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The Relationship between Serum Markers of Collagen Turnover and Cardiovascular Outcome in the Elderly: The Cardiovascular Health Study

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Abstract

Background—The deposition of collagen fibrils in the myocardial extracellular matrix increases with age and plays a key role in the pathophysiology of heart failure (HF). We sought to determine the predictive value of serum markers of collagen turnover for incident HF and cardiovascular (CV) morbidity, mortality and all-cause mortality in elderly individuals.

Methods and Results—In 880 participants in the Cardiovascular Health Study (mean age 77 ± 6 yrs, 48% female), serum levels of carboxyl-terminal peptide of procollagen type I (PIP), carboxyl-terminal telopeptide of collagen type I (CITP), and amino-terminal peptide of procollagen type III (PIIINP) were measured in 4 groups: HF with reduced ejection fraction (HFREF; n=146, EF < 55%); HF with preserved EF (HFPEF; n=175, EF $\geq 55\%$), controls with CV risk factors but not HF (CVD; n = 280) and healthy controls free of CV disease (n=279). Relationships between these serum markers and outcome at follow-up of 12 ± 4 years (range, 3-17 years) was determined in six models including those adjusted for conventional risk factors, renal function, NT-proBNP and agents which interfere with collagen synthesis. For the entire cohort, in unadjusted and adjusted models, both PIIINP and CITP were associated with myocardial infarction, incident HF, hospitalization for HF, cardiovascular and all-cause mortality. In healthy controls, CITP and PIIINP were associated with all-cause death. In controls with risk factors, CITP was associated with incident HF, and in participants with HFPEF, CITP was associated with hospitalization for HF. No collagen biomarker was associated with outcome in participants with HFREF, and PIP was not associated with outcome in the cohort or its subgroups.

Conclusions—In both healthy and elderly individuals with CV disease at risk of developing HF, CITP and PIIINP are significantly associated with multiple adverse cardiac outcomes including myocardial infarction, HF and death.

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Keywords

biomarkers; collagen; elderly; heart failure; outcomes

The prevalence and incidence of congestive heart failure (HF) continue to increase in the elderly population (1-2). Although a number of echocardiographic hemodynamic and biological markers have been found useful for risk stratification of patients with both acute and chronic HF (3-5), the ability to predict the occurrence of HF (i.e. incident disease among healthy elderly or those with risk factors for developing HF), remains a challenge.

In spite the fact that the etiologies of heart failure in the elderly with preserved ejection fraction (HFPEF) and HF with reduced ejection fraction (HFREF) can be different (e.g. arterial hypertension and coronary artery disease or dilated cardiomyopathy, respectively), changes in the interstitial matrix are dynamic and might be a common finding in both conditions, with more interstitial than replacement fibrosis in HFPEF, which partially explain the increase in LV diastolic stiffness and left ventricular filling pressures despite preservation of systolic contraction (6).

Excessive deposition of collagen in the myocardial extracellular matrix has a deleterious effect on both mechanical and electrophysiologic properties of the heart muscle and may lead to an unfavorable outcome (7-8). Although non-specific for the myocardium, serum markers of collagen turnover have been proposed to be utilized for identification of myocardial fibrosis (9-11). Type I and III collagens are the major myocardial fibrillar collagens and both are synthesized as procollagens with a small amino terminal and a larger carboxy terminal propeptide. Serum markers of collagen synthesis, carboxy-terminal propeptide of type I procollagen (PIP) and amino-terminal propeptide of type III procollagen (PIIINP), and degradation, carboxy-terminal telopeptide of collagen type I (CITP), result from the hydrolysis of collagen type I fibrils by matrix metalloproteinase (MMP)-1 (9), and reflect intramyocardial collagen turnover (9-11) which is also age related and involves the action of tissue inhibitors of MMPs (TIMPs), with greater serum levels of TIMP-1 in HFPEF than in HFREF and higher MMP-1/TIMP-1 ratio in HFREF than in HFPEF (12, 13).

In a prior study done in the same population, we found a strong association between the increased levels of fibrosis markers and prevalent HF (14). A number of other studies have evaluated the short (15,16) and long term (7, 17) prognostic value of serum biomarkers of collagen turnover in patients with HF of different etiologies or after acute myocardial infarction (18-19), but none enrolled exclusively free-living elderly populations.

We hypothesized that in the elderly, serum markers of collagen turnover are predictors of incident HF and of major cardiovascular (CV) adverse events, including CV and all-cause mortality. To test this hypothesis we analyzed two key markers of collagen I metabolism (PIP and CITP) and one of collagen III synthesis in association with a large number of clinical, echocardiographic and biochemical variables in a selected cohort of participants in the Cardiovascular Health Study (CHS) who were followed for a mean period of 12 ± 4 years (range 3-17 years).

Methods

The Cardiovascular Health Study (CHS) is a prospective, community-based, epidemiologic observational study designed to assess cardiovascular risk factors and outcomes in elderly persons in which 5888 were enrolled (age 65-100 at study entry) of whom 5201 were

recruited in 1989 to 1990, and a supplemental cohort of 687 African-American participants were added in 1992 to 1993. The design, rationale and examination details of the CHS Study have been published elsewhere (20).

Fibrosis markers were assessed in a sub-cohort of the CHS (N = 880), using a case-control design based on HF status. Clinical evaluations, and blood draws were obtained during the same study visit. Participant characteristics are presented in Table 1. The presence of HF was determined by expert adjudication of clinical records as described previously (3). HF status was determined for all CHS participants alive at the 1993–1994 examination, and updated to reflect clinical status in 1996–1997.

All patients who had an adjudicated HF diagnosis were selected from the full CHS cohort (N = 5888) in year 1992–1993 and year 1996–1997 (n = 310) and were compared to two control groups. The following participants were enrolled in this study: (1) 146 with HF and decreased (< 55%) ejection fraction (EF) (HFREF), 175 with HF and normal EF ($\geq 55\%$) (HFPEF) (3) 280 controls with CV disease (CVD) risk factors but without HF, and 279 healthy controls without HF, coronary heart disease, hypertension, diabetes mellitus, peripheral arterial disease, stroke, TIA or use of cardioactive and/or anticoagulant agents.

Selection of participants in each of the two control groups was based on frequency matching for age and sex of the patients with HF. Matching was successful in approximating equivalent age and gender between groups (mean \pm S.D. age for HF = 80.2 ± 5.7 yrs, controls with CVD risk factors 81.3 ± 6.5 yrs, and healthy controls 79.7 ± 5.5 yrs; and 48%, 49%, 50% were female, respectively) even though statistical significant differences between groups exist.

Assays for fibrosis markers were done in blood samples obtained in 1992–1993; (n = 633) or 1996–1997 (n = 237) and samples were analyzed in 2005 at the University of Vermont. All clinical and biochemistry measures were obtained at the same evaluation visit for each patient when fibrosis markers were assessed (i.e., 1992–1993 or 1996–1997, respectively) to ensure simultaneous assessments of fibrosis markers and covariates. There were no differences in clinical characteristics between participants with samples obtained between 1992–1993 and those with samples obtained in 1996–1997 (age-adjusted p-values >0.10).

Diseases relevant to fibrosis markers in the elderly were also recorded, including osteoporosis and arthritis, history of stroke and obstructive peripheral artery disease. Prevalent disease status was updated based on adjudicated incident events throughout the study.

Echocardiograms were used to determine cardiac structural and functional characteristics, and were analyzed at a central core echocardiography laboratory (JSG). Qualitative LVEF was estimated based on echocardiographic data obtained either at the baseline CHS examination and interpreted at the core echocardiography reading laboratory, or at the point of care as abstracted from clinical records.

Covariates

Prevalent CVD was defined as a history of myocardial infarction, stroke, or HF, at the time of the 1994-1995 examination cycle.

Demographic and subject characteristics were age, gender, race, weight, height, body mass index, history of hypertension, diabetes, hyperlipidemia, serum glucose, creatinine, C-reactive protein, cystatin C and NT-pro BNP, ACE and aldosterone receptors- inhibitors. Participants with a history of chronic liver disease or chronic pulmonary disease were

excluded to minimize confounding effects of liver and pulmonary disease on fibrosis markers.

Determination of plasma PCPIIINP, PIP, CITP

Collagen biomarkers were measured from blood draws performed in 1992-3, and 1996-7, and the serum was stored and deep frozen at -70°C .

Phlebotomy methods, blood processing, and handling of samples have been described previously (3). Aliquots were frozen at -70°C until analysis.

PIP was measured using an enzyme immunoassay kit (Takara Mirus Bio Inc., Madison, WI). The assay range is 10 – 640 $\mu\text{g/L}$ with a lower detection limit of 10 $\mu\text{g/L}$. Intra-assay and inter-assay CVs range from 4.5-7.4% and 4.3-6.3%, respectively.

CITP was measured using the CITP RIA from Orion Diagnostica on serum samples. Intra- and inter-assay variability are 3.5-9.5% and 5.6-9.0%, respectively. The lower detection limit is 0.4 $\mu\text{g/L}$.

PIIINP was determined by a coated-tube radioimmunoassay as described previously by Risteli et al (21), using commercial antisera specifically directed against the terminal amino terminal peptide (Orion Diagnostica, Finland). The interassay and intra-assay variations for determining PIIIP are both about 5%. The sensitivity (lower detection limit) is 1.5 ng /mL.

Outcome Events

The primary outcome events were cardiovascular and all-cause death for the whole cohort, and incident HF for the control and healthy control groups. Other outcome events were hospitalization for HF and myocardial infarction.

Throughout the follow-up period, participants were interviewed every 6 months and follow-up examinations were conducted annually at each local center. Outcome events were tabulated on the basis of report of physician diagnosed myocardial infarction or stroke (22). Deaths were confirmed by review of medical records and death certificates, as well as review of data on hospitalizations from the Health Care Financing Administration Medicare database on health care utilization. Cardiovascular death was classified according to criteria published previously (23).

Statistical Analysis

The distributions of PIP, PIIINP, and other clinical variables were described for the study groups: HFREF, HFPEF, controls (no HF) and cardiovascular-healthy participants. Because the distributions of biomarkers were skewed, results were presented as the median value (interquartile range); age was presented as mean \pm SD; categorical variables, such as gender, race, et al, were presented as percentage. Group comparisons were conducted using Kruskal-Wallis test, one-way ANOVA and chi-square test, as appropriate, pairwise comparisons were performed with Bonferroni correction. Two-sided p-values were reported.

Six Cox proportional hazards regression models were performed: model 1 unadjusted, model 2 adjusted for age, gender and race; model 3 adjusted for age, gender, race, hypertension, diabetes, hyperlipidemia; model 4 adjusted for demographics, risk factors, left atrium antero-posterior diameter, left atrium volume, transmitral E/A ratio and LV mass; model 5 adjusted for demographics, risk factors and serum glucose, creatinine, C-reactive protein, cystatin C and NT-pro BNP and model 6, adjusted for demographics, risk factors and agents which interfere with collagen synthesis (ACE and aldosterone receptors-inhibitors). The hazard ratios (95% confidence intervals) for the highest tertile vs the lowest

tertile were reported. A value $p < 0.05$ by the two-tailed test was considered statistically significant. All analyses were performed using version 9.1 of SAS (SAS Institute Inc., Cary, North Carolina).

The study was approved by the institutional review committees of the participating medical centers.

Results

Participant Characteristics

The main demographic and medical history characteristics of the participants' population are given in Table 1. Compared to the healthy control group, there were more white participants in the control and HF groups where there was a higher prevalence of risk factors for atherosclerosis and cardiovascular morbidity. Compared to the participants with HFPEF, the participants with HFREF group had a higher prevalence of remote myocardial infarction (64% vs. 38%, $p < 0.0001$) with a similar prevalence of hypertension and diabetes. The median values, lower and upper quartiles of fibrosis biomarkers in the study groups are illustrated in Table 2. CITP and PIIINP serum levels were higher in the participants with HF compared to control groups ($p < 0.0001$ for both biomarkers). When the serum fibrosis markers were compared by demographics and clinical characteristics (Table 3), PIP serum level was only related to gender (higher in women). In general there was a weak correlation between fibrosis markers and age and only in the healthy control, control and HFPEF groups for CITP ($r = 0.19$, $p = 0.001$, $r = 0.27$, $p = 0.0001$, $r = 0.15$, $p = 0.05$, respectively), in HFPEF for PIP ($r = 0.25$, $p = 0.001$) and in the control group for PIIINP ($r = 0.12$, $p = 0.04$). Overall, higher serum levels of CITP and PIIINP were found in those participants with CVD than in the healthy control group.

Cardiovascular Morbidity, Incident HF, and Hospitalization for HF

During a mean follow-up of 12 ± 4 yrs (range 3-17 yrs), of the 880 participants, 151 had myocardial infarction, 128 new (incident) HF, 132 strokes and 31 had transient ischemic attacks. Of all the participants with HF, 259 had hospitalization for HF exacerbation (131 in HFPEF vs. 128 in HFREF group, $p = 0.43$). The highest events rate was in HFREF, followed by HFPEF, controls with risk factors, and healthy controls. Hazard ratios between the lowest and highest tertiles of the fibrosis markers for outcome events in the combined cohort are presented in Table 4. In models further adjusted for serum glucose, creatinine, cystatin C, C-reactive protein and NT-proBNP in addition to demographics and risk factors, CITP and PIIINP remained modestly associated with myocardial infarction (AUC = 0.58 for both) and PIIINP with hospitalization for HF (AUC = 0.58). Finally in models adjusted for demographics, risk factors and agents which interfere with collagen synthesis (e.g. ACE and aldosterone receptors –inhibitors), CITP remained associated with myocardial infarction and HF (AUC = 0.63 and 0.59, respectively) and PIIINP with myocardial infarction, HF and hospitalization for HF (AUC = 0.62, 0.58 and 0.78, respectively).

In the healthy control group, no associations were found between fibrosis markers and incident HF or myocardial infarction. In the control group, CITP was associated with incident HF in unadjusted, adjusted for demographics and adjusted for demographics and risk factors models (HR = 2.9, 95% CI: 1.7, 4.8, HR = 2.3, 95% CI: 1.2, 4.0, and HR = 2.3, 95% CI: 1.2, 3.6) and PIIINP was associated in unadjusted models with incident HF (HR = 2.3, 95% CI: 1.2, 3.6). In the participants with HFPEF, CITP was associated with hospitalization for HF in both demographic and demographic and risk factors adjusted models (HR = 2.0, 95% CI: 1.2, 3.2, and HR = 2.3, 95% CI: 1.2, 4.0, respectively). In the HFREF group, no significant associations were found in any model.

All-Cause and Cardiovascular Death

At the end of the follow-up period, all-cause death was recorded in 564 and cardiovascular mortality in 242 participants. The highest mortality rate was recorded in the HFREF group (92.4% for all-cause and 59.2% for cardiovascular death) and the lowest in the healthy control group (all-cause and cardiovascular mortality, 53.4% and 14.5%, respectively).

Hazard ratios of the fibrosis markers for all-cause and cardiovascular death in the combined cohort are given in Table 4. In models adjusted for demographics, risk factors, ACE and aldosterone receptors – inhibitors, CITP was modestly associated with all cause-death and cardiovascular death (AUC = 0.59 and 0.52 respectively) whereas PIIINP was associated only with all-cause death (AUC = 0.59). Adjustments for the biochemical variables described above eliminated the association of CITP and PIIINP with death.

In per group analyses, in the healthy control group, CITP and PIIINP were associated with all-cause death: CITP in unadjusted models (HR = 1.7, 95% CI: 1.2, 2.6) and PIIINP in models 1-3 (unadjusted: HR=2.0, 95% CI: 1.2, 2.9, adjusted for demographics, HR= 1.7, 95% CI: 1.2, 2.9, and adjusted for demographics and risk factors, the same HR). In the control group, CITP and PIIINP were associated only in unadjusted models with all-cause death (HR=2.0, 95% CI: 1.2,2,9 and HR = 1.7, 95% CI: 1.2, 2.6, respectively). In both HF groups, the fibrosis markers were not associated with death. Finally, the addition of parameters of LV diastolic function (left atrial size and transmitral E/A ratio) and a potential substrate for its alteration (LV mass) to the demographic and risk factors variables, denied the association of collagen turnover markers with both cardiovascular morbidity and mortality in every group.

Discussion

The principal finding of this study is that in community-dwelling elderly individuals, CITP and PIIINP were significantly associated with multiple adverse cardiac outcomes including myocardial infarction, HF and death. Notably these findings included subgroups of participants who are CV healthy (i.e. without prevalent CVD, hypertension, subclinical disease or risk factors), as well as those without HF, but with subclinical disease and/or risk factors. Moreover, in participants with HFPEF, CITP is associated with increased hospitalization for HF. These results provide further support for the hypothesis that fibrosis is an important contributor to CVD and outcomes among the elderly and serum markers of collagen turnover are predictors of incident HF and of major CV adverse events, including cardiovascular and all-cause mortality.

Of the three biomarkers analyzed PIP had the weakest association with the cardiovascular morbidity and mortality. While in smaller series enrolling younger patients were reported associations of PIP with HF (8, 10, 24), other studies found a lack of association between PIP with prevalent HF (11, 14, 17) possibly because of predominance of type I collagen destruction over its synthesis, a shift from type I to type III collagen synthesis, or plateau of collagen type I synthesis in the elderly individuals. These findings strengthen our previous observation where using similar Cox regression models, we found an association between CITP and PIIINP, but not PIP, with prevalent HF (14).

The predictive value of serum fibrosis biomarkers for adverse events was previously reported in patients with acute myocardial infarction (18, 19), arterial hypertension and hypertrophic cardiomyopathy (25). A limited number of studies enrolling smaller and younger number of patients than in the present study have evaluated the prognostic value of these markers in patients with HF. Cicoira et al reported on 101 individuals with HF (mean age 62 ± 9 years) and found that in patients with a LVEF < 31%, a serum level >4.7 mcg/L

of PIIINP was associated with a higher mortality and rate of hospitalization for HF as compared with the other patients (7). Ruiz-Ruiz et al found in 111 patients (age = 73 ± 8 years) with decompensated HF that a higher level of PIP was associated with a increased mortality and readmission rate (26), and Rossi et al reported on 106 patients (age = 64 ± 6 years) with dilated cardiomyopathy that a higher serum level of PIIINP was associated with a restrictive mitral inflow pattern and 2-fold higher mortality than those with a non-restrictive pattern (27). In RALES (16), which enrolled 261 elderly patients with EF < 35% in NYHA class III and IV, those with baseline PIIINP > 3.85 $\mu\text{g/L}$ had a higher relative risk of death and /or HF hospitalization.

Recently, investigators from the Framingham study described the association of PIIINP and tissue inhibitor of matrix metalloproteinase inhibitor-1 (TIMP-1) with mortality and incident CVD in community-based participants (28). Another recent study reported the value of a large number of biomarkers panel for identification of LVH and HFPEF including NT pro-BNP, PIIINP and CITP concluding that multi biomarkers panels performed better than any single biomarker for this purpose (29).

The present study confirms the association of PIIINP with incident disease and mortality, and extends those findings by additionally evaluating CITP and PIP. Moreover, our population differs in evaluating older subjects, not selected by echocardiographic criteria and who were stratified based on CV health status including subgroups that were CV healthy, had prevalent CVD without HF, as well as prevalent HFREF, and prevalent HFPEF. In addition, rather than using a single CVD endpoint, we evaluated separate CVD endpoints including CV mortality, myocardial infarction, incident HF and hospitalization for HF.

Our findings support the hypothesis that myocardial fibrosis plays an important role in the development of CVD and its presence is associated with adverse outcomes. Significant efforts towards finding antifibrotic treatments are under way. Three large completed clinical studies utilizing aldosterone antagonists also support the role of fibrosis in HF and the impact of these agents on decreasing collagen formation which was translated in a better outcome (16, 30, 31), while an ongoing NHLBI sponsored trial addresses the RAAS inhibition on the outcome of patients with HFPEF: TOPCAT (Treatment of Preserved Cardiac Function Heart Failure With an Aldosterone Antagonist).

Strength and Limitations

The large cohort of well characterized community-dwelling population based on elderly age range subjects, the large number of prospectively assessed covariates available for entry in the statistical models, the formal adjudication and detailed information on events, the unusual long follow-up period with a low rate of loss and the presence of a healthy comparison cohort are the main strengths of this study. In spite the fact that the fibrosis markers investigated are not myocardial specific, they have been used before as serum surrogates for the excessive collagen presence in the myocardial extracellular matrix (10, 11). Moreover, participants with conditions known to elevate serum collagen markers (e.g. liver disease, severe pulmonary disease, metabolic bone disease) were not enrolled in the study and we previously showed failure of bone mineral density to affect the relationship of fibrosis markers to prevalent HF (14). While this investigation was limited to evaluation of only 3 biomarkers of collagen metabolism, research is ongoing of many biomarkers which reflect multiple facets of the pathophysiology of HF, including inflammation, cell death and injury, interstitial repair, and diastolic load. It is likely that use of multiple biomarkers will in the future prove particularly valuable for prediction of incident HF and of the outcome of prevalent HF (29, 32).

Conclusions

Serum markers of collagen synthesis turnover (PIINP, CITP) are associated with CV morbidity, mortality and all-cause mortality. Among the 3 biomarkers evaluated, only CITP and PIINP are predictors of both incident CVD, hospitalization for HF exacerbation in those participants with HFPEF, and death. In the elderly, healthy or with CVD, increased serum level of biomarkers of collagen turnover suggest that fibrosis may be a key mechanism of new CVD and adverse outcomes. These results have specific therapeutic implications, some of which are being tested currently in clinical trials.

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Table 1

The Demographics and the Prevalent Diseases of the Enrolled Participants

Variable	Healthy Control N = 279	Control N = 280	HFPEF N = 175	HFrEF N = 146	p-value	Pairwise comparison
Age (years)	78±6	76 ± 6	76 ± 5	77 ± 6	0.01	2*
Male n (%)	144 (51)	146 (52)	79 (45)	92 (63)	0.02	3*, 5*, 6*
White n(%)	269 (95)	154 (55)	149 (85)	128 (88)	<0.0001	1 [†] , 2 [‡] , 3 [‡] , 4 [‡] , 5 [‡]
Diabetes n (%)	0	59 (21)	47 (27)	44 (30)	0.17	1 [‡] , 2 [‡] , 3 [‡] , 4 [‡] , 5 [‡]
Hypertension n (%)	3 (1)	160 (57)	93 (53)	70 (48)	0.0003	1 [‡] , 2 [‡] , 3 [‡] , 4 [‡] , 5 [‡]
Hypertlipidemia n (%)	127 (45)	148 (53)	84 (48)	61 (42)	0.11	NS
Stroke or TIA n (%)	0	36 (13)	37 (21)	42 (29)	0.001	1 [‡] , 2 [‡] , 3 [‡] , 4 [‡] , 5 [‡]
Myocardial Infarction n(%)	0	39 (14)	67 (38)	93 (64)	<0.0001	1 [‡] , 2 [‡] , 3 [‡] , 4 [‡] , 5 [‡] , 6 [‡]
Incidence rate of hospitalization for heart failure n (%)	-	-	131 (75)	128 (88)	<0.0001	N/A

1 indicates healthy control versus control; 2, healthy control versus DHF; 3, healthy control versus SHF; 4, control versus SHF; 5, control versus DHF; 6, DHD versus SHF.

* P<0.05;

[†] P<0.01;

[‡] P<0.001

Table 2
The Median Values, Lower and Upper Quartiles of Fibrosis Biomarkers in the Study Groups

Variable	Healthy Control N = 279	Control N = 280	HFPEF N = 175	HFrEF N = 146	p-value*
PIP (µg/l)	403 (336, 491)	384 (321, 467)	395 (329, 503)	406 (353, 477)	0.25
CITP (µg/l)	3.8 (2.8, 5.3)	4.5 (3.2, 5.9)	5.9 (4.3, 8.6)	5.7 (4.0, 8.3)	<0.0001
PIIINP (ng/ml)	3.1 (2.4, 4.0)	3.8 (2.8, 5.0)	4.2 (3.0, 6.9)	4.7 (3.4, 7.2)	<0.0001

* p-values for pair comparisons of CITP and PIIINP < 0.0001 for all the groups with the exception of HF groups in which were not significant (CITP = 0.55 and PIIINP = 0.24)

Table 3

The Median Values, Lower and Upper Quartiles for Fibrosis Biomarkers in Relation to the Demographics and Prevalent Clinical Characteristics

Variable	PIP (µg/l)	CITP (µg/l)	PIIINP (µg/l)
Gender			
Female (426)	408 (343, 500)	4.7 (3.4, 6.4)	3.5 (2.6, 4.6)
Male (454)	386 (321, 462)	4.7 (3.4, 6.3)	4.0 (2.9, 5.4)
p-value	0.003	NS	<0.0001
Race			
White (693)	397 (333, 478)	4.7 (3.3, 6.4)	3.6 (2.7, 5.0)
African American (181)	388 (324, 494)	4.8 (3.6, 6.2)	4.1 (3.0, 5.4)
p-value	NS	NS	0.04
Hypertension			
Yes (412)	391 (325, 477)	5.0 (3.7, 6.9)	3.9 (2.9, 5.7)
No (467)	395 (333, 481)	4.3 (3.1, 5.9)	3.4 (2.2, 4.8)
p-value	NS	<0.0001	<0.0001
Diabetes			
Yes (217)	381 (324, 476)	5.1 (3.7, 7.4)	4.0 (2.9, 5.8)
No (655)	399 (333, 479)	4.6 (3.2, 5.9)	3.6 (2.7, 5.0)
p-value	NS	<0.0001	0.01
Stroke			
Yes (114)	426 (332, 518)	5.9 (4.2, 7.3)	4.4 (3.2, 6.5)
No (713)	391 (328, 647)	4.5 (3.2, 6.1)	3.6 (2.7, 4.9)
p-value	NS	<0.0001	<0.0001
Hyperlipidemia			
Yes (449)	391 (323, 471)	4.6 (3.3, 6.2)	3.6 (2.7, 4.8)
No (425)	396 (336, 487)	4.8 (3.4, 6.6)	3.8 (2.8, 5.3)
p-value	NS	NS	0.02
Stroke or TIA			
Yes (154)	390 (324, 475)	4.7 (3.4, 6.2)	3.7 (2.7, 5.1)
No (686)	392 (332, 480)	4.6 (3.3, 6.3)	3.6 (2.7, 5.1)
p-value	NS	NS	NS
Myocardial Infarction			
Yes (151)	407 (341, 477)	4.9 (3.5, 6.7)	4.0 (2.9, 6.5)
No (615)	391 (332, 481)	4.5 (3.3, 6.0)	3.5 (2.7, 4.8)
p-value	NS	0.03	0.003

Table 4
The Association of Fibrosis Biomarkers with Cardiovascular Morbidity, All-cause and Cardiovascular Mortality for the Entire Cohort

Outcome Variable	PIP HR (95% CI)*	PIP HR (95% CI)†	PIP HR (95% CI)‡	CITP HR (95% CI)*	CITP HR (95% CI)†	CITP HR (95% CI)‡	PIINP HR (95% CI)*	PIINP HR (95% CI)†	PIINP HR (95% CI)‡
Myocardial Infarction	1.2 (0.8, 1.7)	1.2 (0.8, 1.7)	1.2 (0.8, 2.0)	2.0 (1.2, 2.9)	2.0 (1.2, 2.9)	1.7 (1.2, 2.6)	2.0 (1.4, 3.2)	2.3 (1.4, 3.2)	2.0 (1.4, 3.9)
Heart Failure§	1.0 (0.6, 1.7)	1.2 (1.0, 1.7)	1.0 (0.6, 1.4)	2.6 (1.4, 4.0)	4.4 (3.2, 5.8)	1.7 (1.2, 2.9)	2.6 (1.7, 4.0)	3.2 (2.6, 4.4)	2.0 (1.2, 3.2)
Hospitalization for HF¶	1.2 (1.0, 1.7)	1.2 (0.8, 1.4)	1.2 (1.0, 1.7)	2.9 (2.3, 3.6)	2.6 (2.0, 3.6)	2.3 (1.7, 3.2)	2.9 (2.3, 3.6)	2.6 (2.0, 3.6)	2.6 (2.0, 3.6)
All-Cause Death	1.2 (1.0, 1.4)	1.0 (0.8, 1.4)	1.0 (0.8, 1.4)	2.0 (1.7, 2.6)	2.0 (1.4, 2.3)	1.7 (1.4, 2.0)	2.0 (1.4, 2.3)	2.0 (1.4, 2.3)	1.7 (1.4, 2.3)
Cardiovascular Death	1.2 (1.0, 1.7)	1.2 (0.8, 1.7)	1.2 (0.8, 1.7)	2.0 (1.4, 2.9)	2.0 (1.4, 2.6)	1.4 (1.0, 2.0)	1.7 (1.2, 2.3)	1.7 (1.2, 2.3)	1.4 (1.0, 2.0)

* unadjusted

† adjusted by age, gender and race

‡ adjusted by age, gender, race, hypertension, diabetes, hyperlipidemia

§ control and healthy control groups

¶ pertains only to the HF groups