Original Article

Clinically Significant Novel Biomarkers for Prediction of First Ever Myocardial Infarction

The Tromsø Study

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Background—Identification of individuals with high risk for first-ever myocardial infarction (MI) can be improved. The objectives of the study were to survey multiple protein biomarkers for association with the 10-year risk of incident MI and identify a clinically significant risk model that adds information to current common risk models.

Methods and Results—We used an immunoassay platform that uses a sensitive, sample-efficient molecular counting technology to measure 51 proteins in samples from the fourth survey (1994) in the Tromsø Study, a longitudinal study of men and women in Tromsø, Norway. A case control design was used with 419 first-ever MI cases (169 females/250 males) and 398 controls (244 females/154 males). Of the proteins measured, 17 were predictors of MI when considered individually after adjustment for traditional risk factors either in men, women, or both. The 6 biomarkers adjusted for traditional risk factors that were selected in a multivariable model (odds ratios [OR] per standard deviation) using a stepwise procedure were apolipoprotein B/apolipoprotein A1 ratio (1.40), kallikrein (0.73), lipoprotein a (1.29), matrix metalloproteinase 9 (1.30), the interaction term IP-10/CXCL10×women (0.69), and the interaction term thrombospondin 4×men (1.38). The composite risk of these biomarkers added significantly to the traditional risk factor model with a net reclassification improvement of 14% (P=0.0002), whereas the receiver operating characteristic area increased from 0.757 to 0.791, P=0.0004.

Conclusions—Novel protein biomarker models improve identification of 10-year MI risk above and beyond traditional risk factors with 14% better allocation to either high or low risk group. (***Circ Cardiovasc Genet*. 2015;8:363-371. DOI: 10.1161/CIRCGENETICS.113.000630.)**

Key Words: biomarker ■ cardiovascular disease ■ epidemiology ■ follow-up study ■ myocardial infarction

Several predictive models are currently being used for risk stratification and clinical decision-making in cardiovascular medicine and primary healthcare.[1,2] Most models are based on the traditional cardiovascular risk factors (TRF), that is, age, sex, blood pressure, total cholesterol, high-density lipoprotein cholesterol, and smoking status, and with the estimated 10-year risk of either cardiovascular mortality or event rate as outcome. However, it is evident that the traditional risk factors do not adequately reflect all cardiovascular risk because the majority of individuals who experience a first time cardiovascular event have adverse levels in <2 traditional risk factors and are misidentified as being at low risk.[3] Both the successes and shortcomings of the traditional risk factors have stimulated research into identifying additional biomarkers, that is, biological signals, which can be used to improve on current cardiovascular disease risk models, or are indicators of progressive subclinical disease and, as such, would have utility in predicting cardiovascular event risk, improve on traditional predictive models, and lead to more accurate treatment decisions. Blood-based biomarkers that can be easily integrated into patient management in the primary care setting are particularly desirable. Of the <60 different proteins screened to date, only 3, C-reactive protein (CRP), N-terminal prohormone of brain natriuretic peptide, and cardiac troponin I, have been shown, in combination only, to add incremental value to TRF-based predictive models of first-time CVD.[4] However, their clinical utility in preventive cardiology has not been clearly established. CRP, like other acute phase proteins, such as fibrinogen, is widely recognized to be a marker of a general inflammatory state that contributes to cardiovascular

Clinical Perspective on p 371

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disease, but its role in a specific causal pathway has yet to be defined. The use of cardiac troponin I has revealed a high prognostic potential of low troponin concentrations, but their clinical value in risk prediction has not been established.[5] On the other hand, elevated blood levels of N-terminal prohormone of brain natriuretic peptide indicate that individuals already have cardiovascular disease, and yet do not, as individual biomarkers, add any information beyond the TRFs in terms of unaccounted risk or new risk associated with unrecognized events.

Assessment of the clinical utility of these and other novel biomarkers across populations is complicated by differences in study sample characteristics and, perhaps most importantly, by the definition of the outcome. Using a composite end point, such as ischemic stroke, myocardial infarction (MI), coronary ischemia requiring revascularization, heart failure, and cardiovascular death, may increase the power of the study to detect signal, but can result in a loss of information from biomarkers associated with specific disease pathogenesis.

To address these issues, we applied a high throughput, microfluidics immunoassay platform[6] for discovery and verification of protein biomarkers associated with specific CVDs using study sera from the large population-based Tromsø Study in Norway.[7] The Tromsø Study provides a unique opportunity combining these 2 activities because of a high participation rate, the comprehensiveness of the study's clinical examination and metadata, and the relatively high incidence of adjudicated hard coronary heart disease events (myocardial infarction and sudden cardiovascular death). The Tromsø Study provides an unusual opportunity to compare differences in biomarker profiles between males and females without the confounding effects introduced by using a composite end point.

The objective of the present study is to identify blood-based protein markers that add significantly to the prediction of incident 10-year MI adjusted for traditional risk factors and to select a multivariable biomarker model that improves model fit, discrimination, and reclassification beyond that of the traditional risk factor model in nondiabetic men and women.

Methods and Materials

Study Population

Serum samples were drawn from a subset of participants in the fourth survey of the Tromsø Study (1994–1995). The Tromsø Study is a single-center prospective, population-based study with repeated health surveys of officially registered inhabitants in the municipality of Tromsø, Norway.[7] Eligible for the present study were all women aged 50 to 74 years and men aged 55 to 74 years and 5% to 10% samples of other subjects aged 25 to 85 years with valid written consent (n=7895). The Tromsø Study was approved by the regional committee for medical research ethics.

Cases and controls were drawn from the Tromsø cohort to form a traditional case–control study. Cases were defined as all participants with no previous MI, ischemic stroke, coronary artery bypass grafting, percutaneous coronary intervention, or self-reported angina at baseline and who experienced a first-ever MI (n=419) within 10 years of follow-up. Controls (n=398) were randomly selected from the entire group of participants completing 10-year follow-up without an event of interest and using the same inclusion criteria as for the cases. We excluded subjects with self-reported diabetes mellitus or who had nonfasting glucose level ≥200 mg/dL or HbA_{1c} ≥6.5% at baseline (42 cases and 8 controls).

End Point Assessment

Incident cardiovascular events and mortality among the participants were recorded from the date of enrollment in 1994–1995 through to the end of follow-up, 31 December 2005. Adjudication of hospitalized and out-of hospital events was performed by an independent end point committee based on data from hospital and out-of hospital journals, autopsy records, and death certificates. The Norwegian national 11-digit identification number allowed linkage to national and local diagnosis registries. Cases of incident MI were identified by linkage to the discharge diagnosis registry at the University Hospital of North Norway with search for ICD 9 codes 410 to 414, 798, and 799 in the period 1994–1998 and thereafter ICD 10 codes I20–I25, R96, R98, and R99. University Hospital of North Norway is the only hospital in the area serving the Tromsø population. Modified WHO MONICA/MORGAM criteria for MI were used and included clinical symptoms and signs, findings in electrocardiograms, values of cardiac biomarkers, and autopsy reports when applicable (http://www.ktl.fi/publications/morgam/manual/followup/form22.htm). Linkage to the National Causes of Death Registry at Statistics Norway allowed identification of fatal incident cases of MI that occurred as out-of-hospital deaths, including deaths that occurred outside of Tromsø, as well as information on all-cause mortality. Information from the death certificates was used to collect relevant information of the event from additional sources, such as autopsy reports and records from nursing homes, ambulance services, and general practitioners. The Norwegian Registry of Vital Statistics provided information on emigration and death.

Data From the Baseline Clinical Examination

Information about smoking habits, prevalent diabetes mellitus, angina pectoris, previous MI, stroke, and use of antihypertensive and lipid-lowering drugs was collected from self-administered questionnaires. The baseline examination comprised 2 visits with an interval of 4 to 12 weeks. At each visit, standardized measurements of height and weight were taken, nonfasting blood samples were collected, and specially trained personnel recorded blood pressure with an automatic device (Dinamap Vital Signs Monitor, Tampa, Fla). Three readings were recorded with 1-minute intervals, and the average of the final 2 readings from each visit was used in the analyses. The nonfasting blood samples were collected from an antecubital vein and serum prepared by centrifugation after 1 hour respite at room temperature. Serum total cholesterol and triglycerides were analyzed by enzymatic colorimetric methods with commercial kits (CHOD-PAP for total cholesterol and GPO-PAP for triglycerides; Boehringer-Mannheim). Serum high-density lipoprotein cholesterol was measured after the precipitation of lower-density lipoprotein with heparin and manganese chloride. The average of the serum lipid values from the 2 visits was used in analyses. Plasma glucose was measured by a hexokinase method. HbA_{1c} was measured by an immuno-turbidimetric method on a COBAS Mira Plus Chemistry Analyzer (Roche Diagnostics, Basel, Switzerland) with reagents from the same company. Blood analyses were performed by the Department of Clinical Chemistry, University Hospital North-Norway, Tromsø.

Candidate Protein Biomarker Selection

A literature search for proteins associated with cardiovascular disease generated a list of >900 potential biomarker candidates. Candidate proteins were prioritized according to their association with the pathophysiology of coronary heart disease and atherogenesis, including lipid/sterol trafficking, endothelial activation, vascular remodeling, foam cell development, plaque destabilization, inflammation/infection, vascular tone and hypertension, thrombosis/fibrinolysis, platelet activation, and lipid oxidation. Of the 165 prioritized markers, availability of reagents—capture and detection antibodies and standard analyte—and successful development of assays further reduced the number of screening candidates to 59. Data analyses included results from the 51 assays whose performance characteristics met internal quality standards (see Methods and Materials in the Data Supplement and Tables I and II in the Data Supplement).

Assay Development and Data Production Runs

Serum processing, assay development, and assay production runs were performed at a single site with dedicated staff and equipment. Individual sandwich-format immunoassays were developed for data production on a research platform that integrates an automated assay plate processing system with the molecular counting technology of the Erenna™ System. This approach to biomarker discovery as well as the molecular counting technology detection technology has been described previously.[6,8,9] The campaign approach to assay development and production is described in detail in the Data Supplement. Briefly, capture and detection antibodies and standard analytes for each target protein were acquired from commercial sources (Table I in the Data Supplement) and assay conditions, such as serum dilution buffer, serum dilution factor, and concentrations of capture and detection antibodies, were optimized to generate a standard curve within the biological range for each assay. Only assay reagents and protocols that met minimum criteria for sensitivity, specificity, and dynamic range were used in the data production runs (see Methods and Materials in the Data Supplement).

Statistical Analysis

Data Preprocessing

All statistical analyses were performed using STATA version 13.0 (Stata corporation, College station, TX, USA) or SAS software 9.4 (SAS Institute Inc., Cary, NC, USA). Skewed numeric variables were transformed (log10, square root) to approximate a normal distribution. Less than 5% of values were missing for all but 3 of 51 production assays, which all had <9% missing. Data were assumed to be missing at random, and the ICE command in STATA was used to impute 20 data sets. Rubin's rule was used to combine the results for the imputed data sets.

Association of Individual Markers With MI

We used logistic regression models to assess the association between each protein and MI with and without sex interaction, alone and adjusted for the traditional risk factors, and the interaction terms age×sex and blood pressure×blood pressure medication. All risk factors were included as continuous variables in the models, except for the binary variables sex, smoking, and blood pressure medication. OR were reported per 1 standard

Table 1. Baseline Characteristics.* The Tromsø Study

	Women			Men		
Characteristic	Cases, n=169	Controls, n=244	*P* Value	Cases, n=250	Controls, n=154	*P* Value
Age, y	65.6 (7.2)	59.6 (8.4)	<0.001	63.3 (8.2)	59.3 (8.8)	<0.001
Systolic blood pressure, mm Hg	151.3 (25.5)	137.1 (21.8)	<0.001	146.4 (19.5)	139.3 (20.7)	<0.001
Diastolic blood pressure, mm Hg	84.3 (12.8)	78.8 (12.2)	<0.001	84.8 (11.4)	81.6 (11.6)	0.006
Smoking, n (%)	77 (45.6)	90 (36.9)	0.077	115 (46.0)	59 (38.3)	0.13
Blood pressure medication, n (%)	23 (13.6)	20 (8.2)	0.077	31 (12.5)	6 (3.9)	0.004
Total cholesterol, mmol/L	7.39 (1.37)	6.81 (1.16)	<0.001	6.80 (1.13)	6.57 (1.15)	0.049
HDL C, mmol/L	1.61 (0.46)	1.68 (0.44)	0.12	1.35 (0.39)	1.43 (0.39)	0.065
Lipid medication, n (%)	4 (2.4)	2 (0.8)	0.20	3 (1.2)	3 (1.2)	0.55
HbA1c, %	5.48 (0.34)	5.40 (0.32)	0.012	5.42 (0.38)	5.35 (0.35)	0.075
BMI, kg/m^2	26.4 (4.7)	25.5 (4.4)	0.048	26.6 (3.5)	25.7 (3.2)	0.007
Apolipoprotein B 100, μg/mL	16.9 (11.4–26.3)	13.6 (9.6–21.4)	0.012	18.3 (13.0–27.0)	14.6 (9.9–21.8)	0.002
ApoBApoA1	0.009 (0.006–0.017)	0.007 (0.005–0.012)	0.007	0.011 (0.008–0.018)	0.009 (0.005–0.015)	<0.001
Complement C3, mg/mL	213.6 (167.4–275.7)	229.5 (176.7–294.8)	0.10	223.6 (165.4–280.8)	210.1 (165.6–264.3)	0.35
Complement C3B, μg/mL	2.76 (2.14–3.48)	2.92 (2.33–3.46)	0.093	2.75 (2.23–3.35)	2.58 (2.09–3.10)	0.18
Carboxypeptidase B2, μg/mL	27.3 (23.9- 31.0)	26.6 (23.4- 29.9)	0.46	26.8 (22.7- 31.9)	24.8 (21.8- 29.2)	0.030
C-reactive protein, ng/mL	81.2 (32.2–174.5)	46.7 (21.0–104.4)	0.027	84.8 (40.7–186.2)	56.0 (27.2–131.4)	0.84
IP-10, ng/mL	0.036 (0.029–0.049)	0.036 (0.028–0.047)	0.73	0.035 (0.028–0.047)	0.033 (0.027–0.041)	0.47
Heat shock protein 70, ng/mL	2.97 (2.06–5.34)	2.41 (1.84–3.76)	0.001	3.36 (2.33–5.65)	2.97 (2.21–4.20)	0.13
Kallikrein, plasma, μg/mL	29.1 (25.0–34.5)	30.0 (24.8–35.2)	0.25	26.7 (22.6–32.8)	28.8 (24.0–33.3)	0.16
Lipoprotein (a), ng/mL	160.2 (84.3–496.2)	135.2 (67.1–341.5)	0.034	150.6 (64.4–513.7)	101.1 (54.9–189.4)	0.002
Matrix metalloproteinase 3, ng/mL	7.4 (5.6- 9.8)	6.7 (5.2- 9.1)	0.18	12.1 (8.9- 17.2)	11.1 (8.6- 15.1)	0.022
Matrix metalloproteinase 8, ng/mL	11.5 (7.8–18.0)	9.0 (6.4–15.3)	<0.001	14.8 (9.2–22.5)	11.6 (7.5–19.0)	0.007
Matrix metalloproteinase 9, ng/mL	406.2 (290.0–596.8)	355.8 (241.4–480.9)	<0.001	487.9 (340.4–662.7)	382.9 (268.0–545.7)	<0.001
Myeloperoxidase, ng/mL	53.0 (40.6–76.9)	47.1 (31.8–64.7)	<0.001	60.8 (41.0–90.4)	54.7 (38.8–79.2)	0.23
Brain natriuretic peptide Pro NT, ng/mL	0.14 (0.09–0.27)	0.17 (0.10–0.31)	0.63	0.16 (0.09–0.31)	0.14 (0.09–0.22)	0.074
Thrombospondin 4, μg/mL	0.75 (0.53–1.19)	0.71 (0.47–1.09)	0.83	0.83 (0.60–1.29)	0.68 (0.44–0.90)	0.16
Tissue inhibitor metalloproteinase 4, ng/mL	5.09 (3.99–7.20)	4.65 (3.60–5.98)	0.010	4.34 (3.50–5.37)	3.78 (3.00–4.61)	<0.001

Values are mean (standard deviation), median (interquartile range), or number (percentage).

*Serum concentrations shown for proteins with test for difference between cases and controls by Students *t* test.

deviation change in the predictor, where the standard deviation was calculated from the distribution in the control group.

Marker Correlation

To explore marker interdependence, we calculated pairwise spearman correlation coefficients between all variables (biomarkers and traditional risk factors). The resulting correlation matrix was plotted as a heat map.

Multivariable Logistic Regression Models to Predict 10-Year Incidence of MI

Two multivariable models were fitted and compared with our benchmark model, Model 1, which comprised the TRFs only. Each of the Models 2 and 3 were finalized by stepwise selection of new markers that minimized the Bayesian Information Criterion. All markers significantly associated with MI after adjustment for TRFs were candidates for Model 2. All variables that were selected for Model 2 were entered in Model 3. Candidates for the stepwise selection into Model 3 were the interaction terms (markers by sex) that were significant in a model that was adjusted for the TRFs. No marker by sex interaction term improved model 3 when the main effect of the marker was included in the model. However, after removing the main effect of the marker, a few markers by sex interaction terms improved model 3. This suggests that the marker effect was only present for one of the sexes. The sex for which the marker had an effect was indicated in the interaction term as (female or male) ×biomarker. False discovery rates (FDR)[10] were calculated from the *P* values from the variables included in model 2 and model 3. The FDR in model 2 were calculated based on all assessed protein main effects, n=52, and the FDR in model 3 was based on additional 52 tests because of the assessed tests of interaction with sex. Model fit was assessed by Bayesian Information Criteria, area under the receiving operating characteristic curve, and Net reclassification improvement (NRI).[11,12] Because the study design was enriched for cases, the intercept term of each model was corrected such that the mean risk predicted by the model reflected incidence of MI in the cohort (8.4%). To calculate NRI, the thresholds for moderate and high risk were set at 10% and 20%, respectively. Model calibration was tested with the Hosmer–Lemeshow test using 10 risk groups.

Results

Median (interquartile range) levels of all biomarkers in men and women are shown in Table III in the Data Supplement.

Twenty-four individual markers plus the APOB100 to APOA1 ratio (ApoBApoA1) showed a crude association with MI ($P<0.05$) in men and women combined (Table IVA

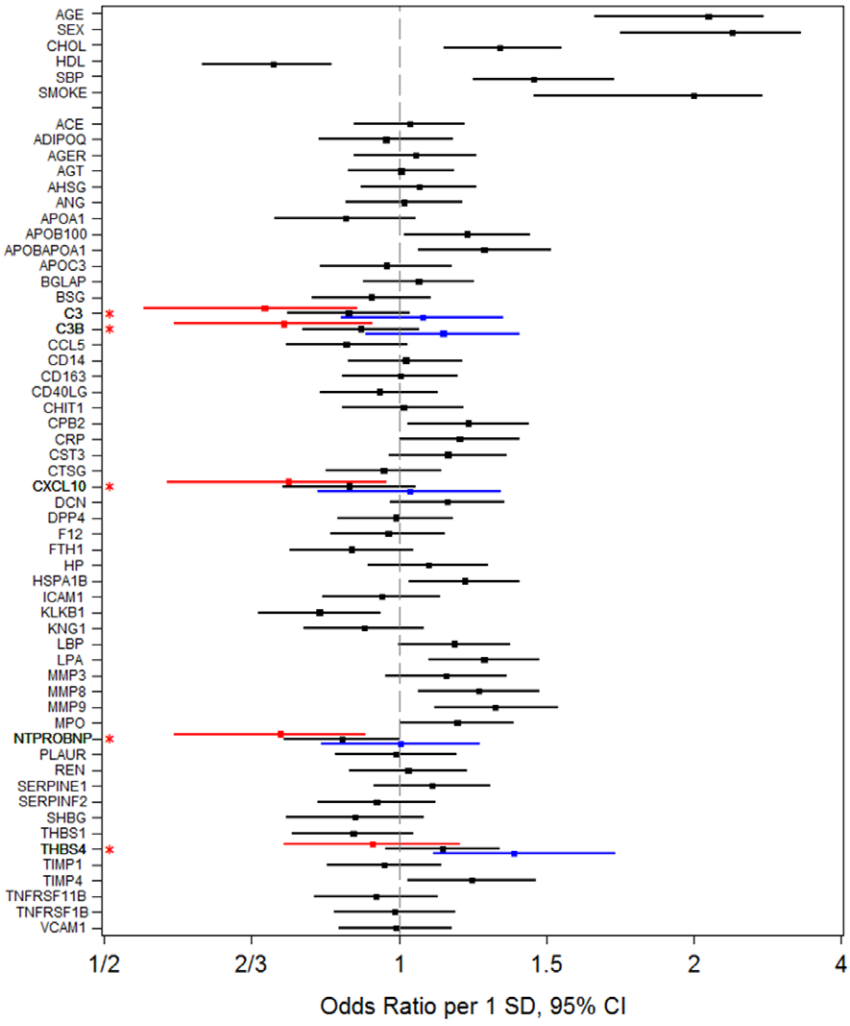

Figure 1. Standardized odds ratios (ORs) for incident myocardial infarction (MI) in 10 years of follow-up. ORs are of traditional risk factors and of 52 serum protein biomarkers adjusted for age, sex, age×sex, systolic blood pressure (SBP), SBP×blood pressure medication, smoking status, total cholesterol (CHOL), HDL cholesterol (HDL) in women and men (black bars). Red asterisks indicate biomarkers with significant differences in ORs between women (red bars) and men (blue bars). ORs were estimated by logistic regression and expressed per 1-SD increase of values in controls. The Tromsø Study.

in the Data Supplement), and three additional markers were significant in the sex-specific models (Table IVB and IVC in the Data Supplement). After adjustment for TRFs, 17 variables were significantly associated with incident MI in men, women, or in both combined (Table 1 and Figure 1; Tables IVA–IVC in the Data Supplement). All significant ORs and *P* values in Table IVA–IVC in the Data Supplement are shown in bold.

As summarized in Table 2, 11 variables were significantly associated with MI in the multivariable adjusted analyses of men and women combined: apolipoprotein B (ApoB100, OR=1.21), the ApoBApoA1 (OR=1.26), carboxypeptidase B2 (OR=1.21), CRP(OR=1.18), heat shock 70kDa protein 1B (OR=1.20), plasma kallikrein (KLKB1, OR= 0.81), lipoprotein (a) (LPa, OR=1.26), matrix metalloproteinase 8 (MMP8, OR=1.25), matrix metalloproteinase 9 (MMP9, OR=1.30), myeloperoxidase (MPO, OR=1.17), and tissue inhibitor of metallopeptidase 4 (TIMP4, OR=1.22; Table 2; Table IVA in the Data Supplement).

Five biomarkers were significantly different between men and women (test for interaction $P<0.05$; Figure 1; Table IVa in the Data Supplement). Complement C3 and C3b, CXCL10, and N-terminal prohormone of brain natriuretic peptide were protective in women and not significant among men (Table IVA and IVB in the Data Supplement). Conversely, thrombospondin-4 (THBS4) conferred significant risk in men but not in women.

As shown in Figure 2, a moderate to high degree of biomarker interdependence were observed for 18 pairs of biomarkers with spearman correlations >0.5. Seven were in the range 0.50 to 59, 4 in the range 0.60 to 69 (CTSG with MMP8, MMP9 and MPO, and ICAM1 with VCAM1), 5 in the range 0.70 to 0.79 (CD14 with VCAM1 and ICAM1, and the pairs of MMP8, MMP9, and MPO), and 2 in the range 0.80 to 0.90 (CCL5 with THBS1 and APOB100 with ApoBApoA1). One correlation coefficient had a negative value <–0.50 (APOA1 with ApoBApoA1, r =–0.501).

Figure 3 shows the AUC comparing the TRF-based model with the 2 selected multivariable risk prediction models (with and without interaction terms with sex) that were selected based on the Bayesian Information Criterion. Models 2 and 3 increased the AUCs by 0.027 and 0.035, respectively (*P* values 0.002 and 0.0004). The protein markers selected by the stepwise procedure not including sex interaction terms (Model 2) were ApoBApoA1, KLKB1, MMP9, and LPa. Model 3 was expanded from Model 2 with the addition of CXCL10 in women and THBS4 in men only (the interaction terms female×CXCL10 and male×THBS4). As shown in Table 3, both models improved net reclassification with NRI=8.5% (P=0.024) and NRI=14.2% (P=0.0002), resulting from a slightly higher net distribution of cases classified up than noncases classified down. Table 4 presents the ORs for the 2 models. All ORs are equal to or have improved slightly compared with the TRF-adjusted estimates presented in Table IV in the Data Supplement. In model 2, the FDRs for the 4 selected proteins ranged between 0.021 and 0.0048. In model 3, the total number of candidate variables doubled from 52 to 104 because of the inclusion of interaction terms with sex. Consequently, the FDRs were increased showing the highest values for CXCL10 in females (FDR=0.18) and THBS4 in males (FDR=0.16). Model calibration analyses did not show any significant deviation between predicted and observed risk. The Hosmer–Lemeshow test *P* values in the 20 imputed data sets ranged from 0.52 to 0.76 for model 2 and 0.14 to 0.91 for model 3.

Discussion

We evaluated 51 novel blood-based proteins for predicting 10 year risk of MI in a case–control study drawn from the population-based Tromsø Study. After adjustment for TRFs,

Table 2. Significant Odds Ratios for MI in Multivariable Adjusted Models*. The Tromsø Study

	Crude		Age Adjusted		Multivariable Adjusted†	
	OR (95% CI)	*P* Value	OR (95% CI)	*P* Value	OR (95% CI)	*P* Value
APOB100	1.45 (1.25–1.68)	<0.001	1.41 (1.21–1.65)	<0.001	1.21 (1.01–1.44)	0.034
ApoBApoA1	1.53 (1.31–1.77)	<0.001	1.49 (1.27–1.74)	<0.001	1.26 (1.06–1.52)	0.011
CPB2	1.18 (1.02–1.37)	0.031	1.21 (1.03–1.42)	0.020	1.21 (1.02–1.43)	0.026
CRP	1.49 (1.28–1.72)	<0.001	1.39 (1.19–1.62)	<0.001	1.18 (1.00–1.39)	0.048
HSPA1B	1.36 (1.19–1.57)	<0.001	1.29 (1.12–1.50)	<0.001	1.20 (1.03–1.40)	0.020
KLKB1	0.83 (0.72–0.96)	0.012	0.86 (0.73–1.00)	0.055	0.81 (0.68–0.95)	0.012
LPa	1.22 (1.07–1.40)	0.003	1.23 (1.07–1.42)	0.004	1.26 (1.09–1.47)	0.003
MMP8	1.45 (1.26–1.68)	<0.001	1.38 (1.19–1.62)	<0.001	1.25 (1.05–1.47)	0.011
MMP9	1.53 (1.33–1.77)	<0.001	1.46 (1.25–1.69)	<0.001	1.30 (1.10–1.54)	0.002
MPO	1.36 (1.18–1.56)	<0.001	1.26 (1.09–1.46)	0.002	1.17 (1.00–1.37)	0.045
TIMP4	1.27 (1.11–1.46)	<0.001	1.17 (0.99–1.38)	0.063	1.22 (1.02–1.46)	0.026

APOB indicates apolipoprotein B; ApoBApoA1, APOB100 to APOA1 ratio; CI, confidence intervals; CPB2, carboxypeptidase B2; CRP, C-reactive protein; HDL, high-density lipoprotein; HSPA1B, heat shock 70kDa protein 1B; KLKB1, plasma kallikrein; LPa, lipoprotein (a); MMP8, matrix metalloproteinase 8; MMP9, matrix metalloproteinase 9; MPO, myeloperoxidase; OR, odds ratios; and TIMP4, tissue inhibitor of metallopeptidase 4.

*ORs per 1 standard deviation change of transformed concentrations calculated in control subjects.

†Adjusted for age, sex, age×sex, blood pressure, blood pressure×blood pressure medication, total cholesterol, HDL cholesterol, and daily smoking.

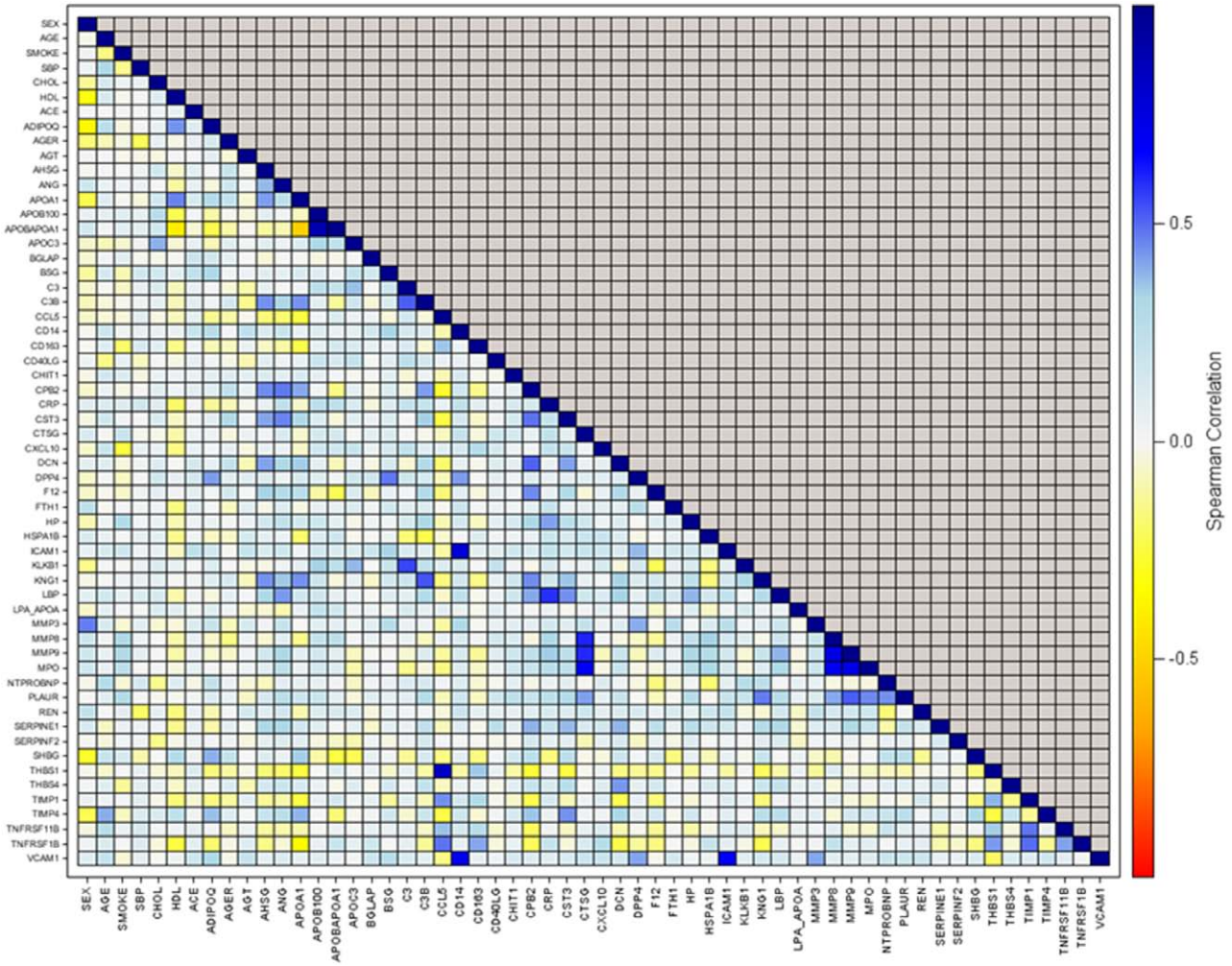

Figure 2. Spearman correlation matrix of traditional risk factors and 52 serum protein biomarkers. The Tromsø Study.

17 biomarker variables significantly improved discrimination and model fit. The discrimination, model fit, and reclassification were further improved by adding multiple biomarkers to the TRF-based model. A composite of ApoB/ApoA1, KLKB1, LPa, and MMP9 increased the AUC by 0.027 with an NRI of 9%, and a further inclusion of sex-specific terms of TBHS4 for men and CXCL10 for women increased the AUC by 0.035 and the NRI to 14%.

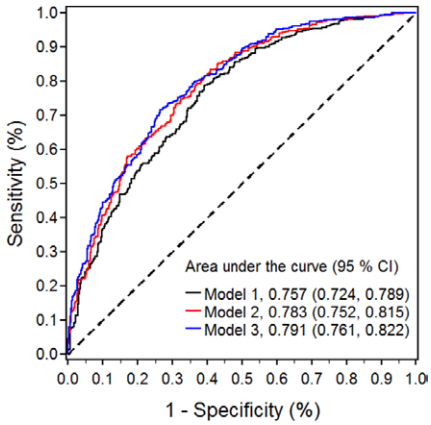

Figure 3. Receiver operator characteristics curves for incident first ever myocardial infarction in 10 years of follow-up. The Tromsø Study.

Surprisingly, KLKB1, the principal activating protease of the plasma kallikrein/kinin pathway, showed a strong protective and independent association with MI in men and women. KLKB1 was the only single protein that borderline significantly improved the discrimination as determined by the AUC. Two other plasma kallikrein/kinin pathway proteins, Factor XII (F12) and kininogen, were neither positively nor negatively associated with MI. The role of the plasma kallikrein/kinin pathway in cardiovascular disease remains unclear. Several studies analyzed the relationship of F12 with vascular thrombosis and cardiovascular events,[13,14] but only 1 study also evaluated KLKB1.[15] The comparison of results is complicated by differing study designs, blood matrices, outcomes, and the use of different measures of the analytes, which include detection of activated enzymes or of inhibitor-bound complexes, and activity assays. The anti-KLKB1 antibody reagents used in the present study recognize both prekallikrein (inactive) and kallikrein (activated); whether the detected analyte is complexed with inhibitors is not known.

The protein markers MMP8, MMP9, and MPO were all significantly associated with MI. Their enzymatic activities have been localized histochemically in vulnerable plaque phenotypes[16–18]

Table 3. Net Reclassification of Study Participants Who Did (MI=Yes) and Those Who Did Not (MI=No) Experience a Myocardial Infarction for Models 2 and 3. The Tromsø Study

		Model 2			Model 3			Percent in Risk Class
	Risk Class	<10%	10–<20%	≥20%	<10%	10–<20%	≥20%	
MI=Yes								
Model 1	<10%	104	26	6	97	27	12	32.5%
	10–<20%	32	76	40	29	74	45	35.3%
	≥20%	1	22	112	3	20	112	32.2%
	Percent in Risk Class	32.7%	29.6%	37.7%	30.8%	28.9%	40.3%	
		NRI_{YES}=0.048, *P*=0.09			NRI_{YES}=0.080, *P*=0.0071			
MI=No								
Model 1	<10%	251	16	0	252	14	1	67.1%
	10–<20%	36	49	12	43	43	11	24.4%
	≥20%	0	10	24	1	9	24	8.5%
	Percent in Risk Class	72.1%	18.8%	9.0%	74.4%	16.6%	9.0%	
		NRI_{NO}=0.037, *P*=0.11			NRI_{NO}=0.062, *P*=0.0062			
		$NRI_{overall}$=0.085, *P*=0.024			$NRI_{overall}$=0.142, *P*=0.0002			

Model 1: Traditional risk factors only (TRFs). Model 2: TRFs+ApoBApoA+KLKB1+LPa+MMP9. Model 3: TRFs+ApoBApoA+KLKB1+LPa+MMP9+Females×CXCL10+Males×THBS4.

ApoBApoA1 indicates APOB100 to APOA1 ratio; CXCL10, C-X-C motif chemokine 10; KLKB1, plasma kallikrein; LPa, lipoprotein (a); MI, myocardial infarction; MMP9, matrix metalloproteinase 9; NRI, Net reclassification improvement; THBS4, thrombospondin-4; and TIMP4, tissue inhibitor of metallopeptidase 4.

and have been associated with first ever MI.[4,19,20] However, in the stepwise selection procedure, MMP9 was the only that was selected. This reflects a high degree of interchangeability between these markers as showed in the correlation matrix (Figure 2). The high degree of interdependence may suggest a common tissue source or immune response to an atherogenic exposure.

Our results support previous findings regarding ApoBApoA1[4,21] and LPa.[22] These biomarkers have been evaluated in many populations, using standardized reagents and formats which we could not adapt to the research platform used in the present study. As a consequence, the absolute mass levels measured for these analytes in this study are not necessarily comparable to those reported in other populations. We did select antibody reagents with desirable epitope specificities where possible. For example, although apolipoproteins A1 and B100 blood levels are considered insensitive to fasting state, we used ApoB100-specific assay antibody reagents (R&D) for screening in this nonfasting population to avoid possible confounding by postprandial changes in ApoB48 levels. To measure LPa, we acquired reagents to quantify protein levels independently of the Kringle 4 domain repeat isoforms, using a commercially available set of antibodies directed to the apo(a) moiety.[23]

We found that higher CXCL10 levels protected against myocardial infarction in women. Chemokines are inflammatory cytokines which cause directed migration of leukocytes into inflamed tissue, and increased levels have been found in atherosclerotic lesions.[24] In a small cross-sectional study on 49 patients with acute MI and 44 healthy controls, a combination of 7 chemokines, among them CXCL10, markedly improved prediction of disease.[25] Although the sex-specific associations between CXCL10 and first-ever MI have not previously been studied in prospective population-based studies,

Table 4. Odds Ratios for Myocardial Infarction in 2 Models*. The Tromsø Study

	Model 2			Model 3		
Term	OR (95% CI)	*P* Value	FDR	OR (95% CI)	*P* Value	FDR
ApoBApoA1	1.34 (1.10, 1.64)	0.0037	0.048	1.40 (1.14, 1.71)	0.0012	0.062
KLKB1	0.72 (0.60, 0.86)	0.0004	0.021	0.73 (0.61, 0.88)	0.0011	0.114
LPa	1.27 (1.09, 1.49)	0.0027	0.047	1.29 (1.10, 1.51)	0.0020	0.069
MMP9	1.31 (1.10, 1.55)	0.0018	0.047	1.30 (1.10, 1.54)	0.0023	0.060
Female* CXCL10	…	…	…	0.69 (0.52, 0.91)	0.0085	0.177
Male*THBS4	…	…	…	1.38 (1.08, 1.77)	0.0094	0.163

ApoBApoA1 indicates APOB100 to APOA1 ratio; CI, confidence intervals; CXCL10, C-X-C motif chemokine 10; FDR, false discovery rate; KLKB1, plasma kallikrein; LPa, lipoprotein (a); MMP9, matrix metalloproteinase 9; OR, odds ratios; and THBS4, thrombospondin-4.

*ORs per 1 standard deviation change of transformed concentrations calculated in control subjects. Adjusted for age, sex, age×sex, blood pressure, blood pressure×blood pressure medication, total cholesterol, HDL cholesterol, and daily smoking.

the MONICA/KORA study showed no significant association in women and men combined, in agreement with our results.[26]

We are not aware of population-based studies showing the association between Thrombospondin 4 and cardiovascular disease. However, THBS4 is a matricellular protein expressed by endothelial and smooth muscle cells and may be important in regulation of vascular inflammation. Our finding supports the suggestion that the thrombospondin proteins and their single-nucleotide polymorphisms play a significant role in cardiovascular pathology.[27]

Adjusted for the TRFs, CRP improved model fit in the total sample and in men, but not in women. The insignificant result in women is in agreement with the conclusion by the systematic review by Shah et al, in that CRP does not perform better than the Framingham risk equation.[28] Their conclusion is also supported by our stepwise selection procedure that did not include CRP.

The variables included in our risk prediction models have all been linked to CHD but to some extent with conflicting res ults.[4,15,17,21,22,24,27] Our study is the first to demonstrate multivariable prediction models, including these variables adjusted for TRFs. Furthermore, we have assessed 1 model with and 1 without biomarker interaction terms with sex, indicating a possible improved discrimination and reclassification by including sex-specific biomarker terms. The NRI of 14% in model 3 resulting from net 8% cases classified up and net 6% of noncases classified down indicate a highly relevant improvement compared with the TRF model. However, the robustness of our findings would be increased by replication in an independent cohort. The amount of reclassification presented here, which is dependent on calibration of the models, is likely to represent an upper bound in the number of cases and controls reshifted among risk categories that can be expected in an independent cohort. Additionally, we cannot rule out the possibility of spurious associations because of sampling or experimental bias. The use of Bayesian Information Criterion as criterion in our selection process is equivalent with using a likelihood ratio test with P value threshold 0.01 (when the sample size is n=817). A 1% threshold implies an expected false-positive finding of <1 biomarker (out of 52) in model 2 and ≈1 false-positive in model 3 (out of 104 possible terms in the regression model). However, the observed FDR was <0.047 for all 4 included proteins in model 2, indicating the expected number of false positives to be 4×0.047=0.19. In model 3, the highest FDR of the 6 included proteins was 0.177, which indicate 1 false-positive (6×0.177=1.06).

The high attendance rate, adjudication of events from records of the only local hospital, and negligible loss of participants to follow-up are strengths of this study, as are the single site sample collection with standardized clinical exams and laboratory analyses, storage and documentation, and a high number of events. The use of frozen serum samples represent a limitation because it can have influenced the biomarker levels and thereby the absolute risk estimates. Furthermore, the Tromsø population is a relatively homogenous middle-aged white population, and the results may not be applicable to other ethnic or age groups.

Conclusions

Ten-year risk estimation of MI was improved by adding novel protein biomarkers to the traditional risk factor model. The net reclassification was improved by 9% by adding ApoBApoA1, KLKB1, LPa, and MMP9 to the risk score model and further improved to 14% by including sex-specific terms of TBHS4 for men and CXCL10 for women.

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Disclosures

A. Patwardhan, M.W. Rowe, J. Sudduth-Klinger, and S. Hamren have been employed by Tethys Bioscience. The other authors report no conflicts.

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CLINICAL PERSPECTIVE

Several predictive models are currently being used to estimate 10-year risk of either cardiovascular morbidity or mortality. Most models are based on traditional cardiovascular risk factors. However, it is evident that the traditional risk factors do not adequately reflect all cardiovascular risk as the majority of individuals who experience a first time cardiovascular event have adverse levels in <2 traditional risk factors and are misidentified as being at low risk. We aimed to survey 51 blood-based protein markers to improve on traditional predictive models, which may lead to more accurate treatment decisions and add significantly to the prediction of incident 10-year myocardial infarction. Data in nondiabetic men and women from the Tromsø Study identified 2 models that improved 10-year prediction of MI beyond that of the traditional risk factors. The combination of apolipoprotein B/A1, kallikrein, matrix metalloproteinase 9, and lipoprotein (a) improved net reclassification of 8.5% to either low, median, or high-risk group. The net reclassification improvement increased to 14.2% by adding sex-specific terms of thrombospondin 4 for men and C-X-C motif chemokine 10 for women to the model.

Clinically Significant Novel Biomarkers for Prediction of First Ever Myocardial Infarction: The Tromsø Study

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SUPPLEMENTAL MATERIAL

Supplemental Methods

Blood and sample processing

The study serum samples underwent no more than three freeze/thaw cycles from time of receipt to protein data production. All sera were kept at 4º C between sample dilutions, and were otherwise stored at -80º C until assay production.

Assay Development and Data Production Campaigns

Assay Reagents

Immunoassay components were obtained from commercial sources, including R&D Systems, Inc. (Minneapolis, USA), United States Biological (Swampscott, USA), Abcam, Inc. (Cambridge, USA), Hytest Ltd. (Turku, Finland), Academy Biomedical (Houston, USA), AbD Serotec (Raleigh, USA), Novus Biologicals (St. Charles, USA), Mabtech (Cincinnati, USA), Biodesign (Memphis, USA), EMD Calbiochem (Billerica, USA), Mercodia, AB (Uppsala, Sweden), and Affinity BioReagents (Golden, USA). See Supplemental Table 1. Two human serum pools were used as controls for assay development and data production: Pooled normal human serum (NHS) from VWR International, LLC (Radnor, USA), and a Tromsø Study Pool (TSP) created by combining aliquots from 10%-16% of the study samples.

Assay Development

Antibody concentration, diluents, blocking agents and washes were optimized for each biomarker using factorial analysis, evaluating parameters of signal-to-noise ratio, lower limit of detection, upper limit of detection, parallelism, recovery and specificity. Acceptable assay performance criteria required the standard curve be near 4-5 log Event Photons at the lower limit of detection, and 6-7 log Event Photons at the upper limit of detection, with a minimum of 2 logs linear range. A sample spiked with analyte, titrated within the linear range was required to have a slope parallel to that of the standard curve, and 80-120% recovery of the analyte was required for acceptance. Variance between with-in plates replicates was required to be <20%.

To identify the appropriate dilution range for the samples for each biomarker, the Tromsø Study Pool was diluted in assay buffer with 5-fold serial dilutions (5- to $1.9x10^6$- fold dilution range). The dilution closest to the midpoint of the standard curve was selected for data production. Once the MRD was identified, study serum samples were placed in an 8x12 array, diluted with assay specific buffers and then stored at -80°C until ready to add to the immunoassay plates.

For over 90% of the immunoassays, the anti-biomarker detection antibody was directly conjugated with AlexaFluor 647 carboxylic acid, succinimidyl ester from Life Technologies (Carlsbad, USA) and conjugates were purified by ultrafiltration with Micron YM-30 from Millipore Corporation (Billerica, USA). Where a detection antibody was not available in unlabeled form, a biotinylated anti-biomarker antibody and AlexaFluor 647-conjugated streptavidin from Life Technologies was used for detection. All immunoassays were performed in 384-well NUNC Maxisorp plates sealed with pierceable heat sealing tape.

Biomarker immunoassays/data production

Sandwich-format immunoassays were performed in a total volume of 10 µL/well. Plates were prepared by adding 10 µL capture antibody in diluent to each well and incubating overnight at room temperature (RT). Wells were washed and blocked with 60 µL of assay specific blocking agent for 2-h at RT.

Analyte for each standard curve was serially diluted to eight levels in assay buffer. Controls included replicates of the NHS and the TSP diluted to the same concentration as study samples, and a negative control (dilution buffer only). Diluted samples, standards, and controls were added (10 µL/well) to the coated wells and incubated overnight at RT.

After washing wells, anti-biomarker antibody was diluted in the appropriate buffer and dispensed into each well (10 µL/well) and incubated for 2-h at RT. For immunoassays using biotinylated anti-biomarker antibody, an additional step followed with a wash, and the addition of 10 µL/well AlexaFluor 647-conjugated streptavidin at 1 ng/ml in assay buffer, then incubated for 2-h at RT. Wells were then washed and the antibody-analyte complexes were released from wells by adding 20 µL/well of 4 M Urea, 10 mM Boric Acid, 0.15 M NaCl, 0.001% BSA, 0.02% Triton X-100. This solution was used directly for molecular counting - based quantification *(Bioanalysis*. 2011 Oct;3(19):2233-51) .

Molecular Counting

Detection of AlexaFluor 647-labeled antibodies was performed on the Errena™ System (Singulex, Inc., Alameda, CA), which aspirates liquid from each well through an interrogation space within a capillary flow cell. Laser light (639 nm) is directed into the interrogation space, and the resulting emission from each labeled antibody (668 nm) is measured via a confocal microscope with a photon detector. The photon detector transmits an electronic pulse for each photon detected, and pulses are counted in 1-ms bins. Only binned pulses that exceeded a 6-SD threshold above background are counted, so photons emitted from individual dye molecules are distinguished from background. Binned pulses are summed over a 1-min interval or until 1000 pulses are detected and recorded as photons/minute.

Only one biomarker was measured per plate. Each plate included three replicates of each sample and controls, and six replicates of an eight-point standard curve generated from dilutions of known quantities of the specific biomarker. The concentration of biomarker in each sample was determined by interpolation of the mean of the replicates from the standard curve. Production assay data that met the following criteria were entered into the study database: >70% samples detected, >2 logs standard curve linear range, and <20% replicate coefficient of variance (CV) between with-in plate replicates

Assay Performance Metrics and Power Estimates

Inter-assay (interplate) and total CVs were calculated for each assay run using the pooled serum controls: the TSP and the NHS in campaign 1, and two replicates of TSP in campaign 2. See Supplemental Table 2.

Power to detect an odds ratio of a given magnitude was estimated for each assay through simulation. A simulated population consisting of 100,000 measurements of a normally distributed variable was generated. Outcomes were assigned to each observation such that the percentage of positive outcomes in the population matched the prior probability in Tromsø (3.3%), and such that higher values of the variable were associated with outcome with a specified odds ratio. For a given assay, the values of the variable were scaled to the same mean and SD as observed in the study, and Gaussian noise was added corresponding to the total CV for that assay. Samples were then randomly drawn from this population with the same number of cases and controls as in the present study, and a logistic regression model of the outcome was fit in each sample. A total of 1,000 samples was drawn for each odds ratio and each assay, and the power was estimated as the fraction of samples where the coefficient of the fit was

significant ($p<0.05$). This was done over 100 odds ratios covering a range between 1 and 2.5, equally spaced on a logarithmic scale. The odds ratios corresponding to 50% and 95% power were then estimated for each assay from a spline fit of the resulting power vs. OR curves.

Supplemental Table 1. Biomarker Immunoassay Reagents and Vendors. The Tromsø Study

Gene Symbol	Protein Name	Capture Vendor	Capture Catalogue #	Analyte Control Vendor	Analyte Control Catalogue #	Detection Vendor	Detection Catalogue#
ACE	angiotensin I converting enzyme 1	R&D Systems	AF929	R&D Systems	929-ZN-010	R&D Systems	841366
ADIPOQ	adiponectin	R&D Systems	MAB10651	R&D Systems	1065-AP-050	R&D Systems	AF1065
AGER	advanced glycosylation end-product receptor	R&D Systems	MAB11451	R&D Systems	1145-RG-050	R&D Systems	AF1145
AGT	angiotensingen	R&D Systems	MAB3156	VWR	80050-234	R&D Systems	AF3156
AHSG	alpha-2-HS-glycoprotein/Fetuin A	R&D Systems	MAB1184	R&D Systems	1184-P1-050	R&D Systems	AF1184
ANG	angiogenin	R&D Systems	840307	R&D Systems	840309	R&D Systems	840308
APOA1	apolipoprotein A-I	Abcam	ab17278	Biodesign	A95120H	Abcam	ab7613
APOB	apolipoprotein B	Mabtech	3715-3-1000	Biodesign	A50220H	Mabtech	3715-5-250
APOB100	apolipoprotein B 100	R&D Systems	MAB4124	Biodesign	A50220H	R&D Systems	AF3260
APOC3	Apolipoprotein C-III	Academy Biomedical	33A-G2b	Academy Biomedical	33P-UP202	Academy Biomedical	33A-R1b
BGLAP	osteocalcin	Hytest	4OC8-6F9	Novus Biologicals	H00000632-Q01	Hytest	4OC8-3H8
BSG	CD147/EMMPRIN	R&D Systems	MAB972	R&D Systems	972-EMN-050	R&D Systems	AF972
C3	complement C3	USBio	C7850-14	abD Serotec	2222-5704	USBio	C7850-10A
C3b	complement C3b	USBio	C7850-14	abD Serotec	2222-5909	USBio	C7850-10A
CCL5	RANTES	R&D Systems	840216	R&D Systems	840218	R&D Systems	840217
CD14	CD14	R&D Systems	MAB3833	R&D Systems	383-CD-050/CF	R&D Systems	AB383

Gene Symbol	Protein Name	Capture Vendor	Capture Catalogue #	Analyte Control Vendor	Analyte Control Catalogue #	Detection Vendor	Detection Catalogue#
CD163	CD163	R&D Systems	MAB1607	R&D Systems	1607-CD-050	R&D Systems	AF1607
CD40LG	CD40 ligand	Hytest	4CD40	Cell Sciences	CRC800B	Hytest	4CD40
CHIT1	chitinase 1 (chitotriosidase)	R&D Systems	MAB3559	R&D Systems	3559-GH	R&D Systems	AF3559
CPB2	carboxypeptidase B2	Hytest	4TA1-13D5	Hytest	8TA1	Hytest	4TA1-13H4
CRP	C-reactive protein	USBio	C7907-09	USBio	C7907-26A	USBio	C7907-10
CST3	cystatin C	Hytest	4CC1	Hytest	8CY5	Hytest	PCC2
CTSG	Cathepsin G	USBio	N2257	Affinity BioReagents	RP-77525	USBio	C2097-52
CXCL10	IP-10	R&D Systems	MAB266	R&D Systems	266-IP-050/CF	R&D Systems	AF266-na
DCN	decorin	R&D Systems	MAB1432	R&D Systems	143-DE-100	R&D Systems	AF143
DPP4	dipeptidyl-peptidase 4	R&D Systems	MAB1180	R&D Systems	1180-SE	R&D Systems	AF1180
F12	coagulation factor XII	USBio	F0019-03	USBio	F0019-15	USBio	F0019-06
FTH1	ferritin	USBio	F4015	USBio	F4015-21	USBio	F4015-17
HP	haptoglobin	USBio	H1820-05	USBio	H1820-03	USBio	H1820-06
HSPA1B	heat shock 70kDa protein 1B	R&D Systems	MAB1663	R&D Systems	CUSTOM 02	R&D Systems	AF1663
ICAM1	intercellular adhesion molecule 1	R&D Systems	MAB720	R&D Systems	ADP4-050	R&D Systems	AF720
KLKB1	Plasma Kallikrein	USBio	P6200-50	EMD Calbiochem	529583-1MG	USBio	P6201
KNG1	kininogen 1	USBio	K1800	R&D Systems	1569-PI-010	R&D Systems	AF1569
LBP	Lipopolysaccharide-binding protein	USBio	L2525-27	R&D Systems	870-LP-025/CF	R&D Systems	AF870
LPa	Lipoprotein (a)	Mercodia	CT1280	Mercodia	20-2517	Mercodia	C1356

Gene Symbol	Protein Name	Capture Vendor	Capture Catalogue #	Analyte Control Vendor	Analyte Control Catalogue #	Detection Vendor	Detection Catalogue#
MMP3	matrix metalloproteinase 3	R&D Systems	841043	R&D Systems	841045	R&D Systems	841044
MMP8	matrix metalloproteinase 8	R&D Systems	MAB908	R&D Systems	908-MP-010	R&D Systems	AF908
MMP9	matrix metalloproteinase 9	R&D Systems	841028	R&D Systems	841030	R&D Systems	841029
MPO	myeloperoxidase	Abcam	ab10164	R&D Systems	3174-MP	R&D Systems	AF3174
NTproBNP	brain natriuretic peptide Pro NT	Hytest	4NT1-15C4	Hytest	8NT1	Hytest	4NT1-13G12
PLAUR	uPAR	R&D Systems	MAB807	R&D Systems	807-UK-100/CF	R&D Systems	AF807
REN	prorenin	R&D Systems	MAB4090	R&D Systems	4090-AS-020	R&D Systems	AF4090
SERPINE1	plasminogen activator inhibitor	R&D Systems	AF4090	R&D Systems	1786-PI-010	R&D Systems	AF1786
SERPINF2	serpin peptidase inhibitor, clade F	R&D Systems	MAB1470	R&D Systems	1470-PI-010	R&D Systems	AF1470
SHBG	sex-hormone binding globulin	R&D Systems	mab2656	USBio	S1012-54	R&D Systems	AF2656
THBS1	thrombospondin 1	R&D Systems	MAB3074	R&D Systems	3074-TH-050	Genetec	GTX22962
THBS4	thrombospondin 4	R&D Systems	MAB2390	R&D Systems	2390-TH-050	R&D Systems	AF2390
TIMP1	tissue inhibitor of metallopeptidase 1	R&D Systems	MAB970	R&D Systems	840296	R&D Systems	840295
TIMP4	tissue inhibitor of metallopeptidase 4	R&D Systems	MAB974	R&D Systems	974-TSF-010	R&D Systems	AF974
TNFRSF11B	osteoprotegerin	R&D Systems	MAB8051	R&D Systems	805-OS-100/CF	R&D Systems	AF805
TNFRSF1B	tumor necrosis factor receptor, 1B	R&D Systems	MAB726	R&D Systems	726-R2-050	R&D Systems	AF726
VCAM1	vascular cell adhesion molecule 1	R&D Systems	MAB809	R&D Systems	ADP5	R&D Systems	AF809

Supplemental Table 2. Performance characteristics of Biomarker Immunoassays. The Tromsø Study

ASSAY	CAMPAIGN	COEFFICIENTS OF VARIATION (%)			minimum sOR detectable @ 95% POWER
		CONTROL	TOTAL	INTER-ASSAY	
ACE	1	HSP/TSP	47%	52%	1.76
ADIPOQ	1	HSP/TSP	31%	27%	1.32
AGER	2	TSP	8%	1%	1.22
AGT	1	HSP/TSP	38%	21%	1.16
AHSG	2	TSP	11%	4%	1.28
ANG	2	TSP	11%	8%	1.28
APOA1	2	TSP	10%	8%	1.28
APOB100	2	TSP	24%	19%	1.32
APOC3	2	TSP	6%	4%	1.28
BGLAP	1	HSP/TSP	24%	24%	1.10
BSG	1	HSP/TSP	9%	8%	1.33
C3	2	TSP	17%	8%	1.21
C3b	2	TSP	15%	12%	1.28
CCL5	1	TSP	27%	na	1.18
CD14	1	HSP/TSP	18%	6%	1.18
CD163	1	HSP/TSP	10%	6%	1.26
CD40LG	2	TSP	25%	24%	1.35
CHIT1	2	TSP	6%	5%	1.18
CPB2	2	TSP	12%	9%	1.29
CRP	2	TSP	9%	8%	1.29
CST3	2	TSP	26%	16%	1.42
CTSG	1	HSP/TSP	18%	9%	1.32
CXCL10	2	TSP	10%	8%	1.32
DCN	2	TSP	9%	8%	1.32
DPP4	1	HSP/TSP	21%	22%	1.41
F12	2	TSP	12%	10%	1.34
FTH1	1	HSP/TSP	23%	24%	1.52
HP	2	TSP	10%	8%	1.24
HSPA1B	2	TSP	19%	13%	1.27
ICAM1	1	HSP/TSP	21%	6%	1.39
KLKB1	2	TSP	9%	8%	1.32
KNG1	2	TSP	11%	9%	1.32

ASSAY	CAMPAIGN	COEFFICIENTS OF VARIATION (%)			minimum sOR detectable @ 95% POWER
		CONTROL	TOTAL	INTER-ASSAY	
LBP	2	TSP	4%	3%	1.10
LPa	2	TSP	13%	12%	1.28
MMP3	1	HSP/TSP	22%	22%	1.34
MMP8	2	TSP	7%	5%	1.12
MMP9	2	TSP	7%	5%	1.12
MPO	2	TSP	10%	9%	1.14
NTproBNP	2	TSP	13%	13%	1.20
PLAUR	2	TSP	13%	9%	1.19
REN	1	HSP/TSP	26%	24%	1.32
SERPINE1	2	TSP	13%	8%	1.24
SERPINF2	2	TSP	15%	12%	1.30
SHBG	2	TSP	23%	15%	1.38
THBS1	1	TSP	38%	na	1.56
THBS4	2	TSP	19%	15%	1.26
TIMP1	1	HSP/TSP	24%	17%	1.32
TIMP4	2	TSP	6%	4%	1.12
TNFRSF11B	1	HSP/TSP	27%	20%	1.35
TNFRSF1B	1	HSP/TSP	16%	12%	1.25
VCAM1	1	HSP/TSP	27%	23%	1.37

Supplemental Table 3. Baseline characteristics in MI cases and controls by sex. The Tromsø Study

	Women		Men	
Characteristic	n	Median (IQR)*	n	Median (IQR)*
ACE (ng/ml)	400	806.64 (529.29-1120.1)	393	820.71 (542.22-1132.7)
ADIPOQ (µg/ml)	411	6.45 (4.14- 9.60)	399	3.81 (2.53- 5.57)
AGER (ng/ml)	397	0.41 (0.30- 0.52)	392	0.35 (0.26- 0.45)
AGT (ng/ml)	413	590.77 (230.79-1557.6)	403	624.76 (231.71-1711.0)
AHSG (µg/ml)	396	902.47 (750.07-1048.2)	391	888.72 (764.81-1049.0)
ANG (ng/ml)	380	195.14 (160.71-234.93)	387	218.27 (176.53-256.85)
APOA1 (µg/ml)	394	1730.8 (1285.8-2094.9)	390	1505.2 (1153.2-1836.9)
APOB (µg/ml)	396	14.77 (9.92- 23.37)	391	16.82 (11.96- 24.39)
APOBAPOA1 (ng/ml)	393	0.008 (0.005- 0.014)	389	0.011 (0.007- 0.016)
APOC3 (µg/ml)	382	242.43 (199.10-303.89)	376	235.49 (196.92-284.94)
BGLAP (ng/ml)	412	5559.3 (4304.3-7682.6)	402	5388.2 (4192.0-7234.4)
BSG (ng/ml)	410	45.34 (36.91- 53.82)	403	42.24 (35.76- 48.78)
C3 (mg/ml)	366	223.66 (172.68-280.46)	372	217.10 (165.52-275.06)
C3B (µg/ml)	394	2.85 (2.25- 3.46)	392	2.70 (2.19- 3.28)
CCL5 (ng/ml)	413	102.41 (47.77-212.94)	403	86.37 (47.36-184.99)
CD14 (ng/ml)	410	233.52 (185.51-309.58)	403	228.88 (182.45-294.98)
CD163 (ng/ml)	413	89.66 (52.62-165.71)	404	84.54 (56.21-153.24)
CD40LG (ng/ml)	395	11.24 (7.99- 15.25)	392	11.35 (8.19- 15.76)
CHIT1 (ng/ml)	396	41.88 (27.08- 59.04)	391	42.94 (27.37- 61.00)
CPB2 (µg/ml)	397	26.99 (23.49- 30.59)	392	26.09 (22.29- 30.69)
CRP (ng/ml)	397	56.03 (23.84-137.66)	391	68.06 (33.94-157.81)
CST3 (ng/ml)	396	567.21 (475.71-750.01)	390	591.36 (470.04-727.64)
CTSG (ng/ml)	413	28.59 (17.76- 45.35)	402	35.15 (21.64- 53.55)
CXCL10 (ng/ml)	396	0.04 (0.03- 0.05)	389	0.03 (0.03- 0.05)
DCN (ng/ml)	397	12.78 (10.89- 15.00)	391	13.18 (11.41- 15.41)
DPP4 (ng/ml)	410	984.98 (728.92-1192.0)	403	912.96 (734.70-1158.8)
F12 (µg/ml)	396	23.39 (18.34- 30.11)	392	23.02 (17.64- 28.75)
FTH1 (ng/ml)	411	122.48 (63.40-241.56)	403	208.40 (110.46-422.90)
HP (µg/ml)	393	758.16 (532.90-1077.8)	392	677.68 (399.11-1056.7)
HSPA1B (ng/ml)	396	2.60 (1.91- 4.23)	391	3.16 (2.29- 4.97)
ICAM1 (ng/ml)	410	31.69 (24.70- 43.56)	403	33.12 (25.34- 44.09)
KLKB1 (µg/ml)	394	29.63 (24.84- 34.90)	391	27.22 (23.05- 33.00)
KNG1 (µg/ml)	394	83.23 (69.42- 99.48)	392	83.50 (68.32-102.85)
LBP (ng/ml)	397	920.05 (744.96-1097.6)	392	976.19 (778.83-1163.0)

Supplemental Table 3. Baseline characteristics in MI cases and controls by sex. (cont'd)

		Women		Men
Characteristic	n	Median (IQR)*	n	Median (IQR)*
LPa (ng/ml)	396	141.41 (71.77-377.27)	391	129.09 (60.38-377.99)
MMP3 (ng/ml)	410	7.02 (5.27- 9.24)	403	11.52 (8.73- 16.36)
MMP8 (ng/ml)	396	10.26 (6.64- 16.11)	391	13.24 (8.81- 21.06)
MMP9 (ng/ml)	397	372.96 (260.67-521.62)	392	444.39 (315.32-615.68)
MPO (ng/ml)	397	50.12 (34.02- 70.20)	392	58.62 (40.62- 85.97)
NTPROBNP (ng/ml)	396	0.16 (0.10- 0.29)	391	0.15 (0.09- 0.26)
PLAUR (ng/ml)	396	1.60 (1.25- 2.09)	392	1.61 (1.21- 2.18)
REN (ng/ml)	412	0.44 (0.28- 0.67)	403	0.58 (0.37- 0.87)
SERPINE1 (ng/ml)	394	36.43 (28.97- 44.40)	390	39.58 (31.11- 48.24)
SERPINF2 (ng/ml)	396	2608.8 (1480.6-3902.7)	390	2431.1 (1663.3-3580.2)
SHBG (ng/ml)	397	1820.9 (1300.8-2493.8)	392	1344.9 (1013.8-1849.5)
THBS1 (µg/ml)	409	34.87 (19.69- 59.59)	399	33.99 (20.07- 55.60)
THBS4 (ng/ml)	396	737.53 (490.61-1136.6)	392	755.08 (512.42-1161.3)
TIMP1 (ng/ml)	413	55.54 (40.17- 73.81)	404	58.30 (41.85- 75.38)
TIMP4 (ng/ml)	396	4.73 (3.74- 6.18)	392	4.10 (3.27- 5.18)
TNFRSF11B (ng/ml)	413	43.73 (32.66- 55.71)	404	41.61 (31.22- 53.83)
TNFRSF1B (ng/ml)	413	18.18 (14.08- 22.21)	403	18.25 (14.58- 22.50)
VCAM1 (ng/ml)	410	118.73 (89.06-152.65)	403	125.32 (94.95-162.48)

* Values are median (interquartile range)

Supplemental Table 4a. Odds ratios for MI*. The Tromsø Study

Variable	Crude		Sex and age adjusted		Multivariable adjusted†		p-value for sex diff.‡
	OR (95% CI)	p-value	OR (95% CI)	p-value	OR (95% CI)	p-value	
AGE	**1.80 (1.55-2.10)**	**<0.001**	**1.87 (1.60-2.19)**	**<0.001**	**2.15 (1.65-2.79)**	**<0.001**	0.14
SEX	**2.34 (1.77-3.10)**	**<0.001**	**2.59 (1.92-3.48)**	**<0.001**	**2.40 (1.73-3.32)**	**<0.001**	Na
CHOL	**1.29 (1.12-1.47)**	**<0.001**	**1.32 (1.14-1.53)**	**<0.001**	**1.32 (1.13-1.55)**	**<0.001**	0.60
HDL-C	**0.74 (0.65-0.85)**	**<0.001**	**0.73 (0.63-0.85)**	**<0.001**	**0.71 (0.61-0.83)**	**<0.001**	0.84
SYSBP	**1.64 (1.42-1.90)**	**<0.001**	**1.44 (1.23-1.69)**	**<0.001**	**1.45 (1.23-1.71)**	**<0.001**	0.61
SMOKE	**1.41 (1.07-1.87)**	**0.015**	**1.82 (1.33-2.47)**	**<0.001**	**2.00 (1.45-2.77)**	**<0.001**	0.35
ACE	1.03 (0.90-1.18)	0.65	1.03 (0.90-1.19)	0.65	1.03 (0.89-1.20)	0.70	0.052
ADIPOQ	0.88 (0.76-1.01)	0.060	**0.83 (0.71-0.98)**	**0.030**	0.97 (0.80-1.16)	0.71	0.67
AGER	0.87 (0.75-1.01)	0.059	0.99 (0.84-1.15)	0.85	1.05 (0.89-1.24)	0.59	0.10
AGT	1.00 (0.88-1.14)	0.99	0.98 (0.85-1.13)	0.79	1.01 (0.87-1.16)	0.93	0.19
AHSG	1.13 (0.98-1.30)	0.090	1.14 (0.98-1.32)	0.080	1.06 (0.90-1.24)	0.50	0.72
ANG	**1.19 (1.03-1.37)**	**0.017**	1.08 (0.93-1.26)	0.33	1.01 (0.86-1.19)	0.86	0.48
APOA1	**0.78 (0.67-0.92)**	**0.002**	**0.78 (0.66-0.93)**	**0.005**	0.87 (0.71-1.05)	0.14	0.86
APOB	**1.45 (1.25-1.68)**	**<0.001**	**1.41 (1.21-1.65)**	**<0.001**	**1.21 (1.01-1.44)**	**0.034**	0.33
APOBAPOA1	**1.53 (1.31-1.77)**	**<0.001**	**1.49 (1.27-1.74)**	**<0.001**	**1.26 (1.06-1.52)**	**0.011**	0.43
APOC3	1.02 (0.88-1.17)	0.81	1.11 (0.95-1.29)	0.18	0.97 (0.81-1.16)	0.71	0.19
BGLAP	1.06 (0.93-1.21)	0.39	1.08 (0.93-1.25)	0.31	1.06 (0.91-1.23)	0.48	0.49
BSG	1.02 (0.89-1.17)	0.80	0.99 (0.85-1.15)	0.89	0.93 (0.79-1.09)	0.37	0.60
C3	0.93 (0.81-1.08)	0.37	0.96 (0.82-1.12)	0.57	0.87 (0.74-1.03)	0.11	**0.015**
C3B	0.92 (0.80-1.06)	0.26	0.97 (0.84-1.13)	0.70	0.90 (0.77-1.06)	0.20	**0.009**
CCL5	0.89 (0.77-1.03)	0.12	0.93 (0.80-1.08)	0.36	0.87 (0.74-1.02)	0.091	0.39
CD14	1.13 (0.98-1.30)	0.092	1.04 (0.89-1.20)	0.64	1.02 (0.87-1.19)	0.81	0.51
CD163	1.13 (0.99-1.30)	0.079	1.08 (0.93-1.25)	0.31	1.01 (0.86-1.18)	0.95	0.92
CD40LG	0.90 (0.78-1.04)	0.16	0.96 (0.82-1.12)	0.60	0.95 (0.81-1.11)	0.52	0.67
CHIT1	1.11 (0.96-1.28)	0.18	1.02 (0.87-1.20)	0.77	1.01 (0.86-1.20)	0.88	0.72
CPB2	**1.18 (1.02-1.37)**	**0.031**	**1.21 (1.03-1.42)**	**0.020**	**1.21 (1.02-1.43)**	**0.026**	0.12
CRP	**1.49 (1.28-1.72)**	**<0.001**	**1.39 (1.19-1.62)**	**<0.001**	**1.18 (1.00-1.39)**	**0.048**	0.50
CST3	**1.28 (1.10-1.47)**	**<0.001**	**1.20 (1.03-1.40)**	**0.018**	1.14 (0.97-1.35)	0.11	0.51
CTSG	1.10 (0.96-1.26)	0.16	1.04 (0.90-1.21)	0.56	0.96 (0.82-1.12)	0.60	0.32
CXCL10	1.02 (0.88-1.18)	0.82	0.91 (0.77-1.07)	0.26	0.87 (0.73-1.05)	0.15	**0.048**
DCN	**1.24 (1.08-1.42)**	**0.003**	1.16 (1.00-1.34)	0.057	1.14 (0.98-1.34)	0.093	0.43
DPP4	0.98 (0.86-1.13)	0.79	1.00 (0.87-1.16)	0.97	0.99 (0.85-1.16)	0.92	0.70
F12	0.96 (0.84-1.11)	0.58	1.00 (0.86-1.15)	0.95	0.97 (0.83-1.14)	0.72	0.52

Supplemental Table 4a. Odds ratios for MI (cont'd)

Variable	Crude		Sex and age adjusted		Multivariable adjusted†		p-value for sex diff.‡
	OR (95% CI)	p-value	OR (95% CI)	p-value	OR (95% CI)	p-value	
FTH1	1.07 (0.93-1.24)	0.33	0.96 (0.82-1.13)	0.63	0.88 (0.74-1.04)	0.14	0.87
HP	**1.19 (1.04-1.37)**	**0.013**	**1.25 (1.08-1.45)**	**0.003**	1.08 (0.92-1.28)	0.33	0.54
HSPA1B	**1.36 (1.19-1.57)**	**<0.001**	**1.29 (1.12-1.50)**	**<0.001**	**1.20 (1.03-1.40)**	**0.020**	0.91
ICAM1	**1.16 (1.01-1.34)**	**0.035**	1.06 (0.91-1.23)	0.47	0.95 (0.81-1.12)	0.57	0.87
KLKB1	**0.83 (0.72-0.96)**	**0.012**	0.86 (0.73-1.00)	0.055	**0.81 (0.68-0.95)**	**0.012**	0.73
KNG1	0.97 (0.84-1.13)	0.71	0.98 (0.84-1.14)	0.75	0.91 (0.77-1.07)	0.25	0.10
LBP	**1.43 (1.24-1.64)**	**<0.001**	**1.30 (1.13-1.51)**	**<0.001**	1.16 (1.00-1.36)	0.054	0.76
LPa	**1.22 (1.07-1.40)**	**0.003**	**1.23 (1.07-1.42)**	**0.004**	**1.26 (1.09-1.47)**	**0.003**	0.58
MMP3	**1.39 (1.21-1.59)**	**<0.001**	1.08 (0.93-1.27)	0.31	1.14 (0.96-1.34)	0.13	0.16
MMP8	**1.45 (1.26-1.68)**	**<0.001**	**1.38 (1.19-1.62)**	**<0.001**	**1.25 (1.05-1.47)**	**0.011**	0.96
MMP9	**1.53 (1.33-1.77)**	**<0.001**	**1.46 (1.25-1.69)**	**<0.001**	**1.30 (1.10-1.54)**	**0.002**	0.66
MPO	**1.36 (1.18-1.56)**	**<0.001**	**1.26 (1.09-1.46)**	**0.002**	**1.17 (1.00-1.37)**	**0.045**	0.20
NTPROBNP	1.01 (0.88-1.15)	0.90	**0.84 (0.72-0.97)**	**0.019**	0.86 (0.73-1.00)	0.057	**0.037**
PLAUR	1.15 (1.00-1.32)	0.054	1.10 (0.94-1.27)	0.23	0.99 (0.84-1.17)	0.93	0.33
REN	**1.15 (1.00-1.32)**	**0.045**	1.05 (0.90-1.21)	0.54	1.03 (0.87-1.21)	0.76	0.86
SERPINE1	**1.18 (1.03-1.37)**	**0.021**	1.19 (1.02-1.38)	0.029	1.10 (0.93-1.29)	0.27	0.46
SERPINF2	0.93 (0.81-1.08)	0.35	0.94 (0.81-1.10)	0.45	0.94 (0.80-1.11)	0.46	0.32
SHBG	**0.85 (0.74-0.99)**	**0.035**	**0.81 (0.69-0.96)**	**0.015**	0.89 (0.74-1.07)	0.22	0.61
THBS1	0.91 (0.79-1.05)	0.21	0.94 (0.81-1.10)	0.46	0.88 (0.75-1.04)	0.14	0.84
THBS4	**1.21 (1.05-1.39)**	**0.007**	1.16 (1.00-1.34)	0.052	1.13 (0.97-1.32)	0.13	**0.013**
TIMP1	1.09 (0.95-1.25)	0.21	1.05 (0.91-1.22)	0.50	0.96 (0.82-1.12)	0.62	0.13
TIMP4	**1.27 (1.11-1.46)**	**<0.001**	1.17 (0.99-1.38)	0.063	**1.22 (1.02-1.46)**	**0.026**	0.18
TNFRSF11B	**1.17 (1.01-1.35)**	**0.035**	1.01 (0.86-1.18)	0.94	0.94 (0.80-1.11)	0.47	0.91
TNFRSF1B	**1.20 (1.04-1.38)**	**0.012**	1.12 (0.96-1.30)	0.15	0.99 (0.84-1.17)	0.89	0.74
VCAM1	1.15 (1.00-1.32)	0.051	0.97 (0.84-1.12)	0.68	0.99 (0.85-1.16)	0.91	0.69

OR, Odds Ratios; CI, Confidence Intervals.

*OR's per 1 standard deviation change of transformed concentrations calculated in control subjects.

†Adjusted for age, sex, age*sex, blood pressure, blood pressure*blood pressure medication, total cholesterol, HDL cholesterol and daily smoking.

‡Test of interaction between sex and each independent variable in the multivariable adjusted model.

Supplemental Table 4b. Odds ratios for MI in men*. The Tromsø Study

	Crude		Age adjusted		Multivariable adjusted†	
	OR (95% CI)	p-value	OR (95% CI)	p-value	OR (95% CI)	p-value
AGE	**1.61 (1.30-1.99)**	**<0.001**	**1.61 (1.30-1.99)**	**<0.001**	**1.69 (1.33-2.14)**	**<0.001**
SEX	na		na		na	
CHOL	**1.23 (1.01-1.51)**	**0.044**	**1.25 (1.01-1.54)**	**0.039**	**1.29 (1.03-1.61)**	**0.024**
HDL-C	**0.82 (0.67-1.00)**	**0.047**	**0.74 (0.60-0.92)**	**0.006**	**0.72 (0.57-0.89)**	**0.003**
SYSBP	**1.49 (1.20-1.86)**	**<0.001**	**1.35 (1.07-1.69)**	**0.011**	**1.31 (1.03-1.66)**	**0.025**
SMOKE	1.37 (0.91-2.07)	0.13	**1.64 (1.07-2.53)**	**0.024**	**1.71 (1.09-2.67)**	**0.019**
ACE	0.93 (0.77-1.12)	0.44	0.94 (0.77-1.14)	0.52	0.90 (0.73-1.11)	0.32
ADIPOQ	1.02 (0.84-1.25)	0.84	0.88 (0.71-1.09)	0.26	1.02 (0.80-1.29)	0.87
AGER	1.07 (0.85-1.35)	0.56	1.12 (0.88-1.42)	0.37	1.24 (0.95-1.61)	0.11
AGT	0.93 (0.77-1.11)	0.42	0.92 (0.76-1.11)	0.37	0.91 (0.75-1.11)	0.34
AHSG	1.09 (0.89-1.34)	0.40	1.12 (0.91-1.38)	0.29	1.04 (0.83-1.31)	0.72
ANG	1.17 (0.95-1.44)	0.13	1.18 (0.95-1.46)	0.14	1.09 (0.87-1.36)	0.46
APOA1	0.87 (0.70-1.10)	0.24	0.83 (0.66-1.05)	0.13	0.91 (0.69-1.19)	0.47
APOB	**1.57 (1.24-1.98)**	**<0.001**	**1.54 (1.21-1.95)**	**<0.001**	**1.33 (1.02-1.72)**	**0.034**
APOBAPOA1	**1.58 (1.25-1.99)**	**<0.001**	**1.58 (1.24-2.01)**	**<0.001**	**1.35 (1.03-1.76)**	**0.027**
APOC3	1.10 (0.90-1.33)	0.37	1.22 (0.99-1.50)	0.062	1.10 (0.87-1.40)	0.41
BGLAP	1.04 (0.86-1.26)	0.67	1.06 (0.87-1.29)	0.56	1.00 (0.82-1.23)	0.98
BSG	1.06 (0.86-1.30)	0.61	1.02 (0.83-1.27)	0.82	0.99 (0.79-1.24)	0.95
C3	1.12 (0.91-1.37)	0.28	1.15 (0.93-1.41)	0.20	1.07 (0.86-1.33)	0.56
C3B	1.14 (0.94-1.38)	0.19	1.18 (0.97-1.44)	0.11	1.13 (0.91-1.40)	0.26
CCL5	0.92 (0.75-1.14)	0.46	0.93 (0.75-1.15)	0.51	0.94 (0.75-1.17)	0.57
CD14	1.14 (0.94-1.39)	0.17	1.03 (0.84-1.26)	0.76	0.98 (0.80-1.21)	0.85
CD163	1.06 (0.87-1.28)	0.58	1.03 (0.84-1.26)	0.78	0.99 (0.80-1.23)	0.93
CD40LG	0.90 (0.73-1.11)	0.32	0.94 (0.76-1.16)	0.57	0.92 (0.74-1.16)	0.49
CHIT1	1.10 (0.88-1.38)	0.42	1.00 (0.78-1.27)	0.98	1.00 (0.78-1.28)	1.00
CPB2	**1.35 (1.07-1.69)**	**0.011**	**1.41 (1.11-1.79)**	**0.005**	**1.43 (1.11-1.84)**	**0.005**
CRP	**1.55 (1.23-1.94)**	**<0.001**	**1.51 (1.20-1.90)**	**<0.001**	**1.30 (1.02-1.65)**	**0.035**
CST3	**1.35 (1.08-1.69)**	**0.008**	**1.29 (1.03-1.61)**	**0.029**	1.23 (0.97-1.57)	0.084
CTSG	0.96 (0.79-1.17)	0.72	0.98 (0.80-1.20)	0.83	0.90 (0.73-1.12)	0.36
CXCL10	1.11 (0.88-1.40)	0.39	1.00 (0.79-1.27)	0.98	1.03 (0.80-1.32)	0.82
DCN	1.23 (1.00-1.51)	0.051	1.23 (0.99-1.52)	0.059	1.24 (0.99-1.54)	0.056
DPP4	0.99 (0.82-1.19)	0.89	1.01 (0.84-1.23)	0.88	1.03 (0.85-1.26)	0.74
F12	0.99 (0.81-1.23)	0.96	1.07 (0.86-1.33)	0.56	1.03 (0.82-1.29)	0.79

	Crude		Age adjusted		Multivariable adjusted†	
	OR (95% CI)	p-value	OR (95% CI)	p-value	OR (95% CI)	p-value
FTH1	0.88 (0.73-1.07)	0.20	0.94 (0.77-1.14)	0.51	0.90 (0.73-1.11)	0.31
HP	**1.31 (1.07-1.61)**	**0.009**	**1.32 (1.07-1.63)**	**0.008**	1.18 (0.93-1.50)	0.17
HSPA1B	**1.28 (1.05-1.57)**	**0.017**	**1.30 (1.05-1.60)**	**0.016**	1.21 (0.97-1.50)	0.090
ICAM1	1.21 (0.99-1.48)	0.065	1.11 (0.90-1.37)	0.33	1.00 (0.80-1.25)	0.99
KLKB1	0.86 (0.70-1.06)	0.16	0.86 (0.70-1.06)	0.16	0.83 (0.67-1.04)	0.10
KNG1	1.07 (0.87-1.32)	0.54	1.10 (0.88-1.36)	0.40	1.05 (0.84-1.32)	0.66
LBP	**1.43 (1.16-1.77)**	**0.001**	**1.38 (1.11-1.71)**	**0.004**	1.24 (0.99-1.55)	0.065
LPa	**1.36 (1.12-1.65)**	**0.002**	**1.30 (1.07-1.58)**	**0.009**	**1.32 (1.07-1.62)**	**0.009**
MMP3	**1.20 (1.00-1.45)**	**0.048**	1.14 (0.94-1.37)	0.18	**1.24 (1.02-1.52)**	**0.033**
MMP8	**1.37 (1.11-1.69)**	**0.004**	**1.42 (1.14-1.78)**	**0.002**	**1.28 (1.00-1.64)**	**0.047**
MMP9	**1.52 (1.23-1.88)**	**<0.001**	**1.55 (1.25-1.93)**	**<0.001**	**1.42 (1.11-1.80)**	**0.004**
MPO	1.17 (0.96-1.44)	0.12	1.19 (0.97-1.46)	0.10	1.09 (0.87-1.37)	0.44
NTPROBNP	1.18 (0.98-1.42)	0.085	1.01 (0.82-1.24)	0.94	1.01 (0.81-1.25)	0.96
PLAUR	**1.28 (1.03-1.59)**	**0.024**	1.21 (0.97-1.51)	0.091	1.10 (0.86-1.41)	0.44
REN	1.03 (0.84-1.25)	0.79	1.05 (0.85-1.28)	0.66	1.03 (0.83-1.28)	0.80
SERPINE1	1.17 (0.95-1.44)	0.13	**1.24 (1.00-1.55)**	**0.049**	1.17 (0.94-1.47)	0.16
SERPINF2	0.98 (0.81-1.19)	0.86	1.00 (0.82-1.22)	0.99	1.01 (0.82-1.25)	0.90
SHBG	1.05 (0.85-1.30)	0.65	0.85 (0.67-1.08)	0.19	0.88 (0.68-1.14)	0.34
THBS1	0.87 (0.70-1.08)	0.22	0.89 (0.72-1.12)	0.33	0.87 (0.69-1.10)	0.24
THBS4	**1.38 (1.11-1.70)**	**0.003**	**1.36 (1.10-1.68)**	**0.004**	**1.37 (1.10-1.72)**	**0.005**
TIMP1	0.96 (0.79-1.17)	0.71	0.92 (0.75-1.12)	0.41	0.85 (0.69-1.06)	0.14
TIMP4	**1.50 (1.22-1.86)**	**<0.001**	**1.32 (1.05-1.65)**	**0.017**	**1.37 (1.07-1.74)**	**0.011**
TNFRSF11B	1.14 (0.92-1.40)	0.23	0.99 (0.79-1.24)	0.94	0.94 (0.75-1.19)	0.61
TNFRSF1B	1.18 (0.95-1.46)	0.13	1.11 (0.89-1.38)	0.37	1.00 (0.79-1.27)	0.99
VCAM1	1.14 (0.94-1.37)	0.18	1.02 (0.84-1.24)	0.82	1.03 (0.84-1.26)	0.78

OR, Odds Ratios; CI, Confidence Intervals.

*OR's per 1 standard deviation change of transformed concentrations calculated in control subjects.

†Adjusted for age, sex, age*sex, blood pressure, blood pressure*blood pressure medication, total cholesterol, HDL cholesterol and daily smoking.

Supplemental Table 4c. Odds ratios for MI in women*. The Tromsø Study

	Crude		Age adjusted		Multivariable adjusted†	
	OR (95% CI)	p-value	OR (95% CI)	p-value	OR (95% CI)	p-value
AGE	**2.24 (1.77-2.84)**	**<0.001**	**2.24 (1.77-2.83)**	**<0.001**	**2.14 (1.63-2.80)**	**<0.001**
SEX	na		na		Na	
CHOL	**1.53 (1.26-1.86)**	**<0.001**	**1.35 (1.09-1.66)**	**0.005**	**1.38 (1.11-1.72)**	**0.004**
HDL-C	0.84 (0.70-1.01)	0.060	**0.74 (0.60-0.90)**	**0.003**	**0.73 (0.60-0.90)**	**0.003**
SYSBP	**1.78 (1.45-2.18)**	**<0.001**	**1.49 (1.20-1.86)**	**<0.001**	**1.58 (1.26-2.00)**	**<0.001**
SMOKE	1.43 (0.96-2.13)	0.078	**2.06 (1.32-3.22)**	**0.002**	**2.35 (1.47-3.78)**	**<0.001**
ACE	1.15 (0.94-1.40)	0.18	1.14 (0.92-1.40)	0.23	1.22 (0.98-1.53)	0.082
ADIPOQ	1.02 (0.84-1.24)	0.86	0.80 (0.64-1.00)	0.054	0.91 (0.70-1.17)	0.46
AGER	0.83 (0.69-1.00)	0.055	0.89 (0.72-1.09)	0.25	0.92 (0.74-1.14)	0.43
AGT	1.07 (0.88-1.29)	0.52	1.06 (0.86-1.30)	0.60	1.13 (0.91-1.41)	0.27
AHSG	1.18 (0.97-1.44)	0.10	1.15 (0.93-1.42)	0.21	1.10 (0.88-1.38)	0.40
ANG	1.05 (0.86-1.28)	0.65	0.97 (0.78-1.20)	0.77	0.97 (0.77-1.23)	0.82
APOA1	0.85 (0.69-1.04)	0.12	0.74 (0.58-0.93)	0.012	0.85 (0.66-1.11)	0.23
APOB	**1.33 (1.09-1.62)**	**0.004**	**1.34 (1.09-1.65)**	**0.006**	1.11 (0.88-1.41)	0.37
APOBAPOA1	**1.37 (1.13-1.66)**	**0.002**	**1.44 (1.17-1.77)**	**<0.001**	1.18 (0.93-1.50)	0.18
APOC3	1.00 (0.81-1.22)	0.96	0.98 (0.79-1.21)	0.84	0.85 (0.66-1.10)	0.21
BGLAP	1.13 (0.93-1.37)	0.23	1.08 (0.88-1.33)	0.47	1.11 (0.89-1.39)	0.36
BSG	1.09 (0.89-1.32)	0.40	0.94 (0.76-1.16)	0.57	0.88 (0.70-1.12)	0.30
C3	0.83 (0.67-1.02)	0.083	**0.79 (0.63-1.00)**	**0.045**	**0.69 (0.54-0.89)**	**0.004**
C3B	**0.80 (0.66-0.98)**	**0.035**	**0.80 (0.64-0.99)**	**0.042**	**0.73 (0.57-0.93)**	**0.011**
CCL5	0.90 (0.74-1.11)	0.33	0.93 (0.75-1.16)	0.54	0.79 (0.62-1.01)	0.060
CD14	1.13 (0.92-1.38)	0.23	1.06 (0.86-1.31)	0.59	1.07 (0.85-1.34)	0.55
CD163	**1.22 (1.01-1.49)**	**0.044**	1.12 (0.90-1.38)	0.31	1.04 (0.82-1.31)	0.76
CD40LG	0.87 (0.71-1.07)	0.18	0.99 (0.80-1.23)	0.95	0.98 (0.78-1.25)	0.89
CHIT1	1.14 (0.94-1.39)	0.18	1.06 (0.86-1.30)	0.59	1.04 (0.83-1.29)	0.76
CPB2	1.11 (0.91-1.37)	0.31	1.01 (0.81-1.26)	0.91	1.07 (0.84-1.35)	0.59
CRP	**1.38 (1.13-1.68)**	**0.002**	**1.29 (1.04-1.59)**	**0.020**	1.13 (0.89-1.43)	0.32
CST3	**1.27 (1.04-1.54)**	**0.018**	1.12 (0.91-1.38)	0.28	1.10 (0.88-1.39)	0.41
CTSG	1.14 (0.93-1.39)	0.20	1.11 (0.90-1.36)	0.35	1.04 (0.83-1.30)	0.73
CXCL10	1.00 (0.81-1.22)	0.97	0.82 (0.65-1.03)	0.095	**0.74 (0.57-0.97)**	**0.028**
DCN	1.19 (0.98-1.45)	0.086	1.06 (0.85-1.32)	0.58	1.09 (0.86-1.37)	0.49
DPP4	1.02 (0.84-1.25)	0.81	0.97 (0.78-1.21)	0.80	0.95 (0.75-1.21)	0.69
F12	0.98 (0.80-1.18)	0.80	0.89 (0.72-1.10)	0.29	0.94 (0.75-1.18)	0.59

	Crude		Age adjusted		Multivariable adjusted†	
	OR (95% CI)	p-value	OR (95% CI)	p-value	OR (95% CI)	p-value
FTH1	1.06 (0.85-1.31)	0.62	0.95 (0.76-1.20)	0.69	0.85 (0.67-1.10)	0.21
HP	1.19 (0.97-1.46)	0.092	1.17 (0.95-1.46)	0.15	1.02 (0.80-1.29)	0.90
HSPA1B	**1.34 (1.11-1.62)**	**0.002**	**1.27 (1.04-1.55)**	**0.019**	1.21 (0.98-1.50)	0.079
ICAM1	1.08 (0.88-1.31)	0.47	1.03 (0.83-1.28)	0.77	0.93 (0.74-1.17)	0.52
KLKB1	0.88 (0.72-1.09)	0.25	0.85 (0.68-1.07)	0.17	**0.77 (0.60-0.99)**	**0.044**
KNG1	0.90 (0.73-1.11)	0.32	0.84 (0.66-1.06)	0.13	0.79 (0.61-1.01)	0.060
LBP	**1.38 (1.13-1.67)**	**0.001**	1.23 (1.00-1.51)	0.052	1.13 (0.90-1.42)	0.29
LPa	1.18 (0.97-1.43)	0.095	1.19 (0.96-1.46)	0.11	1.21 (0.97-1.52)	0.093
MMP3	1.13 (0.94-1.37)	0.20	1.01 (0.82-1.24)	0.93	1.02 (0.82-1.27)	0.85
MMP8	**1.37 (1.12-1.67)**	**0.002**	**1.32 (1.07-1.63)**	**0.011**	1.22 (0.96-1.54)	0.098
MMP9	**1.40 (1.15-1.71)**	**<0.001**	**1.34 (1.08-1.66)**	**0.007**	1.24 (0.98-1.56)	0.069
MPO	**1.41 (1.16-1.71)**	**<0.001**	**1.30 (1.07-1.60)**	**0.010**	**1.28 (1.03-1.60)**	**0.027**
NTPROBNP	0.87 (0.71-1.05)	0.15	**0.71 (0.57-0.88)**	**0.002**	**0.72 (0.57-0.91)**	**0.006**
PLAUR	1.04 (0.86-1.27)	0.68	1.01 (0.82-1.24)	0.94	0.90 (0.71-1.13)	0.36
REN	1.06 (0.88-1.29)	0.52	1.03 (0.84-1.26)	0.78	1.04 (0.84-1.30)	0.71
SERPINE1	1.08 (0.89-1.32)	0.43	1.12 (0.91-1.39)	0.28	1.04 (0.83-1.31)	0.73
SERPINF2	0.89 (0.72-1.10)	0.28	0.88 (0.71-1.10)	0.28	0.86 (0.67-1.10)	0.22
SHBG	0.89 (0.72-1.08)	0.24	0.82 (0.66-1.02)	0.071	0.92 (0.72-1.18)	0.53
THBS1	0.95 (0.78-1.17)	0.65	0.99 (0.80-1.23)	0.92	0.89 (0.70-1.14)	0.36
THBS4	1.08 (0.89-1.32)	0.43	0.95 (0.76-1.19)	0.66	0.93 (0.73-1.18)	0.56
TIMP1	1.19 (0.98-1.45)	0.085	1.23 (0.99-1.52)	0.057	1.09 (0.87-1.36)	0.47
TIMP4	**1.36 (1.12-1.66)**	**0.002**	1.01 (0.81-1.27)	0.90	1.10 (0.86-1.42)	0.44
TNFRSF11B	**1.24 (1.01-1.52)**	**0.039**	1.01 (0.81-1.27)	0.92	0.95 (0.74-1.21)	0.66
TNFRSF1B	1.21 (0.99-1.47)	0.066	1.13 (0.92-1.40)	0.24	0.98 (0.77-1.24)	0.86
VCAM1	1.07 (0.87-1.31)	0.52	0.92 (0.74-1.15)	0.48	0.96 (0.77-1.22)	0.76

OR, Odds Ratios; CI, Confidence Intervals.

*OR's per 1 standard deviation change of transformed concentrations calculated in control subjects.

†Adjusted for age, sex, age*sex, blood pressure, blood pressure*blood pressure medication, total cholesterol, HDL cholesterol and daily smoking.