Relations of Biomarkers of Distinct Pathophysiological Pathways and Atrial Fibrillation Incidence in the Community

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Background—Biomarkers of multiple pathophysiological pathways have been related to incident atrial fibrillation (AF), but their predictive ability remains controversial.

Methods and Results—In 3120 Framingham cohort participants (mean age 58.4±9.7 years, 54% women), we related 10 biomarkers that represented inflammation (C-reactive protein and fibrinogen), neurohormonal activation (B-type natriuretic peptide [BNP] and N-terminal proatrial natriuretic peptide), oxidative stress (homocysteine), the renin-angiotensin-aldosterone system (renin and aldosterone), thrombosis and endothelial function (D-dimer and plasminogen activator inhibitor type 1), and microvascular damage (urinary albumin excretion; n=2673) to incident AF (n=209, 40% women) over a median follow-up of 9.7 years (range 0.05 to 12.8 years). In multivariable-adjusted analyses, the biomarker panel was associated with incident AF ($P<0.0001$). In stepwise-selection models ($P<0.01$ for entry and retention), log-transformed BNP (hazard ratio per SD 1.62, 95% confidence interval 1.41 to 1.85, $P<0.0001$) and C-reactive protein (hazard ratio 1.25, 95% confidence interval 1.07 to 1.45, $P=0.004$) were chosen. The addition of BNP to variables recently combined in a risk score for AF increased the C-statistic from 0.78 (95% confidence interval 0.75 to 0.81) to 0.80 (95% confidence interval 0.78 to 0.83) and showed an integrated discrimination improvement of 0.03 (95% confidence interval 0.02 to 0.04, $P<0.0001$), with 34.9% relative improvement in reclassification analysis. The combined analysis of BNP and C-reactive protein did not appreciably improve risk prediction over the model that incorporated BNP in addition to the risk factors.

Conclusions—BNP is a predictor of incident AF and improves risk stratification based on well-established clinical risk factors. Whether knowledge of BNP concentrations may be used to target individuals at risk of AF for more intensive monitoring or primary prevention requires further investigation. **(*Circulation*. 2010;121:200-207.)**

Key Words: atrial fibrillation ■ biomarkers ■ epidemiology ■ arrhythmia ■ risk assessment

It is anticipated that over the next 4 decades, the prevalence of atrial fibrillation (AF) will increase dramatically owing to an aging population, improved therapies, and longer survival with heart disease.[1,2] AF is associated with higher rates of stroke and hospitalization,[3,4] diminished quality of life,[5] and significant mortality.[6] The identification of risk factors for developing AF is an important epidemiological task with potential implications for public health,[7,8] and research in this respect has been prioritized by the National Heart, Lung, and Blood Institute (http://www.nhlbi.nih.gov/meetings/workshops/prevent-af.htm).

Clinical Perspective on p 207

Well-established clinical risk factors for AF other than age and sex are body mass index, hypertension, and cardiovascular disease, including valvular disease and heart failure[9–11]; however, these risk factors do not explain all cases of AF, which

Received May 24, 2009; accepted October 20, 2009.

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The online-only Data Supplement is available with this article at http://circ.ahajournals.org/cgi/content/full/CIRCULATIONAHA.109.882241/DC1.

Guest Editor for this article was Michael E. Cain, MD.

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Circulation **is available at http://circ.ahajournals.org** **DOI: 10.1161/CIRCULATIONAHA.109.882241**

suggests a need for improvement in risk prediction and understanding of the pathophysiology of AF.[12] Blood and urinary biomarkers are potential tools to enhance AF risk prediction and to provide insights into the pathophysiology of the disease. On the basis of biological plausibility and prior reports, biomarkers were chosen to represent distinct pathophysiological pathways, including inflammation (C-reactive protein [CRP] and fibrinogen),[13,14] neurohormonal activation (B-type natriuretic peptide [BNP] and N-terminal proatrial natriuretic peptide [N-ANP]),[15,16] oxidative stress and endothelial dysfunction (homocysteine),[17] the renin-angiotensin-aldosterone system (renin and aldosterone),[16] thrombosis and endothelial function (D-dimer and plasminogen activator inhibitor type 1),[18,19] and microvascular damage (urinary albumin excretion).[20] We hypothesized that the combined analysis of these biomarkers identifies a small panel of distinct biomarkers that are associated with new-onset AF and will improve risk stratification beyond clinical risk factors.

Methods

Study Sample

The Framingham Offspring Study enrolled 5124 individuals in the early 1970s with regular follow-up every 4 to 8 years.[21] Participants (n=3532) who attended the sixth examination cycle (1995–1998) were eligible for analysis. For the present study, attendees were excluded on the basis of any missing biomarker measurements (n=270), incomplete or missing follow-up (n=1), prevalent AF (n=106), serum creatinine >2 mg/dL (n=18), or missing covariate data (n=17). As a result, data on 3120 participants were available for analysis (n=2673 for those with a urinary albumin measurement). The Boston University Medical Center Institutional Review Board approved the study protocols, and participants provided informed consent at each examination.

Clinical Evaluations

Regular cardiovascular health assessments at the Framingham Heart Study clinic include cardiac risk factor documentation during a physician-administered interview and physical examination. Valvular heart disease was considered present if a systolic murmur louder than grade 3 (on a 6-point scale) or any diastolic murmur was detected on auscultation. Heart failure was diagnosed by the endpoint adjudication committee on the basis of previously published criteria.[22] Hypertension medication was determined by self-report. The average of 2 seated systolic blood pressure measurements obtained by a Framingham Heart study physician constituted the examination blood pressure.

AF Verification

The participants' physician office visits and hospitalization records were collected. The diagnosis of AF was based on AF or atrial flutter present on ECG tracings and information from hospital or outpatient records or Framingham Study clinic examinations. For Framingham Offspring participants, biennial health history updates included a routine question on AF. Incident AF cases underwent review, and 2 Framingham cardiologists had to agree on the diagnosis.[12]

Biomarker Determination

Blood samples were obtained routinely from fasting participants and processed immediately. The measurement characteristics of the biomarkers have been described previously.[23] Plasma biomarkers comprised high-sensitivity CRP, D-dimer, fibrinogen, BNP, N-ANP, renin, plasminogen activator inhibitor type 1, and homocysteine. Aldosterone was measured from serum. Urinary albumin and creatinine were determined with a spot morning specimen. Assay details are provided in the online-only Data Supplement. Mean interassay coefficients of variation were 13% for natriuretic peptides and 10% for other biomarkers.

Echocardiography

Attendees at examination cycle 6 routinely received transthoracic echocardiography. Echocardiographic measurements such as M-mode left atrial diameter, wall thickness (sum of diastolic interventricular septum and left ventricular posterior wall) and a measure of systolic function (left ventricular fractional shortening)[24] were available at the baseline examination on 2289 attendees.

Statistical Analyses

Biomarkers were transformed by the natural logarithm and were standardized (mean of 0 and SD of 1) for analyses. For multivariable-adjusted models, we selected AF risk factors that have been reported in association with incident AF and that have been incorporated recently in a weighted risk score for individualized risk prediction of AF. The present sample constitutes a subsample of the risk-score–derivation sample. The variables of the risk algorithm comprised age (at baseline examination 6), sex, body mass index, systolic blood pressure, ECG PR interval, hypertension treatment, heart valve disease (heart murmur), and heart failure.[12] Multivariable-adjusted proportional hazards regression models were estimated to relate the biomarkers to incident AF.[25] The proportional hazards assumption was examined with a Kolmogorov-type supremum test based on cumulative sums of Martingale-based residuals over follow-up times and covariate values.[26] For primary analyses, we used a stepwise procedure to select biomarkers associated with AF at a conservative 2-sided significance threshold of $P<0.01$ for entry and retention in the model,[27] with age, sex, and clinical covariates forced into the model. The regression coefficients presented are per SD increase in log-transformed biomarkers. For the final model, covariate-adjusted cumulative AF incidence estimates for tertiles of biomarker score (calculated as coefficient-weighted sums of standardized biomarkers associated with AF incidence) were estimated and plotted graphically. We assessed C-statistics to describe discrimination of the baseline model and the model that included selected biomarkers.[28] Calibration was calculated for deciles of risk with a modified Hosmer-Lemeshow statistic for survival analysis.[29] We assessed net reclassification improvement for predefined 10-year AF risk categories (<5%, 5% to 10%, and >10%),[12] integrated discrimination improvement, and the relative integrated discrimination improvement and reclassification calibration.[30] The statistical metrics to assess reclassification are an area of intense development. We also tested the newly introduced reclassification calibration, a method that also accounts for censored data.[31]

To establish the value of the retained biomarker(s) as a potential clinical tool in risk prediction, we reran the proportional hazards models using as a baseline "covariate" a recently developed risk-score function for 10-year incidence of AF (http://www.framinghamheartstudy.org/risk/atrial.html). Any event outside the 10-year time frame was censored (n=6 cases). Censored data were treated as nonevents.

Secondary Analyses

For the final model that incorporated the biomarkers that were significantly associated with incident AF, we assessed potential effect modification by age and sex by a global likelihood ratio test. We further explored whether the association of the selected biomarkers with incident AF was mediated by heart murmur or interim heart failure. In addition, bivariate correlation coefficients for the (log) biomarkers were calculated. We also present the data for each biomarker analyzed separately in multivariable-adjusted models. For the final model, we adjusted for echocardiographic variables (left atrial size, left ventricular wall thickness, and left ventricular fractional shortening) to explore whether the relations between biomarkers and incident AF were mediated by cardiac structure and function measures. Analyses were conducted with SAS version 8.1 (Cary, NC). The authors had full access to and take full responsibility

Table 1. Baseline Characteristics by Incident AF Status

	AF Status in Follow-Up	
	No AF (n=2911)	Incident AF (n=209)
Clinical characteristics*		
Age, y	57.8±9.5	66.3±8.6
Women, n (%)	1608 (55)	84 (40)
Body mass index, kg/m^2	27.9±5.2	28.7±5.9
Systolic pressure, mm Hg	128±18	137±22
Hypertension treatment, n (%)	751 (26)	105 (50)
ECG PR interval, ms	163±23	170±27
Significant murmur, n (%)	68 (2)	14 (7)
Prevalent heart failure, n (%)	12 (<1)	5 (2)
Biomarkers, median (25th, 75th percentile)		
CRP, mg/L	2.0 (0.9, 4.6)	3.0 (1.3, 7.1)
Fibrinogen, mg/dL	329 (288, 378)	351 (306, 401)
BNP, pg/mL	7.7 (4.0, 16.7)	21.3 (8.2, 46.0)
N-ANP, pmol/L	311 (218, 444)	459 (323, 753)
Renin, mU/L	12.0 (7.0, 21.0)	10.0 (5.0, 21.0)
Aldosterone, ng/dL	10.0 (7.0, 14.0)	10.0 (7.0, 14.0)
Homocysteine, mmol/L	9.0 (7.4, 11.0)	9.8 (8.2, 12.3)
D-dimer, ng/mL	311 (200, 462)	435 (300, 636)
Plasminogen activator inhibitor type 1, mg/mL	22.6 (14.2, 33.8)	26.3 (19.3, 39. 6)
Urinary albumin-to-creatinine ratio†	6.1 (2.7, 14.2)	8.4 (3.0, 20.0)
Echocardiographic variables‡		
Fractional shortening	0.37±0.06	0.36±0.07
Left atrial size, cm	3.92±0.50	4.27±0.63
Left ventricular wall thickness, mm	1.89±0.24	2.04±0.33

Age was at the beginning of the follow-up period.

*Clinical characteristics expressed as mean±SD or n (%).

†Available for a subset of 2507 subjects without and 166 subjects with incident AF.

‡Available for a subset of 2154 subjects without and 135 subjects with incident AF.

for the integrity of the data. All authors have read and agree to the manuscript as written.

Results

Participant Characteristics

The study sample had an overall mean age of 58.4±9.7 years, and 54% of the participants were women. In Table 1, the baseline characteristics for individuals who developed AF and those free of AF during follow-up are provided. During a median 9.7 years of follow-up (maximum 12.8 years) until November 2007, 209 incident AF cases occurred (40.0% among women; n=166 in the subset with a urinary albumin-to-creatinine ratio available).

Biomarkers and AF Incidence

We confirmed the validity of the proportionality of hazards assumption for the variables in the selected models. In models that were adjusted for established risk factors, the biomarkers as a panel were associated with incident AF ($P<0.0001$). In multivariable models for single biomarkers in relation to incident AF, N-ANP, BNP, and CRP were associated with outcome (Table 2). In the stepwise-selection procedure, BNP (hazard ratio [HR] per SD=1.62, 95% confidence interval [CI] 1.41 to 1.85, $P<0.0001$) and CRP (HR 1.25, 95% CI 1.07 to 1.45, $P=0.004$) met the inclusion criterion (online-only Data Supplement Table I). The biomarker selection was similar in the subsample of individuals with a urinary albumin-to-creatinine ratio available (BNP HR 1.63, 95% CI 1.40 to 1.89, $P<0.0001$; CRP HR 1.31, 95% CI 1.11 to 1.55, $P=0.002$). The urinary albumin-to-creatinine ratio was not significantly associated with incident AF. The addition of BNP alone, CRP alone, or both simultaneously to the model that contained the clinical risk factors increased the χ^2 statistic from 223 to 303, 229, and 310, respectively. Cumulative event rates according to tertiles of the biomarker score that incorporated BNP and CRP revealed an increase in AF events, with the highest AF incidence observed in the top biomarker score tertile (Figure 1).

Table 2. Multivariable-Adjusted Proportional Hazards Regression Models for AF, With Each Log-Transformed Biomarker Examined Separately

Variable	HR	95% CI	P
Inflammation			
CRP	1.25	1.07–1.46	0.004
Fibrinogen	1.09	0.94–1.26	0.26
Natriuretic peptides			
BNP	1.62	1.42–1.86	<0.0001
N-ANP	1.50	1.28–1.75	<0.0001
Renin-angiotensin-aldosterone system			
Aldosterone	1.05	0.92–1.19	0.50
Renin	0.89	0.77–1.02	0.08
Oxidative stress			
Homocysteine	1.08	0.94–1.24	0.28
Thrombosis, endothelial function			
D-dimer	1.11	0.92–1.32	0.28
Plasminogen activator inhibitor type 1	1.13	0.96–1.33	0.15
Microvascular damage			
Urinary albumin-to-creatinine ratio*	1.09	0.93–1.28	0.29

Biomarker concentrations are natural log-transformed measures.

HRs are provided per 1-SD increase in log-biomarker concentration. Models are adjusted for age, sex, body mass index, systolic blood pressure, hypertension treatment, PR interval, auscultatory valvular heart disease, and heart failure.

*Urinary albumin-to-creatinine ratio was available for 2673 subjects.

When we assessed biomarkers in addition to the risk factors identified as part of the recently developed AF risk score, the risk information derived from BNP increased the C-statistic from 0.78 (95% CI 0.75 to 0.81) to 0.80 (95% CI 0.78 to 0.83; online-only Data Supplement Table II) and improved net reclassification (Figure 2A). The analysis method of net reclassification has been developed to assess the putative clinical utility of a novel risk factor. It is based on prespecified risk categories. A clinically useful biomarker would help to optimize risk classification beyond the model

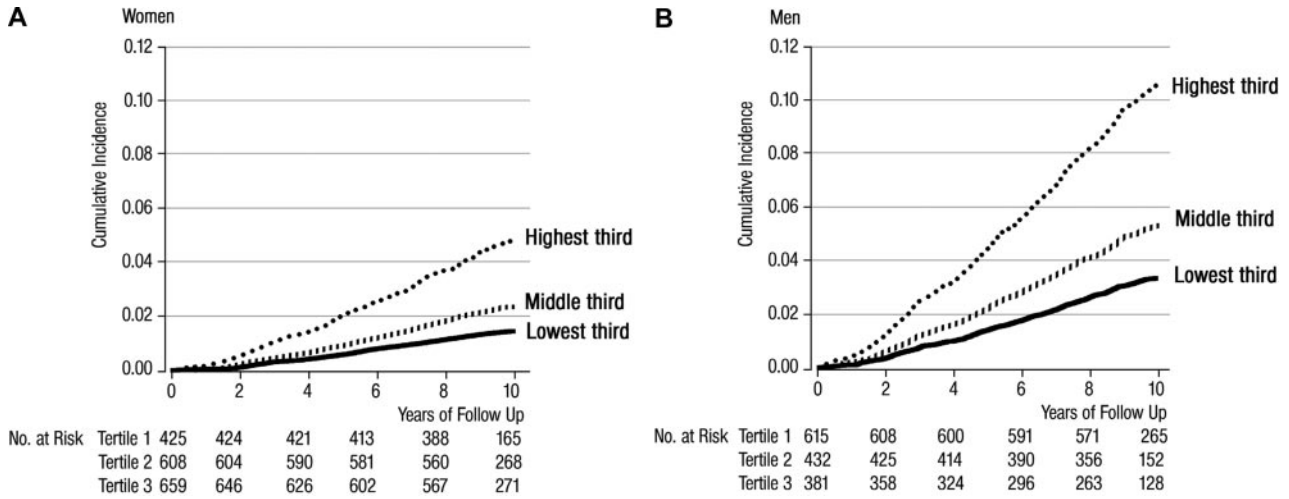

Figure 1. Covariate-adjusted AF cumulative incidence curves for tertiles of the biomarker score (including BNP and CRP) in women (A) and men (B). Mean values of the biomarkers for each tertile of biomarker score were used in creating the cumulative incidence estimates.

that included known risk factors. Ideally, the novel marker would reclassify all individuals with a future event into the high-risk category and all individuals without the outcome into the lowest-risk category. Among those participants who developed AF in the present study sample, the inclusion of BNP concentrations resulted in 25 individuals being reclassified into higher-risk categories (correct direction, green-shaded cells), but 28 were inappropriately classified into a lower-risk category (red-shaded cells). Conversely, among individuals who did not develop AF during the 10 years of follow-up, BNP concentrations would have led to undesirable reclassification of risk upward in 217 individuals, whereas the inclusion of BNP concentrations would have appropriately reclassified 444 individuals into lower-risk categories. The net reclassification improvement, which in the present case indicates the overall reclassification in the desirable direction, was 0.06 (95% CI −0.01 to 0.14, $P=0.09$); reclassification occurred predominantly in the intermediate-risk group. A clinically less intuitive method to assess reclassification is the calculation of the integrated discrimination improvement,

A) Reclassification based on BNP.				
Without BNP	With BNP			
	<5%	5-10%	>10%	Total
Participants who developed atrial fibrillation				
<5%	23 (69.7)	10 (30.3)	0	33
5-10%	11 (21.6)	25 (49.0)	15 (29.4)	51
>10%	1 (0.8)	16 (13.5)	102 (85.7)	119
Total	35	51	117	203
Participants who did not develop atrial fibrillation				
<5%	1557 (92.5)	118 (7.0)	9 (0.5)	1684
5-10%	252 (40.5)	280 (45.0)	90 (14.5)	622
>10%	11 (1.8)	181 (29.6)	419 (68.6)	611
Total	1820	579	518	2917
Net reclassification improvement was 0.06 (95% CI -0.01-0.14, *P*=0.09).				
B) Reclassification based on CRP.				
Without CRP	With CRP			
	<5%	5-10%	>10%	Total
Participants who developed atrial fibrillation				
<5%	28 (84.9)	5 (15.1)	0	33
5-10%	5 (9.8)	39 (76.5)	7 (13.7)	51
>10%	0	6 (5.0)	113 (95.0)	119
Total	33	50	120	203
Participants who did not develop atrial fibrillation				
<5%	1617 (96.0)	67 (4.0)	0	1684
5-10%	92 (14.8)	461 (74.1)	69 (11.1)	622
>10%	0	55 (9.0)	556 (91.0)	611
Total	1709	583	625	2917
Net reclassification improvement 0.009, (95% CI -0.04-0.06, *P*=0.72)				
C) Reclassification based on both biomarkers, BNP and CRP simultaneously.				
Without biomarkers	With both biomarkers (BNP and CRP)			
	<5%	5-10%	>10%	Total
Participants who developed atrial fibrillation				
<5%	22 (66.7)	10 (30.3)	1 (3.0)	33
5-10%	10 (19.6)	22 (43.1)	19 (37.3)	51
>10%	1 (0.8)	12 (10.1)	106 (89.1)	119
Total	33	44	126	203
Participants who did not develop atrial fibrillation				
<5%	1546 (91.8)	127 (7.5)	11 (0.7)	1684
5-10%	264 (42.4)	261 (42.0)	97 (15.6)	622
>10%	23 (3.8)	177 (29.0)	411 (67.3)	611
Total	1833	565	519	2917
Net reclassification improvement was 0.11 (95% CI 0.04-0.19, *P*=0.002).				

Figure 2. Reclassification based on biomarkers. Individuals in the unshaded diagonal boxes did not change classification with the additional biomarkers. Green shading indicates the number and percent of individuals who were reclassified in a desirable direction when the new biomarkers were added to the baseline model; red shading indicates individuals who were reclassified in an undesirable direction. Data in parentheses are row percents.

which does not rely on prespecified risk categories but represents a continuous measure; this was 0.03 (95% CI 0.02 to 0.04, $P<0.0001$), with 34.9% relative improvement.

An even newer metric to evaluate novel biomarkers is the reclassification calibration test introduced by Cook et al,[31] which also takes into account censored data. The χ^2 statistic for the model with risk-score variables only (23.58, $P=0.0003$) decreased to 8.78 with the addition of BNP ($P=0.12$), which indicates a better fit (for these lack-of-fit statistics, a lower value indicates better fit, and nonsignificance is desirable). CRP (net reclassification improvement 0.009, 95% CI −0.04 to 0.06, $P=0.72$; integrated discrimination improvement 0.005, 95% CI 0.0002 to 0.01, $P=0.04$; relative integrated discrimination improvement 5.9%) achieved only a very small improvement in reclassification calibration (Figure 2B). The reclassification calibration χ^2 decreased slightly from 7.26 ($P=0.20$) to 7.05 ($P=0.22$) when CRP was added. Figure I in the online-only Data Supplement provides plots of the estimated risk from the models with and without the biomarkers in addition to the risk factors. To create these plots, we computed risk of AF for each person at each event time (n=203), first from the model with only the clinical risk factors and then from the model with the addition of BNP and CRP. We then averaged each model-specific set of risk estimates by event status, which resulted in the set of sample average predicted risks for AF events and nonevents seen in the plots. The addition of the information derived from BNP and CRP led to a greater separation of the event curves, primarily through a modest increase in estimated incidence for the AF event group.

The reclassification when the combination of both biomarkers was used in addition to the model comprising only the clinical covariates was clearly driven by BNP (net reclassification improvement 0.11, 95% CI 0.04 to 0.19, $P=0.002$; integrated discrimination improvement 0.04, 95% CI 0.02 to 0.05, $P<0.0001$; relative integrated discrimination improvement 39.1%; Figure 2C). The C-statistic did not change appreciably (0.81, 95% CI 0.78 to 0.84) and the calibration χ^2 statistic increased slightly when CRP was added to the model that included BNP.

When the risk algorithm for 10-year incidence of AF was used (n=203 events), the final stepwise selection resulted in a similar model that incorporated BNP ($P<0.0001$) and CRP ($P=0.003$). The reclassification statistics for the variables combined in a risk score may inflate the reclassification and discrimination statistics for biomarkers compared with assessment of the risk factors separately. In our case, the net reclassification improvement for BNP was 0.08 ($P=0.04$). The integrated measure was 0.04 ($P<0.0001$), with 48.2% relative improvement. Further details on the results when the risk score was used are provided in the online-only Data Supplement.

Secondary Analyses

We did not observe statistically significant age or sex interactions with BNP or CRP for the final model (global $P=0.38$). When adjusted for interim development of heart murmur or heart failure, the coefficients and significance of the estimates for BNP and CRP did not change materially (online-only Data Supplement Table IV). The exclusion of individuals (n=17) with prevalent heart failure at baseline did not change the final model appreciably (data not shown). The use of a more parsimonious model with the strongest risk factors (age, sex, hypertension, and heart failure) yielded discrimination statistics comparable to the model that incorporated the risk factors from the Framingham risk score (online-only Data Supplement Table V). The use of the broader range of risk factors moderately increased calibration and fit of the model. The strongest correlations for biomarkers were observed between BNP and N-ANP (Pearson correlation coefficient $r=0.66$) and between fibrinogen and D-dimer ($r=0.45$; online-only Data Supplement Table VI). CRP and BNP had a low positive correlation ($r=0.04$, $P=0.02$).

After adjustment of the final model that incorporated both BNP and CRP for echocardiographic measures (left atrial diameter, left ventricular wall thickness, and fractional shortening), the association of BNP with AF remained robust (HR 1.52, 95% CI 1.28 to 1.81, $P<0.0001$). However, CRP was no longer significantly associated with incident AF (HR 1.10, 95% CI 0.91 to 1.34, $P=0.33$).

Discussion

Principal Findings

In a prospective, middle-aged to elderly community-based cohort, we examined the association of 10 biologically plausible biomarkers with incident AF over a median of 9.7 years. The neurohumoral marker BNP emerged as the strongest predictor of incident AF. When used in addition to a risk score for AF incidence, it improved discrimination and resulted in a substantive net reclassification improvement of 7.9% and a relative integrated discrimination improvement of almost 50%, which remained strong (35%) even after we accounted for potential inflation of the results. The inflammatory biomarker CRP also was statistically significantly associated with the outcome but did not markedly improve risk prediction beyond BNP. We observed that the final models were not substantively altered by the analysis of cardiac disease as a time-dependent variable or by the incorporation of echocardiographic features. Furthermore, we did not observe significant effect modification by sex or age in the models that incorporated BNP and CRP.

BNP as an indicator of cardiac stress is a highly plausible candidate biomarker for AF risk. Manifest AF is accompanied by elevated natriuretic peptide concentrations,[32] even in paroxysmal AF[33,34] and in the absence of overt heart failure.[32] Intuitively, the prohormone fragment of atrial natriuretic peptide, which is predominantly expressed in the atria, might be the member of the natriuretic peptide family that should have strongest predictive power for incident AF. Both natriuretic peptides are elevated in AF patients,[35] and the atria may be a main source for BNP even in the absence of ventricular dysfunction.[36] Correlates of BNP concentrations are left atrial size and left ventricular ejection fraction.[37] The present data demonstrated that even after we accounted for potential intermediate mechanisms by adjusting for interim cardiac disease or echocardiographic measures of left atrial dimensions and systolic function, BNP retained its strength of

association with AF. Thus, BNP appears to provide risk information for AF beyond that provided by noninvasively assessed cardiac structure and function.

Recent investigations over a shorter follow-up period and with fewer AF cases, including a study by Framingham investigators that examined natriuretic peptides in relation to multiple cardiovascular outcomes (68 AF cases), have suggested an association of natriuretic peptides with incident AF.[15,38,39] We now demonstrate that BNP provides additional risk information compared with known strong clinical risk factors for AF and with multiple other biomarkers that have been related to AF. The net reclassification improvement and relative integrated discrimination improvement, which takes into account the number of variables in the basic model and the gain of information by the addition of the novel variable, support the strength of BNP in addition to the clinical risk factors.

Manifest AF is accompanied by systemic inflammatory activity and increased oxidative stress.[40,41] We confirmed prior investigations that related the inflammatory biomarker CRP to incident AF,[42] but we did not find an association with fibrinogen that reached statistical significance.[14] The magnitude of association we observed for CRP in the present study was similar to that observed previously. However, CRP did not perform as well as BNP in improving risk classification, and application of CRP as a risk indicator in clinical practice is unlikely to be resource effective. Even if CRP does not improve risk prediction substantially, the observed relation may help to elucidate the underlying mechanisms of AF and to identify therapeutic targets. Pleiotropic effects of statins have been shown to decrease inflammatory activity, and antiinflammatory treatment might be a rationale for AF prevention on the basis of the consistent association of CRP with AF.[43] Prior literature relating homocysteine to AF suggested that homocysteine is an indicator of endothelial dysfunction and susceptibility to thromboembolic events in manifest AF.[17] Data have remained inconsistent,[44,45] and after multivariable-adjustment, we did not identify a significant association between homocysteine and occurrence of AF.

Strengths and Limitations

Some limitations merit consideration. Inherent to the study design, we may have missed asymptomatic AF episodes. We cannot exclude the possibility that baseline BNP concentrations may have been influenced in part by clinically undetected paroxysmal AF. Furthermore, it would be a useful future research endeavor to investigate whether BNP concentrations can be used to predict risk of AF burden (both duration and number of episodes).

Framingham Offspring participants are almost exclusively of European ancestry, which may limit the generalizability of the present findings to other races/ethnicities. The mean age of the sample at baseline was 58.4 years, and the strength of association of the risk factors and biomarkers may differ in younger individuals or patients with lone AF. Conversely, the risk score was derived from an ambulatory, community-based sample. We acknowledge that the generalizability to a referral-based sample with a higher prevalence of heart failure is uncertain. The risk score may need to be recalibrated if the prevalence of AF risk factors varies substantively from that observed in the present sample. We had a modest number of AF cases; hence, we cannot exclude the possibility that with more events, biomarkers with more modest effect sizes also would have been related to AF onset. On the other hand, a larger number of AF cases might also have led to a regression toward the mean of BNP concentrations in individuals who developed AF.

The present results will need confirmation in independent prospective samples. The utility of the determination of BNP needs to be demonstrated, and potential preventive interventions must be tested. The benefit of prediction algorithms ultimately depends on the demonstration of improved outcomes, ie, a reduction in incidence of AF. At present, no strong preventive measures for AF have been established. BNP is an attractive candidate biomarker, but an observational study design cannot prove a causal relation. However, a better understanding of the relation of BNP to incident AF might provide valuable insights into its pathophysiology and help to identify targets for intervention. In the Framingham cohort, the correlation of N-ANP with BNP was moderately high at 0.66, and after incorporation of both natriuretic peptides into the model, BNP emerged as the stronger biomarker. Of note, the mean BNP concentrations in individuals developing AF were higher than in individuals free of manifest AF at follow-up but fell within the clinical range of normal BNP concentrations. Mildly elevated BNP thus shows a higher susceptibility for incident AF, yet the present data clearly demonstrate that measurement of BNP alone is not sufficient for AF risk evaluation. The present study results can only suggest that BNP, in addition to careful assessment of clinical risk factors, may be able to refine risk prediction.

A major concern with respect to the validity of the present reclassification results and conclusions remains the arbitrary choice of cutoffs for risk categories, because to date, no established risk-prediction scheme has been implemented for AF. We used the same risk classes as in the original publication of the risk algorithm.[12] A different definition of cut points may result in changes in the net reclassification. For this reason, we also provide data on integrated discrimination improvement, which is not dependent on specified risk categories. The results of both analyses showed the same direction, with a borderline improvement after the addition of BNP to the baseline model.

The strengths of the present study are the well-characterized community-based sample with routine ascertainment of clinical risk factors and potential confounders, strict quality control of biomarker measurements, continuous collection of information on outcomes over a comparatively long follow-up time frame, and rigorous ascertainment of incident AF cases. The availability of routine echocardiographic measures at the same examination cycle allowed us to explore mechanistic questions as to whether the relation of BNP to AF was mediated solely through cardiac remodeling. A great advantage of the present investigation is the ability to explore a broad range of pathophysiologically distinct biomarkers and to compare them directly for their strength of association.

In conclusion, the neurohormone BNP and the inflammatory biomarker CRP revealed significant associations with outcome in multivariable-adjusted analyses. BNP was the strongest single biomarker in relation to AF occurrence and significantly improved risk prediction beyond a risk score based on known clinical risk factors.

Sources of Funding

This study was supported by National Institutes of Health/National Heart, Lung, and Blood Institute contracts N01-HC-25195 and 6R01-NS 17950; National Institutes of Health grants 1R01HL092577-01A1 (Drs Ellinor and Benjamin), HL064753, HL076784, and AG028321 (Dr Benjamin), and 1 RO1HL71039 (Dr Vasan); National Institutes of Health Research career award K24 HL04334 (Dr Vasan) and K24, DK080140 (Dr Meigs); an American Diabetes Association career development award (Dr Meigs); and Deutsche Forschungsgemeinschaft (German Research Foundation) research fellowship SCHN 1149/1-1 (Dr Schnabel).

Disclosures

None.

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CLINICAL PERSPECTIVE

The prevalence of atrial fibrillation (AF) is expected to increase owing to an aging population, improved therapies, and longer survival with heart disease. Many pathophysiological pathways have been examined in animal and human studies within the context of AF. We report the prospective association of a broad panel of blood and urinary biomarkers representing inflammation (C-reactive protein and fibrinogen), neurohormonal activation (B-type natriuretic peptide and N-terminal proatrial natriuretic peptide), oxidative stress and endothelial dysfunction (homocysteine), the renin-angiotensin-aldosterone system (renin and aldosterone), thrombosis (D-dimer and plasminogen activator inhibitor), and microvascular damage (urinary albumin excretion) in a community-based cohort with long-term incidence of AF. A recently published risk score for long-term incidence of AF combines several well-established clinical risk factors for AF such as age, sex, body mass index, hypertension, and cardiovascular disease, including valvular disease and heart failure. We tested the predictive value of the strongest biomarkers in addition to the clinical variables combined in the risk algorithm. The neurohormone B-type natriuretic peptide and the inflammatory biomarker C-reactive protein revealed significant associations with outcome in multivariable-adjusted analyses. B-type natriuretic peptide was the strongest single biomarker in relation to AF occurrence and significantly improved risk prediction based on the risk algorithm. Whether determination of B-type natriuretic peptide contributes to strategies to prevent AF must be established in future studies. Our findings may also provide valuable insights into the pathophysiology of AF.