Metabolic, Inflammatory and Imaging Biomarkers in Evaluation of Coronary Arteries Anatomical Stenosis in Patients with Stable Coronary Artery Disease

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Aim. To reveal the statistically significant determinants of the coronary artery (CA) stenosis ⩾70% in patients with chronic stable CA disease receiving drug therapy.

Material and methods. The study included 68 patients (aged 59.6±6.4 years) with stable CA disease and optimal cardioactive therapy. Coronary angiography was performed in all patients. Basic serum parameters of carbohydrate and lipid metabolism were evaluated; serum concentration of cytokines, adipokines and high sensitive C-reactive protein (hsCRP) were determined by ELISA. The epicardial adipose tissue (EAT) thickness was measured by B-mode echocardiography.

Results. The patients' classification model was created. It allowed to determine probability *P* for CA stenosis of 70% or more for each patient using formula $P\frac{1}{1+e^{-L}}$, where L=0.89-1.09×gender+ 0.51×triglycerides−0.28×HDL+0.24×hsCRP (HDL – high density lipoproteins). If calculated *P* value falls into interval (0; 0.228) the patient should be classified into the group with the risk of CA stenosis ⩾70%, while if calculated *P* value falls into interval (0.228; 1), the patient should be classified into group with CA stenosis below 70%. Even though EAT thickness was indistinguishable determinant of CA stenosis ⩾70% in our study, its inclusion into the model as a fifth variable allowed to increase the model quality: area under ROC-curve (AUC) in the model without EAT thickness constituted 0.708 (p=0.009), and increased up to 0.879 (p=0.011) after EAT thickness inclusion.

Conclusions. Male sex, level of triglycerides, HDL and hsCRP are statistically significant determinants of CA stenosis ⩾70%. The presence of the triglycerides level in the created model underscores an important contribution of this lipid fraction, even when elevated only up to the moderate values, into modulation of the residual cardiovascular risk in patients receiving statins.

Keywords: coronary atherosclerosis, triglycerides, fibrates, high density lipoprotein cholesterol, epicardial adipose tissue thickness.

For citation: Koshelskaya O.A., Suslova T.E., Kologrivova I.V., Margolis N.Y., Zhuravleva O.A., Kharitonova O.A., Vinnitskaya I.V. Metabolic, Inflammatory and Imaging Biomarkers in Evaluation of Coronary Arteries Anatomical Stenosis in Patients with Stable Coronary Artery Disease. *Rational Pharmacotherapy in Cardiology* 2020;16(1):4-9. DOI:10.20996/1819-6446-2020-01-01

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Received: 09.07.2019
Accepted: 22.08.2019

Evaluation of the role of new biomarkers in atherogenesis progression is hoped to extend insight into its molecular and biochemical mechanisms [1]. While contribution of low-density lipoprotein cholesterol (LDL) as the risk factor for atherosclerosis and coronary artery disease (CAD) development had been convincingly demonstrated, results of studies concerning high-density lipoprotein cholesterol (HDL) and triglycerides (TG) are rather contradictory [2]. Echocardiographic epicardial adipose tissue (EAT) thickness is considered to be one of the informative visual markers of atherosclerosis [3]. In accordance with many authors' data the epicardial obesity may be regarded as a surrogate marker of coronary atherosclerosis [4], however diagnostic value of EAT thickness as an instrument for individual risk stratification in CAD patients has not yet evaluated. There is no comprehensive information concerning relationships between EAT thickness, types of lipid profile disturbances, activity of chronical subclinical inflammation and presence of significant coronary artery (CA) stenosis.

The aim of the study was to reveal statistically significant determinants of ⩾70% CA anatomical stenosis in patients with chronic stable CAD under drug treatment.

Material and methods

The study was approved by the local Ethic Committee of the Cardiology Research Institute. The study enrolled the 40-70-year old patients with stable CAD and documented coronary atherosclerosis with different degree of manifestation according to the coronary angiography data. Majority of patients received optimal medical treatment. Clinical characteristics of the patients are listed in the Table 1. CA stenosis ⩾70% was revealed in 53 (78%) of 68 patients. Exclusion criteria were the following: no angiographic signs of CA atherosclerosis, acute atherosclerotic complications during the last 6 months, any inflammatory diseases, diabetes mellitus with Hb_{A1c} >10% or glycaemia during a day >11 mmol/l, chronic kidney disease more severe than 3b stage, left ventricle ejection fraction <40%, oncologic, hematological and immune diseases. Many patients were smokers and met the criteria of metabolic syndrome [5].

Selective coronary angiography was conducted in all the patients with the help of the Cardio-scop-V angiographic complex and the Digitron-3NAC computer system, Siemens (Germany) in a department of X-ray surgery.

Epicardial adipose tissue thickness was measured as an echo-negative space between the free wall of the right ventricle myocardium and pericardial visceral layer perpendicular to the right ventricle free wall in the B-mode ultrasound imaging [6]. The measurements were performed during 3 consecutive cardiac cycles with calculation of the mean value.

Anthropometric measurements were also conducted, at that general obesity and abdominal obesity were evaluated by the body mass index (BMI) calculation and waist circumference measurement, respectively.

Table 1. Clinical characteristics of the enrolled patients (n=68)

Parameter	
Men, n (%)	38 (55.9)
Age, years	59.5 (55.0; 65.0)
Patients with history of myocardial infarction, n (%)	29 (42.7)
CAD duration, years	2 (1; 5)
Patients with type 2 diabetes mellitus, n (%)	22 (32.4)
Diabetes duration, years	7 (2; 14)
Arterial hypertension duration, years	10 (5; 15)
Systolic BP, mm Hg	121 (112; 126)
Diastolic BP, mm Hg	71 (66; 76)
Smokers, n (%)	31 (45.6)
Body mass index, kg/m^2	29.5 (27.1; 32.2)
Overweight patients, n (%)	31 (45.6)
Obese patients, n (%)	31 (45.6)
Waist circumference, cm	100.0 (93.0 106.0)
Hips circumference, cm	103.0 (100.0; 108.0)
Visceral obesity index	2.19 (1.51; 3.89)
LDL, mmol/l	2.53 (1.92; 3.44)
HDL, mmol/l	1.1 (0.88; 1.24)
TG, mmol/l	1.49 (1.13; 2.02)
Treatment with statins, n (%)	55 (81)
HbA_{1c} (diabetic patients), %	6.94 (6.17; 8.32)

Data are presented as Me ($Q_{25\%}$; $Q_{75\%}$), unless indicated otherwise

BP – blood pressure, CAD – coronary artery disease, TG – triglycerides, HDL – high-density lipoprotein cholesterol, LDL – low-density lipoprotein cholesterol

The enzyme-immunoassay method was used to assess serum levels of C-reactive protein by highly-sensitivity test (hsCRP), (Biomerica, Germany), insulin (AccuBind, США), interleukin-6 (IL)-6 (Vector-BEST, Russia), tumor necrosis factor-alpha (TNF)-α (Affymetrix, eBioscience, США), resistin, leptin (Mediagnost, Германия), adiponectin (Assaypro, США). Blood lipids (levels of total cholesterol, TG, HDL, LDL were analyzed using "Diakon-DS" kits (Russia) and levels of apolipoprotein A1, apolipoprotein B were analyzed using DiaSys kits (Germany).

Statistical analysis of the data was performed with the help of the Statistica 10.0 software application package (Statsoft Inc., the USA). Distribution of quantitative attributes was checked for normality. To describe values with non-normal distribution median and interquartile range (25^{th} and 75^{th} percentiles) were used. Quantitative attributes were compared with the help of the Mann-Whitney test. To evaluate relationships between the attributes the Spearman's correlation coefficient (r_S) was used. For multivariable estimation of the prognostic value of the attributes predicting ⩾70% CA stenosis, the model of classification of the patients into groups of CA stenosis <70% and ⩾70% was built. The results of the statistical analysis were considered as significant at $p \leqslant 0.05$.

Results and discussion

We have analyzed potential correlations between coronary atherosclerosis with ⩾70% stenosis in one or more CA and studied clinical characteristics and biomarkers. CA stenosis of ⩾70% correlated with TG (r_S=0.31) and HDL (r_S=-0.28) levels and with male sex (r_S=0.24). The following independent indices demonstrated statistically significant intergroup distinctions (group 1 – patients with ⩾70% CA stenosis, group 2 – patients with <70% CA stenosis): sex (p=0.049), TG (p=0.011), HDL (p=0.023) and hsCRP (p=0.050) (Table 2).

Building a model of multiple linear regression was found to be impossible due to non-normality of residuals at the preliminary analysis of the statistical data. We have built a model of two-class classification of the patients in the groups 1 and 2 (model of logistic regression) in the form of a single-layer neural network with sigmoidal function of activation. The model ascertains the probability (*P*) of CA stenosis ⩾70% according to each patient's characteristics by the formula: $P\frac{1}{1+e^{-L}}$, with

$L=\beta 0-\beta 1\times sex+\beta 2\times TG-\beta 3\times HDL+\beta 4\times hsCRP$.

The level of significance of the model was p=0.009. Scores of the model's parameters were the following: β0=0.89; β1=1.09; β2=0.51; β3=0.28; β4=0.24. The two-valued variable of sex was specified as: sex=1, if a patient was man and sex=2, if a patient was woman. All the other variables in the model were quantitative.

On the ground of the ROC-analysis of the model the ROC-curve was built (Fig. 1A) with evaluation of the threshold probability of ⩾70% anatomic stenosis presence or absence, which was equal to 0,228. If calculated value of *P* falls in the interval of 0-0.228 a patient should be subsumed to the group of ⩾70% CA stenosis risk, while at calculated value of *P* from 0.228 to 1 a patient should be subsumed to the group of <70% stenosis.

Although according to our data the variable of EAT thickness itself was not a statistically significant determinant of ⩾70% CA anatomic stenosis, the inclusion of this index in the described above classification model was noted to improve its quality. So, in accordance with the ROC-analysis results the area under the model's ROC-curve was 0.708 (p=0.009), while at inclusion of EAT thickness as a fifth variable the area under the ROC-curve increased to 0.879 (p=0.011; Fig. 1B).

The received data demonstrate the male sex, serum levels of TG, HDL and hsCRP to be the independent determinants of ⩾70% CA stenosis in patients with documented CAD due to atherosclerosis on optimal medical treatment. According to the built model the most important contribution to the development of significant anatomic stenosis was made by male sex, increased TG and decreased HDL levels, which ratio is known to be a surrogate marker of insulin resistance. Along with that our study results indicate that in the settings of decreased level of LDL due to statins intake, high residual risk for development of severe coronary stenosis depends on TG and HDL metabolism disturbances under condition of concomitant moderate increase in hsCRP concentration. We believe that precisely this combination of

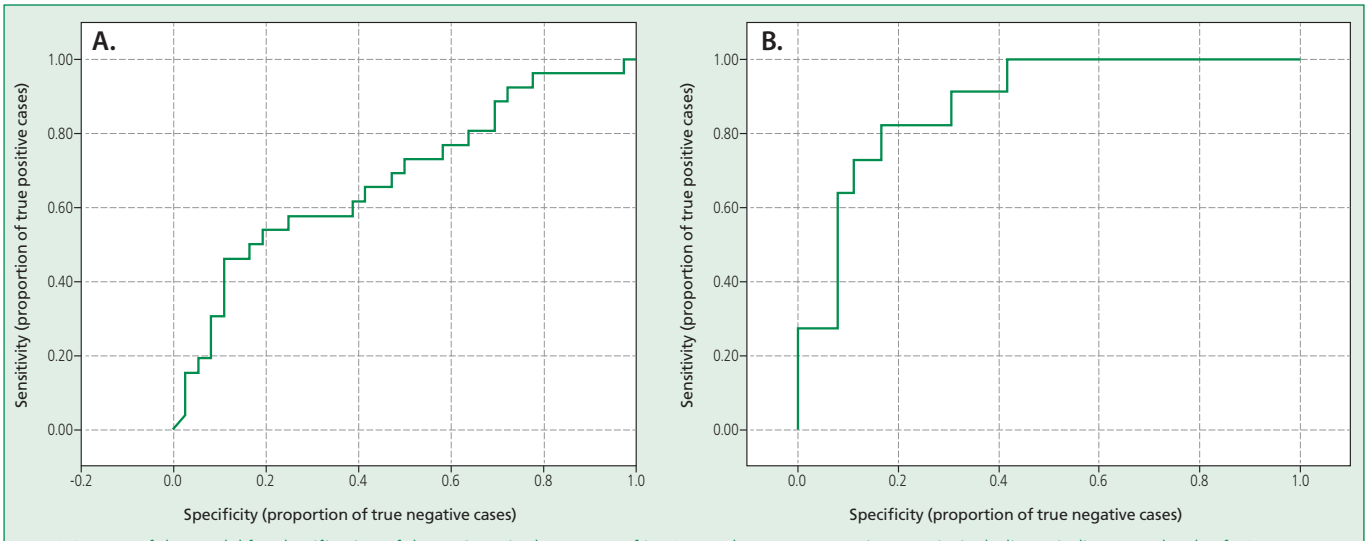
Table 2. Comparison of clinical and biochemical characteristics of the patients with and without ≥70% coronary stenosis

Parameter	Group 1 (n=53)	Group 2 (n=15)	p
Sex (men/women)	35/18	12/23	0.049
Age, years	59 (55; 65)	64 (56; 66)	>0.05
Body mass index, kg/m^2	29,6 (26.3; 32.3)	29.3 (27.4; 31.6)	>0.05
Patients with obesity, n (%)	25 (47.2%)	7 (46.7%)	>0.05
WC/HC	0.97 (0.92; 1.01)	0.92 (0.90; 1.02)	>0.05
EATT, mm	5.0 (4.0; 6.1)	4.9 (4.0; 6.3)	>0.05
TG, mmol/l	1.57 (1.17; 2.32)	1.21 (0.86; 1.51)	0.011
LDL, mmol/l	2.72 (2.11; 3.67)	2.07 (1.65; 3.08)	>0.05
HDL, mmol/l	1.03 (0.86; 1.17)	1.30 (0.89; 1.37)	0.023
hsCRP, mg/l	2.52(1.14; 4.00)	1.47 (0.90; 3.03)	0.050
HOMA-IR, standard units	2.95 (2.21; 5.71)	3.70 (3.10; 6.54)	>0.05
Treatment with statins, n (%)	45 (75.5%)	11 (73.3%)	>0.05

Data are presented as Me ($Q_{25\%}$; $Q_{75\%}$), unless indicated otherwise

WC – waist circumference, HC – hips circumference, EATT – epicardial adipose tissue thickness, hsCRP – high sensitive C-reactive protein, TG – triglycerides, LDL – low-density lipoprotein cholesterol, HDL – high-density lipoprotein cholesterol, HOMA-IR – Homeostasis Model Assessment of Insulin Resistance

factors can enable penetration of the TG-rich particles in the intima and realization of their proatherogenic effects [7,8]. In that context the data of genome-wide studies, which demonstrated that influence on lipoprotein-associated lipase (the key enzyme hydrolyzing TG-rich particles) can contribute to additional decrease in cardiovascular risk in patients already received LDL-lowering therapy, are of great importance [2]. A reduction in TG serum level by 35-50% and increase in HDL level by 5-20% under the



A. ROC-curve of the model for classification of the patients in the groups of ⩾70% and <70% anatomic stenosis, including 4 indices: sex, levels of TG, HDL and hsCRP (the area under the curve 0.708; threshold 0.228)

B. ROC-curve of the model for classification of the patients in the groups of ⩾70% and <70% anatomic stenosis, including 5 indices: sex, levels of TG, HDL, hsCRP and EAT thickness (the area under the curve 0.879; threshold 0.281)

TG – triglycerides, HDL – high-density lipoprotein cholesterol, hsCRP – high sensitive C-reactive protein, EAT – epicardial adipose tissue

Figure 1. ROC-curves of the models for classification of the patients in the groups of ≥70% and <70% anatomic stenosis

influence of fenofibrate was proved to decrease accumulation of visceral adipose tissue and levels of pro-inflammatory adipokines, restrict the processes of inflammation, insulin resistance and oxidative stress, which inhibits atherogenesis [8,9]. According to the quantitative analysis of angiography results the DAIS clinical trial had revealed capability of fenofibrate to slow down progression of coronary atheroma in patients with type 2 diabetes even at the normal serum level of TG. This is considered as a result of pleiotropic antiatherogenic effects of the drug [9].

It must be noted that in accordance with the data of our study, which included men and women with different degree of manifestation of coronary atherosclerosis and metabolic disturbances, the mere fact of EAT thickening had not significantly contributed in severe coronary stenosis development, as distinct from the data received in a sample of obese men [10]. However, inclusion of this index in the model of classification allowed to improve its quality. These data do not contradict the existing concept of pathologic effect of the EAT depot on atherogenesis [11], but evidently demonstrate that not the EAT accumulation as much as its dysfunction can determine significance of coronary stenosis in CAD patients under conventional treatment as a result of increased serum levels of hsCRP and TG as well as decreased concentration of HDL.

Our study was limited by its single-step design, the small-scale sample of patients, which made it impossible to reveal potential gender distinctions and absence of statins in a treatment regimen of 19% of the patients which could anyhow influence the EAT function.

Conclusion

Statistically significant determinants of ⩾70% CA anatomic stenosis in CAD patients under medical treatment are the following: male sex, blood levels of TG, HDL and hsCRP, while quantitative estimation of EAT thickness can be only used as additional marker of coronary atherosclerosis significance.

Presence of the TG serum level in the built model of logistic regression underlines important contribution of this lipid fraction in cardiovascular risk in patients under statins treatment. Prospective research is necessary to estimate anti-atherogenic effects of fibrates and their potential favorable impact on the adipose tissue reallocation.

Disclosures. All authors have not disclosed potential conflicts of interest regarding the content of this paper.

Funding. The paper was prepared in the frame of the theme № AAAA-A15-115123110026-3 of fundamental studies.

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