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

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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, fibrinogen, interleukin-6, intercellular adhesion molecule-1, lipoprotein-associated phospholipase-A2 [mass and activity], monocyte chemoattractant protein-1, myeloperoxidase, CD40 ligand, P-selectin, and 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. C-reactive protein (hazard ratio [HR] reported per SD 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.

Conclusions—Of 11 inflammatory biomarkers tumor necrosis factor receptor II was related to cardiovascular disease and mortality in the Framingham Heart Study. The combination of TNFRII with C-reactive protein 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. **(*Arterioscler Thromb Vasc Biol*. 2013;33:1728-1733.)**

Key Words: cardiovascular disease ■ cohort ■ epidemiology ■ inflammation ■ mortality

Cardiovascular diseases (CVDs), diabetes mellitus, 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 proinflammatory 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 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 3 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 (eg, tumor necrosis factor receptor II [TNFRII]) and chemokines (monocyte chemoattractant protein-1) induce leukocyte recruitment and the acute-phase response.[10] Proinflammatory 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 antimicrobial defense.

Received on: January 14, 2013; final version accepted on: April 18, 2013.

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The online-only Data Supplement is available with this article at http://atvb.ahajournals.org/lookup/suppl/doi:10.1161/ATVBAHA.112.301174/-/DC1.

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Table 1. Baseline Characteristics of the Study Sample

	Overall Cohort (n=3035)
Age, y	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 mellitus, %	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 (%) for dichotomous variables.

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.

Materials and Methods

Materials and Methods are available in the online-only 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 (Table I in the online-only Data Supplement).

Prediction of CVD

Table II in the online-only Data Supplement presents the multivariable-adjusted association of the separate inflammatory biomarkers with major CVD incidence among the 2827 participants free of major CVD at the seventh examination cycle. TNFRII and CRP were individually associated with major incident CVD. With stepwise selection, CRP (hazard ratio [HR] per ln-biomarker SD, 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 2 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 to 0.010; P=0.11). The Hosmer–Lemeshow statistic showed adequate calibration between observed and predicted mortality risk of the final model (χ^2=13.2; df=10; P=0.15).

Table 2. Final Model From Stepwise Selection Analysis for Inflammatory Biomarkers in Relation to Cardiovascular Disease

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 mellitus, and hypertension treatment. Participants with prevalent major cardiovascular disease were excluded from this analysis. Hazard ratios are per 1 SD increase in ln-biomarker concentration.

We also performed reclassification analyses for CVD risk categories >10 years (Table 3). Net-reclassification improvement (NRI) was not statistically significant (−1.1%; P=0.62). The relative integrated discrimination improvement was 7.9% (P=0.004) and the integrated discrimination improvement was 0.9% (P=0.0039). Figure I in the online-only Data Supplement shows sex-specific–adjusted survival curves by tertile of the biomarker score incorporating the top 2 inflammatory markers. Decreased survival was observed across tertiles.

Prediction of Mortality

Table III in the online-only Data Supplement provides the HRs per SD of natural log-transformed biomarkers for multivariable-adjusted single biomarker associations with mortality. Four of the 11 markers were individually associated

Table 3. Reclassification Matrix for Cardiovascular Disease During 10 Years of Follow-Up

	With TNFRII, CRP			
Without Biomarkers	<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. CRP indicates C-reactive protein; CVD, cardiovascular disease; and TNFRII, tumor necrosis factor receptor II.

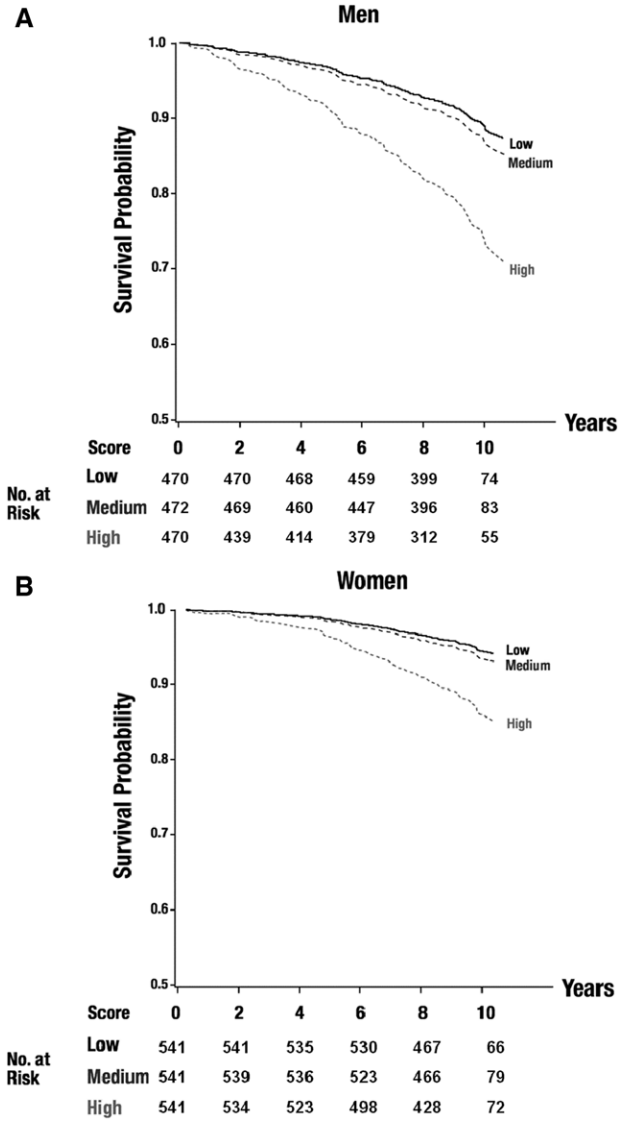

Figure. Adjusted survival curves for tertiles of the biomarker score (including tumor necrosis factor receptor II, interleukin-6, and intercellular adhesion molecule-1) for men (**A**) and women (**B**), $P_{log\text{-}rank}$<0.0001 for both. The numbers of participants at risk are displayed in the figure.

with mortality: CRP, ICAM-1, interleukin-6, and TNFRII (all *P*<0.0001). In the stepwise biomarker model, the 3 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. The Figure shows sex-specific–adjusted survival curves by tertile of the biomarker score incorporating the top 3 inflammatory markers. Decreased survival was observed across tertiles. Whereas the 2 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, 0.765–0.813] rose to 0.799 [95% CI, 0.776–0.823]). The 3 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, 0.788–0.834) after adding biomarkers. The estimated increment of c-statistic was 0.022 (95% CI, 0.011–0.033; *P*<0.0001). The Hosmer–Lemeshow statistic showed adequate calibration between observed and predicted mortality risk in the final model (χ^2=12.5; *df*=10; *P*=0.19). The relative integrated discrimination improvement index was 25.7% (*P*<0.0001) and integrated discrimination improvement was 5.1% (*P*<0.0001). For the top biomarker, TNFRII alone, the relative integrated discrimination improvement index was 17.9% (*P*<0.0001) and integrated discrimination improvement was 3.6% (*P*<0.0001).

We tested reclassification for 3 categories of risk of death >10 years: <5%, 5% to 10%, and >10% (Table 5). The NRI

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/high-density lipoprotein-cholesterol, hypertension treatment, diabetes mellitus, and interim CVD (time-dependent variable). Hazard ratios are per 1 SD increase in log-biomarker concentration. CVD indicates cardiovascular disease.

Table 5. Reclassification Matrix for 10-Year Mortality

	With TNFRII, ICAM-1, and Interleukin-6			
Without Biomarkers	<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). ICAM-1 indicates intercellular adhesion molecule-1; and TNFRII, tumor necrosis factor receptor II.

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%). 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 2 biomarkers in relation to incident CVD and the 3 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 3 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 ≈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 3 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. TNF-α 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 TNF-α system. In the cardiovascular system, TNF-α signaling via TNFRII is protective in postischemic 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-TNF–α 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 mellitus 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, 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 because of 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 2 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 3 markers in addition to traditional risk factors of mortality. Because 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. Because of a moderate number of outcomes we did not have the power to perform subgroup

analyses for cause-specific mortality (eg, cardiovascular mortality, cancer mortality). Because of limited numbers of individuals with cardiovascular medication, such as statins, aspirin, and hormone replacement therapy, and a variety of biases (eg, 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 nonreplication of findings from large consortia as seen for lipoprotein-associated phospholipase A2.[47] Apart from overall small effect sizes, nonreplication of published associations of inflammatory biomarkers, such as CD40 ligand, monocyte chemoattractant protein-1, myeloperoxidase, and P-selectin, in multivariable models may be because of our rigorous cardiovascular risk factor adjustment, the sample structure of community-based individuals compared with 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 3 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 11 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 use 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, ie, how alterations of the inflammatory system contribute to immunosenescence, frailty, and increased risk for death.[49] Our findings may stimulate future research at the epidemiological and 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.

Acknowledgments

Lipoprotein-associated phospholipase A2 activity measurements were provided by GlaxoSmithKline and mass measurements by diaDexus at no cost to the Framingham Heart Study. The researchers are indebted to the Framingham Heart Study participants and staff.

Sources of Funding

This work was supported by National Institutes of Health (NIH)/National Heart, Lung, and Blood Institute contract N01-HC-25195, 6R01-NS17950, and NIH grants HL064753, HL076784, AG028321 (E.J. Benjamin), and 1 RO1HL71039 (R.S. Vasan); NIH Research career award K24 HL04334 (R.S. Vasan); Deutsche Forschungsgemeinschaft (German Research Foundation) Research Fellowship SCHN 1149/1-1 and Emmy Noether Program SCHN 1149/3-1 (R.B. Schnabel); 1K23HL083102, Doris Duke Charitable Foundation Clinical Scientist Development Award (S. Kathiresan).

Disclosures

None.

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Significance

There is an ongoing discussion on the clinical use 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 was nominally related to CVD and significantly predictive of mortality. The magnitude and strength of association were modest. Even the combination of the 2 strongest biomarkers, tumor necrosis factor receptor II and C-reactive protein for incident CVD and tumor necrosis factor receptor II 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 tumor necrosis factor receptor II and other inflammatory biomarkers in the process of aging, CVD risk, and mortality.