Paper 2

Nyrnes A, Njølstad I, Mathiesen EB, Wilsgaard T, Hansen JB, Skjelbakken T, Jørgensen L, Løchen ML.

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Gend Med. 2012 Dec;9(6):536-547.e2. Epub 2012 Oct 6.

Inflammatory Biomarkers as Risk Factors for Future Atrial Fibrillation. An Eleven-Year Follow-Up of 6315 Men and Women: The Tromsø Study

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ABSTRACT

Background: Inflammatory biomarkers are reported as risk factors for atrial fibrillation (AF), but their impact is uncertain.

Objective: We investigated the associations between inflammatory biomarkers and future AF in a large general cohort.

Methods: Available markers were white blood cells (WBCs) with subgroups, fibrinogen, high-sensitivity C-reactive protein (hs-CRP), and osteoprotegerin (OPG). A total of 6315 men and women from a population survey in Tromsø, Norway in 1994 to 1995 were followed for a mean of 10.9 years. Mean age at baseline was 60 years. Measurements of height, weight, blood pressure, heart rate, total cholesterol, high-density lipoprotein (HDL) cholesterol, WBC count, and information on diabetes, angina, myocardial infarction, and antihypertensive treatment, were obtained at baseline. Fibrinogen, hs-CRP, and OPG were obtained at a follow-up visit. The outcome measure was first-ever AF, documented on an electrocardiogram. The Cox proportional hazards regression model was used to estimate hazard ratios of AF.

Results: In the multivariable analysis, adjusted for traditional cardiovascular risk factors and other inflammatory biomarkers, hs-CRP was associated with AF in men only (hazard ratio = 1.14 for a 1 SD increase; 95% CI, 1.02–1.28). There was a significant increase in AF across quartiles of WBCs in men (P = 0.007) and in the total study population (P = 0.004). OPG was associated with AF in patients free of coronary heart disease at baseline. Fibrinogen and subgroups of WBCs showed no significant association with AF.

Conclusion: This population-based cohort study showed that hs-CRP was independently associated with AF in men, but apparently not in women, and that patients with WBCs in the upper quartile had increased risk of AF. (*Gend Med.* 2012;9:536–547)

Key words: atrial fibrillation, cohort study, gender, inflammation, risk factors.

INTRODUCTION

Inflammatory biomarkers have been shown in several studies to be associated with atrial fibrillation (AF), but results are inconsistent. Whether inflammation is an initiator, a consequence, or merely an association of AF is debated. However, many studies support the concept that inflammation contributes to at least some types of AF.[1,2] The exact mechanisms are still unclear. Atrial biopsies from patients with AF have confirmed the presence of

Accepted for publication September 9, 2012.

http://dx.doi.org/10.1016/j.genm.2012.09.001
1550-8579/$ - see front matter

inflammation, with myocyte loss and fibrosis replacement. This leads to electrical and structural remodelling of the atria, thus creating a substrate that permits abnormal, multiple wavelets of excitation.[3]

A prospective cohort study is therefore highly warranted to elucidate whether inflammation contributes directly to the development of AF. The aim of our study was to investigate selected inflammatory biomarkers as risk factors for incident AF in a large, general population. Available biomarkers were white blood cell counts (WBCs; total and subgroups), fibrinogen, high-sensitivity C-reactive protein (hs-CRP), and osteoprotegerin (OPG).

METHODS

The Tromsø Study[4] is a prospective study of the general population in the municipality of Tromsø, Norway. The study started in 1974, with a main focus on determinants and distribution of cardiovascular diseases (CVDs). The study design includes repeated surveys to which total birth cohorts and random samples are invited, and involves a large proportion of the municipality's adult population. The surveys have been approved by the Regional Committee for Medical and Health Research Ethics, the Data Inspectorate, and the Norwegian Directorate of Health.

Study Population

Participants of the 1994 to 1995 survey, conducted by the University of Tromsø in collaboration with the National Health Screening Service, were eligible for the present study. All inhabitants ≥25 years old were invited. A total of 27,158 persons (77% of the eligible population) participated in the first visit of the survey. All participants 55 to 74 years old and 5% to 10% samples in other 5-year age groups (25–54 years and 75–85 years) were invited to a second visit, to which 74% of invited men and 77% of invited women attended. These participants constituted the study population of the present analysis. The participants were almost exclusively of white, Caucasian origin. Informed, written consent was obtained from all participants. Persons who did not consent to medical research, and persons not officially registered as inhabitants of Tromsø on the day of participation were excluded from the analyses, as were persons with AF diagnosed before the study (n =115; 1.8% of study population) or those with inconclusive baseline AF data (n = 7). We also excluded participants with measured hs-CRP >10 mg/L (n= 271) or missing values of hs-CRP (n = 119), leaving 6315 participants (3222 women and 3093 men; aged 25 to 84 years), who were included in our final study population.

Baseline Demographic Characteristics

Information on diabetes, angina, myocardial infarction, use of antihypertensive treatment, physical activity (on a 4-level scale), smoking, and alcohol was obtained from self-reported questionnaires. Coronary heart disease (CHD) was defined as previous myocardial infarction and/or prevalent angina. Smoking habits were classified as daily current smoker versus nonsmoker. Height and weight were measured with participants wearing light clothing and no shoes. Body mass index (BMI) was calculated as weight (kilograms) divided by squared height (meters squared). Blood pressure (BP) and heart rate were recorded 3 times at 1-minute intervals after 2 minutes of seated resting, by specially trained technicians using an automatic device (Dinamap Vital Signs Monitor 1846; Criticon, GE Healthcare Home, Little Chalfont, United Kingdom). The mean of the second and third BP reading was used in the analyses.

Laboratory Measurements

WBC counts were analyzed within 12 hours in an automated blood cell counter (Coulter Counter; Coulter Electronics, Luton, United Kingdom) in 5 mL blood drawn into vacutainer tubes containing EDTA as an anticoagulant (K_3– EDTA 40 μL, 0.37 mol/L^{-1} per tube). Nonfasting serum total cholesterol was analyzed by enzymatic colorimetric methods with commercial kits (CHOD-PAP; Boehringer-Mannheim, Barcelona, Spain). Serum high-density lipoprotein (HDL) cholesterol was measured after the precipitation of lower density lipoprotein with heparin and manganese chloride. Fibrinogen was analyzed by a clotting assay with reagents from IL (Instrumentation Laboratory, Mi-

lano, Italy) on an ACL 3000 coagulation analyzer (Block Scientific Inc, Bohemia, New York). OPG and hs-CRP concentrations were analyzed in serum aliquots stored at −70°C. OPG was analyzed by an ELISA assay (R&D Systems, Abingdon, United Kingdom) with mouse antihuman OPG as capture antibody. Biotinylated goat antihuman OPG and streptavidin horseradish peroxidase were used for detection. The OPG assay was performed according to the manufacturer's instructions. The detection limit was 62.5 pg/mL. The intra- and interassay coefficients of variation in our laboratory were 3.2% and 6.8%, respectively. Between-assay variation in OPG was adjusted for by using an internal standard. hs-CRP was measured by a particle-enhanced immunturbidimetric assay on a Modular P autoanalyzer (Roche Diagnostics, Mannheim, Germany) with reagents from the manufacturer. The analyses were done at the Department of Clinical Chemistry, University Hospital of North Norway, Tromsø, Norway.

Definition and Ascertainment of AF Cases and Follow Up

All Tromsø study participants were followed up with respect to incident cases of AF documented on an electrocardiogram. The national 11-digit identification number allowed linkage to national and regional diagnosis registers. The participant list of the Tromsø Study in 1994 to 1995 was linked to the diagnosis registry at the University Hospital of North Norway (diagnoses from the outpatient clinic were included), which is the only hospital in this area, and to the Causes of Death Registry at Statistics Norway, using the following diagnostic codes: *International Classification of Diseases-9th Revision* (ICD-9) codes 427.0 to 427.99 and ICD-10 codes I47 to I48. In addition, we manually searched paper versions of hospital records (used until February 2001) for notes on AF, and performed text searches with the term "atrial fibrillation" in the electronic hospital records of Tromsø Study participants with diagnoses of cerebrovascular or cardiovascular events. Adjudication of hospitalized and out-of-hospital events was performed by an independent end point committee and based on data from hospital and out-of-hospital records. Patients with transient AF that occurred only during an acute myocardial infarction or in connection with a cardiac surgery procedure, and patients with AF documented only in the terminal phase of life, defined here as the last 7 days

Table I. Baseline demographic characteristics: The Tromsø Study, 1994 to 2007.

Characteristics	Women (n = 3222)	Men (n = 3093)	*P*
Age, y	60.5 (10.3)	59.5 (10.0)	<0.0001
Body mass index, kg/m^2	25.9 (4.4)	26.0 (3.3)	0.10
Systolic blood pressure, mm Hg	144.7 (24.3)	144.6 (20.3)	0.88
Heart rate, beats/min	73.9 (12.5)	69.8 (13.0)	<0.0001
Cholesterol, total, mmol/L	6.93 (1.34)	6.57 (1.20)	<0.0001
Alcohol, glasses/2 wks	2.5 (3.6)	5.5 (7.3)	<0.0001
Tea-totaller, %	26.1	12.9	<0.0001
Smoking, %	31.0	34.7	0.002
Physical activity, %	28.2	47.1	<0.0001
Coronary heart disease, %	8.3	15.0	<0.0001
Diabetes, %	3.9	4.4	0.30
Antihypertensive treatment, %	13.1	12.8	0.79
Inflammatory markers			
White blood cells, 10^9/L	6.77 (1.77)	7.06 (1.93)	<0.0001
Fibrinogen, g/L	3.39 (0.75)	3.25 (0.78)	<0.0001
hs-CRP, mg/L	1.65 (1.66)	1.85 (1.81)	<0.0001
Osteoprotegerin, ng/mL	3.41 (1.06)	3.20 (1.10)	<0.0001

hs-CRP = high-sensitivity C-reactive protein.
Data are presented as means (SD) or percentages.

Table II. Multivariable-adjusted* risk factor levels at baseline in women and men, stratified by future atrial fibrillation status: The Tromsø Study, 1994 to 2007.

	Women			Men		
	Atrial Fibrillation (n = 259)	No Atrial Fibrillation (n = 2963)	*P*	Atrial Fibrillation (n = 307)	No Atrial Fibrillation (n = 2786)	*P*
Age,† y	67.1	60.0	<0.0001	65.4	58.9	<0.0001
Body mass index, kg/m^2	26.5	25.8	0.007	26.6	26.0	0.001
Systolic blood pressure, mm Hg	147.5	144.3	0.020	147.0	144.3	0.023
Heart rate, beats/min	73.1	74.0	0.26	68.4	69.9	0.06
Cholesterol, total, mmol/L	6.68	6.96	0.001	6.39	6.59	0.010
Alcohol, glasses/2 wks	2.8	2.5	0.30	5.5	5.5	0.59
Tee-totaller, %	24.0	23.3	0.81	9.3	11.4	0.21
Smoking, %	26.1	26.3	0.95	25.9	31.3	0.12
Physical activity, %	28.9	26.2	0.41	49.8	47.0	0.39
Coronary heart disease, %	6.4	4.9	0.16	17.2	10.7	<0.0001
Diabetes, %	2.8	2.1	0.27	3.8	2.7	0.16
Antihypertensive treatment, %	13.3	8.0	0.001	10.3	8.9	0.37
Inflammatory markers						
White blood cells, 10^9/L	6.84	6.76	0.46	7.17	7.05	0.28
Fibrinogen, g/L	3.43	3.39	0.29	3.19	3.26	0.07
hs-CRP, mg/L	1.55	1.65	0.33	1.99	1.85	0.17
Osteoprotegerin, ng/mL	3.53	3.40	0.024	3.21	3.18	0.68

*Adjusted for age, systolic blood pressure, body mass index, total cholesterol, smoking, coronary heart disease, diabetes, antihypertensive treatment and inflammatory markers.
†Unadjusted values.

(n = 13), were not classified as having AF. Individuals were followed from the date of examination in 1994 to 1995 until the date of first documented AF, date of censoring due to migration or death, or end of follow-up at December 31, 2007, whichever came first. Those who died or emigrated from Tromsø during follow up were identified through the Population Register of Norway.

Statistical Analyses

Characteristics of the study population are presented as means (SD) or percentages. Differences between groups were assessed by *t*-tests and χ^2 tests. Multivariable adjustments of baseline risk factors were performed in the general linear model and the generalized linear model. Age-adjusted and multivariable Cox proportional hazards regression models were used to estimate hazard ratios (HRs) for AF with 95% CIs. The analyses were conducted separately for men and women, as well as for the total study population. The following, traditional cardiovascular risk factors and symptoms were assessed: age, BMI, systolic BP, heart rate, total cholesterol, HDL cholesterol, current smoking, physical activity, prevalent CHD, diabetes, and antihypertensive treatment. Variables not associated with AF in either sex in the age-adjusted analyses (heart rate, HDL cholesterol, physical activity, smoking, and alcohol consumption) were not included in the multivariable models. The inflammatory biomarkers were analyzed both as categorized (in quartiles) and as continuous variables. Cross-product terms between sex and inflammation variables were added to the models to assess interaction. No significant interaction was found. The proportional hazard assumption was assessed by visual inspection of log minus log plots of the survival curves. Analyses were conducted with SPSS version 19.0 for Windows (SPSS, Inc, Chicago, Illinois). A 2-sided *P* value < 0.05 was considered statistically significant.

Table III. Hazard ratio (95% CI) of atrial fibrillation in quartiles and per 1 SD increase in inflammatory biomarkers: The Tromsø Study, 1994 to 2007.

	Per 1 SD hs-CRP Increase	*P*	hs-CRP Quartile 1	2	3	4	*P* for trend
			All				
hs-CRP, mg/L			0.01–0.58	0.59–1.13	1.14–2.25	2.26–9.97	
Events (n = 566)			n = 95	n = 156	n = 147	n = 168	
Model 1	1.17 (1.09–1.25)	<0.0001	1.0	1.37 (1.07–1.77)	1.25 (0.97–1.62)	1.55 (1.21–1.99)	0.003
Model 2	1.08 (1.00–1.17)	0.058	1.0	1.22 (0.94–1.58)	1.04 (0.80–1.36)	1.19 (0.92–1.56)	0.44
Model 3*	1.07 (0.97–1.16)	0.17	1.0	1.18 (0.91–1.54)	1.00 (0.76–1.31)	1.13 (0.85–1.50)	0.77
			Women				
hs-CRP (mg/L)			0.01–0.53	0.54–1.06	1.07–2.12	2.13–9.97	
Events (n = 259)			n = 44	n = 67	n = 65	n = 83	
Model 1	1.17 (1.05–1.31)	0.005	1.0	1.22 (0.84–1.79)	1.14 (0.78–1.67)	1.51 (1.05–2.18)	0.042
Model 2	1.06 (0.94–1.20)	0.36	1.0	1.10 (0.75–1.62)	0.91 (0.61–1.35)	1.10 (0.75–1.62)	0.85
Model 3*	0.98 (0.85–1.13)	0.79	1.0	1.03 (0.70–1.52)	0.79 (0.52–1.19)	0.89 (0.59–1.35)	0.37
			Men				
hs-CRP (mg/L)			0.01–0.63	0.64–1.21	1.22–2.41	2.42–9.88	
Events (n = 307)			n = 53	n = 88	n = 81	n = 85	
Model 1	1.17 (1.06–1.29)	0.002	1.0	1.50 (1.07–2.11)	1.35 (0.96–1.91)	1.57 (1.12–2.22)	0.032
Model 2	1.10 (0.99–1.22)	0.07	1.0	1.34 (0.95–1.89)	1.17 (0.82–1.66)	1.28 (0.89–1.83)	0.37
Model 3*	1.14 (1.02–1.28)	0.024	1.0	1.33 (0.93–1.89)	1.21 (0.84–1.75)	1.37 (0.92–2.02)	0.22

	Per 1-SD OPG increase	*P*	OPG Quartile 1	2	3	4	*P* for Trend
			All				
OPG, ng/mL			0.458–2.589	2.590–3.149	3.150–3.813	3.814–17.529	
Events (n = 566)			n = 69	n = 109	n = 166	n = 222	
Model 1	1.12 (1.02–1.22)	0.016	1.0	0.91 (0.67–1.25)	1.10 (0.82–1.49)	1.20 (0.88–1.65)	0.06
Model 2	1.08 (0.98–1.18)	0.11	1.0	0.93 (0.68–1.27)	1.11 (0.82–1.50)	1.18 (0.86–1.63)	0.10
Model 3†	1.08 (0.98–1.19)	0.11	1.0	0.94 (0.68–1.29)	1.15 (0.84–1.58)	1.23 (0.89–1.71)	0.06
			Women				
OPG, ng/mL			0.894–2.711	2.712–3.268	3.269–3.921	3.922–11.037	
Events (n = 259)			n = 27	n = 46	n = 80	n = 106	
Model 1	1.19 (1.04–1.35)	0.010	1.0	0.88 (0.54–1.43)	1.27 (0.80–2.01)	1.26 (0.78–2.04)	0.09
Model 2	1.11 (0.97–1.26)	0.14	1.0	0.89 (0.54–1.44)	1.20 (0.75–1.92)	1.12 (0.69–1.82)	0.35
Model 3†	1.11 (0.97–1.27)	0.14	1.0	0.98 (0.59–1.63)	1.32 (0.81–2.15)	1.26 (0.76–2.10)	0.20
			Men				
OPG, ng/mL			0.458–2.475	2.476–3.019	3.020–3.668	3.669–17.529	
Events (n = 307)			n = 42	n = 58	n = 84	n = 123	
Model 1	1.06 (0.93–1.20)	0.39	1.0	0.86 (0.58–1.29)	0.91 (0.61–1.35)	1.13 (0.75–1.70)	0.29
Model 2	1.06 (0.93–1.20)	0.37	1.0	0.87 (0.58–1.30)	0.94 (0.63–1.41)	1.21 (0.80–1.83)	0.15
Model 3†	1.06 (0.93–1.21)	0.36	1.0	0.86 (0.56–1.31)	0.98 (0.65–1.48)	1.23 (0.80–1.89)	0.13

	Per 1 SD Fibrogen. Increase (new)	*P*	Fibrinogen Quartile 1	2	3	4	New *P* for Trend
			All				
Fibrinogen, g/L			1.0–2.7	2.8–3.2	3.3–3.7	3.8–7.8	
Events (n = 556)			n = 92	n = 145	n = 155	n = 164	
Model 1	1.05 (0.96–1.14)	0.31	1.0	1.09 (0.84–1.42)	1.35 (1.04–1.76)	1.17 (0.90–1.51)	0.14
Model 2	1.02 (0.93–1.12)	0.65	1.0	1.04 (0.80–1.35)	1.31 (1.01–1.71)	1.08 (0.82–1.41)	0.38
Model 3‡	0.98 (0.89–1.08)	0.65	1.0	1.03 (0.79–1.34)	1.26 (0.96–1.65)	0.96 (0.72–1.29)	0.96

(continued)

Table III (continued).

	Per 1 SD Fibrogen. Increase (new)	*P*	Fibrinogen Quartile 1	2	3	4	New *P* for Trend
			Women				
Fibrinogen, g/L			1.0–2.8	2.9–3.3	3.4–3.8	3.9–7.8	
Events (n = 254)			n = 42	n = 59	n = 69	n = 84	
Model 1	1.14 (1.01–1.30)	0.04	1.0	0.99 (0.67–1.47)	1.29 (0.88–1.89)	1.41 (0.97–2.05)	0.02
Model 2	1.10 (0.96–1.26)	0.16	1.0	0.91 (0.61–1.35)	1.17 (0.80–1.73)	1.26 (0.86–1.85)	0.09
Model 3‡	1.10 (0.95–1.27)	0.21	1.0	0.92 (0.61–1.38)	1.15 (0.77–1.71)	1.28 (0.85–1.92)	0.10
			Men				
Fibrinogen, g/L			1.1–2.6	2.7–3.1	3.2–3.7	3.8–7.5	
Events (n = 302)			n = 44	n = 84	n = 111	n = 63	
Model 1	0.98 (0.87–1.10)	0.69	1.0	1.28 (0.89–1.85)	1.66 (1.17–2.36)	1.00 (0.67–1.47)	0.82
Model 2	0.98 (0.86–1.10)	0.69	1.0	1.21 (0.84–1.75)	1.63 (1.14–2.34)	0.96 (0.64–1.43)	0.89
Model 3‡	0.90 (0.78–1.03)	0.12	1.0	1.17 (0.80–1.70)	1.49 (1.03–2.16)	0.74 (0.47–1.15)	0.35
	Per 1 SD WBC Increase	*P*	WBC Quartile 1	2	3	4	*P* for Trend
			All				
WBC, 10^9/L			2.2-5.5	5.6-6.6	6.7-7.9	8.0-34.0	
Events (n = 551)			n = 117	n = 134	n = 158	n = 142	
Model 1	1.12 (1.03–1.21)	0.005	1.0	1.11 (0.87–1.43)	1.35 (1.06–1.72)	1.57 (1.23–2.01)	<0.0001
Model 2	1.08 (1.00–1.17)	0.050	1.0	1.07 (0.84–1.38)	1.28 (1.00–1.63)	1.49 (1.15–1.93)	0.001
Model 3§	1.07 (0.99–1.16)	0.09	1.0	1.08 (0.84–1.39)	1.24 (0.97–1.59)	1.44 (1.11–1.88)	0.004
			Women				
WBC, 10^9/L			2.2–5.5	5.6–6.5	6.6–7.7	7.8–16.6	
Events (n = 252)			n = 53	n = 64	n = 71	n = 64	
Model 1	1.14 (1.00–1.30)	0.055	1.0	1.43 (0.99–2.06)	1.46 (1.02–2.08)	1.57 (1.09–2.25)	0.018
Model 2	1.10 (0.95–1.27)	0.20	1.0	1.32 (0.92–1.91)	1.33 (0.92–1.92)	1.40 (0.95–2.05)	0.11
Model 3§	1.07 (0.92–1.25)	0.36	1.0	1.31 (0.90–1.89)	1.24 (0.85–1.79)	1.32 (0.89–1.95)	0.23
			Men				
WBC, 10^9/L			2.9–5.7	5.8–6.7	6.8–8.0	8.1–34.0	
Events (n = 299)			n = 74	n = 64	n = 81	n = 80	
Model 1	1.11 (1.00–1.22)	0.049	1.0	0.90 (0.64–1.25)	1.26 (0.92–1.72)	1.50 (1.09–2.07)	0.003
Model 2	1.07 (0.97–1.17)	0.16	1.0	0.90 (0.64–1.26)	1.22 (0.89–1.68)	1.53 (1.09–2.15)	0.006
Model 3§	1.07 (0.97–1.17)	0.18	1.0	0.88 (0.63–1.24)	1.23 (0.89–1.70)	1.52 (1.07–2.16)	0.007

hs-CRP = high-sensivity C-reactive protein; OPG = osteoprotegerin; WBC = white blood cell.
Model 1: adjusted for age and sex.‖
Model 2: adjusted for age, sex,‖ systolic blood pressure, body mass index, total cholesterol, smoking, coronary heart disease, diabetes, and antihypertensive treatment.
*Model 3: adjusted for age, sex,‖ systolic blood pressure, body mass index, total cholesterol, smoking, coronary heart disease, diabetes, antihypertensive treatment, and inflammatory biomarkers (WBC, OPG, and fibrinogen).
†Model 3: adjusted for age, sex,‖ systolic blood pressure, body mass index, total cholesterol, smoking, coronary heart disease, diabetes, antihypertensive treatment and inflammatory biomarkers (hs-CRP, WBC, and fibrinogen).
‡Model 3: adjusted for age, sex,‖ systolic blood pressure, body mass index, total cholesterol, smoking, coronary heart disease, diabetes, antihypertensive treatment, and inflammatory biomarkers (hs-CRP, OPG, and WBC).
§Model 3: adjusted for age, sex,‖ systolic blood pressure, body mass index, total cholesterol, smoking, coronary heart disease, diabetes, antihypertensive treatment and inflammatory biomarkers (hs-CRP, OPG, and fibrinogen).
‖Not in sex-specific models.

RESULTS

We identified 259 women (8.0%) and 307 men (9.9%) with incident AF during a mean follow-up of 10.9 (0–13.3) years (68,820 person-years). The incidence rate of AF per 1000 person-years was 7.23 (95 % CI, 6.34–8.12) in women and 9.45 (95 % CI, 8.42–10.48) in men. The baseline crude characteristics for men and women are shown in **Ta-**

ble I. Women were, on average, 1 year older than men and had a significantly higher heart rate, higher levels of total cholesterol, fibrinogen, and OPG, and lower levels of WBCs and hs-CRP. A lower proportion of women had prevalent CHD compared with men. Women reported lower alcohol consumption and were less physically active than men.

We also adjusted the baseline characteristics for age and cardiovascular risk factors, and present these data in **Table II**. Patients who later developed AF were significantly older, had higher BMI and systolic BP, and lower cholesterol (both sexes). Women who developed AF were more often on antihypertensive medication and had higher OPG levels. Men who developed AF had a higher frequency of CHD.

The distribution of baseline risk factors across quartiles of inflammatory biomarkers for the total study population is shown in the **Supplemental Table** (see in the online version at http://dx.doi.org/10.1016/j.genm.2012.09.001.) WBCs showed a weak, but significant negative correlation with age (Pearson's r −0.10 [women] and −0.08 [men]). The other inflammatory biomarkers were positively correlated with age (Pearson's r: OPG 0.51, fibrinogen 0.25, and hs-CRP 0.11, with almost identical coefficients in women and men; data not shown).

Table III shows HRs of AF in quartiles and per 1 SD increase for each of the inflammatory biomarkers, for the total study population, and for women and men separately.

We show 3 different models, with adjustment in Model 1 only for age (and sex, for the total group), and in Model 2 with the addition of traditional cardiovascular risk factors. In Model 3, we show the multivariable Cox proportional hazards regression analysis, with adjustment for traditional cardiovascular risk factors and the other inflammatory biomarkers. hs-CRP was a predictor of AF in men, with a HR of 1.14 (95 % CI, 1.02–1.28).

Exclusion of patients with prevalent myocardial infarction at baseline and adjustment for ascertained incident myocardial infarction during follow up did not change the results. The estimates were similar, with a HR of 1.17 ($P = 0.02$) in Model 3 (data not shown).

OPG was a predictor of AF in the total study population when patients with baseline CHD were excluded, with a HR of 1.13 (95 % CI, 1.01–1.26).

The risk of AF increased significantly across quartiles of WBCs in men ($P = 0.007$) and in the total study population ($P = 0.004$). Patients with WBC counts in the upper quartile (more than 8.0×10^9) had a 44% increased risk of AF compared with those with WBC counts in the lowest quartile (less than 5.5×10^9). However, the association between WBCs and AF did not reach statistical significance in the linear model (HR = 1.07; 95% CI, 0.99–1.16). None of the subgroups of WBCs (monocytes, lymphocytes, neutrophils, eosinophils, and basophils) showed any significant association with AF in the multivariable analyses. Fibrinogen was associated with incident AF in women in the age-adjusted analysis, but not significantly so in the multivariable analysis.

DISCUSSION

In this study of inflammatory biomarkers in a general population, we found that hs-CRP was associated with incident AF in men, but not in women. This seems to be in conflict with other studies, which reported hs-CRP to be an independent risk factor for future AF in both sexes, although with varying strength of association.[2,5–7] However, most studies adjusted for sex in the multivariable analyses, but did not perform sex-specific analyses. A report from the Women's Health Study also found increasing risk with increasing hs-CRP in multivariable analysis adjusted for established cardiovascular risk factors.[8] To our knowledge, a sex difference in the association between hs-CRP and AF was not reported previously. In our population, men had significantly higher levels of hs-CRP than women. This could reflect a higher level of CHD in men. However, we found no significant interaction between sex and hs-CRP, and it was therefore not possible to draw any absolute conclusions about the sex differences. It was important that the absolute number of incident AF cases was 18% higher in men compared with women. This might

have influenced the difference between the sexes, by attenuating the result for women.

hs-CRP, a marker of low-grade inflammation, was proven to be a risk factor for cardiovascular disease and also for AF in many studies.[2,9] CRP, in general, is considered to reflect underlying disease processes associated with AF, and is thought to reflect the burden of CVD. Prevalent CVD is an established risk factor for AF. We excluded subjects with hs-CRP >10 mg/L from analysis, in an attempt to avoid cases of AF precipitated by an acute condition (eg, an infection). We defined a cutoff value that was in accordance with other cardiovascular research studying the influence of inflammation.

It was suggested that the association between hs-CRP and AF could be mediated by coexisting CHD. This did not appear to be the case in our study, as we investigated this in a model where we excluded patients with prevalent myocardial infarction at baseline. This did not change the results.

Whether CRP also contributes directly to the development of AF is being debated, as there are studies both to support and reject this.[10–12] A report from the Copenhagen City Heart Study found that CRP was associated with AF, but genetically elevated CRP did not confer any elevated risk. This could suggest that elevated CRP per se does not increase AF risk.[12] In contrast, a recent study from Taiwan demonstrated a possible role of the CRP gene as a predisposing factor to AF in patients with mainly structural heart disease.[13]

We found that OPG was associated with incident AF in a model adjusted for age and sex, and in an age-adjusted model for women. This relation lost its significance with further adjustment for CHD and other confounders. However, we also performed analyses excluding patients with prevalent CHD at baseline and adjustment for ascertained incident myocardial infarction during follow up. This showed a significant association between OPG and AF in the total study population, which remained significant in the multivariable analysis. Excluding patients with baseline myocardial infarction rendered similar estimates and *P* values (data not shown). This was partly in contrast with the findings from the Framingham Offspring Cohort, published in 2009, where OPG, when adjusted for established risk factors, was significantly associated with incident AF in a community-based sample. However, this relation lost its significance when myocardial infarction and heart failure were taken into account.[14]

OPG is a novel biomarker that has gained increasing interest as a marker of CVD. OPG is associated with inflammation, vascular calcification, endothelial function, and atherosclerosis. Recent studies indicated that high plasma OPG was a strong predictor of CVD and mortality in high risk and general populations.[15,16] OPG is a member of the tumor necrosis factor receptor superfamily, and was first identified in 1997 as a strong inhibitor of bone resorption. Further research confirmed that OPG is widely distributed in human tissue, including the cardiovascular system, where it is expressed in endothelial and smooth muscle cells.[17] Elevated OPG levels were shown to reflect the prevalence and severity of coronary artery disease and the degree of coronary calcification.[17] We recently found that OPG was associated with future risk of myocardial infarction, ischemic stroke, total mortality, cardiovascular mortality, and nonvascular mortality in the general population, independent of traditional cardiovascular risk factors.[16]

An association between OPG and future AF could be a result of the burden of concomitant vascular disease, and not by OPG as a cause of AF. However, our recent findings could indicate a direct relation between OPG and AF in individuals free of CHD.

To our knowledge, prospective studies on the impact of WBCs in a general population are lacking. Our study showed a significant increase in AF across quartiles of WBCs. The role of WBCs in AF development remains undefined. WBCs were found to infiltrate the atrial myocardium both in patients with lone AF[3] and in patients with AF and underlying structural heart disease.[18,19] Studies also showed that preoperative WBC elevation was a predictor of postoperative AF after coronary artery bypass surgery[20,21] and postablation AF recurrence.[22] WBCs might promote structural and elec-

trical remodeling of the atria in multiple ways. Activated WBCs release a number of cytokines that activate intracellular inflammatory cascades in fibroblasts, cardiomyocytes, and leukocytes themselves. Thus, inflammation is a initiator of atrial fibrosis, as shown in biopsies from AF patients.[3,18,19]

The distribution of age across quartiles of WBCs was paradoxical compared with all other inflammatory markers (ie, patients of higher age had lower WBC counts) (**Supplement Table**). This phenomenon was probably explained by immunosenescence, the aging of the immune system. This does not affect all immune processes equally, as also suggested by our data. All immune cells originate from hematopoietic stem cells in the bone marrow, and there is a general decline in the total bone marrow hematopoietic tissue with aging. Total circulating WBC counts do not change with age in healthy older people, but the function of several cell types is reduced. Thus, the inflammatory responses in the elderly often only render a moderate rise in WBCs. In contrast, older adults display cytokine profiles that are consistent with a chronic low-level inflammatory state, which is sometimes referred to as "inflammaging." In our study population, WBCs showed a weak, but significant negative correlation with age. The other inflammatory biomarkers were positively correlated with age.

Fibrinogen is also a marker of inflammation, but has been less studied in the context of AF. We found no association between baseline fibrinogen and future AF in the multivariable analysis. Fibrinogen was found to predict future AF in the Copenhagen City Heart Study,[23] the Atherosclerosis Risk in Communities study,[24] and the Women's Health Study,[8] but not in the Framingham Offspring Study[9], the Malmö Preventive Study,[25] or the Rotterdam Study.[26] The conflicting results of these studies could partly be explained by differences in the age groups studied and different risk factor adjustment, but also by the different effects of fibrinogen on men and women.

The possible effect of statins on AF has been debated. An anti-inflammatory and CRP-lowering effect was demonstrated, as well as a stabilizing effect on endothelial cells and autonomic function.[27] Observational studies showed lower occurrence of AF in statin-treated groups, but trials yielded conflicting results.[27] In our study population, only patients <70 years old were asked about lipid-lowering medication at baseline. Only 1.9% of women and 2.3% of men indicated taking such medication, but this was not specified to statins or other substances. We had no information about lipid-lowering medication in the older subjects or new statin users during follow up. Therefore, our data did not allow any consideration about the effect of statins.

Also, angiotensin-converting enzyme inhibitors and angiotensin receptor blockers (ARBs) are considered to have anti-inflammatory effects, and showed an inverse association with AF in some studies,[6,27] but results were conflicting. We had no information about the use of angiotensin-converting ezyme inhibitors at baseline in this study population. However, the ARBs were not commonly used in Norway at that time, and any ARB effect in this group would be negligible at baseline, but might have increased during follow up.

We chose a set of adjustment variables that allowed comparisons with other studies. Especially, we wanted to compare our findings with those of the Framingham Offspring Study, the only study, until now, that assessed the possible effect of OPG on AF.[14] The traditional cardiovascular risk factors were shown to be correlated with incident AF.[28,29]

During recent years, sex differences have been increasingly acknowledged in several health-related issues (eg, in CVD). In all medical research, it is important to investigate whether there are sex differences. If so, the next issue will be to decide whether the differences are of practical, clinical relevance. In our study, the sex differences came out modest, and most did not reach statistical significance. Nonetheless, our sex-specific analysis represented an important supplement to previously published reports, which did not address sex issues.

Strengths and Limitations

Our study was performed in a large, general population with a high attendance rate, which we

considered a major strength. We had a fairly long observation period (11 years) and a middle-aged population of a mean age of 60 years at baseline. Many other studies were performed in smaller, selected populations, in other age groups, or in 1 sex only. Until now, there had been no study of AF in a larger, general Norwegian population. Also, we had a high number of events (AF cases) compared with similar studies. Another strength was our rigorous case validation and thorough search methods, utilizing specific word search ("atrial fibrillation") in electronic hospital records in addition to hospital diagnosis coding. This ensured the detection of a large proportion of known (diagnosed) AF cases.

Some limitations should be mentioned. The population was Caucasian, and the results might not be generalizable to other ethnic groups. Risk factor levels might have changed during (the 11 year) follow up. Only a few inflammatory markers were measured in this survey. Blood samples were analyzed once. Serum samples were kept frozen for 12 years at −70°C without any freezing–thawing cycles before measurement of OPG and CRP. However, long-term stability of OPG was reported.[30]

Unfortunately, we were not able to adjust for congestive heart failure, because these data were not available. This introduced a possible bias. Heart failure is an important risk factor for AF, and is included in the Framingham risk score.[31]

The real number of AF cases might be understated, because there might still be many persons in our community with undiagnosed AF. Some people have asymptomatic AF, and many with the paroxysmal form fail to get their arrhythmia documented on an electrocardiogram. Some patients might be taken care of by their general practitioner without the need of hospital contact. A study from Great Britain in 1997 found that this applied to as many as one-third of AF patients in primary care.[32] We had no corresponding data for Norway.

CONCLUSIONS

In summary, this prospective, population-based study of inflammatory biomarkers showed that hs-CRP was associated with incident AF in men, but apparently not in women, and that patients with WBCs in the upper quartile had increased risk of AF. As far as we know, our study was the first to indicate a sex difference regarding the prediction of hs-CRP for AF. More investigation in this field is needed to explore the clinical significance of our findings.

ACKNOWLEDGMENTS

This work was supported by the Norwegian EXTRAFoundation for Health and Rehabilitation through EXTRA funds, The Norwegian Heart and Lung Patient Organization, the Simon Fougner Hartmann's Family Foundation, and the Norwegian Health Association. Dr. Nyrnes acquired, analyzed and interpreted the data, performed statistical analyses, and drafted the manuscript. Dr. Løchen conceived and designed the research, handled funding, acquired and interpreted the data, and made critical revision of the manuscript. Drs. Mathiesen, Njølstad, and Hansen conceived and designed the research, acquired and interpreted the data, and made critical revision of the manuscript. Dr. Wilsgaard analyzed and interpreted the data, performed statistical analyses, and made critical revision of the manuscript. Dr. Skjelbakken acquired and interpreted the data and made critical revision of the manuscript. Dr. Jørgensen contributed to funding, interpreted the data, and made critical revision of the manuscript. All authors approved the final version of the manuscript.

CONFLICTS OF INTEREST

The authors have indicated that they have no conflicts of interest regarding the content of this article.

SUPPLEMENTAL MATERIAL

Supplemental material accompanying this article can be found in the online version at http://dx.doi.org/10.1016/j.genm.2012.09.001.

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Supplemental Table. Distribution of baseline risk factors across quartiles of inflammatory biomarkers, total study population: The Tromsø Study, 1994 to 2007.

	WBC Quartile				
WBC	1	2	3	4	*P* for Trend
WBC, 10^9/L	2.2–5.5	5.6–6.6	6.7–7.9	8.0–34.0	
Age, y	61.0 (9.5)	60.4 (10.0)	60.0 (10.4)	58.6 (10.3)	<0.0001
Body mass index. kg/m^2	25.7 (3.6)	26.1 (3.9)	26.2 (4.0)	25.7 (4.0)	0.51
Systolic blood pressure, mm Hg	143.4 (21.9)	144.6 (22.5)	144.9 (21.8)	145.6 (23.1)	0.009
Heart rate, beats/min	69.7 (12.2)	70.5 (12.8)	72.3 (12.5)	74.9 (13.5)	<0.0001
Cholesterol, total, mmol/L	6.75 (1.30)	6.75 (1.28)	6.75 (1.31)	6.77 (1.26)	0.63
Smoking, % (n)	11.9 (174)	20.8 (325)	36.4 (578)	61.5 (921)	<0.0001
Coronary heart disease, % (n)	10.6 (154)	11.1 (173)	12.9 (204)	12.2 (182)	0.076
Diabetes, % (n)	3.2 (46)	2.8 (44)	5.3 (84)	5.3 (80)	<0.0001
Antihypertensive treatment, % (n)	11.9 (173)	13.7 (214)	12.9 (205)	13.2 (198)	0.41
	Fibrinogen Quartile				
Fibrinogen	1	2	3	4	*P* for Trend
Fibrinogen, g/L	1.0–2.7	2.8–3.2	3.3–3.7	3.8–7.8	
Age, years	55.4 (11.7)	60.1 (9.6)	61.4 (9.2)	63.0 (83)	<0.0001
Body mass index, kg/m^2	25.6 (3.6)	26.0 (3.7)	26.1 (4.0)	26.1 (4.3)	0.001
Systolic blood pressure, mm Hg	140.1 (20.5)	144.2 (21.8)	145.0 (22.8)	149.0 (23.5)	<0.0001
Heart rate, beats/min	70.0 (12.7)	70.7 (12.7)	72.4 (12.9)	74.5 (13.1)	<0.0001
Cholesterol, total, mmol/L	6.40 (1.27)	6.71 (1.20)	6.86 (1.28)	7.01 (1.33)	<0.0001
Smoking, % (n)	21.2 (312)	24.4 (410)	36.6 (515)	48.1 (806)	<0.0001
Coronary heart disease, % (n)	7.6 (111)	11.8 (198)	12.3 (172)	14.5 (242)	<0.0001
Diabetes, % (n)	2.5 (37)	3.3 (56)	5.3 (74)	5.7 (96)	<0.0001
Antihypertensive treatment, % (n)	8.6 (126)	12.2 (205)	13.7 (193)	16.7 (279)	<0.0001
	hs-CRP Quartile				
hs-CRP	1	2	3	4	*P* for Trend
hs-CRP, mg/L	0.01–0.58	0.59–1.13	1.14–2.25	2.267–9.97	
Age, years	57.3 (11.4)	59.9 (10.0)	61.3 (8.9)	61.7 (9.4)	<0.0001
Body mass index, kg/m^2	24.4 (3.2)	25.7 (3.6)	26.6 (4.0)	27.0 (4.3)	<0.0001
Systolic blood pressure, mm Hg	138.9 (21.1)	144.2 (21.5)	146.4 (22.4)	149.2 (23.2)	<0.0001
Heart rate, beats/min	70.5 (12.6)	71.1 (12.9)	71.9 (12.9)	74.1 (12.9)	<0.0001
Cholesterol, total, mmol/L	6.57 (1.33)	6.71 (1.28)	6.86 (1.26)	6.86 (1.26)	<0.0001
Smoking, % (n)	28.2 (442)	28.1 (447)	35.8 (566)	39.1 (617)	<0.0001
Coronary heart disease, % (n)	6.7 (105)	11.0 (175)	12.4 (195)	16.3 (256)	<0.0001
Diabetes, % (n)	1.6 (25)	3.5 (55)	4.6 (73)	7.0 (110)	<0.0001
Antihypertensive treatment, % (n)	6.8 (107)	11.6 (185)	14.7 (232)	18.6 (294)	<0.0001
	OPG Quartile				
OPG	1	2	3	4	*P* for Trend
OPG, ng/mL	0.458–2.589	2.590–3.149	3.150–3.813	3.814–17.529	
Age, y	51.6 (10.6)	59.1 (8.2)	62.6 (7.6)	66.9 (6.9)	<0.0001
Body mass index, kg/m^2	25.8 (3.5)	26.2 (3.7)	26.0 (3.9)	25.8 (4.5)	0.53

(continued)

Supplemental Table (continued).

	OPG Quartile				
OPG	1	2	3	4	*P* for Trend
Systolic blood pressure, mm Hg	135.8 (18.2)	142.4 (20.7)	146.9 (23.0)	153.7 (23.4)	<0.0001
Heart rate, beats/min	70.2 (12.6)	71.3 (12.4)	72.5 (12.9)	73.6 (13.5)	<0.0001
Cholesterol, total, mmol/L	6.35 (1.25)	6.76 (1.26)	6.90 (1.28)	6.99 (1.28)	<0.0001
Smoking, % (n)	34.5 (543)	31.3 (495)	34.5 (545)	31.0 (489)	0.17
Coronary heart disease, % (n)	6.6 (104)	10.1 (159)	12.7 (200)	17.0 (268)	<0.0001
Diabetes, % (n)	1.5 (24)	2.7 (42)	4.1 (64)	8.4 (133)	<0.0001
Antihypertensive treatment, % (n)	6.5 (102)	11.7 (184)	13.8 (218)	19.9 (314)	<0.0001

hs-CRP = high-sensitivity C-reactive protein; OPG = osteoprotegerin; WBC = white blood cells.
Data are presented as means (SD) for continuous variables or percentages (number of cases) for categorical variables.