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Citation

Agarwal, Isha, Nicole L. Glazer, Eddy Barasch, Mary L. Biggs, Luc Djousse, Annette L. Fitzpatrick, John S. Gottdiener, et al. 2014. "Fibrosis-Related Biomarkers and Incident Cardiovascular Disease in Older Adults." *Circulation: Arrhythmia and Electrophysiology* 7 (4): 583–89. <https://doi.org/10.1161/circep.114.001610>.

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Fibrosis-Related Biomarkers and Incident Cardiovascular Disease in Older Adults

The Cardiovascular Health Study

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Background—Fibrotic changes in the heart and arteries have been implicated in a diverse range of cardiovascular diseases (CVD), but whether circulating biomarkers that reflect fibrosis are associated with CVD is unknown.

Methods and Results—We determined the associations of 2 biomarkers of fibrosis, transforming growth factor- β (TGF- β), and procollagen type III N-terminal propeptide (PIIINP), with incident heart failure, myocardial infarction, and stroke among community-living older adults in the Cardiovascular Health Study. We measured circulating TGF- β (n=1371) and PIIINP (n=2568) from plasma samples collected in 1996 and ascertained events through 2010. Given TGF- β 's pleiotropic effects on inflammation and fibrogenesis, we investigated potential effect modification by C-reactive protein in secondary analyses. After adjustment for sociodemographic, clinical, and biochemical risk factors, PIIINP was associated with total CVD (hazard ratio [HR] per SD=1.07; 95% confidence interval [CI], 1.01–1.14) and heart failure (HR per SD=1.08; CI, 1.01–1.16) but not myocardial infarction or stroke. TGF- β was not associated with any CVD outcomes in the full cohort but was associated with total CVD (HR per SD=1.16; CI, 1.02–1.31), heart failure (HR per SD=1.16; CI, 1.01–1.34), and stroke (HR per SD=1.20; CI, 1.01–1.42) among individuals with C-reactive protein above the median, 2.3 mg/L (*P* interaction <0.05).

Conclusions—Our findings provide large-scale, prospective evidence that circulating biomarkers of fibrosis, measured in community-living individuals late in life, are associated with CVD. Further research on whether TGF- β has a stronger fibrogenic effect in the setting of inflammation is warranted. (*Circ Arrhythm Electrophysiol*. 2014;7:583–589.)

Key Words: cardiovascular diseases ■ collagen ■ epidemiology ■ heart failure

Left ventricular¹ and vascular² remodeling are dynamic structural alterations that occur in response to growth factors, vasoactive mediators, and hemodynamic stimuli. Both types of remodeling are strong risk factors for cardiovascular disease (CVD).^{1,2} Transforming growth factor- β (TGF- β) is a matrix cytokine that modulates key features of remodeling, including cell growth, proliferation, collagen biosynthesis, and the release of collagen byproducts such as procollagen type III N-terminal propeptide (PIIINP). Circulating levels of PIIINP are strongly associated with collagen type III content in cardiovascular vessels and the myocardium.^{3,4}

TGF- β and PIIINP are associated with several CVD risk factors, including age, obesity,⁵ and hypertension.⁶ In prospective studies, TGF- β and PIIINP have been inconsistently associated with incident CVD events.^{5,7–12} Most of these studies have been conducted among hospitalized patients or patients with preexisting CVD. A few studies have been conducted among community-living individuals,^{9,11,13} but sample sizes have been modest, with the largest to date examining 922 individuals over a mean of 9.9 years.⁹ Larger studies among community-living individuals of both TGF- β and PIIINP on the incidence of CVD are clearly needed. In addition, although TGF- β has dual roles as a profibrotic and anti-inflammatory molecule, the interrelationships of fibrosis-related biomarkers

Clinical Perspective on p 589

Received January 23, 2014; accepted May 16, 2014.

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Guest Editor for this article was Gerhard Hindricks, MD.

The Data Supplement is available at <http://circep.ahajournals.org/lookup/suppl/doi:10.1161/CIRCEP.114.001610/-/DC1>.

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Circ Arrhythm Electrophysiol is available at <http://circep.ahajournals.org>

DOI: 10.1161/CIRCEP.114.001610

with inflammation and CVD have yet to be clarified.¹⁴ We evaluated the prospective associations of TGF- β and PIIINP with incident heart failure, myocardial infarction (MI), and stroke among community-living elderly individuals enrolled in the Cardiovascular Health Study.

Methods

Study Design

The design, rationale, and examination details of Cardiovascular Health Study (CHS) have been published elsewhere.¹⁵ Briefly, 5201 participants were recruited to CHS from Medicare eligibility lists in Forsyth County, NC; Sacramento County, CA; Washington County, MD; and Pittsburgh, PA in 1989 to 1990, and a supplemental cohort of 687 black participants was added in 1992 to 1993. To meet eligibility criteria, individuals had to be ≥ 65 years old, living in the community, expected to remain in the current community for ≥ 3 years after baseline, not under active cancer treatment, and able to provide written informed consent. Follow-up interviews were conducted at annual visits through 1998 to 1999 and at interim 6-month telephone calls, which are still ongoing. Our analysis included follow-up through 2010. All participants in our study provided written informed consent, and the institutional review board at each center approved the study protocol.

Exposure Assessment

TGF- β and PIIINP were measured in 2011 to 2012 from stored EDTA plasma samples from the 1996 to 1997 CHS visit, which is the baseline for these analyses. TGF- β was measured by ELISA (Quantikine Human TGF- β 1 Immunoassay; R&D Systems, Minneapolis, MN). PIIINP was measured by the UniQ Intact N terminal Propeptide of Type III Procollagen radioimmunoassay kit manufactured by Orion Diagnostics (Fountain Hills, AZ). Inter- and intra-assay coefficients of variation were between 1.9% and 2.9% and 6.4% to 9.3%, respectively, for TGF- β . For PIIINP, inter- and intra-assay coefficients of variation were both $< 7.2\%$.

Because platelet contamination in plasma samples can artificially elevate levels of TGF- β ,¹⁶ we conducted pilot studies that identified probable platelet contamination at 2 of our 4 clinic sites. Hence, we measured TGF- β only at the 2 remaining sites, a priori. Characteristics of participants between sites that were included and excluded did not differ substantially, including PIIINP measurements (Table 1 in the Data Supplement). Our final analysis included 1371 individuals free from heart failure, MI, and stroke with measured levels of TGF- β , 2568 individuals with measured levels of PIIINP, and 1330 individuals with measured levels of both TGF- β and PIIINP.

Outcome Assessment

Cardiovascular events, including heart failure, MI, and stroke were centrally adjudicated by a CHS outcome assessment committee on the basis of patient reports, physician diagnoses, medical records, and medication use, as previously described.^{17,18} Left ventricular ejection fraction was estimated from echocardiograms for patients with heart failure collected at the time of the incident event.¹⁹ Heart failure with reduced ejection fraction was defined as heart failure with left ventricular ejection fraction $< 55\%$, and heart failure with normal ejection fraction was defined as a heart failure with left ventricular ejection fraction $\geq 55\%$.²⁰

Covariate Assessment

When possible, we used covariate measurement from the 1996 to 1997 visit. If necessary, we carried measurements forward from the most recent visit before 1996 to 1997 with available data. We relied on self-reported age, sex, race, smoking history, leisure time physical activity (kilocalories/week), and alcohol intake (drinks/week). Use of oral hypoglycemic agents, insulin, antihypertensive medications (including diuretics, β -blockers, angiotensin-converting enzyme inhibitors, calcium channel blockers, and other antihypertensive medications), or statins was verified using a validated medication inventory.²¹ We

imputed 685 missing values of pack years based on age, sex, and smoking status. Trained study personnel measured systolic blood pressure. Height and weight were measured to calculate body mass index (the weight in kilograms divided by the square of the height in meters). We obtained blood and urine samples for measurement of fasting glucose, 2-hour glucose tolerance test, total cholesterol, C-reactive protein (CRP), N-terminal type B probrain natriuretic peptide (NT-proBNP), high-sensitivity troponin-T, urine albumin/creatinine ratio (ACR), and estimated glomerular filtration rate based on measured levels of cystatin-C. We defined diabetes mellitus as fasting glucose ≥ 126 mg/dL and use of insulin or oral hypoglycemic medications.

Statistical Analysis

We examined covariate distributions by quintile of TGF- β and PIIINP and evaluated Spearman correlation coefficients. We used Cox proportional hazards models to examine associations of TGF- β and PIIINP with heart failure, MI, and stroke, and a combined end point of first CVD event, using follow-up time since the 1996 to 1997 visit as the time scale. We evaluated hazard ratios for TGF- β and PIIINP as continuous variables (per SD) and across quintiles of biomarkers. To evaluate joint associations of the biomarkers with outcomes, we created a combined variable of TGF- β and PIIINP, which was the sum of the standardized (z score) measurements for each biomarker.

Depending on the outcome of interest, individuals with prevalent disease at baseline were excluded. Model 1 adjusted for age (strata), sex, race, and clinic site. Model 2 additionally adjusted for smoking status (current, former, never), pack years of smoking, body mass index, systolic blood pressure, leisure time physical activity (quintiles), alcohol use, CRP (log), total cholesterol (quintiles), diabetes mellitus, antihypertensive medications, statins, and prevalent forms of other CVD (heart failure, MI, and stroke, depending on the outcome) at baseline. We found no violations of the proportional hazards assumption in fully adjusted models, using an interaction term for exposure and follow-up time. In sensitivity analyses, we additionally adjusted for variables that could potentially be affected by fibrosis and variables that were not available in the full cohort, including estimated glomerular filtration rate, urine ACR, NT-proBNP, high-sensitivity troponin-T, and 2-hour glucose tolerance test. These markers, including urine ACR²² and NT-proBNP,²³ are strong predictors of all-cause and cardiovascular mortality in older adults and may be either upstream or downstream of fibrogenic pathways. We adjusted for these variables to determine whether fibrosis-related biomarkers were independently associated with CVD risk.

We assessed multiplicative interaction between TGF- β and PIIINP and by sex, race, diabetes mellitus status, and CRP. Because we detected a significant interaction with CRP, we conducted stratified analyses that dichotomized CRP at its median value (2.3 mg/L).

For individuals with incident heart failure, we determined separate associations with heart failure with reduced ejection fraction, heart failure with normal ejection fraction, and unclassified heart failure and used Lunn & McNeil competing risks models to compare associations across competing outcomes formally.²⁴

All analyses were conducted in SAS (SAS Institute, Cary, NC). $P < 0.05$ was considered statistically significant for all analyses, including interaction terms.

Results

Participant Characteristics

Demographic, clinical, and laboratory characteristics of participants according to quintiles of TGF- β and PIIINP are shown in Table 1. Individuals with higher levels of either TGF- β or PIIINP were more likely to be black, less physically active, and have higher CRP. Individuals with higher levels of either biomarker were also more likely to have prevalent diabetes mellitus.^{25–27} NT-proBNP levels were higher among individuals with higher PIIINP but not among individuals with higher TGF- β . TGF- β levels were weakly positively correlated with PIIINP levels (Spearman $r = 0.08$; $P = 0.001$).

Table 1. Characteristics of Cardiovascular Health Study Participants by Quintile of Each Biomarker, 1996 to 1997

	Quintile 1	Quintile 2	Quintile 3	Quintile 4	Quintile 5
Among 1627 individuals with measured levels of TGF- β					
TGF- β , ng/L	1489 (267)	2288 (279)	3482 (401)	5447 (830)	10 927 (4498)
PIIINP, ng/mL	4.7 (1.6)	4.9 (1.8)	4.9 (1.6)	5.0 (1.8)	5.2 (2.0)
Age, y	77 (4)	78 (5)	78 (5)	78 (4)	78 (5)
Male, %	39	41	41	45	37
Black race, %	20	17	19	20	31
Former smoking, %	37	52	50	49	44
Current smoking, %	12	5	7	9	13
BMI, kg/m ²	27 (5)	27 (5)	27 (5)	27 (4)	27 (5)
SBP, mm Hg	134 (19)	136 (21)	133 (20)	133 (21)	136 (21)
Physical activity, kilocalories/wk*	780 (1353)	767 (1358)	698 (1335)	549 (1373)	613 (1122)
Alcohol use, drinks/wk*	0.0 (0.5)	0.0 (0.5)	0.0 (1.0)	0.0 (1.0)	0.0 (0.5)
CRP, mg/L*	2.2 (3.8)	2.4 (4.1)	2.4 (3.5)	2.2 (4.3)	3.3 (6.3)
Total cholesterol, mg/dL	195 (38)	202 (39)	198 (37)	200 (41)	203 (41)
Diabetes mellitus, %	14	14	15	17	20
Antihypertensive medications, %	47	55	58	57	62
Statins, %	12	10	11	10	10
eGFR, mL/min per 1.73 m ²	74 (16)	72 (22)	70 (20)	71 (20)	70 (19)
Urine ACR, mg/g*	9 (10)	10 (16)	9 (14)	10 (16)	9 (17)
NT-proBNP, pg/mL*	107 (144)	119 (186)	122 (185)	103 (167)	105 (144)
Among 3237 individuals with measured levels of PIIINP					
TGF- β , ng/L	4312 (3549)	4862 (4726)	4693 (3820)	4820 (3900)	5001 (3695)
PIIINP, ng/mL	3.0 (0.4)	3.9 (0.2)	4.4 (0.2)	5.3 (0.3)	7.5 (2.0)
Age, y	77 (4)	78 (5)	78 (5)	78 (5)	79 (5)
Male, %	33	39	39	45	45
Black race, %	11	14	14	16	22
Former smoking, %	42	43	45	49	46
Current smoking, %	10	8	7	7	7
BMI, kg/m ²	26 (4)	27 (4)	27 (5)	27 (5)	28 (5)
SBP, mm Hg	137 (21)	137 (21)	136 (20)	137 (21)	137 (21)
Physical activity, kilocalories/wk*	863 (1428)	814 (1459)	698 (1466)	660 (1423)	540 (1155)
Alcohol use, drinks/wk*	0.0 (1.0)	0.0 (1.0)	0.0 (1.0)	0.0 (1.0)	0.0 (0.5)
CRP, mg/L*	2.2 (3.8)	2.3 (3.9)	2.3 (4.1)	2.5 (4.2)	2.7 (4.2)
Total cholesterol, mg/dL	206 (38)	204 (38)	204 (40)	198 (39)	199 (42)
Diabetes mellitus, %	13	12	14	16	21
Antihypertensive medications, %	54	55	55	61	65
Statins	10	9	9	11	10
eGFR, mL/min per 1.73 m ²	77 (18)	74 (18)	71 (19)	67 (19)	63 (19)
Urine ACR, mg/g*	10 (14)	9 (13)	9 (14)	11 (23)	12 (30)
NT-proBNP, pg/mL	116 (143)	118 (148)	116 (166)	136 (189)	138 (220)

Except where indicated, continuous variables are reported as mean (SD). Dichotomous and categorical variables are reported as %. ACR indicates albumin/creatinine ratio; BMI, body mass index; CI, confidence interval; CRP, C-reactive protein; eGFR, estimated glomerular filtration rate; NT-proBNP, N-terminal type B probrain natriuretic peptide; PIIINP, procollagen type III N-terminal propeptide; SBP, systolic blood pressure; and TGF- β , transforming growth factor- β .

*Reported as median (interquartile range).

Risk of CVD

In age-, sex-, race-, and clinic-adjusted models, PIIINP was associated with risk of total CVD, heart failure, and stroke (Table 2) as was the combined measure of TGF- β and PIIINP. Most associations remained statistically significant in fully

adjusted models; however, the associations between PIIINP and stroke and between the combined measure and heart failure were attenuated. When tested across extreme quintiles, the highest quintile of PIIINP was associated with $\approx 30\%$ higher risk of total CVD relative to the lowest (hazard ratio=1.34; 95% confidence

Table 2. Multivariable-Adjusted Hazard Ratios (per SD) for Incident CVD Among Cardiovascular Health Study Participants

	TGF- β				PIIINP				Combined			
	n (Cases/Total)	HR	95% CI	P Value	n (Cases/Total)	HR	95% CI	P Value	n (Cases/Total)	HR	95% CI	P Value
Total CVD												
Model 1*	540/1371	1.08	0.99–1.19	0.08	1017/2568	1.11	1.05–1.18	<0.001	525/1330	1.15	1.06–1.26	0.002
Model 2†	540/1371	1.06	0.97–1.17	0.19	1017/2568	1.07	1.01–1.14	0.03	525/1330	1.12	1.02–1.23	0.02
Heart failure												
Model 1*	432/1555	1.06	0.96–1.17	0.28	855/2940	1.13	1.06–1.21	<0.001	422/1577	1.11	1.00–1.23	0.04
Model 2†	432/1555	1.05	0.94–1.17	0.39	855/2940	1.08	1.01–1.16	0.03	422/1577	1.07	0.96–1.19	0.20
Myocardial infarction												
Model 1*	225/1510	1.04	0.90–1.20	0.57	452/2882	1.05	0.95–1.16	0.32	216/1465	1.00	0.87–1.17	0.91
Model 2†	225/1510	1.04	0.90–1.20	0.61	452/2882	1.03	0.93–1.14	0.60	216/1465	0.99	0.85–1.15	0.86
Stroke												
Model 1*	249/1600	1.13	0.99–1.28	0.07	454/3036	1.10	1.01–1.20	0.04	241/1549	1.22	1.08–1.39	0.002
Model 2†	249/1600	1.12	0.98–1.28	0.10	454/3036	1.07	0.98–1.17	0.16	241/1549	1.22	1.08–1.39	0.002

1 SD=3951.75 ng/L (TGF- β); 1.78 ng/mL (PIIINP). CVD indicates cardiovascular disease; CI, confidence interval; HR, hazard ratio; PIIINP, procollagen type III N-terminal propeptide; and TGF- β , transforming growth factor- β .

*Adjusted for age, sex, race, and clinic.

†Adjusted for age, sex, race, clinic, smoking status, pack years, body mass index, systolic blood pressure, physical activity, alcohol use, C-reactive protein, total cholesterol, diabetes mellitus, hypertension medications, and statins.

interval, 1.09–1.65). TGF- β was not associated with risk of incident CVD overall nor did we observe an interaction between levels of TGF- β and PIIINP on risk (P interaction=0.10).

In sensitivity analyses conducted among the 60% of individuals without missing values of estimated glomerular filtration rate, urine ACR, NT-proBNP, high-sensitivity troponin-T, and 2-hour glucose tolerance test, additional adjustment for these variables did not substantially change regression coefficients.

Stratified Analyses

We did not observe significant effect modification by sex, race, or diabetes mellitus status (P interactions all >0.05). However, CRP modified the associations of TGF- β with total CVD and heart failure (P interactions both <0.05). Associations of TGF- β , PIIINP, and the combined measure of TGF- β and PIIINP with total CVD, heart failure, and stroke were generally statistically significant among individuals with higher CRP (>2.3 mg/L) and consistently larger in magnitude for individuals with higher CRP than those with lower CRP (Table 3). CRP level was modestly but positively correlated with both TGF- β (Spearman $r=0.08$; $P=0.002$) and PIIINP (Spearman $r=0.09$; $P<0.001$).

Associations With Heart Failure Subtypes

Using a competing risks model in the full cohort, we did not detect significant differences in the associations of either biomarker across categories of heart failure ($P=0.27$ for TGF- β , $P=0.15$ for PIIINP). There were also no clear differences across categories of heart failure among individuals with higher CRP ($P=0.58$ for TGF- β , $P=0.49$ for PIIINP).

Discussion

In this prospective, community-based study of older adults, circulating levels of fibrosis-related biomarkers were associated with multiple adverse cardiovascular outcomes. Associations for TGF- β with total CVD, heart failure, and stroke were

statistically significant among individuals with higher CRP, with a similar but nonsignificant pattern observed for PIIINP. Our findings provide further evidence for the hypothesis that fibrosis is an important contributor to CVD among older adults, and in the case of TGF- β , particularly when combined with systemic inflammation.

We observed a significant association between PIIINP and heart failure in the entire cohort, and PIIINP levels were higher among individuals with higher levels of NT-proBNP, a marker of LV diastolic strain. Although chronic kidney disease is prevalent in older adults and an important contributor to circulating levels of PIIINP, observed associations between PIIINP and CVD were similar after adjustment for renal function (estimated glomerular filtration rate and urine ACR). In previous studies, PIIINP has been associated with multiple indicators of cardiac structure,²⁸ including interventricular septum thickness and left atrial diameter,¹¹ as well as measures of cardiac dysfunction, including E/A ratio²⁹ and peak E wave velocity.¹¹ PIIINP has also previously been associated with the risk of incident heart failure in community-living individuals⁹ and older adults.¹¹ Our study confirms these findings and extends them to a larger cohort of community-based individuals. Because we observed a much larger number of incident cardiovascular events compared with previous studies, we are able to report separate associations for heart failure, MI, and stroke, which revealed notable differences between cardiovascular end points. In addition, we defined CVD according to validated, objective clinical end points (heart failure, MI, stroke), whereas previous studies⁹ included subjective end points, such as angina and transient ischemic attack, which could bias their results.

We observed an association between TGF- β and heart failure but only among individuals with higher levels of CRP. In a previous nested case-control study of ≈ 200 individuals in CHS, TGF- β was associated with risk of heart failure without stratification by CRP.⁸ There are several possible explanations for our inability

Table 3. Multivariable-Adjusted Hazard Ratios (per SD) for Incident CVD Among Cardiovascular Health Study Participants Stratified by CRP

	TGF- β					PIIINP					Combined				
	n (Cases/Total)	HR	95% CI	P Value	P-Int	n (Cases/Total)	HR	95% CI	P Value	P-Int	n (Cases/Total)	HR	95% CI	P Value	P-Int
Total CVD					0.05					0.29					0.24
High CRP	285/673	1.16	1.02–1.31	0.02		535/1248	1.14	1.04–1.25	0.006		278/648	1.21	1.06–1.38	0.005	
Low CRP	255/698	0.96	0.82–1.13	0.60		482/1320	1.02	0.92–1.12	0.71		247/682	1.04	0.90–1.20	0.60	
Heart Failure					0.05					0.61					0.30
High CRP	233/776	1.16	1.01–1.34	0.04		467/1457	1.10	1.00–1.21	0.05		228/749	1.14	0.99–1.32	0.08	
Low CRP	199/779	0.93	0.77–1.12	0.43		388/1483	1.05	0.94–1.17	0.37		194/758	1.01	0.85–1.19	0.94	
Myocardial infarction					0.59					0.89					0.71
High CRP	124/760	1.09	0.90–1.33	0.38		260/1433	1.08	0.94–1.24	0.27		122/734	1.05	0.85–1.29	0.67	
Low CRP	101/750	1.02	0.81–1.30	0.84		192/1449	0.97	0.82–1.14	0.68		94/731	0.98	0.77–1.24	0.87	
Stroke					0.39					0.24					0.12
High CRP	140/806	1.20	1.01–1.42	0.04		244/1515	1.14	1.00–1.29	0.05		135/777	1.36	1.15–1.61	<0.001	
Low CRP	109/794	1.04	0.82–1.33	0.73		210/1521	1.03	0.90–1.18	0.69		106/772	1.10	0.88–1.37	0.43	

1 SD=3951.75 ng/L (TGF- β); 1.78 ng/mL (PIIINP). High vs low CRP is dichotomized at CRP's median value, 2.3 mg/dL. *P* interactions were calculated using CRP as a continuous variable. Adjusted for age, sex, race, clinic, smoking status, pack years, body mass index, systolic blood pressure, physical activity, alcohol use, CRP, total cholesterol, diabetes mellitus, hypertension medications, statins, and prevalent cardiovascular disease (year 9). CVD indicates cardiovascular disease; CI, confidence interval; CRP, C-reactive protein; HR, hazard ratio; PIIINP, procollagen type III N-terminal propeptide; and TGF- β , transforming growth factor- β .

to detect an overall association compared with the previous study. First, the mean age at which TGF- β was measured in our study was 78 years compared with 75 years among controls in the previous study. It is possible that the balance of TGF- β 's profibrotic and anti-inflammatory effects³⁰ changes over time, with greater opportunity for the latter effect to suppress leukocyte recruitment and prevent the formation of unstable leukocyte-rich atherosclerotic lesions later in life.^{10,12,26,31} Second, our study relies on an updated assay for TGF- β , which is more reproducible than the assay used in the previous study. Third, participants in our study had a median follow-up of 7.6 years before the occurrence of incident heart failure, whereas participants in the previous study had an average follow-up of 5.4 years; a single measurement of TGF- β may be insufficient to capture the true association between fibrosis and heart failure over the longer period of follow-up in our study. Finally, although we did not detect a statistically significant association between TGF- β and heart failure, the confidence intervals of our estimates and those of the previous study are likely to overlap. As such, we cannot exclude the possibility of an association of TGF- β with heart failure in the present analysis. Notably, several studies outside of CHS have also failed to demonstrate an association between TGF- β and adverse cardiovascular outcomes.^{10,12,26,31} In 1 prospective study of 155 patients on hemodialysis, a 1 ng/mL reduction in TGF- β concentration was actually associated with a 9% increase in the risk of cardiovascular events.¹²

The ultimate association of TGF- β and CVD remains complicated because of TGF- β 's pleiotropic activity. In the cardiovascular system, inflammatory and injurious stimuli such as shear stress, oxidized cholesterol, hemodynamic overload, and CRP³² stimulate lymphocytes, monocytes, and other inflammatory cells to secrete TGF- β .³³ Although transient TGF- β signaling is part of the normal wound healing response, sustained TGF- β signaling can lead to excess deposition of connective tissue elements, progressive remodeling, and scar formation. Because of

TGF- β 's multifunctional roles in both normal wound healing and pathological fibrosis, TGF- β is unlikely to be a potential therapeutic target for CVD. However, targeting downstream regulators of fibrosis, such as the Smad receptors, may allow selective diminution of TGF- β 's profibrotic effects.³⁴

We found significant associations of fibrosis-related biomarkers and incident stroke, but not incident MI, even in minimally adjusted models. The absence of an association between PIIINP and MI in our study contrasts with a nested case-control study conducted earlier in the course of CHS, which used a different PIIINP assay and shorter follow-up period.¹³ Among identified risk factors that are common between stroke and MI, those that are more likely to lead to stroke than to MI may be disproportionately related to fibrosis.³⁵ Specifically, fibrosis has been implicated in both hypertension³⁶ and cardioembolism/atrial fibrillation,³⁷ 2 particularly strong risk factors for stroke.^{38–40} Mechanistically, TGF- β increases the collagen content of arteries, resulting in arterial stiffening, reduced compliance, and increased blood pressure.⁴¹ TGF- β may also contribute to fibrotic remodeling of the atria, although associations of TGF- β with atrial fibrillation have been mixed.^{30,42,43} Furthermore, higher circulating levels of PIIINP have been associated with a lower probability of maintaining sinus rhythm among patients with paroxysmal atrial fibrillation.⁴⁴ The differential association of fibrosis-related biomarkers with these risk factors for stroke versus MI may explain, in part, why TGF- β and PIIINP were associated with stroke but not MI in this study.

The associations we observed in this study were consistently greatest in magnitude, and generally statistically significant, among individuals with higher levels of CRP. Emerging evidence points to a potential role for CRP in fibrosis and fibrosis-related organ damage. CRP has been shown to directly stimulate TGF- β and induce other genes that contribute to collagen deposition.⁴⁵ CRP also reflects several activated pathways in systemic inflammation.^{46,47} TGF- β acts via several different types of receptors, which have different effects on extracellular

matrix deposition, and its predominant signaling pathway may depend on the inflammatory milieu.³¹ Although the biological plausibility of an interaction between fibrosis and inflammation is attractive, our finding requires confirmation in other cohorts.

Our investigation is novel in several respects. We conducted our study in a large, well-characterized, community-based population and an average follow-up time of >10 years. We were able to minimize the possibility of confounding by measuring a diverse range of covariates and including these covariates in our multivariable-adjusted models. In addition, whereas previous studies of fibrosis have limited their analysis to markers of collagen homeostasis (eg, matrix metalloproteinases, their tissue inhibitors, and byproducts of collagen turnover), or other isolated biomarkers, we included 2 complementary biomarkers of fibrosis, TGF- β , and PIIINP. The combination of TGF- β and PIIINP into a combined measure further allowed us to explore putative associations.

Our study has several important limitations. First, we had limited statistical power to detect associations of TGF- β with CVD because of the limited number of TGF- β measurements.

Second, plasma levels of both biomarkers were measured at a single point, and it is possible that the longitudinal trajectory of change in TGF- β or PIIINP may provide additional information, independent of the baseline level, on the future risk of CVD. Third, associations in this study may be biased toward the null because of measurement error resulting from the long hiatus between sample collection in 1996 to 1997 and biomarker measurement in 2005. Fourth, neither TGF- β nor PIIINP is specific to cardiac fibrosis, and thus associations with CVD outcomes may be partly shadowed by fibrotic processes in other organs.

Although both TGF- β and PIIINP have previously been used as markers of tissue fibrosis in epidemiological studies,⁴⁸ they are imperfect measures of underlying tissue fibrosis. The gold standard for evaluation of myocardial or vascular fibrosis is tissue biopsy, but even then fibrosis can be missed if it is not homogeneously distributed.⁴⁹ Several imaging modalities can also detect organ fibrosis with high sensitivity and specificity, but their use in longitudinal human studies has not been widespread.²⁸ Plasma biomarkers such as TGF- β and PIIINP provide a readily available and noninvasive assessment of fibrosis appropriate for cohort studies. However, given the relatively modest observed hazard ratios for TGF- β and PIIINP and their measurement issues, neither biomarker is likely to be immediately useful for prognosis in clinical care. Nonetheless, because these markers only imperfectly reflect underlying fibrosis, the potential benefits of targeting fibrosis may be larger than the observed hazard ratios for TGF- β and PIIINP might suggest. Given that several antifibrotic agents are already in development or testing,^{46,50} clinical trials to specifically target fibrosis and determine its effects on CVD such as heart failure and stroke may be feasible in the near future.

In conclusion, PIIINP and, in the setting of high CRP, TGF- β are associated with some forms of CVD among older adults. Our findings provide support for future research on fibrosis as a potentially targetable pathway to reduce cardiovascular morbidity and mortality. In the setting of clinical trials, TGF- β and PIIINP could potentially be used to identify target populations for interventions and to monitor response to therapy. Further research on whether TGF- β has a stronger adverse effect on CVD in the setting of increased inflammation is warranted.

Sources of Funding

This research was supported by contracts HL118775, HL094555, N01HC85084, N01HC35129, N01HC85085, N01HC45133, HHSN268201200036C, HHSN268200800007C, N01HC55222, N01HC85079, N01HC85080, N01HC85081, N01HC85082, N01HC85083, N01HC85086, and grant HL080295 from the National Heart, Lung, and Blood Institute (NHLBI), with additional contribution from the National Institute of Neurological Disorders and Stroke. Additional support was provided by AG023629 from the National Institute on Aging. A full list of principal CHS investigators and institutions can be found at CHS-NHLBI.org.

Disclosures

None.

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CLINICAL PERSPECTIVE

Vascular and myocardial fibrosis have been implicated in numerous forms of cardiovascular disease (CVD) associated with arrhythmias, but whether circulating biomarkers of fibrosis are associated with CVD is unknown. Transforming growth factor- β (TGF- β) is a matrix cytokine with profibrotic and anti-inflammatory effects; procollagen type III N-terminal propeptide is a byproduct of type III collagen production. TGF- β and procollagen type III N-terminal propeptide (PIIINP) have been associated with CVD risk factors, but limited and inconsistent data are available on prospective associations with incident CVD. We determined the associations of TGF- β and PIIINP with incident heart failure, myocardial infarction, and stroke among community-living older adults in the Cardiovascular Health Study. After adjustment for sociodemographic, clinical, and biochemical risk factors, PIIINP was associated with total CVD and heart failure but not myocardial infarction or stroke. TGF- β was not associated with any CVD outcomes in the full cohort but was associated with total CVD, heart failure, and stroke among individuals with CRP above the median, 2.3 mg/L. Our findings provide large-scale, prospective evidence that circulating biomarkers of fibrosis, measured in community-living individuals late in life, are associated with CVD. Further research on whether TGF- β has a stronger fibrogenic effect in the setting of inflammation is warranted. Pharmacological agents to reduce fibrosis are already in development and may play a role in preventing the progression of CVD.