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A Multimarker Approach for the Prediction of Coronary Artery Disease: Cost-Effectiveness Analysis

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ABSTRACT

Objectives: Coronary artery disease (CAD), as the leading cause of death, poses a huge economic burden on health-care systems. We used a multimarker approach to explore discriminative abilities of several lipid, inflammatory, and oxidative stress/antioxidative defense markers as CAD predictors. We assessed their cost-effectiveness compared with the Framingham risk score (FRS).

Methods: Using a decision model, we evaluated the costs, accuracy, and cost-effectiveness of each model. The FRS was used as the baseline model. Other models were formed with the consecutive addition of selected markers: apolipoprotein A-I (apoA-I), apolipoprotein B (apoB), apolipoprotein (a) [apo(a)] isoform, lipoprotein (a), high-sensitivity C-reactive protein, malondialdehyde, superoxide dismutase (SOD), sulfhydryl, and superoxide anion (O_2^-). A best-case model was formed from a combination of diagnostic markers to yield the best patient stratification algorithm. All models were assessed by their predictive probabilities using receiver operating characteristic curves. To accomplish our goals, we recruited 188 CAD patients (verified by coronary angiography) and 197 asymptomatic CAD-free subjects for comparison. The analysis was performed from a third-party payer perspective.

Results: Only two strategies had outstanding discriminative abilities: the best-case model (FRS, SOD, and O_2^-) and FRS plus SOD with area under the curve (AUC) values of 0.924 and 0.906, respectively. The cost-effectiveness ratio varied between €593 per AUC for the baseline model to €2425 per AUC for FRS plus apo(a) isoform. Strategies involving oxidative stress/antioxidative defense markers were more cost-effective than strategies involving lipid or inflammatory markers. All results were robust.

Conclusion: Our results support the feasibility of a multimarker approach for CAD screening. The introduction of oxidative stress/antioxidative defense markers in the clinical laboratory would be convenient and cost-effective.

Keywords: coronary artery disease, cost-effectiveness, multimarker approach, risk prediction.

Introduction

Coronary artery disease (CAD) is the major cause of death worldwide [1]. CAD mortality in Eastern European countries is much higher than the European average as it reaches a value of 650 of 100,000 in some countries [2]. The direct health-care cost of CAD in the European Union was just under €23 billion in 2003 of which inpatient expenditure represented 62% of the total costs. CAD is estimated to represent one quarter of the overall cost of cardiovascular disease management and treatment [3].

Atherosclerosis is the underlying cause in most CAD cases with its typical clinical manifestations being myocardial infarction, angina, and stroke. Atherosclerosis is a complex disease caused by a variety of risk factors such as dyslipidemia, inflammation, and/or oxidative stress, and many individual biomarkers have been used for CAD risk assessment. The association of total cholesterol (TC), low-density lipoprotein cholesterol (LDL-c), and high-density lipoprotein cholesterol (HDL-c) levels with coronary diseases has been proven, and such parameters are commonly measured in current diagnostic practice. Other lipid-related measurements such as apolipoprotein A-I (apoA-I), apolipoprotein B (apoB) [4], apolipoprotein (a) [apo(a)] isoform, or lipoprotein (a) (Lp(a)) [5,6] have been correlated with coronary diseases and may add more predictive power to more commonly used lipid markers. The prognostic utility of high-sensitivity C-reactive protein (hs-CRP) and its ability to predict future coronary events has been reported in clinical studies [7–11]. Markers of oxidative stress and antioxidative defense risk parameters, mainly malondialdehyde (MDA), superoxide anion (O_2^-), superoxide dismutase (SOD), and sulfhydryl (SH) groups, are closely related to endothelial dysfunction, which as a function of time progresses to symptomatic CAD [12–15]. Nevertheless, none of the aforementioned risk factors used by them as a single biomarker is able to predict CAD with sufficient accuracy. Therefore, measurement of multiple biomarkers may be an effective strategy to improve prediction of cardiovascular events [16,17].

The Framingham risk score (FRS) is nowadays widely used as a tool for coronary risk stratification. Several predictors are included in the FRS: age, gender, TC, HDL-c, blood pressure, antihypertensive therapy, and cigarette smoking. Nevertheless, sensitivity and specificity of the FRS do not exceed 70% and 82%, respectively, if all the risk factors are included [18]. Limitations of the FRS (narrow section of the population) restricts FRS applicability [19,20] and poses a challenge to researchers in predicting the risk for coronary events. One of the ways to overcome this limitation is the introduction of additional biological markers that enhance FRS predictive power.

Bearing in mind that CAD is a global problem, it is also very important that laboratory markers, especially those used for screening and diagnostic purposes, are cost-effective. The results of economic evaluation of lipid and/or inflammatory markers have been reported in a limited number of studies [21–23]. Nevertheless, so far, there have been no reports regarding cost-effectiveness of oxidative stress and antioxidative markers for CAD risk prediction. In our present study, we used a multimarker approach, first, to explore the abilities of several lipid, inflammatory, and oxidative stress/antioxidative defense markers

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to enhance FRS prediction of cardiovascular risk. Second, we analyzed the cost-effectiveness of adding different individual markers to the FRS to identify the most cost-effective strategy for CAD prediction.

Materials and Methods

Study Participants

We recruited 188 CAD patients (122 males and 66 females, mean age 56.0 ± 9.08 years) from the Institute of Cardiovascular Disease, Clinical Centre of Serbia, Belgrade, and 197 asymptomatic CAD-free subjects (113 males and 84 females, mean age 55.7 ± 10.37 years) who attended a regular annual medical checkup at various public health centers in Belgrade for comparison purposes. The presence of clinically significant CAD (stenosis ≥50%) in at least one of the three major coronary vessels (the left main or the left anterior descending artery, the left circumflex artery, and right coronary artery) was verified by coronary angiography. Two cardiologists unaware that the patients were enrolled in the study reviewed all the angiograms as a validation procedure for correct diagnosis. For the purpose of the current study, patients with coronary artery stenosis of subclinical significance (<50%) were not included. The absence of clinically significant CAD in the group of healthy controls was verified by normal electrocardiogram (ECG) and exercise test results, but subclinical stenosis could not be ruled out.

All enrolled study participants completed a questionnaire that contained numerous risk-related issues. The following factors were recorded for all participants: body mass index, smoking habits, and systolic and diastolic blood pressure (SBP and DBP, respectively). Hypertension was defined in subjects with SBP ≥140 mmHg, a DBP of ≥90 mmHg, or being on antihypertensive medication. We excluded subjects with a history of recent clinical infection or hs-CRP concentration >10 mg/L; concurrent major renal, hepatic, or malignant disease; surgery; or major trauma during the month prior to study entry. Diabetic patients (patients with a fasting glucose level ≥7.0 mmol/L or patients who were receiving oral hypoglycemic agents or insulin medication) and patients receiving any antihyperlipidemic medication were also excluded from the study.

All patients gave informed consent before their enrolment in the study. The study was planned according to the ethical guidelines following the Declaration of Helsinki. The Ethics Committee for Clinical Research at the Faculty of Pharmacy approved our study protocol.

Design

The National Cholesterol Education Program Adult Treatment Panel III guidelines recommend calculation of the FRS for cardiovascular risk assessment. The baseline model included FRS and calculation of LDL-c because the concentration of LDL-c is needed for adequate monitoring and assessment of therapy. For the LDL-c calculation, assessment of triglycerides (TG) is required because of that fact it is incorporated into the baseline model. Other models were devised with the consecutive addition of selected markers: apoA-I, apoB, apo(a) phenotype, Lp(a), hs-CRP, MDA, SOD, SH, and O_2^- to the baseline model. A best-case model was formed according to the combination of diagnostic markers providing the best capabilities for patient stratification. The design is given in Figure 1.

Diagnostic Analytical Methods

Peripheral venous blood (obtained from all individuals after overnight fasting) was drawn into collection tubes containing heparin. Plasma was separated, and multiple aliquots of each sample were stored at −80°C until analysis. The samples were thawed immediately before analyses. For MDA determination, butylated hydroxytoluene (0.05% w/v) was immediately added to the plasma, and the mixture was stored at −80°C until analysis. O_2^- was measured in fresh plasma samples. All the assays were performed blindly.

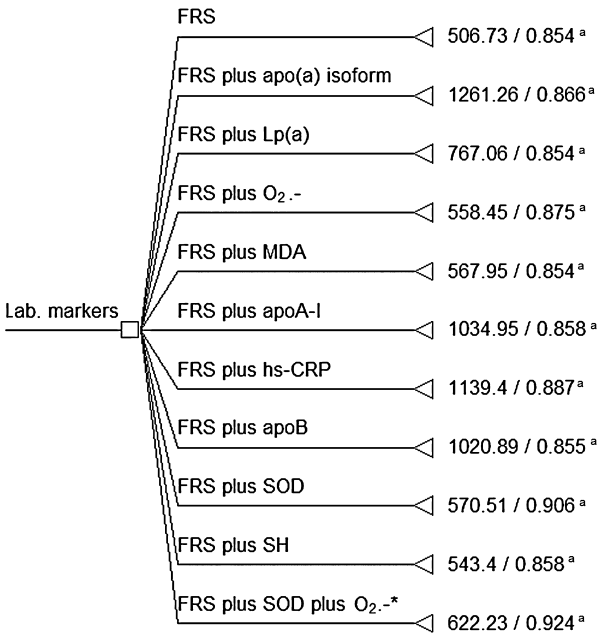

Figure 1 Schematic representation of the decision model. Framingham risk score (FRS): gender, age, total cholesterol, triglycerides, high-density lipoprotein cholesterol, calculation of low-density lipoprotein cholesterol, systolic blood pressure, treatment for hypertension, and cigarette smoking. *FRS plus superoxide dismutase (SOD) plus superoxide anion (O_2^-): best-case model according to logistic regression analysis. [a]Output values for cost/effectiveness for each model. apo(a), apolipoprotein (a); Lp(a), lipoprotein (a); MDA, malondialdehyde; hs-CRP, high-sensitivity C-reactive protein; SH, sulfhydryl.

Lipid status parameters were measured in heparin plasma samples. TC, HDL-c, TG, apoA-I, and apoB were assayed by standard laboratory procedures. The concentration of LDL-c was calculated using the Friedewald formula [24]. The sizes of apo(a) isoforms were determined by high-resolution sodium dodecyl sulfate agarose gel electrophoresis followed by immunoblotting. Calibration was performed using standards of known number of K4 repeats provided by Technoclone (Vienna, Austria). Lp(a) was measured using immunoturbidimetry (BIOKIT, Barcelona, Spain). Serum hs-CRP was quantitated using a latex-enhanced immunoturbidimetric method employing a Quantex hs-CRP kit (BIOKIT). Plasma MDA concentration was measured using the thiobarbituric acid-reactive substances (TBARS) assay previously described by Girotti et al. [25]. TBARS concentrations were measured at 535 nm, and the data were expressed as MDA equivalents using MDA-bis(diethylacetal) (1–10 μmol/L) as a standard (Merck, Darmstadt, Germany). The rate of nitroblue tetrazolium reduction was used to measure the level of O_2^- as described by Auclair and Voisin [26]. The absorbance was recorded every 15 seconds during the first minute of the reaction. The molar absorption coefficient of 15×10^3 M/cm was used. Plasma SOD activity was measured according to a previously published method by Misra and Fridovich [27]. The concentration of SH groups in

plasma was determined using 0.2 mmol/L of 5,5′-dithiobis (2-nitrobenzoic acid) reported by Ellman [28].

Most of the laboratory tests were measured using an ILAB 600 analyzer (Instrumentation Laboratory, Milan, Italy). Apo(a) isoform analysis required sodium dodecyl sulfate agarose gel electrophoresis and immunoblotting using a Submarine Electrophoresis Unit (Hoeffer Pharmacia Biotech, Uppsala, Sweden). MDA was determined manually because of the various steps required during the analysis.

Due to intraindividual variability, values for all parameters (except for the apo(a) phenotype) were estimated as the mean of three measurements performed at 1 to 2-week intervals. Our laboratory performed strict internal and external quality control procedures. Reagents for instrument calibration were supplied by the manufacturers.

Costs

All the costs for diagnostic reagents, consumables, and labor are summarized in Table 1. For the laboratory markers that can be evaluated on the automated analyzer and are reimbursed by the Republic Institute for Health Insurance (RIHI), the costs (service fee) were derived from the RIHI price list [29]. All the costs indicated were from 2008. Since then, the price of all the biochemical markers has not changed. For the biochemical markers not on the RIHI list but can be analyzed automatically (O_2^-, SOD, and –SH), the service fee price was calculated according to the following: cost for test reagents, specimen collection, and fee for technical services. For example, for the O_2^- costs for reagents were calculated in the following manner: For 100 samples and sample blank preparation, the required quantities of reagents were the following: 101 ml of the working reagents (1 mmol/L nitroblue tetrazolium dissolved in freshly prepared phosphate buffer of 0.05 mmol/L with the addition of 0.1 mg/ml gelatin). For this quantity of reagents, according to the 2008 Acros Organics catalog, the cost for O_2^- evaluation was 14.84 euros. The cost of consumables for collection tubes containing heparin with needles was calculated as an average price recognized by the RIHI. The fee for technical service was calculated as time needed for specimen collection and average time needed by the analyzer to process 100 samples multiplied by the laboratory technician's salary. The cost of MDA and apo(a) isoform determination was calculated as the sum of time needed for sample preparation and manual evaluation of the markers. The cost of test reagents included the cost of chemicals needed for reagent preparation, calibration, standards, and controls based on 100 analyses. Costs were calculated based on market prices from the 2008 Acros Organics catalog of fine chemicals. The average monthly salary of a laboratory technician was obtained from the RIHI [29]. The total cost was calculated by adding up all the expenditures of all direct analytical and labor resources consumed. All costs are presented in euros (the average exchange rate in 2008 was 1 euro = 81.91 RSD).

Table 1 Total costs for laboratory markers based on 100 analyses (all costs are presented in euros, 2008 value)

Markers	Test reagents*	Specimen collection†	Fee for technical services‡	Total cost§
Total cholesterol	—	—	—	156.09¶
Triglycerides	—	—	—	159.54¶
High-density lipoprotein cholesterol	—	—	—	191.10¶
Apolipoprotein A-I	—	—	—	528.22¶
Apolipoprotein B	—	—	—	514.16¶
High-sensitivity C-reactive protein	—	—	—	632.67¶
Lipoprotein (a)	—	—	—	260.33¶
Apolipoprotein (a) isoform	622.59	28.87	103.07	754.54
Malondialdehyde	3.9	28.87	28.45	61.22
Superoxide anion	14.84	28.87	8.01	51.72
Superoxide dismutase	0.06	28.87	34.85	63.78
Sulfhydryl	0.19	28.87	7.61	36.67

*Cost for test reagents, plus costs for controls (pathology or/and normal), calibration, and standards based on 100 analyses.
†Cost for 100 collection tubes containing heparin.
‡Salary for laboratory personnel (for specimen collection) and for technical service, based on 100 analyses.
§Sum of all previously mentioned costs.
¶Marker is on the Republic Institute for Health Insurance price list, and the cost covers test reagents, collection tubes, personnel costs, and fee for technical service.

Administrative costs (e.g., electricity) were excluded from the calculations because these costs are the same for all compared strategies. Non-health-care costs and indirect costs were not included in the analysis. The outcomes were analyzed from the perspective of a third-party payer—RIHI, as the leading health-care payer, responsible for health care of almost the entire Serbian population (7.5 million).

For the baseline model, the costs of TC, TG, and HDL-c were required. The cost of LDL-c (required for FRS calculation of 10-year risk) was not included as the LDL-c concentration was calculated manually using the Friedewald formula. The costs of other models were equal to the sum of costs for the baseline model and costs for additionally included laboratory markers.

The effectiveness of the models as CAD predictors was evaluated using receiver operating characteristic (ROC) curves with the predictive probabilities from different logistic regression models.

The results are presented as the cost for 100 analyses of laboratory markers and the incremental cost-effectiveness ratio (ICER) for each marker. The ICER is defined as the difference in cost divided by the difference in effectiveness between competing strategies [30]. In this case, the ICER was defined as incremental cost per additional accuracy in CAD prediction, obtained by inclusion of a new laboratory marker between the two diagnostic models.

Statistical and Sensitivity Analysis

Multiple logistic regression analysis was used to investigate association between the laboratory tests (entered as continuous variables) and the presence of CAD (a categorical dependent variable) within different models. Controls were coded with 0, and CAD patients were coded with 1. ROC curves with predictive probabilities from logistic regression models were used to calculate the areas under the curves (AUCs) or C-index for the baseline model and all other constructed models. We also entered all laboratory parameters into forward stepwise logistic regression analysis to determine the best-case model. The latter only included those variables that emerged as statistically significant. By using Hosmer and Lemeshow's rule for the logistic models, the discriminative abilities of the models were classified according to their AUC values as poor ($0.5 \leq AUC < 0.7$), acceptable ($0.7 \leq AUC < 0.8$), excellent ($0.8 \leq AUC < 0.9$), or outstanding ($AUC \geq 0.9$) [31].

In addition, the jackknife technique [32] was used to assess the impact of potentially influential outliers that might meaningfully alter the interpretation of model results. The stability of a calculated AUC was confirmed by comparing the calculated t value for the jackknifed coefficient with the t critical value.

All calculations were performed using Microsoft Excel, EduStat 2.01 (2005, Alpha Omnia, Belgrade, Serbia), and

Table 2 The results of ROC analysis and jackknife method for discriminating CAD (+) from CAD (–) patients

Strategy	AUC (confidence interval)	Standard error‡	Jackknifed coefficients (confidence interval)
FRS model*	0.854 (0.813–0.896)	0.021	0.824§ (0.780–0.861)
FRS model plus SH	0.858 (0.802–0.915)	0.029	0.826* (0.760–0.893)
FRS model plus O_2^-	0.875 (0.836–0.914)	0.020	0.891§ (0.837–0.945)
FRS model plus MDA	0.854 (0.810–0.898)	0.021	0.876§ (0.824–0.927)
FRS model plus SOD	0.906 (0.871–0.914)	0.018	0.939§ (0.864–1.014)
Best-case model†	0.924 (0.894–0.955)	0.015	0.947§ (0.909–0.986)
FRS model plus Lp(a)	0.853 (0.818–0.896)	0.022	0.852§ (0.802–0.902)
FRS model plus apoB	0.855 (0.814–0.896)	0.021	0.880§ (0.834–0.925)
FRS model plus apoA-I	0.858 (0.817–0.899)	0.021	0.875§ (0.827–0.923)
FRS model plus hs-CRP	0.887 (0.839–0.915)	0.019	0.892§ (0.846–0.935)
FRS model plus apo(a) isoform	0.866 (0.824–0.907)	0.021	0.880§ (0.833–0.927)

*Framingham risk score (FRS): gender, age, total cholesterol, triglyceride, high-density lipoprotein cholesterol, calculation of low-density lipoprotein cholesterol, systolic blood pressure, treatment for hypertension, and cigarette smoking. Best-case model: FRS, SOD, and O_2^-.
†Best-case model: FRS plus SOD and O_2^-.
‡Standard error for AUC.
§A jackknifed coefficient was stable because its calculated *t* values exceed the *t* critical value.
ROC, receiver operating characteristic; CAD, coronary artery disease; AUC, area under the curve; SH, sulfhydryl; O_2^-, superoxide anion; MDA, malondialdehyde; SOD, superoxide dismutase; Lp(a), lipoprotein (a); apoB, apolipoprotein B; apoA-I, apolipoprotein A-1; hs-CRP, high-sensitivity C-reactive protein.

MedCalc for Windows version 9.6.3 (Mariakerke, Belgium). The minimal statistical significance was set at $P < 0.05$.

For cost-effectiveness analysis, TreeAge Module Healthcare version 1.5.2 (TreeAge Software, Inc., Williamstown, MA) was used. To examine the robustness of the results, we performed two types of sensitivity analysis. One-way sensitivity analysis was performed by varying the total cost within the ±30% interval and effectiveness in the AUC within the 95% confidence interval (CI). Two-way sensitivity analysis was performed by simultaneously varying the total costs and effectiveness within the same intervals [33].

Results

The accuracy of all the models was assessed by calculating their AUC values and is presented in Table 2. The best-case model (consisting of FRS, SOD, and O_2^-) had outstanding discriminative ability with an AUC value of 0.924. Only one other strategy (FRS plus SOD) had an outstanding AUC value (0.906). All other comparative strategies had excellent discriminative abilities (AUC values between 0.854 and 0.887). Diagnostic models with outstanding discriminative abilities (AUC higher than 0.9) can be interpreted as models that have a probability greater than 90% for correct classification of patients. FRS accuracy was 85.4% (AUC = 0.854), and newly defined diagnostic models (best-case and FRS plus SOD models) had better probabilities for patient classifications.

Table 2 shows the internal validation results in which the jackknife method was used. The jackknifed coefficients implied that there was little overexpression in the estimated predictive accuracy of the baseline model, FRS and SH model, and FRS and Lp(a) model. The performance of the remaining models showed little improvement after internal validation. The results obtained using the jackknife technique indicate that our original model estimates were stable and were not affected by influential outlier cases within the study sample.

Table 3 shows cost-effectiveness of risk assessment markers for 100 CAD patients (100 analyses). The cost-effectiveness ratio varied between €593 per AUC for the baseline model and €2425 for FRS plus apo(a) isoform. A strategy of combined FRS plus MDA evaluation was dominated (higher costs and worse effectiveness) by FRS plus O_2^- evaluation. The best-case model was dominant (lower costs and better effectiveness) compared with all markers that are on the RIHI price list. Of the remaining five strategies, evaluation of FRS plus SH groups and FRS plus O_2^- evaluation were excluded because of extended (or weak) domi-

Table 3 Costs, effectiveness, and incremental cost-effectiveness for a model for CAD prediction based on 100 analyses

Strategy	Cost (€)	Incremental cost (€)	Effectiveness (AUC)	Incremental effectiveness (AUC)	C/E (€/AUC)	ICER (€/AUC)
FRS model*	506.7	—	0.854	—	593	—
FRS model plus SH	543.4	36.7	0.858	0.004	633	9167
FRS model plus O_2^-	558.5	15.1	0.875	0.017	638	885
FRS model plus MDA	568.0	9.5	0.854	–0.021	665	Dominated
FRS model plus SOD	570.5	12.1	0.906	0.031	630	389
Best-case model†	622.2	51.7	0.924	0.018	673	2873
FRS model plus lipoprotein (a)	767.1	144.8	0.854	–0.070	899	Dominated
FRS model plus apolipoprotein B	1020.9	398.7	0.855	–0.069	1194	Dominated
FRS model plus apolipoprotein A-I	1035.0	412.7	0.858	–0.066	1206	Dominated
FRS model plus CRP	1139.4	517.2	0.887	–0.037	1285	Dominated
FRS model plus apolipoprotein (a) isoform	1261.3	639.0	0.866	–0.058	1456	Dominated

*FRS model: gender, age, total cholesterol, triglyceride, high-density lipoprotein cholesterol, calculation of low-density lipoprotein cholesterol, systolic blood pressure, treatment for hypertension, and cigarette smoking.
†Best-case model: FRS plus SOD and O_2^-.
Dominated strategy: strategy with higher cost and lower effectiveness than another strategy (second best).
CAD, coronary artery disease; FRS, Framingham risk score; SH, sulfhydryl; O_2^-, superoxide anion; MDA, malondialdehyde; SOD, superoxide dismutase; CRP, C-reactive protein; AUC, area under the curve; ICER, incremental cost-effectiveness ratio.

Table 4 Costs, effectiveness, and incremental cost-effectiveness for CAD laboratory markers after elimination of dominant strategies based on 100 analyses

Strategy	Cost (€)	Incremental cost (€)	Effectiveness (AUC)	Incremental effectiveness (AUC)	C/E (€/AUC)	ICER (€/AUC)
FRS model*	506.7	—	0.854	—	593	—
FRS model plus SOD	570.5	63.8	0.906	0.052	630	1227
Best-case model†	622.2	51.7	0.924	0.018	673	2873

*FRS model: gender, age, total cholesterol, triglycerides, high-density lipoprotein cholesterol, calculation of low-density lipoprotein cholesterol, systolic blood pressure, treatment for hypertension, and cigarette smoking.
†Best-case model: FRS, SOD, and O_2^-.
CAD, coronary artery disease; SOD, superoxide dismutase; AUC, area under the curve; ICER, incremental cost-effectiveness ratio; FRS, Framingham risk score.

nance, meaning that both strategies were dominated by a linear combination of baseline models and FRS plus SOD evaluation.

The ICER calculation for the remaining three strategies: baseline, FRS plus SOD evaluation, and the best-case model, is presented in Table 4. For the FRS plus SOD, the ICER (compared with the baseline model) was €1227 per additional accuracy calculated for 100 analyses. The ICER for the best-case model (compared with the baseline model) was €1650 per additional accuracy calculated for 100 analyses.

The efficiency frontier composed of strategies that had the lowest cost and the highest effectiveness available is presented in Figure 2. It consists of three strategies (the baseline model, FRS plus SOD evaluation, and the best-case model) with costs that ranged from €506 to €622 per 100 analyses. All other strategies lie on the left and above the frontier, indicating that the costs of these strategies was higher and accuracy was lower compared with strategies on the frontier.

Sensitivity analysis indicated that results were robust. The costs of diagnostic markers have greater potential effects on results compared with the same marker effectiveness. Only the costs of FRS, SOD, O_2^-, SH groups, and MDA posed a potential to influence results. Sensitivity analyses using the different cost values (within the ±30% range) for these variables showed a range of €585.6 to €1594.5 per AUC gained for the FRS and SOD model. The cost of FRS was identified as the most sensitive variable, and it represented part of the cost of all other models. Therefore, it was not reasonable to expect a significant change in the results.

Discussion

We have analyzed the cost-effectiveness of selected CAD laboratory markers from a third-party payer perspective. One of the tools for identification of individuals at high risk for coronary events is the FRS that has been used for the creation of the baseline model for our study. The FRS has serious limitations [19,20], which can be overcome using population-adjusted equations or by a multimarker approach. In our study, the effectiveness of each model was estimated by AUC calculations after construction of ROC curves, which are valuable tools for the assessment of diagnostic accuracy of laboratory tests [34]. A multimarker approach enhances risk stratification of patients and adds to the overall prediction of risk based on the FRS [16,17]. The AUC of the baseline model was among the lowest AUC values when compared with other models tested (Table 2). This is in agreement with results from other studies regarding the accuracy of the FRS (AUC values vary between 0.640 and 0.813) [35–37]. In our study, only two strategies showed outstanding discriminative abilities: the model consisting of FRS plus SOD and the best-case model (FRS, SOD plus O_2^-), with AUC values

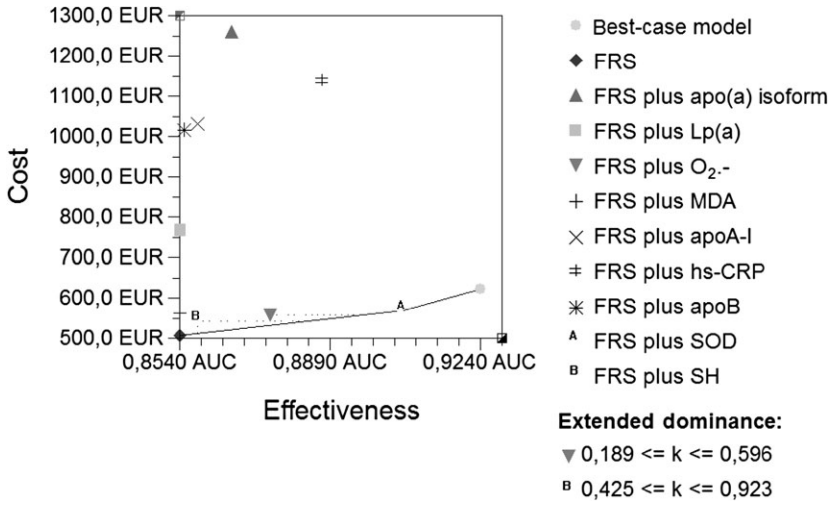

Figure 2 Efficiency frontier of the models for coronary artery disease risk assessment. Framingham risk score (FRS): gender, age, total cholesterol, triglycerides, high-density lipoprotein cholesterol, calculation of low-density lipoprotein cholesterol, systolic blood pressure, treatment for hypertension, and cigarette smoking. Best-case model: FRS, superoxide dismutase (SOD), and superoxide anion (O_2^-). All models were constructed by the addition of one laboratory marker to the FRS, except the best-case model, which was determined from the logistic regression analysis. AUC, area under the curve; apo(a), apolipoprotein (a) isoform; Lp(a), lipoprotein (a); apoA-I, apolipoprotein A-I; apoB, apolipoprotein B; MDA, malondialdehyde; hs-CRP, high-sensitivity C-reactive protein; SH, sulfhydryl.

of 0.906 and 0.924, respectively (Table 2). This finding implies that the addition of oxidative stress/antioxidative defense markers to traditional cardiovascular risk factors provides enhanced overall predictive power and is consistent with our earlier observations [38,39]. According to our results, the addition of lipid markers to the FRS did not improve patient stratification (Table 2). The accuracies of the model consisting of FRS plus SOD and the best-case model were similar to the accuracy of quantitative computed tomography (CT) angiography (AUC of 0.93 [95% CI 0.9–0.9]) for detecting or ruling out stenoses ≥50% [40].

Desirable features of biomarkers depend on their intended use (screening, diagnostic, or treatment biomarkers), whereas cost-effectiveness is an important feature for all of them [41]. Economic evaluation of lipid and/or inflammatory markers has been performed in a limited number of studies [21–23,37]. Nevertheless, cost-effectiveness analysis of oxidative stress and antioxidative markers has not been published. To our knowledge, this is the first study that has incorporated lipid, inflammatory, and markers of oxidative stress and the oxidative system, their diagnostic accuracies, and economic considerations for CAD risk evaluation.

Our study suggests that evaluation of the FRS plus SOD was the most cost-effective model for CAD prediction (Table 3). The model provides additional diagnostic accuracy (increase in AUC) compared with current risk assessment based on the FRS. The cost (just €63.78 for 100 analyses) makes it suitable for laboratory use. Additional costs per gained diagnostic accuracy would be €1227/AUC for 100 analyses compared with conventional risk assessment based solely on the FRS (Table 4). The combined determination of FRS, SOD, and O_2^- (the best-case model) provided better diagnostic accuracy with an AUC value of 0.924 and an extra cost of €52 per 100 analyses. Ridker and colleagues [42,43] showed that the addition of hs-CRP determination significantly improved clinical prediction of the models based on lipids alone, which is in agreement with our finding that the model that included FRS and hs-CRP had greater accuracy compared with the baseline model (Table 2). Nevertheless, the model was not found to be cost-effective (Table 3). In contrast, Blake and coworkers [22] noted that a strategy involving hs-CRP could be relatively cost-effective in middle-aged patients without overt hyperlipidemia. In addition, economic evaluations conducted in Germany and Italy showed that hs-CRP was a cost-effective marker, and it improved increased cardiovascular risk prediction, especially among individuals aged 45 years and older, and it improved the effectiveness of currently available therapies by selecting patients at higher risk [21]. Schnell-Inderst et al.'s study evaluated risk prediction models after adding hs-CRP to traditional risk factors. Adding hs-CRP to the prediction models increased AUC by up to 0.027, but both the clinical relevance and cost-effectiveness of this improvement remain unclear [37]. Such differences could be explained by different study designs. Our study explored cost-effectiveness of different models in terms of their abilities to predict CAD. In contrast, improved outcomes and reduced costs of cardiovascular events and statin therapy have also been considered in the other aforementioned studies.

Bearing in mind that the estimated direct costs of heart disease in the United States in 2009 most likely reached US$106.3 billion (representing cost per capita of US$347) [44] and that in the EU in 2006, the cost of CAD was just under €24 billion (representing cost per capita of €49) [45], possible savings in CAD diagnostics and therapy could be made. According to a cost-effectiveness study of coronary CT angiography, the ICER was US$17,516 per correct diagnosis [46]. Our model that incorporated laboratory markers had a lower ICER value, but as the outcomes were not expressed in the same units, we could not make suitable comparisons between the two studies.

Our ROC curve results reflected the ability of the examined models to discriminate between CAD cases and CAD-free subjects. Therefore, the results permitted us to evaluate the usefulness of models as screening tools for clinically significant CAD. To assess the predictive value of models as diagnostic tools, patients presenting with similar symptoms but without any angiographically visible stenoses should be a control group rather than apparently healthy CAD-free subjects. Nevertheless, as coronary angiography is an aggressive method, it is never performed without good reason. Accordingly, it was not possible for us to recruit a sufficient number of patients with 0% stenosis that would have allowed us to perform meaningful statistical analyses.

The oxidative stress/antioxidative defense markers included in this study do not have defined thresholds (cutoff values), and they have not generally been included in routine laboratory analysis. Prospective studies including a large number of subjects are needed to elucidate the accuracy of the oxidative stress/antioxidative defense markers in terms of test sensitivity and specificity, likelihood ratios, and predictive values, and to assess their cost-effectiveness in clinically more informative measures [47]. The best diagnostic model for response to therapy still remains to be conclusively identified. Nevertheless, our study has illustrated that the use of AUC analysis and ICER enables decision-makers to choose a model among different diagnostic models with similar diagnostic importance but with different associated costs. Diagnostic models with outstanding discriminative function (AUC > 0.9) had probability >90% for correct classification of disease. From a diagnostic point of view, if a diagnostic model has better accuracy, it has a higher probability for early CAD diagnosis. This would be very important for better identification of individuals with CAD risk and immediate therapeutic intervention, which would save future health-care cost expenditure. In addition, the inclusion of other laboratory markers (including N-terminal pro-brain natriuretic peptide, brain natriuretic peptide, total oxidative stress status, total antioxidant status, pro-oxidative–antioxidative balance, and soluble tumor necrosis factor-like weak inducer of apoptosis [sTWEAK]) would add strength to the CAD prediction model.

The power of our data analysis was limited by study sample size. We used the c statistic for discrimination ability and the jackknife method for internal validation because there were no other similar data for us to validate our models. There is a general demand for further research aimed at probabilistic, instead of discriminating, analysis to evaluate diagnostic models with biomarkers and risk factors that can also be applied easily by clinicians and interpreted in a meaningful way.

It should be noted that markers on the RIHI price list levy service fees, whereas for markers that are not on the list, we determined service fees as the sum of time needed for sample and test preparation, and for automatic or manual evaluation of the 100 samples. Finally, our study incorporated only direct medical costs as the study perspective was that of a third-party payer. We did not take into account costs for equipment/analyzers for marker evaluation because we assumed that the clinical laboratory already had adequate analyzers. In the case of a different scenario, the included costs and results would differ substantially. We can make suitable comparisons with other studies that noted the cost of hs-CRP. In our study, the calculated cost for hs-CRP was €6.32 per patient. The cost to measure C-reactive protein per patient in Italy and Germany was €3.8 and €7.5, respectively [21], whereas the cost in the United States was €18 (US$25) [22].

In conclusion, our results support the feasibility of using additional biomarkers (especially the FRS plus SOD model) in

CAD screening. In times of sparing economic resources, the introduction of oxidative stress/antioxidative defense markers into the clinical laboratory would be highly convenient because they are easy to perform and do not require dedicated personnel and equipment. Prospective studies validating the benefit of oxidative stress/antioxidative defense, inflammatory, and lipid markers in combination with traditional markers to fully determine the most cost-effective strategy for coronary risk assessment are currently lacking.

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