Title: Inflammation-sensitive proteins and risk of atrial fibrillation: A population-based cohort study.

Running title: Inflammation-sensitive proteins and AF

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Abstract

Low-grade inflammation has been repeatedly associated with cardiovascular diseases but the relationship with incidence of atrial fibrillation (AF) remains unclear. We explored the association between elevated plasma levels of inflammation-sensitive proteins (ISPs) and incidence of AF in a population-based cohort.

Plasma levels of five ISPs (fibrinogen, haptoglobin, ceruloplasmin, α_1-antitrypsin and orosomucoid) and two complement factors (C3 and C4) were measured in 6031 men (mean age 46.8 years) without history of myocardial infarction, heart failure, stroke or cancer. Incidence of hospitalizations due to AF during a mean follow-up of 25 years was studied both in relation to individual inflammatory proteins and the number of elevated ISPs.

During follow-up, 667 patients were hospitalized with a diagnosis of AF. After adjustment for potential confounding factors, the hazard ratios (HR) for AF were 1.00 (reference), 1.08 (95% CI: 0.88-1.31), 1.07 (CI: 0.84-1.36), and 1.40 (CI: 1.12-1.74), respectively, in men with none, one, two and three or more ISPs in the 4th quartile (p for trend = 0.007). Ceruloplasmin was the only individual ISP significantly associated with incidence of AF after adjustment for confounding factors (HR 1.17 per standard deviation, 95% CI: 1.08-1.26).

In conclusion, a score of five ISPs was associated with long-term incidence of hospitalizations due to AF in middle-aged men. Of the individual ISPs, a significant association was observed for ceruloplasmin, a protein previously associated with copper metabolism and oxidative stress.

Keywords: Atrial fibrillation; Inflammation, Epidemiology

Abbreviations:

AF – Atrial fibrillation

BMI – Body Mass Index

CRP – C-reactive protein

CVD – Cardiovascular disease

ISPs – Inflammatory sensitive proteins

LDL – Low-density lipoprotein

Introduction

Atrial fibrillation (AF) is the most common cardiac arrhythmia in the general population, and a major cause of morbidity and mortality (1-4). Lifetime risk for development of AF has been estimated to 1 in 4 in individuals aged 40 years or more (5). High age, hypertension, obesity, myocardial infarction and heart failure are major risk factors for AF in the general population (6-8).

It is now widely accepted that systemic low-grade inflammation is a risk factor for cardiovascular disease (CVD). Many studies have reported relationships between raised plasma levels of inflammatory markers and incidence of myocardial infarction and stroke (9-13). In studies of patients with AF, inflammation has been associated with perpetuation of AF and poor conversion rates (14). However, whether inflammation is associated with incidence of AF remains unclear. C-reactive protein (CRP) was associated with increased incidence of AF in a study of elderly Americans (15) and in the Framingham study (16). A study of healthy subjects reported that CRP was associated with incidence of AF, but only in the presence of high C3 and C4 levels (17). A recent study reported that a panel of 12 inflammatory markers predicted incidence of AF. However, no individual marker predicted AF when incidence of myocardial infarction was taken into account (18).

Studies from the Malmö Preventive Study, Sweden, have shown that elevated plasma levels of five inflammation-sensitive proteins (ISPs; fibrinogen, haptoglobin, ceruloplasmin, α_1-antitrypsin and orosomucoid) are risk factors for myocardial infarction, heart failure and stroke. These proteins have been associated with incidence of cardiovascular disease, both when studied individually, and as a composite score of inflammation (9, 19). The purpose of this population-based cohort study was to explore whether these ISPs are associated with incident AF during a long-term follow-up among middle-aged men without history of CVD.

Methods

Between 1974 and 1984, 22444 men, aged 26 to 61 years, participated in a screening program for the detection of individuals at high risk for CVD. The participation rate was 71%. As a part of the program, plasma levels of five ISPs were determined for a random sample of 6193 men. After exclusion of men with a history of cancer, atrial fibrillation, heart failure, myocardial infarction or stroke, and 17 subjects with missing data on blood pressure and cholesterol, 6031 men remained. Mean age was 46.8 ± 3.7 years.

The health service authority of Malmö approved and funded the screening program. The regional ethics committee approved the data linkage with the Swedish population- and hospital registers. Participants provided informed consent.

Baseline examination

Blood samples were drawn after an overnight fast. Levels of serum cholesterol were determined using standard methods at the laboratory of Malmö University hospital. Diabetes was defined as fasting whole blood glucose ≥6.1 mmol/l, 2-h post-glucose load ≥10.0 mmol/l, or self-reported diabetes (20). Body mass index (BMI) was calculated as weight/height^2. Blood pressure (mm Hg) was measured twice in the right arm using a sphygmomanometer after 10 minutes of rest. Data on smoking habits and antihypertensive treatment was ascertained from a questionnaire. Physical inactivity in spare time was assessed using the question 'Are you mostly engaged in sedentary activities in spare time, for example, watching TV, reading, going to the movie?'

Subjects who reported a physician's diagnosis of angina pectoris or who used nitrates were considered to have angina pectoris. The question 'Do you use any heart drugs?' assessed use of heart medications.

Alcohol consumption was assessed using the modified shortened version of the Michigan Alcoholism Screening Test (21). Men with more than two of nine affirmative answers were considered to have high alcohol consumption.

ISPs, complement factors

Plasma levels of five ISPs and two complement factors were assessed by means of an electroimmunometric assay (22). The analysis was performed consecutively at the time of study entry. The lower detection limits were 20 mg/L for ceruloplasmin, 50 mg/L for α_1-antitrypsin and 350 mg/L for orosomucoid, haptoglobin and fibrinogen, respectively. C3 and C4 were originally expressed as the percentages of the mean values from a reference population of blood donors. In order to facilitate the interpretation of the C3 and C4 values, the percentages have been converted into grams/litre (C3 100% = 0.98 g/L, C4 100% = 0.20 g/L)(23). The inter-assay coefficient of variation was <5%.(22)

In accordance with previous studies from this cohort, relations with incidence of AF are presented in relation to the number of raised ISPs (fibrinogen, orosomucoid, α1-antitrypsin, haptoglobin, and ceruloplasmin) in the top quartile (9, 19, 23). We have previously shown that all ISPs are associated with incidence of CVD, with largely the same relative risks for all individual ISPs (9). The reliability in terms of internal consistency was fully adequate for this composite score (Cronbach's a=0.65). Median values for the ISPs were 3.46 g/L (interquartile range, 3.0 to 4.0 g/L) for fibrinogen, 0.80 g/L (0.67 to 0.93 g/L) for orosomucoid, 1.28 g/L (1.09 to 1.42 g/L) for a_1-antitrypsin, 1.30 g/L (0.89 to 1.75 g/L) for haptoglobin, and 0.30 g/L (0.26 to 0.35 g/L) for ceruloplasmin.

Of the complement factors, median (interquartile range) was 0.98 g/L (0.86 to 1.15) for C3 and 0.23 g/L (0.18-0.28) for C4.

Follow-up

All men were followed from the baseline examination until first hospitalization due to AF, death, emigration from Sweden, or December 31, 2006. AF was defined as a diagnosis of either atrial fibrillation or atrial flutter as in previous studies (6-8, 24), given the close relationship between these diseases (25). Subjects were considered to have AF if diagnosed with a primary or contributory hospital discharge diagnosis code 427.92 (ICD-8), 427D (ICD-9) or I48 (ICD-10). Since inflammation is associated with increased incidence of myocardial infarction, which could cause AF, we performed secondary analyses, in which cases with incident myocardial infarction (ICD codes I21 or 410) during follow-up were followed until the day of the infarction and censored after that. The Swedish Hospital Discharge register was used for case-retrieval. A validation study has shown that case misclassification of AF in national registers is small (8).

Statistics

One-way ANOVA and Pearson χ^2 were used to compare men with and without AF during follow-up. Cox proportional hazards regression was used to compare incidence of AF between men in different categories of ISPs and to estimate hazard ratios (HR) adjusted for potential confounding factors. In accordance with previous studies (9, 19), incidence of AF was first analysed in relation to the number of elevated ISPs, and secondly, in relation to the individual proteins. The fit of the proportional hazards model was confirmed by plotting the AF incidence rates over time for different categories of risk factors, and by introducing time-dependent variables in the model. The model was adjusted for cardiovascular risk factors associated with AF. All analyses were performed in SPSS version 17 or in PASW Statistics 18 (SPSS Inc, Chicago, Illinois, USA).

Results

During a mean follow-up of 25 years, 667 men (11%, 4.4 per 1000 person years) were diagnosed with AF. Baseline levels of ceruloplasmin were significantly higher in men who were hospitalized due to AF during follow-up ($p<0.05$). Age, BMI, blood pressure, anti-hypertensive medication, diabetes, angina, and high alcohol consumption were also significantly higher in men who developed AF (Table 1).

Incidence of atrial fibrillation in relation to ISPs

Incidence of AF was significantly associated with the number of elevated ISPs (Table 2). The ISPs were elevated several years before the incident events of AF (Figs. 1, 2). The relationship remained significant after adjustments for possible confounding factors. Other independent predictors for AF in the risk factor adjusted analysis were age (HR per year: 1.09, 95% confidence interval (CI): 1.07-1.12), BMI (HR per kg/m^2: 1.06, 95% CI: 1.04-1.09), systolic blood pressure (HR per mm Hg: 1.008, 95% CI: 1.003-1.013), anti-hypertensive medication (HR: 1.38, 95% CI 1.02-1.86), smoking (HR: 1.23, 95% CI: 1.04-1.46) and high alcohol consumption (HR: 1.44, 95% CI: 1.16-1.79). No significant relationship was observed for cholesterol, diabetes or physical activity.

In secondary analyses, censoring was performed at incident myocardial infarction. A total of 528 men had incident AF without previous or concomitant myocardial infarction. The association between ISPs and AF was essentially unchanged. After adjustment for risk factors, the HRs were 1.00 (reference), 1.13 (95% CI: 0.91-1.41), 1.16 (95% CI: 0.89-1.53) and 1.51 (95% CI: 1.18-1.93), respectively, for men with none, 1, 2, and 3 or more ISPs in the top quartile (p for trend=0.002).

Incidence of atrial fibrillation in relation to individual ISPs

When expressed as age-adjusted HR per standard deviation increase of plasma concentration, all ISPs except α_1-antitrypsin showed significant associations with incidence of AF. After adjustments for confounding factors, ceruloplasmin was the only protein which remained significantly associated with incidence of AF (Table 3). This association was unchanged when subjects with myocardial infarction were censored from the analysis.

Complement factors and incidence of AF

There was no significant association between complement C3 or C4 and incidence of AF (Table 3). Incidence of AF was explored for different combinations of high complement C3 or C4 and high ISP levels. There was no indication that C3 or C4 modified the relationship between the ISPs and incidence of AF.

Discussion

It is now widely accepted that systemic low-grade inflammation is a risk factor for cardiovascular disease and many studies have reported relationships between inflammatory markers and incidence of myocardial infarction and stroke. However, whether inflammation is associated with incidence of AF remains unclear. The present study showed that a composite score of five acute phase proteins was significantly associated with incidence of AF. When considered individually, only ceruloplasmin showed a significant association with AF. These relationships remained after adjustment for several potential confounding factors.

Recent studies of different groups of patients suggest that inflammation has a role in initiating and perpetuating AF, and that inflammation correlates with duration of AF and cardioversion success rate (14). However, there is limited data from prospective population-

based studies. In general, the relationship between inflammation and AF does not seem to be as strong as the associations with myocardial infarction, heart failure and stroke (9, 19).

Schnabel et al recently reported that a panel of 12 inflammatory markers predicted incidence of AF. They found only osteoprotegerin to be significantly associated with AF, and no individual marker predicted AF when incidence of myocardial infarction was taken into account. Fibrinogen, which was the only biomarker also examined in the present study, did not show any significant relationship with AF in that study (18). A study of 5806 men and women (mean age 73 ± 5 years) who were followed during a mean time of 6.9 years reported increased incidence of AF in subjects with high C-reactive protein (CRP)(15). A study of 1032 middle-aged healthy subjects, followed over four years, reported that CRP was associated with AF only in the presence of raised levels of complement C3 (17). Recent reports from the Framingham Heart study and from the Malmö Diet and Cancer study found an independent association between CRP and AF, but no improvement in disease discrimination (16, 24). Mean age of these studies were approximately 58 years, as compared to the mean age of 46.8 years in the present study. A recent 'Mendelian randomization study' including 47000 individuals showed that elevated plasma CRP was associated with increased risk of AF. Importantly, genetically elevated CRP levels were not associated with AF in that study. This suggests that plasma CRP per se does not increase AF risk (26). However, there could still be an important role for other markers of the underlying systemic inflammation. To our knowledge, there is no previous study on ceruloplasmin in relation to incidence of atrial fibrillation.

The results in the present study indicate that elevated levels of acute-phase proteins, in particular ceruloplasmin, may precede AF. The association between AF and ceruloplasmin is markedly stronger than the relationship with the other ISPs in our study. Ceruloplasmin has

strong oxidant effects and has been shown to induce LDL oxidation (27). The protein contains seven copper atoms per molecule, accounting for approximately 95% of the total circulating copper in healthy adults (28). Removal of one of the copper atoms completely blocks the oxidative activity of ceruloplasmin (27). Turnlund et al showed that long-term high copper intake resulted in significantly elevated ceruloplasmin enzymatic activity (29). Emerging evidence implicates a role of oxidative stress in the initiation and maintenance of AF, in which oxidative stress may cause remodelling of the atrium (30-32). This could be a possible link between ceruloplasmin and incidence of AF.

It has been suggested that raised levels of inflammatory markers may not be a reflection of the arrhythmia itself but a result of confounding by underlying cardiac pathology (33). However, mean age was rather low in this study, individuals with known cardiovascular diseases were excluded and incidence of AF occurred many years after the baseline examination. It therefore seems unlikely that the present results can be explained by reverse causation. Several pieces of evidence also indicate that inflammation could have a causal role in the initiation and perpetuation of AF, possibly by inducing structural remodeling of the atrium. This hypothesis is partly based on histological studies which demonstrated inflammatory infiltrates in AF patients and in animal models of AF (34-36).

Hypertension and obesity are the two major risk factors for AF, and inflammation has been suggested as a mediator of these associations (37). Although the model was adjusted for baseline levels of these factors, it is still possible that some men developed hypertension and obesity during the follow-up period. Longitudinal studies have shown that low-grade systemic inflammation is a risk factor for development of hypertension and obesity (38, 39).

Some methodological issues need to be considered. The endpoints of this study were retrieved using the Swedish hospital discharge register. Some cases of atrial fibrillation might only be treated in primary care, and are therefore not included in this study. However, the

estimates of prevalence and incidence in a recent validation study from this population were largely comparable with other epidemiological studies of AF (8). Since all diagnoses were settled during hospital stay, most cases are assumed to be valid. A validation study of cases retrieved from the Swedish national registers showed that the validity of the diagnosis is very high (8).

Another limitation is that electrocardiographic information at baseline was not available, suggesting that some cases may have had AF already when entering the study. However, since mean age at baseline was only 47 years, and considering the fact that AF is strongly related to age, the number of cases with AF at baseline is assumed to be small. CRP was not used in clinical practice at the time when this study started and was therefore not available in our study.

Mean age in this study was rather low, and very few participants used any heart drug at the time when the study started. We do not know how many received anti-arrhythmic drugs during the follow-up period. If anything, however, anti-arrhythmic drugs would weaken the association between ISPs and atrial fibrillation, thereby increasing the risk of a false negative result.

We do not know if the incident cases were classified as paroxysmal, persistent or permanent AF. However, the subcategories of AF overlap to a great extent. Most cases of paroxysmal AF seem to evolve into persistent and permanent AF (40). The arrhythmia is discovered in different phases in different patients depending on what symptoms the patient experience.

As plasma levels were only measured at baseline, the levels of ISPs may have changed before disease manifestation. This is a limitation. However, this “regression dilution” might result in an underestimation of the actual risk (41).

Previous studies have shown that the ISPs studied here are risk factors for developing myocardial infarction (9). One question is whether the increased risk of AF could be explained by the increased incidence of myocardial infarction in men with low-grade inflammation. However, when excluding cases with incident myocardial infarctions during follow-up, risk estimates remained significant and even increased slightly, which indicates that the relationship between ISPs and AF is independent of myocardial infarction. However, we were unable to account for patients with silent myocardial infarction, i.e. who were not hospitalized.

It should be mentioned that the primary analysis using a score of all five ISPs was decided a priori based on the methods used in previous studies (9, 19). The analyses of individual ISPs could therefore be considered as secondary analyses. However, the p-value for the relationship between ceruloplasmin and incidence of atrial fibrillation remains significant even after a Bonferroni-correction.

In conclusion, our study shows that a score of five inflammation sensitive proteins is associated with long-term incidence of hospitalizations due to atrial fibrillation in middle-aged men. When inflammatory markers were considered individually, a significant association was observed with ceruloplasmin.

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Conflicts of interest

Gunnar Engström is employed as senior epidemiologist by AstraZeneca R&D, Lund, Sweden. Samuel Adamsson Eryd, J. Gustav Smith, Olle Melander and Bo Hedblad have no potential conflicts of interest.

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Text tables

Table 1. Baseline characteristics in subjects with and without atrial fibrillation during follow-up

	Atrial fibrillation		
	No (*n*=5364)	Yes (*n*=667)	*p*
Age (years)	46.7 ± 3.7	47.8 ± 3.4	<0.001
BMI (kg/m^2)	24.9 ± 3.3	25.7 ± 3.5	<0.001
Systolic BP (mmHg)	128.7 ± 15.5	132.2 ± 16.4	<0.001
Diastolic BP (mm Hg)	86.9 ± 10.0	89.1 ± 10.7	<0.001
Anti-hypertensive medication (%)	4.2	7.8	<0.001
Cholesterol (mmol/L)	5.69 ± 1.04	5.73 ± 0.98	0.37
Triglycerides (mmol/L) [#]	1.58 ± 1.11	1.58 ± 0.90	0.87
Smokers (%)	48	48	0.33
Diabetes (%)	4.7	4.9	<0.001
Angina (%)	1.2	1.6	<0.001
Heart drug (%)	0.4	0.3	0.72
Physical inactivity (%)	57	54	0.058
High alcohol consumption (%)	13	15	<0.001
Heart rate [#]	68 ± 10	67 ± 10	0.19
ISPs (g/L)			
Fibrinogen	3.5 ± 0.80	3.6 ± 0.80	0.12
Haptoglobin	1.38 ± 0.67	1.39 ± 0.73	0.76
Ceruloplasmin	0.316 ± 0.07	0.322 ± 0.07	0.015
α_1-Antitrypsin	1.27 ± 0.27	1.28 ± 0.27	0.50
Orosomucoid	0.82 ± 0.20	0.83 ± 0.21	0.18
Complement factors			
C3 [#]	103.0 ± 22.7	103.8 ± 22.6	0.43
C4 [#]	120.9 ± 40.2	120.6 ± 40.2	0.89

[#] Results on C3, C4, triglycerides and heart rate was based on 5817, 5813, 6019 and 5984 subjects, respectively.

Table 2. Incidence of atrial fibrillation in relation to number of elevated ISPs

	Number of elevated ISPs				*p* for trend
	None *n* = 2437	One *n* = 1555	Two *n* = 891	Three or more *n* = 1148	
Atrial fibrillation *n* (%)	253 (10.4)	173 (11.1)	96 (10.8)	145 (12.6)	
AF per 1000 person-years	4.00	4.46	4.48	5.54	
Age adjusted HR	1.0	1.16 (0.96-1.41)	1.19 (0.94-1.51)	1.61 (1.31-1.98)	<0.001
+ risk factors[a]	1.0	1.08 (0.88-1.31)	1.07 (0.84-1.36)	1.39 (1.12-1.74)	0.007

[a]Hazard ratios (95% CI) adjusted for age, BMI, systolic blood pressure, anti-hypertensive medication, angina, total cholesterol, smoking, diabetes, physical activity and alcohol consumption.

Table 3. Incidence of atrial fibrillation in relation to individual inflammation-sensitive proteins and complement factors.

	Age adjusted HR	*p*	+ risk factors*	*p*
Fibrinogen	1.13 (1.05-1.22)	0.002	1.07 (0.99-1.16)	0.11
Haptoglobin	1.13 (1.05-1.22)	0.001	1.08 (1.00-1.17)	0.056
Ceruloplasmin	1.17 (1.08-1.26)	<0.001	1.13 (1.04-1.22)	0.003
α_1-Antitrypsin	1.07 (0.99-1.16)	0.087	1.04 (0.96-1.12)	0.34
Orosomucoid	1.13 (1.05-1.22)	0.001	1.06 (0.98-1.15)	0.14
C3	1.10 (1.02-1.19)	0.020	1.01 (0.93-1.09)	0.90
C4	1.01 (0.93-1.09)	0.80	0.96 (0.89-1.05)	0.37

Presented as hazards ratios (HR) per standard deviation increase of the plasma protein concentration.

Standard deviation values for the plasmaproteins were 0.80 g/L for fibrinogen, 0.68 g/L for haptoglobin, 0.067 g/L for ceruloplasmin, 0.27 g/L for α_1-antitrypsin, 0.20 g/L for orosomucoid 0.22 g/L for C3, 0.080 g/L for C4.

*Adjusted for age, BMI, systolic blood pressure, anti-hypertensive medication, angina, total cholesterol, smoking, diabetes, physical activity and alcohol consumption.

Figures

Figure 1. Incidence of hospitalizations due to AF over a mean follow-up of 25 years, in relation to the number of elevated ISPs.

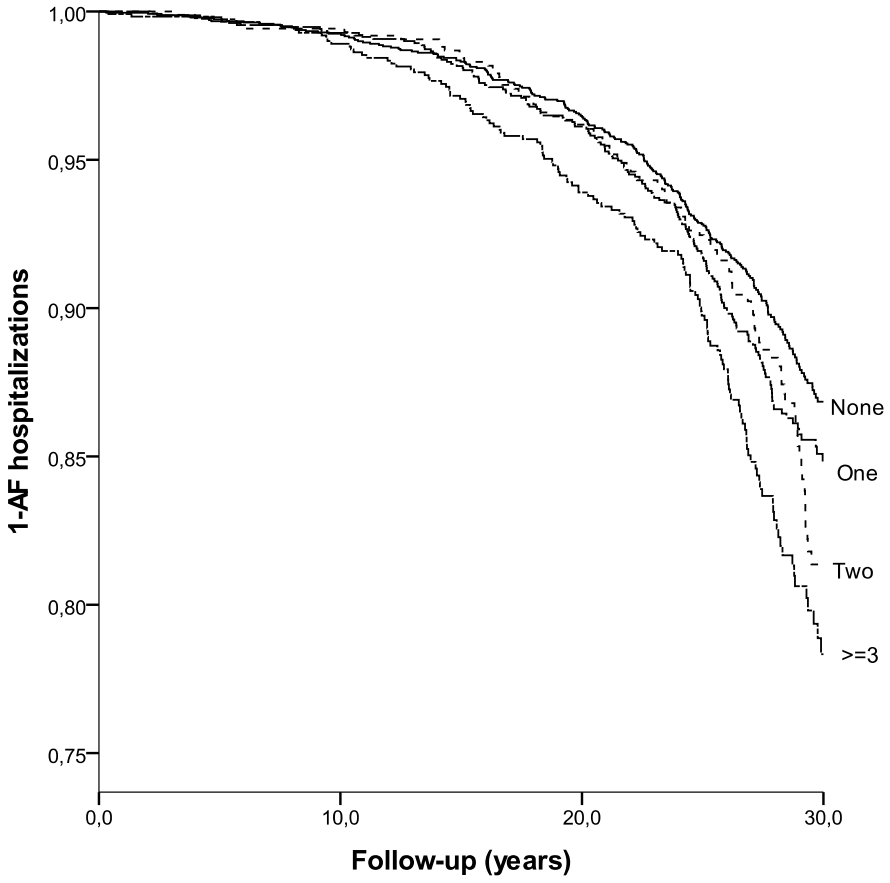

Figure 2. Incidence of hospitalizations due to AF over a mean follow-up of 25 years, in relation to quartiles of ceruloplasmin.