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Myocardial Collagen Cross-Linking Is Associated With Heart Failure Hospitalization in Patients With Hypertensive Heart Failure

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ABSTRACT

BACKGROUND Excessive myocardial collagen cross-linking (CCL) determines myocardial collagen's resistance to degradation by matrix metalloproteinase (MMP)-1 and interstitial accumulation of collagen fibers with impairment of cardiac function.

OBJECTIVES This study sought to investigate whether CCL and a newly identified biomarker of this alteration are associated with hospitalization for heart failure (HHF) or cardiovascular death in patients with HF and arterial hypertension in whom other comorbidities were excluded.

METHODS Endomyocardial biopsies and blood samples from 38 patients (invasive study), and blood samples from 203 patients (noninvasive study) were analyzed. Mean follow-ups were 7.74 ± 0.58 years and 4.72 ± 0.11 years, respectively. Myocardial CCL was calculated as the ratio between insoluble and soluble collagen. The ratio between the C-terminal telopeptide of collagen type I (CITP) and matrix metalloproteinase-1 (CITP:MMP-1) was determined in blood samples.

RESULTS Invasive study: CCL was increased ($p < 0.001$) in patients compared with controls. Patients were categorized according to normal or high CCL values. Patients with high CCL exhibited higher risk for subsequent HHF (log-rank test $p = 0.022$), but not for cardiovascular death. CITP:MMP-1 was inversely associated with CCL ($r = -0.460$; $p = 0.005$) in all patients. Receiver operating characteristic curves rendered a CITP:MMP-1 cutoff ≤1.968 (80% sensitivity and 76% specificity) for predicting high CCL. Noninvasive study: Patients were categorized according to CITP:MMP-1 ratio values as normal ratio (>1.968) or low ratio (≤1.968). Patients with a low ratio exhibited higher risk for HHF (log-rank test $p = 0.014$), which remained significant after adjustment for relevant covariables (adjusted hazard ratio: 2.22; 95% CI: 1.37 to 3.59, $p = 0.001$). In addition, CITP:MMP-1-based categorization yielded significant integrated discrimination and net reclassification improvements ($p = 0.003$ and $p = 0.009$, respectively) for HHF over relevant risk factors. CITP:MMP-1 was not associated with the risk of cardiovascular death.

CONCLUSIONS Excessive myocardial CCL is associated with HHF in hypertensive patients with HF. In this population, the serum CITP:MMP-1 ratio identifies patients with increased CCL and high risk of HHF. (J Am Coll Cardiol 2016;67:251-60) © 2016 by the American College of Cardiology Foundation.

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ABBREVIATIONS AND ACRONYMS

CCL = collagen cross-linking

CI = confidence interval

CITP = C-terminal telopeptide of collagen type I

CVF = collagen volume fraction

DM = diabetes mellitus

HF = heart failure

HHF = hospitalization for heart failure

IDI = integrated discrimination improvement

IHD = ischemic heart disease

LV = left ventricle/ventricular

LVEF = left ventricular ejection fraction

MMP = matrix metalloproteinase

NRI = net reclassification index

NT-proBNP = N-terminal pro-B-type natriuretic peptide

ROC = receiver-operating characteristic

TIMP = tissue inhibitor of matrix metalloproteinases

Due to its adverse consequences on left ventricular (LV) mechanics and function, electrical activity, and coronary microcirculation, myocardial fibrosis is involved in the pathophysiology and clinical course of heart failure (HF) of different etiologies (1). For instance, myocardial fibrosis, evaluated on biopsy samples and quantitatively defined by the increase in volume of myocardial tissue occupied by collagen fibers (namely, collagen type I), is associated with increased LV stiffness and diastolic dysfunction (2), impaired LV contraction and systolic dysfunction (3), and long-term mortality (4,5) in treated HF patients.

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As demonstrated in different experimental models of pressure overload, myocardial fibrosis is characterized, not only by excessive deposition of collagen fibers, but also by increased cross-linking of collagen fibrils within the fibers (i.e., collagen cross-linking [CCL]) (6–8). CCL is a process whereby collagen fibrils are covalently linked to one another by the enzyme lysyl oxidase (LOX), thus forming insoluble fibers with increased thickness and material stiffness (9). In addition, CCL determines the resistance of collagen fibers to degradation by matrix metalloproteinase (MMP)-1 (10), resulting in diminished cleavage of a small C-terminal telopeptide of the fiber (CITP in the case of collagen type I fibers, where it represents 1 of the 2 major cross-link sites) and a large amino-terminal telopeptide.

Although recent clinical studies point to an excess of CCL as a major determinant of LV diastolic and systolic dysfunction in patients with HF (2,11,12), no information on its prognostic significance in these patients is available. We therefore hypothesized that an excess of myocardial CCL is associated with long-term outcomes in patients with chronic HF attributable to hypertension. We also hypothesized that a diminished circulating level of CITP (corrected by total or unbound MMP-1 availability) may be a biomarker of reduced collagen type I degradation due to increased CCL. To test these hypotheses, in an invasive study, the association of myocardial CCL with clinical outcomes (i.e., hospitalization for heart failure [HHF] and cardiovascular death) and with CITP:MMP-1 and CITP:(MMP-1: tissue inhibitor of matrix metalloproteinase 1 [TIMP-1]) ratios were analyzed in a small cohort of patients with HF of hypertensive etiology. In a noninvasive study, associations between the CITP:MMP-1 ratio and the same clinical outcomes were analyzed in another larger cohort of patients with HF of hypertensive etiology.

METHODS

All of the subjects gave written informed consent to participate in the study, and the institutional review committee approved the study protocol. The study conformed to the principles of the Helsinki Declaration.

STUDY SUBJECTS. Patients were consecutively enrolled between 2002 and 2010. The patient population of the invasive and the noninvasive studies consisted of 38 and 203 hypertensive patients with a previous clinical diagnosis of chronic stage C HF, respectively. None of the patients exhibited ischemic heart disease (IHD), diabetes mellitus (DM), or stages 3 to 5 chronic kidney disease (CKD). Blood samples from the coronary sinus and the antecubital vein, and 3 transvenous endomyocardial biopsies from the middle area of the interventricular septum were taken from each invasive study patient during a cardiac catheterization procedure. Blood samples from the antecubital vein were taken in each patient from the noninvasive study.

Septal endomyocardial biopsies were obtained from autopsies of 10 age- and sex-matched subjects with no macroscopic and microscopic cardiac lesions to assess control reference values for histomorphological myocardial parameters. For further details, see the Online Appendix.

CARDIAC AND BIOCHEMICAL STUDIES. Two-dimensional echocardiographic, pulsed Doppler, and tissue Doppler imaging were performed in all patients. LV mass and dimensions, and parameters assessing systolic and diastolic function were measured. For further details, see the Online Appendix.

Plasma N-terminal pro-B-type natriuretic peptide (NT-proBNP), and serum CITP, MMP-1, and TIMP-1 were measured by enzyme-linked immunosorbent assay methods in all patients. CITP, MMP-1, and TIMP-1 values were expressed in molarity and CITP:MMP-1 and CITP:(MMP-1/TIMP-1) ratios were calculated in each patient. For further details, see the Online Appendix.

HISTOMORPHOLOGICAL STUDIES. To assess myocardial fibrosis, the fraction of myocardial volume occupied by collagen fibers or collagen volume fraction (CVF) was determined by morphometry in sections stained with collagen-specific picrosirius red. Immunohistochemical analysis of collagen types I and III was performed on formalin-fixed,

paraffin-embedded sections. The fraction of myocardial volume with positive staining for either collagen type I or collagen type III fibers ($C_I VF$ and $C_{III} VF$, respectively) was analyzed by morphometry.

To distinguish between cross-linked or insoluble collagen and non-cross-linked or soluble collagen, colorimetric and enzymatic procedures were used (13,14). The concentration of each form of collagen was corrected by the total amount of protein. CCL was calculated as the ratio between the insoluble and soluble collagen. For further details, see the Online Methods.

STUDY OUTCOME. The primary outcome was first HHF after enrollment and was identified by medical record review. HHF was defined as worsening signs and symptoms of HF that required urgent therapy and resulted in hospitalization (15). The secondary outcome was death from cardiovascular causes (i.e., congestive HF, acute myocardial infarction, malignant arrhythmias, sudden death, stroke, cardiorespiratory arrest). Vital status was ascertained by Social Security Medical Registries, and in the cases where any patient failed to appear at the scheduled review, his/her relatives were contacted by phone. Two board-certified cardiologist investigators blinded to the patients' histomorphological and biochemical data adjudicated outcomes according to the pre-specified criteria.

STATISTICAL ANALYSIS. Values are expressed as mean ± SEM and 95% confidence interval (CI), and categorical variables are expressed as numbers and percentages. Invasive study patients were categorized in 2 subgroups according to values of myocardial CCL within (subgroup with normal CCL) and above (subgroup with high CCL) the upper limit of normality (established as mean + 1.96 SD obtained in control subjects and equal to 2.96). To analyze differences between patients from the 2 subgroups in the invasive study, a Student *t* test for unpaired data was performed once normality was demonstrated (Shapiro-Wilks test); otherwise, a nonparametric test (Mann-Whitney *U* test) was used. Categorical variables were analyzed by the chi-square test or Fisher exact test, when necessary. The correlation between continuously distributed variables was tested using the Pearson and Spearman correlation coefficients and univariate regression analysis.

Receiver-operating characteristic (ROC) curves allowed determination of the overall performance of CITP:MMP-1 and CITP:(MMP-1:TIMP-1) ratios determined in antecubital vein blood for identifying abnormally high CCL in the invasive study patient population. The cutoff point (1.968) established by ROC analysis for the CITP:MMP-1 ratio in the invasive study was used to categorize patients from the noninvasive study into 2 subgroups: those with values >1.968 (normal CITP:MMP-1 ratio) and those with values ≤1.968 (low CITP:MMP-1 ratio).

Although these studies were originally designed as case-control studies, the percentages of patients free of the considered outcomes were estimated by the Kaplan-Meier method; unadjusted differences were assessed with log-rank tests. Only the first outcome was considered for the analysis and patients without an outcome were censored at the date of their last follow-up. Multiple Cox regression analysis was used to calculate hazard ratios and corresponding 95% CIs for the risk of future outcomes, adjusting for relevant covariables. In the invasive study, the covariables selected were peak early velocity of the transmitral flow divided by the peak early diastolic velocity of the mitral annulus displacement (E:E′) ratio, LV ejection fraction (LVEF), and NT-proBNP. In the noninvasive study, covariables were selected by forward stepwise Cox regression analysis, retaining those covariables that remained significant at $p < 0.05$ (systolic blood pressure, estimated glomerular filtration rate, LV mass index, LVEF, NT-proBNP, and treatment with beta-blockers and with calcium antagonists), along with age and sex. Backward stepwise analysis yielded the same 7 covariables. The proportional hazard assumption was verified using Schoenfeld's residuals.

Finally, to estimate the ability of the CITP:MMP-1 ratio-based classification to improve risk prediction in the noninvasive study, we calculated the integrated discrimination improvement (IDI) and the continuous net reclassification index (NRI). We predicted in each subject the risk for outcomes from a Cox regression basic model or the basic model extended with the predictor variable (categorization by level of the CITP:MMP-1 ratio > or ≤1.968), and the continuous NRI and IDI were calculated as described by Pencina et al. (16).

Statistical analyses were performed using SPSS (version 15.0, SPSS, Armonk, New York) and STATA (version 12.1, Stata Corp., College Station, Texas). NRI and IDI were calculated with the help of a STATA add-on (17).

RESULTS

INVASIVE STUDY. Classification of patients on the basis of CCL. CCL was increased ($p < 0.001$) in the whole group of patients compared with controls (3.31 ± 0.14 vs. 1.43 ± 0.29). Taking into account the criteria mentioned previously (Methods section, Statistical Analysis subsection), 11 patients

exhibited normal CCL values (subgroup with normal CCL 2.30 ± 0.11) and 27 patients exhibited high CCL values (subgroup with high CCL 3.73 ± 0.13). The clinical and echocardiographic characteristics of the 2 patient subgroups are shown in Online Table 1. No differences in histologically assessed collagen tissue were observed between patients with normal and high CCL (CVF: 7.21 ± 0.75% vs. 7.64 ± 0.63%; $C_I VF:C_{III} VF$ ratio: 7.62 ± 1.83 vs. 6.71 ± 0.84).

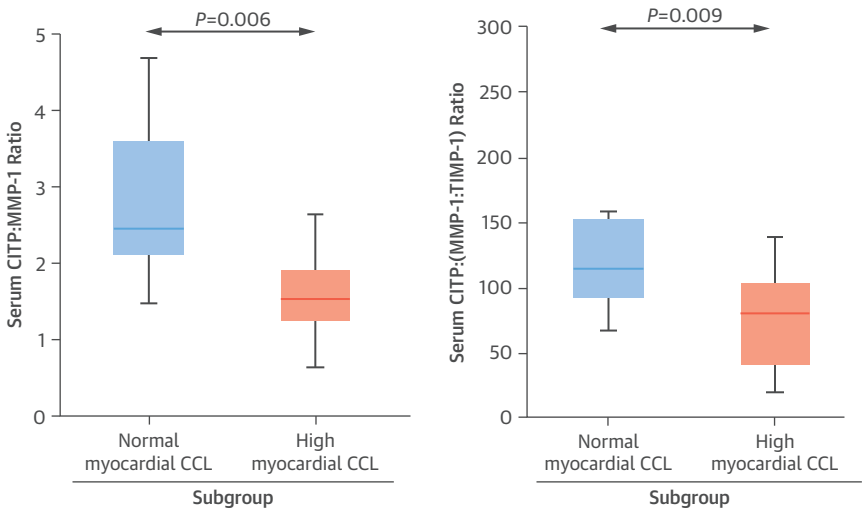
The CITP:MMP-1 ratio was reduced in patients with high CCL, compared with patients with normal CCL, in both coronary sinus blood (1.38 ± 0.15 vs. 1.77 ± 0.15, p = 0.026) and antecubital vein blood (1.61 ± 0.14 vs. 2.83 ± 0.35, p = 0.006) (Figure 1). In addition, the CITP:(MMP-1:TIMP-1) ratio was lower in patients with high CCL than in patients with normal CCL in both coronary sinus blood (79.04 ± 10.17 vs. 111.55 ± 14.21, p = 0.043) and antecubital vein blood (79.96 ± 8.84 vs. 142.17 ± 20.24, p = 0.009) (Figure 1).

CCL was directly correlated with E:E′ (r = 0.625, p < 0.001) and inversely correlated with LVEF (r = −0.430, p = 0.007) in all patients. In addition, CCL was directly correlated with NT-pro-BNP (r = 0.421, p = 0.010) in all patients. Inverse correlations were found between the CITP:MMP-1 ratio in antecubital vein blood and myocardial CCL (r = −0.460, p = 0.005) (Figure 2) and insoluble collagen (r = −0.452, p = 0.006), and between the CITP:(MMP-1:TIMP-1) ratio in antecubital vein blood and myocardial CCL (r = −0.387, p = 0.022) (Figure 2) and insoluble collagen (r = −0.425, p = 0.011) in all patients.

Analysis of ROC curves. The ROC curves allowed for the determination of the overall performance of serum CITP:MMP-1 and CITP:(MMP-1:TIMP-1) ratios determined in antecubital vein blood for identification of high myocardial CCL in HF patients. The area under the ROC curve was similar for the CITP:MMP-1 ratio and the CITP:(MMP-1:TIMP-1) ratio (Figure 3, Table 1), and both curves were higher than 0.50 (Table 1). From the ROC curves, cutoff reference values for serum CITP:MMP-1 and CITP:(MMP-1:TIMP-1) ratios were calculated. The sensitivity and specificity of each of these 2 values for identifying high CCL are presented in Table 1. Overall, the cutoff value of the serum CITP:MMP-1 ratio showed better specificity and sensitivity. This parameter was therefore chosen as a potential biomarker of myocardial CCL.

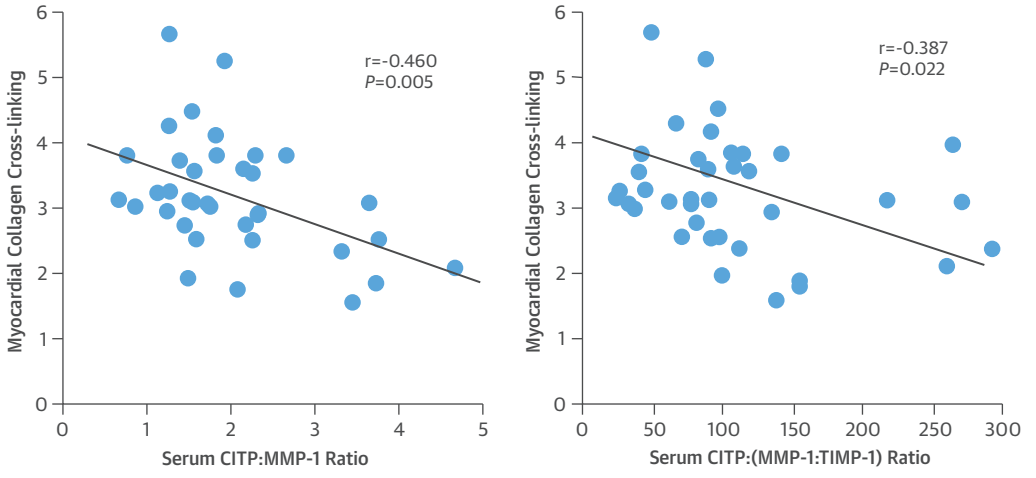
Follow-up and outcomes. The mean follow-up in patients with normal and high CCL was 9.48 ± 1.08 years (range 0.72 to 12.05 years) and 7.22 ± 0.65 years (range: 1.19 to 11.39 years), respectively. Two patients (18%) with normal CCL presented with HHF, as compared with 17 patients (63%) with high CCL (chi-square test p = 0.012; log-rank test p = 0.022). Multivariate Cox regression analysis, including the

FIGURE 1 Distribution of Potential Circulating Biomarkers of CCL in Patients With HF of Hypertensive Etiology From the Invasive Study



Box plots show the 5th and 95th **(vertical lines)**, 25th and 75th **(boxes)**, and 50th **(horizontal line)** percentile values for serum ratios of CITP to total MMP-1 (CITP:MMP-1) **(left panel)** and CITP to unbound MMP-1 [CITP:(MMP-1:TIMP-1)] **(right panel)**, determined in blood from the antecubital vein in patients with normal or high myocardial CCL, as defined in the text. CCL = collagen cross-linking; CITP = C-terminal telopeptide of collagen type I; HF = heart failure; MMP = matrix metalloproteinase; TIMP = tissue inhibitor of matrix metalloproteinase.

FIGURE 2 Association of Potential Circulating Biomarkers of CCL With Myocardial CCL in Patients With HF of Hypertensive Etiology From the Invasive Study



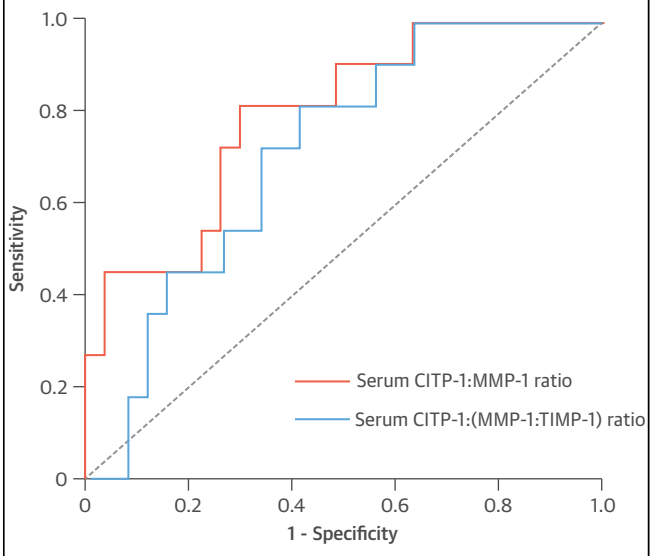
Inverse correlations between the serum ratio of CITP to total MMP-1 (CITP:MMP-1) ($y = -0.43x + 4.14$) **(left panel)** and the serum ratio of CITP to unbound MMP-1 [CITP:(MMP-1:TIMP-1)] ($y = -0.006x + 3.86$) **(right panel)** determined in blood from the antecubital vein and myocardial collagen cross-linking in all patients. Abbreviations as in Figure 1.

covariables E:E′ ratio, LVEF, and NT-proBNP, showed a significantly increased risk of HHF for patients with high CCL, as compared with patients with normal CCL, with a hazard ratio of 5.42 (95% CI: 1.11 to 26.37, p = 0.036). Additional adjustments were not considered because of the low number of patients. A total of 2 deaths (18%) in the subgroup with normal CCL and 9 (33%) in the subgroup with high CCL were due to cardiovascular causes (chi-square test p = 0.350; log-rank test p = 0.292).

NONINVASIVE STUDY. Classification of patients on the basis of the CITP:MMP-1 ratio. Taking into account the criteria mentioned previously (Methods section, Statistical Analysis subsection), patients in this study were classified into 2 subgroups: those with CITP:MMP-1 ratio values >1.968, predicting a normal myocardial CCL (subgroup with normal CITP:MMP-1 ratio n = 89) and patients with CITP:MMP-1 ratio values ≤1.968, predicting high myocardial CCL (subgroup with low CITP:MMP-1 ratio n = 114). Clinical and echocardiographic characteristics of the 2 patient subgroups are shown in Online Table 2.

Follow-up and outcomes. The mean follow-up in patients with normal and low CITP:MMP-1 ratios was 4.58 ± 0.18 years (range: 0.38 to 6.60 years) and 4.84 ± 0.14 years (range 0.24 to 7.21 years), respectively. Thirty patients (34%) with normal CITP:MMP-1 ratios presented with HHF, as compared with 62 patients

FIGURE 3 ROC Curves for Determining Abnormally High Myocardial CCL in the Invasive Study



ROC curves for the ratios of CITP to total MMP-1 (CITP:MMP-1) and CITP to unbound MMP-1 [CITP:(MMP-1:TIMP-1)] determined in blood from the antecubital vein, plotted for various cutoff values, for determining high myocardial CCL, as defined in the text. ROC = receiver-operating characteristic; other abbreviations as in Figure 1.

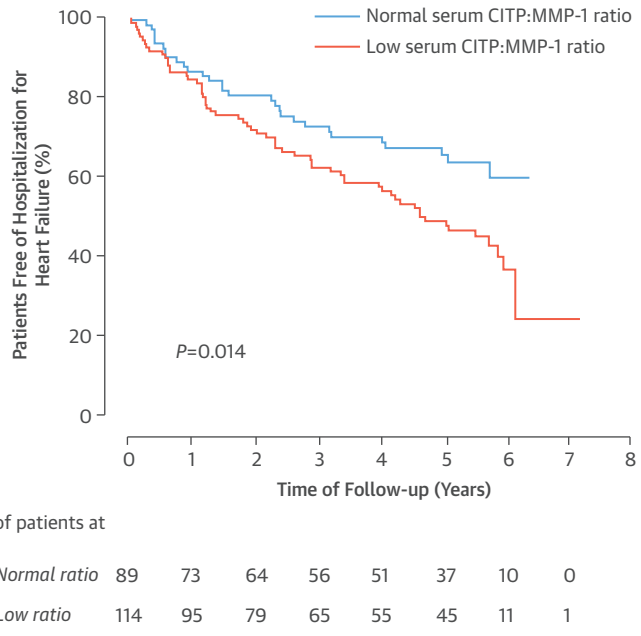
TABLE 1 Overall Performance of the 2 Serum Parameters for Predicting High Myocardial CCL According to ROC Curves

	Serum CITP:MMP-1 Ratio	Serum CITP:(MMP-1:TIMP-1) Ratio
AUC	0.798	0.724
95% CI	0.648–0.948	0.560–0.887
p Value	0.004	0.032
Cutoff	1.968	94.83
Sensitivity, %	80	73
Specificity, %	76	67
Chi-square test	8.61	5.30
p Value	0.003	0.021

AUC = area under the curve; CCL = collagen cross-linking; CI = confidence interval; CITP = C-terminal telopeptide of collagen type I; MMP-1 = matrix metalloproteinase-1; ROC = receiver-operating characteristic; TIMP-1 = tissue inhibitor of MMP-1.

(54%) with low CITP:MMP-1 ratios (chi-square test p = 0.003). Longitudinal analysis performed by Kaplan-Meier curves showed that patients with low CITP:MMP-1 ratios had a higher risk of HHF than patients with normal CITP:MMP-1 ratios (log-rank test p = 0.014) (Figure 4). Multivariate Cox regression analysis, including relevant covariables (age, sex, systolic blood pressure, estimated glomerular filtration rate, LV mass index, LVEF, NT-proBNP and treatment with beta-blockers and calcium antagonists), showed a significantly increased risk of HHF for patients with low CITP:MMP-1 ratios, as compared with patients with normal CITP:MMP-1 ratios, with a hazard ratio of 2.22 (95% CI: 1.37 to 3.59, p = 0.001). A total of 15 (17%) deaths in the subgroup with normal CITP:MMP-1 ratio and 22 (19%) in the subgroup with low CITP:MMP-1 ratio were due to cardiovascular causes (chi-square test p = 0.654; log-rank test p = 0.935).

FIGURE 4 Kaplan-Meier Curves for HHF in the Noninvasive Study According to Study Subgroups



Unadjusted Kaplan-Meier analysis and log-rank test show the percentage of patients free of HHF. (Normal CITP:MMP-1 ratio, patients with values of the serum ratio of C-terminal telopeptide of collagen type I [CITP] to total matrix metalloproteinase-1 [MMP-1] >1.968, n = 89; low CITP:MMP-1 ratio, patients with CITP:MMP-1 ratio values ≤1.968, n = 114). The ratios were determined in blood from the antecubital vein. HHF = hospitalization for heart failure; other abbreviations as in Figure 1.

Improvement of risk prediction. IDI and continuous NRI analyses indicated that addition of the categorical variable CITP:MMP-1 ratio ≤1.968 to a model that included the previously mentioned relevant covariables improved risk prediction of HHF, although it did not influence risk of death from cardiovascular causes (Table 2).

DISCUSSION

The novelty of our study is that we report independent associations between an excess of myocardial CCL and HHF in a cohort of patients with HF of hypertensive etiology. In addition, we describe, for the first time, that the serum CITP:MMP-1 ratio associates with myocardial CCL in the same cohort of patients and predicts the risk of HHF in an independent, larger cohort of patients with HF of hypertensive etiology. Furthermore, we prove the incremental prognostic value of this ratio over important risk factors, as confirmed by 2 measures of improvement in discrimination: IDI and continuous NRI.

ASSOCIATION OF MYOCARDIAL CCL WITH THE RISK OF HHF. We found that approximately two-thirds of patients with HF of hypertensive etiology exhibit high CCL. Of interest, compared with patients with normal CCL, patients with high CCL exhibited more severe LV diastolic and systolic dysfunction, but similar CVF and $C_I VF:C_{III} VF$ ratio. These findings suggest that an altered organization of the collagen fibril to form the collagen fiber may be more determinant than the amount of fibers per se in the detrimental impact of myocardial fibrosis on LV function in HF patients of hypertensive etiology.

To the best of our knowledge, this is the first report to demonstrate the prognostic impact of myocardial CCL in HF patients. In particular, high CCL was associated with increased risk of HHF in patients with HF of hypertensive etiology. Under a pathophysiological point of view, it can be argued that high myocardial CCL may reduce diastolic reserve and

thus, in conditions of fluid overload or exercise, the subsequent acute elevation of filling pressures may lead to reduced diastolic filling and produce HF symptoms resulting in hospitalization.

HHF is a growing public health problem. Despite initial improvement during hospitalization, subsequent rehospitalization, morbidity, and mortality after discharge remain high (18-20). Yet outcomes have not improved, despite a number of trials with drugs directed against neurohormonal activity, but not against targets directly involved in the structural remodeling of the myocardial collagen matrix (21). In this conceptual framework, it has been reported that myocardial extracellular volume fraction (a parameter assessed using T1-mapping cardiac magnetic resonance that closely reflects histological diffuse myocardial fibrosis) is associated with HHF in diabetic patients and in patients with nonischemic dilated cardiomyopathy (22,23). Our finding that an excess of CCL in patients with HF of hypertensive origin, treated as recommended by the guidelines, is associated with increased risk of HHF adds further support to the notion that myocardial fibrosis predisposes to HHF in patients with HF of different etiologies. In addition, our finding points to mechanisms involved in CCL as potential targets to treat HF. It must be noted that associations of enhanced myocardial LOX expression with increased CCL have been reported in animals with genetic (24) or induced (25,26) hypertension, as well as in hypertensive patients with HF (27). Furthermore, addition of torasemide to standard HF therapy in patients with HF of hypertensive etiology resulted in normalization of LOX, CCL, and LV chamber stiffness, and improvement of LV function and New York Heart Association class (27). Of interest, torasemide has been shown to inhibit bone morphogenetic protein-1, a protease that activates LOX, in the myocardium of patients with HF of hypertensive etiology (28).

ASSOCIATION OF A POTENTIAL BIOMARKER OF CCL WITH THE RISK OF HHF. Despite its recognition as a major public health problem in cardiovascular medicine, HHF is difficult to predict (18). Here, we report that the serum CITP:MMP-1 ratio may be useful as both a biomarker of abnormally high CCL and a predictor of HHF (**Central Illustration**). As shown in the invasive study, the CITP:MMP-1 ratio fulfills the criteria proposed for a circulating parameter to be a biomarker of alterations in myocardial collagen in HF patients (29). First, it is associated with histologically determined myocardial CCL and the association is pathophysiologically coherent. In fact, the higher the myocardial CCL, the lower the serum CITP:MMP1 ratio, suggesting that highly cross-linked collagen type I fibers are resistant to degradation by MMP-1 (10,12). Secondly, as shown by the ROC curve analysis, the serum CITP:MMP-1 ratio is a sensitive and specific parameter for the identification of high myocardial CCL. Finally, patients with serum CITP:MMP-1 ratio values ≤1.968 have a significantly higher probability of presenting with abnormally high myocardial CCL than patients with serum CITP:MMP-1 ratios above this value.

TABLE 2 IDI and NRI for Outcomes by Adding the Serum CITP:MMP-1 Ratio to a Model Including Covariables

	IDI			NRI		
Outcome Variable	IDI (%)	95% CI	p Value	NRI (%)	95% CI	p Value
Hospitalization for HF	5.07	1.70 to 8.44	0.003	38.7	9.50 to 67.9	0.009
Death from cardiovascular causes	0.46	−1.05 to 3.93	0.55	11.2	−26.5 to 48.8	0.56

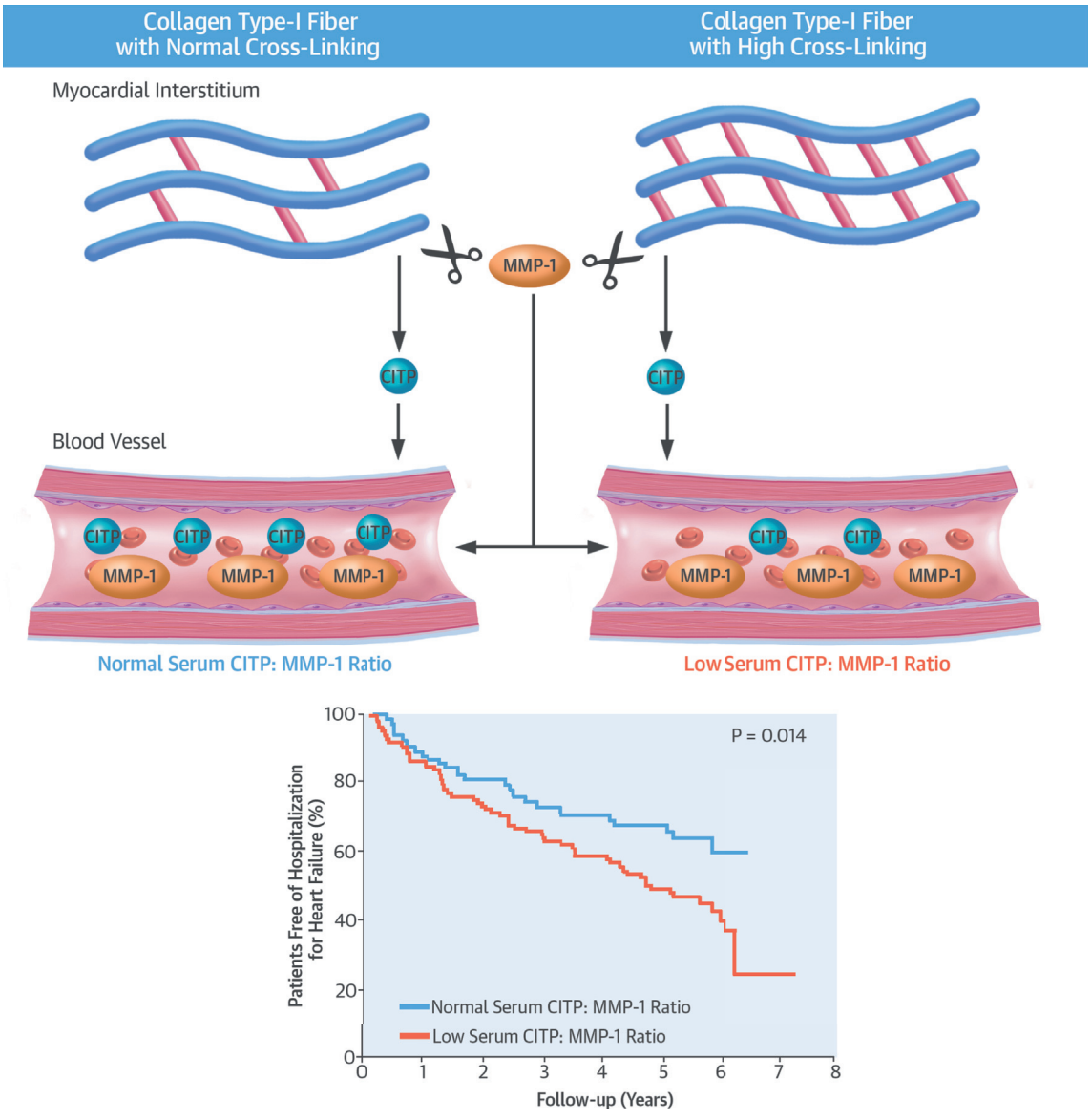
The IDI is the difference between the discrimination slopes of basic models (including age, sex, systolic blood pressure, estimated glomerular filtration rate, LV mass index, LVEF, NT-proBNP, and treatment with beta-blockers and calcium antagonists) and basic models extended with the predictor variable. The discrimination slope is the difference in predicted probabilities (%) between patients with and without event. The NRI reflects the improvement in discriminative power by adding the serum CITP:MMP-1 ratio (cutoff = 1.968) to a model already including relevant covariables (see above).

HF = heart failure; IDI = integrated discrimination improvement; LV = left ventricular; LVEF = left ventricular ejection fraction; NRI = net reclassification improvement; NT-proBNP = N-terminal pro-B-type natriuretic peptide; other abbreviations as in Table 1.

In addition, in the noninvasive study we provide evidence that the serum CITP:MMP-1 ratio is associated with the risk of presenting with HHF. In fact, HF patients with serum CITP:MMP-1 ratio values ≤1.968 have an approximately 2-fold higher probability of presenting with HHF than patients with a serum CITP:MMP-1 ratio above this value. It is noteworthy that the ratio predicts the risk of HHF independent of several relevant HHF risk factors (19). We also demonstrate that the serum CITP:MMP-1 ratio significantly increased the basic model's diagnostic utility for HHF, as quantified by the IDI and the continuous NRI.

STUDY LIMITATIONS. First, because they are descriptive in nature, the associations found between myocardial alterations and clinical outcomes in the invasive study do not establish causality. Secondly, the limited numbers of events recorded in the same study constrained risk adjustment and statistical power, and did not allow us to adjust for every difference in baseline characteristics. We did adjust for several clinically-relevant variables encountered in practice, although we cannot exclude overfitting. Thirdly, we performed biopsies of the right side of the interventricular septum to assess tissue collagen characteristics. However, as we have shown previously (30), the septum is representative of the free wall in the human failing hypertensive heart in terms of

CENTRAL ILLUSTRATION **CCL and Hypertensive HF: Identification of a Circulating Biomarker of Collagen Type I Cross-Linking With Prognostic Utility in HF of Hypertensive Origin**



López, B. et al. J Am Coll Cardiol. 2016; 67(3):251–60.

In the process of degradation of the collagen type I fiber within the myocardial interstitium, the enzyme collagenase or matrix metalloproteinase (MMP)-1 cleaves a small C-terminal telopeptide of the fiber or CITP (that represents 1 of the 2 major cross-linking sites in the collagen type I molecule) and a large N-terminal telopeptide. Because cross-linking between collagen type I molecules determines the resistance of the collagen type I fiber to degradation by MMP-1, the higher the cross-linking, the lower the generation of CITP. Both MMP-1 and CITP reach the systemic circulation and thus can be measured by specific enzyme-linked immunosorbent assay methods. We show for the first time that a low serum CITP:MMP-1 ratio, determined in blood from the antecubital vein, identifies patients with high myocardial collagen type I cross-linking and high risk of hospitalization for heart failure (HF) in a population of patients with HF of hypertensive etiology. **Magenta symbols** represent cross-links. CCL = collagen cross-linking.

collagen fiber deposition. Fourthly, although in the noninvasive study we analyzed a larger cohort to maximize generalizability of the data observed in the small first cohort, our data reflect only 1 center's experience and cannot be extrapolated to patients with HF of etiologies other than arterial hypertension. Furthermore, comorbidities frequently associated with hypertension (e.g., IHD, DM, stages 3 to 5 CKD) were absent in the studied populations. Finally, although HHF carries a significantly high mortality risk (31), we did not find an association of CCL with cardiovascular mortality in the invasive study, or of the serum CITP:MMP-1 ratio with cardiovascular mortality in the noninvasive study. However, it must be mentioned that the rates of cardiovascular death were low in patients from both the invasive (3.75 per 100 patients per year) and the noninvasive (3.86 per 100 patients per year) studies. Still, adjudication for cause of death can be challenging, controversial, and biased.

CONCLUSIONS

For the first time, we show that excessive myocardial CCL identifies those patients with prevalent HF of hypertensive etiology prone to present with HHF. Albeit preliminary, our results substantiate the potential usefulness of the serum CITP:MMP-1 ratio to identify these patients. Furthermore, we demonstrate that this ratio can add incremental prognostic value, improving the classification of patients at risk and improving model prediction beyond relevant clinical risk factors.

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PERSPECTIVES

COMPETENCY IN MEDICAL KNOWLEDGE: Excessive myocardial CCL is associated with decompensated systolic and diastolic heart failure HF in patients with hypertensive heart disease.

TRANSLATIONAL OUTLOOK: Additional studies are needed to understand the mechanisms by which disorganization of collagen fibrils progresses to myocardial fibrosis and deterioration of ventricular function in patients with HF of hypertensive etiology and to assess the prognostic value of incorporating measurements of the ratio of biomarkers of CCL and matrix metalloproteinase activity in the clinical management of patients with HF.

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KEY WORDS biomarker, cardiovascular death, heart failure of hypertensive etiology, myocardial fibrosis

APPENDIX For an expanded Methods section and supplemental references and tables, please see the online version of this article.