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Original Contribution

Fibrosis-Related Biomarkers and Risk of Total and Cause-Specific Mortality

The Cardiovascular Health Study

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Fibrosis has been implicated in diverse diseases of the liver, kidney, lungs, and heart, but its importance as a risk factor for mortality remains unconfirmed. We determined the prospective associations of 2 complementary biomarkers of fibrosis, transforming growth factor-β (TGF-β) and procollagen type III N-terminal propeptide (PIIINP), with total and cause-specific mortality risks among community-living older adults in the Cardiovascular Health Study (1996–2010). We measured circulating TGF-β and PIIINP levels in plasma samples collected in 1996 and ascertained the number of deaths through 2010. Both TGF-β and PIIINP were associated with elevated risks of total and pulmonary mortality after adjustment for sociodemographic, clinical, and biochemical risk factors. For total mortality, the hazard ratios per doubling of TGF-β and PIIINP were 1.09 (95% confidence interval (CI): 1.01, 1.17; $P = 0.02$) and 1.14 (CI: 1.03, 1.27; $P = 0.01$), respectively. The corresponding hazard ratios for pulmonary mortality were 1.27 (CI: 1.01, 1.60; $P = 0.04$) for TGF-β and 1.52 (CI: 1.11, 2.10; $P = 0.01$) for PIIINP. Associations of TGF-β and PIIINP with total and pulmonary mortality were strongest among individuals with higher C-reactive protein concentrations (P for interaction < 0.05). Our findings provide some of the first large-scale prospective evidence that circulating biomarkers of fibrosis measured late in life are associated with death.

biomarkers; fibrosis; inflammation; mortality

Abbreviations: CHS, Cardiovascular Health Study; CI, confidence interval; CITP, carboxy-terminal telopeptide of collagen type I; CRP, C-reactive protein; HR, hazard ratio; PIIINP, procollagen type III N-terminal propeptide; TGF-β, transforming growth factor-β.

Fibrosis, the excessive accumulation of extracellular matrix components, is a pathological result of severe or repetitive tissue injury (1). Triggers of fibrosis are diverse and include chronic inflammatory or autoimmune disease, hypertension, poorly controlled diabetes, exposure to toxins/irritants (including smoking), and aging (1).

Fibrosis has been increasingly implicated as a major cause of morbidity and mortality (2); however, few large-scale epidemiologic studies have been conducted to evaluate these hypotheses. Although promising imaging modalities can detect organ fibrosis with high sensitivity and specificity, the use of these modalities in longitudinal human studies has been limited by high cost, technical difficulty, and low availability (3). Plasma biomarkers of collagen biosynthesis provide a reliable, noninvasive assessment of fibrosis that can be easily implemented in large-scale epidemiologic studies (3). Two complementary biomarkers of collagen biosynthesis are transforming growth factor-β (TGF-β) and procollagen type III N-terminal propeptide (PIIINP). Strong correlations exist between changes in these plasma biomarker levels and ongoing organ fibrosis (4).

TGF-β is a multifunctional cytokine and key driver of fibrosis (5). TGF-β regulates cell proliferation, collagen formation, and the release of collagen propeptides such as PIIINP (6, 7). In laboratory rats, adenoviral-mediated gene delivery of TGF-β has been shown to induce severe pulmonary fibrosis

(8), whereas inhibition of TGF-β has been shown to prevent progression of liver fibrosis (9) and ameliorate chronic progressive nephritis (10). In humans, elevated circulating levels of both TGF-β and PIIINP have been cross-sectionally associated with diverse diseases that have a fibrotic component, including liver failure, kidney failure, pulmonary fibrosis, asthma, chronic obstructive pulmonary disease, cancer, rheumatic disease, and heart failure (1, 11–15).

Given the broad ramifications of fibrosis, we hypothesized that circulating levels of the profibrotic biomarkers TGF-β and PIIINP might be associated with the risks of total and cause-specific mortality. No large-scale studies have examined the prospective associations of TGF-β with total or cause-specific mortality risk, and only a few smaller studies have examined associations of PIIINP and mortality, including 1 nested case-control study of congestive heart failure in the Cardiovascular Health Study (CHS) (16).

METHODS

Study design

The design, rationale, and examination details of CHS have been published previously (17). Briefly, participants were recruited from Medicare eligibility lists in Forsyth County, North Carolina; Sacramento County, California; Washington County, Maryland; and Pittsburgh, Pennsylvania. A cohort of 5,201 participants was recruited in 1989–1990, and a supplemental cohort of 687 mostly black participants was added in 1992–1993. Eligible individuals were at least 65 years of age, living in the community, expected to remain in the current community for at least 3 years, not under active cancer treatment, and able to provide written informed consent. Follow-up was conducted at 4 CHS field centers: Wake Forest University School of Medicine, Winston-Salem, North Carolina; the University of California, Davis, Davis, California; Johns Hopkins University, Hagerstown, Maryland; and University of Pittsburgh, Pittsburgh, Pennsylvania. Follow-up interviews for events were done at annual visits through 1998–1999; interim 6-month telephone calls are still ongoing. The present analysis was limited to follow-up through 2010. All participants in our study provided written informed consent, and the institutional review board at each study center approved our study protocol.

Exposure assessment

TGF-β and PIIINP levels were measured in stored plasma samples from the 1996–1997 CHS follow-up visit, which is the baseline for these analyses. TFG-β was measured using an enzyme-linked immunosorbent assay (Human TGF-beta 1 Quantikine ELISA Kit, R&D Systems, Minneapolis, Minnesota). PIIINP was measured using a radioimmunoassay kit (UniQ PIIINP RIA; Orion Diagnostics, Fountain Hills, Arizona). Inter- and intra-assay coefficients of variation were between 1.9%–2.9% and 6.4%–9.3%, respectively, for TFG-β. For PIIINP, inter- and intra-assay coefficients of variation were both less than 7.2%.

Platelets are a significant source of TGF-β, and platelet contamination in plasma samples can lead to artificially elevated TGF-β measurements (18). We conducted pilot studies that confirmed probable platelet contamination at 2 of our 4 clinic sites. Hence, we measured TGF-β levels only in samples taken at the 2 remaining sites. Participant characteristics (including PIIINP measurements) did not differ substantially between sites that were included and excluded (Web Table 1, available at http://aje.oxfordjournals.org/). Our final analysis included 1,443 persons for whom we had measured levels of TGF-β, 2,726 persons for whom we had measured levels of PIIINP, and 1,399 persons for whom we had measured levels of both TGF-β and PIIINP, all of whom were free from myocardial infarction and stroke.

Total and cause-specific mortality

Methods for classifying total and cause-specific deaths have previously been described in detail (19). In summary, total and cause-specific deaths were assessed, investigated, and adjudicated by a centralized events committee using available data from interviews, information provided by next of kin, death certificates, and medical records, including diagnostic tests and consultations. Cause of death was first determined to be cardiovascular or noncardiovascular. Cardiovascular deaths included those from atherosclerotic coronary disease, cerebrovascular disease (stroke), other atherosclerotic disease (such as aortic aneurysm), and other vascular disease (such as valvular heart disease or pulmonary embolism). The noncardiovascular deaths were first classified into 19 disease and organ system categories and then collapsed into 4 categories based on the most common causes of death: pulmonary (e.g., pneumonia, obstructive lung disease), cancer, neurologic (e.g., dementia, amyotrophic lateral sclerosis, Parkinson's disease), or other.

Covariate assessment

When possible, covariate measurements were taken from the data obtained at the 1996–1997 visit. If 1996–1997 data were unavailable, we carried forward using the most recent available measurements. Participants self-reported age, sex, race, marital status, smoking history, leisure time physical activity level, alcohol consumption, and use of insulin, oral hypoglycemic agents, statins, or antihypertension medications (including angiotensin-converting enzyme inhibitors, angiotensin receptor blockers, or aldosterone receptor inhibitors) using a validated medication inventory (20). Trained study personnel measured systolic blood pressure, fasting and 2-hour glucose levels, total cholesterol level, C-reactive protein (CRP) level, forced expiratory volume in 1 second, N-terminal pro-brain natriuretic peptide level, troponin-T level, urine albumin/creatinine ratio, and estimated glomerular filtration rate based on measured levels of cystatin-C and interviewed participants to assess depressive symptoms (Center for Epidemiologic Studies-Depression scale) and cognitive function (Mini-Mental State Examination). We used technician-measured height and weight to calculate body mass index (weight in kilograms divided by the square of the height in meters). We imputed 584 missing values of pack-years of smoking based on age, sex, and smoking status.

Statistical analysis

We examined the distribution of the above-listed covariates by quintile of TGF-β and PIIINP levels and evaluated Spearman correlation coefficients. We used Cox proportional hazards models to examine associations of TGF-β and PIIINP with total and cause-specific mortality, using follow-up time since the 1996–1997 visit as the time scale. We evaluated hazard ratios for TGF-β and PIIINP as continuous variables. Logarithmic transformation (base 2) appeared to maximize model fit for the continuous associations; this transformation provides estimates per doubling in each biomarker. We also tested estimates per standard deviation of the $\log_2$-transformed biomarkers and across quintiles of the untransformed biomarkers.

We created sequential models, choosing covariate forms to maximize model likelihood statistics. Our analytical objective was to examine the relationship of fibrosis-related biomarkers with mortality risk after adjustment for confounding variables. As such, our multivariate models were adjusted for factors likely to be both associated with and upstream from fibrosis, as well as potential risk factors for mortality. Model 1 was adjusted for age (strata), sex, race, and clinic site. Model 2 was additionally adjusted for body mass index (quintiles), smoking status (current, former, never), pack-years of smoking, leisure time physical activity (kilocalories, quintiles), alcohol consumption (drinks/week), marital status, systolic blood pressure (mm Hg), fasting glucose level, total cholesterol (quintiles), CRP (log), and use of oral hypoglycemic agents, insulin, statins, or antihypertension medications. Model 3 was additionally adjusted for variables that could potentially be affected by fibrosis (i.e., variables that could be either confounders or mediators), including estimated glomerular filtration rate and Center for Epidemiologic Studies-Depression symptom score. In sensitivity analyses, we further adjusted for potential confounders for which information was not available in the full cohort, including N-terminal pro-brain natriuretic peptide (log), troponin-T (log), 2-hour glucose level, urine albumin/creatinine ratio (quintiles), and Mini-Mental State Examination score (individually in separate models), and we compared regression coefficients of adjusted and unadjusted models in comparison populations of the same size. We checked the proportional hazards assumption using an interaction term for exposure and follow-up time and found no violations.

To assess the possibility of joint effects, we fit a model that was mutually adjusted for both TGF-β and PIIINP and assessed the multiplicative interaction between these 2 biomarkers. Analyses of possible joint effects were conducted only in individuals for whom we had measured levels of both biomarkers.

For cause-specific mortality, we used competing risks models to formally compare associations across competing outcomes (21). We allowed regression coefficients for key determinants of fibrosis-related biomarkers to vary across competing outcomes; these included sex, race, CRP level, fasting glucose level, and use of insulin or oral hypoglycemic agents.

We assessed the multiplicative interactions of TGF-β and PIIINP with sex, race, CRP level, and diabetes status. When we found evidence of interaction, we used graphical methods to examine the shape of the interaction and conducted stratified analyses accordingly.

All tests of statistical significance are 2-sided. All analyses were conducted in SAS, version 9.2 (SAS Institute, Inc., Cary, North Carolina). We interpreted $P < 0.05$ as statistically significant.

RESULTS

Participant characteristics

Table 1 shows the demographic, clinical, and laboratory characteristics of participants according to quintiles of TGF-β and PIIINP. Individuals with higher levels of either TGF-β or PIIINP were more likely to be black, to be either current or former smokers, and to have lower estimated glomerular filtration rates and higher CRP levels. Consistent with the hypothesis that glycemic dysregulation promotes fibrosis, individuals with higher levels of either TGF-β or PIIINP were more likely to have higher fasting glucose levels and to be taking insulin or hypoglycemic agents (22–24). TGF-β level was modestly but positively correlated with PIIINP level (Spearman $r = 0.08$, $P = 0.005$).

Risk of total mortality

There were 946 deaths over a median follow-up of 10.8 (range, 0.1–14.5) years among individuals with measured levels of TGF-β. There were 1,788 deaths over 10.9 (range, 0.1–14.6) years among individuals with measured levels of PIIINP. In multivariable-adjusted models, $\log_2$(TGF-β) and $\log_2$(PIIINP) were both individually associated with higher total incident mortality (Table 2). Hazard ratios per doubling of TGF-β and PIIINP were similar to hazard ratios per standard deviation of the log-transformed biomarkers (per standard deviation of $\log_2$(TGF-β), hazard ratio (HR) = 1.09, 95% confidence interval (CI): 1.02, 1.18; per standard deviation of $\log_2$(PIIINP), HR = 1.07, CI: 1.01, 1.12). When tested across extreme quintiles, elevated levels of untransformed TGF-β were associated with an approximately 40% higher risk of total mortality (HR = 1.38, CI: 1.08, 1.66); elevated levels of untransformed PIIINP were associated with an approximately 30% higher risk of total mortality (HR = 1.27, CI: 1.09, 1.49).

Associations with total mortality risk were only modestly changed by additional adjustment for N-terminal pro-brain natriuretic peptide, troponin-T, 2-hour glucose levels, urine albumin/creatinine ratio, or Mini-Mental State Examination score in separate models (Web Table 2). Sample sizes for these sensitivity analyses ranged from 1,054 to 1,443 for TGF-β and from 1,995 to 2,726 for PIIINP.

Joint effects

To provide comparable estimates for TGF-β and PIIINP in joint models, we examined both biomarkers in per–standard-deviation units. After mutual adjustment, standardized levels of TGF-β remained associated with incident total mortality, as did standardized levels of PIIINP (Web Table 3). We did

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

Characteristic	Quintile of Biomarker									
	1		2		3		4		5	
	Mean (SD)	%	Mean (SD)	%	Mean (SD)	%	Mean (SD)	%	Mean (SD)	%
	*Individuals With Measured Levels of TGF-β (*n = *1,443)*									
TGF-β level, ng/L	1,467 (268)		2,244 (267)		3,417 (409)		5,408 (822)		10,893 (4,424)	
PIIINP level, ng/mL	4.7 (1.6)		4.9 (1.8)		4.8 (1.6)		4.9 (1.8)		5.2 (2.0)	
Age, years	77.4 (4.4)		77.4 (4.5)		78.0 (4.6)		77.9 (4.2)		77.6 (4.9)	
Male sex		36		37		38		42		36
Black race		21		18		19		19		30
Field center										
North Carolina		83		68		47		32		25
California		0		0		0		0		0
Maryland		0		0		0		0		0
Pennsylvania		17		32		53		68		75
Body mass index[a]	26.6 (5.0)		26.7 (4.6)		26.9 (4.5)		26.6 (4.3)		27.4 (4.9)	
Former smoking		34		49		46		47		43
Current smoking		13		5		6		10		13
Pack-years of smoking	15 (26)		19 (26)		14 (21)		17 (22)		18 (22)	
Leisure time physical activity, kilocalories	1,325 (1,605)		1,194 (1,364)		1,290 (1,700)		1,112 (1,553)		1,060 (1,303)	
Alcohol consumption, drinks/week	1.8 (5.5)		1.5 (3.9)		2.1 (5.0)		2.1 (4.9)		1.9 (5.2)	
Married		52		55		60		55		44
Systolic blood pressure, mm Hg	134 (19)		138 (21)		133 (20)		134 (22)		136 (20)	
Fasting glucose level, mg/dL	102 (29)		104 (29)		103 (27)		106 (34)		109 (35)	
Total cholesterol, mg/dL	195 (38)		203 (39)		201 (37)		201 (42)		203 (41)	
CRP level, mg/L	4.3 (7.3)		4.0 (5.2)		4.3 (6.2)		4.5 (6.9)		6.4 (11.8)	
Medication use										
Insulin or hypoglycemic agents		10		7		9		12		13
Statins		9		9		9		9		10
Antihypertension medications		53		52		55		54		59
eGFR, mL/min/1.73 m^2	75 (16)		73 (22)		70 (18)		72 (20)		72 (19)	
CES-D	5.5 (4.6)		6.0 (5.1)		5.4 (4.6)		6.1 (4.8)		5.9 (5.1)	
FEV1, L	1.9 (0.6)		1.9 (0.6)		1.9 (0.6)		1.9 (0.6)		1.9 (0.6)	

Table continues

not observe an interaction between levels of TGF-β and PIIINP (P-interaction = 0.82).

Risk of cause-specific mortality

In multivariable-adjusted cause-specific models, TGF-β and PIIINP were both significantly associated with incident pulmonary mortality (Table 3). Adjustment for forced expiratory volume in 1 second in the single-outcome model did not materially alter the relationships between TGF-β and total mortality (HR = 1.08, CI: 1.00, 1.16) or PIIINP and total mortality (HR = 1.15, CI: 1.03, 1.28) compared with comparison populations of the same size, suggesting that forced expiratory volume in 1 second may not be an important mediator of the association between fibrosis and mortality. In competing risk models, formal comparisons of the hazard ratios for TGF-β and PIIINP across competing causes of death were not significant (both $P > 0.05$).

Stratified analyses

We did not observe significant effect modification for associations with incident total mortality by sex, race, or diabetes status (P for interaction > 0.05). However, CRP modified the associations of both TGF-β (P for interaction = 0.05) and PIIINP (P for interaction = 0.03) with incident total mortality. TGF-β was modestly but positively correlated with CRP level (Spearman $r = 0.07$, $P = 0.01$), as was PIIINP (Spearman $r = 0.06$, $P < 0.001$). Associations of TGF-β and PIIINP with both incident total mortality and pulmonary

Table 1. Continued

Characteristic	Quintile of Biomarker									
	1		2		3		4		5	
	Mean (SD)	%	Mean (SD)	%	Mean (SD)	%	Mean (SD)	%	Mean (SD)	%
	Individuals With Measured Levels of PIIINP (n = 2,726)									
TGF-β level, ng/L	4,153 (2,977)		4,952 (4,905)		4,684 (3,895)		4,478 (3,922)		4,942 (3,740)	
PIIINP level, ng/mL	3.0 (0.4)		3.9 (0.2)		4.5 (0.2)		5.3 (0.3)		7.3 (1.9)	
Age, years	77.2 (4.3)		77.8 (5.0)		77.8 (4.5)		78.1 (4.7)		78.5 (5.1)	
Male sex		31		35		36		43		43
Black race		12		13		15		16		23
Field center										
North Carolina		21		22		28		27		30
California		29		27		29		28		28
Maryland		26		24		19		18		15
Pennsylvania		25		27		23		27		27
Body mass index[a]	25.6 (4.1)		26.6 (4.2)		27.4 (4.9)		27.3 (4.6)		28.0 (5.1)	
Former smoking		40		42		43		48		49
Current smoking		10		8		7		8		7
Pack-years of smoking	15 (22)		15 (23)		15 (22)		16 (23)		16 (23)	
Leisure time physical activity, kilocalories	1,387 (1,679)		1,423 (1,789)		1,294 (1,709)		1,334 (1,770)		1,089 (1,458)	
Alcohol consumption, drinks/week	2.4 (5.7)		2.1 (5.4)		2.0 (5.3)		2.0 (5.1)		1.7 (5.1)	
Married		52		57		60		55		52
Systolic blood pressure, mm Hg	137 (21)		137 (20)		136 (20)		137 (21)		137 (21)	
Fasting glucose level, mg/dL	104 (32)		102 (22)		104 (27)		107 (35)		109 (34)	
Total cholesterol, mg/dL	207 (38)		206 (38)		204 (40)		199 (38)		199 (42)	
CRP level, mg/L	4.6 (7.9)		4.4 (7.9)		4.4 (7.5)		4.8 (8.4)		5.0 (7.8)	
Medication use										
Insulin or hypoglycemic agents		8		9		8		11		12
Statins		9		7		8		10		7
Antihypertension medications		50		50		52		57		62
eGFR, mL/min/1.73 m^2	78 (18)		75 (18)		72 (19)		69 (18)		64 (19)	
CES-D	5.9 (4.9)		5.3 (4.7)		5.4 (4.6)		5.6 (4.8)		6.2 (5.1)	
FEV1, L	1.9 (0.6)		1.9 (0.6)		1.9 (0.6)		2.0 (0.6)		1.9 (0.6)	

Abbreviations: CES-D, Center for Epidemiologic Studies-Depression symptoms score; CRP, C-reactive protein; eGFR, estimated glomerular filtration rate; FEV1, forced expiratory volume in 1 second; PIIINP, procollagen type III N-terminal propeptide; SD, standard deviation; TGF-β, transforming growth factor-β.

[a] Weight (kg)/height $(m)^2$.

mortality were strongest among individuals with higher CRP (Table 4).

DISCUSSION

We found TGF-β and PIIINP to be independently associated with incident total mortality, and particularly pulmonary mortality, in a community-based cohort of older adults. Associations with both total mortality and pulmonary mortality were strongest among individuals with higher CRP levels. Our study provides some of the first large-scale evidence to support the hypothesis that fibrosis plays an important role in diseases of aging.

We observed significant associations of the fibrosis-related biomarkers TGF-β and PIIINP with total mortality. Interstitial and vascular fibrosis are the result of increased collagen turnover (synthesis and degradation) and can be measured by markers of collagen turnover such as PIIINP and carboxy-terminal telopeptide of collagen type I (CITP). In a previously conducted nested case-control study in the CHS, PIIINP and CITP were both associated with an increased risk of mortality (16). Our study confirms the association

Table 2. Hazard Ratios for Total Mortality per Doubling of TGF-β and PIIINP, Cardiovascular Health Study, 1996–2010

Model	TGF-β					PIIINP				
	No. of Deaths	Total No. of Participants	HR	95% CI	*P* Value	No. of Deaths	Total No. of Participants	HR	95% CI	*P* Value
1[a]	946	1,443	1.08	1.00, 1.15	0.04	1,788	2,726	1.27	1.15, 1.41	<0.001
2[b]	946	1,443	1.11	1.03, 1.19	0.006	1,788	2,726	1.23	1.11, 1.37	<0.001
3[c]	946	1,443	1.09	1.01, 1.17	0.02	1,788	2,726	1.14	1.03, 1.27	0.01

Abbreviations: CI, confidence interval; HR, hazard ratio; PIIINP, procollagen type III N-terminal propeptide; TGF-β, transforming growth factor-β.

[a] Adjusted for age, sex, race, and clinic.

[b] Adjusted for the variables in model 1 and body mass index, smoking status, pack-years of smoking, exercise level, alcohol consumption, marital status, systolic blood pressure, fasting glucose level, total cholesterol level, C-reactive protein concentration, and use of oral hypoglycemic agents, insulin, statins, or antihypertension medications.

[c] Adjusted for the variables in model 2 and estimated glomerular filtration rate and Center for Epidemiologic Studies-Depression symptoms score.

between PIIINP and mortality and extends it to a larger cohort of older individuals. We did not examine CITP in the present study, as it was not re-measured in the full CHS cohort like PIIINP was, but we did examine TGF-β, an upstream and potentially more central driver of fibrosis than either PIIINP or CITP. In contrast to what was done in the previous study, we evaluated separate cause-specific mortality end points, including cardiovascular, pulmonary, cancer, and neurologic causes of death, rather than examining mortality as a single end point; this proved to be useful, as the results strongly implicated pulmonary causes of death in the outcome of fibrosis. Additionally, we demonstrated that adjustment for a marker of tissue microinjury, troponin-T, did not significantly affect the observed associations between biomarkers of fibrosis and mortality.

Given the relatively modest observed hazard ratios for TGF-β and PIIINP and their specialized natures, it is unlikely that either biomarker will be immediately useful for prognosis or prediction in clinical care. However, the potential benefits of targeting fibrosis could be larger than the relatively modest observed hazard ratios for TGF-β and PIIINP might suggest. Although neither biomarker is a perfect measure of organ fibrosis, the observed associations of TGF-β and PIIINP with total and pulmonary mortality were consistent, statistically significant, robust, and precise. More sensitive and specific biomarkers of organ fibrosis, if they become available, could have even stronger associations with total and pulmonary mortality. As seen in Table 1, neither TGF-β nor PIIINP is strongly correlated with traditional risk factors for death. This observation suggests that fibrosis represents a novel and largely independent pathway leading to chronic disease beyond standard pathways. Given that several antifibrotic agents are already in development or testing (2, 25), clinical trials to specifically target fibrosis and determine its effects on chronic disease and aging may be feasible in the near future.

Table 3. Hazard Ratios for Cause-Specific Mortality[a] per Doubling of TGF-β and PIIINP, Cardiovascular Health Study, 1996–2010[b]

Cause of Death	TGF-β					PIIINP				
	No. of Deaths	Total No. of Participants	HR	95% CI	*P* Value	No. of Deaths	Total No. of Participants	HR	95% CI	*P* Value
Pulmonary	97	1,443	1.27	1.01, 1.60	0.04	193	2,726	1.52	1.11, 2.10	0.01
Cardiovascular	299	1,443	1.13	0.99, 1.29	0.06	592	2,726	1.07	0.89, 1.28	0.49
Cancer	231	1,443	1.07	0.92, 1.24	0.38	378	2,726	1.23	0.97, 1.56	0.08
Neurologic	174	1,443	1.08	0.91, 1.28	0.39	331	2,726	1.00	0.78, 1.29	0.99
Other	145	1,443	0.95	0.79, 1.15	0.61	295	2,726	1.16	0.89, 1.52	0.27

Abbreviations: CI, confidence interval; HR, hazard ratio; PIIINP, procollagen type III N-terminal propeptide; TGF-β, transforming growth factor-β.

[a] Cause of death was classified as cardiovascular (e.g., coronary, cerebrovascular, other cardiovascular), neurologic (e.g., dementia, amyotrophic lateral sclerosis, Parkinson's disease), pulmonary (e.g., pneumonia, obstructive), cancer, or other.

[b] Adjusted for age, race, sex, clinic, body mass index, smoking status, pack-years of smoking, exercise level, alcohol consumption, marital status, systolic blood pressure, fasting glucose level, total cholesterol level, C-reactive protein concentration, use of oral hypoglycemic agents, insulin, statins, or antihypertension medications, estimated glomerular filtration rate, and Center for Epidemiologic Studies-Depression symptoms score.

Table 4. Hazard Ratios for Total and Pulmonary Mortality per Doubling of TGF-β and PIIINP by Stratum[a] of CRP, Cardiovascular Health Study, 1996–2010[b]

CRP Stratum by Cause of Death	TGF-β					PIIINP				
	No. of Deaths	Total No. of Participants	HR	95% CI	*P* Value	No. of Deaths	Total No. of Participants	HR	95% CI	*P* Value
Total mortality										
High CRP	514	727	1.12	1.01, 1.24	0.04	937	1,364	1.24	1.07, 1.45	0.005
Low CRP	432	716	1.06	0.95, 1.18	0.29	852	1,362	1.06	0.90, 1.24	0.49
Pulmonary mortality										
High CRP	57	727	1.60	1.12, 2.29	0.01	103	1,364	1.70	1.08, 2.67	0.02
Low CRP	40	716	1.22	0.84, 1.77	0.29	90	1,362	1.38	0.84, 2.27	0.20

Abbreviations: CI, confidence interval; CRP, C-reactive protein; HR, hazard ratio; PIIINP, procollagen type III N-terminal propeptide; TGF-β, transforming growth factor-β.

[a] CRP was dichotomized at its median value (2.3 mg/L).

[b] Adjusted for age, race, sex, clinic, body mass index, smoking status, pack-years of smoking, exercise level, alcohol consumption, marital status, systolic blood pressure, fasting glucose level, total cholesterol level, C-reactive protein concentration, use of oral hypoglycemic agents, insulin, statins, or antihypertension medications, estimated glomerular filtration rate, and Center for Epidemiologic Studies-Depression symptoms score.

Among the causes of death that we studied, incident pulmonary mortality was the one most strongly associated with TGF-β and PIIINP. Previous studies have implicated TGF-β as a factor in several highly prevalent pulmonary diseases, including asthma and chronic obstructive pulmonary disease (14). Both asthma and chronic obstructive pulmonary disease are characterized by chronic inflammation, epithelial damage, and excessive extracellular matrix deposition (26, 27). Endobronchial biopsies from the airways of severely asthmatic individuals have shown elevated expression of TGF-β (28), as have airway epithelium samples from patients with chronic obstructive pulmonary disease (26). In rodent models, administration of adenovirus expressing TGF- β is sufficient to drive significant lung fibrosis (29). Although we did not have access to tissue samples in the present study, studies with lung imaging are needed to further evaluate our findings.

In this study, associations involving TGF-β and PIIINP were present in the entire population but were strongest 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 other genes that contribute to collagen I/III and α-smooth muscle actin deposition in the extracellular matrix (30). CRP is also a marker for general levels of inflammation. Several families of proinflammatory cytokines can stimulate genes involved in extracellular matrix production and deposition, including collagens, proteoglycans, and matrix metalloproteins (25, 31). Inflammatory cells, including circulating monocytes, tissue macrophages, neutrophils, mast cells, and eosinophils, are important sources of TGF-β and other profibrotic signaling molecules (1). The observed consistency of the interaction with CRP for both TGF-β and PIIINP is reassuring, as is the biological plausibility of such an interaction; however, our finding requires confirmation in other cohorts.

Our study has several strengths. It was conducted in a large, well-characterized, community-based population with an average follow-up time of more than 10 years. We measured a diverse range of covariates and included these covariates in our multivariable-adjusted models to minimize the possibility of confounding. Together, TGF-β and PIIINP provide complementary snapshots of the process of collagen biosynthesis and accumulation. Indeed, the consistency of associations across TGF-β and PIIINP lends support to the hypothesis that these 2 biomarkers reflect a common underlying pathway.

Our study also faces several limitations. Although both TGF-β and PIIINP have previously been used as markers of tissue fibrosis in epidemiologic studies (32), they are imperfect measures of underlying tissue fibrosis. Information about organ-specific fibrosis, for example, biological specimens from multiple organs, could confirm and potentially strengthen our conclusions. Another potential limitation of the present study is the unknown stability of TGF-β and PIIINP over time. Plasma levels of both biomarkers were measured at a single time point; it is possible that associations with total and cause-specific would be stronger with repeated measures.

In conclusion, we found that TGF-β and PIIINP are associated with incident total mortality, in particular pulmonary mortality, among older adults. Associations were stronger in individuals with higher levels of CRP. Our findings provide support for future research on fibrosis as a potentially targetable pathway to reduce morbidity and mortality, particularly for diseases that occur late in life. Further studies to elucidate the mechanisms by which TGF-β and PIIINP are associated with pulmonary mortality are warranted.

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