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Circulating fibrosis biomarkers and risk of atrial fibrillation: The Cardiovascular Health Study (CHS)

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Abstract

Background—Cardiac fibrosis is thought to play a central role in the pathogenesis of atrial fibrillation (AF). Retrospective studies have suggested that circulating fibrosis biomarkers are associated with AF, but prospective studies are limited.

Methods—We measured circulating levels of 2 fibrosis biomarkers, procollagen type III, N-terminal propeptide (PIIINP) and transforming growth factor β1 among participants of the CHS, a population-based study of older Americans. We used Cox proportional hazards and competing risks models to examine adjusted risk of incident AF over a median follow-up of 8.8 years.

Results—Levels of PIIINP were assessed in 2,935 participants, of whom 767 developed AF. Compared with the median PIIINP level (4.45 μg/L), adjusted hazard ratios (95% CIs) were 0.85 (0.72–1.00) at the 10th percentile, 0.93 (0.88–0.99) at the 25th percentile, 1.04 (0.95–1.04) at the 75th percentile, and 1.07 (0.90–1.26) at the 90th. Transforming growth factor β1 levels, assessed in 1,538 participants with 408 cases of incident AF, were not associated with AF risk.

Conclusion—In older adults, PIIINP levels were associated with risk of incident AF in a complex manner, with an association that appeared to be positive up to median levels but with little relationship beyond that. Further studies are required to confirm and possibly delineate the mechanism for this relationship.

Background

Atrial fibrillation (AF) is the most common sustained cardiac arrhythmia and causes significant morbidity and mortality. Conventionally, AF has been categorized as an electrical disorder, in which disorganized electrical conduction in the atria results in mechanical dysfunction (ie, fibrillation). However, abnormalities in atrial structure, termed *remodeling*, can also play an important role in the development of AF. Among the most consistently described of these structural abnormalities is the development of atrial fibrosis.[1]

Atrial fibrosis is the process in which collagen and extracellular matrix are deposited within the atria, often resulting in heterogenous conduction and impaired contraction. It has been described in a range of conditions including aging,[2,3] heart failure,[4,5] valve disease,[6] hypertension,[7,8] and myocardial infarction (MI).[9] The association between atrial fibrosis and AF has been observed in both animal models of AF[5,10,11] and in humans.[12,13] However, in general, most of these studies have been performed in vitro or on explanted tissue, with limited clinical application. Although efforts to examine atrial fibrosis using cardiac imaging have shown promise,[14] our ability to quantify atrial fibrosis in a manner useful for disease prediction remains limited.

Although, ultimately, fibrosis is a result of deposition of collagen and other extracellular matrix proteins within a given tissue, a number of upstream pathological processes are involved, including increased activation of the renin-angiotensin-aldosterone system,[15] activation of inflammatory pathways,[16,17] and transforming growth factor β1 (TGF-β1)

activation.[11] As such, circulating markers of collagen, extracellular matrix proteins, or other proteins involved in these various processes may permit noninvasive assessment of fibrosis. If found, a fibrosis biomarker could potentially be useful in predicting risk of AF and possibly targeted for treatment; however, although a number of small studies have shown mixed results, studies in large prospective populations have been limited. In this study, we examine 2 biomarkers of fibrosis, TGF-β1, an upstream modulator of profibrotic pathways and procollagen type III N-terminal propeptide (PIIINP), a peptide released with deposition of collagen III. We measured these complementary markers in the CHS, a large prospective cohort of older adults with prolonged follow-up for risk of incident AF.

Methods

Population

The design and objectives of the CHS have been described previously.[18] In brief, CHS is a longitudinal study of men and women 65 years or older, randomly selected from Medicare lists in Pittsburgh, PA; Forsyth County, NC; Sacramento, CA; and Hagerstown, MD. The original cohort of 5,201 participants was enrolled in 1989 to 1990; a second cohort of mostly 687 African Americans was recruited in 1992 to 1993, providing a total of 5,888 participants. The institutional review board at each center approved the study, and each participant gave informed consent.

Baseline and annual follow-up examinations included a standardized questionnaire assessing a variety of risk factors, including smoking, alcohol intake, history of stroke, coronary heart disease, and heart failure; self-reported health status; and medication use. Methods of determining prevalent cardiovascular disease were previously validated by Psaty et al.[19] The physical examination included measurements of standing height, weight, blood pressure,[19] and resting 12-lead electrocardiogram (ECG). Fasting laboratory measurements included cholesterol, glucose, C-reactive protein, cystatin C, serum creatinine,[20] and N-terminal prohormone of brain natriuretic peptide.[21] Glomerular filtration rate (GFR) was calculated as a metric of kidney function using cystatin, as has been described elsewhere.[22] Participants were contacted every 6 months for follow-up, alternating between a telephone interview and a clinic visit for the first 10 years and by telephone interview only after that.

For these analyses, we included participants who provided blood samples in 1996 to 1997. Of the original 5,888 participants, 1,179 individuals were deceased by the year 9 (1996–1997) clinic visit. Two hundred ninety-six individuals did not participate in the year 9 clinic visit, and an additional 1,004 individuals did not have blood collected at that time. For PIIINP, 106 individuals had blood drawn but did not have a PIIINP result, 1 individual had an “out of range” result, and 367 participants had prevalent AF at the time, leaving a study population of 2,935 individuals. For TGF-β1, 1,687 individuals had blood drawn but did not have a TGF-β1 result, and 184 participants had prevalent AF, leaving a study population of 1,538 individuals. A total of 1,490 individuals without prevalent AF had both PIIINP and TGF-B1 levels measured. Characteristics of the 893 individuals participating in the year 9 clinic visit who had clinical data but did not have PIIINP or TGF-B1 measured are shown in online Appendix Supplementary Table I.

Determination of incident AF

An annual resting ECG was obtained yearly through the 9th year of follow-up, and discharge diagnoses for all hospitalizations were collected. We identified cases of AF in 2 ways. Annual outpatient study ECGs were interpreted by the EPICARE ECG reading center, where the diagnoses of AF or atrial flutter were verified.[23] In addition, hospital discharge diagnoses (International Classification of Diseases, Ninth codes 427.3, 427.31, 427.32) that included codes for AF and flutter were also included, although AF or flutter diagnoses that were made during the same hospitalization as coronary artery bypass surgery or heart valve surgery were not counted. Prior evaluation in CHS determined the positive predictive value of hospital discharge diagnosis to be 98.6% for diagnosis of AF,[23] and a Holter substudy identified that only 1 (0.1%) in 819 subjects had persistent or intermittent AF not identified by the above measures.[24] Ascertainment of incident AF was complete through June 2009.

Biomarker measurements

Phlebotomy methods, blood processing, and handling of samples have been described previously.[18,25,26] Aliquots were frozen at −70 °C until analysis.

Levels of TGF-β1 and PIIINP were measured using stored EDTA plasma obtained at the year 9 CHS clinic visit in 1996 to 1997. Procollagen type III, N-terminal propeptide was assessed in 2,935 individuals from all 4 sites. Because platelets are a major source of TGF-β1, measurement may be susceptible to artificial increases in plasma levels due to platelet degranulation if the plasma is not platelet poor. Because plasma from 2 of the clinic sites was not platelet poor, TGF-β1 was measured only in 1,538 participants from the other 2 sites. Levels of TGF-β1 were measured by use of a commercially available standard quantitative sandwich enzyme-linked immunosorbent assay kit (R&D Systems Inc, Minneapolis, MN)[25] and run according to the manufacturer's recommendations. Procollagen type III, N-terminal propeptide was measured by a coated-tube radioimmunoassay[27] using commercial antisera specifically directed against the terminal amino-terminal peptide of procollagen III (Orion Diagnostica, Espoo, Finland). Interassay and intra-assay coefficients of variation were 8.6% and 3.3% for TGF-β and approximately 5% for PIIINP from an established in-house sample.

Analysis

We present the characteristics of participants according to biomarker levels in tertiles. For analysis of PIIINP, we used Cox proportional hazards regression. Time-to-event was calculated as the interval between the 1996 to 1997 examination (when the blood specimen was obtained) and the development of incident AF, loss to follow-up, death, or end of AF follow-up (June 2009). Unadjusted, population-adjusted (age, sex, race, and clinic site), and risk factor–adjusted models were examined, although only adjusted analyses are presented. Models were assessed for violation of the proportional hazards assumption using Schoenfeld residuals, and no meaningful violations were found. Because spline plots indicated that the association of PIIINP and risk of AF was nonlinear, we modeled PIIINP continuously using a natural cubic spline with 2 knots at the 33.3rd and 66.6th percentiles of PIIINP (3.96 and 5.06 μg/L, respectively) and calculated hazard ratio (HR) estimates for the 10th, 25th, 75th, and 90th percentiles, setting the median as the referent. Given the association of PIIINP with

risk of mortality,[28–30] we also examined the association of PIIINP with risk of AF in the presence of competing risk of death using the method of Fine and Gray.[31] We used similar methods for TGF-β1 analysis (as well as for brain natriuretic peptide and C-reactive protein in Table I), with log base-2 transformation used because it allows a clinically familiar interpretation, as the HR for each unit increase on this scale reflects the risk associated with a doubling of the biomarker level.

For post hoc analysis of PIIINP, we examined 2 separate models above and below the median PIIINP value, with both population adjustment and risk factor adjustment (see Results for details). We tested for interactions with age, sex, and kidney function (GFR) using risk factor–adjusted models, with *P* values based on the model plus the respective interaction term. (Note: GFR based on cystatin was not included in the original risk factor model, so to test for this interaction, the variable was also added to the model).

Results

Demographic, clinical, and biological characteristics of CHS participants at the 1996 to 1997 examination (at the time of blood collection), by levels of PIIINP and TGF-β1, are shown in Table I. Online Appendix Supplementary Table II also displays the combined data for each biomarker.

Procollagen type III, N-terminal propeptide

The mean (SD) PIIINP level was 4.8 μg/L (1.7 μg/L). Among participants in whom PIIINP was available (n = 2,935), 767 developed incident AF. The mean followup time was 8.2 years (median 8.8 years, maximum 13.1 years). Procollagen type III, N-terminal propeptide, modeled continuously, displayed a nonlinear relationship with the risk of incident AF both before and after adjustment (Table II). We observed a linear relationship between risk of AF and PIIINP approximately up to the median value, beyond which point we did not detect a significant association. Sub-HR estimates for PIIINP from the Fine and Gray model were of similar magnitude to the HR estimates from the Cox cause-specific hazards model (online Appendix Supplementary Figure 1).

Post hoc analyses of this relationship was performed by fitting a linear spline model with a knot at the median (median of PIIINP 4.45 μg/L), with adjustment for age, sex, race, and clinic site. Within this model, the HR for the linear relationship between PIIINP and AF below the median was 1.19 (95% CI 1.04–1.37, *P* = .01), whereas the HR above the median was 1.01 (95% CI 0.95–1.06, *P* = .83). When this model was adjusted for age, sex, race, clinic, systolic blood pressure, hypertension medications, height, body mass index (BMI), BMI-squared, congestive heart failure (CHF), MI, and diabetes, the HR below the median was 1.18 (95% CI 1.02–1.36, *P* = .02), and that above the median was 0.99 (95% CI 0.94–1.05, *P* = .77). We observed no effect after adjustment for individual classes of antihypertensive medications (online Appendix Supplementary Table III). We observed no significant interaction between PIIINP and age (*P* = .55), sex (*P* = .84), diabetes (*P* = .22), obesity (*P* = .94), or GFR (*P* = .27).

Adjusting for incident CHD or CHF as a time-varying covariate, we found a minimal effect on the PIIINP HR estimates. Using the post hoc fully adjusted model with splines below and above the median, the estimates additionally adjusting for incident CHD as a time-varying covariate are as follows: for PIIINP below the median, HR = 1.18 (1.03–1.36, *P* = .02), and above the median, HR = 0.99 (0.94–1.05, *P* = .87). In addition, estimates adjusting for incident CHF as a time-varying covariate are as follows:, for PIIINP below the median, HR = 1.19 (1.03–1.36, *P* = .02), and above the median, HR = 0.99 (0.94–1.05, *P* = .81).

Transforming growth factor β1

Transforming growth factor β1 levels were available in 1,538 participants, with 408 cases of incident AF. The mean (SD) TGF-β1 level was 4,730.3 pg/mL (3,917.7 pg/mL). We did not find an association between TGF-β1 level and incident AF in unadjusted or adjusted models (Table III).

Discussion

In this prospective cohort study of circulating fibrosis biomarkers, circulating levels of the propeptide PIIINP appeared to display a nonlinear association with incident AF. This relationship was generally linear up to the median level, after which there was no clear association. We found no association, nonlinear or otherwise, between circulating TGF-β1 levels and incident AF.

The link between atrial fibrosis and AF has been demonstrated in a wide range of settings, from animal models[5,11,32] to human studies.[12,14] In one study of atrial tissue obtained from autopsy specimens of patients with and without AF, not only did atrial fibrosis correlate with both a history of AF and the pattern of AF (more fibrosis in permanent AF hearts), but atrial fibrosis did not correlate with advanced age,[12] which itself had previously been thought to be a risk factor for atrial fibrosis.[33] However, despite its apparent importance in the development of AF, detection of atrial fibrosis remains a challenge. Although the use of delayed enhancement of late gadolinium on cardiac magnetic resonance has shown some promise,[34] limitations in spatial resolution in the atria compared with the ventricles (where it has been very useful) make broad application of this technique difficult.

Given these limitations, circulating biomarkers of fibrosis represent a potentially promising noninvasive approach to detecting atrial fibrosis.[35,36] Thus far, a number of potential circulating biomarkers have been proposed to detect cardiac fibrosis, including TGF-β1,[37] metalloproteinase (TIMP),[38] procollagen type I amino-terminal peptide,[30] PIIINP,[26,30] carboxy-terminal peptide of procollagen type I,[26] carboxy-terminal telopeptide of collagen type I,[26] and osteopontin.[30] Many of these biomarkers were examined in acute settings, such as postoperatively or at the time of electrical cardioversion, and thus application to the ambulatory population may be less relevant.

Among the 2 biomarkers we examined, PIIINP levels had been noted to be lower in individuals more likely to maintain sinus rhythm after treatment with candesartan.[39] However, in other studies no correlation between plasma PIIINP and AF duration was observed.[40] The mixed results of these smaller studies are reflected in the results from our

large prospective study, in which the association between PIIINP and incident AF appeared to be linear up to the median level, but flat above the median. The CIs were quite wide, and thus, our results should be interpreted with caution. Interpretation of this complex association is not clear, but one possible explanation is simply that a cut point exists beyond which increased levels no longer increase risk. Other explanations could be related to the specific timing of circulating PIIINP within the fibrotic process, for which further studies, perhaps with serial PIIINP measurements, would be informative about the specific timing of elevations in circulating PIIINP in the overall fibrosis process, and development of AF. For example, circulating PIIINP could be elevated relatively early in the process of cardiac fibrosis, with release of other markers later on as fibrosis develops.

Our observation that circulating TGF-β1 levels have no clear association with incident AF is equally important given that a number of smaller studies[37,38,41–43] have shown mixed results for this factor that is known to play a central role in cardiac fibrosis.[25,42,44] Our results suggest that although it is important in the biological process of fibrosis, measurement of circulating TGF-β1 appears unrelated to incident AF.

Biomarkers in general have great potential because, if calibration and discrimination are adequate, they can be objectively quantified and applied clinically. Because experimental studies implicate atrial fibrosis in the etiology of AF, the continued search for circulating fibrosis biomarkers will be important in the future of discovering possible treatments and preventive measures for AF.

Our study was limited in several ways. For one, we only had measurements of each biomarker at one point in time. There is limited information available regarding the dynamic changes in these biomarkers in time, and thus, additional measurements would have been informative about the interindividual variation that might exist. Future studies might benefit from this information; however, that we did not detect a significant effect with TGF-β1 and a highly nonlinear one with PIIINP suggests that these efforts might not have a great deal of merit. An additional limitation, true of most population-based studies of AF, is that limited information is available about the clinical scenarios leading to the development of AF in terms of management of risk factors. In addition to subclinical AF, which is an emerging issue in detection,[45] the overall pattern of AF might have been different among biomarker levels, which would not have been appreciated. That subclinical AF may have been present at the time of enrollment is always a concern in population studies, which we could not address in this analysis.

In conclusion, in a large prospective cohort of older adults, TGF-β1 was not associated with risk of incident AF, whereas PIIINP appeared to be associated in a graded manner up to the median, with a threshold above this level. Future studies are needed to validate this finding and to explore other potential circulating fibrosis biomarkers in the risk of incident AF.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Table I

Demographic, clinical, and biological characteristics of CHS participants at the 1996–97 examination, by tertile of PIIINP and TGF-β1

	PIIINP			TGF-β1		
	Tertile 1 (0.54–3.96 μg/L)	**Tertile 2 (3.96–5.06 μg/L)**	**Tertile 3 (5.06–17.50 μg/L)**	**Tertile 1 (619–2409 pg/mL)**	**Tertile 2 (2409–4914 pg/mL)**	**Tertile 3 (4914–38,184 pg/mL)**
Demographics						
n	979	978	978	513	512	513
Age (y)	77.3 ± 4.4	78.0 ± 4.7	78.5 ± 5.0	77.4 ± 4.3	78.1 ± 4.7	77.8 ± 4.7
Male sex, n (%)	334 (34.1%)	383 (39.2)	399 (40.8)	185 (36)	200 (39)	198 (39)
African American race, n (%)	128 (13.1)	152 (15.5)	214 (21.9)	110 (21)	99 (19)	145 (28)
Current smoking, n (%)	94 (9.6)	65 (6.7)	69 (7.1)	45 (9)	35 (7)	61 (12)
Height (cm)	162.7 ± 9.3	163.6 ± 9.5	163.5 ± 9.4	163.7 ± 9.3	163.4 ± 9.6	162.8 ± 9.6
Medical history						
Treated HTN, n (%)	526 (53.7)	528 (54.0)	584 (59.8)	285 (56)	276 (54)	305 (59)
Diabetes, n (%)	125 (12.8)	128 (13.2)	179 (18.5)	87 (17)	80 (16)	121 (24)
CHF, n (%)	62 (6.3)	54 (5.5)	91 (9.3)	29 (6)	27 (5)	39 (8)
MI, n (%)	85 (8.7)	86 (8.8)	111 (11.4)	43 (8)	54 (11)	48 (9)
Stroke, n (%)	53 (5.4)	43 (4.4)	69 (7.1)	16 (3)	29 (6)	29 (6)
ECG						
Heart rate (beats/min)	63.3 ± 10.1	63.3 ± 10.2	63.9 ± 10.5	62.4 ± 9.6	63.1 ± 10.7	64.2 ± 10.5
PR interval (ms)	170.3 ± 30.4	174.0 ± 30.5	174.4 ± 31.4	171.6 ± 30.4	171.9 ± 31.2	173.1 ± 32.6
QRS interval (ms)	93.4 ± 18.7	94.1 ± 18.8	95.2 ± 19.1	94.3 ± 18.3	93.1 ± 17.4	94.0 ± 18.9
Cardiac						
LA volume (mL)	30.3 ± 12.7	33.4 ± 13.4	34.2 ± 15.4	33.6 ± 14.5	35.4 ± 15.4	33.2 ± 14.1
Normal LVEF, n (%)	811 (86.7)	814 (87.6)	796 (87.9)	452 (92)	435 (89)	425 (88)
Biomarkers						
Log2BNP (pg/mL)	6.7 ± 1.4	6.8 ± 1.5	6.9 ± 1.5	6.7 ± 1.3	6.9 ± 1.5	6.6 ± 1.4
Log2CRP (mg/L)	1.2 ± 1.6	1.3 ± 1.6	1.4 ± 1.6	1.2 ± 1.6	1.2 ± 1.6	1.4 ± 1.7
Cystatin C (mg/L)	1.1 ± 0.3	1.1 ± 0.3	1.3 ± 0.5	1.1 ± 0.3	1.2 ± 0.4	1.2 ± 0.4
GFR-cystatin (mL/min per 1.73 m2)	76.5 ± 18.3	71.8 ± 18.9	65.4 ± 19.3	65.4 ± 19.3	65.4 ± 19.3	71.5 ± 18.5

Values are shown are mean ± SD, unless otherwise specified.

Abbreviations: *HTN*, Hypertension; *LA*, left atrial; *LVEF*, left ventricular ejection fraction; *BNP*, brain natriuretic peptide; *Log2*, the base-2 logarithm; *CRP*, C-reactive protein; *GFR-cystatin*, GFR estimate using cystatin C.

Table II

HRs and 95% CIs for incident AF according to PIIINP level

PIIINP	BM, HR (95% CI)	BM + RF model, HR (95% CI)
10% (3.07 μg/L)	0.84 (0.72–0.99)	0.85 (0.72–1.00)
25% (3.69 μg/L)	0.92 (0.87–0.98)	0.93 (0.88–0.99)
50% (4.45 μg/L)	1.0 (referent)	1.0 (referent)
75% (5.45 μg/L)	1.07 (0.98–1.18)	1.04 (0.95–1.14)
90% (6.72 μg/L)	1.13 (0.95–1.34)	1.07 (0.90–1.26)

BM covariates: age, sex, race, clinic site. RF model: BM + systolic blood pressure, hypertensive medications, BMI, BMI-squared, height, smoking status, history of CHF, MI, or prevalent diabetes. Modeled using natural cubic spline with *df* = 3 and 2 knots at 33.3rd and 66.6th quantiles of PIIINP (3.96 and 5.06 μg/L, respectively). Each estimate is the HR for an individual with that value of PIIINP compared with the median value (predicted from model).

Abbreviations: *BM*, Base model; *RF*, risk factor.

Table III

Incident AF and TGF-β1 level

	HR (log_2TGF-β1)	95% CI	*P*
BM	1.07	0.96–1.18	.23
BM + RF	1.05	0.95–1.17	.36

BM covariates: age, sex, race, clinic site. RF model: BM + systolic blood pressure, hypertensive medications, BMI, BMI-squared, height, smoking status, history of CHF, MI, or prevalent diabetes.

Abbreviations: *BM*, Base model; *RF*, risk factor.