Calcification markers and long-term outcomes of coronary artery bypass grafting

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Aim. To assess the long-term outcomes of coronary artery bypass grafting (CABG) and their association with calcification biomarkers.

Material and methods. The study included 129 men (mean age, 61,5±7,5 years) with coronary atherosclerosis who were admitted for CABG surgery. Patients were divided into 2 groups: with favorable and unfavorable (death, myocardial infarction, stroke, surgery) 5-year prognosis after surgery. Before the surgery, the blood concentrations of calcification biomarkers (osteoprotegerin, osteopontin, osteonectin and osteocalcin) were determined in all patients.

Results. Long-term outcomes of myocardial revascularization were studied in 92 patients (71%). An unfavorable long-term 5-year period was identified in 28 men (30,4%). In men with an unfavorable 5-year prognosis, the blood osteocalcin level before CABG was 1,2 times higher than in men with a favorable one. Multivariate linear regression showed that the risk of a 5-year unfavorable prognosis for coronary atherosclerosis after myocardial revascularization was associated with the blood osteocalcin concentration, determined before CABG (B=0,018, R^2=0,285, p=0,008).

Conclusion. The data obtained indicate the relevance of continuing studies on osteocalcin, including with respect to its contribution to coronary atherosclerosis and calcification.

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Atherosclerosis is the pathomorphological basis of cardiovascular diseases (CVDs), including coronary atherosclerosis (CAS) and coronary artery disease (CAD). It is CVDs, in particular, CAD, that are the leading cause of death in the world today. Coronary artery bypass graft (CABG) surgery remains the most important method of myocardial revascularization. A large number of studies have been devoted to evaluating long-term outcomes after CABG. Significant factors affecting a poor prognosis are diabetes [1], smoking [2, 3], familial hypercholesterolemia [4]. The influence of sex and age on the short- and long-term survival of patients after CABG is also analyzed [5].

There is no doubt about the association of coronary artery calcification with CVD [6, 7]. In a multiethnic prospective cohort study, multislice computed tomography revealed an association of coronary artery calcification with an almost twofold increased risk of cardiovascular episodes independent of statin therapy [8].

Today, the following calcification biomolecules are being actively studied: osteoprotegerin, osteopontin, osteocalcin and osteonectin. It has been determined that osteoprotegerin and osteopontin are important factors in vascular remodeling and the progression of atherosclerosis.

The concentration of osteopontin in patients with coronary artery disease correlates with the severity of coronary atherosclerosis [9]. Associations were found between the concentration of osteopontin in the blood and the presence of coronary artery disease [10], as well as between the blood concentration of osteoprotegerin and CAD severity [11]. It has been shown that osteocalcin is a predictor of CAS severity [12]. A decrease in the blood concentration of osteocalcin is associated with a high risk of developing carotid atherosclerotic plaques in patients with type 2 diabetes [13]. The study of calcification biomolecules for assessing risk and predicting complications of atherosclerosis seems to be very relevant.

The aim of our study was to assess the long-term outcomes of CABG surgery in patients with CAS, as well as to search for associations of an unfavorable 5-year prognosis with the blood content of calcification biomarkers before surgery.

Material and methods

The study was carried out within joint research work of the Research Institute of Internal and Preventive Medicine — branch of the Federal Research Center Institute of Cytology and Genetics and the Meshalkin National Medical Research Center. The study was approved by the ethical committees of these centers. The study included 129 men aged 42-77 years (mean age, 61,5±7,5 years) with CAS verified by coronary angiography, with stable exertional angina, who were admitted for surgical treatment to the Meshalkin National Medical Research Center. All patients received standard CAD therapy before and after CABG surgery, including statins, antiplatelet agents, angiotensin-converting enzyme inhibitors, and β-blockers. The exclusion criteria were myocardial infarction (MI) <6 months old, acute and exacerbation of chronic infectious and inflammatory diseases, renal failure, active liver disease, cancer, hyperparathyroidism. All patients signed written informed consent. All 129 patients underwent CABG. The mean time of on-pump CABG was 50 minutes, while there was 3 grafts on average. Cardioplegia in CABG was performed with Custodiol solution. CABG was performed according to the standard technique.

Before CABG surgery, venous blood was received from all patients for basic biochemical tests, including assessing the concentration of calcification biomolecules. In the blood, the concentration of osteoprotegerin, osteopontin (ELISAs Bender MedSystems test systems), osteocalcin, osteonectin (ELISAs Immunodiagnostic Systems Ltd test systems) was determined using an ELISA Multiscan EX analyzer (Thermo, Finland). Also, following biomarkers of endothelial dysfunction were determined in blood by ELISA method: monocyte chemoattractant protein 1 (MCP-1), soluble vascular cell adhesion molecule (sVCAM), E-selectin (ELISAs Bender MedSystems test systems).

Long-term outcomes of myocardial revascularization were studied by us 5 years after CABG surgery. The following endpoints of an unfavorable long-term period were assessed: cardiovascular death, MI, stroke, additional surgical interventions (percutaneous transluminal coronary angioplasty, carotid endarterectomy, etc). To analyze the long-term outcomes, we used discharge summaries of patients who were undergoing reexamination and treatment at the Meshalkin National Medical Research Center. The direct telephone interviews were also used.

The statistical processing was performed using the SPSS software (17.0). The distribution normality was determined using the Kolmogorov-Smirnov test. The significance of differences was assessed using the Mann-Whitney test. In order to reveal the associations, multivariate linear regression was carried out. Differences were considered significant at p<0,05.

Results

Long-term outcomes of myocardial revascularization were studied 5 years after CABG in 92

Table 1

Comparison of blood concentration of calcification biomolecules between groups of men with different long-term prognosis of CAS (Me [25;75])

Parameters	Unfavorable prognosis, n=28	Favorable prognosis, n=64
Osteoprotegerin, pg/ml	51,4 [33,5; 79,3]	52,3 [34,2; 77,3]
Osteocalcin, ng/ml	14,0 [9,0; 21,8]*	11,8 [7,7; 15,1]
Osteopontin, ng/ml	20,2 [17,8; 49,8]	28,9 [16,0; 38,0]
Osteonectin, µg/ml	7,4 [9,2; 10,2]	8,8 [7,9; 10,9]
sVCAM, ng/ml	788,7 [627,4; 1058,6]	841,2 [697,0; 1038,1]
Е-селектин, ng/ml	49,9 [33,6; 62,1]	47,6 [33,2; 60,0]
MCP-1, pg/ml	443,5 [249,5; 537,3]	456,6 [322,1; 588,8]

Note: * — difference between groups at p=0,035

Abbreviations: MCP-1 — monocyte chemoattractant protein 1, sVCAM — soluble vascular cell adhesion molecule.

Table 2

Results of multivariate regression analysis of the association of blood concentration of calcification biomolecules with the risk of an unfavorable prognosis for CAS after myocardial revascularization

Parameters	B coefficient	R^2	p
Osteoprotegerin, pg/ml	0,013	0,296	0,053
Osteocalcin, ng/ml	0,018	0,285	0,008
Osteopontin, ng/ml	0,249	0,084	0,114
Osteonectin, µg/ml	0,213	0,116	0,095

patients, which is 71% of all patients included in the study. Within 5 years, 5 men (5,4%) had cardiovascular death (including 1 case of fatal MI), 6 (6,5%) — non-fatal MI, 5 (5,4%) — stroke, 12 — (13,0%) underwent additional surgery.

After analyzing the data, 2 groups of patients were formed: group 1 — 64 men (69,6%) with a favorable 5-year course of the disease, group 2 — 28 men (30,4%) with an unfavorable 5-year course of the disease.

Coronary artery calcification is associated with CVDs [6, 7]. Therefore, we searched for associations of an unfavorable 5-year prognosis of CAS after CABG with calcification biomolecules, which were measured in the blood before CABG. The results of a comparative intergroup analysis are presented in Table 1. We found that in men with an unfavorable 5-year prognosis of CAS, the blood level of osteocalcin before CABG was 1,2 times higher than in men with a favorable 5-year prognosis of the disease.

The following multivariate linear regression analysis with the dependent variable "favorable/ unfavorable prognosis" and independent variables "biomarkers of calcification and endothelial dysfunction" also showed a significance of osteocalcin levels (Table 2).

We found that the risk of a 5-year unfavorable prognosis for CAS after myocardial revascularization is associated with the blood concentration of osteocalcin, determined before CABG (B=0,018, R^2=0,285, p=0,008).

Discussion

The relationship between the progression of CAS and the long-term disease prognosis remains poorly understood. We assessed the relationship between the poor prognosis of CAS and some biochemical markers. Diabetes [1], smoking [2], familial hypercholesterolemia [4] are often a risk factors (RFs) for CAS progression and an unfavorable prognosis.

It is known that the risk of coronary calcification increases with age. The progression of coronary artery calcification, analyzed over 10 years of follow-up using computed tomography, demonstrated a relationship with cardiovascular RFs. It has been proposed to use longitudinal coronary artery calcium progression to assess CVD RFs [6]. The limitation of our study is the lack of data on multislice computed tomography, which has a high sensitivity and specificity in the diagnosis and quantitative assessment of coronary calcification.

We analyzed the relationship between biochemical markers of calcification, which have been actively studied in recent years, with an unfavorable 5-year prognosis for CAS after myocardial revascularization.

Osteopontin and osteonectin are glycoproteins of a cell-matrix protein class known as regulators of metalloproteinase activity. Osteopontin is a multifunctional protein involved in the production of cytokines, regulation of cell migration, adhesion and differentiation of various cells, including macrophages, endothelial cells, smooth muscle cells, lymphocytes and fibroblasts [10]. Osteoprotegerin and osteopontin are one of the key factors in both vascular remodeling and the progression of atherosclerosis. It has been shown that in patients with CAD, the level of both osteoprotegerin and osteopontin is increased [14]. The concentration of osteopontin in patients with CAD correlates with CAD severity and parameters of left ventricular remodeling [9]. Osteoprotegerin is a glycoprotein from to the tumor necrosis factor receptor family, which inhibits osteoclastogenesis by acting as a decoy receptor for the receptor activator of nuclear factor-κB ligand. A direct relationship between osteoprotegerin and CAD severity was shown [11]. The relationship between the blood level of osteoprotegerin and coronary calcium has been demonstrated in patients with type 2 diabetes [15].

In our study, we did not find a significant relationship between the blood concentration of osteopontin, osteonectin, and osteoprotegerin and the 5-year prognosis of patients with CAS after myocardial revascularization. Perhaps this is due to the insufficient number of compared groups of men.

Osteocalcin is a hydroxyapatite-binding protein synthesized by osteoblasts, which contains 3 gamma-carboxyglutamic acid residues, which are responsible for the protein's calcium-binding properties. Osteocalcin, known as a bone turnover marker, is used in clinical practice to assess the efficacy and treatment of osteoporosis [16]. In addition, osteocalcin acts as a hormone that controls the metabolism of glucose and energy in the pancreatic β-cells, adipose and muscle tissues [17]. According to some reports, osteocalcin also acts as a permanent inhibitor of vascular calcification [15].

However, findings about the relationship between blood levels of osteocalcin and cardiac function are inconsistent. Clinical study data have shown that blood levels of osteocalcin correlate with heart function. The authors reported on the relationship between blood osteocalcin levels and left ventricular ejection fraction, finding that lower blood osteocalcin levels correlated with a higher risk of left ventricular systolic dysfunction [18]. Results have been published indicating that coronary calcification is independently associated with blood levels of osteocalcin [11].

In our study, we showed that in men with an unfavorable 5-year prognosis for CAS after myocardial revascularization, the blood level of osteocalcin before CABG was 1,2 times higher than in men with a favorable 5-year prognosis. In addition, we found that the risk of a 5-year unfavorable prognosis for in patients with CAS after myocardial revascularization was associated with the blood concentration of osteocalcin determined before CABG (B=0,018, R^2=0,285, p=0,008).

Conclusion

Our findings for osteocalcin are consistent with some studies but inconsistent with others. This reflects the contradictory data accumulated to date in literature on this calcification biomolecule. Since calcification biomolecules continue to be actively studied in the world today, it is undoubtedly relevant to continue research on the influence of these biomolecules on the prognosis of CVDs and their complications.

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