Multiple Inflammatory Biomarkers in Relation to Cardiovascular Events and Mortality in the Community

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Abstract

Objective—Evidence suggests that chronic low-grade inflammation and oxidative stress are related to cardiovascular disease (CVD) and mortality.

Approach and Results—We examined 11 established and novel biomarkers representing inflammation and oxidative stress (C-reactive protein [CRP], fibrinogen, interleukin-6, intercellular adhesion molecule-1 [ICAM-1], lipoprotein-associated phospholipase A2 (mass and activity), monocyte chemoattractant protein-1, myeloperoxidase, CD40 ligand, P-selectin, tumor necrosis factor receptor II [TNFRII]) in relation to incident major CVD and mortality in the community. We studied 3035 participants (mean age 61±9 years, 53% women). During follow-up (median 8.9 years), 253 participants experienced a CVD event and 343 died. CRP (hazard ratios [HR] reported per standard deviation ln-transformed biomarker, 1.18, 95% confidence interval [CI] 1.02-1.35; nominal P=0.02) and TNFRII (HR 1.15, 95% CI; 1.01-1.32; nominal P=0.04) were retained in multivariable-adjusted models for major CVD, but were not significant after adjustment for multiple testing. The biomarkers related to mortality were TNFRII (HR 1.33, 95% CI: 1.19-1.49; P<0.0001); ICAM-1 (HR 1.24, 95% CI: 1.12-1.37; P<0.0001), and interleukin-6 (HR 1.25, 95% CI: 1.12-1.39; P<0.0001). The addition of these markers to the model including traditional risk factors increased discrimination and reclassification for risk of death (P<0.0001), but not for CVD.

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Conclusions—Of 11 biomarkers, TNFRII was associated nominally with incident major CVD, and significantly with all-cause mortality, which renders it an interesting target for future research. The combination of TNFRII with CRP in relation to CVD and with interleukin-6 to mortality increased the predictive ability in addition to CVD risk factors for total mortality but not for incident CVD.

Keywords

mortality; cardiovascular disease; inflammation; epidemiology; cohort

Cardiovascular diseases (CVD), diabetes, chronic lower respiratory disease, arthritis, and cancer are the major causes of associated morbidity and mortality in aging populations such as the United States (http://www.cdc.gov/nchs/nhis.htm). Each of these conditions is associated with a pro-inflammatory state.[1-3] On the contrary, avoidance of CVD risk factors and the absence of low-grade chronic inflammation delay or prevent onset of disease and are strongly related to survival and successful aging.[4,5]

Circulating fibrinogen, C-reactive protein (CRP) and white blood count were the first inflammatory biomarkers investigated in large-scale studies of initially healthy individuals, mostly in relation to cardiovascular events.[6-9] All three biomarkers are relatively nonspecific and represent the common final path at the end of the inflammatory cascade. More recently other biomarkers representing different stages of the inflammatory pathway and oxidative stress have become available. CD40 ligand, intercellular adhesion molecule-1 (ICAM-1) and P-selectin initiate cell adhesion and transmigration; cytokines (e.g. tumor necrosis factor receptor II (TNFRII)) and chemokines (monocyte chemoattractant protein-1) induce leukocyte recruitment and the acute-phase response.[10] Pro-inflammatory actions and oxidative stress are reflected by lipoprotein-associated phospholipase A2 activity and mass, whereas myeloperoxidase originates from the oxidative burst that is part of anti-microbial defense.

To further elucidate the role of inflammatory biomarkers representing distinct pathophysiological pathways, we examined the association of diverse inflammatory blood biomarkers together and individually with risk of CVD and mortality in the community-based Framingham Heart Study.

MATHERIALS AND METHODS

Details on materials and methods are provided in the online Supplement.

RESULTS

Participant Characteristics

The baseline characteristics of our sample are shown in Table 1. The cohort consisted of middle-aged to older adults (mean age 61±9 years) and 53% women. At baseline, 7% of the study sample had major CVD. During a median follow-up of 8.9 years (maximum 11.3 years), 253 participants experienced incident major CVD events and 343 died (CVD death=80, cancer death n=142, other or unknown n=121). Baseline characteristics of individuals excluded from analysis were similar to participants who entered analyses (Supplementary Table I).

Prediction of CVD

Supplementary Table II presents the multivariable-adjusted association of the separate inflammatory biomarkers with major CVD incidence among the 2827 participants free of

major CVD at the 7th examination cycle. TNFRII and CRP were individually associated with major incident CVD. With stepwise selection CRP (hazard ratio [HR] per ln biomarker standard deviation 1.18, 95% confidence interval [CI]: 1.02-1.35; nominal P=0.02) and TNFRII (HR, 1.15, 95% CI: 1.01-1.32; nominal P=0.04) were retained as predictors of major CVD (Table 2). After Bonferroni correction on the number of principal components, none of the biomarkers retained statistical significance.

The two selected biomarkers did not significantly improve the discrimination ability for CVD of the model comprising traditional risk factors (c-statistic of 0.769, 95% CI: 0.743-0.796 before, and 0.773, 95% CI: 0.746-0.80 after adding the biomarkers). The estimated increment of c-statistic was 0.0038 (95% CI: −0.0023-0.010, P=0.11). The Hosmer-Lemeshow statistic showed adequate calibration between observed and predicted mortality risk of the final model (X^2=13.2, df=10, P=0.15).

We also performed reclassification analyses for CVD risk categories over 10 years (Table 3). Net-reclassification improvement was not statistically significant (-1.1%, P=0.62).The relative integrated discrimination improvement was 7.9%; P=0.004, the integrated discrimination improvement, 0.9%; P=0.0039. Supplementary Figure I shows sex-specific adjusted survival curves by tertile of the biomarker score incorporating the top two inflammatory markers. Decreased survival was observed across tertiles.

Prediction of Mortality

Supplementary Table III provides the HRs per standard deviation of natural log-transformed biomarkers for multivariable-adjusted single biomarker associations with mortality. Four of the 11 markers were individually associated with mortality: CRP, ICAM-1, interleukin-6, and TNFRII (all P<0.0001). In the stepwise biomarker model the three markers selected were ICAM-1, interleukin-6, and TNFRII (all P<0.0001) (Table 4). TNFRII revealed the highest HR (HR 1.33, 95% CI: 1.19-1.49).

The additional adjustment for interim CVD marginally decreased the estimated HRs, but the same set of biomarkers was selected in the final model. Figure 1 shows sex-specific adjusted survival curves by tertile of the biomarker score incorporating the top three inflammatory markers. Decreased survival was observed across tertiles. Whereas the two lower tertiles differed little, the event rate clearly was highest in the upper tertile.

The most statistically significant biomarker, TNFRII alone in the model for death incorporating clinical risk factors had higher model discrimination (c-statistic of 0.789 (95% CI, 0.765 to 0.813) rose to 0.799 (95% CI, 0.776 to 0.823). The three selected biomarkers combined only marginally improved the discrimination ability of the model for death beyond traditional risk factors, with c-statistic 0.811 (95% CI, 0.788 to 0.834) after adding biomarkers. The estimated increment of c-statistic was 0.022 (95% CI, 0.011 to 0.033, P<0.0001). The Hosmer-Lemeshow statistic showed adequate calibration between observed and predicted mortality risk in the final model (X^2=12.5, df=10, P=0.19). The relative integrated discrimination improvement index was 25.7%; P<0.0001; integrated discrimination improvement 5.1%, p<0.0001. For the top biomarker, TNFRII, alone, the relative integrated discrimination improvement index was 17.9%, P<0.0001, integrated discrimination improvement 3.6%, P<0.0001.

We tested reclassification for three categories of risk of death over 10 years <5%, 5-10% and >10% (Table 5). The net-reclassification improvement was 9.4%, P<0.0001. The NRI table shows that there is substantial downward reclassification in those who survived (net downward 212/2697=8.0%), but little reclassification in those who died (net upward 5/338=1.5%). We have shaded cells representing favorable reclassification green and

unfavorable reclassification red to further emphasize the utility of reclassification metrics. Thus, net reclassification was mainly driven by a downward reclassification of those who survived, whereas there was little upward reclassification of those who died.

Secondary Analyses

We did not observe significant interactions by age or sex for the two biomarkers in relation to incident CVD and the three biomarkers that were associated with mortality. Accounting for estimated glomerular filtration rate, hormone replacement, aspirin or lipid treatment did not change the associations of the three markers with mortality markedly (data not shown).

DISCUSSION

Principal Findings

In our large contemporary community-based cohort, circulating TNFRII was associated with both CVD events and mortality over a follow-up period of about 10 years. CRP and TNFRII were nominally associated with major CVD, but not after adjustment for multiple testing. Furthermore, they did not substantively improve the risk prediction model based on classical CVD risk factors. TNFRII, ICAM-1 and interleukin-6 were selected into the final model for death using a stepwise procedure. We showed significant discrimination and reclassification increments for mortality when TNFRII was assessed in addition to classical risk factors. The combined incorporation of the top three biomarkers only modestly improved the c-statistic and reclassification metrics. The relation between the selected markers and mortality was similar in models accounting for interim CVD, suggesting that they provide risk information beyond CVD. Overall, our data on a large panel of biomarkers representing varying aspects of inflammation extend the knowledge on the association of inflammation with incident CVD and death in the community.[9,11-13]

The mechanisms relating TNFRII to CVD and mortality are uncertain. Tumor necrosis factor-alpha cytokine is central to the inflammatory cascade that promotes the acute-phase response.[14] The more stable TNFRII may be a good indicator of the biological effects of the tumor necrosis factor-alpha system. In the cardiovascular system, tumor necrosis factor-alpha signaling via TNFRII is protective in post-ischemic recovery in adult myocardium.[15] In mice, endothelial TNFRII plays a critical role in arteriogenesis and ischemia-mediated adaptive angiogenesis.[16] TNFRII blood concentrations have been related to autoimmune diseases, their prognosis and the response to anti-tumor necrosis factor-alpha therapy.[17-20] Higher concentrations of circulating TNFRII have been reported with advancing age[21] and in overweight individuals.[21] We and others found an association of TNFRII with mean arterial pressure, hypertension treatment, arterial stiffness, endothelial function, ankle-brachial index, and kidney disease, all of which are strong predictors of overall mortality.[22-27] More specifically, moderately increased TNFRII concentrations are observed in atherosclerosis[28] and coronary artery disease,[29] and TNFRII has been related to the risk of CVD in women with diabetes and in smaller study samples.[30,31] However, the relation with CVD is not completely consistent; no significant relation was observed in a cross-sectional study of older adults.[32] Our findings extend the epidemiological evidence relating TNFRII to CVD and death in an unselected community-based sample.

The glycoprotein ICAM-1 is expressed in different cell types. The membrane-bound form on endothelial cells is involved in early stages of inflammatory processes by facilitating the transmigration of leukocytes through the interaction with lymphocyte function-associated antigen.[33] A strong relation has been demonstrated with viral infections,[34] graft rejection after transplantation,[35] endothelial function,[36] CVD,[37] and cancer.[38] Elevated ICAM-1 concentrations have been reported to be associated with increased all-cause mortality in a

smaller sample of individuals aged 65 years or older, which may be explained by the relation to CVD.[39] With our current findings, we can extend these observations to middle-age to older community-dwelling adults in the context of other inflammatory biomarkers.

An association of the correlated biomarkers CRP and interleukin-6 with overall mortality has been reported.[40-42] CRP was not selected into the final model for the outcome of death, likely due to its correlation with the already selected markers, especially interleukin-6 (r=0.47 in our sample). Most inflammatory processes end in the downstream activation of the interleukin-6, CRP pathways. Thus, they are relatively nonspecific acute-phase markers induced by infections, cancer, tissue damage, and inflammation.[43] Whereas some studies found CRP improved risk prediction for CVD,[9,44] some studies reported otherwise after accounting for standard risk factors.[7,45] Our well-characterized sample provides supportive evidence for the role of the moderately correlated CRP and interleukin-6 as risk predictors of mortality and CVD. Our results do not indicate a clear superiority of either of the two biomarkers.

Overall, several easily measurable inflammatory biomarkers were related to all-cause mortality. The exact mechanisms through which TNFRII, ICAM-1 and interleukin-6 are associated with increased risk of death are not well-established. Further research is needed to unravel the pathophysiology of inflammation in healthy aging and disease. A better characterization on the community level is needed to identify causal pathways and define interventional strategies.

Strengths and Limitations

Strengths of the study are the well-characterized community-based sample with routine ascertainment of potential clinical confounders, near complete follow-up, and a comprehensive and standardized adjudication process for outcomes. A broad range of biomarkers representing different phases and pathways of inflammation measured with strict quality control also were assets. We were able to demonstrate significant improvement in model discrimination and reclassification by incorporating the three markers in addition to traditional risk factors of mortality. Since widely applied risk categories for mortality are not available from the literature,[46] we defined them to ensure that there were adequate numbers of events in each category (without reference to the inflammatory biomarkers). We concede that the risk categories for mortality lack external validation, proof of clinical relevance, and might vary in other datasets. Due to a moderate number of outcomes we did not have the power to perform subgroup analyses for cause-specific mortality (e.g., cardiovascular mortality, cancer mortality). Due to limited numbers of individuals with cardiovascular medication such as statins, aspirin and hormone replacement therapy and a variety of biases (e.g. indication) in a community-based sample, our analyses adjusting for cardiac medications remain exploratory and need refinement in better suited samples. Furthermore, despite long-term follow-up with carefully adjudicated outcomes, the sample size does not permit us to identify smaller associations. Lack of statistical power may be one of the reasons of non-replication of findings from large consortia as seen for Lp-PLA2.[47] Apart from overall small effect sizes, non-replication of published associations of inflammatory biomarkers such as CD40 ligand, monocyte chemoattractant protein-1, myeloperoxidase, and P selectin in multivariable models may be due to our rigorous cardiovascular risk factor adjustment, the sample structure of community-based individuals compared to often smaller clinical samples, presenting with acute disease states.

We acknowledge that the clinical relevance of our data is uncertain because it may not be cost-effective to measure three biomarkers as long as a specific, targeted intervention to reduce mortality based on this knowledge is not available. In addition, our findings will need to be replicated in external cohorts. Finally, the pathophysiological background for our

findings and potential causal mechanisms need to be examined, which will require further investigation in the experimental setting.

In conclusion, we report estimates of the associations of eleven distinct circulating inflammatory biomarkers in a community-based cohort with CVD and all-cause mortality during nearly a decade of follow-up. Multivariable-selection analyses revealed TNFRII was nominally predictive of CVD and significantly predictive of mortality. However, the magnitude and strength of association were modest. Even the combination of information on TNFRII and CRP for incident CVD and TNFRII and interleukin-6 for mortality increased the predictive ability of a model consisting of classical CVD risk factors only modestly, if at all. Embedded in the ongoing discussion of the clinical utility of the measurement of circulating inflammatory biomarkers[48] in addition to classical CVD risk factors for risk prediction in the community, our results thus speak against a direct clinical application for risk determination. Rather, our data may be of interest to clinicians and scientists concerned about "inflammaging", i.e. how alterations of the inflammatory system contribute to immunosenescence, frailty and increased risk for death.[49] Our findings may stimulate future research at the epidemiologic as well as basic science level, to establish the pathophysiological role of the inflammatory marker TNFRII as a risk marker for CVD events and mortality in the middle-aged to older individuals.

MATERIALS AND METHODS

Study Sample

Individuals (n=5124) were recruited to the Framingham Offspring cohort, in the early 1970s and were followed by examinations every four to eight years.(1) The seventh examination cycle was attended by 3539 participants (1998 2001); inflammatory biomarkers were measured in 3325 attendees. For the present investigation, we excluded individuals missing any inflammatory biomarker measurements (n=286), incomplete or missing follow up (n=2), and missing covariate data (n=2), leaving 3035 participants (85.8% of all examination 7 attendees) for analysis. The study protocols were reviewed and approved by the Boston University Medical Center Institutional Review Board. Participants provided informed consent at every examination cycle. All authors have read and agreed to the manuscript as written.

Clinical Evaluations & Follow-up

Health history updates were performed biennially by Framingham staff. At the Framingham clinic questionnaires, physician administered interviews and physical examinations, and laboratory assessments provided information on CVD risk factors. Assessment of current smoking, alcohol consumption, and medications relied on self report. Clinic physicians performed blood pressure measurements with seated participants; two systolic blood pressure values were averaged. Diabetes was defined as an elevated fasting blood glucose 126 mg/dL or the use of diabetes drugs.

Follow up was cutoff at December 31, 2009. Routine collection of participants' physician office visit and hospitalization records was performed by Framingham Heart Study staff. For death reviews official death certificates and medical records were acquired. If medical records were unavailable, next of kin were contacted. Major CVD events, defined as myocardial infarction, coronary insufficiency (prolonged angina and electrocardiographic alterations), heart failure, or stroke, including deaths resulting from these diseases, were regularly adjudicated by a committee of three investigators using standardized written criteria.(2)

Biomarker determination

Venous blood samples were obtained from fasting participants and immediately processed and stored at −80°C. Eleven inflammatory markers were measured: plasma CD40 ligand, CRP, fibrinogen, lipoprotein associated phospholipase A2 activity and mass, P selectin, and TNFRII, and serum ICAM 1, interleukin 6, monocyte chemoattractant protein 1, and myeloperoxidase. Characteristics of measurement kits, test properties and blood specimens have been published.(3) Mean inter assay coefficients of variation were less than 10% for all markers. Correlations among biomarkers, clinical correlates, heritability and genetic variation in inflammatory SNPs in relation to the biomarker panel have been reported previously.(4)

Statistical Analyses

We applied natural logarithmical (ln) transformation to biomarker values to normalize their skewed distributions and we carried out sex pooled standardization (mean 0, standard deviation [SD] 1) prior to analyses. We used multivariable adjusted Cox proportional hazards regression models to relate inflammatory biomarkers to occurrence of CVD events and death after confirming that the assumption of proportionality of hazards was met.(5) We forced clinical covariates for the multivariable prospective analyses of incident CVD and mortality into the model. We based the covariate selection on prior publications.(6) We included age, sex, current smoking, body mass index, systolic blood pressure, total/high density lipoprotein cholesterol, diabetes, hypertension treatment, as an indicator of elevated blood pressure, and prevalent major CVD. Since we were interested in the prediction of first onset of CVD, participants with prevalent major CVD were excluded from analyses of incident major CVD. We further investigated whether the association of inflammatory markers with mortality was due to occurrence of interim major cardiovascular events. We performed the final inflammatory marker stepwise selection model by incorporating CVD as a time dependent variable. Regression coefficients were calculated for one SD increase in ln transformed biomarker concentrations.

We calculated the c statistics before and after adding the significant biomarkers to examine model discrimination with respect to risk of CVD and death over 10 years. We further assessed net reclassification improvement for risk categories <6%, 6 20%, >20% risk for CVD,(7) and categories of <5%, 5 10%, >10% risk for mortality(8) during follow up. Mortality risk categories were chosen to ensure that there were adequate numbers of events in each category independent of the relation to the biomarkers. We calculated integrated discrimination improvement using a recently published method.(7)

We used a stepwise procedure to select biomarker(s) associated with outcomes with two sided P<0.05 for retention. A biomarker score was created for each participant as a sum of (biomarker*coefficient) for biomarkers retained in the final models for CVD and mortality. Adjusted survival curves were calculated using corrected group prognosis method(9) implemented in a SAS macro by Zhang et al.(10) Event free survival curves were generated by tertile of the biomarker score for each sex.

Secondary Analyses

The biomarkers retained in the final models were tested for their interactions with age and sex. In addition, we examined whether adjustment for estimated glomerular filtration rate calculated according to the Modification of Diet in Renal Disease Study formula,(11) hormone replacement therapy, aspirin intake or lipid treatment substantively changed our results.

The 11 biomarkers were correlated. In a covariate adjusted principal component analysis, we found that among them there were only 4 principal components with eigenvalues greater than one. To account for multiple testing, we used Bonferroni correction approach on the number of principal components. A P value <0.0125 (0.05/4) was the significance criterion for both endpoints – CVD and mortality. Analyses were performed using SAS version 9.2 (Cary, North Carolina, http://www.sas.com/presscenter/guidelines.html) statistical software.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

Abbreviations

CVD	cardiovascular disease
CI	confidence Interval
CRP	C-reactive protein
HR	hazard ratio
ICAM-1	intercellular adhesion molecule-1
SD	standard deviation
TNFRII	tumor necrosis factor receptor II

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SIGNIFICANCE

There is an ongoing discussion on the clinical utility of the measurement of circulating inflammatory biomarkers for cardiovascular risk prediction. In our middle-aged to older community-based cohort we report the associations of 11 distinct circulating inflammatory biomarkers with first major cardiovascular disease [CVD] events and all-cause mortality during long-term follow-up. In multivariable-adjusted analyses tumor necrosis factor receptor II [TNFRII] was nominally related to CVD and significantly predictive of mortality. The magnitude and strength of association were modest. Even the combination of the two strongest biomarkers, TNFRII and C-reactive protein for incident CVD and TNFRII and interleukin-6 for mortality increased the predictive ability of a model consisting of classical CVD risk factors only modestly. Our findings may stimulate future research to understand the pathophysiological role of TNFRII and other inflammatory biomarkers in the process of aging, CVD risk and mortality.

Men

Score	0	2	4	6	8	10
Low	470	470	468	459	399	74
Medium	472	469	460	447	396	83
High	470	439	414	379	312	55

No. at Risk

Women

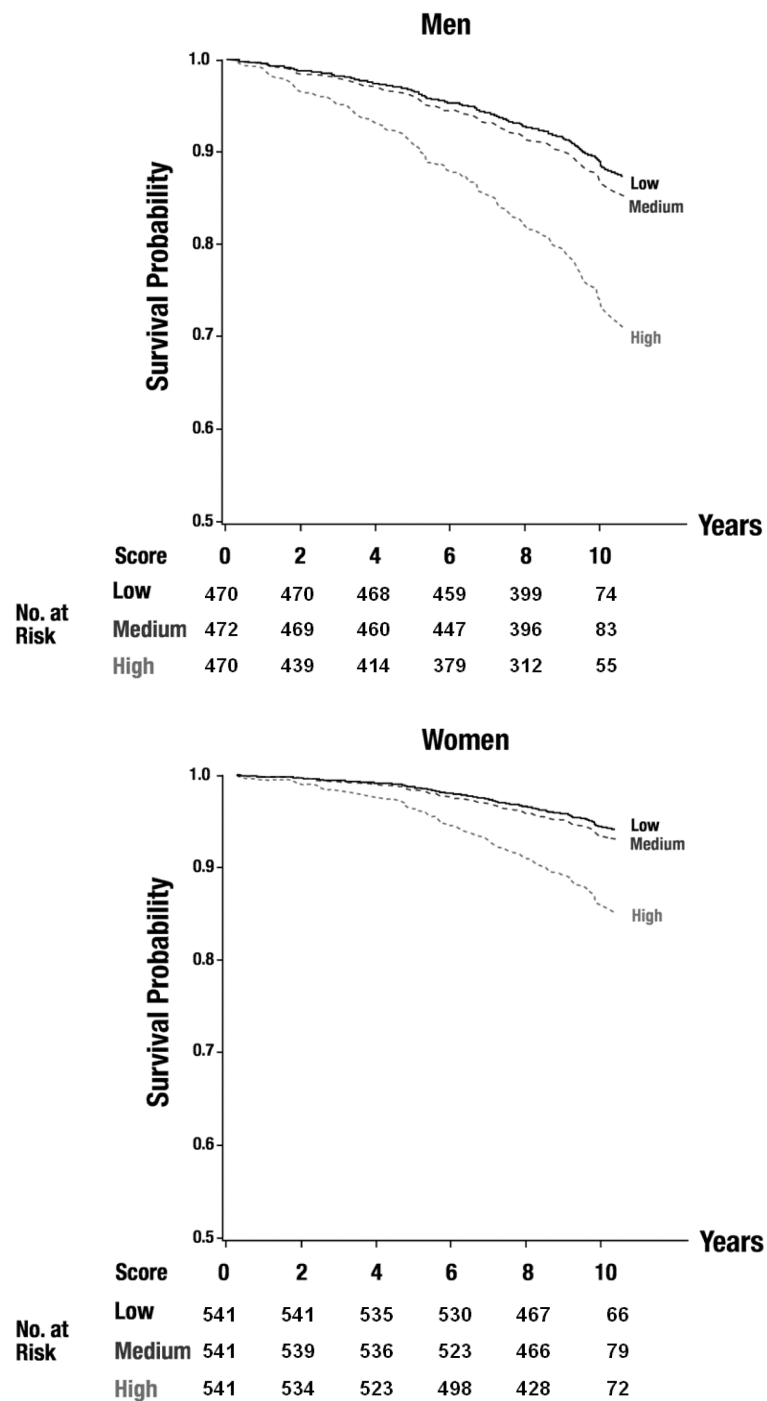| Score	0	2	4	6	8	10
Low	541	541	535	530	467	66
Medium	541	539	536	523	466	79
High	541	534	523	498	428	72

No. at Risk

Figure 1.
Adjusted survival curves for tertiles of the biomarker score (including TNFRII, interleukin-6, ICAM-1) for men (A) and women (B), $P_{\text{log rank}}<0.0001$ for both. The numbers of participants at risk are displayed in the figure.

Table 1

Baseline Characteristics of the Study Sample

	Overall cohort (n=3035)
Age, years	61±9
Women, %	1623 (53)
Cigarette smoking, %	398 (13)
Body mass index, kg/m^2	28.1±5.3
Systolic blood pressure, mm Hg	127±19
Diabetes, %	400 (13)
Total/high density lipoprotein cholesterol	4.1±1.3
Hypertension treatment, %	1028 (34)
Lipid treatment, %	622 (20)
Aspirin ≥3 per week, %	963 (32)
Hormone replacement therapy, %	499 (16)
Prevalent cardiovascular disease, %	208 (7)

Data are presented as mean±SD for continuous variables and n (percentage) for dichotomous variables.

Table 2

Final Model from Stepwise Selection Analysis for Inflammatory Biomarkers in Relation to CVD

Variable	Hazard Ratio	95% Confidence	Interval	Nominal *P*-Value
C-reactive protein	1.18	1.02	1.35	0.02
Tumor necrosis factor receptor II	1.15	1.01	1.32	0.04

The covariates included age, sex, current smoking, body mass index, systolic blood pressure, total/high density lipoprotein cholesterol, diabetes, and hypertension treatment. Prevalent major CVD were excluded from this analysis.

Hazard ratios are per one standard deviation increase in ln-biomarker concentration.

Table 3

Reclassification Matrix for CVD During 10 Years of Follow-up

Without Biomarkers	With TNFRII, CRP			
	<6%	6-20%	>20%	Total
Participants without CVD events				
<6%	1142 (94.5)	67 (5.5)	0 (0.0)	**1209**
6-20%	73 (7.1)	904 (88.5)	45 (4.4)	**1022**
>20%	0 (0.0)	41 (12.0)	302 (88.1)	**343**
Total	**1215**	**1012**	**347**	**2574**
Participants with CVD events				
<6%	27 (90.0)	3 (10.0)	0 (0.0)	**30**
6-20%	7 (6.5)	91 (84.3)	10 (9.3)	**108**
>20%	0 (0.0)	9 (7.8)	106 (92.2)	**115**
Total	**34**	**103**	**116**	**253**

Analyses were censored at 10-year follow-up. Provided are the number of individuals and row percent. Net-reclassification improvement was −1.1%, P=0.62. Participants with prevalent major CVD were excluded from analyses of incident major CVD resulting in N=253 individuals for analysis. Green shaded cells represent favorable reclassification, red shaded cells unfavorable reclassification.

Table 4

Final Model from Stepwise Selection Analysis for Inflammatory Biomarkers in Relation to Overall Mortality

Variable	Hazard Ratio	95% Confidence Interval		Nominal P-Value
Multivariable model				
Tumor necrosis factor receptor II	1.33	1.19	1.49	<0.0001
Intercellular adhesion molecule-1	1.24	1.12	1.37	<0.0001
Interleukin-6	1.25	1.12	1.39	<0.0001
Multivariable Model + Interim CVD				
Tumor necrosis factor receptor II	1.29	1.15	1.44	<0.0001
Intercellular adhesion molecule-1	1.23	1.11	1.36	<0.0001
Interleukin-6	1.23	1.10	1.38	0.0002

The covariates included age, sex, smoking, systolic blood pressure, body mass index, total/HDL cholesterol, hypertension treatment, diabetes and interim CVD (time-dependent variable).

Hazard ratios are per one standard deviation increase in log-biomarker concentration.

Table 5

Reclassification matrix for 10-year mortality

Without Biomarkers	With TNFRII, ICAM-1, interleukin-6			
	<5%	5-10%	>10%	Total
Participants surviving				
<5%	989 (92.3)	73 (6.8)	9 (0.8)	**1071**
5-10%	188 (31.1)	335 (55.4)	82 (13.6)	**605**
>10%	9 (0.9)	179 (17.5)	833 (81.6)	**1021**
Total	**1186**	**587**	**924**	**2697**
Participants who died				
<5%	19 (73.1)	4 (15.4)	3 (11.5)	**26**
5-10%	5(13.2)	19 (50.0)	14 (36.8)	**38**
>10%	1 (0.4)	10(3.6)	263 (96.0)	**274**
Total	**25**	**33**	**280**	**338**

Analyses were censored at 10-year follow-up. Provided are the number of individuals and row percent. Relative Net-reclassification improvement was 9.4%, *P*<0.0001.

Green shaded cells represent favorable reclassification, red shaded cells unfavorable reclassification.