

Влияние факторов роста фибробластов и конечных продуктов гликирования на толщину комплекса интима-медиа у больных с ишемической болезнью сердца и сахарным диабетом 2 типа

Иванникова Е.В., Калашников В.Ю., Смирнова О.М., Кузнецов А.Б., Терёхин С.А., Ильин А.В.

ФГБУ Эндокринологический научный центр, Москва
(директор — академик РАН И.И. Дедов)

Цель. Определение уровней трансформирующего фактора роста фибробластов (TGFβ1), основного фактора роста фибробластов (β-FGF), маркеров неспецифической воспалительной реакции (интерлейкина-6 (IL-6), С-реактивного белка (CRP)), конечных продуктов гликирования (AGE) и их рецепторов (RAGE) и изучение их влияния на толщину комплекса интима-медиа (КИМ) у больных с ишемической болезнью сердца (ИБС) и сахарным диабетом 2 типа (СД2) в зависимости от компенсации углеводного обмена.

Материалы и методы. У 37 пациентов с ИБС было проведено общеклиническое обследование, анализ показателей углеводного и липидного обмена, функции почек, а также инструментальные методы исследования (эхокардиография, коронароангиография и дуплексное сканирование брахиоцефальных артерий). Для определения уровня исследуемых показателей всем больным забор крови осуществлялся из аорты во время проведения коронароангиографии и параллельно из кубитальной вены.

Результаты. Установлено, что наличие СД2 у больных ИБС ассоциировано с более тяжелым атеросклеротическим поражением коронарных и брахиоцефальных сосудов. Выявлена прямая корреляционная взаимосвязь между степенью стенозирования и уровнем факторов роста фибробластов, провоспалительных факторов, конечных продуктов гликирования. Получена прямая корреляция TGFβ1 и AGE с показателями липидного обмена. Выявлено достоверное повышение уровня исследуемых показателей в артериальной и венозной крови у пациентов с СД.

Заключение. Полученные данные подтверждают наличие взаимосвязи нарушений со стороны соединительной ткани и липидного обмена в патогенезе атеросклероза. Продemonстрировано негативное влияние гипергликемии на атеросклеротические изменения стенки сосудов.

Ключевые слова: факторы роста фибробластов; комплекс интима-медиа; конечные продукты гликирования; сахарный диабет; атеросклероз

The effect of fibroblast growth factors and advanced glycation end-products on the intima-media complex thickness in patients with coronary heart disease and type 2 diabetes

Ivannikova E.V., Kalashnikov V.Yu., Smirnova O.M., Kuznetsov A.B., Terekhin S.A., Il'in A.V.
Endocrinology Research Centre, Moscow, Russian Federation

Objective. To determine the levels of fibroblast transforming growth factor (TGFβ1), basic fibroblast growth factor (β-FGF), markers of nonspecific inflammatory response (interleukin-6 (IL-6)), C-reactive protein (CRP), advanced glycation end-products (AGEs) and their receptors (RAGEs) and to study their effect on the intima-media complex (IMC) thickness in patients with coronary heart disease (CHD) and type 2 diabetes, depending on carbohydrate metabolism compensation.

Materials and Methods. 37 patients with CHD underwent a general clinical examination, analysis of the carbohydrate and lipid metabolism parameters and the renal function, and also were evaluated with instrumental methods of analysis (echocardiography, coronary angiography and duplex scanning of the brachiocephalic arteries). To determine the level of the analyzed parameters, blood samples were taken from the aorta during coronary angiography and concomitantly from the cubital vein in all patients.

Results. The presence of diabetes mellitus (DM) in patients with CHD was found to be associated with a more severe atherosclerotic disease of the coronary and brachiocephalic vessels. A direct correlation between the degree of stenosis and the level of fibroblast growth factors, inflammatory factors, and advanced glycation end-products was found. A direct correlation between AGE and TGFβ1 and the lipid metabolism parameters was established. A statistically significant elevation of the studied parameters in the arterial and venous blood of patients with DM was revealed.

Conclusion. These findings confirm the relationship between connective tissue disorders and lipid metabolism in the pathogenesis of atherosclerosis. A negative effect of hyperglycaemia on atherosclerotic changes of the vascular wall was demonstrated.

Keywords: fibroblast growth factors; intima-media complex; advanced glycation end-products; diabetes; atherosclerosis

DOI: 10.14341/DM2014247-55

The use of the simple non-invasive method of ultrasonography (USG) allows identification of the changes in the vascular wall at the early stages [1]. Thickening of the intima–media complex (IMC) of large peripheral vessels is considered as an important characteristic of systemic changes in atherosclerosis [2]. Pignoli was one of the first researchers who demonstrated that the IMC thickness of the common carotid artery (CCA) obtained using USG was identical to that measured in a post-mortem examination [3]. Subsequently, a series of studies were conducted where a negative effect of various atherosclerosis risk factors on the IMC thickness was revealed [4]. According to some studies, even in young patients with a low risk for the development of cardiovascular diseases by the Framingham Risk Score (FRS) (<5%), early atherosclerotic changes were revealed upon USG of the carotid arteries, which may be indicative of atherosclerosis progression in the coronary arteries [5]. Therefore, these findings demonstrated that an examination of the extracranial portion of the brachiocephalic arteries by measuring the IMC thickness was the method of choice for non-invasive screening for subclinical manifestations of atherosclerosis.

In 2007, Lorenz et al analysed the results of eight studies that included 37,197 patients [6]. This analysis found a regular increase in the thickness of the IMC by 0.1 mm with an increase in the risk of acute coronary syndrome (ACS) from 10% to 15% and in the risk of acute cerebrovascular disease (ACVD) from 13% to 18% [7]. According to Bots et al (Rotterdam study), an increase in the IMC thickness even in the range of normal values (0.75–0.91 mm) was accompanied by a 4.8-fold increase in the relative risk of the first ACVD [8]. In the Second Manifestation of Arterial Disease (SMART) study, USG of the carotid arteries in 2,374 patients with proven vascular diseases revealed that thickening of the IMC was associated with a high risk for the development of cardiovascular pathology [9]. The IMC thickness of the CCA has been closely related to age, gender, smoking habits and presence of arterial hypertension (AH) [10, 11]. In the Kuopio Ischemic Heart Disease Study among 1,165 males aged 42–65 years, the frequency of IMC thickening was 2.61 times higher in people with hypertension than in those with a normal arterial pressure (AP). This parameter has also been shown to be dependent on total cholesterol levels and body mass index (BMI) [12, 13].

It is known that the arterial wall is a three-layer structure [14]. The first layer is the intima, which is primarily composed of endothelial cells. The medial layer is represented by smooth muscle that is protected by the adventitia from above. These are separated by the internal and external elastic membranes [6]. On USG, the IMC in a healthy person appears as a two-layer structure, with the hyperechoic layer of the intima adjacent to the lumen and the subjacent hypoechoic layer of the media. The cells of each layer perform unique functions. For example, adventitial fibroblasts actively synthesise one of the most important components of the extracellular matrix, collagen. Smooth

muscle cells are responsible for elastin production [16]. Collagen gradually accumulates in the intima of the vascular wall with age, resulting in its thickening and structural changes in the other layers [17–19]. On USG of the thickened IMC, the differentiation of the layers disappears, and heterogeneity and roughness of the surface appear [15]. A number of studies have demonstrated that collagen is deposited in all three layers, which lends a special rigidity to the vessels. With ageing, cultured endothelial cells and fibroblasts of the adventitial layer synthesise type 1 collagen several times more actively [20]. With age, particularly under hyperglycaemic conditions, the concentration of fibroblasts activating the synthesis of various growth factors is also increased [19].

These growth factors include the basic fibroblast growth factor (β -FGF), transforming growth factor (TGF- β 1) and platelet-derived growth factor (PDGF-AA) [21]. Their role in cytokine production in response to stimulation with interleukins (ILs) has been recognised [22]. β -FGF is a potent modulator of cell differentiation, proliferation and motility [23]. TGF- β 1 enhances or suppresses (depending on the cell type) the response of most cells to other growth factors, regulates their differentiation and β -FGF activity and results in the incorporation of fibrillin protein in the intercellular matrix, thus activating myofibroblasts. Fibroblasts are also activated directly under hyperglycaemic conditions because of the acceleration of polyol shunting, activation of protein kinase C, oxidative stress and glycation of fibroblast growth factors and formation of advanced glycation end products (AGEs) [18, 24, 25]. This leads to uncontrolled pathological activity of fibroblasts; they begin to actively proliferate, destroy collagen and synthesise new material, which facilitates the restructuring of the vascular wall to develop fibrosis [18, 26–28].

OBJECTIVE

The objective of this study was to investigate the activity of growth factors TGF- β 1 and β -FGF, non-specific inflammatory response markers [IL-6 and C-reactive protein (CRP)] and AGEs and their receptors (RAGEs) as well as the relationships of these parameters with the IMC thickness in patients with coronary heart disease (CHD) depending on the carbohydrate metabolism status.

MATERIALS AND METHODS

This cross-sectional cohort study included patients with CHD who had been examined at the Endocrinology Research Centre in 2012–2013. The first group included 17 patients (11 males) without carbohydrate metabolism disorders; the second group included 20 patients (14 males) with type 2 diabetes mellitus (T2DM). The groups were comparable in terms of age, CHD duration, BMI and renal function status. In order to compare the levels of the studied parameters in the venous and arterial blood, blood samples were collected from the aorta during coronary angiography (CAG) and concomitantly from the median cubital vein in

all patients. The samples were centrifuged (15,000 rpm) and deep-frozen at -70°C . The studied parameters (TGF- β 1, β -FGF, IL-6, CRP, AGEs and RAGEs) were determined by the enzyme-linked immunosorbent assay (ELISA) method using standard kits purchased from eBioscience and Biomedica. The expected values were provided in the manufacturer's protocols.

Ultrasound Doppler examination and duplex scanning were performed on a Siemens device (Germany) using a broadband probe with phased array at a frequency of 7.5–10.5 MHz in duplex and Doppler modes (B-mode, colour Doppler mapping, spectral analysis of the Doppler frequency shift). The upper edge of the thyroid cartilage served as a guide for the identification of the distal portion (bifurcation) of the CCA. Duplex scanning of vessels was conducted with a linear probe operating in the frequency range of 7.5–10 MHz. When scanning the carotid arteries, the probe was placed along the front and rear edges of the sternocleidomastoid muscle. Scanning was performed in three planes (two longitudinal and one transverse). The IMC thickness of the CCA was measured 1.5–2 cm proximal to the bifurcation along the artery wall outermost from the probe. Upon diagnostic scanning, the IMC thickness of the CCA, internal carotid artery (ICA) and external carotid artery (ECA) were evaluated at the site of maximum thickening as determined visually [29].

Characteristics such as lifestyle, diet, smoking, disease duration, heredity and physical activity levels were taken into account when collecting anamnestic data. A general examination was performed and the body weight (with BMI calculation) and AP were measured. The levels of lipids, blood glucose throughout the day and glycated haemoglobin were evaluated, and the degree of atherosclerotic lesions of other systems (arteries of the lower limbs and neck) was determined. All patients signed an informed consent form to participate in the study.

Statistica 6.0 for Windows and BioStat for Windows (Primer of Biostatistics, 4th Edition, S.A. Glantz, McGraw-Hill) software were used for statistical processing of the data. The data are presented as a median (25; 75 percentiles). The relationship among various parameters was established using Spearman's rank correlation (Spearman R). Probability (p) values of <0.05 were considered statistically significant.

RESULTS AND DISCUSSION

The median age of patients was 67 years (61.5; 73) in the first group and 67.5 years (60.5; 73.7) in the second group. The median BMI in the first group was 27.2 kg/m² (26.8; 29.8), which was comparable to that of 27 kg/m² (28.1; 35.4) in the second group of patients with T2DM. At the time of enrolment, 81.1% (n = 30) patients received permanent lipid-lowering therapy, 64.8% (n = 24) received aspirin therapy, 32.4% (n = 12) received dual antiplatelet therapy and 5.4% (n = 2) received anticoagulant therapy. The median duration of T2DM in patients in the second group was 11.5 years (9.5; 18.5). Oral antihyperglycaemic therapy was prescribed for 40% (n = 8) patients, which was

Table 1

Clinical profiles of the groups		
	First group (n = 17)	Second group (n = 20)
Age, years	67 [61,5;73]	67,5 [60,5;73,7]
Males/females, %	64,7/35,3	70/30
Smokers, %	70,5	65,3
BMI, kg/m ²	27,2 [26,8;29,8]	27 [28,1;35,4]
HbA _{1c} , %	5,6 [5;5,8]	7,6 [7,3;8]*
Cholesterol, mmol/L	4,25 [3,8;5]	4,5 [3,7;4,85]
Creatinine, $\mu\text{mol/L}$	82,6 [75,2;88]	71 [67,5;84,3]
GFR, mL/min/1.73 m ²	85[90;75]	96[95;79]
EF, %	57,5 [56;61,2]	51 [44,5;55]
LVMI, g	182 [123,5;234]	193,5 [139,7;235,7]
ACVD, %	17,6 (n=3)	20 (n=4)
ACS without ST-segment elevation, %	29,4 (n=5)	35 (n=7)
Post-infarction cardiosclerosis, %	23,5 (n=4)	25 (n=5)
Exertional angina:		
functional class II, %	41,7 (n=7)	40 (n=8)
functional class III, %	58,8 (n=10)	60 (n=12)
CHD duration, years	4,9 [2,3;10,2]	5,5 [2,8;9,5]
CAG:		
one vessel, %	17,5	4
two vessels, %	21,5	15
three vessels, %	61*	81*
left coronary artery trunk, %	19*	26*

*p<0,05

combined with metformin in 25% (n = 5) patients; 60% (n = 12) patients received intensified insulin therapy. In the assessment of carbohydrate metabolism compensation, HbA_{1c} levels were 7.6% (7.3; 8) in the second group and 5.6% (5; 5.8) in the first group. Lipid metabolism parameters did not differ in both groups; on average, the median cholesterol level was 4.5 mmol/L (3.7; 4.85) in the presence of T2DM and 4.25 mmol/L (3.8; 5) without T2DM. Evaluation of renal function was performed on the basis of the creatinine level data [82.6 $\mu\text{mol/L}$ (75.2; 88) in the first group and 71 $\mu\text{mol/L}$ (67.5; 84.3) in the second group]; the median glomerular filtration rate (GFR) was 72 mL/min/1.73 m² (67.7; 75.7) and 84.2 mL/min/1.73 m² (67.5; 94), respectively (Table 1).

The average duration of CHD was 4.9 years (2.3; 10.2) in the first group and 5.5 years (2.8; 9.5) in the second group. The number of patients who had suffered from ACVD was 17.6% (n = 3) in the first group and 20% (n = 4) in the second group. ACS without ST-segment elevation was diagnosed in 29.4% (n = 5) patients in the first group and 35% (n = 7) in the second group. It is noteworthy that 15% (n = 3) patients had painless myocardial ischemia. One patient in the first group and two in the second group had previously undergone coronary artery bypass surgery. Fifteen patients in the first group and 19 in the second group were treated, either previously or at the time of the study, with angioplasty of the coronary arteries. When conducting analysis according to special methods, echocardiography (EchoCG) revealed that the median left ventricular ejection fraction (EF) was

Table 2

Data on the degree of stenosis of the carotid arteries according to ultrasound Doppler examination

Stenosis, %		CCA		ICA		ECA	
		First group	Second group	First group	Second group	First group	Second group
<50	on the left	95 (n=19)	64,7 (n=11)	90 (n=18)	76,4 (n=13)	80 (n=16)	76,4 (n=13)
	on the right	80 (n=16)	64,7 (n=11)	80 (n=16)	70,5 (n=12)	85 (n=17)	76,4 (n=13)
50–75	on the left	0	23,5 (n=4)	5 (n=1)	17,6 (n=3)	15 (n=3)	17,6 (n=3)
	on the right	15 (n=3)	17,6 (n=3)	15 (n=3)	11,7 (n=2)	5 (n=1)	11,7 (n=2)
>75	on the left	5 (n=1)	11,7 (n=2)	5 (n=1)	5,8 (n=1)	5 (n=1)	5,8 (n=1)
	on the right	5 (n=1)	17,6 (n=3)	5 (n=1)	17,6 (n=3)	11,7 (n=2)	11,7 (n=2)
IMC thickness, mm	on the left	0,9 [0,6; 1,3]	1,3 [1; 1,6]*	0,5 [0,5; 0,7]	1 [0,5; 1,2]*	0,5 [0,5; 0,6]	1 [0,5; 1,2]
	on the right	0,8 [0,6; 1,3]	1,4 [0,8; 1,6]*	0,5 [0,5; 1,4]	0,9 [0,5; 1]	0,5 [0,5; 1,4]	1 [0,5; 1,2]

*p < 0.01 vs. first group

Table 3

The levels of the studied factors in the venous and arterial blood of patients with CHD depending on the presence of T2DM.

Parameter under study		Expected values	First group (n = 17)	Second group (n = 20)
β-FGF, pg/ml	artery	0–13	22,71* [14,5; 37,5]	35,88* [19,04; 89,01]
	vein		9,45* [9,2; 32,6]	11,63* [2,3; 18,86]
IL-6, pg/ml	artery	0–8,7	0,44* [0,37; 6,25]	2,07* [1,57; 2,4]
	vein		3,11* [1,55; 6,7]	4,22* [2,02; 4,37]
CRP, mg/L	artery	0–5	5,9 [3,4; 8,5]	6,9 [1,8; 15,36]
	vein		8,35 [6,13; 28,4]	12,04 [2,7; 13,85]
TGFβ1, pg/ml	artery	4639–14757	6351* [4104,4; 10805]	8536,3* [6031,5; 19519]
	vein		24618* [14137; 27111]	28099* [15359; 29691]
AGEs, pg/ml	artery	0–33	22,87 [19,3; 25,14]	81,3* [32,3; 110,1]
	vein		39,1 [9,69; 87,06]	46,3 [9,6; 67,7]
RAGEs, pg/ml	artery	368–4354	1076,4 [1056,4; 3330,7]	6883 [2278,3; 11482]
	vein		1236 [1366; 2200]	7161,8 [1872,8; 14964]

*p₁₋₂ < 0,01

57.5% (56; 61.2) in the first group and 51% (44.5; 55) in the second group. The median left ventricular mass index (LVMI) was 182 g (123.5; 234) in the first group and 193.5 g (139.7; 235.7) in the second group. According to CAG, a more severe coronary artery disease rate of three-vessel disease: 61% in the first group and 81% in the second group (p < 0.005) was noted in the group of patients with T2DM.

According to the recommendations of the European Society of Hypertension (ESH) and the European Society of Cardiology (ESC) for arterial hypertension, when diagnosing a lesion of target organs, the value of 0.9 mm was selected as the upper limit of normal IMC thickness, 0.9–1.3 mm indicated thickening and >1.3 mm represented

severe atherosclerosis [29]. Based on the USG data for the brachiocephalic arteries, patients were divided into three groups depending on the degree of stenosis of the CCA:

- group A: <50%, which was considered non-clinically significant narrowing
- group B: >50% but <75%, which did not require surgical treatment
- group C: >75%.

In analysing the data, more severe atherosclerotic lesions of the carotid arteries, which required surgical treatment, were observed in the second group (Table 2).

The IMC thickness of the CCA was significantly different between patients in the second group with T2DM and those in the first group without T2DM (1 + 0.6 and 0.9 + 0.3 mm, respectively) (Fig. 1).

A previous study has elucidated on the important role of inflammation in atherosclerosis progression [24]. Patients with T2DM show signs of diffuse dysfunction of the vascular endothelium that develops in the smooth muscle and endothelial cells under conditions of hyperglycaemia and pathological lipid peroxidation. In the present study, patients with T2DM had higher levels of inflammatory markers. For example, the levels of IL-6 were statistically significantly higher in second group [2.07 pg/mL (1.57; 2.4) in the arterial blood and 4.22 pg/mL (2.02; 4.37) in the venous blood] than in the first group [0.44 pg/mL (0.37; 6.25) and 3.11 pg/mL (1.55; 6.7), respectively] (r = 0.543, p < 0.05). CRP levels in patients with T2DM were significantly higher

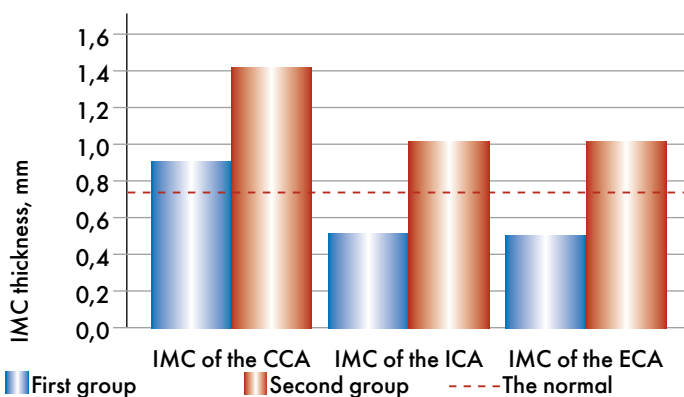


Fig. 1. The IMC (mm) in patients with CHD depending on the presence of T2DM (p < 0.05).

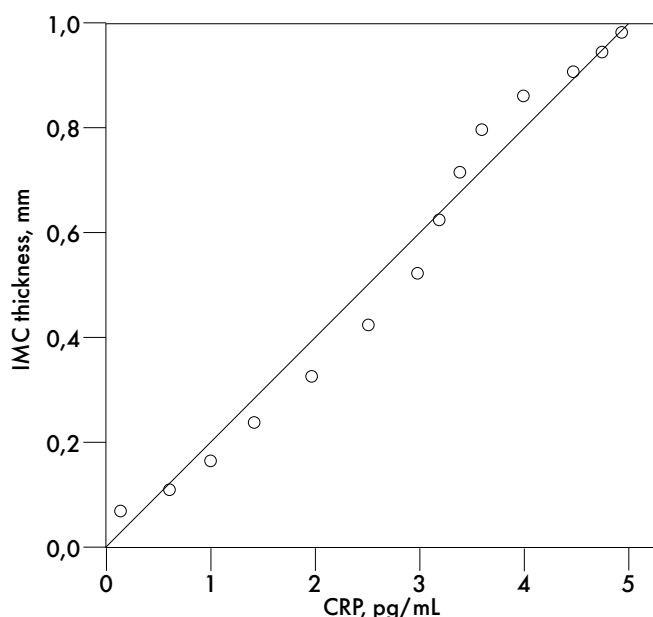


Fig. 2. Correlation between CRP levels in the venous blood and IMC thickness ($r = 0.531$, $p < 0.01$).

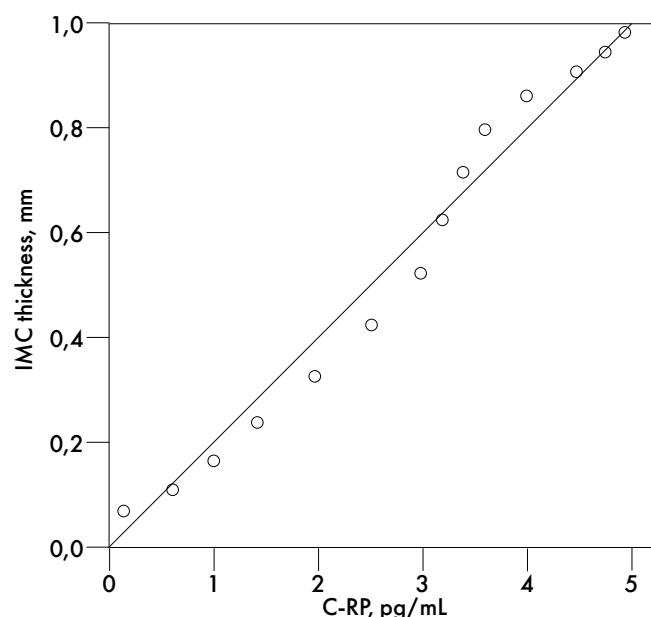


Fig. 3. Correlation between TGF- β 1 levels in the venous blood and IMC thickness ($r = 0.864$, $p < 0.05$).

in both the arterial blood [6.9 mg/L (1.8; 15.36)] and venous blood [12.04 mg/L (2.7; 13.85)]. In the group of patients with CHD, CRP levels were 5.9 mg/L (3.4; 8.5) in the arterial blood and 8.35 mg/L (6.13; 28.4) in the venous blood (Table 3). The impact of ACS on this significant increase in CRP levels should not be excluded; however, a separate statistical analysis of this cohort of patients was not possible because of the small sample size. The CRP level elevation in both groups of patients was possibly due to the severity and prevalence of atherosclerotic lesions in several vascular systems. In addition, the groups themselves were heterogeneous with respect to CHD severity (in particular, the groups included patients with both acute and chronic CHD). Meanwhile, the number of patients with ACS in both groups was approximately the same (30% and 35%, respectively), which, in our opinion, allowed comparison between the groups.

We analysed the relationship between the IMC thickness and levels of inflammatory markers. A direct correlation was observed between the IMC thickness of the CCA ($r = 0.401$, $p < 0.05$) and the degree of its stenosis and CRP levels in the venous blood of patients with T2DM (Fig. 2). A relationship between IL-6 levels in the venous blood and the IMC thickness of the ICA ($r = 0.814$, $p < 0.01$) was also established.

T2DM duration and HbA_{1c} levels were directly correlated with the levels of IL-6 and CRP, which may indicate an increase in the local cardiac synthesis of fibroblast growth factors and inflammatory markers. A strong direct correlation was observed between CRP and IL-6 levels and the degree of lesions of the coronary vessels ($r = 0.638$; $p < 0.05$), which was consistent with the results reported previously [30].

The levels of TGF- β 1 were significantly higher in the second group of patients [8,536.3 pg/mL (6,031.5; 19,519) in the arterial blood and 28,099 pg/mL (15,359; 29,691)

in the venous blood] than in the first group [6,351 pg/mL (4,104.4; 10,805) in the arterial and 24,618 pg/mL (14,137; 2711) in the venous blood] ($p < 0.05$). A significant increase in TGF- β 1 levels was observed in both the arterial and venous blood depending on the duration of T2DM ($r = -0.322$, $p < 0.05$). A direct correlation between TGF- β 1 levels and patients' age ($p < 0.01$) as well as lipid metabolism parameters, such as the levels of LDL cholesterol ($r = 0.549$, $p < 0.05$) and triglycerides ($r = 0.421$, $p < 0.05$), was identified. This confirmed the involvement of the disturbances of the inflammatory processes and the cell ageing rate, uncontrolled synthesis of fibroblast growth factors and lipid metabolism in atherosclerotic lesions in patients with T2DM [25, 26] and also the role of fibroblast growth factors in the thickening of the IMC (Fig. 3). A similar role was also demonstrated by positive correlations of the studied parameters in both groups of patients.

β -FGF levels were significantly higher in patients with T2DM [35.88 pg/mL (19.04; 89.01) in the arterial blood and 11.63 pg/mL (2.3; 18.86) in the venous blood] than in those without T2DM were [22.77 pg/mL (14.5; 37.5) in the arterial blood and 9.45 pg/mL (9.2; 32.6) in the venous blood] ($p < 0.05$). A positive correlation was observed between β -FGF levels and T2DM duration ($r = 0.522$, $p < 0.05$). A sustained correlation was noted between β -FGF levels in the aortic blood in patients with T2DM and the IMC thickness of the CCA ($r = 0.554$, $p < 0.05$), the degree of its stenosis ($r = 0.435$, $p < 0.05$), the IMC thickness of the ICA ($r = 0.442$, $p < 0.005$) and the degree of stenosis of the ECA ($r = 0.557$, $p < 0.005$). A direct correlation was found between β -FGF levels in the aortic blood and the degree of disease of the right coronary artery (RCA) ($r = 0.478$, $p < 0.05$) and the anterior interventricular artery (AIVA) ($r = 0.533$; $p < 0.05$) in the second group of patients.

AGE levels was significantly higher in patients with T2DM [81.3 pg/mL (32.3; 110.1) in the arterial blood

and 46.3 pg/mL (9.6; 67.7) in the venous blood] than in those without T2DM [22.8 pg/mL (19.3; 25.14) in the arterial blood and 39.1 pg/mL (9.69; 87.06) in the venous blood]. These data were consistent with the results of many studies. Glycation end products in DM have been known to accumulate in cardiomyocytes and large blood vessel walls [24]. Proteins modified under glucotoxic conditions interact actively with RAGEs; their levels in the present study were 1,076.4 pg/mL (1,056.4; 3,330.7) in the arterial blood and 1,236 pg/mL (1,366; 2,200) in the venous blood in the first group of patients and 6,883 pg/mL (2,278.3; 11,482) in the arterial blood and 7,161.8 pg/mL (1,872.8; 14,964) in the venous blood in the second group.

An inverse relationship between AGE and RAGE levels and T2DM duration ($r = -0.120$; $p = 0.009$) as well as a direct positive relationship between AGE levels and total cholesterol levels ($r = 0.708$, $p < 0.01$) were established. A sustained relationship between AGE levels in the arterial blood and the degree of stenosis of the ICA ($r = 0.513$, $p < 0.01$) was also observed, which was consistent with the results reported in the literature [61]. A direct positive correlation between TGF- $\beta 1$ and AGE levels in the aortic blood was revealed in patients with T2DM and CHD ($r = 0.423$, $p = 0.05$). In addition, there was a relationship between TGF- $\beta 1$ levels in both the venous ($r = 0.498$, $p < 0.01$) and aortic blood ($r = 0.502$, $p < 0.01$) and LVMI values in both patient groups.

Previous *in vivo* studies have suggested the fundamental relationship between AGE and RAGE levels and the degree of heart failure (HF) [21]. The enhanced AGE production promotes intracellular protein dysfunction. In addition, AGEs change the properties of proteins involved in gene transcription regulation [32]. AGEs can freely enter a cell and not only disrupt its structure but also change the signal communication with other cells [33]. AGEs rapidly accumulate in endothelial cells, which activates the synthesis of a large number of regulatory molecules involved in cell cycle and further cell activity. For example, the pathological activation of fibroblast growth factors by AGEs leads to the overproduction of collagen, which, in turn, leads to the thickening of the basal membrane and the development of chronic inflammation in the vascular wall [34]. Uncontrolled synthesis of insulin-like and PDGFs activates the division of fibroblasts and smooth muscle cells and increases thrombosis [35]. Therefore, a decrease in elastic properties, narrowing of the vessel lumen and a change in the response to external factors are observed, which leads to acceleration of the atherosclerotic process [36]. AGEs have been shown to affect the synthesis of short-lived apolipoprotein A-I (ApoA-I) [37, 38]. In a previous study, patients with decompensated DM were divided depending on the statin therapy performed [39] and positive correlations with AGE levels were obtained in both groups of patients compared with the healthy group. Furthermore, an increase in AGE levels in the blood serum has been shown to affect the properties and structure of the extracellular matrix and to disrupt intercellular communications [40].

Several studies have shown a correlation between serum AGE levels and the development of HF [41]. A negative

effect of AGEs on the myocardium was due to reduction of vascular wall elasticity, diffuse intimal thickening, endothelial dysfunction and accumulation of collagen in the interstitium. These changes disrupt blood flow in the aorta and carotid arteries [42]. Previous studies have demonstrated that AGEs had a direct negative effect on the myocardium [43, 44]. Under normal conditions, diastolic atrial pressure increases under the action of the backward wave; as the rigidity of blood vessels increases, the wave velocity increases and the peak occurs at the end of systole [45]. This leads to an increase in the systolic pressure and afterload, which is one of the factors for the development of cardiovascular diseases. An inverse correlation between AGE levels in the pericardial fluid and left ventricular EF has been found [45, 46]. In DM, the AGE accumulation rate is several times higher; that is, the natural ageing processes occur several times faster. The results of a 4.5-year study, which included 559 females of post-menopausal age, confirmed the involvement of AGEs and RAGEs in the development of cardiovascular diseases [47, 48]. The high levels of AGEs and RAGEs circulating in the blood [95% confidence interval (CI): 1.08–3.48, $p = 0.026$ and 95% CI: 0.98–1.65, $p = 0.07$, respectively] were associated with a high mortality rate among females of the elder age group with signs of carbohydrate metabolism disorder. Another study conducted by Koyama demonstrated that serum RAGE levels correlated with NYHA functional class and low EF [49]. Presumably, RAGEs are an independent factor in the development of diastolic dysfunction. Similarly, Raposeiras-Roubin et al demonstrated that RAGEs are a highly sensitive and specific marker for the prognosis of HF decompensation in patients with DM [50, 51]. This and similar studies have shown that determination of AGE and RAGE levels in the blood serum of patients with CHD may be an independent predictor of the risk of developing HF.

CONCLUSION

In summary, we found that thickening of the IMC was affected by a combination of factors, including the presence of T2DM, its duration and the extent of its compensation. The increase in TGF- $\beta 1$ and β -FGF levels in T2DM confirmed the stimulating effect of hyperglycaemia on the amount and properties of fibroblasts, which accelerated the atherosclerotic processes. The resulting inverse correlation between AGE and fibroblast growth factor levels ($p < 0.005$) and the presence and duration of uncompensated T2DM and the degree of stenosis of the coronary and brachiocephalic arteries reflected vascular lesion severity in T2DM [10].

FUNDING AND DISCLOSURE INFORMATION

The authors declare no conflict of interest associated with the manuscript.

Study was supported by Endocrinology Research Centre (Moscow). The Study Protocol was approved by local ethical committee (#10-08 on 11-Dec-2008).

REFERENCES

1. Ajani UA, Ford ES. Has the Risk for Coronary Heart Disease Changed Among U.S. Adults. *Journal of the American College of Cardiology*. 2006;48(6):1177–1182. DOI: <http://dx.doi.org/10.1016/j.jacc.2006.05.055>
2. Chin SO, Hwang JK, Rhee SY, Chon S, Hwang Y, Oh S, et al. Risk factors for the progression of intima-media thickness of carotid arteries: a 2-year follow-up study in patients with newly diagnosed type 2 diabetes. *Diabetes Metab J*. 2013;37(5):365–374. DOI: <http://dx.doi.org/10.4093/dmj.2013.37.5.365>
3. Pignoli P, Tremoli E, Poli A, Oreste P, Paoletti R. Intimal plus medial thickness of the arterial wall: a direct measurement with ultrasound imaging. *Circulation*. 1986;74(6):1399–1406. DOI: <http://dx.doi.org/10.1161/01.CIR.74.6.1399>
4. Bots ML, Baldassarre D, Simon A, de Groot E, O'Leary DH, Riley W, Kastelein JJ, Grobbee DE. Carotid intima-media thickness and coronary atherosclerosis: weak or strong relations. *Eur Heart J*. 2007;28(4):398–406.
5. Eleid MF, Lester SJ, Wiedenbeck TL, Patel SD, Appleton CP, Nelson MR, et al. Carotid Ultrasound Identifies High Risk Subclinical Atherosclerosis in Adults with Low Framingham Risk Scores. *Journal of the American Society of Echocardiography*. 2010;23(8):802–808. DOI: <http://dx.doi.org/10.1016/j.echo.2010.06.003>
6. Lorenz MW, Markus HS, Bots ML, Rosvall M, Sitzer M. Prediction of Clinical Cardiovascular Events With Carotid Intima-Media Thickness: A Systematic Review and Meta-Analysis. *Circulation*. 2007;115(4):459–467. DOI: <http://dx.doi.org/10.1161/CIRCULATIONAHA.106.628875>
7. Tarzamni MK, Salehi R, Givian F, Farhang S. Association of carotid intima-media thickness with the presence and severity of coronary artery disease. *Neurosciences (Riyadh)*. 2006;11(4):308–311. PubMed PMID: 22266443.
8. Bots ML, Hoes AW, Koudstaal PJ, Hofman A, Grobbee DE. Common Carotid Intima-Media Thickness and Risk of Stroke and Myocardial Infarction: The Rotterdam Study. *Circulation*. 1997;96(5):1432–1437. DOI: <http://dx.doi.org/10.1161/01.CIR.96.5.1432>
9. Simons PC, Algra A, van de Laak MF, Grobbee DE, van der Graaf Y. Second manifestations of ARTERial disease (SMART) study: rationale and design. *European Journal of Epidemiology*. 1999;15(9):773–781. DOI: <http://dx.doi.org/10.1023/A:1007621514757>
10. Белоусов ЮБ, Несмараев ЖН. Эндотелиальная дисфункция как причина атеросклеротического поражения артерий при артериальной гипертензии. *Фарматека*. 2004;6(84):62–72 [Belousov YuB, Namsaraev ZhN. Endotelial'naya disfunktsiya kak prichina ateroskleroticheskikh porazheniy arteriy pri arterial'noy gipertenzii: metody korrektsii. *Farmateka*. 2004;(6):62–72.]
11. Campistol JM, Inigo P, Jimenez W, Lario S, Clesca PH, Oppenheimer F, et al. Losartan decreases plasma levels of TGF-beta 1 in transplant patients with chronic allograft nephropathy. *Kidney Int*. 1999;56(2):714–719. DOI: <http://dx.doi.org/10.1046/j.1523-1755.1999.00597.x>
12. Herder M, Johnsen SH, Arntzen KA, Mathiesen EB. Risk Factors for Progression of Carotid Intima-Media Thickness and Total Plaque Area: A 13-Year Follow-Up Study: The Tromso Study. *Stroke*. 2012;43(7):1818–1823. DOI: <http://dx.doi.org/10.1161/STROKEAHA.111.646596>
13. Ingelsson E, Sullivan LM, Murabito JM, Fox CS, Benjamin EJ, Polak JF, et al. Prevalence and Prognostic Impact of Subclinical Cardiovascular Disease in Individuals With the Metabolic Syndrome and Diabetes. *Diabetes*. 2007;56(6):1718–1726. DOI: <http://dx.doi.org/10.2337/db07-0078>
14. Ziemann SJ, Melenovsky V, Clattenburg L, Corretti MC, Capriotti A, Gerstenblith G, et al. Advanced glycation endproduct crosslink breaker (alagebrium) improves endothelial function in patients with isolated systolic hypertension. *Journal of Hypertension*. 2007;25(3):577–583. DOI: <http://dx.doi.org/10.1097/HJH.0b013e328013e7dd>
15. Балахонова ТВ, Трипотень МИ, Погорелова ОА. Ультразвуковые методы оценки комплекса интима-медиа артериальной стенки. *SonoAce-Ultrasound*. 2010;21:57–63. [Balakhonova TV, Tripoten' MI, Pogorelova OA. Ul'trazvukovyye metody otsenki kompleksa intima-media arterial'noy stenki. *SonoAce-Ultrasound*. 2010;(21):57–63.]
16. Sauvage M, Hinglais N, Mandet C, Badier C, Deslandes F, Michel JB, et al. Localization of elastin mRNA and TGF-β1 in rat aorta and caudal artery as a function of age. *Cell and Tissue Research*. 1998;291(2):305–314. DOI: <http://dx.doi.org/10.1007/s004410051000>
17. Fleenor BS, Seals DR, Zigler ML, Sindler AL. Superoxide-lowering therapy with TEMPOL reverses arterial dysfunction with aging in mice. *Aging Cell*. 2012;11(2):269–276. DOI: <http://dx.doi.org/10.1111/j.1474-9726.2011.00783.x>
18. Fleenor BS, Sindler AL, Eng JS, Nair DP, Dodson RB, Seals DR. Sodium nitrite de-stiffening of large elastic arteries with aging: Role of normalization of advanced glycation end-products. *Experimental Gerontology*. 2012;47(8):588–594. DOI: <http://dx.doi.org/10.1016/j.exger.2012.05.004>
19. Fleenor BS, Marshall KD, Durrant JR, Lesniewski LA, Seals DR. Arterial stiffening with ageing is associated with transforming growth factor-1-related changes in adventitial collagen: reversal by aerobic exercise. *The Journal of Physiology*. 2010;588(20):3971–3982. DOI: <http://dx.doi.org/10.1113/jphysiol.2010.194753>
20. Fleenor BS, Marshall KD, Rippe C, Seals DR. Replicative Aging Induces Endothelial to Mesenchymal Transition in Human Aortic Endothelial Cells: Potential Role of Inflammation. *J Vasc Res*. 2012;49(1):59–64. DOI: <http://dx.doi.org/10.1159/000329681>
21. Tiyyagura SR, Pinney SP. Left ventricular remodeling after myocardial infarction: past, present, and future. *Mt Sinai J Med*. 2006;73(6):840–851. PubMed PMID: 17117309.
22. Keeley EC, Mehrad B, Strieter RM. Fibrocytes: bringing new insights into mechanisms of inflammation and fibrosis. *Int J Biochem Cell Biol*. 2009;42(4):535–542. DOI: <http://dx.doi.org/10.1016/j.biocel.2009.10.014>
23. Jandeleit-Dahm K, Cooper M. The Role of AGEs in Cardiovascular Disease. *CPD*. 2008;14(10):979–986. DOI: <http://dx.doi.org/10.2174/138161208784139684>
24. Baumann M, Richart T, Sollinger D, Pelisek J, Roos M, Kouznetsova T, et al. Association between carotid diameter and the advanced glycation endproduct Nepsilon-Carboxymethyllysine (CML). *Cardiovasc Diabetol*. 2009;8(1):45. DOI: <http://dx.doi.org/10.1186/1475-2840-8-45>
25. Steppan J, Tran H, Benjo AM, Pellakuru L, Barodka V, Ryoo S, et al. Alagebrium in combination with exercise ameliorates age-associated ventricular and vascular stiffness. *Experimental Gerontology*. 2012;47(8):565–572. DOI: <http://dx.doi.org/10.1016/j.exger.2012.04.006>

26. Basta G. Advanced Glycation End Products Activate Endothelium Through Signal-Transduction Receptor RAGE: A Mechanism for Amplification of Inflammatory Responses. *Circulation*. 2002;105(7):816–822. DOI: <http://dx.doi.org/10.1161/hc0702.104183>
27. Halushka MK, Selvin E, Lu J, Macgregor AM, Cornish TC. Use of human vascular tissue microarrays for measurement of advanced glycation endproducts. *J Histochem Cytochem*. 2009;57(6):559–566. DOI: <http://dx.doi.org/10.1369/jhc.2009.953273>
28. Kass DA, Shapiro EP, Kawaguchi M, Capriotti AR, Scuteri A, deGroof RC, et al. Improved Arterial Compliance by a Novel Advanced Glycation End-Product Crosslink Breaker. *Circulation*. 2001;104(13):1464–1470. DOI: <http://dx.doi.org/10.1161/hc3801.097806>
29. Coll B, Feinstein SB. Carotid intima-media thickness measurements: Techniques and clinical relevance. *Curr Atheroscler Rep*. 2008;10(5):444–450. DOI: <http://dx.doi.org/10.1007/s11883-008-0068-1>
30. Барбараш ОЛ, Авраменко ОЕ, Осокина АВ, Сумин АН, Веремеев АВ. Роль провоспалительных факторов в оценке прогноза больных с прогрессирующей стенокардией в сочетании с сахарным диабетом 2 типа. *Сахарный диабет*. 2012;4:39–45. [Barbarash O, Sumin A, Avramenko O, Osokina A, Veremeev A. Prognostic significance of proinflammatory factors in patients with progressive angina pectoris and type 2 diabetes mellitus. *Diabetes mellitus*. 2012;(4):39–45]. DOI: <http://dx.doi.org/10.14341/2072-0351-5536>
31. Hartog JW, Voors AA, Schalkwijk CG, Scheijen J, Smilde TDJ, Damman K, et al. Clinical and prognostic value of advanced glycation end-products in chronic heart failure. *European Heart Journal*. 2007;28(23):2879–2885. DOI: <http://dx.doi.org/10.1093/eurheartj/ehm486>
32. Lautenslager GT, Shearman CW, Hud E, Cohen MP. Effects of nonenzymatic glycation and fatty acids on functional properties of human albumin. *Metabolism*. 2011;60(12):1683–1691. DOI: <http://dx.doi.org/10.1016/j.metabol.2011.03.022>
33. Yan SF, D'Agati V, Schmidt AM, Ramasamy R. Receptor for Advanced Glycation Endproducts (RAGE): a formidable force in the pathogenesis of the cardiovascular complications of diabetes & aging. *Curr Mol Med* 2007;7(8):699–710. PubMed PMID: 18331228.
34. Nin JW, Jorsal A, Ferreira I, Schalkwijk CG, Prins MH, Parving HH, et al. Higher Plasma Levels of Advanced Glycation End Products Are Associated With Incident Cardiovascular Disease and All-Cause Mortality in Type 1 Diabetes: A 12-year follow-up study. *Diabetes Care*. 2011;34(2):442–447. DOI: <http://dx.doi.org/10.2337/dc10-1087>
35. Hegab Z, Gibbons S, Neyes L, Mamas M. Role of advanced glycation end products in cardiovascular disease. *World J Cardiol*. 2012;4(4):90–102. DOI: <http://dx.doi.org/10.4330/wjc.v4.i4.90>
36. Reddy GK. AGE-related cross-linking of collagen is associated with aortic wall matrix stiffness in the pathogenesis of drug-induced diabetes in rats. *Microvascular Research*. 2004;68(2):132–142. DOI: <http://dx.doi.org/10.1016/j.mvr.2004.04.002>
37. Lapolla A, Brioschi M, Banfi C, Tremoli E, Cosma C, Bonfante L, et al. Nonenzymatically glycated lipoprotein ApoA-I in plasma of diabetic and nephropathic patients. *Ann N Y Acad Sci*. 2007;1126:295–299. DOI: <http://dx.doi.org/10.1196/annals.1433.005>
38. Hoang A, Murphy AJ, Coughlan MT, Thomas MC, Forbes JM, O'Brien R, et al. Advanced glycation of apolipoprotein A-I impairs its anti-atherogenic properties. *Diabetologia*. 2007;50(8):1770–1779. DOI: <http://dx.doi.org/10.1007/s00125-007-0718-9>
39. Low H, Hoang A, Forbes J, Thomas M, Lyons JG, Nestel P, et al. Advanced glycation end-products (AGEs) and functionality of reverse cholesterol transport in patients with type 2 diabetes and in mouse models. *Diabetologia*. 2012;55(9):2513–2521. DOI: <http://dx.doi.org/10.1007/s00125-012-2570-9>
40. Basta G. Advanced glycation end products and vascular inflammation: implications for accelerated atherosclerosis in diabetes. *Cardiovascular Research*. 2004;63(4):582–592. DOI: <http://dx.doi.org/10.1016/j.cardiores.2004.05.001>
41. Linden E, Cai W, He JC, Xue C, Li Z, Winston J, et al. Endothelial Dysfunction in Patients with Chronic Kidney Disease Results from Advanced Glycation End Products (AGE)-Mediated Inhibition of Endothelial Nitric Oxide Synthase through RAGE Activation. *Clinical Journal of the American Society of Nephrology* 2008;3(3):691–698. DOI: <http://dx.doi.org/10.2215/CJN.04291007>
42. Sugimoto K, Yasujima M, Yagihashi S. Role of Advanced Glycation End Products in Diabetic Neuropathy. *CPD* 2008;14(10):953–961. DOI: <http://dx.doi.org/10.2174/138161208784139774>
43. Hou FF, Ren H, Owen WF, Guo ZJ, Chen PY, Schmidt AM, et al. Enhanced expression of receptor for advanced glycation end products in chronic kidney disease. *J Am Soc Nephrol* 2007;15(7):1889–1896.
44. Uribarri J, Cai W, Peppas M, Goodman S, Ferrucci L, Striker G, et al. Circulating glycotoxins and dietary advanced glycation endproducts: two links to inflammatory response, oxidative stress, and aging. *J Gerontol A Biol Sci Med Sci* 2007;62(4):427–433. PubMed PMID: 17452738.
45. Hartog JW, Voors AA, Bakker SJL, Smit AJ, van Veldhuisen DJ. Advanced glycation end-products (AGEs) and heart failure: Pathophysiology and clinical implications. *European Journal of Heart Failure*. 2007;9(12):1146–1155. DOI: <http://dx.doi.org/10.1016/j.ejheart.2007.09.009>
46. Simm A, Wagner J, Gursinsky T, Nass N, Friedrich I, Schinzel R, et al. Advanced glycation endproducts: A biomarker for age as an outcome predictor after cardiac surgery. *Experimental Gerontology*. 2007;42(7):668–675. DOI: <http://dx.doi.org/10.1016/j.exger.2007.03.006>
47. Semba RD, Ferrucci L, Sun K, Beck J, Dalal M, Varadhan R, et al. Advanced glycation end products and their circulating receptors predict cardiovascular disease mortality in older community-dwelling women. *Aging Clin Exp Res* 2009;21(2):182–190. PubMed PMID: 19448391.
48. Lopes-Virella MF, Hunt KJ, Baker NL, Lachin J, Nathan DM, Virella G. Levels of Oxidized LDL and Advanced Glycation End Products-Modified LDL in Circulating Immune Complexes Are Strongly Associated With Increased Levels of Carotid Intima-Media Thickness and Its Progression in Type 1 Diabetes. *Diabetes*. 2010;60(2):582–589. DOI: <http://dx.doi.org/10.2337/db10-0915>
49. Koyama Y, Takeishi Y, Niizeki T, Suzuki S, Kitahara T, Sasaki T, et al. Soluble Receptor for Advanced Glycation End Products (RAGE) is a Prognostic Factor for Heart Failure. *Journal of Cardiac Failure*. 2008;14(2):133–139. DOI: <http://dx.doi.org/10.1016/j.cardfail.2007.10.019>
50. Raposeiras-Roubin S, Rodiño-Janeiro BK, Grigorian-Shamagian L, Moure-González M, Seoane-Blanco A, Varela-Román A, et al. Relation of Soluble Receptor for

Advanced Glycation End Products to Predict Mortality in Patients With Chronic Heart Failure Independently of Seattle Heart Failure Score.

The American Journal of Cardiology. 2011;107(6):938–944.
DOI: <http://dx.doi.org/10.1016/j.amjcard.2010.11.011>

INFORMATION ABOUT THE AUTHORS

Ekaterina V. Ivannikova	PhD student, Endocrinology Research Centre, Dmitriya Ulyanova Str., 11, Moscow, Russian Federation, 117036 E-mail: doc.ivannikova@gmail.com
Viktor Yu. Kalashnikov	MD, PhD, Head of the Emergency and Interventional Cardiology Department, Endocrinology Research Centre, Moscow, Russian Federation
Olga M. Smirnova	MD, PhD, Professor, Chief Research Scientist, Department of Programmed Learning and Treatment, Endocrinology Research Centre, Moscow, Russian Federation
Aleksandr B. Kuznetsov	MD, PhD, Physician at the Cardiology Department, Endocrinology Research Centre, Moscow, Russian Federation
Sergey A. Terekhin	MD, PhD, Head of the X-Ray Endovascular Diagnosis and Treatment Room, Endocrinology Research Centre, Moscow, Russian Federation
Aleksandr V. Il'in	Head of the Laboratory, Endocrinology Research Centre, Moscow, Russian Federation