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PREDICTIVE VALUE OF UPDATING THE SCORE CARDIOVASCULAR RISK ASSESSMENT ENGINE WITH NOVEL BIOMARKERS IN A TYPE 2 DIABETES POPULATION

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Abstract

Background and Aims: *The aim of the study was to estimate the predictive value of some new biomarkers in the assessment of cardiovascular disease (CVD) risk in a type 2 diabetes (T2DM) population and to perform a correlation between the SCORE risk results and the risk profile estimated by the use of these biomarkers. Finally, we aimed to establish if the CVD risk assessment can be improved by adding the biomarkers into the SCORE risk equation.* ***Material and Methods:*** *In the study population the CVD risk assessment was performed using the SCORE High Risk Chart. The new individual biomarkers were: estimated glomerular filtration rate (eGFR), urinary albumin excretion (UAE) rate, albumin/creatinine ratio (UACR), cystatin C, plasminogen activator inhibitor-1 (PAI-1), high-sensitivity C-reactive protein (hs-CRP), high density lipoprotein cholesterol (HDL) and apolipoprotein B (apo-B).* ***Results:*** *The SCORE risk prediction model results were significantly altered by adding in the equation apo-B and HDLc values. An increase of one standard deviation of the apo-B values caused the increase of the SCORE results with 0.19 standard deviations while an increase of one standard deviation of the HDLc values decreased the SCORE results with 0.26 standard deviations.* ***Conclusions:*** *Advanced lipid testing, including the measurement of apo-B, provides a more comprehensive cardiac risk assessment and should be used in the development of specifically designed risk-scores for T2DM individuals.*

key words: *type 2 diabetes, cardiovascular (CV) risk assessment, novel biomarkers, multimarker risk score*

Background and Aims

Clinical practice guidelines recommend a multifactorial approach for the management of cardiovascular disease (CVD) risk in type 2 diabetes (T2DM) [1,2]. Even-though treatment targets have been established for parameters such as glycated hemoglobin (HbA1c), blood pressure (BP), low-density lipoprotein cholesterol (LDLc), large clinical endpoint trials

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provided evidence that in most subjects a residual risk of CVD events still exists after achieving the optimal values, suggesting that there are nontraditional risk factors which need to be addressed [3,4]. Therefore clinical management decisions in subjects with T2DM should be based on a comprehensive assessment and quantification of the CVD risk of the individual patient. One of the emerging issues implies the reclassification of the patients with intermediate level of risk estimated based on the conventional risk factors. Especially in this category of patients, an advanced lipoprotein testing method should be applied as well as other laboratory assessments, as detailed below [5]. Detection and staging of diabetic renal impairment through the measurement of albuminuria, estimated Glomerular Filtration Rate (eGFR) and cystatin C, as well as detection of markers of atherosclerotic processes such as inflammation, including C-reactive protein (CRP) measured with a high sensitivity assay. All these parameters independently predict CV risk and provide additional prognostic information [6].

The objectives of the current study are: 1) to estimate the predictive value of novel biomarkers in the assessment of CVD risk in a T2DM population; 2) to establish if there is a correlation in-between the results obtained by applying the SCORE prediction model and the CVD risk profile estimated by the use of the novel biomarkers; 3) to verify if the CVD risk assessment can be improved by adding the novel biomarkers into the SCORE risk equation, especially for the T2DM subjects classified as low or intermediate risk individuals. The final aim is to identify novel biomarkers-related therapeutic strategies which may be beneficial in reducing the CVD estimated risk.

Material and methods

The clinical research protocol was reviewed and approved by the local Ethics Committee. A number of 188 subjects, men and women, with a diagnosis of T2DM, at least 18 years of age, registered at the Diabetes Centre in Oradea, Bihor County, agreed to participate and were assessed for eligibility. Before inclusion, the following criteria had to be fulfilled: stable standard of care T2DM treatment (≥ 3 months) as well as stable background lipid lowering treatment, antihypertensive drugs and antiplatelet agents (≥ 3 months). Subjects presenting with any of the following were not included in the study: type 1 diabetes (T1DM), diabetes secondary due to endocrine or pancreatic disorders, uncontrolled hypertension defined as systolic BP ≥ 180 mmHg or hypotension defined as systolic BP≤ 100 mmHg (requiring antihypertensive therapy adjustments), previous major ischemic cardiovascular events as well as revascularization procedures. Excluded were also patients currently being treated with systemic corticosteroids or weight loss medication. A history of rapidly progressing renal disease as well as anemia, known to interfere with HbA1c methodology, were also exclusion criteria.

From the 188 prescreened patients, 137 subjects were selected and after being provided with study-related information, signed the informed consent form (ICF) before any study specific procedures. The following anthropometric and clinical parameters have been assessed: height, weight, body mass index (BMI), BP. The body height and weight were measured using a Seca Medical Digital Scale, the BMI was calculated as kg/m^2= weight (kg)/height (m^2) and the BP using the 705CP-II Omron Digital Automatic Blood Pressure Monitor. The following laboratory samples have been collected: hematology, standard lipid

profile, apolipoprotein B (apo-B), HbA1c, fasting plasma glucose (FPG), 2 hour postprandial glucose (PPG) after ingestion of a standardized meal, serum creatinine, eGFR estimated by the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation, high-sensitivity C-reactive protein (hs-CRP), plasminogen activator inhibitor-1 (PAI-1), cystatin C, first morning void for urinary albumin excretion (UAE) rate and albumin/creatinine ratio (UACR). Regarding LDLc, the Friedwald equation was applied: LDLc= total cholesterol- HDLc- triglycerides /5, if triglycerides level was < 400 mg/dl. HbA1c was measured using Clover A1c Analyser available at the study site, all other laboratory parameters being performed at a local laboratory. Subjects were evaluated for the above mentioned parameters at baseline, 2 weeks and 12 weeks after visit 1. A number of 120 subjects out of 137 completed all the 3 scheduled visits, 17 were disqualified based on the BP values criteria or laboratory tests abnormalities such as hemoglobin level or rapid decline in eGFR.

Statistical analysis: For data processing purposes, SPSS v 17.0 software package was used (SPSS Inc Chicago, IL, USA). The Spearman correlation coefficient was used to measure the strength and direction of association between two variables, the individually analyzed biomarkers and the SCORE risk assessment values. To depict the performance of a biomarker in predicting the CVD risk in a T2DM population, equivalent to a higher than 5 SCORE risk engine result, a receiver-operator characteristic (ROC) curve has been constructed. To evaluate the biomarker performance by ROC analysis the following characteristics have been assessed: sensitivity, specificity, positive and negative predictive values. Multiple regression analysis using SPSS statistics was used in order to determine whether the value of a dependent variable- the SCORE risk equation results can be influenced by the values of multiple independent variables - the CVD risk biomarkers. P values < 0.05 were considered statistically significant.

Results

Finally 120 type 2 diabetes subjects (62.5% males/37.5% females) with a median age of 60 years were evaluated. The distribution on age groups revealed no statistically significant differences between genders, as shown in Table 1.

Table 1. Distribution of study subjects according to age.

	Whole study group	Men	Women	p
40-49 years of age	13 (10.8%)	10 (13.3%)	3 (6.6%)	0.522
50-59 years of age	53 (44.2%)	32 (42.7%)	21 (46.7%)	
60-69 years of age	54 (45.0%)	33 (44.0%)	21 (46.7%)	

A 6 years median length of diabetes duration was found, with no statistically significant difference between men or women as shown in Table 2.

Table 2. Distribution of study subjects according to disease duration.

	Whole study group	Men	Women	p
0-4 years	40 (33.3%)	24 (32.0%)	16 (35.6%)	0.791
5-9 years	52 (43.3%)	32 (42.7%)	20 (44.4%)	
≥ 10 years	28 (23.4%)	19 (25.3%)	9 (20.0%)	

Two most common cardiovascular risk patterns were identified in the study population, as assessed by applying the SCORE risk chart, with a first peak in the range of 1-3% and a

second local maximum corresponding to the range of 5-7% SCORE values, resulting in a bimodal distribution. The frequency of the SCORE values is outlined in Table 3. As shown below, a SCORE value result <5% was reported in a majority (60.8%) out of the total number of subjects.

Table 3. The SCORE risk values in the study population.

		Frequency	Percentage of the total number	Cumulative frequency
SCORE	0.00%	5	4.2	4.2
	1.00%	22	18.3	22.5
	2.00%	17	14.2	36.7
	3.00%	22	18.3	55.0
	4.00%	7	5.8	60.8
	5.00%	15	12.5	73.3
	6.00%	10	8.3	81.7
	7.00%	12	10.0	91.7
	8.00%	6	5.0	96.7
	9.00%	3	2.5	99.2
	13.00%	1	.8	100.0
	Total	120	100.0	

Table 4. Correlation of biomarkers with the SCORE risk results in the study population.

	r	p
apo-B (g/l)	0.129	0.160
hs-CRP (mg/l)	0.012	0.897
PAI-1 (mg/l)	-0.006	0.952
AER (mg/l)	0.157	0.088
UACR (mg/g)	0.197	0.031
e GFR (ml/min/1.73m²)	-0.154	0.094
Cystatin C (mg/l)	0.126	0.172
The Spearman correlation coefficient was used for the measurement of the strength and direction of association between the two variables		

A significant positive correlation was identified in-between the SCORE risk values and the UACR values (r=0.197; p=0.031). A marginally but not significant correlation was identified in-between the SCORE prediction model results, the UAE rate (r=0.157; p=0.088) and the eGFR (r=-0.154; p=0.094). No other statistically significant relationships in-between the SCORE results and the other individually analyzed biomarkers have been reported (Table 4).

Regarding the predictive value of novel biomarkers, in detection of patients with a SCORE risk higher than 5%, data regarding the predicting performance- sensitivity, specificity. positive and negative predictive value - was obtained for apo-B, CRP, PAI-1 and cystatin C as detailed in Figures 1, 2, 3 and 4.

To determine whether in a T2DM population, the SCORE risk equation results can be modified by the values of multiple independent variables, including lipid profile, renal impairment and inflammatory biomarkers, a multivariate regression model was designed. The SCORE risk prediction model results were significantly altered by adding in the equation apo-B and HDLc values. Regarding the apo-B values an increase of one single standard

deviation caused the increase of the SCORE CV risk results with 0.19 standard deviations. Referring to the HDLc values an increase of one single standard deviation decreased the SCORE CV risk results with 0.26 standard deviations, as shown in Table 5.

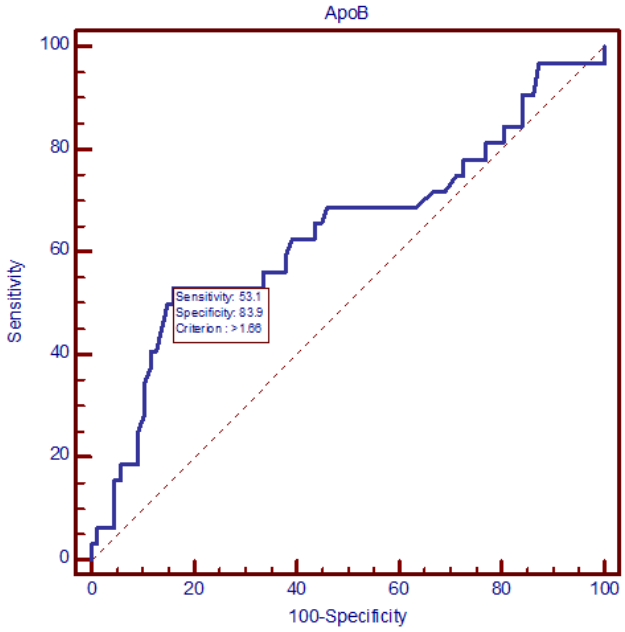

Figure 1. *Evaluation of the apo-B predictive performance for CVD risk using a receiver operating characteristic analysis (ROC curve)*

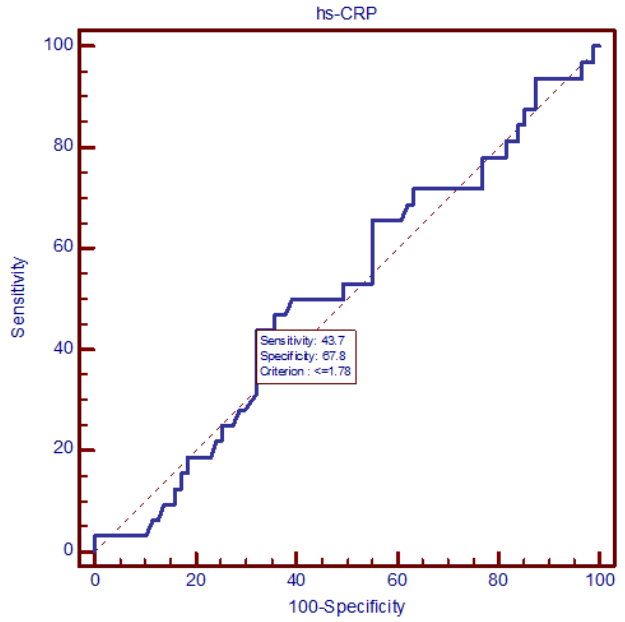

Figure 2. *Evaluation of the hs CRP prediction value for CVD risk assessment using ROC analysis*

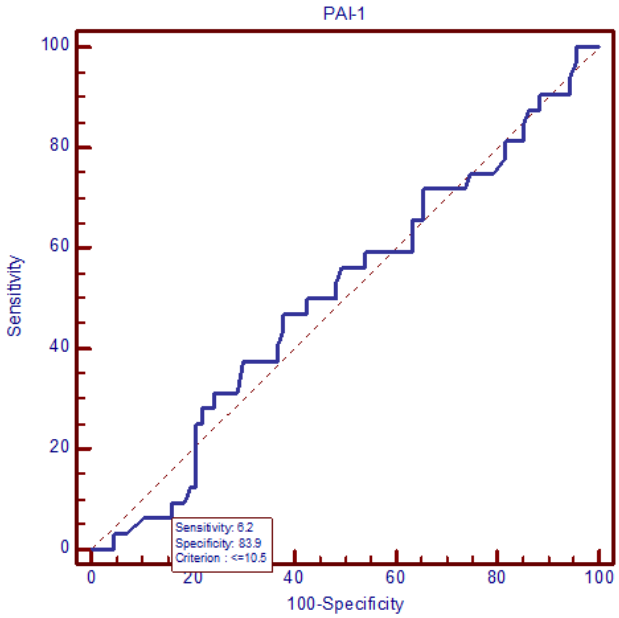

Figure 3. *Evaluation of the PAI-1 prediction value for CVD risk assessment using ROC analysis*

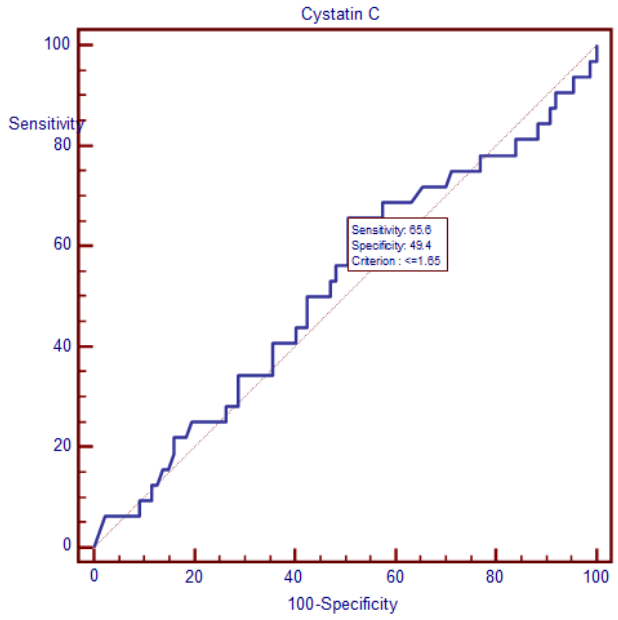

Figure 4. *Evaluation of cystatin C prediction value for CVD risk assessment using ROC analysis*

Discussions

Our results showed that after applying the SCORE CVD risk chart, the majority of T2DM subjects were classified as low or intermediate risk, even though according to clinical research data, the presence of T2DM is equivalent to that of a previous myocardial infarction [7]. A systematic review evaluated the performance of

17 different CVD risk scores in T2DM individuals, the majority being validated in the general population, a smaller number being specifically designed for individuals with diabetes [8]. The results showed that the prediction models developed based on traditional CVD risk factors, when applied to subjects with diabetes underestimated the CVD risk [8]. Regarding the predictive value of diabetes-specific risk engines, the results varied considerably among different clinical trials cohorts, the conflicting data delaying the expanding use of the newly developed diabetes-related risk scores and the replacement of the established conventional risk factors based methods of risk assessment.

Table 5. A multivariate regression model designed for the estimation of the impact of multiple independent variables on a dependent variable: SCORE risk equation.

	Non standardized coefficients		Standardized coefficients	t	p
	Slope of the regression line	Standard error	Beta		
Apo-B	0.843	0.399	0.191	2.114	0.037
HDLc	-0.064	0.023	-0.260	-2.768	0.007
hs-CRP	-0.022	0.044	-0.055	-0.496	0.621
PAI-1	-0.011	0.021	-0.057	-0.536	0.593
Albumin/creatinin ratio	0.000	0.001	-0.032	-0.316	0.752
Cystatin C	0.129	0.281	0.048	0.461	0.646
Dependent variable: SCORE					

Regarding the clinical utility of assessing biomarkers for CVD risk estimation, our results showed that high risk SCORE charts results significantly positively correlated with UACR, which implies that T2DM subjects with higher SCORE estimated CV disease risk tend to have higher UACR values. Even though up-to-date hs-CPR is the only clinically validated biomarker of CV risk assessment [9], no statistically significant correlation in-between the SCORE results and the hs-CPR value has been reported in our study.

It is current knowledge that in T2DM as well as other high CVD risk subjects, performing a standard lipid panel underestimates the risk profile. Advanced lipid testing, including the direct measurement of plasma apo-B, which has also been assessed in our current study, seems to provide a more comprehensive cardiac risk assessment. In a meta-analysis of clinical studies using LDL-c, non-HDLc and apo-B as CVD biomarkers, apo-B was found to be the most reliable predictor of ischemic cardiovascular events [10]. The results showed that over a 10-year period, the mean relative risk ratio was 12% higher than for LDL-c and 6% greater than for non-HDLc [10]. In a large epidemiological study - AMORIS (Apoprotein Related Mortality Risk) - apo-B demonstrated a higher sensitivity and specificity than LDL-c as a predictive variable of fatal myocardial infarction, irrespective of gender or age [11].

Our data showed that in T2DM subjects, for an accurate prediction of high CVD risk equivalent with an > than 5% SCORE result, apo-B sensitivity percentage was equal with 53.1%, high specificity of 83.9%, positive predictive value of 54.8%, negative predictive value of 83%. Furthermore in our study the SCORE risk prediction model results were significantly altered by adding in the equation apo-B and HDLc values. For apo-B, a higher value caused a significant increase of the SCORE CV risk results, while for HDLc a higher value caused a decrease of the SCORE CV risk results.

LDLc is still being considered the primary target of lipid lowering therapy but the hypothesis of using the apo-B and HDLc values instead for therapy initiation as well as for treatment intensification receives more and more evidence and support [12].

Conclusions

In predicting the CVD risk in a T2DM population, UACR values are as valuable as applying the high risk SCORE chart based on multiple traditional CV risk factors. For T2DM subjects classified as low or intermediate risk based on the SCORE chart, adding in the equation apo-B and HDLc values improved the prediction. Therefore an HDLc and apo-B based strategy for evaluating and treating excess CVD risk should be implemented in high-risk patients. Advanced lipid testing, including the measurement of HDLc sub-fractions and apo-B lipoproteins provides a more comprehensive cardiac risk assessment and should be used in the development of specifically designed risk-scores for T2DM individuals.

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