Original Article

Relationship between Cystatin C, Retinol-binding Protein 4 and Framingham Risk Score in Healthy Postmenopausal Women

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Abstract

Background: We aimed to examine the relationship between high levels of cystatin C, retinol-binding protein 4 (RBP4) and cardiovascular risk score [determined by Framingham Risk Score (FRS)] in postmenopausal women.

Methods: A total of apparently healthy 129 postmenopausal women (mean age 57.1 ± 4.6 years) were included. Serum cystatin C, RBP4, glucose, lipid parameters, creatinine, uric acid and high sensitivity C-reactive protein (hsCRP) were determined. Anthropometric parameters and blood pressure were also obtained. FRS was calculated. Multiple linear regression analysis (MLR) was performed to identify independent factors affecting FRS and to estimate the final predictors of its variability. Receiver Operating Characteristic (ROC) curve analysis was used with the purpose of testing discriminatory potential of a group of parameters selected in MLR analysis, with FRS level as dependent variable.

Results: We found significantly higher levels of both proteins, cystatin C (P = 0.001) and RBP4 (P = 0.006), in the FRS higher (medium and high) risk groups (FRS ≥ 10%) compared to low risk FRS group (FRS < 10%). MLR revealed the best model consisting of 4 parameters (e.g., body mass index (BMI) (P < 0.001), triglycerides (TG) (P = 0.004), RBP4 (P = 0.021), and cystatin C (P = 0.046), R^2-adjusted = 0.347) for FRS prediction. Construction of a model consisted of those 4 FRS formula independent parameters (BMI, TG, cystatin C and RBP4) using logistic regression analysis showed that new ROC curve had excellent discriminatory capability (area under the curve = 0.820).

Conclusion: High cystatin C and retinol-binding protein 4 may contribute significantly to cardiovascular risk burden in addition to traditional cardiovascular markers.

Keywords: Cystatin C, Framingham risk score, postmenopausal women, retinol-binding protein 4

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Introduction

Postmenopausal women are at increased risk of cardiovascular disease (CVD) compared with women prior to menopause.[1] In the light of this fact, early detection of cardiovascular risk burden is of utmost importance for preventing adverse cardiovascular outcomes in this population group.

Although the risk for CVD [as estimated using the 10-year Framingham risk score (FRS)] includes some of the well-established parameters (e.g., gender, age, total cholesterol level, high-density lipoprotein cholesterol level, systolic blood pressure, antihypertensive therapy, diabetes mellitus, smoking), there are variables that are not included in FRS, which might contribute significantly to CV risk assessment.[2]

Cystatin C and retinol-binding protein (RBP4) are small proteins (13.3 kDa and 21.1 kDa, respectively), and due to their low-molecular weight, they are filtered freely through the glomeruli and reabsorbed in the proximal tubules.[3,4] Therefore, cystatin C and RBP4 have emerged as sensitive markers for early detection of renal impairment.[5] Recently, cystatin C and RBP4 have also been proposed as markers of metabolic abnormalities.[6,7]

It was generally assumed that some cell types express cystatin C much more than others, such as macrophages.[8] The recent discovery that cystatin C is expressed in human adipose tissue has also confirmed the association between obesity and elevated serum cystatin C in humans.[9]

Retinol-binding protein 4, mainly secreted from hepatocytes and adipocytes, has been suggested to be a central regulator of insulin sensitivity.[10] Insulin resistance was found to be accompanied by down-regulation of the insulin responsive glucose transporter-4 (GLUT4) which might be the signal for RBP4 secretion from adipocytes and development of systemic insulin resistance.[10] Therefore, high levels of RBP4 and cystatin C have also been shown to predict the incidence of type 2 diabetes mellitus.[11,12]

Moreover, these proteins have been proposed to be markers of cardiovascular disturbances.[13,14] Previous studies have shown that serum RBP4 and cystatin C levels were significantly elevated in patients with coronary artery disease (CAD) compared to non-CAD patients, and were significantly increased with the number of stenotic vessels.[13,14]

However, none of these studies have evaluated the effect of high levels of both these proteins on CVD risk in apparently healthy postmenopausal women. Therefore, we aimed to examine the relationship between high levels of cystatin C, RBP4 and cardiovascular risk score (determined as Framingham Risk Score) in healthy postmenopausal women.

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Materials and Methods

Study population

The study enrolled a total of 129 postmenopausal women (mean age 57.1 ± 4.6 years) who volunteered to participate in this cross-sectional study. Participants were consecutively recruited in the study when seeking gynecologic healthcare in the Department of Gynecology in Primary Health Care Center in Podgorica, Montenegro for their regular check-up, over a period from October 2012 to May 2013. Menopause is defined as the absence of menstrual bleeding for more than one year. All the participants completed a questionnaire including demographic characteristics, somatic illnesses, smoking history and current medication use. Medical history and clinical examinations were performed on the same day.

Inclusion criteria were participants with fasting glucose < 7.0 mmol/L. In addition, all participants with fasting glucose ≥ 5.6 mmol/L, but ≤ 6.9 mmol/L, underwent a two-hour oral glucose tolerance test (OGTT) with 75 g anhydrous glucose dissolved in 250 mL of water in order to exclude diabetes. Participants with 2-hour postload glucose ≥ 11.1 mmol/L were excluded from the study.[15] The inclusion criteria also entailed participants with normal glomerular filtration rate (creatinine-based estimated glomerular filtration rate (eGFR) ≥ 90 mL/min/1.73 m² calculated using the Chronic Kidney Disease Epidemiology Collaboration Equation (CKD-EPI)),[16] without signs and symptoms of acute inflammatory disease, with no history or presence of malignancy, hypo- or hyperthyroidism,[17] and non-smokers. Participants who had diabetes mellitus (fasting glucose ≥ 7.0 mmol/L or 2-hour postload glucose ≥ 11.1 mmol/L), renal dysfunction (creatinine-based eGFR< 90 mL/min/1.73 m²), hepatic dysfunction, cardiovascular disorders, and C-reactive protein levels (CRP) > 10 mg/L[18] were excluded from the study, as well as those who had been using any medications[19,20] (antihypertensive, lipid-lowering, hypoglycemic, anti-inflammatory medications or hormonal replacement therapy) in the last six months. We excluded all these participants since RBP4 and cystatin C can be influenced additionally by all the states mentioned above.[5,11,12,17–20]

Participants were instructed not to perform any vigorous physical activity the day before the blood samples were taken. All the participants provided written informed consent. The study protocol was approved by the Ethical Committee of Primary Health Care Center in Podgorica and the research was carried out in compliance with the Declaration of Helsinki.[21]

Anthropometric measurements

Basic anthropometric measurements, body height (cm), body weight (kg) and waist circumference (WC) (cm), were performed in the morning. Weight was measured to the nearest 0.1 kg on a balance beam scale, with the subjects barefoot and with light clothing. Height was measured to the nearest 0.1 cm using a wall-mounted stadiometer, without shoes. Waist circumference was measured with non-stretchable tape over the unclothed abdomen at the midpoint between the lowest rib and the iliac crest. The tape was parallel to the floor and did not compress the skin. Body mass index (BMI) was calculated as weight in kilograms divided by height in meters squared (kg/m^2). Blood pressure was measured with a sphygmomanometer after the subject had been seated for 15 minutes. The average of three measurements on the right arm was recorded. All measurements were made by the same trained evaluator.

The Framingham risk score (FRS) is based on gender, total cholesterol, high-density lipoprotein cholesterol, smoking status, presence of diabetes, and systolic blood pressure. The Framingham risk score (FRS) was calculated for each individual subject in accordance with the score sheet available as on-line calculator at https://www.cvdriskchecksecure.com/framinghamriskscore.aspx.

Thereafter, the cohort of apparently healthy postmenopausal women involved in this study, were divided into low (FRS < 10%) and higher Framingham Risk Score (FRS ≥ 10%).[22,23]

Biochemical analyses

The blood samples were taken between 7–9 hours a.m., after 12–14 hours of overnight fasting. Samples were left to clot for 30 minutes and then centrifuged at 3000 rpm for 10 minutes. Serum samples were divided into aliquots and stored at -20ºC, without prior thawing and re-freezing before analyses, except for glucose, which was determined immediately after the blood was drawn. Serum levels of glucose, creatinine, uric acid, total cholesterol (TC), high density lipoprotein cholesterol (HDL-c), low density lipoprotein cholesterol (LDL-c) and triglycerides (TG) were measured using standardized enzymatic procedures using a spectrophotometer (Roche Cobas 400, Mannheim, Germany). Cystatin C, RBP4, and high sensitivity C-reactive protein (hsCRP) levels were determined using a nephelometric assay (Behring Nephelometer Analyzer, Marburg, Germany). GFR was estimated using the Chronic Kidney Disease Epidemiology Collaboration Equation (CKD-EPI).[16]

Statistical analysis

Statistical analysis was performed using SPSS statistical package (version 15.0 for Windows, SPSS, Chicago, IL, USA). The necessary sample size was calculated using RBP4 and cystatin C as the two primary endpoints to detect a difference of about 20% in both parameters between the analyzed groups at a power of 80% and a *P* value of 0.05. Data are presented as mean ± standard deviation, or median (interquartile range), or counts and percentages. Differences between groups were evaluated with a Student's t test for normally distributed, or Mann-Whitney test for non-normally distributed parameters. A Pearson's (r) correlation coefficient was used to determine the relationships between FRS and other variables. Multiple linear regression analysis (MLR) was performed to identify independent factors affecting FRS and to estimate the final predictors of its variability. Receiver Operating Characteristic (ROC) curve analysis was used with the purpose of testing discriminatory potential of a group of parameters selected in MLR analysis, with FRS as the dependent variable. Construction of a model consisted of FRS formula independent parameters using logistic regression analysis. In all analyses, *P* values < 0.05 were considered as statistically significant.

Results

Table 1 shows the general clinical and biochemical characteristics of apparently healthy postmenopausal women involved in this study. Significantly higher fasting glucose ($P = 0.034$), SBP, and DBP ($P < 0.001$, respectively), but lower HDL-c ($P < 0.001$) were observed in women with higher risk score level as compared with low risk score level group. Furthermore, we found significant difference in several parameters, which are independent of FRS calculation, i.e., significantly higher anthropometric parameters-

Table 1. General characteristics of studied women divided according to Framingham Risk Score status.

Characteristics	Women with low FRS (<10%) (n = 75)	Women with higher FRS (≥10%) (n = 54)	P-Value
Age (years)	55.9 ± 4.56	58.6 ± 4.27	0.001
BMI (kg/m²)	25.4 ± 3.62	29.2 ± 4.12	<0.001
WC (cm)	86.3 ± 10.17	96.8 ± 10.30	<0.001
Fasting glucose (mmol/L)	5.30 ± 0.44	5.50 ± 0.61	0.034
TC (mmol/L)	6.39 ± 1.08	6.68 ± 1.00	0.129
HDL-cholesterol (mmol/L)#	1.80 ± 0.39	1.42 ± 0.33	<0.001
LDL-cholesterol (mmol/L)	4.13 ± 1.02	4.64 ± 0.89	0.004
TG (mmol/L)#	1.11 (0.91–1.46)	1.71 (1.31–2.28)	<0.001
Uric acid (μmol/L)	259 ± 65.1	291 ± 62.7	0.007
hsCRP (mg/L)#	0.81 (0.60-1.08)	1.86 (1.17–2.53)	<0.001
SBP (mm Hg)	122 ± 18.7	152 ± 12.4	<0.001
DBP (mm Hg)	80.0 ± 11.28	95.2 ± 7.39	<0.001
Creatinine (μmol/L)	55.1 ± 6.46	56.3 ± 6.39	0.315
eGFR(mL/min/1.73m²)	101 (100–103)	99 (95–99)	0.013
Cystatin C (mg/L)	0.75 ± 0.09	0.80 ± 0.10	0.001
RBP4 (mg/L)	40.3 ± 8.88	44.7 ± 8.84	0.006
Time since menopause (years)	5.00 (3.00–9.00)	7.50 (4.00–10.00)	0.218
FRS (%)	5.92 ± 2.01	14.77 ± 4.17	<0.001

Data are presented as mean ± standard deviation or#median (interquartile range); FRS-Framingham Risk Score; BMI-Body mass index; WC-Waist circumference; TC-total cholesterol; HDL-cholesterol-High density lipoprotein cholesterol; LDL-cholesterol-Low density lipoprotein cholesterol; TG-Triglycerides; SBP-Systolic blood pressure; DBP-Diastolic blood pressure; hsCRP-high sensitivity C–reactive protein; eGFR-Estimated glomerular filtration rate using the Chronic Kidney Disease Epidemiology Collaboration Equation; RBP4-Retinol-binding protein 4

BMI and WC ($P < 0.001$, respectively), higher level of uric acid ($P = 0.007$), hsCRP ($P < 0.001$), cystatin C ($P = 0.001$), and RBP4 ($P = 0.006$) among women in higher risk group, as compared with women in low risk group. Women in higher risk group also had higher LDL-c ($P = 0.004$), TG ($P < 0.001$), but lower eGFR ($P = 0.013$). No significant difference was found with respect to duration of menopause between these two groups.

Thereafter, we performed Pearson's correlation to examine the potential relationship between FRS level and cardiometabolic parameters independent of FRS calculation (BMI, WC, LDL-c, TG, cystatin C, RBP4, creatinine, eGFR, hsCRP and uric acid level) in all apparently healthy postmenopausal women.

Pearson's correlation revealed significant positive relationships between FRS level and BMI, WC, LDL-c, TG cystatin C, RBP4, uric acid level (all $P < 0.001$), and hsCRP ($P = 0.029$), as well as a significant negative relationship between FRS and eGFR ($P < 0.001$) (Table 2).

Multiple linear regression (MLR) analysis was performed to identify which of the measured markers had the best association with FRS. All variables found to have a significant predictive value in Pearson's correlation (e.g., BMI, TG, eGFR, uric acid, hsCRP, cystatin C and RBP4) were further analyzed in MLR analysis for FRS prediction. The backward selection enabled us to find the best model consisting of 4 parameters (e.g., BMI ($P < 0.001$), TG ($P = 0.004$), RBP4 ($P = 0.021$), and cystatin C ($P = 0.046$)) which are shown in Table 3. Adjusted R^2 for the best model was 0.347, which means that 34.7% of variation in FRS could be explained by this model.

Thereafter, we conducted a receiver operating characteristic (ROC) analysis of selected parameters to test their discriminatory ability regarding FRS status (low *vs.* higher risk). Additionally, we constructed a Model consisting of those 4 parameters (BMI, TG, cystatin C and RBP4) using logistic regression analysis generated predictive probabilities. Figures 1 and 2 show the ROC curve graph and Table 4 shows the most important ROC parameters: area under the curve (AUC) with 95% confidence interval (CI) of selected parameters and the Model.

ROC curves comparison showed that all separate curves have comparable discriminatory capability towards risk level status. Construction of a model consisting of those 4 FRS formula independent parameters (BMI, TG, cystatin C and RBP4) using logistic regression analysis showed that the new ROC curve had excellent discriminatory capability (AUC = 0.820, according to Hosmer and Lemeshow's rules).[24]

Table 2. Pearson's correlation (r) of Framingham Risk Score status and examined parameters independent of FRS calculation.

Variable	R	*P*-Value
BMI (kg/m²)	0.480	<0.001
WC (cm)	0.429	<0.001
LDL-c (mmol/L)	0.381	<0.001
TG (mmol/L)	0.498	<0.001
Uric acid (µmol/L)	0.300	<0.001
hsCRP (mg/L)	0.192	0.029
Creatinine (µmol/L)	0.150	0.089
eGFR (mL/min/1.73m²)	-0.325	<0.001
Cystatin C (mg/L)	0.314	<0.001
RBP4 (mg/L)	0.312	<0.001
Time since menopause (years)	0.172	0.057

FRS-Framingham Risk Score; BMI-Body mass index; WC-Waist circumference; LDL-cholesterol-Low density lipoprotein cholesterol; TG-Triglycerides; hsCRP-high sensitivity C–reactive protein; eGFR-Estimated glomerular filtration rate using the Chronic Kidney Disease Epidemiology Collaboration Equation; RBP4-Retinol-binding protein 4.

Table 3. Multiple linear regression standardized β coefficients, 95% Confidence Interval and P values for the parameters in the best-fit model for the association of several parameters with FRS, backward selection.

Coefficients	Standardized Coefficients Beta	95% Confidence Interval for β	*P*-Value
BMI (kg/m²)	0.335	0.212–0.631	<0.001
TG (mmol/L)	0.242	0.606–3.017	0.004
RBP4 (mg/L)	0.182	0.016–0.198	0.021
Cystatin C (mg/L)	0.144	-0.835–16.673	0.046

BMI-Body mass index; TG-Triglycerides; RBP4-Retinol-binding protein 4.

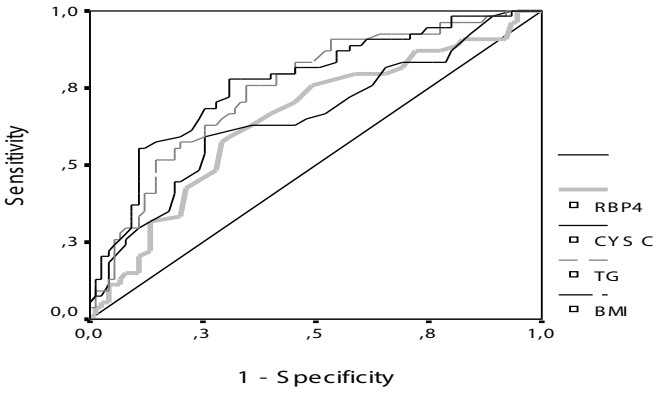

Figure 1. ROC curves of selected parameters' discriminatory ability regarding Framingham Risk Score status (low *vs.* higher risk).

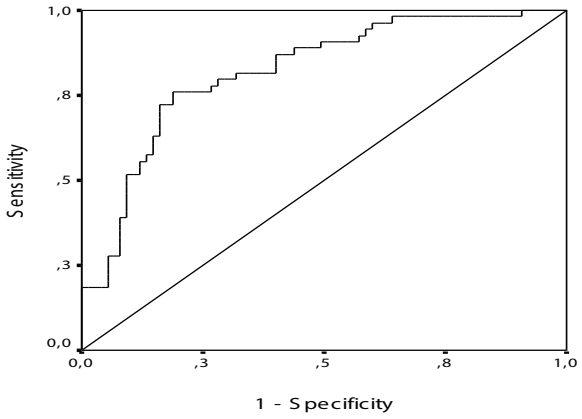

Figure 2. ROC curve of Model consisted of 4 selected parameters' discriminatory ability regarding Framingham Risk Score status (low *vs.* higher risk)

Table 4. Area under the curve, 95% Confidence Interval and Standard error for the parameters of selected parameters' discriminatory ability regarding Framingham Risk Score status (low *vs.* higher risk); Pairwise comparison of the areas under ROC curves (AUCs) for Model and separate parameters.

Parameter	AUC	95% CI	SE	*P**
Model (4 parameters)	0.820	0.747–0.894	0.037	<0.001
BMI	0.768	0.685–0.852	0.043	<0.001
TG	0.749	0.664–0.834	0.043	<0.001
Cystatin C	0.657	0.560–0.754	0.049	0.002
RBP4	0.649	0.552–0.764	0.050	0.004

AUC - area under ROC curve; CI- Confidence interval; SE-standard error; Model: BMI-Body mass index; TG-Triglycerides; Cystatin C, and RPB4-Retinol-binding protein 4. * - P from pairwise comparison for AUC differences between Model and separate parameter.

Discussion

To our knowledge, this is the first study investigating the new biomarkers, such as cystatin C and RBP4 simultaneously, in order to evaluate the contribution to CV risk assessment. We found significantly higher levels of both proteins, cystatin C and RBP4, in the FRS higher (medium and high) risk groups (FRS ≥ 10%) compared to low risk FRS group (FRS < 10%). This confirmed, at least partially, the association of these two proteins with general cardiovascular risk in postmenopausal women in our study.

Besides the expected positive relationship between FRS and BMI and TG, which are well known traditional CV risk factors, we also revealed significant relationship between FRS and RBP4 and cystatin C in the group of apparently healthy postmenopausal women. Therefore, we conducted a ROC analysis of selected parameters to test their discriminatory ability regarding FRS status (low vs. higher risk). Additionally, construction of a model consisting of those 4 FRS formula independent parameters (BMI, TG, cystatin C and RBP4) using logistic regression analysis showed that the new ROC curve (Figure 2) had excellent discriminatory capability.[24]

Our results are also in accordance with Won *et al*.[25] who found a strong positive relationship between RBP4 and FRS in healthy males and females. They showed that RBP4 increased with visceral fat accumulation and was associated with CVD risk factors, suggesting that RBP4 could be a mediator between harmful effects of visceral obesity and the increased risk of CVD independent of traditional risk factors.

Indeed, the obese state, especially the visceral type, is accompanied by low-grade chronic inflammation, which is characterized by infiltration of macrophages in adipocytes and increased expression of inflammatory cytokines.[26] The inflammatory response promotes the activation of transcriptional factors and pro-inflammatory cytokines and adipokines (e.g., RBP4), which may affect insulin action by suppressing insulin receptor signaling[27] and lead to high risk for adverse cardiovascular outcomes.[28] Moreover, infiltration of macrophages secreting inflammatory cytokines, can also affect the serum cystatin C concentration,[8] since besides expression in adipocytes, cystatin C is expressed in preadipocytes, endothelial cells, and macrophages.[9]

Qing *et al*.[29] reported cystatin C as an independent risk factor for development and severity of asymptomatic CAD in subjects with metabolic syndrome and normal creatinine-based eGFR. Some previous studies have also reported significantly higher serum RBP4 and cystatin C levels in patients with CAD compared to non-CAD patients, and also noticed significant increase of these proteins with the number of stenotic vessels.[13,14]

On the other hand, a study conducted by Huang *et al*.[30] only weakly supports the possibility that perturbations in RBP4 homeostasis may be an additional risk factor for subclinical coronary atherosclerosis in healthy, recently postmenopausal women, suggesting that both low and high RBP4 levels may be associated with subclinical coronary atherosclerosis.

Ito *et al*.[31] showed that cystatin C remained significantly associated with incident CVD events after adjustment for Framingham risk score variables (FRSVs) in adults without baseline clinical CVD. However, the addition of cystatin C to FRSVs did not substantially affect CVD risk prediction in that cohort.

Our results confirmed that some other markers, such as hsCRP and uric acid were not independently associated with CV risk score, suggesting that some traditional factors (such as obesity) might attenuate this association.

High sensitivity CRP is a nonspecific marker of systemic inflammation. Observational studies, although inconclusive, have suggested that hsCRP only has a small, or no incremental contribution to cardiovascular risk prediction compared to traditional risk factors.[2,32]

In a meta-analysis of 83 studies in patients with CAD, an elevated CRP increased the adjusted relative risk of only 1.19 fold.[33] In a study conducted by Eapen *et al*.[32] an elevated CRP level increased risk by a higher, but still modest, 1.6-fold.

Some other studies have failed to demonstrate the predictive power of CRP to CV risk. In the Women's Health Initiative study including 27,347 post-menopausal women, aged 50 to 79 years, among the 18 biomarkers measured, CRP level did not significantly improve CVD prediction either alone or in combination with other biomarkers.[2]

On the other hand, many population-based studies in subjects free of known CAD have found that CRP adds to risk prediction above standard risk factor assessment.[34,35]

Moreover, in the current study after MLR, the association between FRS and uric acid was not statistically significant any more. On the contrary, Nam *et al*.[36] showed an increased uric acid concentration associated with an increase in coronary heart disease risk calculated from the FRS in apparently healthy Korean adults. Similar results were also shown by Lee *et al*.[37]

A large number of novel biomarkers have been identified to be associated with CV risk. However, controversy still remains

with regard to their utility in CVD risk assessment.[38] In a nested case-control study, Kim *et al*.[2] analyzed 18 biomarkers previously associated with CAD in 321 patients with CAD and 743 control postmenopausal women. Five (von Willebrand factor, factor VIII, homocysteine, Interleukin-6, and D-dimer) of the 18 biomarkers tested were associated with CAD, but only D-dimer improved the C-statistic compared with traditional risk factors.

Mansur *et al*.[39] demonstrated that inclusion of lipoprotein (a) and lymphotoxin-alfa mutations in the set of conventional risk factors showed an additive, but small, increase in risk prediction of premature coronary disease, contrary to Kim *et al*.[2] who reported no association of lioprotein (a) with CAD.

Blakenberg *et al*.[40] analyzed the risk prediction of CAD associated with 30 biomarkers in two middle-aged European populations. The study showed that adding any single biomarker separately to the established risk model did not improve risk estimation in either population.

In the Uppsala Longitudinal Study of Adult Men, the C-statistic for CVD death prediction increased when four markers (troponin I, N-terminal pro-brain natriuretic peptide, cystatin C, and CRP) were added to established markers in all participants.[41]

Considering the fact that CVD is a complex phenotype involving multiple biological pathways and since biomarkers can also provide insight about pathophysiologic abnormalities that precede overt CVD, search for biomarkers that reflect distinct pathways, including cardiac stress, inflammation, atherosclerosis, vascular structure and function, and metabolism is needed.[42] In line with this, Halim *et al*.[43] used a nested case-control design to examine the association of 53 circulating proteins with the risk of death or myocardial infarction in a high-risk cohort. They identified a set of 6 biomarkers (intercellular adhesion molecule-1, matrix metalloproteinase-3, N-terminal pro-B-type natriuretic peptide, interleukin-6, soluble CD40 ligand, and insulin-like growth factor binding protein-2) strongly associated with death or myocardial infarction.

In the light of all these facts, even with the multiple biomarker approach, a reliable set of biomarkers has yet to be found that improves CVD risk stratification sufficiently.

Limitations of the present study must be considered. Since we included only postmenopausal women in our study, all of whom were non-smokers, we reported lower score values than the previous study conducted by Won *et al*.[25] Furthermore, a causal relationship between FRS and serum RBP4, as well as cystatin C levels in postmenopausal women, could not be established.

In conclusion, to our knowledge, the present study is the first one to examine the association of both, RBP4 and cystatin C with FRS and to evaluate their effect on CV risk burden in apparently healthy postmenopausal women. Postmenopausal women with higher FRS displayed higher serum RBP4 and cystatin C levels than women with low FRS. Our results also show that cystatin C and RBP4 were independent predictors of FRS, in addition to traditional CV risk factors (e.g., BMI and TG) suggesting that high cystatin C and RBP4 may add significant contribution to higher cardiovascular risk. Therefore, determination of these proteins may be beneficial in early detection of postmenopausal cardiovascular disturbances.

Conflict of Interest Statement

The authors have declared no conflicts of interest.

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