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Multimarker Prediction of Coronary Heart Disease Risk

The Women's Health Initiative

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Objectives The aim of this study was to investigate whether multiple biomarkers contribute to improved coronary heart disease (CHD) risk prediction in post-menopausal women compared with assessment using traditional risk factors (TRFs) only.

Background The utility of newer biomarkers remains uncertain when added to predictive models using only TRFs for CHD risk assessment.

Methods The Women's Health Initiative Hormone Trials enrolled 27,347 post-menopausal women ages 50 to 79 years. Associations of TRFs and 18 biomarkers were assessed in a nested case-control study including 321 patients with CHD and 743 controls. Four prediction equations for 5-year CHD risk were compared: 2 Framingham risk score covariate models; a TRF model including statin treatment, hormone treatment, and cardiovascular disease history as well as the Framingham risk score covariates; and an additional biomarker model that additionally included the 5 significantly associated markers of the 18 tested (interleukin-6, D-dimer, coagulation factor VIII, von Willebrand factor, and homocysteine).

Results The TRF model showed an improved C-statistic (0.729 vs. 0.699, $p = 0.001$) and net reclassification improvement (6.42%) compared with the Framingham risk score model. The additional biomarker model showed additional improvement in the C-statistic (0.751 vs. 0.729, $p = 0.001$) and net reclassification improvement (6.45%) compared with the TRF model. Predicted CHD risks on a continuous scale showed high agreement between the TRF and additional biomarker models (Spearman's coefficient = 0.918). Among the 18 biomarkers measured, C-reactive protein level did not significantly improve CHD prediction either alone or in combination with other biomarkers.

Conclusions Moderate improvement in CHD risk prediction was found when an 18-biomarker panel was added to predictive models using TRFs in post-menopausal women. (J Am Coll Cardiol 2010;55:2080–91) © 2010 by the American College of Cardiology Foundation

The predictive capacities of the major cardiovascular risk factors, including age, sex, cigarette smoking, high blood pressure, elevated total cholesterol (TC) level, low high-density lipoprotein cholesterol (HDL-C) level, and diabetes mellitus, have been well established. Using these major risk factors in a combined manner, the Framingham risk score was developed in an effort to assist clinicians in risk assessment and treatment planning (1). While the Framing-

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ham risk score is considered a standard and generally acceptable approach to risk prediction (2), newer biomarkers, which reflect inflammation, endothelial function, fibrin formation and fibrinolysis, oxidative stress, renal function, ventricular function, and even myocardial cell damage, have also been associated with cardiovascular risk, and their predictive values have been studied (3–12). A multimarker risk prediction approach, that is, the inclusion of several newer biomarkers simultaneously, also has been studied with the goal of improving the accuracy and clinical utility of cardiovascular risk prediction (13–16). Some studies have suggested that adding several newer biomarkers can substantially improve risk classification (15,16), but others have observed only minimal improvement in the ability to classify cardiovascular risk by adding biomarkers (13,14).

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In current medical practice, the accurate assessment of cardiovascular risk is considered essential for clinical decision making, because the benefits, risks, and costs of alternative management strategies must be weighed to choose the best treatment for individual patients. However, controversy remains both as to the utility of new markers in cardiovascular risk assessment, especially in women, and also as to the best statistical methods to use in assessing the incremental value of new biomarkers. Blood biomarkers have been studied as risk factors and predictors of coronary heart disease (CHD) in the Women's Health Initiative participants (17–19). However, the predictive values of combining traditional risk factors (TRFs) and newer biomarkers have not been studied thoroughly. To determine whether new biomarkers are useful in clinical practice, their performance in disease prediction should be assessed by various indexes (20). In light of ongoing uncertainty in these areas, we investigated whether multiple biomarkers yield a better assessment of cardiovascular risk in post-menopausal women compared with a standard risk assessment incorporating major TRFs alone.

Methods

In the present study, we used datasets from a nested case-control biomarker study and an 8.6% subsample study in the WHI-HT (Women's Health Initiative Hormone Trials). Details of the study design, data collection, intervention, and outcome ascertainment in the WHI-HT, including CONSORT diagrams, have been published previously (21–23).

Study populations. The WHI-HT enrolled 27,347 post-menopausal women ages 50 to 79 years from 1993 to 1998 at 40 U.S. clinical centers. Post-menopausal women with prior hysterectomies (n = 10,739) were randomly assigned to receive conjugated equine estrogen 0.625 mg/day (CEE) or placebo, and those with a uterus (n = 16,608) were randomly assigned to receive CEE with medroxyprogesterone acetate 2.5 mg/day (MPA) or placebo. The CEE and CEE + MPA trials were stopped after mean follow-up periods of 7.1 and 5.6 years, respectively (24,25). Because of early adverse effects of hormone therapy on cardiovascular events in the Women's Health Initiative, a nested case-control study for biomarkers was performed. All centrally adjudicated cases of CHD, stroke, and venous thromboembolism occurring during the first 4 years of follow-up were included in biomarker studies. Controls were matched on age, randomization date, hysterectomy status, and prevalent cardiovascular disease (CVD) at baseline. Matching on CVD history was specific to the case type, but all controls for the 3 case types were used after excluding any with incident CHD, stroke, or venous thromboembolism. Eventually, the CHD biomarker study included 359 patients with CHD and 820 controls. Of the 359 participants with CHD, 11 also had strokes, 9 had venous thromboembolism, and 1 had all 3 events. The present study was restricted to 321 patients and 743 controls who were either white or African American and had complete data for blood pressure, TC, HDL-C, fasting glucose, and current smoking status. Five-year incidence of CHD was calculated for all the WHI-HT participants of white and African-American ethnicity. Additionally, an 8.6% subsample study of the WHI-HT participants was used for the estimation of mean risk factor levels and also for the validation of CHD risk prediction models. This component of the present study included 1,261 white and 678 African-American women, among whom 39 incident CHD events were observed (Online Appendix 1).

Outcome ascertainment. Clinical outcomes were identified by semiannual questionnaires and classified by centrally trained local adjudicators following medical record review. All locally adjudicated cases of CHD were reviewed by central adjudicators. CHD included nonfatal myocardial infarction, CHD death, and incident silent myocardial infarction. Definite and probable nonfatal myocardial infarction required overnight hospitalization and was defined according to an algorithm based on standardized criteria using cardiac pain, cardiac enzymes levels, and electrocardiographic findings and included myocardial infarction occurring during surgery or other procedures (26). CHD death was defined as death consistent with CHD as the underlying cause plus 1 or more of the following: hospitalization for myocardial infarction within 28 days before death, previous angina or myocardial infarction, death due

Abbreviations and Acronyms

ABM = additional biomarker
CEE = conjugated equine estrogen 0.625 mg/day
CHD = coronary heart disease
CRP = C-reactive protein
CVD = cardiovascular disease
FRSN = Framingham risk score with new coefficients
FRSO = Framingham risk score with original coefficients
HDL-C = high-density lipoprotein cholesterol
MPA = medroxyprogesterone acetate 2.5 mg/day
TC = total cholesterol
TRF = traditional risk factor

to a procedure related to CHD, or a death certificate consistent with the underlying cause as atherosclerotic CHD. Definite silent myocardial infarction was diagnosed by clear changes from baseline to year 3 or year 6 electrocardiograms (Novacodes 5.1 and 5.2) (27).

Risk factors and biomarker measurements. Demographic and general health characteristics were based on self-report. Current smokers were those who had ever smoked at least 100 cigarettes and were currently smoking. Nondrinkers were those who reported fewer than 12 drinks of any kind of alcoholic beverage in their lifetimes. Medications and supplement use were ascertained by a computer-driven inventory system at the first screening visit. Systolic and diastolic blood pressures were measured twice after a 5-min rest period using a conventional mercury sphygmomanometer (22). Blood samples were collected and processed at baseline and were stored at a central biorepository at −70°C. Analyses were run in single batches including patients and controls and 10% blind duplicates within 8 years of collection. Blood analyses included fasting glucose, lipid profile, and a panel of 18 biomarkers: lipoprotein(a), homocysteine, insulin, C-reactive protein (CRP), E-selectin, interleukin-6, matrix metalloproteinase-9, fibrin D-dimer, factor VIII, plasminogen activator inhibitor-1 antigen, prothrombin fragment 1.2, plasmin-antiplasmin complex, thrombin-activatable fibrinolysis inhibitor, von Willebrand factor, fibrinogen, hematocrit, and leukocyte and platelet counts. Fasting glucose, insulin, lipid profile, lipoprotein(a), fibrinogen, hematocrit, and leukocyte and platelet counts were available for the case-control sample and the 8.6% random subsample, but other biomarkers were measured only for the case-control sample. Detailed methods for physical assessment and biomarker measurements have been described elsewhere (18,22).

Statistical analysis. Multiple logistic regression models were used for the assessment of independent relationships of risk factors and biomarkers to CHD incidence. The first logistic models were adjusted for age, systolic blood pressure, TC, HDL-C, diabetes (fasting glucose ≥126 mg/dl or current treatment), and smoking status. The second models were additionally adjusted for statin use, active hormone treatments, and history of CVD at baseline. Coefficients were calculated for each categorical risk factor or a 1-SD increment of each continuous risk factor. Associations between the 18-biomarker panel and CHD risk were assessed with and without logarithmic transformation. The logarithmic scale was selected for 14 biomarkers (except for fibrinogen, leukocytes, platelets, and hematocrit) because they had skewed distributions and showed stronger associations with CHD when log transformed.

We developed 4 prediction equations for 5-year CHD risk. The first equation (Framingham risk score with original coefficients [FRSO]) used coefficients from the original Framingham risk score (1). The second equation (Framingham risk score with new coefficients [FRSN]) included systolic blood pressure, TC, and HDL-C in continuous forms and diabetes and current smoking, and the coefficients were obtained from the nested case-control study. The third equation (TRF) included statin treatment, hormone treatment (CEE and CEE + MPA), and history of CVD at baseline, which were independently associated with CHD, in addition to the variables in the FRSN equation. The fourth equation (additional biomarker [ABM]) additionally included biomarkers that were significantly associated with CHD even after adjustment for TRFs (equations are presented in Online Appendix 2).

All blood biomarkers were additionally considered for risk prediction by adding each single biomarker to the TRF model. The mean level of each risk factor was obtained from the subsample of WHI-HT, except for biomarkers that were available only in the case-control study (Online Table 1). We included hormone treatments as covariates in the models. Previous studies reported that CEE + MPA treatment was associated with increased CHD risk (hazard ratio: 1.24; 95% confidence interval: 1.00 to 1.54) (25), but hormone treatment had no significant interaction with other risk factors (25) or biomarkers (18) except for baseline low-density lipoprotein cholesterol.

The discriminatory power of each model was assessed by the C-statistic (area under the receiver-operating characteristic curve), and its difference between models was tested using a nonparametric method (28). We also calculated the Yates slope (the difference between predicted risk between patients and controls; larger values indicate better discrimination), the Brier score (the sum of squared difference between the observed outcome and fitted probability; smaller values indicate better fit), and the integrated discrimination improvement (29,30). The increased discriminative value of adding TRFs and biomarkers was further examined with reclassification tables. This method is based on the difference between 2 models in the individual estimated probability that a case subject will be categorized as a case subject. The net reclassification improvement (NRI) was calculated for those changes in estimated prediction probabilities that imply a change from 1 category to another according to the method described by Pencina et al. (30). We used risk categories of <5%, 5% to <10%, and ≥10%, because the present study predicted 5-year CHD risk. To assess the agreements of predicted risks between different models, we plotted scatterplots and calculated unweighted and weighted kappa coefficients (for risk categories) and Spearman's coefficients (for continuous risks).

As a calibration analysis, the mean predicted risk and observed actual risk were compared across quintiles of predicted CHD risk in the subsample study. The significance of difference between predicted and actual risks was tested using the Hosmer-Lemeshow chi-square test. Because the subsample study had relatively low incidence and small numbers of outcome events, we also compared predicted and actual risks by 3 predicted risk categories

(<5%, 5% to <10%, and ≥10%). Statistical analyses were performed using SAS version 9.1 (SAS Institute Inc., Cary, North Carolina) without adjustment for multiple testing.

Results

The incidence of CHD was 3.48 per 1,000 person-years in the entire WHI-HT and 3.67 per 1,000 person-years in the subsample study (data are presented in Online Table 2). Baseline characteristics of the case-control biomarker study are presented separately for patients and controls (Table 1). The proportion of African-American women was not different between patients and controls. Cigarette smoking, physical inactivity, CVD history, treated diabetes, and the use of antihypertensive drugs, antidiabetic drugs, statins, and aspirin were more common in patients, but alcohol intake was more common in controls. TRFs such as systolic blood pressure, TC, HDL-C, smoking, diabetes, and CVD history as well as statin and active hormone treatments were independently associated with CHD. However, alcohol intake, antihy-

Table 1 Baseline Characteristics of the Patients With CHD and Controls

Characteristic	Controls (n = 743), n (%)	Patients (n = 321), n (%)	p Value for Chi-Square Test
African-American ethnicity	81 (10.9)	35 (10.9)	0.9994
Hysterectomy	300 (40.4)	138 (43.0)	0.4265
CVD history at baseline	103 (13.9)	85 (27.2)	<0.0001
Current smoker	64 (8.6)	67 (20.9)	<0.0001
Nondrinker	345 (46.8)	182 (57.4)	0.0016
Physical inactivity	113 (17.4)	65 (23.6)	0.0302
Treated diabetes mellitus	36 (4.9)	57 (17.8)	<0.0001
Antihypertensive drug use	195 (26.2)	128 (39.9)	<0.0001
Antidiabetic drug use	30 (4.0)	44 (13.7)	<0.0001
Statin use	65 (8.8)	49 (15.3)	0.0016
Aspirin use	162 (21.8)	101 (31.5)	0.0008
CEE treatment	147 (19.8)	78 (24.3)	0.0979
CEE + MPA treatment	226 (30.4)	113 (35.2)	0.1241
	Mean ± SD	**Mean ± SD**	**p for *t* Test**
Age (yrs)	66.7 ± 6.7	66.7 ± 6.9	0.9630
BMI (kg/m^2)	28.4 ± 5.6	29.5 ± 5.8	0.0055
SBP (mm Hg)	129.6 ± 17.4	136.5 ± 18.8	<0.0001
DBP (mm Hg)	75.1 ± 9.2	76.6 ± 10.5	0.0205
TC (mg/dl)	226.4 ± 36.9	234.8 ± 38.8	0.0008
HDL-C (mg/dl)	54.9 ± 14.2	50.1 ± 13.6	<0.0001
LDL-C (mg/dl)	141.0 ± 33.5	150.1 ± 33.7	<0.0001
Fibrinogen (mg/dl)	308.2 ± 84.3	331.1 ± 92.4	0.0002
Leukocytes (kcell/ml)	6.0 ± 1.6	6.5 ± 1.9	<0.0001
Platelets (kcell/ml)	248.4 ± 56.8	247.0 ± 59.3	0.7114
Hematocrit (%)	40.5 ± 2.7	40.8 ± 3.0	0.1461
	Median (IQR)	**Median (IQR)**	**p for Wilcoxon's Test**
Triglycerides (mg/dl)	132 (99–182)	150 (113–217)	<0.0001
Lipoprotein(a) (mg/dl)	20 (9–41)	22 (10–46)	0.2465
Fasting glucose (mg/dl)	97 (90–107)	100 (91–119)	<0.0001
Fasting insulin (μIU/ml)	7.6 (4.9–12.1)	9.3 (5.9–14.8)	<0.0001
CRP (μg/ml)	2.2 (1.0–4.8)	3.1 (1.5–6.5)	<0.0001
E-selectin (ng/ml)	44 (32–57)	46 (32–62)	0.1662
Interleukin-6 (pg/ml)	2.8 (2.0–4.1)	3.4 (2.5–5.2)	<0.0001
MMP-9 (ng/ml)	220 (160–305)	235 (166–337)	0.0396
D-dimer (μg/ml)	0.3 (0.2–0.5)	0.4 (0.2–0.7)	<0.0001
Factor VIII (%)	104.5 (73.0–137.0)	119.5 (82.5–158.0)	<0.0001
PAI-1 antigen (ng/ml)	40.0 (21.9–70.3)	47.5 (26.1–77.9)	0.0553
Prothrombin fragment 1.2 (nmol/l)	1.3 (1.1–1.5)	1.4 (1.1–1.7)	0.0426
PAP (nmol/l)	4.5 (3.5–5.7)	4.4 (3.5–6.0)	0.8370
TAFI (μg/ml)	5.1 (3.9–6.4)	5.1 (3.8–6.3)	0.3365
vWF (%)	90 (66–119)	99 (73–139)	<0.0001
Homocysteine (μmol/l)	8.1 (6.6–10.1)	8.5 (7.0–10.8)	0.0129

BMI = body mass index; CEE = conjugated equine estrogen 0.625 mg/day; CHD = coronary heart disease; CRP = C-reactive protein; CVD = cardiovascular disease; DBP = diastolic blood pressure; HDL-C = high-density lipoprotein cholesterol; IQR = interquartile range; LDL-C = low-density lipoprotein cholesterol; MMP = matrix metalloproteinase; MPA = medroxyprogesterone acetate 2.5 mg/day; PAI = plasminogen activator inhibitor; PAP = plasmin-antiplasmin complex; SBP = systolic blood pressure; TAFI = thrombin-activatable fibrinolysis inhibitor; TC = total cholesterol; vWF = von Willebrand factor.

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pertensive treatment, and aspirin use were not significantly associated after adjustment for other risk factors. Among the 18-biomarker panel, only interleukin-6, D-dimer, factor VIII, von Willebrand factor, and homocysteine levels were independently associated with CHD (Table 2).

Figure 1 shows the distribution of predicted CHD risks by 4 different models separately in cases and controls. From the first (FRSO) through the fourth (ABM) models, the difference of predicted risks between cases and controls gradually increased or the discrimination improved, but simultaneously, the distribution of predictive risks also widened even within cases or controls.

Predicted CHD risks using both a continuous scale and risk categories were compared between FRSO and FRSN, between FRSN and TRF, and between TRF and ABM models. Agreement of predicted absolute risks between different prediction models was good (Spearman's coefficient = 0.816, 0.888, and 0.918), but the agreement of risk categories was relatively poor (simple kappa = 0.372, 0.493, and 0.623). The scatterplots with cutoff lines show that the absolute differences were minimal to moderate in most

Table 2 Adjusted ORs for CHD

	Adjusted for Age, SBP, TC, HDL-C, Diabetes, Smoking		Adjusted for Age, SBP, TC, HDL-C, Diabetes, Smoking, Statin and Hormone Treatments, CVD History		Adjusted for Age, SBP, TC, HDL-C, Diabetes, Smoking, Statin and Hormone Treatments (Excluding Women With CVD History)	
Variable	OR (95% CI)	p Value	OR (95% CI)	p Value	OR (95% CI)	p Value
Framingham covariates						
Age (per SD)	1.04 (0.90–1.21)	0.5874	1.00 (0.86–1.16)	0.9754	0.98 (0.82–1.16)	0.7887
BMI (per SD)	1.10 (0.95–1.28)	0.2057	1.07 (0.91–1.25)	0.4034	1.05 (0.88–1.26)	0.5606
Waist (per SD)	1.17 (1.00–1.36)	0.0547	1.13 (0.96–1.33)	0.1304	1.13 (0.94–1.35)	0.1915
SBP (per SD)	1.46 (1.27–1.69)	<0.0001	1.49 (1.29–1.73)	<0.0001	1.54 (1.30–1.83)	<0.0001
TC (per SD)	1.33 (1.15–1.52)	<0.0001	1.40 (1.21–1.62)	<0.0001	1.35 (1.15–1.59)	0.0003
HDL-C (per SD)	0.74 (0.64–0.86)	<0.0001	0.73 (0.63–0.86)	<0.0001	0.73 (0.61–0.87)	0.0005
Diabetes (yes/no)	2.32 (1.60–3.37)	<0.0001	2.09 (1.42–3.08)	0.0002	2.01 (1.27–3.18)	0.0027
Current smoking (yes/no)	3.38 (2.25–5.09)	<0.0001	3.39 (2.23–5.17)	<0.0001	3.58 (2.24–5.71)	<0.0001
Other risk factors (yes/no)						
African American	0.96 (0.61–1.51)	0.8539	0.95 (0.59–1.53)	0.8297	0.97 (0.55–1.72)	0.9119
Physical inactivity	1.24 (0.85–1.81)	0.2594	1.27 (0.87–1.86)	0.2207	1.03 (0.66–1.62)	0.8830
Alcohol intake	0.72 (0.54–0.96)	0.0253	0.75 (0.56–1.02)	0.0629	0.75 (0.53–1.05)	0.0879
CVD at baseline	2.25 (1.58–3.20)	<0.0001	2.03 (1.40–2.93)	0.0002		
Antihypertensive treatment	1.53 (1.12–2.08)	0.0072	1.18 (0.84–1.65)	0.3519	0.99 (0.67–1.47)	0.9610
Statin treatment	1.96 (1.28–3.00)	0.0019	1.68 (1.07–2.63)	0.0238	1.29 (0.70–2.39)	0.4116
Aspirin treatment	1.57 (1.15–2.15)	0.0048	1.28 (0.91–1.80)	0.1582	1.11 (0.74–1.68)	0.6122
CEE treatment	1.44 (1.00–2.07)	0.0480	1.46 (1.01–2.11)	0.0452	1.55 (1.02–2.38)	0.0418
CEE + MPA treatment	1.57 (1.13–2.16)	0.0065	1.62 (1.16–2.26)	0.0046	1.54 (1.06–2.24)	0.0224
Blood biomarkers (per SD)*						
CRP (log)	1.16 (1.00–1.35)	0.0509	1.15 (0.99–1.35)	0.0756	1.18 (0.99–1.41)	0.0652
E-selectin (log)	0.96 (0.82–1.11)	0.5363	0.92 (0.79–1.08)	0.3045	0.95 (0.80–1.13)	0.5642
Interleukin-6 (log)	1.27 (1.10–1.47)	0.0016	1.27 (1.09–1.48)	0.0025	1.25 (1.05–1.48)	0.0129
MMP-9 (log)	1.06 (0.92–1.22)	0.4599	1.03 (0.89–1.19)	0.7248	1.06 (0.89–1.25)	0.5231
Leukocytes	1.10 (0.95–1.27)	0.2208	1.09 (0.94–1.27)	0.2441	1.09 (0.92–1.30)	0.3076
D-dimer (log)	1.32 (1.14–1.54)	0.0003	1.33 (1.14–1.56)	0.0003	1.20 (1.01–1.44)	0.0444
Factor VIII (log)	1.30 (1.12–1.52)	0.0006	1.30 (1.11–1.52)	0.0014	1.17 (0.98–1.39)	0.0790
PAI-1 antigen (log)	0.97 (0.83–1.13)	0.6604	0.96 (0.82–1.13)	0.6221	1.00 (0.83–1.20)	0.9805
Prothrombin fragment 1.2 (log)	1.05 (0.92–1.21)	0.4637	1.06 (0.92–1.23)	0.4095	0.95 (0.80–1.13)	0.5575
PAP (log)	1.12 (0.96–1.31)	0.1401	1.13 (0.97–1.33)	0.1226	1.16 (0.97–1.38)	0.1140
TAFI (log)	0.91 (0.79–1.04)	0.1608	0.90 (0.78–1.04)	0.1612	0.89 (0.75–1.05)	0.1510
vWF (log)	1.26 (1.09–1.46)	0.0014	1.29 (1.11–1.49)	0.0008	1.21 (1.02–1.43)	0.0257
Fibrinogen	1.17 (1.01–1.34)	0.0331	1.11 (0.96–1.28)	0.1643	1.13 (0.96–1.33)	0.1436
Platelets	0.97 (0.84–1.12)	0.6837	0.96 (0.82–1.11)	0.5378	1.00 (0.85–1.19)	0.9667
Hematocrit	0.94 (0.81–1.08)	0.3659	0.94 (0.81–1.09)	0.4410	0.97 (0.82–1.15)	0.7134
Lipoprotein(a) (log)	1.05 (0.91–1.22)	0.4785	1.06 (0.91–1.23)	0.4625	1.01 (0.86–1.20)	0.8705
Homocysteine (log)	1.19 (1.04–1.37)	0.0130	1.20 (1.04–1.38)	0.0117	1.18 (1.00–1.39)	0.0539
Fasting insulin (log)	1.17 (0.99–1.39)	0.0658	1.10 (0.92–1.31)	0.3047	1.09 (0.90–1.34)	0.3787

*Each biomarker was assessed separately.

CI = confidence interval; OR = odds ratio; other abbreviations as in Table 1.

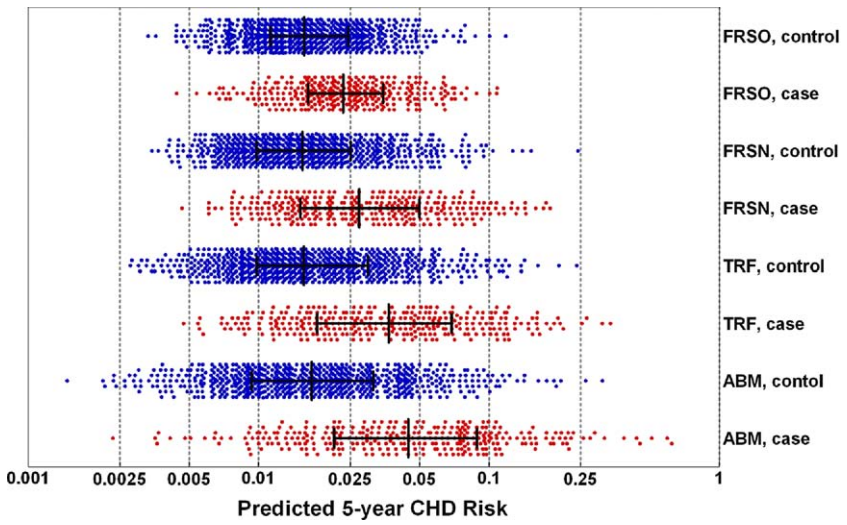

Figure 1 **Discrimination Between Cases and Controls by Different Prediction Models**

Blue and red dots indicate predicted coronary heart disease (CHD) risk in controls and cases, respectively, and **black bars** indicate medians and interquartile ranges. C-statistics were 0.679 (95% confidence interval [CI]: 0.645 to 0.714) for the Framingham risk score with original coefficients (FRSO) model, 0.699 (95% CI: 0.665 to 0.734) for the Framingham risk score with new coefficients (FRSN) model, 0.729 (95% CI: 0.695 to 0.762) for the traditional risk factor (TRF) model, and 0.751 (95% CI: 0.718 to 0.784) for additional biomarker (ABM) model.

instances, even if they were classified into different risk categories (Fig. 2).

Table 3 summarizes various indexes for discrimination, reclassification, improved discrimination, and calibration in the nested case-control dataset and the subsample dataset. The ABM model could not be validated in the subsample study, because ABMs were available only for the case-control study. We did not exclude women with CVD histories at baseline, but we repeated the analysis in the subgroup of women without histories of CVD. Overall, the model coefficients for risk factors were similar, but the improvement and reclassification by ABMs in this subgroup were smaller than those in all eligible participants. When we included 5 newer biomarkers that we found to be significantly associated with CHD in addition to the TRFs, NRI was 8.07% in the case-control sample and 6.45% in the subgroup that was free of CVD at baseline (more data are presented in Online Table 3). Among the CVD-free subgroup, 7 women who developed CHD were reclassified from lower or intermediate-risk groups (5-year CHD risk $<$10%) to the higher risk group ($\geq$10%). When we included women with CVD histories, 20 women were reclassified similarly. The Hosmer-Lemeshow chi-square test by risk quintiles and 3 risk categories did not show a significant difference between predicted risk and actual risk (data are presented in Online Table 4). This indicates good calibration.

Because log CRP was associated with CHD with borderline significance ($p = 0.077$), we also assessed a CHD prediction model that included CRP as well as the 5 significant biomarkers. However, the 5-biomarker model and the CRP-added 6-biomarker model were almost identical (p value for the difference of C-statistic = 0.520, Spearman's coefficient for absolute risk = 0.999, kappa for risk category = 0.967). CRP levels were also analyzed in linear, log-linear, quadratic, and dichotomous forms, but CRP in any form was not significantly associated with CHD after adjustment for TRFs (data are presented in Online Table 5). We also evaluated 18 separate risk prediction models, which included a single biomarker in addition to the TRFs. Only the D-dimer-included model had significantly better discriminative power ($p = 0.042$) than the TRF model (Table 4). The association between D-dimer and CHD risk was independent from other cardiovascular risk factors and medication use. However, in a subgroup analysis by the hormone treatment assignments, the association between D-dimer and CHD was marginally stronger among the women who received active CEE than among those who received CEE placebo (adjusted odds ratio: 1.69 vs. 1.27; p value for interaction = 0.097), but results for the CEE + MPA and CEE + MPA placebo groups were similar (adjusted odds ratio: 1.35 vs. 1.23; p value for interaction = 0.688).

Discussion

We investigated the potential usefulness of traditional cardiovascular risk factors and 18 ABMs for CHD prediction among post-menopausal women age 50 to 79 years. The addition of CVD history and medication use to the Framingham risk score covariates increased the model C-statistic from 0.699 to 0.729. With addition of 5 inflammatory and hemostatic biomarkers, the C-statistic further increased to 0.751, and the corresponding NRI was 6.45%

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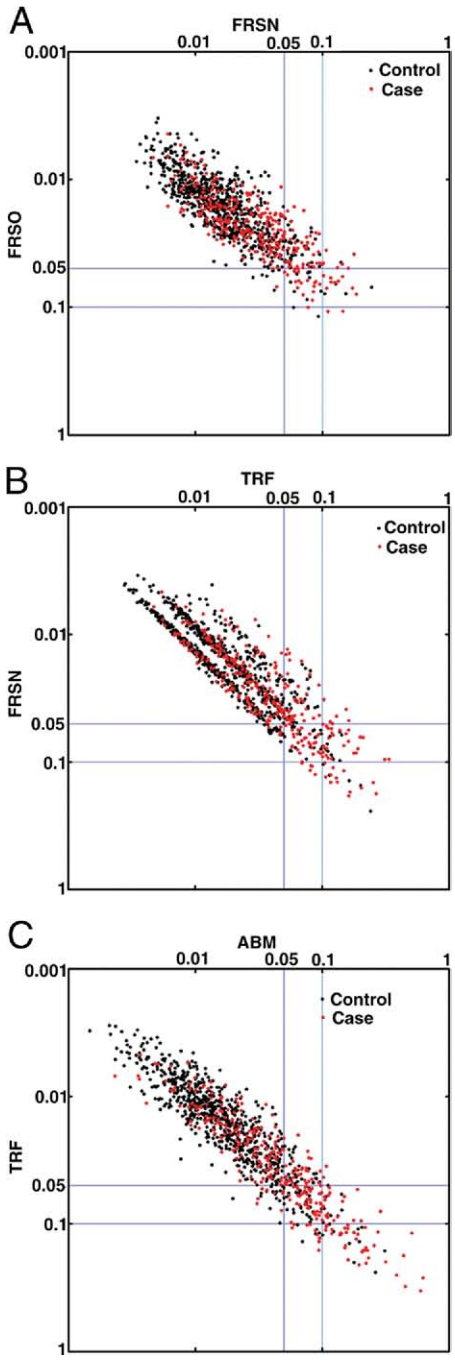

Figure 2 **Comparisons of Predicted 5-Year CHD Risk Between Different Prediction Models**

(A) Risks predicted by FRSO and FRSN models: simple kappa = 0.372 (95% CI: 0.291 to 0.452); weighted kappa = 0.419 (95% CI: 0.340 to 0.497); Spearman's coefficient = 0.816 ($p < 0.0001$). **(B)** Risks predicted by the FRSN and TRF models: simple kappa = 0.493 (95% CI: 0.432 to 0.555); weighted kappa = 0.579 (95% CI: 0.522 to 0.636); Spearman's coefficient = 0.888 ($p < 0.0001$). **(C)** Risks predicted by the TRF and ABM models: simple kappa = 0.623 (95% CI: 0.569 to 0.676); weighted kappa = 0.709 (95% CI: 0.664 to 0.755); Spearman's coefficient = 0.918 ($p < 0.0001$). **Black and red dots** indicate controls and cases, respectively. A logarithmic scale is used on both axes. CHD = coronary heart disease; other abbreviations as in Figure 1.

in women who were free of CVD at baseline. However, our data also suggest that improved model discrimination does not guarantee a better risk stratification for individual women, because as the number of predictors increased, the overlap between the 2 distributions did not diminish as expected from the separation of the means (31).

One strategy that has been proposed to improve on the limitations of individual biomarkers is to combine multiple biomarkers into an integrated score or algorithm. But the effects of multiple biomarkers in addition to the TRFs have been nonsignificant or minimal in many studies. In the Framingham Heart Study, a multimarker score (combining B-type natriuretic propeptide, CRP, urinary albumin/creatinine ratio, homocysteine, and renin) moderately improved the C-statistic by 0.02 in CVD death prediction and by 0.01 in CVD event prediction (13). In the Cardiovascular Health Study, the addition of 6 biomarkers (CRP, fibrinogen, factor VIIIc, interleukin-6, lipoprotein[a], and hemoglobin) did not improve discrimination beyond established risk factors among subjects with (difference = 0.01, $p = 0.15$) or without (difference = 0.01, $p = 0.72$) chronic kidney disease (32). In the Quebec Cardiovascular Study, an inflammation score based on interleukin-6 and fibrinogen levels moderately improved C-statistics (difference = 0.008, $p = 0.03$) for a CHD prediction model (33). Ridker et al. (15,34) proposed the "Reynolds risk score," which included CRP, glycosylated hemoglobin (in women), and parental history of myocardial infarction in women (15) and men (34). The incremental C-statistic was 0.017 compared with the risk predicted by Framingham covariates and 0.003 when compared with the risk predicted by Adult Treatment Panel III covariates. Including more biomarkers, such as apolipoprotein A-I, apolipoprotein B-100, and lipoprotein(a), did not improve the C-statistic further (15). In a Swedish cohort study, the addition of multiple biomarkers improved the C-statistic for CVD prediction by 0.007 ($p = 0.04$) and for CHD prediction by 0.009 ($p = 0.08$) (35). In the present study among post-menopausal women, 5 ABMs improved the C-statistic for CHD prediction by 0.022 ($p = 0.001$) in all women and by 0.016 ($p = 0.027$) in a subgroup without CVD history. In contrast, in an elderly male cohort study (the Uppsala Longitudinal Study of Adult Men), the C-statistic for CVD death prediction increased by 0.11 ($p < 0.001$) when 4 markers (troponin I, N-terminal pro-brain natriuretic peptide, cystatin C, and CRP) were added to established markers in all participants and by 0.06 ($p = 0.03$) in the subgroup that was free of CVD at baseline (16). This large improvement in the Uppsala study might be explained at least in part by the fact that the investigators included cystatin C, troponins, and pro-brain natriuretic peptide, which reflect existing cardiac or renal damage (16,36).

In the present study, among the 5 inflammatory markers evaluated, individually, CRP, interleukin-6, and matrix metalloproteinase-9 levels and leukocyte count (but not E-selectin) were positively associated with CHD. However,

Table 3 Summary of Model Evaluation

Variable	FRSO	FRSN	TRF	ABM
Discrimination in the case-control sample (n = 1,064)				
Yates slope	0.0086	0.0173	0.0283	0.0442
Brier score	0.0131	0.0131	0.0129	0.0129
C-statistic	0.6793	0.6993 (p = 0.081*)	0.7285 (p = 0.001†)	0.7510 (p = 0.001‡)
Discrimination in the CVD-free case-control sample (n = 864)				
Yates slope	0.0072	0.0166	0.0183	0.0238
Brier score	0.0108	0.0109	0.0109	0.0111
C-statistic	0.6774	0.7067 (p = 0.029*)	0.7148 (p = 0.171†)	0.7308 (p = 0.027‡)
Calibration in the 8.6% random sample (n = 1,939)				
Hosmer-Lemeshow chi-square for quintile risk groups	1.7409 (p = 0.628)	3.1228 (p = 0.373)	3.3755 (p = 0.337)	
Hosmer-Lemeshow chi-square for 3 risk categories	2.4016 (p = 0.121)	1.8234 (p = 0.339)	2.8056 (p = 0.094)	
		FRSO to FRSN	**FRSN to TRF**	**TRF to ABM**
Reclassification in the CVD-free case-control sample				
Patients moved to higher		34/228 (14.9%)	19/228 (8.3%)	26/224 (11.6%)
Patients moved to lower		2/228 (0.9%)	4/228 (1.8%)	9/224 (4.0%)
Controls moved to higher		23/636 (3.6%)	10/636 (1.6%)	12/614 (2.0%)
Controls moved to lower		3/636 (0.5%)	9/636 (1.4%)	5/614 (0.8%)
Net reclassification improvement		10.89%	6.42%	6.45%
Integrated discrimination improvement		0.0094 (p < 0.001)	0.0017 (p = 0.015)	0.0055 (p < 0.001)

*Compared with FRSO model. †Compared with FRSN model. ‡Compared with TRF model.
ABM = additional biomarker; CVD = cardiovascular disease; FRSN = Framingham risk score with new coefficients; FRSO = Framingham risk score with original coefficients; TRF = traditional risk factor.

only interleukin-6 was significantly associated with CHD after adjustment for traditional cardiovascular risk factors and medication use. Model discrimination was not significantly improved by any of these 5 inflammatory markers. CRP has been most frequently studied as a potential biomarker that can improve CHD risk prediction in women (37–40) or in both sexes (8,14,41,42), but the predictive effects of including CRP have been inconsistent. In the Women's Health Study, CRP has been strongly associated with the risk for CVD and CHD and also with improved

Table 4 Effects of Adding Single Biomarkers to the TRF Model

	Association With CHD*		Improvement of C-Statistic†	
Biomarker	Coefficients per 1 SD	p Value	Difference	p Value
Inflammation				
CRP (log)	0.1413	0.0767	0.0032	0.2962
E-selectin (log)	−0.0763	0.3197	0.0027	0.3355
Interleukin-6 (log)	0.2337	0.0027	0.0060	0.2156
MMP-9 (log)	0.0265	0.7245	0.0000	0.9507
Leukocytes	0.0898	0.2455	0.0003	0.8543
Hemostasis				
D-dimer (log)	0.2713	0.0004	0.0099	0.0423
Factor VIII (log)	0.2533	0.0016	0.0087	0.0855
PAI-1 antigen (log)	−0.0422	0.5981	0.0006	0.7581
Prothrombin fragment 1.2 (log)	0.0622	0.3973	0.0009	0.6855
PAP (log)	0.1241	0.1158	0.0019	0.5204
TAFI (log)	−0.1028	0.1612	0.0019	0.3895
vWF (log)	0.2455	0.0010	0.0090	0.0503
Fibrinogen	0.1035	0.1651	0.0023	0.3103
Platelets	−0.0451	0.5430	0.0003	0.7400
Hematocrit	−0.0584	0.4394	0.0009	0.4959
Others				
Lipoprotein(a) (log)	0.0563	0.4625	0.0006	0.7210
Homocysteine (log)	0.1802	0.0122	0.0049	0.2139
Insulin (log)	0.0905	0.3101	0.0007	0.7354

*Adjusted for SBP, TC, HDL-C, diabetes, smoking, CVD history, statin treatment, and hormone treatment. †Compared with traditional risk factor (TRF) model (C-statistic = 0.729).
Abbreviations as in Table 1.

disease prediction (37,38,43). A nested case-control analysis in the Nurses' Health Study and the Health Professional Follow-Up Study observed that CRP was significantly associated with CHD risk after adjustment for metabolic disorders in men but not in women (40). In the British Women's Heart and Health Study, CRP was not significantly associated with either CHD or CVD, and it did not improve discrimination (39). In the Women's Health Study, CRP levels ≥3 mg/l were significantly associated with CVD risk independent of metabolic abnormalities (37). However, that relationship was not observed in the nested case-control study in the Nurses' Health Study (40). In our present analysis in the WHI-HT, CRP was not significantly associated with CHD after adjustment for TRFs and did not improve the discriminative power of CHD prediction. A recent study by Shah et al. (44), which consisted of a new analysis of 2 prospective cohorts and a systematic review of 31 published prospective studies, found that while CRP is consistently associated with CHD risk, measurement of CRP provides more limited information for risk prediction than tests of association alone might suggest. A large case-control study in Denmark observed that genetic variants that are strongly associated with lifelong increases of CRP levels are not associated with ischemic heart disease or stroke (45). This finding suggests that increased CRP levels may not be causally related with CHD.

Interleukin-6 is another frequently studied biomarker as a potential predictor of CHD (46–49). Interleukin-6 is known to stimulate hepatic synthesis of acute phase reactants such as CRP and fibrinogen and also to be associated with atherosclerosis and arterial thrombosis (50–52). In our analysis, interleukin-6 was the only inflammatory marker that was independently associated with CHD, but interleukin-6 alone did not improve CHD prediction. Although interleukin-6 has been associated with CHD in some observational studies, its causality remains unclear (49). The British Women's Health and Heart Study observed that interleukin-6 was not associated with CHD after adjusting for established risk factors, and cigarette smoking and lung function (forced expiratory volume) were the main confounders of the association of interleukin-6 and CHD (48). E-selectin, matrix metalloproteinase-9, and leukocyte count have also been associated with CHD risk in other studies, but their independent relationships and additional predictive values are uncertain (53–58).

There is increasing evidence supporting an important role for the hemostatic system in atherosclerotic vascular disease, and abnormal coagulation and fibrinolysis are associated with risk for CHD. Various hemostatic variables have been associated with CHD risk, even after adjustment for TRFs, in prospective studies and meta-analyses (10,14,18,47,59–64), but their causality and predictive power are unclear. In the present study, elevated plasma levels of D-dimer, factor VIII, von Willebrand factor, and fibrinogen were significantly associated with CHD after adjustment for TRFs, and D-dimer, factor VIII, and von Willebrand factor were significant after additional adjustment for medication uses and CVD history. These hemostatic variables have been associated with CHD risk in previous reports (10,14,47,60–62,64,65), and D-dimer has been the strongest factor in some studies (47,64–66). Among the 18 biomarkers analyzed in the present study, only D-dimer significantly increased the model discrimination ($p = 0.042$). The association between D-dimer and CHD risk was independent from other risk factors, but the association was marginally stronger in the active CEE treatment group than in the CEE placebo group. The potential interaction between the D-dimer concentration and hormone treatment needs to be further investigated.

The WHI-HT biomarker study included other biomarkers, such as lipoprotein(a), homocysteine, and fasting insulin levels, which were also associated with CHD in meta-analyses (67–69). Homocysteine level was positively associated with CHD, even after adjustment for TRFs, but did not significantly improve CHD prediction. Observational studies (8,13,14,33,70) and hypothetical analyses (31,71,72) have shown that biomarkers' contributions to a disease prediction might be limited despite their significant associations with the disease.

Study limitations. Some limitations of this study need to be considered. First, the nested case-control design has some disadvantages compared with a cohort study, which is generally preferred for the assessment of risk prediction model. The calibration of a risk prediction model is not possible in a case-control study. Thus, calibration analyses were performed in the random subsample study, which has a prospective cohort design. Risk stratification results should be interpreted with caution in a case-control dataset, because the risk for event or the proportion of cases is artificially fixed. Thus, the distribution of CHD risk should be calculated separately for cases and controls (Online Table 3), and the risk distribution in the combined subjects are different from that in the population (73). The coefficients for individual predictors were estimated by logistic regression analysis in the nested case-control study. Coefficients are assumed to be close between logistic regression analysis and Cox's hazard regression analysis when the disease incidence is low and the risk ratios are constant over time. We also compared the risk ratio from the Framingham studies and odds ratio from the WHI-HT; they were quite similar except for smoking and diabetes, for which the measurements were different between the 2 studies (Online Table 6).

Second, matching on age did not allow estimation of the coefficient for age, so we could not include age as a predictor variable except for the model with the original Framingham risk score coefficients. Thus, the models in this study cannot be directly compared with other age-included prediction models, nor can the study findings be extrapolated to women in other age groups. Even without including age, the predictive models showed acceptable discriminative power (74), presumably be-

cause the study participants have a narrow age range (50 to 79 years) and the coefficients were estimated independently from age. The age-matching might decrease the observed C-statistic for established risk factors and inflate the increments by ABMs.

Third, we did not measure other biomarkers, such as troponins, B-type natriuretic peptide, N-terminal pro-brain natriuretic peptide, cystatin C, and renin, which have been recently reported to improve CVD prediction. Many of those newer biomarkers reflect existing cardiac or renal damage (36) and are more useful in prognostic prediction rather than in risk prediction (75,76).

Fourth, we predicted CHD risk for 5 years, which prevents comparing our results directly with those of other studies with 10-year risk prediction. We also used risk categories of <5%, 5% to 10%, and ≥10% for 5-year CHD risk, instead of <10%, 10% to <19%, and ≥20% for 10-year CHD risk. Previous WHI-HT studies observed that CHD rate was constant at least until 8 years of follow-up (21,23).

Fifth, the validation dataset (8.6% random subsample) was selected from the same population in which the patients and controls were identified. Thus, the calibration results are likely optimistic indicators of what would be found in a completely unrelated population. In addition, the random subsample study measured only a part of biomarkers. Thus calibration analysis of ABM model was unavailable.

Conclusions

In this multimarker cardiovascular risk prediction study, we found modest improvement in CHD risk prediction when 18 biomarkers were evaluated individually and in multimarker predictive models along with traditional cardiovascular risk factors. Women who were reclassified with 5 ABMs from lower or intermediate-risk groups to the higher risk group and who developed CHD constituted <0.1% of the population. We did not find CRP to add significantly to risk prediction in the multimarker model. Our findings, when taken in the context of rapidly expanding research on biomarkers and CHD risk, confirm that the majority of risk prediction content emanates from TRFs and that the ABMs studied here, even when taken together, improve risk prediction only moderately. The hope that existed a few years ago that newer biomarkers could vastly improve cardiovascular risk prediction has not materialized at this time. In addition, this study confirms that newer biomarkers are quite inconsistent from study to study in their ability to improve risk prediction models. This fact also reinforces the ongoing value of the TRFs as the mainstays of CHD risk prediction.

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Key Words: coronary heart disease ■ prediction ■ biomarker.

APPENDIX

For a list of the WHI Investigators, Clinical Coordinating Centers, and Clinical Centers as well as supplemental information and tables, please see the online version of this article.