stroke — by 17%, and coronary events — by 23% [1]. The concept of control and ways to achieve the target LDL level for each category of patients are reflected in the National and European guidelines on dyslipidemia [2, 3]. However, actual clinical practice demonstrates a number of difficulties on the way to controlling RF and achieving the target LDL level. The DA VINCI study showed that in the primary prevention group, only a small number of patients reach the target LDL values according to 2019 [3] and 2016 [4] European guidelines (33% vs 54%). In the group of patients with registered atherosclerotic cardiovascular diseases (CVDs), only 39% and 18% reach the target LDL level according to 2016 and 2019 guidelines, respectively [5]. Based on this, there is an obvious need to implement effective tools aimed at monitoring and providing a personalized approach in managing patients with CVDs, including at the primary health care level and in all CVR categories.

Implementation of digital technologies in actual clinical practice

The widespread introduction of digital technologies in medicine demonstrates successful results in the diagnosis and treatment of a number of diseases [6, 7]. Decision support system (DSS), introduced into healthcare practice, can be considered as a means for making decisions in patient management [8]. DSS is one of the most promising and rapidly developing areas of modern information technologies. The information programmed in them, which integrates the results of major global randomized clinical trials and current clinical guidelines, can serve as a tool for generating evidence-based decisions when using patient-centered approaches.

One of the first works on DSS introduction into clinical practice, published at the end of 20th century, was a prospective controlled study to assess the use and effectiveness of DSS in hypercholesterolemia treatment. The study was carried out in 25 Birmingham clinics, and the Primed DSS was installed on a desktop computer for each physician. This study evaluated the use of a decision support module for hypercholesterolemia in general clinical practice. The software included a screen for entry of socio-demographic data and CVR factors. In the future, the clinician was asked to enter information from the life history, medical history and cholesterol level. The CVR score was displayed on the screen during data collection. A score higher than 10 was assigned to patients at high risk of CVD over the next five years. After complete data entry, the program offered guidelines on patient management tactics. The system provided detailed information about the recommended dose of the drug, nutrition and physical activity. However, the study has clearly demonstrated that the possibility of widespread introduction of such programs into routine clinical practice depends on the simplicity and convenience of the system. Later, a systematic review [9] evaluating the impact of DSS on physician work and patient outcomes was carried out, which revealed an increase in doctor efficiency in 62 of 97 studies (64%) when DSS was used. Outcomes were assessed in 52 studies, of which 7 studies (13%) reported improved patient outcomes and prognosis. It was noted that studies that asked users to automatically use the system indicated better performance than studies that required users to activate the system themselves. However, the data obtained were based on a comparison of studies carried out in different conditions, with the participation of heterogeneous populations and using different methods.

Interesting data were obtained in a cluster randomized trial conducted in the Netherlands [10]. The study evaluated the effectiveness of electronic alerts versus on-demand DSS in the treatment of patients with dyslipidemia for 12 months. Thirty-eight clinics, 77 doctors and 87886 patients (39433 men aged 18 to 70 years and 48453 women aged 18 to 75 years) took part in the study using the ELIAS electronic health record system. Each clinic was set to automatically receive notifications. There was a function "on demand" or "no notifications". In the alert group, 65% of patients eligible for screening completed it (relative risk (RR) versus controls =1,76; 95% confidence interval (CI), 1,1-2,20) versus 35% of patients in the on-demand group (RR versus control =1,28; 95% CI, 0,98-1,68) and 25% of patients in the control group. In the automatic group, 66% of patients requiring treatment received appropriate therapy (RR versus control =1,40; 95% CI, 1,15-1,70) versus 40% of patients (RR versus control =1,19; 95% CI, 0,94-1,50) in the on-demand group and 36% in the control group. The automatic receipt of DSS alerts has significantly improved

the effectiveness of screening and treatment of dyslipidemia by general practitioners.

In 2010, a pilot study was conducted in Spain [11], the aim of which was to assess the efficacy, safety and cost-effectiveness of HTE-DLP DSS implementation in the treatment of dyslipidemia in high-CVR patients. The follow-up period lasted 2 weeks. Ten medical experts in the field of CVR management were recruited to the study. A total of 77 patients (43 and 34 with very high and high CVR, respectively) with LDL cholesterol >2,5 mmol/L were included in the study. The exclusion criteria were Charlson index >3, patient life expectancy <1 year, and triglyceride levels >4,5 mmol/L. The primary endpoint was an LDL-C level <1,8 mmol/L. HTE-DLP DSS was written in Java using open-source tools (OpenJDK, Netbeans, iText, POI). HTE-DLP provided consistency in clinical decision making, which included the choice of lipid-lowering therapy depending on the patient model and contraindications to therapy. Most of the patients reached an LDL level <1,8 mmol/L (55,0% vs 12,5%, p=0,003; RR: 3,26; CI, 1,16-9,15). High-dose statins and combined lipid-lowering therapy were used more often in the DSS group than in the control group (p=0,001). There were 7 reported adverse effects in the intervention group and 2 in the control group. According to the medical experts, HTE-DL system (86,1%) was useful and was considered easy to use (85%). The use of DSS in high-CVR patients has resulted in significant reductions in LDL-C levels.

It is necessary to note the study [12], the purpose of which was to study the DSS effect on the implementation of measures for secondary prevention in patients with coronary artery disease. The hospitals participating in the study were randomly assigned to 2 groups: intervention (n=56) and conservative care (n=56). The total sample consisted of 7448 patients. The program carried out automatic computer monitoring and provided treatment recommendations. Reminders were sent to primary care physicians in the intervention group every 4 months, updating current patient lipid profiles and recommendations for further treatment. In patients with baseline LDL >3,1 mmol/L, a significant decrease in LDL levels was observed in both groups, but was more pronounced in the intervention one: 3,15±0,88 mmol/L vs 3,21±0,89 mmol/L (P<0,02). A significantly lower rate of readmission due to CVD was recorded in patients who received adequate treatment with lipid-lowering drugs (37% vs 40,9% (p<0,001)). This study has demonstrated that an automated computerized reminder system greatly facilitates adherence and enables clinicians to determine which lipid-lowering therapy option is appropriate for each clinical case.

Chen C, et al. (2010) [13] also demonstrated a positive effect of DSS in the management of dyslipidemia patients. This system allowed the majority of patients to reach the target LDL level in 1 year. In addition, 74% of patients who used DSS consistently achieved target LDL levels. Of those who stopped using DSS, only 57% achieved the LDL goal (odds ratio, 2,1 (1,2, 3,8) (p=0,022)), which indicates the DSS effectiveness in achieving the target LDL level.

When developing a DSS, it is necessary to take into account a large number of factors. In the Netherlands, a study was conducted, which reflected the main characteristics of DSS work in the control and treatment of dyslipidemia according to general practitioners [14]. The CholGate study involved 40 outpatient clinics and 76 general practitioners. A structured questionnaire was sent to each doctor participating in the project. The questionnaire consisted of two parts, the first of which was about user requirements for DSS, while the second one was devoted to checking the knowledge of general practitioners about Netherland's guidelines for dyslipidemia management. The response rate was 71%. As a result, 38% of respondents stated the need to implement DSS in clinical practice. According to the first questionnaire part, the main DSS requirements were as follows: fast speed, convenience and simplicity, beautiful interface, automatic updates via the Internet. As for the second questionnaire part, only 58,8% (standard deviation 13,9) gave correct answers regarding the managing patients with dyslipidemia. Thus, this study demonstrated the main conditions that must be taken into account in the development and implementation of DSS, as well as low compliance to current guidelines in the treatment of patients with dyslipidemia.

Conclusion

Thus, it is obvious that the introduction of DSS system into routine practice has a clinical rationale [15] and can be considered as an effective tool for managing RF, treating CVDs, and predicting cardiovascular events. As part of the preparation of a platform for creating a DSS for lipid metabolism disorders, specialists of the Almazov National Medical Research Center, together with scientists from the University of Milan, created an algorithm for making decisions for dyslipidemia in patients with different CVR levels (available at: https://app.diagrams.net/#G16692nhD8cE6Fu4lXNNtEFdVGK_rsC_XZ).

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References

1. Cholesterol Treatment Trialists' (CTT) Collaboration, Baigent C, Blackwell L, Emberson J, et al. Efficacy and safety of more intensive lowering of LDL cholesterol: a meta-analysis of data from 170,000 participants in 26 randomised trials. Lancet. 2010;376(9753):1670-81. doi:10.1016/S0140-6736(10)61350-5.
2. Atherosclerosis and dyslipidemia. Diagnosis and correction of lipid metabolism disorders for the prevention and treatment of atherosclerosis. Russian recommendations, VII revision. 2020;1(38):7-42. (In Russ.) doi:10.34687/2219-8202.JAD.2020.01.0002.
3. Mach F, Baigent C, Catapano AL, et al. 2019 ESC/EAS Guidelines for the management of dyslipidaemias: lipid modification to reduce cardiovascular risk. Russian Journal of Cardiology. 2020;25(5):3826. (In Russ.) doi:10.15829/1560-4071-2020-3826.
4. Catapano AL, Graham I, De Backer G, et al. 2016 ESC/EAS guidelines for the management of dyslipidaemias. Russian Journal of Cardiology. 2017;(5):7-77. (In Russ.) doi:10.15829/1560-4071-2017-5-7-77.
5. Ray KK, Molemans B, Schoonen WM. EU-Wide Cross-Sectional Observational Study of Lipid-Modifying Therapy Use in Secondary and Primary Care: the DA VINCI study, European Journal of Preventive Cardiology. 2020;zwaa047. doi:10.1093/eurjpc/zwaa047.
6. Gusev AV, Pliss MA, Levin MB, Novitsky RE. Trends and forecasts of the development of medical information systems in Russia. Doctor and information technology. 2019;(2):38-49. (In Russ.)
7. Roshanov PS, Misra S, Gerstein HC, et al. Computerized clinical decision support systems for chronic disease management: a decision-maker-researcher partnership systematic review. Implement Sci. 2011;6:92. doi:10.1186/1748-5908-6-92.
8. Lyamina NP, Kotelnikova EV. Decision support system as a component of the patient-oriented model of cardiological rehabilitation. Doctor.ru. 2017;5(134):42-6. (In Russ.)
9. Garg AX, Adhikari NKJ, McDonald H, et al. Effects of Computerized Clinical Decision Support Systems on Practitioner Performance and Patient Outcomes: A Systematic Review. JAMA. 2005;293(10):1223-38. doi:10.1001/jama.293.10.1223.
10. van Wyk JT, van Wijk MA, Sturkenboom MC, et al. Electronic alerts versus on-demand decision support to improve dyslipidemia treatment: a cluster randomized controlled trial. Circulation. 2008;117(3):371-8. doi:10.1161/CIRCULATIONAHA.107.697201.
11. Zamora A, Fernandez de Bobadilla F, Carrion C, et al. VALIDA Study Group; Network of Lipid Units of Catalonia (XULA). Pilot study to validate a computer-based clinical decision support system for dyslipidemia treatment (HTE-DLP). Atherosclerosis. 2013;231(2):401-4. doi:10.1016/j.atherosclerosis.2013.09.029.
12. Gilutz H, Novack L, Shvartzman P, et al. Computerized community cholesterol control (4C): meeting the challenge of secondary prevention. Isr Med Assoc J. 2009;11(1):23-9.
13. Chen C, Chen K, Hsu CY, et al. A guideline-based decision support for pharmacological treatment can improve the quality of hyperlipidemia management. Comput Methods Programs Biomed. 2010;97(3):280-5. doi:10.1016/j.cmpb.2009.12.004.
14. van Wyk JT, van Wijk MA, Moorman PW, et al. User requirements rating and knowledge-level of general practitioners at the start of CholGate — lipid management decision support project. AMIA Annu Symp Proc. 2005;2005:1146.
15. Lobach D, Sanders GD, Bright TJ, et al. Enabling health care decision-making through clinical decision support and knowledge management. Evid Rep Technol Assess (Full Rep). 2012;(203):1-784.