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Alterations in the Pattern of Collagen Deposition May Contribute to the Deterioration of Systolic Function in Hypertensive Patients With Heart Failure

Begoña López, PHD,* Arantxa González, PHD,* Ramón Querejeta, MD, PHD,† Mariano Larman, MD,‡ Javier Díez, MD, PHD*§

Pamplona and San Sebastián, Spain

OBJECTIVES We sought to assess the distribution of collagen deposits and collagen degradation in hypertensive patients with either systolic heart failure (SHF) or diastolic heart failure (DHF).

BACKGROUND Increased collagen synthesis and deposition have been described in the myocardium of heart failure (HF) hypertensive patients.

METHODS We studied 39 HF hypertensive patients subdivided into two groups: 16 with SHF and 23 with DHF. Endomyocardial biopsies were performed to quantify mysial (i.e., perimysial plus endomysial) and perivascular and scar-related collagen volume fraction (CVF). Matrix metalloproteinase (MMP)-1 and its tissue inhibitor matrix metalloproteinase (TIMP)-1 were analyzed in cardiac samples by Western blot and immunohistochemistry, and in blood samples by enzyme-linked immunosorbent assay.

RESULTS Mysial CVF was lower in SHF hypertensive patients than in normotensive ($p < 0.05$) and DHF hypertensive patients ($p < 0.01$). Perivascular and scar-related CVF was higher ($p < 0.05$) in the two groups of hypertensive patients than in normotensive subjects, and in SHF hypertensive compared with DHF hypertensive patients. The MMP-1:TIMP-1 ratio was increased ($p < 0.05$) in tissue and serum samples from the SHF hypertensive group compared with the other two groups of subjects. The MMP-1 expression was increased ($p < 0.01$) in the interstitium and cardiomyocytes of SHF hypertensive patients compared with DHF hypertensive and normotensive subjects. The serum MMP-1:TIMP-1 ratio was inversely correlated with ejection fraction ($r = -0.510$, $p < 0.001$) and directly correlated with left ventricular end-diastolic diameter ($r = 0.549$, $p < 0.001$) in all subjects.

CONCLUSIONS These findings show that the pattern of collagen deposits and the balance of the MMP-1/TIMP-1 system are different in the myocardium of SHF and DHF hypertensive patients. It is proposed that excessive degradation of mysial collagen may be related to the compromise of systolic function in HF hypertensive patients. (J Am Coll Cardiol 2006;48:89–96)

Hypertension is the major risk factor for heart failure (HF) (1). Myocardial fibrosis has been proposed as a major determinant of altered left ventricular (LV) filling leading to diastolic dysfunction and failure in hypertensive people (2). However, in a recent study we did not find differences in the total fraction of myocardial volume occupied by fibrillar collagen (collagen volume fraction [CVF]) between patients with either diastolic heart failure (DHF) or systolic heart failure (SHF) of hypertensive etiology (3). In addition, we found that collagen type I synthesis was inversely correlated with ejection fraction (EF) in these hypertensive patients (3). Up to now, data about mechanisms involved in extracellular collagen degradation (i.e., the enzyme system of matrix metalloproteinases [MMPs]) and distribution of collagen deposits (i.e., mysial or related to muscle compartments and perivascular and scar-related) in HF hypertensive patients have been unavailable.

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Findings from a number of experimental (4–6) and clinical (7–9) studies suggest an initial inhibition of MMP activity during pressure overload hypertrophy. However, over time, it has been shown in spontaneously hypertensive rats (SHRs) that MMP levels and activity increase within the myocardium and this is associated with development of LV dilation and failure (7,8,10). Interestingly, it has been reported that MMP inhibition attenuates LV enlargement and dysfunction in SHRs with HF (11).

We thus have hypothesized that enhanced MMP-mediated collagen degradation may be a contributor of LV dilation and the deterioration of EF in HF hypertensive patients. To test this hypothesis, we studied the myocardial

From the *Division of Cardiovascular Sciences, Centre for Applied Medical Research, School of Medicine, University of Navarra, Pamplona, Spain; †Division of Cardiology, Donostia University Hospital, San Sebastián, Spain; ‡Division of Hemodynamics, Guipúzcoa Polyclinics, San Sebastián, Spain; and the §Department of Cardiology and Cardiovascular Surgery, University Clinic, University of Navarra, Pamplona, Spain. This work was supported by a grant from the UTE Project FIMA and by grant RECAVA C03/01 from the Fondo de Investigaciones Sanitarias, Ministry of Health, Spain. Drs. López and González contributed equally to this work.

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Abbreviations and Acronyms

ADU	= arbitrary densitometric unit
CVF	= collagen volume fraction
DHF	= diastolic heart failure
EF	= ejection fraction
ELISA	= enzyme-linked immunosorbent assay
HF	= heart failure
LV	= left ventricle/ventricular
LVEDD	= left ventricular end-diastolic diameter
LVESWS	= left ventricular end-systolic circumferential wall stress
LVH	= left ventricular hypertrophy
LVMI	= left ventricular mass index
MMP	= matrix metalloproteinase
NT-proBNP	= amino-terminal pro-brain natriuretic peptide
SHF	= systolic heart failure
SHR	= spontaneously hypertensive rat
TIMP	= tissue inhibitor matrix metalloproteinase

expression of MMP-1 and its tissue inhibitor matrix metalloproteinase (TIMP)-1 and the amount and distribution of fibrillar collagen deposits in hypertensive subjects with either SHF or DHF. In addition, the serum levels of MMP-1 and TIMP-1 were analyzed in the same patients to explore the potential usefulness of the MMP-1:TIMP-1 ratio as a marker of myocardial collagen degradation in these patients.

METHODS

Subjects. All 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.

The hypertensive population consisted of 39 white patients with repeated measurements of systolic blood pressure and diastolic blood pressure of >139 and/or 89 mm Hg, respectively, consecutively enrolled from January 2003 to December 2004. All patients underwent appropriate clinical and laboratory evaluation to exclude secondary hypertension. All patients exhibited hypertensive heart disease as indicated by the presence of left ventricular hypertrophy (LVH) in the echocardiogram (see the text that follows). Other cardiac diseases associated with LVH and coronary artery disease were excluded after complete medical examination, which included a diagnostic cardiac catheterization. All patients had a previous clinical diagnosis of chronic HF based on the presence of at least one major and two minor Framingham criteria (12). After echocardiographic evaluation (see text that follows), the patients were classified into two groups: 16 exhibiting an EF <0.40 (SHF) and 23 presenting an EF ≥0.40 (DHF). None of the patients presented any conditions associated with alterations in serum levels of MMP-1 or TIMP-1 (rheumatoid arthritis, cancer, pulmonary fibrosis, and liver cirrhosis).

Twenty healthy, normotensive subjects (16 men and 4 women; mean age, 51 years; range, 33 to 72 years) were used as control subjects for biochemical parameters. A group of 10 normal hearts (from 6 men and 4 women; mean age, 59 years; range, 40 to 68 years) collected from a total of 100 autopsies at the University Clinic of Navarra during 1998 and 1999 served as controls for myocardial studies after cardiac disease had been excluded. An additional group of five explanted hearts from patients with HF and LV dilation submitted to cardiac transplantation were examined to assess whether interventricular septum is representative of the LV free wall in terms of collagen deposition.

Assessment of LV dimensions, mass, and function. Two-dimensional echocardiographic imaging, targeted M-mode recordings, and Doppler ultrasound measurements were obtained in each patient as previously described (13). The presence of LVH was established when left ventricular mass index (LVMI) was >111 g/m^2 for men and >106 g/m^2 in women (14). The presence of LV dilation was established when left ventricular end-diastolic diameter (LVEDD) was ≥ 56 mm (15). Left ventricular end-systolic circumferential wall stress (LVESWS) was calculated in accordance with Douglas et al. (16).

Histomorphological and immunohistochemical studies. Three transvenous endomyocardial biopsies were taken from the middle area of the interventricular septum with a bioptome (96 cm [7-F]; Cordis Corp., Miami Lakes, Florida), under fluoroscopic guidance after angiographic examination, and CVF was determined with an automated image analysis system in sections stained with collagen-specific picrosirius red, as previously reported (13). Two patterns of collagen deposition were defined according to its localization: mysial (collagen associated with groups of cells or perimysium, and collagen which surrounds and interconnects individual cells or endomysium), and perivascular and scar-related collagen (Fig. 1). Thus, total and perivascular and scar-related CVF were measured, and mysial CVF was calculated as the subtraction of perivascular and scar-related CVF from total CVF.

Immunohistochemical analysis for MMP- and TIMP-1 was performed on formalin-fixed and paraffin-embedded sections. Immunohistochemical staining was performed by the avidin peroxidase-labeled dextran polymer method. Positive staining was visualized with DAB plus (Boehringer Mannheim Corp., Indianapolis, Indiana) and tissues were counterstained with Harris hematoxylin (Sigma, St. Louis, Missouri). A mouse monoclonal antibody against MMP-1 (dilution 1:200; Oncogene, San Diego, California) and TIMP-1 (dilution 1:100; Chemicon, Temecula, California) was used as the primary antibody. A semiquantitative scale was developed to measure the expression of interstitial and cardiomyocyte MMP- and TIMP-1 seen at high power (×40). The amount of these molecules was graded on a scale of 0 to 3+, with 0 representing the absence of deposits, 1+ being mild deposits, 2+ corresponding to moderate deposits, and 3+ being intense deposits.

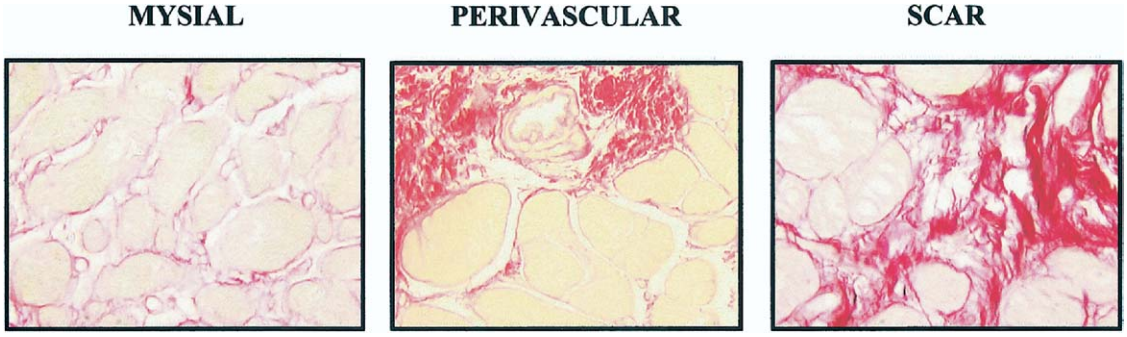

Figure 1. Endomyocardial tissue from one hypertensive patient with heart failure. Sections were stained with picrosirius **red** and collagen fibers were identified in **red** (×40). Collagen tissue was seen as thin bands surrounding individual cardiomyocytes or groups of cardiomyocytes (mysial collagen, **left panel**), as large strands localized around intramyocardial vessels (perivascular collagen, **middle panel**), and as masses or scars diffusely localized within the interstitium (scar collagen, **right panel**).

The histomorphological study was performed by two pathologists blinded to the other characteristics of the patients studied.

Western blot studies. To analyze the expression of MMP- and TIMP-1 proteins, aliquots containing 10 μg of total protein were diluted in 4× sample buffer (40% β-mercaptoethanol, 8% SDS, 40% glycerol, 0.025% bromophenol blue, and 0.25 mmol/l Tris, pH 6.4), separated by electrophoresis on 10% polyacrylamide gel, and transferred onto nitrocellulose membranes. Specific monoclonal antibodies against MMP-1 (Oncogene) and TIMP-1 (Chemicon) were incubated at a dilution of 1:2,000 and 1:200, respectively. Bands were detected by incubation with peroxidase-conjugated anti-mouse IgG (Amersham, Buckinghamshire, United Kingdom) at a dilution of 1:10,000 and 1:5,000, respectively (Fig. 2A). The blots were also tested with a monoclonal β-actin antibody (Sigma) as a control for loading (Fig. 2A). Data are expressed as arbitrary densitometric units (ADUs) relative to β-actin expression.

Biochemical determinations. Venous blood samples were obtained in each patient from the coronary sinus and the left antecubital vein during the cardiac catheterism procedure in which endomyocardial biopsies were performed. Free MMP- and TIMP-1 were determined in serum by enzyme-linked immunosorbent assay (ELISA) methods as previously reported (7). The levels of the amino-terminal pro-brain natriuretic peptide (NT-proBNP) were measured in serum samples by ELISA according to Karl et al. (17).

Statistical analysis. To analyze the differences between the normotensive group and the two groups of hypertensive subjects, a one-way analysis of variance followed by a Student-Newman-Keuls test was performed once normality was checked (Shapiro-Wilks test); otherwise, the nonparametric Kruskal-Wallis test followed by a Mann-Whitney

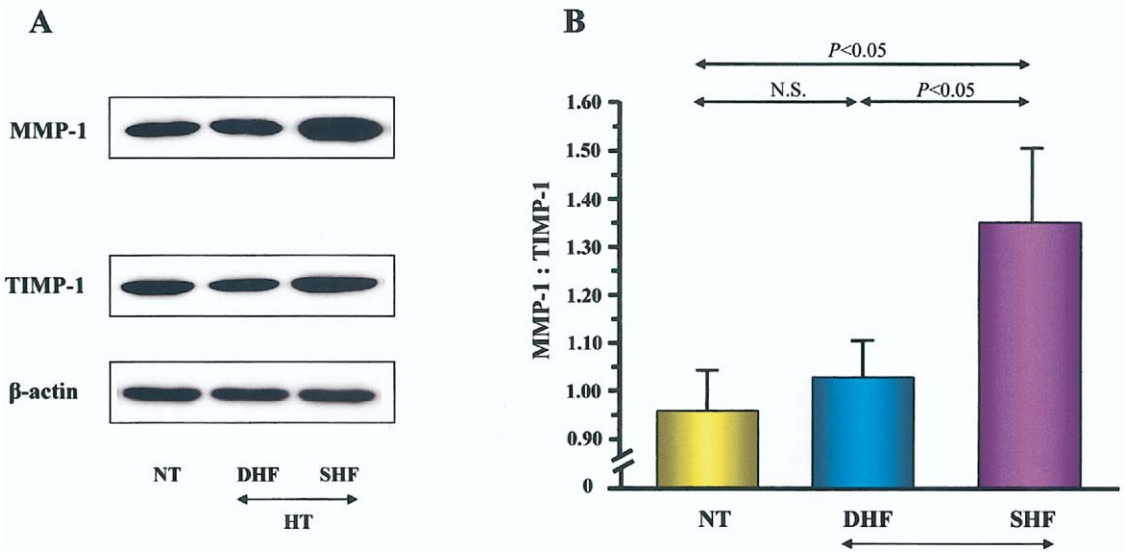

Figure 2. (A) Representative Western blot autoradiogram of the 52-kDa MMP-1 protein and the 28-kDa TIMP-1 protein. Autoradiograms include endomyocardial samples from one normotensive patient, one hypertensive patient with diastolic heart failure, and one hypertensive patient with systolic heart failure. **(B) Bars** represent mean + SEM of the ratio of MMP- to TIMP-1, calculated in endomyocardial samples from the three groups of subjects. DHF = diastolic heart failure; HT = hypertensive; MMP = matrix metalloproteinase; NT = normotensive; SHF = systolic heart failure; TIMP = tissue inhibitor matrix metalloproteinase.

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U test (adjusting the α-level by Bonferroni inequality) was used. Differences between the two groups of hypertensive patients were tested by a Student *t* test for unpaired data once normality was demonstrated; otherwise, a nonparametric test (Mann-Whitney *U* test) was used. Categorical variables were analyzed by the chi-square Fisher exact test when necessary. The MMP-1:TIMP-1 ratio in peripheral vein blood and NT-proBNP were normalized by logarithmic transformation for the correlational analysis. The correlation between continuously distributed variables was tested by univariate regression analysis. Values are expressed as mean ± SEM. A value of $p < 0.05$ was considered statistically significant.

RESULTS

Clinical characteristics of hypertensive patients. Baseline clinical characteristics of the two groups of hypertensive patients are presented in Table 1. Although the duration of HF did tend to be higher in SHF than in DHF hypertensive patients, the difference did not reach statistical significance. No differences were observed between the two groups of patients in the distribution of the different classes of pharmacological compounds. Values of LVMI and relative wall thickness were higher and lower, respectively, in SHF hypertensive patients than in the DHF hypertensive group. As expected, SHF hypertensive subjects exhibited higher values of LV chamber diameters and volumes and lower values of EF than DHF hypertensive patients. Left ventricle dilatation was present in 75% and 35% of patients in the SHF and the DHF groups, respectively, this difference being significant (chi square = 6.109, $p < 0.02$). The level of NT-proBNP was increased ($p < 0.001$) in the two groups of hypertensive patients compared with the normotensive group (36 ± 5 pg/ml). The values of this parameter did tend to be higher in SHF than in DHF hypertensive patients, but the differences did not reach statistical significance.

Table 1. Clinical Parameters Determined in Hypertensive Patients With Chronic Heart Failure

Parameters	DHF Patients	SHF Patients
Age, yrs	62 ± 3	65 ± 3
Gender, male/female	17/6	12/4
Time of diagnosis of HF, days	663 ± 106	967 ± 225
Body mass index, kg/m^2	27.93 ± 0.72	28.90 ± 0.71
NYHA functional class		
I	–	–
II	5	2
III	14	8
IV	4	6
Medications		
ACEIs or ARAs	23	16
Beta-blockers	21	15
Digoxin	11	8
Loop diuretics	23	16
Blood pressure, mm Hg		
Systolic	147 ± 2	140 ± 5
Diastolic	88 ± 1	87 ± 3
Heart rate, beats/min	69.6 ± 3.0	74.1 ± 4.5
LV posterior wall thickness, mm	9.40 ± 0.20	9.80 ± 0.40
IV septal thickness, mm	10.62 ± 0.40	10.93 ± 0.65
LV mass index, g/m^2	133 ± 9	181 ± 16†
Relative wall thickness, mm	0.37 ± 0.01	0.33 ± 0.01*
LV end-diastolic diameter, mm	52 ± 2	61 ± 3†
LV end-systolic diameter, mm	33 ± 2	51 ± 2‡
LV end-diastolic volume, ml	134 ± 10	193 ± 17†
LV end-systolic volume, ml	50 ± 6	130 ± 13‡
LV end-systolic circumferential wall stress, $\times 10^3$ dynes/cm^2	247 ± 11	268 ± 20
V_E:V_A	1.17 ± 0.09	1.49 ± 0.35
Deceleration time, ms	213 ± 11	175 ± 14*
Isovolumic relaxation time, ms	101 ± 3	111 ± 5
Ejection fraction, %	0.60 ± 0.02	0.30 ± 0.01‡
PICP, μg/l	130 ± 7	142 ± 8*
NT-proBNP, pg/ml	830 ± 156	1,196 ± 283*

Values are expressed as number of subjects, and as mean ± SEM. *$p < 0.05$; †$p < 0.01$; ‡$p < 0.001$, compared with DHF patients.

ACEI = angiotensin-converting enzyme inhibitor; ARA = angiotensin receptor antagonist; DHF = diastolic heart failure; HF = heart failure; IV = interventricular; LV = left ventricular; NT-proBNP = amino-terminal pro-brain natriuretic peptide; NYHA = New York Heart Association; PICP = carboxyterminal propeptide of procollagen type I; SHF = systolic heart failure; V_A = maximum late transmitral velocity in diastole; V_E = maximum early transmitral velocity in diastole.

Table 2. Collagen Volume Fraction in Normotensive Subjects and Hypertensive Patients With Chronic Heart Failure

Collagen Volume Fraction (%)	Normotensive Patients	DHF Patients	SHF Patients
Total	1.9 ± 0.7	7.3 ± 0.6†	8.1 ± 0.9†
Mysial	1.8 ± 0.1	2.6 ± 0.5	1.3 ± 0.1*§
Perivascular and scar-related	0.1 ± 0.02	4.7 ± 0.6*	6.8 ± 0.8*‡

Values are expressed as mean ± SEM. *$p < 0.05$; †$p < 0.01$, compared with normotensive patients. ‡$p < 0.05$; §$p < 0.01$, compared with DHF patients.

Abbreviations as in Table 1.

Assessment of cardiac collagen. Total CVF measured in the interventricular septum of failing and dilated hearts was similar to that measured in the free wall of the LV (7.95 ± 0.84% vs. 7.64 ± 0.49%). Thus, interventricular septum samples can be considered as representative for the LV myocardium.

As shown in Table 2, the values of total CVF were higher ($p < 0.01$) in DHF and SHF hypertensive patients than in the normotensive group. No differences in this parameter were found between the two groups of patients. Mysial CVF was decreased in the SHF hypertensive group compared with normotensive ($p < 0.05$) and DHF hypertensive patients ($p < 0.01$). No differences in this parameter were found between normotensive and DHF hypertensive patients. Perivascular and scar-related CVF was higher ($p < 0.05$) in the two groups of hypertensive patients than in the normotensive group. In addition, perivascular and scar-related CVF was increased ($p < 0.05$) in SHF hypertensive patients compared with DHF hypertensive subjects.

Assessment of cardiac MMP-1 and TIMP-1. Figure 2A shows a representative Western blot autoradiogram of MMP- and TIMP-1 proteins in myocardial tissue. The expression of MMP-1 was higher ($p < 0.05$) in the SHF hypertensive group (1.40 ± 0.20 ADU) than in normotensive (0.96 ± 0.16 ADU) and DHF hypertensive patients

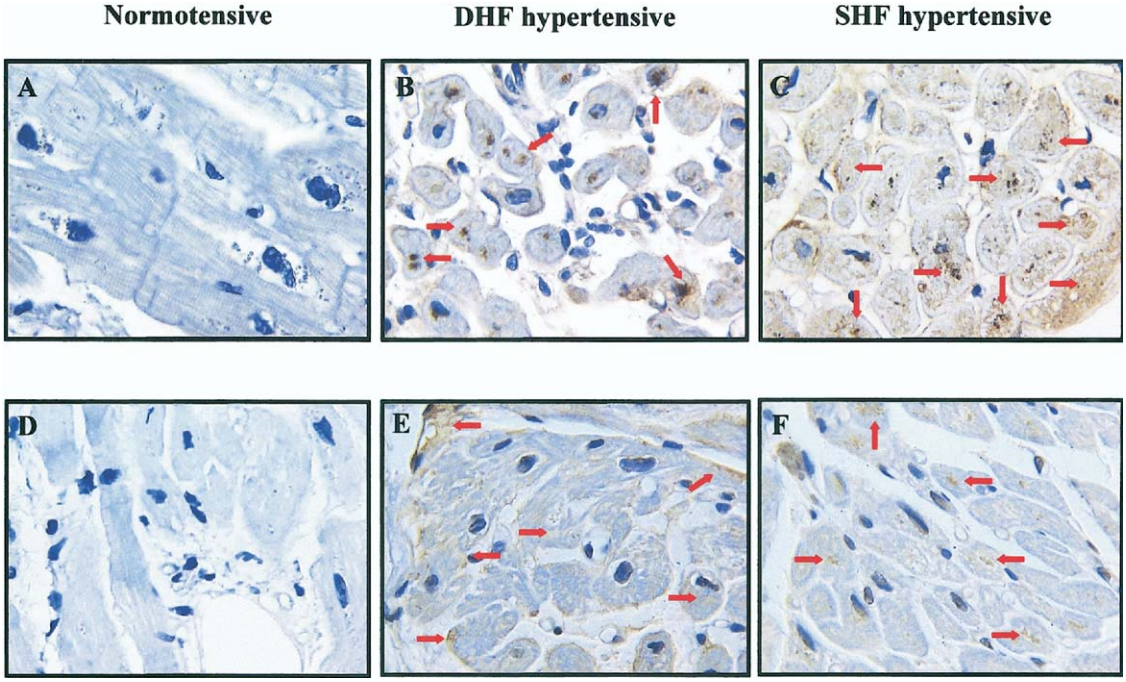

Figure 3. Endomyocardial tissue from one normotensive patient **(A and D)**, one hypertensive patient with diastolic heart failure **(B and E)**, and one hypertensive patient with systolic heart failure **(C and F)**. Sections were immunostained with an antibody anti-MMP-1 **(A, B, and C)** or an antibody anti-TIMP-1 **(D, E, and F)**, and these molecules were identified in **brown** within the cardiomyocytes. **Arrows** highlight specific points. (Magnification: ×100.) Abbreviations as in Figure 2.

(0.97 ± 0.06 ADU). No differences in the expression of TIMP-1 were observed among the three groups of subjects (normotensive 1.05 ± 0.20 ADU; DHF 0.95 ± 0.04 ADU; SHF 1.15 ± 0.12 ADU). As a consequence, the ratio of MMP- to TIMP-1 was significantly enhanced in SHF patients compared with the other two groups of subjects (Fig. 2B).

Although no cardiomyocyte expression of MMP- and TIMP-1 was observed in normotensive subjects, these two proteins were expressed in cardiomyocytes from HF hypertensive patients (Fig. 3). Whereas more patients exhibited low grades of cardiomyocyte MMP-1 expression in the DHF group, more patients exhibited high grades in the SHF group (Table 3). No differences in cardiomyocyte TIMP-1 expression were observed between the two groups of patients.

Interstitial expression of MMP- and TIMP-1 was detected in the three groups of subjects (data not shown). More patients exhibited high grades of interstitial MMP-1 expression in the SHF group than in the DHF group (Table 3). In contrast, more patients exhibited high grades of interstitial TIMP-1 expression in the DHF group than in the SHF group (Table 3).

Assessment of serum free MMP- and free TIMP-1. The values of MMP-1 (15.94 ± 0.86 ng/ml) and TIMP-1 (1,387 ± 79 ng/ml) in coronary sinus blood were higher ($p < 0.001$) than MMP-1 (12.85 ± 0.83 ng/ml) and TIMP-1 (1,194 ± 52 ng/ml) in peripheral vein blood in hypertensive patients, but not in normotensive subjects (coronary sinus blood: MMP-1 4.91 ± 0.55 ng/ml, TIMP-1 290 ± 47 ng/ml; peripheral vein blood: MMP-1 4.82 ± 0.35 ng/ml, TIMP-1 588 ± 22 ng/ml). In addition, direct correlations were found between MMP-1 in coronary sinus blood and peripheral vein blood ($r = 0.641$, $p < 0.001$) and TIMP-1 in coronary sinus blood and peripheral vein blood ($r = 0.625$, $p < 0.001$) in all hypertensive patients.

The level of MMP-1 in peripheral vein blood was increased ($p < 0.001$) in SHF (15.10 ± 1.72 ng/ml) and DHF (11.70 ± 1.00 ng/ml) hypertensive patients compared with the normotensive group (4.82 ± 0.35 ng/ml). In

Table 3. Grade of MMP-1 and TIMP-1 Deposits in Normotensive Subjects and Hypertensive Patients With Chronic Heart Failure

		Normotensive Patients		DHF Patients		SHF Patients	
	Grade	CM	INT	CM	INT	CM	INT
MMP-1	0	100	0	0	0	0	0
	1	0	80	60	20	20	10
	2	0	20	40	40	40	30
	3	0	0	0	40	40	60
p				*	‡	*†	‡§
TIMP-1	0	100	0	20	0	30	0
	1	0	80	40	20	40	40
	2	0	20	20	40	10	40
	3	0	0	0	40	0	20
p				*	‡	*	‡§

Values represent the percentage of patients in each group. *$p < 0.01$ compared with CM in normotensive patients; †$p < 0.01$ compared with CM in DHF patients; ‡$p < 0.05$ compared with INT in normotensive patients; §$p < 0.01$ compared with INT in DHF patients.

CM = cardiomyocytes; INT = interstitium; MMP-1 = matrix metalloproteinase 1; TIMP-1 = tissue inhibitor of MMP-1; other abbreviations as in Table 1.

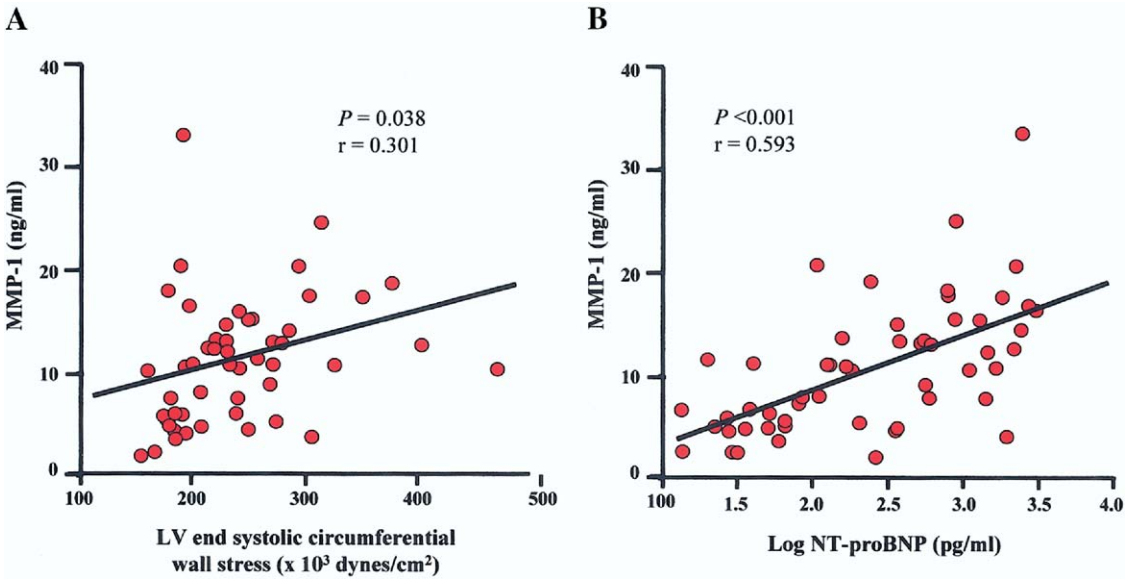

Figure 4. **(A)** Direct correlation (y = 0.03× + 4.75) between left ventricular end-systolic circumferential wall stress and MMP-1 measured in peripheral vein blood in all subjects. **(B)** Direct correlation (y = 5.28× − 1.93) between log amino-terminal pro-brain natriuretic peptide and MMP-1 measured in peripheral vein blood in all subjects. LV = left ventricular; NT-proBNP = amino-terminal pro-brain natriuretic peptide; other abbreviations as in Figure 2.

addition, MMP-1 in peripheral vein blood was higher ($p < 0.05$) in SHF patients than in DHF patients. The level of TIMP-1 in peripheral vein blood was higher ($p < 0.001$) in the two groups of hypertensive patients (SHF 1,076 ± 78 ng/ml; DHF 1,275 ± 73 ng/ml) than in the normotensive group (588 ± 22 ng/ml). In addition, TIMP-1 in peripheral vein blood was decreased ($p < 0.05$) in SHF patients compared with DHF patients. Thus, the ratio of MMP- to TIMP-1 in peripheral vein blood was increased in SHF hypertensive patients (1.51 ± 0.22) compared with both normotensive (0.83 ± 0.06, $p < 0.01$) and DHF hypertensive subjects (0.99 ± 0.11, $p < 0.05$). No differences in this parameter were observed between these two groups of subjects.

Analysis of associations. Direct correlations were found between LVESWS and MMP-1 in peripheral vein blood ($r = 0.301$, $p < 0.05$) (Fig. 4A) and between NT-proBNP and MMP-1 in peripheral vein blood ($r = 0.593$, $p < 0.001$) (Fig. 4B) in all subjects. The ratio of MMP-1 to TIMP-1 in peripheral vein blood was inversely correlated with EF ($r = -0.510$, $p < 0.001$) (Fig. 5A) and directly correlated with LVEDD ($r = 0.549$, $p < 0.001$) (Fig. 5B) in all subjects. Interestingly, significant associations were found between the ratio of MMP-1 to TIMP-1 in periph-

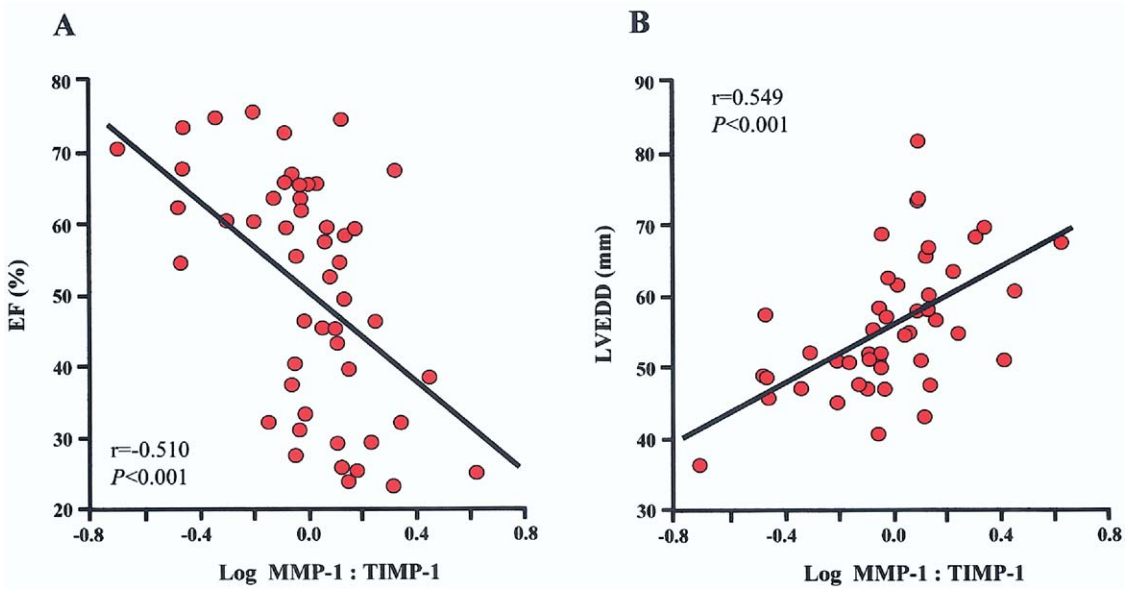

Figure 5. **(A)** Inverse correlation (y = −33.39× + 49.97) between the ratio of MMP-1 to TIMP-1 (log MMP-1:TIMP-1) measured in peripheral vein blood and ejection fraction in all subjects. **(B)** Direct correlation (y = 1.98× + 5.50) between log MMP-1:TIMP-1 measured in peripheral blood and left ventricular end-diastolic diameter in all subjects. EF = ejection fraction; LVEDD = left ventricular end-diastolic diameter; other abbreviations as in Figure 2.

eral vein blood >1.12 and EF <0.40 (chi square = 5.96, $p < 0.02$), and LVEDD ≥56 mm (chi square = 6.01, $p < 0.02$).

DISCUSSION

The main findings of this study are as follows: 1) whereas mysial collagen deposits are diminished, perivascular and scar-related collagen deposits are increased in SHF hypertensive compared with DHF hypertensive patients; 2) an excess of MMP-1 relative to TIMP-1 is present in the myocardium of SHF hypertensive compared with DHF hypertensive patients; 3) up-regulated expression of MMP-1 is detected at both the cardiomyocyte and the interstitial level in SHF hypertensive patients; and 4) increased serum MMP-1:TIMP-1 ratio is associated with LV dilatation and systolic dysfunction in HF hypertensive patients.

Pathophysiological meaning. Besides their rate of synthesis and activation, the actual activity of MMPs depends critically on the balance between active enzyme and TIMPs. Thus, our finding of increased cardiac MMP-1:TIMP-1 ratio in SHF hypertensive subjects suggests that MMP-1 activity is enhanced in these patients. Interestingly, the cardiac MMP-1:TIMP-1 ratio was normal in DHF hypertensive subjects, suggesting that disregulation of the cardiac MMP-1/TIMP-1 system is a characteristic feature of hypertensive patients with SHF. This possibility is in agreement with previous studies showing that in the hearts of patients with aortic stenosis, a pre-dominant expression of MMP-1 over TIMP-1 is present in the hearts of patients with depressed EF compared with patients with preserved EF (18,19).

It is likely that up-regulation of MMP-1 in SHF hypertensive patients is a dynamic process and is determined by the co-occurrence of a number of intracellular and extracellular signals. For instance, a number of humoral factors (i.e., inflammatory mediators, neurohormones, and bioactive peptides) may contribute to MMP-1 up-regulation in SHF hypertensive patients (20). In addition, mechanical signals linked to LV wall stress may result in the induction of myocardial MMP-1 (21). In support of these mechanisms, we found that serum levels of MMP-1 were associated with NT-proBNP and LVESWS.

A clear cause-and-effect relationship between excessive myocardial MMP activity, collagen degradation, and progression to SHF has been shown experimentally through the use of transgenic models and the use of pharmacologic MMP inhibitors. For instance, it has been demonstrated that acute MMP activation and disruption of the fibrillar collagen network in the pressure overload hypertrophied myocardium causes a decrease in systolic performance without changing cardiomyocyte contractility (22). In addition, it has been reported that transgenic mice with cardiac-restricted overexpression of human MMP-1 exhibit loss of collagen network accompanied by a marked deterioration of systolic function (23). In this context, our finding that the reduction of mysial collagen is associated with an intense expression of MMP-1 in cardiomyocytes from SHF patients may be of interest. In fact, experimental evidence has been provided showing that following chronic neurohormonal activation, increased synthesis and release of MMPs into the local extracellular matrix of the cardiomyocyte occurs (24), which in turn could contribute to endomysial and perimysial collagen degradation and disruption. The loss of the mysial collagen network may compromise systolic function through three possible mechanisms (25,26). The first implies discontinuities in the mysial collagen matrix that provides support, geometric alignment, and coordination of adjacent cardiomyocyte fascicle contraction. The second involves the loss of the normal collagen matrix-basement membrane-integrin connections that contribute to the synchrony and synergy of sarcomeres during the contractile process. The third is related to sliding displacements (slippage) of cardiomyocytes leading to a decrease in the number of muscular layers in the ventricular wall and LV dilation, which in turn impairs the working conditions of the LV myocardium.

Clinical application. Some findings reported here may be of clinical interest. The higher concentrations of MMP- and TIMP-1 in coronary sinus blood versus peripheral vein blood, found in hypertensive but not in normotensive patients, and the correlations between their peripheral and coronary sinus levels suggest that circulating MMP- and TIMP-1 detected in HF hypertensive patients may be of cardiac origin and that the peripheral MMP-1:TIMP-1 ratio may be useful as an index of the MMP-1/TIMP-1 balance within the myocardium. The clinical relevance of the MMP-1:TIMP-1 ratio determined in peripheral vein blood is further supported by its associations with parameters assessing LV systolic dysfunction (depressed EF) and remodeling (increased LVEDD). Therefore, because the determination of MMP- and TIMP-1 in serum is simple, reproducible, and low-cost, it might be useful for noninvasive screening for the MMP-1/TIMP-1 system in selected HF hypertensive patients, adding further diagnostic and prognostic information for the assessment of these patients.

Study limitations. This was a study involving a relatively small number of patients, but because of the nature of the goals under investigation, this design is appropriate. In addition, it must be recognized that therapy with different types of drugs may have confounded the findings and their interpretation. Nevertheless, since they are standard therapies for hypertension and HF, it is unreasonable to withdraw them for purposes of this investigation. In addition, no differences in the distribution of the different types of drugs were found between the two groups of patients.

Although we performed biopsies of the interventricular septum to assess changes in collagen in the LV, data here presented indicate that in terms of CVF, the septum is representative of the free wall. This is in agreement with data by Pearlman et al. (27) showing that collagen changes

present in the septum in post-mortem tissue from hypertensive, hypertrophic, non-dilated human hearts are representative of changes existing in the free wall.

Although a number of MMP and TIMP species are expressed within the human myocardium, only MMP- and TIMP-1 were examined in the current study. In addition, no direct assessment of tissue MMP activity (i.e., zymography) was performed. Therefore, further studies are required to identify the complete portfolio of MMPs and TIMPs that are functionally altered in HF hypertensive patients.

Conclusions. We report that an association exists between cardiomyocyte MMP-1 up-regulation, reduction of mysial collagen, and SHF in hypertensive patients. Thus, a new paradigm is proposed in the sense that beyond perivascular and scar-related fibrosis secondary to excessive collagen synthesis, exaggerated perimysial and endomysial collagen degradation may contribute critically to deterioration of systolic function in hypertensive patients.

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Reprint requests and correspondence: Dr. Javier Díez, Área de Ciencias Cardiovasculares, CIMA, Pío XII 55, 31008 Pamplona, Spain. E-mail: jadimar@unav.es.

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