Published in final edited form as:

J Hypertens. 2009 June ; 27(6): 1317–1324. doi:10.1097/HJH.0b013e328329fc20.

Cross-sectional relations of multiple biomarkers representing distinct biological pathways to plasma markers of collagen metabolism in the community

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Abstract

Objective—Hyperhomocysteinemia, neurohormonal activation, inflammation and altered fibrinolysis have been linked to atherothrombosis as well as to myocardial fibrosis and heart failure. Hence, we related a panel of biomarkers representing these pathways to plasma markers of collagen metabolism in a large community-based sample.

Methods—We related nine biomarkers representing select biologic pathways (independent variables: C-reactive protein, B-type natriuretic peptide, N-terminal proatrial natriuretic peptide, aldosterone, renin, fibrinogen, D-dimer, plasminogen activator inhibitor-1 and homocysteine) to three plasma markers of collagen turnover [dependent variables, separate models for each: aminoterminal propeptide of type III collagen, tissue inhibitor of metalloproteinases-1 and matrix metalloproteinase-9 (present versus absent)] in 921 Framingham Heart study participants (mean age 57 years; 58% women). Participants were separated apriori into those with left ventricular end-diastolic dimensions and wall thickness below sex-specific median values (referent group) and either measure at least 90th sex-specific percentile ('remodeled' group). We used stepwise multivariable regression analysis adjusting for cardiovascular risk factors to relate the panel of systemic biomarkers to the three biomarkers of collagen metabolism.

Results—Plasma homocysteine was positively related to all three markers of collagen metabolism in the remodeled group and to aminoterminal propeptide of type III collagen and tissue inhibitor of metalloproteinases-1 in the referent group. Plasminogen activator inhibitor-1 was positively related to aminoterminal propeptide of type III collagen and tissue inhibitor of metalloproteinases-1 in both groups, whereas the natriuretic peptides were associated positively with these collagen markers in the referent group.

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The authors have no disclosures.

Conclusion—In our large community-based sample, plasma homocysteine and plasminogen activator inhibitor-1 were positively related to circulating collagen biomarkers, consistent with experimental studies implicating these pathways in cardiovascular collagen turnover.

Keywords

biomarkers; cardiac remodeling; collagen; hyperhomocysteinemia; matrix

Introduction

Abnormal matrix turnover is implicated in the pathogenesis of cardiovascular disease, including atherosclerosis and adverse cardiac remodeling [1,2]. Various plasma markers representing different stages in the metabolism of the fibrillar collagens types I and III, which are the major collagens present in the myocardium and vascular wall, have been correlated with cardiovascular diseases [3]. Aminoterminal propeptide of type III collagen (PIIINP), a marker that represents total turnover of type III collagen, has been related to cardiac function in pathologic states [4]. Matrix metalloproteinases (MMPs), especially MMP-9, are correlated with cardiovascular tissue remodeling and unstable coronary syndromes [5]. Tissue inhibitor of metalloproteinases-1 (TIMP-1), which has growth-promoting effects in addition to inhibiting MMPs, is also correlated with cardiovascular dysfunction [6].

Collagen turnover, especially in the heart, is regulated by mechanical factors as well as by various homeostatic mechanisms, including the rennin–angiotensin–aldosterone system, natriuretic peptides, plasmin plasminogen system and inflammation [2,7–9]. Recent experimental studies [10,11] show that homocysteine is also an independent stimulus for myocardial fibrosis and dysfunction. It is likely that several of these pathways may be concomitantly perturbed and influence matrix turnover *in vivo*. Accordingly, we examined the relative contributions of several biologic pathways to interindividual variation in collagen metabolism by relating circulating biomarkers representing these pathways to select biomarkers of collagen turnover in a large community-based sample. Biomarkers were chosen to represent biologic pathways known to influence cardiovascular collagen turnover: neurohumoral [N-terminal proatrial natriuretic peptide (NT-ANP), B-type natriuretic peptide (BNP), renin and aldosterone], fibrinolytic/hemostatic factors [fibrinogen, D-dimer and plasminogen activator inhibitor-1 (PAI-1)] and inflammation [C-reactive protein (CRP)], in addition to altered homocysteine metabolism (plasma homocysteine).

Methods

A detailed description of the Framingham Offspring study [12] has been published previously. The Framingham Heart study protocol was approved by the Boston University Medical Center Institutional Review Board. Participants who attended the sixth examination cycle (1995–1998) underwent physical examination and laboratory assessment of cardiovascular disease risk factors. In addition, plasma homocysteine, CRP, BNP, NT-ANP, renin, aldosterone, fibrinogen, PAI-1 and D-dimer concentrations were measured, and a routine echocardiogram was obtained on about 3500 attendees. Additionally, plasma total MMP-9, total TIMP-1 and PIIINP concentrations (referred to as collagen markers) were measured in a subsample (see below).

To examine correlations of the systemic biomarkers with collagen markers separately in participants with relatively ‘normal’ cardiac architecture and those potentially in a phase of cardiac remodeling, we constructed a sampling scheme on the basis of the distribution of the M-mode echocardiographic measurements. Thus, individuals with echocardiographic measurements were stratified into two groups: a ‘referent’ group with left ventricular wall

thickness (LVWT; sum of the diastolic thicknesses of the interventricular septum and the left ventricular posterior wall) and left ventricular end-diastolic dimension (LVEDD) with sex-specific 50th percentile or less, and a 'remodeled' left ventricular group with either LVWT or LVEDD at least sex-specific 90th percentile. This sampling schema has been described in detail in our prior studies [6,13]. Plasma MMP-9, TIMP-1 and PIIINP were measured in the two strata thus defined (they were not measured in all attendees at the examination).

Of 937 participants with both PIIINP and TIMP-1 available, we excluded 16 individuals for the following reasons: prevalent heart failure ($n = 14$), and serum creatinine more than 2 mg/dl ($n = 2$). After exclusions, 921 individuals (mean age 57 ± 10 years; 58% women) remained eligible (535 in referent group, and 386 in remodeled group).

Measurement of collagen markers and systemic biomarkers representing distinct biological pathways

Plasma samples were obtained from fasting participants who had been supine for 5–10 min before venipuncture and stored at -80°C until following assays were performed [6,13–17]: plasma total MMP-9 (intraassay coefficient of variation $<18\%$); plasma total TIMP-1 (Amersham Pharmacia Biotech, Uppsala, Sweden; coefficient of variation $<5\%$); plasma PIIINP concentration (Amersham Pharmacia Biotech; coefficient of variation = 6%); plasma BNP and NT-ANP (Shionogi and Co. Ltd., Osaka, Japan); plasma renin (Nichols assay; Quest Diagnostics, Madison, New Jersey, USA); plasma aldosterone (Quest Diagnostics); CRP (Dade Behring BN100 nephelometer; Dade Behring Inc., Mississauga, Ontario, Canada); plasma total homocysteine level; PAI-1 antigen level; fibrinogen (Diagnostica Stago Reagents; Diagnostica Stago Inc., Parsippany, New Jersey, USA); and D-dimer (Biopool AB, Umea, Sweden). The mean interassay coefficient of variations for the systemic biomarkers were as follows: BNP (12.2%); NT-ANP (12.7%); renin (2.0% for high and 10.0% for low concentrations); aldosterone (4.0% for high and 9.8% for low concentrations); homocysteine (9%); PAI-1 (7.7%); fibrinogen (2.6%); and D-dimer (11.7%).

Statistical analyses

All nine systemic biomarkers, and PIIINP and TIMP-1, were natural logarithmically transformed to normalize their skewed distributions. As plasma MMP-9 was detectable only in approximately 20% of study participants, it was modeled as a binary variable (detectable versus nondetectable) in all statistical analyses. Multivariable linear (for PIIINP and TIMP-1) and logistic (for MMP-9) regression analyses were conducted to determine associations of concentrations of each systemic biomarker (representing distinct biological pathways; independent variables) with collagen markers (dependent variables), adjusting for 10 clinical covariates known to influence circulating concentrations of the collagen markers [i.e., age, sex, serum creatinine, BMI, SBP, antihypertensive treatment, ratio of total/high-density lipoprotein (HDL) cholesterol, cigarette smoking, diabetes mellitus and alcohol consumption] [18]. We analyzed correlations separately in the 'normal' and 'remodeled' groups and also compared the β coefficients for each systemic biomarker within the referent and the remodeled left ventricular groups.

Given the extent of multiple testing (nine systemic biomarkers related to three collagen markers in two groups), we additionally utilized a conservative two-step strategy to gain insights into potential false positive associations resulting from chance. For each collagen marker, we first performed a global test of statistical significance to determine whether the group of nine systemic biomarkers was related to the collagen marker being evaluated. For each collagen marker that was related to the set of systemic biomarkers with a global P value of less than 0.10, we then performed stepwise multivariable regression with backward selection of systemic biomarkers that were significantly associated with the collagen marker of interest. The analyses

were also adjusted for the 10 clinical covariates noted above that were forced into the model. All statistical analyses were conducted with SAS version 8.1 (SAS, Cary, North Carolina, USA), and a *P* value of less than 0.05 was used to define statistical significance.

Results

Table 1 demonstrates the characteristics of our study participants. Participants in the 'remodeled' left ventricular group were older, had a higher BMI and higher prevalence rates of diabetes, hypertension, cardiovascular disease and statin treatment. Among collagen markers, PIIINP and TIMP-1 concentrations were significantly and positively correlated in both left ventricular groups; MMP-9 was not correlated with the other two markers in either left ventricular group (data not shown).

Relations of individual systemic biomarkers to aminoterminal propeptide of type III collagen concentrations

Table 2 demonstrates the results of multivariable regression analyses relating systemic biomarkers to PIIINP, adjusted for 10 clinical covariates known to influence collagen markers. Homocysteine and PAI-1 were correlated positively with PIIINP concentrations in both the referent and the remodeled left ventricular groups, NT-ANP with PIIINP concentrations in the referent group and D-dimer with PIIINP concentrations in the referent group. A statistically significant difference in β coefficient between referent and remodeled groups was seen only in the case of D-dimer. Fibrinogen, aldosterone and CRP were not related to PIIINP in either group.

Relations of systemic biomarkers to tissue inhibitor of metalloproteinases-1 concentrations

In general, TIMP-1 concentrations were more related to systemic biomarkers (relative to PIIINP) as shown in Table 3. Multivariable regression analysis revealed that homocysteine, PAI-1 and BNP were significantly and positively correlated with TIMP-1 concentrations in both the referent and remodeled left ventricular groups. Aldosterone and CRP were positively correlated with TIMP-1 in the remodeled left ventricular group alone, whereas fibrinogen, D-dimer and NT-ANP were correlated positively with TIMP-1 only in the referent group.

Relations of systemic biomarkers to matrix metalloproteinase-9 detectability

MMP-9 was less correlated with systemic biomarkers compared with PIIINP and TIMP-1 (Table 4). Homocysteine and renin were significantly and positively correlated with MMP-9 in the remodeled left ventricular group.

Conjoint associations of systemic biomarkers with collagen markers

In order to determine the relative strength of association of systemic biomarkers to each collagen marker when considered together, and to reduce errors from multiple statistical testing, we performed stepwise multivariable regression with backward selection, including all 10 clinical covariates and all nine systemic biomarkers. Table 5 shows the results of this analysis. Plasma homocysteine and PAI-1 were associated positively with PIIINP and TIMP-1 in both the referent and the remodeled left ventricular groups. Interestingly, homocysteine was the only biomarker that was significantly associated with detectable MMP-9, with a positive and significant relation observed in the remodeled left ventricular group alone. D-dimer was significantly and positively associated with both PIIINP and TIMP-1 in the referent group alone. The inflammatory marker CRP was positively associated only with TIMP-1 in the remodeled left ventricular group. Among markers of neurohormonal activation, NT-ANP was related positively to PIIINP in the referent group, and BNP was positively related to TIMP-1 in the referent group. Renin was negatively correlated with PIIINP in the remodeled left

ventricular group. Interestingly, the relation of renin was stronger when coanalyzed with other biomarkers compared with multivariable regression including only clinical covariates (Table 2). Conversely, the relation of renin to MMP-9 detectability (Table 4) was not noted in stepwise regression analysis including other biomarkers. Aldosterone was not independently related to any collagen marker in either left ventricular group in these conjoint analyses of systemic biomarkers.

Discussion

In this cross-sectional community-based study, we found a significant and positive association of plasma homocysteine with all three markers of collagen metabolism tested and of PAI-1 with two of the markers (PIIINP and TIMP-1). Less consistent patterns of association were noted for neurohumoral markers, CRP and D-dimer, varying based on the presence versus absence of left ventricular remodeling. These associations suggest that a potential reason for the predictive value of these biomarkers as cardiovascular risk factors could be due to their relation to cardiovascular collagen metabolism. Aldosterone and fibrinogen were generally not related to collagen markers in any of the groups.

Collagen markers and cardiovascular disease

Various plasma markers representing different stages in the metabolism of the fibrillar collagens types I and III, which are the major collagens present in the myocardium and vascular wall, have been correlated with cardiovascular diseases [1–3]. PIIINP is a marker that represents total turnover of type III collagen and has been related to cardiac function in hypertension and after a myocardial infarction [4,19]. MMPs, especially MMP-9 are correlated not only with cardiovascular tissue remodeling but potentially also with changes in plaque morphology and stability, as plasma levels have been associated with unstable coronary syndromes [5]. TIMP-1 that has growth-promoting effects in addition to inhibiting MMPs is also correlated with cardiovascular dysfunction [6].

Prior reports from our group have shown an association of circulating MMP-9 and TIMP-1 concentrations with left ventricular structure [6,13]; we did not find any relation of echocardiographic measures with PIIINP concentrations [17]. Even though these plasma markers of collagen metabolism could be altered by collagen turnover in any organ, the degree of correlation demonstrated with alterations in cardiovascular matrix makes them reasonable candidates for investigating cardiovascular matrix remodeling. Several traditional cardiovascular risk factors such as age, sex, dyslipidemia, diabetes, hypertension, obesity, smoking and alcohol intake influence circulating concentrations of collagen markers [18], hence our analyses adjusted for these covariates.

Homocysteine and collagen markers

An elevated plasma homocysteine level or hyperhomocysteinemia has been linked to atherothrombotic cardiovascular disease [20]. In addition, preclinical studies [10,11,21,22] from our laboratory and those of others have shown that hyperhomocysteinemia can also lead to myocardial fibrosis and cardiac failure. Furthermore, epidemiological and clinical studies [16,23,24] from our group as well as others have shown an association of hyperhomocysteinemia with left ventricular remodeling, dysfunction and clinical heart failure. Mechanistically, several studies have linked hyperhomocysteinemia to changes in the extracellular matrix as a pathogenic mechanism of cardiovascular disease. Homocysteine increases collagen production by cultured vascular smooth muscle cells in a dose-dependent manner [25]. Homocysteine has also been reported to induce expression of TIMP-1, an inhibitor of MMPs, in vascular smooth muscle cells [26] as well as of MMP-2 [27].

Both multivariable regression analysis adjusting for clinical covariates known to influence collagen turnover and conjoint analysis showed that plasma homocysteine concentration was significantly and positively related to all three collagen markers in the remodeled left ventricular group and with PIIINP and TIMP-1 in the referent group. Interestingly, among biomarkers analyzed in this study, homocysteine showed the most consistent correlation with plasma markers of collagen metabolism. This was somewhat surprising, given the presence of other well known correlates of collagen markers in the multivariable models. It is possible that the relation of homocysteine to collagen markers is due to homocysteine's effects on collagen turnover in other organs such as bone. We separated participants into referent and remodeled left ventricular groups to address this issue and observed that homocysteine was correlated with collagen markers in the remodeled left ventricular group, and that homocysteine's correlation to MMP-9 was seen only in the remodeled left ventricular group. This would suggest that homocysteine is related to cardiovascular collagen turnover and corroborate the findings of preclinical investigations. However, given our cross-sectional analysis, we cannot infer causality or presume any cardiac specificity of the observed association (i.e., it is possible that this relation is to the sum total of collagen turnover in the cardiovascular system and other organs).

Fibrinolytic markers and collagen turnover

The plasminogen/plasmin system, in addition to its thrombolytic functions, is also involved in tissue remodeling by acting on fibrogenic chemokines and matrix-degrading proteases [8]. PAI-1 is the major inhibitor of plasminogen activation (to plasmin) in tissues, and hence influences tissue remodeling and fibrosis. For example, mice genetically deficient in PAI-1 demonstrate reduced fibrous tissue formation [8], whereas PAI-1 excess promotes fibrosis. However, PAI-1 deficiency has also been associated with cardiac macrophage accumulation and fibrosis in mice [28]. Hence, PAI-1 is an important regulator of cardiovascular collagen metabolism, although the exact mechanisms are likely complex.

In our study, PAI-1 was positively correlated with both PIIINP and TIMP-1 in the referent and the remodeled left ventricular groups. The relations were similar when analysis was conducted accounting for clinical covariates, as well as when PAI-1 was analyzed conjointly with other biomarkers. These findings extend preclinical observations and suggest that PAI-1 is an important correlate of collagen metabolism in the community in participants at both ends of the spectrum of cardiac remodeling. Interestingly, PAI-1 was not correlated with detectable plasma MMP-9 in any analysis. This result is surprising, considering that PAI-1 is thought to influence collagen metabolism mainly by regulating MMP activity. Possible explanations could be that MMP-9 levels were below the limit of detection in most participants, and that we measured MMP-9 level, and not MMP-9 activity.

The fibrinolytic marker D-dimer was significantly correlated with PIIINP and TIMP-1 concentrations only in the referent group. D-dimer has been correlated with collagen markers in conditions such as aortic aneurysms [29]; hence, our results suggest a relation between collagen metabolism and the general fibrinolytic system, with a stronger relation seen with the plasminogen/plasmin system.

Natriuretic peptides and collagen metabolism

Natriuretic peptides are secreted from atria and ventricles in response to load and stretch and have important endocrine and paracrine functions in cardiovascular homeostasis [30]. Acting at a distance on vascular smooth muscle cells and renal tubular cells, these peptides produce vasodilatation and natriuresis. In addition, recent data indicate effects on cardiac fibroblast function, collagen metabolism and myocardial fibrosis [31,7]. Clinical studies [32] have demonstrated a positive relation of plasma natriuretic peptide concentrations with collagen

markers in the setting of myocardial infarction. In our study, the natriuretic peptides were positively related to PIIINP and TIMP-1 in the referent group. Although a significant relation was seen between BNP and TIMP-1 in the remodeled left ventricular group on multivariable analysis of that biomarker alone, this relation was not statistically significant in conjoint analysis of the biomarker panel.

Inflammation and collagen markers

Inflammation influences collagen metabolism, possibly through mediators such as transforming growth factor- β (TGF- β) and MMPs [9]. CRP has been shown to directly induce MMPs [33]. In our investigation, the conjoint analysis with other markers demonstrated that CRP was significantly correlated with TIMP-1 in participants with remodeled hearts. This suggests that inflammation may be an important correlate of collagen metabolism in the context of cardiac remodeling possibly by influencing TIMP-1 levels. As inflammation is also thought to play a major role in atherosclerosis, it was interesting to note that CRP was not related to any collagen marker in the referent group.

Renin angiotensin aldosterone system and collagen markers

Weber and Brilla [2], as well as other groups have shown the regulation of myocardial collagen metabolism by angiotensin II and aldosterone. We have previously demonstrated that serum aldosterone is associated with concentric left ventricular hypertrophy in women [15]. Aldosterone was significantly and positively related to TIMP-1 in the remodeled left ventricular group on multivariable analysis, whereas there was no significant relation with any marker in either group on conjoint analysis. These findings could suggest that the relation of aldosterone to collagen metabolism may be dependent on the presence of cardiovascular risk factors or activation of other biologic pathways. The negative relation of renin to PIIINP in the remodeled left ventricular group could be a surrogate relation, representing a positive correlation between aldosterone and PIIINP due to a potential feedback loop of aldosterone-suppressing renin. However, it is difficult to derive firm conclusions from a cross-sectional study based on a single measurement of hormone levels. Another issue is that antihypertensive treatment can influence renin and aldosterone levels, an effect that depends on the type of agent used, as shown in a recent report from our group [34]. Hence, we addressed this potential covariate by adjusting the regression analysis for the presence of antihypertensive treatment, without separating the type of treatment agents, similar to a recent study [14] conducted by our group on the relation between biomarkers and brachial artery endothelial function.

Strengths and limitations

The use of a large community-based sample, routine assessment of nine systemic biomarkers and three markers of collagen turnover, availability of clinical covariates that are known to influence collagen markers and use of both individual biomarker analyses as well as a conservative stepwise multivariable analytical strategy to reduce errors from multiple statistical testing strengthen our study. Analyses separating participants into those with LVWT and LVEDD with sex-specific 50th percentile or less and at least sex-specific 90th percentile also strengthen the present investigation by allowing us to relate biomarkers separately in participants with relatively normal hearts and those with potentially a greater degree of structural left ventricular remodeling.

However, we recognize several limitations of this study. First, as systemic biomarkers and collagen markers were measured at a given point in time (i.e., they were not measured at different time points along the remodeling process, or after any intervention), no causal inferences can be made. Second, a single measurement of renin and aldosterone may not precisely represent the actual level of activation of a person's rennin-angiotensin-aldosterone axis and could have influenced our findings. Third, the associations we observed were modest

and provide only an indirect measure of the potential and relative contributions of various systemic markers to interindividual variations in collagen metabolism. Fourth, as folic acid fortification was implemented in the middle of the examination cycle from which data used in this study were obtained, levels of homocysteine drawn late in the examination cycle may not reflect long-term homocysteine levels. Fifth, we did not analyze plasma markers of type I collagen; it is possible that associations of biomarkers with PIIINP may be different from associations with plasma markers of type I collagen metabolism. Finally, our study population is predominantly middle-aged to older white individuals of European descent, limiting the generalizability of our observations to other ethnicities.

Conclusion

A comprehensive evaluation of the correlations of a panel of circulating systemic biomarkers representing various biologic pathways to plasma markers of collagen metabolism in our large community-based sample demonstrated that the plasma homocysteine and PAI-1 were correlated with collagen markers, corroborating experimental studies implicating these pathways in cardiovascular collagen turnover. Additional studies are needed to confirm our findings and to determine whether the observed associations are causal.

Acknowledgments

This work was supported by the National Heart, Lung and Blood Institute's Framingham Heart Study (contract No. N01-HC-25195) and NIH grants K23-HL-074077 (Thomas J. Wang), RO1 HL67288, HL080124 and K24-HL04334 (Ramachandran S. Vasan).

Abbreviations

BNPB	type natriuretic peptide
CRPC	reactive protein
MMPmatrix	metalloproteinase
NT-ANPN	terminal proatrial natriuretic peptide
PAI	1plasminogen activator inhibitor-1
PIIIN	Paminoterminal propeptide of type III collagen
TIMP	tissue inhibitor of metalloproteinases

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Table 1

Clinical characteristics of study sample

Clinical characteristics	Referent group (n = 535)	Remodeled group (n = 386)
Age (years)	55.1 (9.1)	60.5 (9.8)
Male (%)	42.4	42.5
BMI (kg/m ²)	25.2 (3.7)	29.8 (5.4)
Smoking (%)	16.4	13.2
Diabetes (%)	3.6	16.1
Hypertension (%)	21.9	54.1
Antihypertensive treatment (%)	13.3	39.4
SBP (mmHg)	121 (17)	134 (21)
DBP (mmHg)	73 (9)	77 (10)
Total/HDL cholesterol ratio	4.2 (1.5)	4.4 (1.3)
Alcoholic drinks/week (%) ^a	10.3	10.1
Prevalent cardiovascular disease (%)	4.5	16.8
Statin treatment (%)	4.5	14.8
Serum creatinine (mg/dl)	1.1 (0.2)	1.1 (0.2)

HDL, high-density lipoprotein.

^aPercentage of heavy drinkers: men who consumed 16 drinks per week or more and women who consumed eight drinks per week or more.

Table 2
Multiple regression analyses (adjusted for 10 clinical covariates) of the relation of biomarkers to aminoterminal propeptide of type III collagen levels

	[0,2-3]Referent		[0,4-5]Remodeled		<i>P</i> (β referent versus β remodeled)
	β (SE)	<i>P</i>	β (SE)	<i>P</i>	
Homocysteine	0.162 (0.055)	0.004	0.106 (0.048)	0.028	0.45
CRP	0.020 (0.058)	0.73	0.026 (0.052)	0.62	0.94
BNP	0.111 (0.062)	0.07	0.076 (0.042)	0.07	0.65
NT-ANP	0.132 (0.064)	0.039	0.063 (0.047)	0.18	0.38
Aldosterone	-0.037 (0.063)	0.55	-0.074 (0.052)	0.15	0.65
Renin	-0.002 (0.062)	0.97	-0.084 (0.045)	0.06	0.29
Fibrinogen	0.040 (0.059)	0.49	-0.018 (0.044)	0.68	0.42
PAI-1	0.137 (0.058)	0.018	0.109 (0.052)	0.038	0.72
D-dimer	0.177 (0.055)	0.001	-0.001 (0.061)	0.99	0.03

All biomarkers are log-transformed.

Clinical covariates include sex, age, creatinine, BMI, SBP, treatment for blood pressure, total and HDL cholesterol ratio, current smoking status, diabetes and alcoholic intake.

BNP, B-type natriuretic peptide; CRP, C-reactive protein; HDL, high-density lipoprotein; NT-ANP, N-terminal proatrial natriuretic peptide; PAI-1, plasminogen activator inhibitor-1; SE, standard error.

Multiple regression analyses (adjusted for 10 clinical covariates) of the relation of biomarkers to tissue inhibitor of metalloproteinases-1 levels

Table 3

	[0,2–3]Referent		[0,4–5]Remodeled		<i>P</i> (β referent versus β remodeled)
	β (SE)	<i>P</i>	β (SE)	<i>P</i>	
Homocysteine	0.169 (0.040)	<0.001	0.186 (0.053)	<0.001	0.80
CRP	0.045 (0.041)	0.28	0.133 (0.056)	0.018	0.20
BNP	0.179 (0.044)	<0.001	0.096 (0.047)	0.041	0.20
NT-ANP	0.104 (0.046)	0.025	0.040 (0.052)	0.45	0.36
Aldosterone	−0.002 (0.045)	0.96	0.111 (0.054)	0.039	0.11
Renin	−0.010 (0.045)	0.83	−0.029 (0.050)	0.56	0.77
Fibrinogen	0.094 (0.042)	0.027	0.070 (0.049)	0.16	0.71
PAI-1	0.137 (0.042)	0.001	0.160 (0.058)	0.006	0.74
D-dimer	0.135 (0.040)	<0.001	0.126 (0.068)	0.065	0.91

All biomarkers are log-transformed.

Clinical covariates include sex, age, creatinine, BMI, SBP, treatment for blood pressure, total and HDL cholesterol ratio, current smoking status, diabetes and alcoholic intake.

BNP, B-type natriuretic peptide; CRP, C-reactive protein; HDL, high-density lipoprotein; NT-ANP, N-terminal proatrial natriuretic peptide; PAI-1, plasminogen activator inhibitor-1; SE, standard error.

Table 4
Multiple regression analyses (adjusted for 10 clinical covariates) of the relation of biomarkers to matrix metalloproteinase-9 detectability

	[0.2–3]Referent		[0.4–5]Remodeled		<i>P</i>	<i>P</i> (β referent versus β remodeled)
	OR (95% CI)	<i>P</i>	OR (95% CI)	<i>P</i>		
Homocysteine	1.00 (0.63–1.59)	0.99	1.43 (1.09–1.88)	0.01		0.20
CRP	1.14 (0.74–1.76)	0.56	1.31 (0.97–1.77)	0.08		0.61
BNP	0.87 (0.51–1.48)	0.60	1.00 (0.79–1.27)	0.99		0.64
NT-ANP	0.74 (0.43–1.27)	0.27	1.00 (0.77–1.31)	0.99		0.32
Aldosterone	1.15 (0.69–1.90)	0.60	1.27 (0.94–1.71)	0.12		0.73
Renin	1.49 (0.95–2.34)	0.09	1.31 (1.02–1.70)	0.038		0.64
Fibrinogen	0.97 (0.60–1.56)	0.90	1.12 (0.87–1.43)	0.39		0.61
PAI-1	1.08 (0.70–1.67)	0.72	1.24 (0.91–1.67)	0.17		0.62
D-dimer	0.87 (0.54–1.42)	0.59	1.05 (0.74–1.48)	0.80		0.56

All biomarkers are log-transformed.

Clinical covariates include sex, age, creatinine, BMI, SBP, treatment for blood pressure, total and HDL cholesterol ratio, current smoking status, diabetes and alcoholic intake.

BNP, B-type natriuretic peptide; CI, confidence interval; CRP, C-reactive protein; HDL, high-density lipoprotein; NT-ANP, N-terminal proatrial natriuretic peptide; OR, odds ratio; PAI-1, plasminogen activator inhibitor-1.

Table 5
Stepwise regression analysis of multiple biomarkers in relation to collagen markers

Collagen marker (dependent)	LV group	Global <i>P</i> value for biomarker panel	Stepwise selection: biomarkers selected	Regression coefficient (SE)	<i>P</i>
Log-PIIINP	Referent	<0.001	log homocysteine	0.147 (0.055)	0.008
			log PAI-1	0.149 (0.057)	0.010
			log D-dimer	0.171 (0.055)	0.002
			log NT-ANP	0.139 (0.063)	0.028
			log homocysteine	0.120 (0.048)	0.013
Log-TIMP-1	Remodeled	0.002	log PAI-1	0.123 (0.052)	0.020
			log renin	-0.112 (0.045)	0.014
			log homocysteine	0.158 (0.039)	<0.001
			log PAI-1	0.135 (0.040)	<0.001
			log D-dimer	0.108 (0.039)	0.006
	Referent	<0.001	log BNP	0.170 (0.043)	<0.001
			log homocysteine	0.184 (0.054)	<0.001
			log PAI-1	0.138 (0.058)	0.018
			log CRP	0.132 (0.055)	0.017
			Stepwise selection: biomarkers selected	OR (95% CI)	<i>P</i>
Collagen marker (dependent)	LV group				
MMP-9 (present or absent)	Referent	0.098	None		
			log homocysteine	1.43 (1.09–1.88)	0.010

BNP, B-type natriuretic peptide; CI, confidence interval; CRP, C-reactive protein; LV, left ventricular; MMP-9, matrix metalloproteinase-9; NT-ANP, N-terminal proatrial natriuretic peptide; OR, odds ratio; PAI-1, plasminogen activator inhibitor-1; PIIINP, aminoterminal propeptide of type III collagen; SE, standard error; TIMP-1, tissue inhibitor of metalloproteinases-1.