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FGF21: A marker of coronary stenosis in non-smoking stable coronary artery disease patients

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Abstract
Aims: To test the relation between FGF21 and stable coronary artery disease and to test the FGF21 in the role of a marker for the presence of hemodynamically significant coronary artery stenosis.
Methods and Results: 203 subjects were divided into subgroups based on the presence of stable coronary artery disease and hemodynamically significant coronary artery stenosis. FGF21 was measured prior coronary angiography was performed. Mean FGF21 concentration was higher (t (201) = 2,082; p = 0,039) in stable coronary artery disease patients (323,16±434,66 pg/ml), than among healthy controls (266,46±417,13 pg/ml). Hierarchical regression was performed to test the FGF21 as a marker of the hemodynamically significant coronary artery stenosis. The contribution of FGF21 to the model accuracy was statistically significant ($\chi^2(4) = 25,606$; $p< 0,001$; n = 123; $R^2 = 0,251$; OR Log10 FGF21 = 2,366). However, moderation interaction of smoking to FGF21 - HSCS relation was identified. Adjustment for smoking substantially improved the predictive capacity of the regression model and FGF21 became a significant contributor in the dependent´s prediction ($\chi^2(3) = 30,778$; $p< 0,001$; n = 81; $R^2 = 0,425$; OR Log10 FGF21 = 7,013).
Conclusions: FGF21 is a clinically useful marker of hemodynamically significant coronary artery stenosis only among non – smoking stable coronary artery disease patients. FGF21 cannot be used as a marker of stable coronary artery disease in general population.

Keywords: Adipokine, fibroblast growth factor 21, coronary stenosis, stable coronary artery disease, smoking

1. Introduction
Fibroblast Growth Factor 21 (FGF21) has been cloned for the first time in 2000 [1]. Together with FGF19 and FGF23, FGF21 is the only fibroblast growth factor known to have endocrine features [2]. It has been shown, that FGF21 affects metabolic adaptation response to the ketogenic diet by the decrease of the adipocyte insulin sensitivity [3]. On the other hand, in the situation of absolute FGF21 insufficiency, mice developed high degree of insulin resistance, possibly as a result of enhanced pancreatic beta cell hyperplasia and enhanced insulin synthesis [4]. Planavila *et al.* linked FGF21 to the antioxidant pathways, which were engaged under inflammatory and hypertrophic conditions within the heart [5]. Subsequently, the role of FGF21 in the settings of coronary artery disease started to be of a great interest. Chow *et al.* found, that serum FGF21 concentration is independently associated with carotid atherosclerosis [6]. Another in - vitro studies revealed, that FGF21 attenuates oxidized LDL - mediated apoptosis in cardiac endothelial cells [7]. Kim *et al.* published results of their study, in which the extent of coronary artery disease, expressed as the Gensini score and Extent score, was independently associated with FGF21 concentration in the whole group, but not in diabetic group [8]. Another study demonstrated, that serum FGF21 concentration is an independent predictor of incident coronary artery disease also in type 2 diabetics [9]. Yet there are studies, in which authors did not find association of FGF21 with current coronary artery disease status [10]. In the study of Ong *et al.* authors didn´t prove the usability of FGF21 as a cardiovascular disease biomarker [11]. From a clinical perspective, it would be interesting to clarify, whether measuring the FGF21 concentration could be utilized in stable coronary artery disease (SCAD) screening and in invasive coronary assessment (ICA) indication.

2. Aims

The first objective was to test, whether there is an independent relation between FGF21 and the diagnosis of SCAD. The second objective was to test the relation between FGF21 and the presence of hemodynamically significant coronary stenosis (HSCS) in SCAD patients. We addressed this objective, particularly with regards to the hypothesis, that FGF21 has a potential to become a biomarker entering the ICA indication algorithm.

3. Materials and Methods

Our study is a transversal design performed on data gathered from 203 subjects divided into the control group and the target group. The controls (n = 80) were enrolled on annual preventive medical check – up. We enrolled 123 SCAD patients scheduled for ICA into the target group. The enrolment phase, which began in March 2014 and lasted until December 2017, was held at the Department of Internal Medicine I., University Hospital in Martin and at clinics belonging to the University Hospital in Martin. The selection of subjects was based on inclusion and exclusion criteria defined in this paper. The study protocol was approved by the Independent Ethics Committee„ IRB00005636 Jessenius Faculty of Medicine, Comenius University in Martin IRB # 1“ and the informed consent was obtained from each study participant. The investigation conformed to the principles outlined in the Declaration of Helsinki.

3.1 Clinical Procedures in the Target Group

After signing the informed consent on study participation, waist circumference (WC), hip circumference, height and weight were measured and body mass index (BMI) and waist to hip ratio (WHR) were calculated. Pre-test probability (PTP) was estimated and ICA was indicated in accordance with the ESC 2013 Guidelines on the Management of SCAD. There were no subjects with PTP lower than 15 % in the target group. The ICA was performed in patients with mid- to high risk of cardiovascular event. ICA was also performed in those low cardiovascular event risk patients, in whom intensified optimal medical therapy did not lead to remission of SCAD symptoms. Transthoracic echocardiography was performed in each study participant. Blood samples were taken at basal conditions after night – long fasting, the same day the ICA was performed. Coronary artery stenting was performed in accordance with the 2014 ESC/EACTS Guidelines on Myocardial Revascularization.

3.1.1 Inclusion criteria: In each patient, single photon emission tomography and/or cardiac stress test had affirmed the diagnosis of ischaemic heart disease, prior the ICA was performed. Only individuals with the evidence of coronary artery disease based on the results of these tests and with symptoms of cardiac ischaemia were enrolled into the study.

3.1.2 Exclusion criteria: Acute forms of ischaemic heart disease, history of myocardial infarction or unstable angina, history of oncological disorders (including remissions), diabetes mellitus type 1, acutely decompensated chronic heart failure, history and symptoms of peripheral artery disease, symptoms and signs of acutely decompensated endocrinopathies, severe pulmonary arterial hypertension, laboratory or clinical signs of acute or chronic infections.

3.2 Clinical Procedures in the Control Group

Basic biometry measures same as in the target group were performed. Blood sampling and biochemical analyses were performed within the same laboratory test conditions as in the target group. Blood samples were taken after night – long fasting, at 7 o´clock in the morning.

3.2.1 Inclusion criteria: Healthy individuals free of laboratory and clinical signs of acute illness or subjective symptoms suggesting acute illness.

3.2.2 Exclusion criteria: Known or suspected coronary artery disease, otherwise same as in the target group.

3.3 Laboratory Procedures

FGF21 level was measured with commercially available enzyme-linked immunosorbent assay (ELISA) kits certified for experimental use (RD191108200R, Human FGF21 ELISA, Bio Vendor Ltd.). Each blood sample had been obtained prior the ICA procedure at the same day ICA procedure was performed. Plasma separation (centrifugation for 15 minutes @ 3500 RPM) was performed immediately after blood sample was drawn. Plasma samples were deep – frozen at minus 20 degrees Celsius without delay and were stored until the ELISA assays were performed in the certified clinical biochemical laboratory. ELISA assays were performed immediately after the samples thawed. Repeating the thaw – freezing cycles was strictly forbidden. Each sample has been processed and measured twice. Only the mean values, obtained by double – testing each sample, entered statistical analyses. The battery of following standard laboratory tests was performed in addition to FGF21 measurement: sodium, potassium, chloride, creatinine and urea concentration measurement, calculation of the estimated glomerular filtration rate (eGFR) using the abbreviated Modification of Diet in Renal Disease formula, triglycerides (TG), low - density lipoprotein (LDL), high - density lipoprotein HDL, total cholesterol (TCH) concentration measurement, C – reactive protein (CRP) level, liver enzymes, blood count differential and basic coagulation parameters.

3.4 Invasive Coronary Assessment

The degree of coronary stenosis and the extent of the stenotic process was visually estimated by the experienced interventional cardiologists. Each individual ICA was double – checked by another pair of eyes. Proximal left anterior descending and left main stenosis of more than 50 % was considered as hemodynamically significant. In other coronary vessels, 70 % narrowing was considered as significant stenosis. If there have been any doubts about the accuracy of visual stenosis quantification, fractional flow reserve (FFR) technique was utilized.

3.5 Variables and Statistical Analyses

The result of ICA entered our statistical analysis in the form of the binomial variable representing the presence or absence of HSCS. The presence or absence of SCAD was represented by the binomial variable „Affiliation“. Diabetes mellitus variable was also coded as binomial variable, based on the presence or absence of diabetes mellitus. Smoking variable was defined based on the presence or absence of habitual smoking at the day of inclusion into the study. However, according to the current Health Care Act in

Slovak Republic, the spa therapy, which follows the coronary artery stenting, can be refunded from public health insurance sources only in non-smokers. Existence of this rule compromise reliability of the anamnestic information regarding the smoking status. Therefore, smoking history was double – checked with the use of medical records of each study participant. When medical record carried the positive information regarding patient´s smoking, we accepted such a patient as a smoker, regardless of orally declared non-smoker status.

Statistical analyses were carried out using the IBM SPSS Statistics v. 20.0 software. In each statistical test performed, the border for statistical significance was p - value ≤ 0,05. In case of serial comparison or serial correlations, Bonferroni correction of the significance threshold was performed. Normality testing was performed using the Shapiro – Wilk test and by visual inspection of a Q – Q plot. In the case of non – normal distribution, data was transformed by the means of commonly used arithmetical techniques: square root, logarithmization plus inversion and inversion in positively skewed data; Reflection and square root, reflection and logarithmization, reflection and inversion in negatively skewed data.

The means of continuous variables were compared using the independent sample t – test and non – parametric Mann – Whitney test, where appropriate. Nominal variables were compared using the chi – square test.

The prediction of FGF21 concentration based on independent variables, along with the calculation of relative contribution of these predictors, was performed using the multiple regression. The definition of an outlier was based on studentized residuals values (threshold was 2,5 x standard deviation). Influential and high leverage points were assessed by Cook´s values and leverage statistics.

In binomial logistic regression, linearity of the continuous variables with respect to the logit of the dependent variable was assessed via the Box – Tidwell procedure. A Bonferroni correction was applied using all terms to assess the statistical significance threshold for non – linearity detection. receiver operating characteristics (ROC) curves were used to visualize crucial properties of the constructed regression models. Interaction analysis was performed to clarify the effect of between – variable interactions.

Kruskal – Wallis H test was conducted to determine, if there were statistically significant differences in FGF21 concentrations between the subgroups based on number of significantly stenotic coronary vessels (NrSSV).

4. Results

Basic characteristics of the target group and the control group are compared in tables 1 and 2. [Table 1] [Table 2] Differences between SCAD subjects divided according to the presence of HSCS are highlighted in tables 3 and 4. In our cohort, there were 61 patients with HSCS and 62 patients without HSCS. There were 155 non – smokers together in both groups. Among the SCAD patients, there were 81 non - smoking patients. The distribution of diabetics between HSCS positive and HSCS negative non – smoking SCAD patients was equal ($\chi^2(1) = 1,260$; p = 0,352). [Table 3] [Table 4]

Addressing the first objective, multiple regression was run to determine the effect of predictors (Age, Gender, Diabetes mellitus, Affiliation, BMI, eGFR) on the FGF21 variable. There was one case with borderline studentized residual (-3,116 standard deviations), which we decided to keep in the regression. The model statistically significantly predicted the FGF21 variable, F (6, 196) = 6,825; $p< 0,001$, adj. R^2 = 0,147. However, the presence or absence of SCAD (Affiliation variable) did not contribute to the dependent´s prediction. Only the Age and Diabetes mellitus variables added significantly to the prediction of the FGF21.

The point – biserial correlation was performed to measure the effect size of Age and Diabetes mellitus variables on FGF21. The coefficients of determination for age and diabetes mellitus were $r_{pb}^2 = 0,0441$ and $r_{pb}^2 = 0,0519$, respectively. Regression coefficients, standard errors, and statistical significance of independent variables in the regression model are summarized in table 5. [Table 5]

Addressing the second objective of the study, a hierarchical binomial regression was performed to ascertain the effect of known predictors and the FGF21 variable on the likelihood, that SCAD patients have HSCS. Age, Gender, Diabetes mellitus and Smoking entered the equation in the first block. Diabetes mellitus variable was subsequently dropped from the regression, as it failed to contribute significantly to the model. FGF21 variable was added in the second block to quantify the additive effect of FGF21 on the predictive capacity of the model. The logistic regression model proved to be statistically significant in the first ($\chi^2(3) = 21,125$; $p< 0,001$; n = 123; Nagelkerke $R^2 = 0,210$) and in the second block ($\chi^2(4) = 25,606$; $p< 0,001$; n = 123; Nagelkerke R^2 = 0,251). The percentage accuracy in classification value of the final model was 67,5 %. The positive predictive value was 67,7 % and specificity was 67,2 %. The area under the curve (AUC) of the second block showed acceptable discrimination of the dependent variable (AUC = 0,749; $p< 0,001$; 95 % CI = 0,66 – 0,837). Adding the FGF21 variable to the model revealed a statistically significant additive effect of FGF21 on the predictive accuracy of the model. However, it increased the AUC value only to a very small extent of Δ Nagelkerke $R^2 = 0,024$. The summary of the regression model is in table 6. [Table 6] The ROC curves of the first and second model blocks with their corresponding AUC are depicted in figures 1 and 2, respectively. [figure 1] [figure 2]

In order to unmask the interaction between variables Smoking status and FGF21, we created the interaction term (FGF21 x Smoking) and we used it in the same binomial regression to quantify its impact on the effect of FGF21 as the predictor of HSCS presence. The interaction between FGF21 and Smoking variable which was added in the second block of the regression model, had a significant impact on the omnibus statistics of the model ($\chi^2(5)_{\text{block 2}}$ = 36,099; Nagelkerke $R^2_{\text{block 2}} = 0,339$; $p_{\text{block 2}} = 0,003$; n = 123). The hierarchical model building strategy revealed that the interaction term entered in block 2 added significantly to the model accuracy ($\chi^2(1)_{\text{block 1}} = 10,493$; Nagelkerke $R^2_{\text{block 1}} = 0,251$; p = 0,001, n = 123). The percentage accuracy in classification value, when the interaction term was added, increased to 70,7 %. The odds ratio of the newly created interaction term was lower than 1 (Exp B = 0,04) which indicates that the effect vector the interaction variable exerted on the HSCS prediction, differs from the effect vectors of the individual component variables (FGF21 and Smoking) entered into the regression model separately (Smoking Exp B = 3,875; FGF21 Exp B = 2,366). This finding substantiates the hypothesis, that the effect of

FGF21 variable on the HSCS prediction differs across different levels of the Smoking variable.

To ascertain whether smoking itself has a direct impact on FGF21, correlation between Smoking and FGF21 was tested. We found that correlation between Smoking and FGF21 variable was non – significant (Kendall T = - 0,033; p = 0,658; n = 123). However, there was statistically significant correlation between Smoking and HSCS variable (Kendall T = 0,234; p = 0,01; n = 123). By the definition, the interaction of Smoking variable with the FGF21 variable, therefore can´t be described as a suppression [12]. Since the FGF21 concentration obviously cannot be causally antecedent to smoking, the Smoking variable also cannot be declared the mediator of the FGF21 variable by the definition. Based on these results, together with the fact, that the interaction term was a significant contributor in the hierarchical regression, we can conclude, that Smoking variable interacts with the FGF21 variable specifically as a moderator.

4.1 Effect of FGF21 on the HSCS Prediction as a Function of the Moderator Variable

Based on the results of analyses described above and based on the distribution of the levels of Smoking variable across SCAD patients (of all non - smoking SCAD patients, 47 were free of HSCS), we decided to perform logistic regression in non - smoking SCAD patients and smoking SCAD patients separately. Prior the analysis was performed, we had statistically verified, that the difference in the proportion of diabetics between HSCS subsets in non - smokers and smokers was statistically non - significant (p = 0,262 and p = 0,197, respectively), so the diabetes prevalence didn´t affect the results.

While testing for the regression model assumptions in the non - smoking subgroup, we found two cases with studentized residuals exceeding 2,5 standard deviations. Since removing these cases did not significantly change the statistics of the regression model, we decided to keep these cases in the equation. In the first block, Age and Gender entered the regression ($\chi^2(2)$ = 18,693; p< 0,001; Nagelkerke R^2 = 0,277; Exp B Age = 1,132; Exp B Gender = 3,609; n = 81). Adding FGF21 variable in the second block ameliorated the predictive capacity of the model substantially ($\chi^2(3)$ = 30,778; p< 0,001; Nagelkerke R^2 = 0,425; Exp B Age = 1,126; Exp B Gender = 4,503; Exp B FGF21 = 7,013; n = 81). The percentage accuracy in classification value was 77,8 %, positive predictive value was 73,5 % and negative predictive value reached 80,1 %. Sensitivity of the model was 73,5 % and specificity reached 80,9 %. The ROC curves of the first and second model block with their corresponding AUC´s are depicted in figure 3 and 4, respectively [figure 3] [figure 4]. Among smoking SCAD patients, the omnibus regression statistic of the analogically built model was non – significant (p = 0,549, n = 42). The HSCS prediction plots based on Age, Gender and FGF21 variables among SCAD patients across different levels of smoking variable is depicted in figure 5. [figure 5] The relationship between the FGF21 concentration and predicted probabilities of HSCS in non - smoking SCAD patients is depicted in figure 6. [figure 6]

There is a positive correlation between smoking and HSCS in our cohort. Therefore, the higher likelihood, that smokers suffer from HSCS, could be the underlying driving force of the moderator effect smoking exerts on the FGF21 – HSCS relation. Should this be the case, the moderating effect of Smoking variable would be spurious. To exclude this possibility, mean levels of FGF21 concentrations across the levels of Smoking variable were compared. The difference in FGF21 concentration between smoking and non – smoking SCAD patients was statistically non - significant (t (121) = 0,370; p = 0,712; n = 123), which means, that observed moderating effect of smoking is not spurious.

Based on above mentioned findings, we tried to predict the Affiliation variable selectively in the non - smoking subgroup of patients. When smoking individuals were removed from the analysis, FGF21 still remained the only non – significant contributor in the model (p = 0,524), while Gender, Diabetes mellitus and Age predicted the SCAD statistically significantly ($\chi^2(3)$ = 47,239; p< 0,001; Nagelkerke R^2 = 0,351; n = 155). This proves, that the FGF21 measurement cannot be used as a SCAD marker in general population, irrespective of smoking status.

4.2 FGF21 and the Extent of the Coronary Atherosclerosis among non-smokers

Despite the fact, that FGF21 predicts the result of the ICA procedure in terms of HSCS prediction, the nature of elevated FGF21 concentration in the presence of HSCS remains unclear. Therefore, we tried to clarify, whether higher FGF21 concentration in the context of HSCS is a reparatory mechanism triggered by coronary atherosclerosis, or a humoral manifestation of the metabolic state tied to the coronary atherosclerosis. Should the elevation of the FGF21 concentration be a manifestation of the reparatory mechanism triggered by coronary atherosclerosis itself, we should be able to detect differences in FGF21 concentrations, that reflect the extent of the coronary atherosclerosis. Therefore, we performed Kruskal – Wallis H test in non - smoking SCAD patients to determine, if there are differences in FGF21 concentrations between the three groups, which differed in *NrSSV*. The „zero“ (n = 47), „one“ (n = 18) and „two and more“ (n = 16) *NrSSV* groups were assessed. When the adjustment for ties was performed, median FGF21 concentrations were significantly different between the *NrSSV* subgroups (χ^2 (2) = 13,471; p = 0,001; n = 81). Dunn´s post – hoc analysis revealed, that „zero“ and „one“ was the only pair of *NrSSV* subgroups, where the differences in FGF21 medians (125,86±455,81 pg/ml; 354,67±564,89 pg/ml, respectively) were statistically significant (adj. p = 0,002), after a Bonferroni correction for multiple comparisons was applied (figure 7). [figure 7]. No other statistically significant between – group differences were found.

5. Discussion

In daily clinical practice, there is a considerable proportion of SCAD patients, in whom the ICA doesn´t reveal any HSCS. The only benefit ICA conveys in these situations, is reassuring the patient about patent coronary arteries. Generally, there is enough space for apprehension - biased decision making when it comes to the problem of the ICA indication. A trial of optimal medical therapy should be performed in low- and intermediate - risk SCAD patients. Despite postponing the ICA in these patients is in full accordance with the ESC guidelines, the theoretical possibility of health complications creates a precondition for erroneous decision - making. In our cohort, HSCS was found only in 50,4 % of all ICA procedures. In this context

we can conclude that in 49,6 % of our SCAD patients, coronary angiography was an invasive procedure of questionable benefit to the patient.

Only the patient´s age and the presence of diabetes mellitus affected the FGF21 concentration significantly in the entire study cohort. The age and diabetes mellitus accounted for 4,41 % and 5,19 % of the variability in FGF21 concentration, respectively. These effects are very small, particularly when put in contrast to the magnitude of the FGF21 difference, found between SCAD patients and SCAD – free subjects (table 1). With respect to the results of the analyses performed, we don´t see the evidence, that FGF21 concentration measurement could be utilized as a screening marker of SCAD among the general population.

Moving into the second aim of this study, we tested the hypothesis, that FGF21 has a potential to enter the ICA indication algorithm. Our results suggest, that adding the FGF21 variable into the regression model carried clinically insignificant improvement of the model accuracy (figure 1 and figure 2), when moderator effect of smoking wasn´t taken into the account.

Further analysis however revealed that smoking alters the effect of FGF21 on the HSCS prediction. Our analysis didn´t rule out the possibility of the reverse interaction. The hypothetical reverse interaction would be represented by the impact of the FGF21 on the simple effect, that moderating variable Smoking exerts on FGF21 – HSCS relation. Reverse interaction could, in theory, play a significant role to the interaction triangle FGF21 – HSCS – Smoking. Reflecting the reverse interaction into the patophysiological field, we could hypothesize, that the ability to respond to the coronary atherosclerosis in the form of elevated FGF21 concentration, might affect the role smoking plays in the coronary atherosclerosis. On the other hand, it is also possible, that smoking itself, by an unknown mechanism, hampers the elevation of FGF21 concentration in the context of coronary atherosclerosis. Although the patophysiological basis of the observed moderation interaction still needs to be clarified, our results prove, that at least among non – smoking SCAD patients, the FGF21 measurement indeed can be used as a marker of the HSCS presence.

As mentioned in the introduction of this article, both reparatory and protective effects exerted by FGF21 in the situation of coronary atherosclerosis, were previously proved. However, there was no evidence backing the hypothesis of linear relationship between the magnitude of the FGF21 elevation and the extent of coronary atherosclerosis. In our study we didn´t see the tendency towards further elevation of the FGF21 concentration when stenotic involvement of multiple coronary arteries took place. Therefore, we hypothesize that the elevation of FGF21 concentration in the context of SCAD reflects engagement of protective general metabolic response, which is indirectly associated with higher odds for having HSCS, rather than the compensatory response, triggered by the extent of coronary atherosclerosis in the coronary arterial tree.

5.1 Study Limitations

There are certain limitations in the study design. There were significant differences in WHR and IVS variables between HSCS subgroups. Nevertheless we believe, that these variables and their respective between – group differences were too small to be clinically relevant, so we didn´t include these variables into the regression models. Also the mean values of HDL and TG differed significantly between HSCS subgroups. However, we didn´t take the hypolipidemic therapy into account because the design of the study precluded this from being performed. Since we did not perform the ICA in the control group, it is also possible, that some of these subjects might had suffered from a clinically silent form of SCAD. This could also affect our findings. Moreover, we didn´t evaluate the effectivity of therapeutic control of diabetes mellitus, nor medical treatment of diabetes. The compliance with the therapy and impact of various types of antihypertensives and their effect on the FGF21 concentration was also omitted from statistical analysis. Another drawback might be sparse utilization of FFR technique, which was limited only to few borderline cases in our study.

Table 1: General Characteristics - Control Group versus Target Group.

Variable	Control group	Target group	Difference sig. (2-tailed)
Age (years)	59,7±7,7	62,5±8,5	p = 0,022
Waist circumference (cm)	102,8±13,6	107,7±11,3	p = 0,006
Hip circumference (cm)	110,2±11,7	107,3±10,1	p = 0,083
Waist to hip ratio	0,933±0,079	1,004±0,061	*p*< 0,001
Body mass index (kg/m^2)	30,23±5,73	30,16±4,72	p = 0,931
LVEF (%)	59,6±4,1	56,7±7	*p*< 0,001
LVEDd (mm)	48,7±3,1	51,3±5,7	*p*< 0,001
IVS (mm)	10,3±1,6	11,3±1,6	*p*< 0,001
LDL (mmol/l)	3,569±0,968	2,607±0,919	*p*< 0,001
HDL (mmol/l)	1,457±0,350	1,300±0,316	*p*< 0,001
TG (mmol/l)	1,775±0,823	1,742±0,964	p = 0,514
TCH (mmol/l)	5,728±1,069	4,643±1,092	*p*< 0,001
eGFR ($ml/min/1.73m^2$)	91,2±22,1	70,4±15	*p*< 0,001
FGF21 (pg/ml)	266,46±417,13	323,16±434,66	p = 0,039

LVEF: left ventricular ejection fraction; LVEDd: left ventricular end - diastolic diameter; IVS: interventricular septal thickness; LDL: low - density lipoprotein; HDL: high - density lipoprotein; TG: triglycerides; TCH: total cholesterol; eGFR: estimated glomerular filtration rate; FGF21: fibroblast growth factor – 21.

Table 2: Nominal Variables Characteristics - Control Group versus Target Group.

Variable	Target group	Control group	Difference sig. (2-tailed)
Gender	Male: 71 (57,7 %) Female: 52 (42,3 %)	Male: 19 (23,8 %) Female: 61 (76,2 %)	*p*< 0,001
Smoking (*Y/N*)	Yes: 42 (34,1 %) No: 81 (65,9 %)	Yes: 6 (7,5 %) No: 74 (92,5 %)	*p*< 0,001
Diabetes mellitus	Yes: 37 (30,1 %) No: 86 (69,9 %)	Yes: 7 (8,8 %) No: 73 (91,2 %)	*p*< 0,001

Table 3: General Characteristics – HSCS Positive versus HSCS Negative Subgroup.

Variable	*HSCS* negative	*HSCS* positive	Difference sig. (2-tailed)
Age (years)	60,8±8	64,1±8,8	p = 0,029
Waist circumference (cm)	106,4±11,6	109,1±10,9	p = 0,199
Hip circumference (cm)	108±10,2	106,7±10,1	p = 0,909
Waist to hip ratio	0,984±0,063	1,023±0,055	*p*< 0,001
Body mass index (kg/m^2)	29,74±4,55	30,50±4,89	p = 0,382
LVEF (%)	57,3±8	55,3±7	p = 0,143
LVEDd (mm)	51,1±6,2	51,5±5,3	p = 0,789
IVS (mm)	10,9±1,7	11,6±1,5	p = 0,025
LDL (mmol/l)	2,648±0,870	2,567±0,971	p = 0,470
HDL (mmol/l)	1,379±0,329	1,225±0,286	p = 0,007
TG (mmol/l)	1,555±0,915	1,922±0,983	p = 0,008
TCH (mmol/l)	4,657±1,029	4,630±1,154	p = 0,902
eGFR (ml/min/1.73m^2)	70,75±12,29	70,11±17,32	p = 0,555
FGF21 (pg/ml)	284,39±450,60	361,31±418,53	p = 0,009

HSCS: hemodynamically significant coronary artery stenosis; LVEF: left ventricular ejection fraction; LVEDd: left ventricular end - diastolic diameter; IVS: interventricular septal thickness; LDL: low - density lipoprotein; HDL: high - density lipoprotein; TG: triglycerides; TCH: total cholesterol; eGFR: estimated glomerular filtration rate; FGF21: fibroblast growth factor - 21.

Table 4: Nominal Variables Characteristics – HSCS Negative versus HSCS Positive Subgroup.

Variable	HSCS negative	HSCS positive	Difference sig. (2-tailed)
Gender	Male: 29 (47,5 %)	Male: 42 (67,7 %)	p = 0,037
	Female: 32 (52,5 %)	Female: 20 (32,3 %)	
Smoking (*Y/N*)	Yes: 14 (23 %)	Yes: 28 (45,2 %)	p = 0,013
	No: 47 (77 %)	No: 34 (54,8 %)	
Diabetes mellitus	Yes: 19 (31,1 %)	Yes: 18 (29 %)	p = 0,846
	No: 42 (68,9 %)	No: 44 (71 %)	

HSCS: hemodynamically significant coronary artery stenosis.

Table 5: Regression Model Summary - Prediction of FGF21 Based on Selected Variables.

Predictor variable	B	Standard error	Beta	p - value
Patient´s age (years)	0,19	0,005	0,269	< 0,001
Patient´s gender	- 0,061	0,083	- 0,052	0,460
Affiliation	0,005	0,097	0,004	0,957
Diabetes mellitus	0,235	0,099	0,164	0,018
BMI (kg/m^2)	0,009	0,008	0,074	0,266
Normalised eGFR (ml/min/1,73 m^2)	- 0,603	0,435	- 0,114	0,266
Constatnt	1,657	0,967		0,088

Diabetes mellitus: Non - diabetics as the reference, number - labelled with lower number; Gender: males number - labelled with lower number, females as the reference. Affiliation: controls as the reference, number - labelled with higher number. Normalised eGFR: logarithmized value of estimated glomerular filtration rate.
FGF21: fibroblast growth factor – 21; B: unstandardized regression coefficient; Beta: standardized regression coefficient.

Table 6: Regression Model Summary - Prediction of HSCS Based on the Age, Gender, FGF21 and Smoking Variables in all of the SCAD Subjects.

Predictor variable	B coefficient	Standard error	Wald	df	p - value	Odds ratio
Age (years)	0,070	0,027	6,818	1	p = 0,009	1,072
NormFGF21	0,861	0,422	4,165	1	p = 0,041	2,366
Gender	0,868	0,419	4,289	1	p = 0,038	2,383
Smoking (*Y/N*)	1,354	0,471	8,266	1	p = 0,004	3,875
Constant	- 7,232	1,906	14,390	1	*p*< 0,001	0,001

HSCS: hemodynamically significant coronary artery stenosis; Gender: females as the reference, males number - labelled with lower number.
Smoking: Non - smoking individuals as the reference, number – labelled with lower number.
Norm FGF21: logarithmized value of Fibroblast Growth Factor - 21 concentration.
B: unstandardized regression coefficient; df: degrees of freedom.

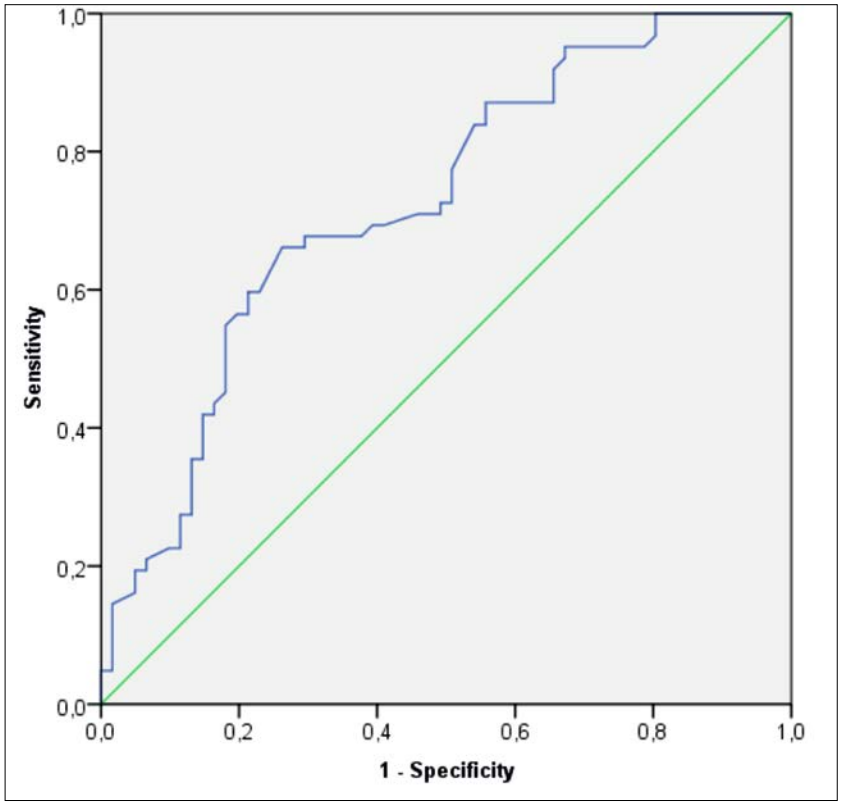

Fig 1: Receiver Operating Characteristic in the First Block of the Regression Model for HSCS Prediction Based on Age, Gender and Smoking Variables in SCAD Patients.

Description: AUC = 0,725; p - value < 0,001; n = 123; 95 % confidence interval = 0,636 – 0,814. HSCS: hemodynamically significant coronary artery stenosis; **SCAD:** stable coronary artery disease; AUC: area under the curve.

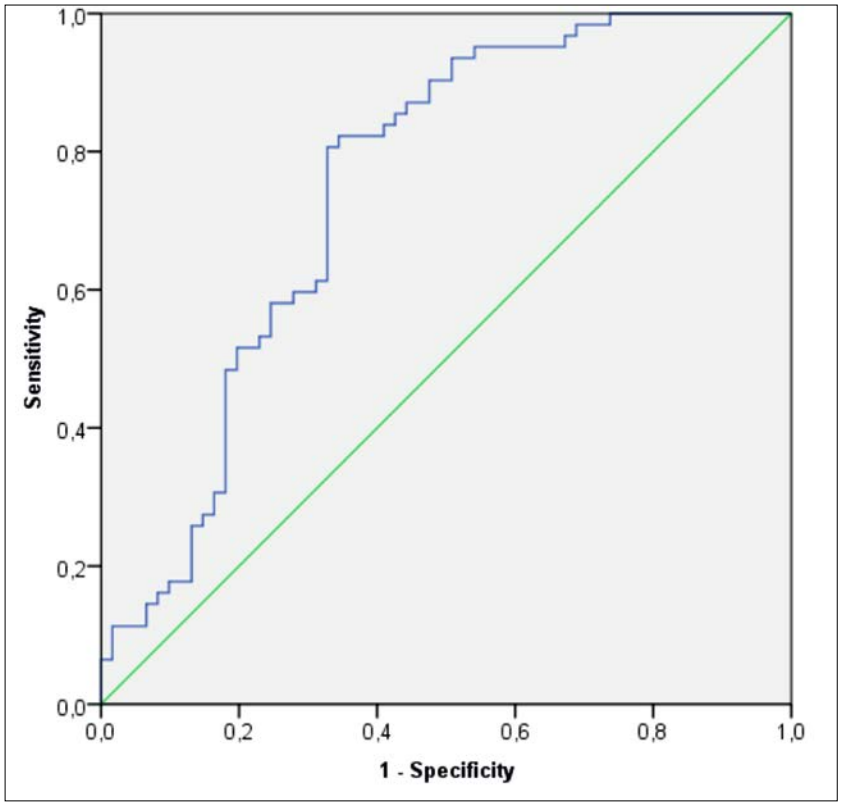

Fig 2: Receiver Operating Characteristic in the Second Block of the Regression Model for HSCS Prediction Based on Age, Gender, Smoking plus the FGF21 Variable in SCAD Patients.

Description: AUC = 0,749; p - value < 0,001; n = 123; 95 % confidence interval = 0,660 – 0,837.

HSCS: hemodynamically significant coronary artery stenosis; SCAD: stable coronary artery disease; AUC: area under the curve.

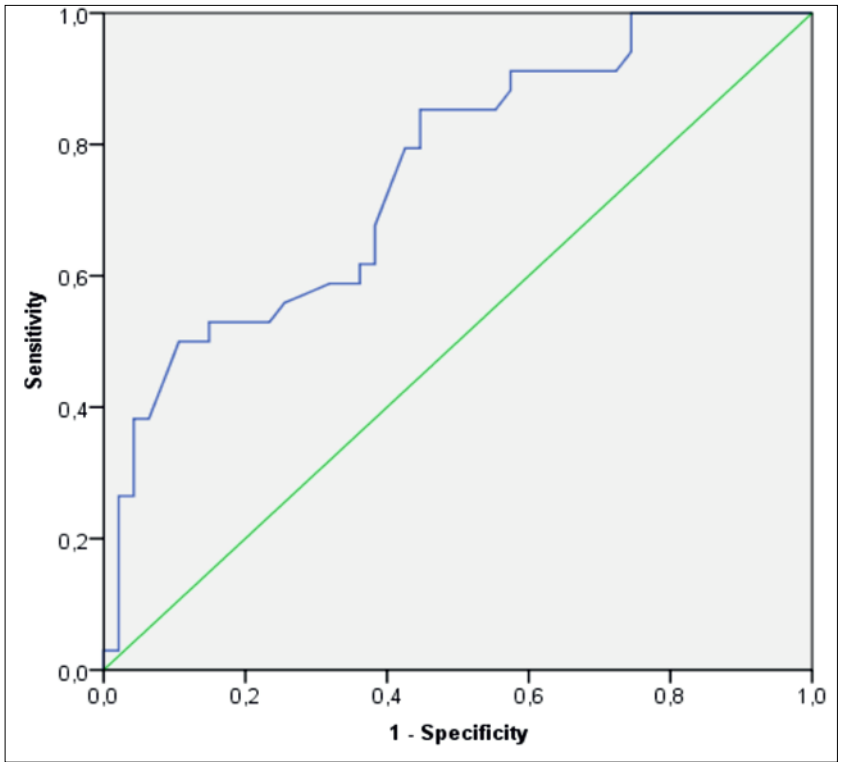

Fig 3: Receiver Operating Characteristic in the First Block of the Regression Model for HSCS Prediction Based on Age and Gender Variables in smoking SCAD Patients.

Description: AUC = 0,754; p - value < 0,001; n = 81; 95 % confidence interval = 0,648 – 0,860.

HSCS: hemodynamically significant coronary artery stenosis; SCAD: stable coronary artery disease; AUC: area under the curve.

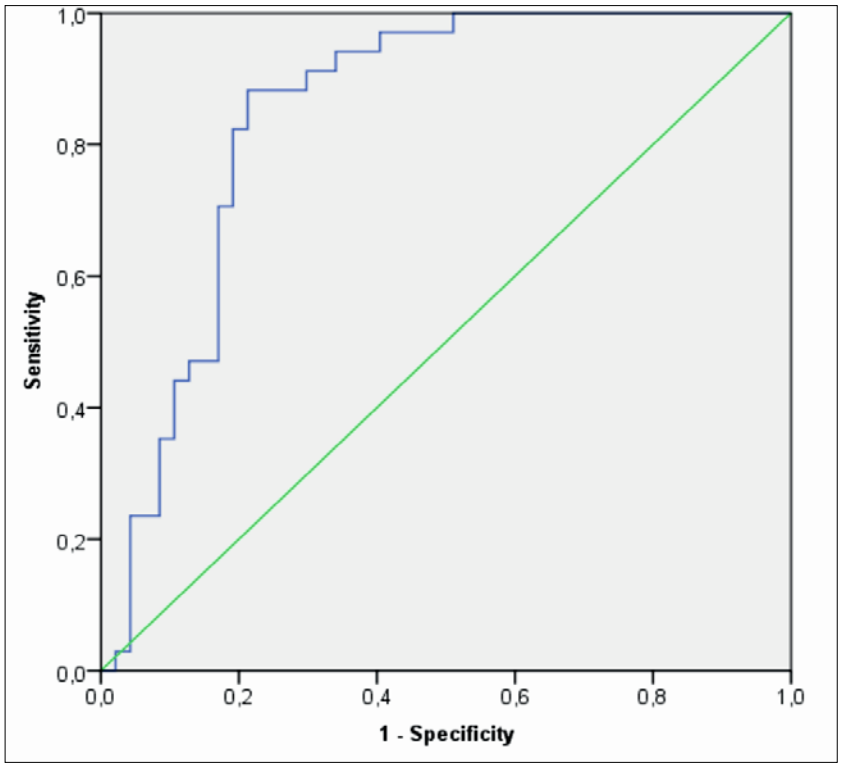

Fig 4: Receiver Operating Characteristic Curve in the Second Block of the Regression Model for HSCS Prediction Based on Age and Gender Variables plus the FGF21 Variable in non - smoking SCAD Patients.

Description: AUC = 0,847; p - value < 0,001; n = 81; 95 % confidence interval = 0,760 – 0,933.

HSCS: hemodynamically significant coronary artery stenosis; SCAD: stable coronary artery disease; AUC: area under the curve.

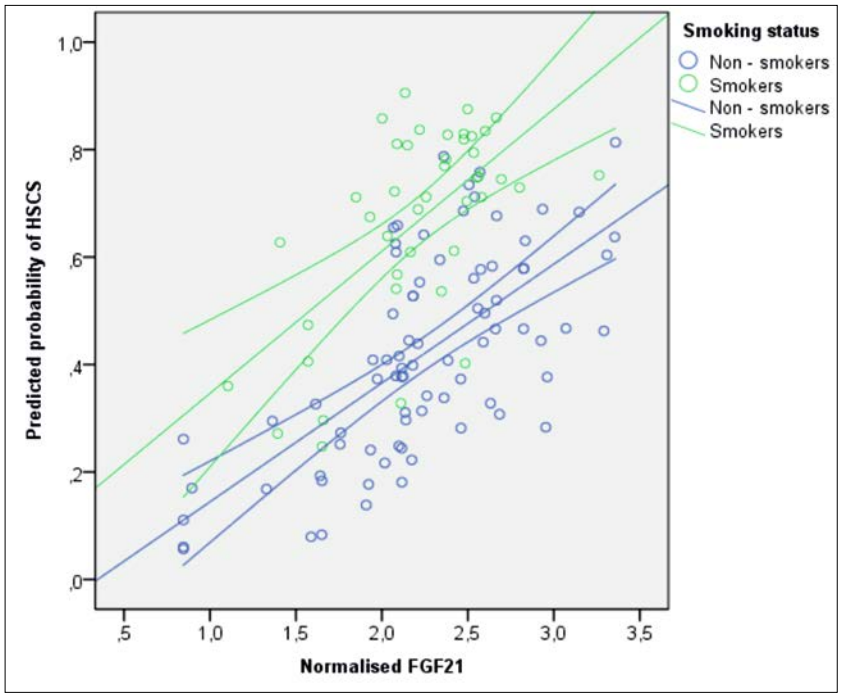

Fig 5: Regression Lines and their 95 % CI of the HSCS Prediction Based on Age, Gender and FGF21 in SCAD Patients across Different Levels of Smoking Variable.

Description: Predicted probability is expressed as a ratio. 95 % confidence intervals are based on mean predicted probability values computed from the regression.

For non – smokers: regression line p - value < 0,001; n = 81;

For smokers: regression line p - value = 0,549; n = 42.

Normalised FGF21: logarithmized value of Fibroblast Growth Factor - 21 concentration.

CI: confidence interval; HSCS: hemodynamically significant coronary artery stenosis; SCAD: stable coronary artery disease.

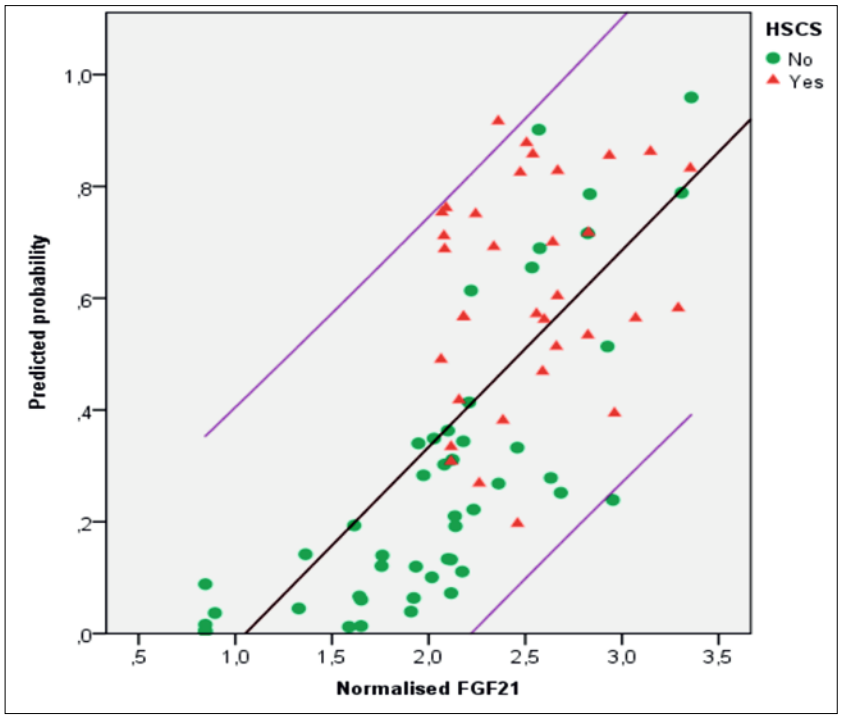

Fig 6: Relation between FGF21 and Predicted Probabilities of HSCS in non - smoking SCAD Patients.

Description: HSCS prediction in regression model based on Age, Gender and FGF21 variables. Number of observations: n = 81. Predicted probability is expressed as a ratio. 95 % confidence intervals are based on individual predicted

probability values computed from the regression. Normalised FGF21: logarithmized value of Fibroblast Growth Factor - 21 concentration.

HSCS: hemodynamically significant coronary artery stenosis; SCAD: stable coronary artery disease.

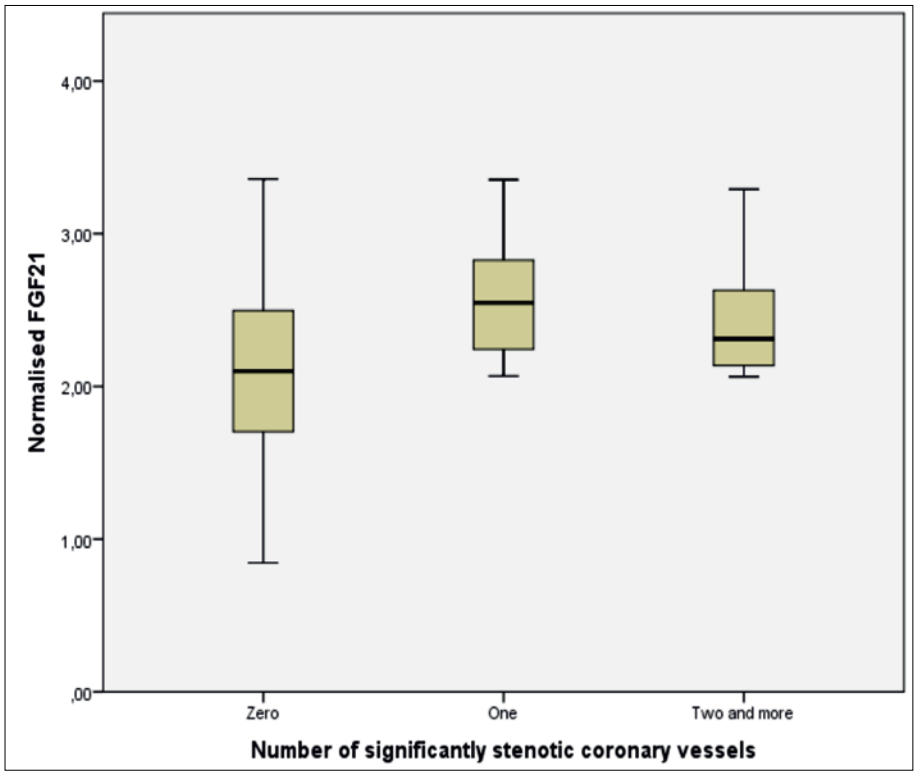

Fig 7: Between - Subgroup Comparison of FGF21 Concentration Medians.

Description: Non - smoking SCAD patients divided into three subgroups according to the NrSSV. The comparison is based on Kruskal – Wallis H test. Number of cases: n = 81. The „Zero" and „One" was the only pair of NrSSV subgroups, where the difference in FGF21 medians was statistically significant (adj. p = 0,002).
NrSSV: Number of significantly stenotic coronary vessels; SCAD: Stable coronary artery disease; Adj. p: Bonferroni correction of the p - value.

6. Conclusions

Although the elevation of the FGF21 concentration is a common finding among patients with SCAD, FGF21 cannot be used as a surrogate marker of SCAD in the general population. FGF21 measurement has a potential to become a part of the ICA indication algorithm, but only among non – smoking SCAD patients.

7. Author Contributions

Stančík conceived the study design, collected plasma samples, performed the laboratory and statistical analyses and drafted the manuscript. Mokáň provided valuable tips concerning the study design.

8. Declaration of Conflicting Interests: None.

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10. Abbreviations

AUC: area under the curve; BMI: body mass index; CRP: C – reactive protein; Exp B: odds ratio; eGFR: estimated glomerular filtration rate; ELISA: enzyme-linked immunosorbent assay; FGF21: fibroblast growth factor – 21; FFR: fractional flow reserve; HDL: high - density lipoprotein; HSCS: hemodynamically significant coronary artery stenosis; ICA: invasive coronary assessment; IVS: interventricular septal thickness; LDL: low - density lipoprotein; LVEF: left ventricular ejection fraction; LVEDd: left ventricular end-diastolic diameter; NrSSV: number of significantly stenotic coronary vessels; NormFGF21: normalised concentrations of fibroblast growth factor – 21; PTP: pre - test probability; ROC: receiver operating characteristics; RPM: revolutions per minute; SCAD: stable coronary artery disease; TG: triglycerides; TCH: total cholesterol; WC: waist circumference; WHR: waist to hip ratio.

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