

# Myocardial Remodeling and Immunologic Activation in Patients With Heart Failure

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**Introduction and objectives.** Immune response-mediated regulation of myocardial collagen remains poorly understood. Our objective was to investigate the relationship between ventricular remodeling and immunologic activation in patients with heart failure (HF) by comparing dilated and ischemic cardiomyopathy.

**Methods.** We studied 94 patients with HF and dilated cardiomyopathy (n=46) or ischemic cardiomyopathy (n=48). We recorded left ventricular (LV) volumes, E/A ratio, and ejection fraction. Plasma concentrations of tumor necrosis factor  $\alpha$  (TNF $\alpha$ ), soluble TNF $\alpha$  receptor I (sTNF-RI), sTNF-RII, interleukin-6 (IL-6), and IL-10 were measured. The serum procollagen type-III amino-terminal propeptide (PIIINP) level was also obtained.

**Results.** Ventricular volumes were greater in the dilated cardiomyopathy than in the ischemic cardiomyopathy group ( $P < .05$ ). However, sTNF-RI, sTNF-RII, and PIIINP levels were higher in the ischemic group ( $P < .05$ ). In this group, there were significant correlations between ventricular volumes and IL-10 and sTNF-RII levels. There was also a significant correlation between PIIINP and sTNF-RII levels ( $r = 0.30$ ;  $P < .05$ ). In the dilated cardiomyopathy group, there was a significant correlation between ventricular volumes and IL-10 level, and between PIIINP level and IL-6 ( $r = 0.32$ ;  $P < .05$ ) and sTNF-RII levels ( $r = 0.32$ ;  $P < .05$ ). Multiple linear regression analysis, which included cytokine levels, age, sex and ventricular function, showed that the sTNF-RII level was an independent predictor of the PIIINP level (adjusted  $r^2 = 0.16$ ;  $P < .0001$ ) and of ventricular volumes (LV end-systolic volume index, adjusted  $r^2 = 0.034$ ;  $P < .05$ ; and LV end-diastolic volume index, adjusted  $r^2 = 0.048$ ;  $P < .05$ ) in both groups.

**Conclusions.** In HF, there is an interaction between proinflammatory cytokines and the extracellular matrix.

Immunologic implications vary according to disease etiology. The elevation in proinflammatory cytokine and PIIINP levels is greater in patients with ischemic cardiomyopathy. Multiple regression analysis showed that the sTNF-RII level was an independent predictor of ventricular remodeling.

**Key words:** *Ventricular remodeling. Cytokines. Collagen. Heart failure.*

## Remodelado miocárdico y activación inmunitaria en pacientes con insuficiencia cardíaca

**Introducción y objetivos.** No se comprende bien la regulación del colágeno miocárdico mediada por la respuesta inmunitaria. Nuestro objetivo fue determinar las relaciones entre remodelado ventricular y activación inmunitaria en pacientes con insuficiencia cardíaca comparando miocardiopatía isquémica y dilatada.

**Métodos.** Estudiamos a 94 pacientes con insuficiencia cardíaca: miocardiopatía dilatada (n = 46) e isquémica (n = 48). Determinamos volúmenes ventriculares, E/A y FE. Medimos las concentraciones de TNF $\alpha$ , sTNF-RI, sTNF-RII, IL-6 e IL-10 y calculamos los valores de PIIINP.

**Resultados.** Los volúmenes ventriculares en la miocardiopatía dilatada fueron superiores a los del grupo isquémico ( $p < 0,05$ ). Sin embargo, los valores de sTNF-RI, sTNF-RII y PIIINP fueron más elevados en el grupo isquémico ( $p < 0,05$ ). En éste, los volúmenes ventriculares se correlacionaron significativamente con IL-10 y sTNF-RII. El PIIINP se correlacionó significativamente con sTNF-RII ( $r = 0,30$ ;  $p < 0,05$ ). En el grupo de miocardiopatía dilatada, los volúmenes ventriculares se correlacionaron significativamente con IL-10 y el PIIINP se correlacionó con IL-6 ( $r = 0,32$ ;  $p < 0,05$ ) y sTNF-RII ( $r = 0,32$ ;  $p < 0,05$ ). La regresión lineal múltiple, que incluyó citocinas, edad, sexo y función ventricular, demuestra que el sTNF-RII es un factor pronóstico independiente del PIIINP ( $r^2$  ajustada = 0,16;  $p < 0,0001$ ) y de los volúmenes ventriculares (IVTSVI,  $r^2$  ajustada = 0,034;  $p < 0,05$ ; IVTDVI,  $r^2$  ajustada = 0,048;  $p < 0,05$ ) en ambos grupos.

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## ABBREVIATIONS

IL: interleukin.  
 LVEDVI: left ventricular end-diastolic volume index.  
 LVESVI: left ventricular end-systolic volume index.  
 PIIINP: procollagen type-III aminoterminal propeptide.  
 TNF $\alpha$ : tumor necrosis factor alpha.  
 TNF-RI and TNF-RII: TNF $\alpha$  receptor types 1 and 2.

**Conclusiones.** En la insuficiencia cardiaca hay una interacción de citocinas proinflamatorias con la matriz extracelular. La implicación inmunitaria es diferente dependiendo de la etiología. Las citocinas proinflamatorias y los valores de PIIINP son más elevados en los pacientes con miocardiopatía isquémica. La regresión múltiple demostró que el sTNF-RII es un factor pronóstico independiente de remodelado ventricular.

**Palabras clave:** Remodelado ventricular. Citocinas. Colágeno. Insuficiencia cardiaca.

## INTRODUCTION

The concept and clinical meaning of left ventricular remodeling have been gradually widened. Cardiac remodeling is relevant in the progression of cardiovascular disease, such as myocardial infarction, valvular heart disease, myocarditis, and dilated cardiomyopathy. Fibroblasts, extracellular matrix proteins,<sup>1</sup> coronary vasculature and cardiac myocytes are involved in the remodeling process. In heart failure, turnover of the extracellular matrix—a three-dimensional structural network of interstitial collagens to which other matrix components are attached—is the main factor determining cardiac remodeling.

The main physiological functions of this network are to maintain tissue integrity and cardiac pump function. Collagen deposition is controlled and can be modulated by hormonal factors, growth factors, cytokines, regulatory proteins, and/or hemodynamic factors. A suitable balance is required between the synthesis and degradation of the extracellular matrix for normal morphogenesis and preservation of the tissue architecture.<sup>2</sup> Excess collagen accumulation leads to ventricular diastolic and systolic dysfunction and eventually contributes to heart failure. Thus, although remodeling may initially be an adaptive response, it slowly becomes maladaptive and leads to progressive decompensation.<sup>1</sup> Collagen synthesis is regulated in cells, whereas deposition of these proteins depends on a balance between the matrix metalloproteinases (MMP) and tissue inhibitors of MMP.<sup>3,4</sup> It seems probable that a paracrine/autocrine

effect of a repertoire of cytokines on inflammatory and myocardial cells can lead to an imbalance and result in a changed myocardial extracellular matrix architecture.<sup>3,5</sup> Thus, measuring cardiac collagen turnover by using serum markers is a useful tool to monitor cardiac tissue repair. In this context, serum concentrations of procollagen type-III aminoterminal propeptide (PIINP) can be measured as a collagen turnover marker in heart failure.<sup>6,7</sup>

It has been shown that proinflammatory cytokines, such as tumor necrosis factor alpha (TNF $\alpha$ ) and interleukin (IL) 6, induce MMP expression which can be measured in plasma and urine.<sup>8</sup> TNF $\alpha$  has multiple physiological functions mediated by its binding to specific receptors. Activated cells have been identified in myocardium, such as macrophages, endothelial cells and myocytes, TNF $\alpha$  producers and TNF $\alpha$  type 1 and 2 receptors (TNF-RI and TNF-RII, respectively).<sup>9</sup> Both receptors can be detected in their soluble forms, sTNF-RI and sTNF-RII, and their concentrations in blood and urine are elevated in severe heart failure.<sup>8,10,11</sup> In contrast, it is known that IL-10, an antiinflammatory cytokine, suppresses proinflammatory cytokine synthesis. In particular, IL-10 could inhibit TNF $\alpha$  synthesis.<sup>12,13</sup>

We put forward the hypothesis that the values of some cytokines and cytokine receptors play an important role in left ventricle (LV) remodeling and functioning in patients with heart failure, although such a role remains poorly understood.<sup>14</sup>

Thus, the purpose of this study was to determine the relationship between ventricular remodeling, as measured biochemically and geometrically, and the degree of immune activation in patients with heart failure, by comparing this relationship in dilated and ischemic cardiomyopathy.

## METHODS

### Patients

Ninety-four patients from the region of Valencia, Spain, diagnosed with heart failure, based on basic data from their medical record: electrocardiogram, chest x-ray, exercise stress test and echo-Doppler ultrasound.<sup>15</sup> The diagnosis of ischemic (n=48) and dilated (n=46) cardiomyopathy was confirmed based on the World Health Organization guidelines (1995),<sup>15-17</sup> and the functional capacity of the patients was defined according to the New York Heart Association. Table 1 shows the clinical characteristics of these patients. Subjects with atrial fibrillation, acute coronary syndromes, acute and chronic liver disease, rheumatoid arthritis, chronic infections, lung disease, or malignant tumors were excluded. There were no significant differences in time elapsed from diagnosis to patient assessment (measured in months) between the

ischemic ( $50.97 \pm 45.61$ ) and dilated cardiomyopathy ( $67.18 \pm 63.73$ ; NS) groups. All patients were undergoing on stable medical therapy, according to the American Heart Association<sup>18</sup> and the European Society of Cardiology guidelines,<sup>19</sup> for at least 1 month before their inclusion in the study, to avoid the possible effect of different cardiovascular interventions; none were taking antiinflammatory drugs on a regular basis. No differences were observed regarding treatment between the 2 groups, except for beta-blockers (63% and 39%) and digoxin (24% and 47%).

Table 2 shows the percentage of patients undergoing each treatment and compares the differences between the 2 study groups. The procedure was approved by the relevant institutional committees or the ethical committees of each center, and the study was done according to good clinical practice guidelines and ethical standards for experiments on human subjects established by the Declaration of Helsinki. Six hospitals in Valencia, Spain, participated.

## Samples

Venous blood was extracted by venopuncture during fasting and after the patient had been in the supine decubitus position for 30 min. The samples were centrifuged immediately, fractionated, and plasma and serum stored at  $-80^{\circ}\text{C}$  in Eppendorf tubes before transporting them to the laboratory to quantify the cytokines and PIIINP concentrations. Echocardiography was done on the same day as blood sampling.

## Cytokine and Cytokine Receptor Measurement

Plasma concentrations of  $\text{TNF}\alpha$  (sensitivity, 0.3 pg/mL), sTNF-RI (sensitivity, 1.2 pg/mL), sTNF-RII (sensitivity, 2.3 pg/mL), IL-6 (sensitivity, 0.7 pg/mL) and IL-10 (sensitivity, 4 pg/mL) were measured in duplicate through an enzyme-linked immunosorbent assay (ELISA) according to the manufacturer's recommendations (Quantikine R&D Systems Inc., Minneapolis, USA). The assays were quantified in a dual microplate reader at a wavelength of 450 nm (Sunrise, TECAN, Austria) supported by Magellan software (version 2.5 TECAN, Austria). The results are expressed in pg/mL.

## Measurement of Procollagen Type-III Aminoterminal Peptide

The concentration of PIIINP (sensitivity, 0.2  $\mu\text{g/L}$ ) was measured with the Orion Diagnostica UniQ PIIINP RIA commercial kit, a quantitative radioimmunoassay designed to measure the concentration of PIIINP in human serum in vitro. The results are expressed as  $\mu\text{g/L}$ .

**TABLE 1. Clinical Characteristics of the Patients Under Study**

	Dilated Cardiomyopathy (n=46)	Ischemic Cardiomyopathy (n=48)	P
Age, y	57 $\pm$ 14	68 $\pm$ 10	<.001
Male/female	35/11	36/12	NS
Systolic blood pressure, mm Hg	123 $\pm$ 17	125 $\pm$ 19	NS
Heart rate, beats/min	79 $\pm$ 13	74 $\pm$ 13	NS
Hypertension	12 (26.7%)	25 (54.3%)	<.01
Diabetes mellitus	10 (21.7%)	26 (54.2%)	<.001
NYHA			
I	11%	6%	NS
II	78%	71%	NS
III	11%	23%	NS
Hematocrit, %	41 $\pm$ 7.9	42 $\pm$ 6.0	NS
Plasma creatinine, mg/dL	1.0 $\pm$ 0.2	1.1 $\pm$ 0.3	NS
Na	139 $\pm$ 2.9	139 $\pm$ 3.2	NS
LVEF	36 $\pm$ 10	35 $\pm$ 9	NS
E/A	0.9 $\pm$ 0.5	1.1 $\pm$ 0.7	NS
LVESVI, mL/m <sup>2</sup>	82 $\pm$ 41	68 $\pm$ 25	<.05
LVEDVI, mL/m <sup>2</sup>	123 $\pm$ 52	104 $\pm$ 32	<.05

\*E/A indicates ratio between flow velocity in early diastole and during atrial contraction; LVEF, left ventricle ejection fraction; LVEDVI, left ventricular end-diastolic volume index; LVESVI, left ventricular end-systolic volume index; NYHA, New York Heart Association functional classes; LV, left ventricle. Data are expressed as mean  $\pm$  standard deviation or percentage.

**TABLE 2. Percentage of Patients Undergoing Each\***

Medication	All Patients	Dilated Cardiomyopathy (n=46)	Ischemic Cardiomyopathy (n=48)	P
Diuretics	80.6	84.8	76.6	NS
ACE inhibitors	77.2	75.6	7.7	NS
Beta-blockers	51.5	39.1	63	<.05
Antialdosterones	45.1	46.7	43.5	NS
Digoxin	35.2	46.7	23.9	<.05
ARB-II	17.6	23.9	11.1	NS
Calcium antagonists	9.9	4.4	15.2	NS

\*ARA-II indicates angiotensin-II receptor blockers; ACE inhibitors, angiotensin-converting enzyme inhibitors.

## Echo-Doppler Ultrasound Study

The study was done using standard echocardiographic systems equipped with 2.5 MHz transducers used in daily clinical practice by the hospitals involved in the study. Echocardiographic examinations were done using standard apical and parasternal views. Echo-Doppler ultrasound images were stored on video tape and the recordings analyzed

in a central laboratory. Ventricular function was assessed in a blinded manner. Bidimensional images, echo-Doppler ultrasound and color-Doppler ultrasound spectra were analyzed using a computerized system (Echo-Dat; Medicine Inc. software). For each patient, 4 consecutive cycles were measured and the mean calculated for each variable.

The area-length method was used to calculate the ejection fraction (LVEF), left ventricular end-systolic volume index (LVESVI)–end-systolic volume of the left ventricle/body–surface area– and left ventricular end-diastolic volume index (LVEDVI)–end-diastolic volume of the left ventricle/body-surface area.<sup>20</sup> Left ventricle ejection fraction was set at 100×[(end-diastolic volume–end-systolic volume)/end-diastolic volume]. Flow velocity in early diastole (E wave), and during atrial contraction (A wave) were measured by pulsed Doppler ultrasound at the valve level, thus obtaining the E/A ratio.

### Statistical Analysis

Data are presented as mean ± standard deviation (SD) or median ± standard error of the mean (SEM) for cytokine and cytokine receptor concentrations. Since the cytokine and PIIINP values did not have a normal distribution, the data underwent logarithmic transformation before all statistical analyses were done. The Pearson product-moment correlation coefficient for logarithmically standardized data was used to correlate LV volumes, changes in the collagen turnover marker, PIIINP, functional parameters (LVEF and E/A) and immune activation. This procedure was used in both groups of heart failure patients with dilated and ischemic cardiomyopathy.

Between-group comparisons of numerical data were done using Student *t* test for independent samples or the Mann-Whitney nonparametric U test for 2 independent samples.

**TABLE 3. Biochemical and Immunological Variables in the 2 Groups of Patients\***

	Dilated Cardiomyopathy (n=46)	Ischemic Cardiomyopathy (n=48)	P
PIIINP, µg/L	3.7±0.3	4.3±0.3	.016
IL-10, pg/mL	16.1±1.9	13.2±1.7	NS
IL-6, pg/mL	2.0±0.5	3.4±1.1	.093
TNFα, pg/mL	3.3±0.8	4.5±1.3	NS
sTNF-RI, pg/mL	1820.7±243.8	2119.8±145.4	.011
sTNF-RII, pg/mL	4221.5±413.2	5115.4±397.7	.049

\*IL indicates interleukin; PIIINP, procollagen type-III aminoterminal propeptide; sTNF-RI, soluble TNFα receptor type I; sTNF-RII, soluble TNFα receptor type II; TNFα, tumor necrosis factor alpha. Data are expressed as mean ± standard error.

Multivariate linear regression analysis (MLRA) was used to test the independent predictive power of cytokine and cytokine receptor values and other variables in ventricular remodeling (PIIINP, LVESVI, and LVEDVI) in heart failure patients with both ischemic and dilated cardiomyopathies.

A step-wise selection procedure was done for the different variables. Best model discrimination was based on the principle of squared minimums and greatest *r*<sup>2</sup>. *P*-values <.05 were considered significant. TNFα, sTNF-RI, sTNF-RII, IL-6, IL-10, age, sex, LVEF, E/A, and diabetes were included in the MLRA with PIIINP as the dependent variable.

The MLRA, with LVESVI and LVEDVI as the dependent variables, included TNFα, sTNF-RI, sTNF-RII, IL-6, IL-10, age, sex, and PIIINP as the independent variables.

All the statistical results were obtained with the SPSS/PC statistical package 10.1 (SPSS, Inc., Chicago, USA). Differences were considered significant when *P*<.05 for all parameters.

### RESULTS

We calculated and compared the biochemical and geometric parameters involved in ventricular remodeling and immunological activation in heart failure patients with ischemic and dilated cardiomyopathy, which, in our patients, yielded high values (Tables 1 and 3). We found that LVESVI and LVEDVI were higher in the dilated cardiomyopathy group than in the ischemic patient group. Cytokine, sTNF-RI, and sTNF-RII concentrations were higher in the ischemic group than in the dilated cardiomyopathy group. There was a statistical trend toward increased values of IL-6 in the ischemic group.

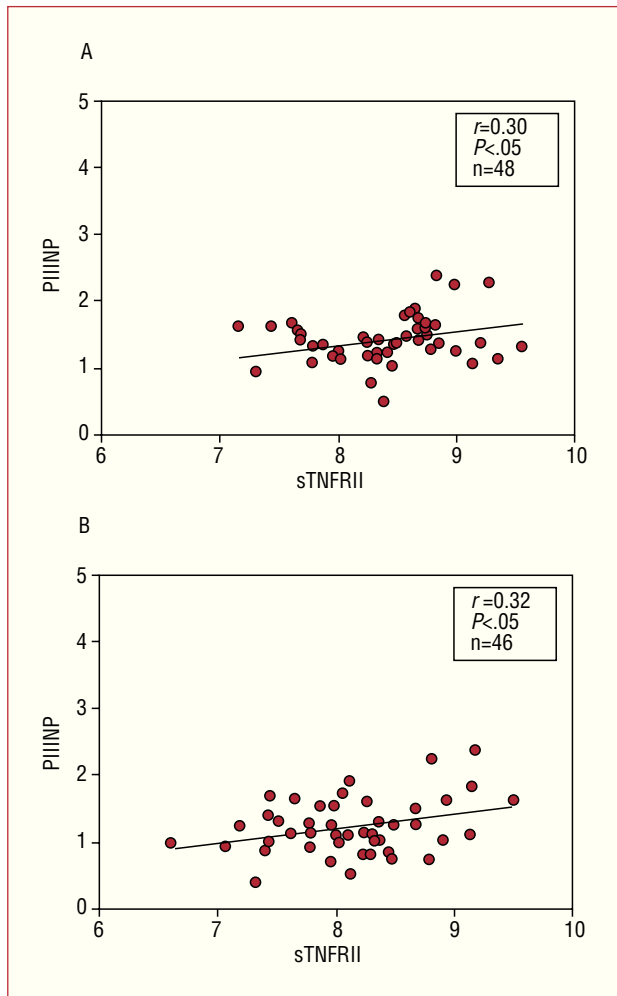
A significant difference was observed in the biochemical matrix turnover marker, PIIINP, with higher figures in ischemic patients.

Pearson correlation of the logarithmically standardized data between the LV volumes and immune parameters was calculated in both groups. In the ischemic group, LVEDVI was correlated negatively with IL-10 (*r*=−0.41; *P*<.05) and sTNF-RII (*r*=−0.31; *P*<.05). No significant correlation was obtained with LVESVI. Procollagen type-III aminoterminal propeptide correlated significantly with sTNF-RII (*r*=0.30; *P*<.05) (Figure 1A).

Finally, we found a negative correlation between the parameter of systolic function, LVEF, and IL-6 (*r*=−0.42; *P*<.01), sTNF-RI (*r*=−0.32; *P*<.05) and sTNF-RII (*r*=−0.33; *P*<.05). However, no significant correlation was obtained when we studied the relationships between the diastolic parameter, E/A, and cytokine concentrations.

In the dilated cardiomyopathy group, LVESVI and LVEDVI correlated with IL-10 (*r*=0.39; *P*<.05,





**Figure.** Pearson correlation of the biochemical matrix turnover marker, PIIINP, and soluble TNF $\alpha$  receptor type II (sTNF-RII) in patient group A: ischemic cardiomyopathy; and patient group B: dilated cardiomyopathy. PIIINP: procollagen type-III aminoterminal propeptide.

and  $r=0.36$ ;  $P<.05$ , respectively). The collagen turnover marker, PIIINP, correlated significantly with IL-6 ( $r=0.32$ ;  $P<.05$ ) and also with sTNF-RII plasma concentrations (Figure 1B) ( $r=0.32$ ;  $P<.05$ ). Results regarding LVEF and E/A were not significant. A multivariate linear regression analysis was done to verify the independent predictive power of cytokines and cytokine receptor values and other variables regarding the biochemical matrix turnover marker, PIIINP, in the heart failure patients. The best model was found when sTNF-RII was associated with PIIINP values ( $P<.0001$ ) (Table 4, point A). An MLRA was also used to investigate the independent predictive power of cytokine and cytokine receptor values in relation to LV volumes. The best model was found when sTNF-RII was associated with LVESVI and LVEDVI as dependent variables ( $P<.05$  and  $P<.05$ , respectively) (Table 4, points B and C).

## DISCUSSION

Ventricular remodeling is a complex and not fully understood process. There appear to be multiple feedback loops that respond to mechanical events, as well as to neurohormonal stimulation and the release of cytokines and other agents, which remain unidentified.<sup>1</sup> Ventricular remodeling progression following these events includes: reduction of myocytes in and thinning of the infarct area, chamber dilatation, fibrosis and scar formation, collagen dissolution and excessive interstitial matrix accumulation, increased wall stress, myocyte hypertrophy, neurohormonal activation, cytokine release, cellular hypertrophy, necrosis and apoptosis and sustained deterioration in cardiac function. It is impossible to sequence the events, because the multiple feedback systems create an interactive process.<sup>21</sup>

In this context, we studied the relationships between ventricular remodeling, measured biochemically and geometrically, and the degree of immune activation in heart failure patients. Although we have to point out that we took the absolute value of LV volumes as the geometric marker of LV remodeling, the concept of LV remodeling involves change in LV volumes over time. We also have to take into account that the differences between normal volumes<sup>22</sup> and those of our patients were sufficiently significant to consider these LV volumes as reliable markers of change.<sup>23</sup> Thus, we studied the relationships between LV volumes, the biochemical matrix turnover marker (PIIINP), and LV function parameters (LVEF, E/A) with circulating cytokine values in ischemic and dilated cardiomyopathy. We chose TNF $\alpha$  and IL-6 as proinflammatory cytokines because previous works have demonstrated their role in this process.<sup>24-26</sup> We

**TABLE 4. Results of Multiple Linear Regression Analysis**

Variable	$\beta$	Standard Error	P
A. Multiple linear regression: predictive value of sTNF-RII on PIIINP values in all patients (adjusted $r^2=0.16$ ; $P<.0001$ )			
Log sTNF-RII	0.326	0.077	.001
B. Linear multiple regression: predictive value of sTNF-RII on LVESVI in all patients (adjusted $r^2=0.034$ ; $P<.05$ )			
Log sTNF-RII	-0.004	0.002	.042
C. Linear multiple regression: predictive value of sTNF-RII on LVEDVI in all patients (adjusted $r^2=0.048$ ; $P<.05$ )			
Log sTNF-RII	-0.006	0.002	.019

\*A: dependent variable log PIIINP. The regression analysis included TNF $\alpha$ , sTNF-RI, sTNF-RII, IL-6, IL-10, age, sex, LVEF, E/A, and diabetes as independent variables, of which only sTNF-RII remained in the final model.

B: dependent variable LVESVI. The regression analysis included TNF $\alpha$ , sTNF-RI, sTNF-RII, IL-6, IL-10, age, sex, and PIIINP as independent variables, of which only sTNF-RII remained in the final model.

C: dependent variable LVEDVI. The regression analysis included TNF $\alpha$ , sTNF-RI, sTNF-RII, IL-6, IL-10, age, sex, and PIIINP as independent variables, of which only sTNF-RII remained in the final model.

also wanted to study the involvement of soluble TNF receptors and selected IL-10 to study the involvement of antiinflammatory cytokines, as its capacity to suppress TNF $\alpha$  is known. We found that there were critical differences in the relationships obtained in both groups.

The results showed that the sTNF-RI and sTNF-RII values were higher in the ischemic group than in the dilated cardiomyopathy group. The fact that the LV volumes are higher in patients with dilated cardiomyopathy demonstrates the higher involvement of these cytokines in cases of ischemic cardiomyopathy than that presented by the even higher cytokine levels in plasma. This could be due to the fact that many factors induce the immune response in this entity, such as vascular inflammation, ischemia-related events and necrosis.<sup>27</sup>

In the ischemic group, LVEDVI was negatively associated with IL-10 and sTNF-RII. Procollagen type-III aminoterminal propeptide correlated with sTNF-RII, and plasma values of IL-6, sTNF-RI and sTNF-RII had a negative influence on LVEF. In contrast, when studying these relationships in dilated cardiomyopathy, we found that LVEDVI and LVESVI were related positively with IL-10.

When analyzing the relationship of IL-10 to LV volume, we also noted that IL-10 and sTNF-RII negatively correlated with LVEDVI in the ischemic patient group. However, IL-10 had a direct relationship with LVEDVI and LVESVI in the dilated cardiomyopathy group.

Given that IL-10 is an antiinflammatory cytokine that could suppress the TNF $\alpha$  production induced by monocyte chemotactic protein,<sup>12</sup> we suggest that different mechanisms are involved in the regulation of ventricular remodeling in both groups of patients. Thus, in the ischemic group, the increase in IL-10 can inhibit progression when suppressing the inflammatory response. In dilated cardiomyopathy, left ventricular volume would not increase in response to IL-10, but its increase would be determined by a common activator: TNF $\alpha$ .

In this context, as occurs with IL-10, the fact that the increase in sTNF-RII values is related to a decrease in LVEDVI in the ischemic group reflects a certain capacity of this receptor to suppress myocardial damage by inhibiting the inflammatory response mediated by TNF $\alpha$ <sup>10</sup> at this level, although this pattern does not hold for LVESVI and EF. No significant correlation was found between the geometric parameters and TNF $\alpha$  receptors in the dilated cardiomyopathy group, where they would have less direct involvement.

Multiple regression analysis was done to ascertain how variables such as age, sex, PIIINP, cytokines, and cytokine receptors have an effect on LV volumes as geometric markers of ventricular remodeling in heart

failure. The results showed that, in the population studied, sTNF-RII had greater relevance than the other parameters under study in its relationship to LVESVI and LVEDVI, given that no additional significant explanatory power was found when the variables TNF $\alpha$ , sTNF-RI, IL-6, IL-10, PIIINP, sex, and age were added as independent variables in the model with LVESVI and LVEDVI as dependent variables. These correlations emphasize the direct involvement of sTNF-RII values in ventricular remodeling in heart failure patients.

Regarding the biochemical marker of ventricular remodeling, PIIINP, sTNFRII was involved in matrix turnover in both groups. This fact shows that this receptor has direct involvement in extracellular matrix remodeling in both cardiomyopathy and that an increase in its concentration is related to PIIINP expression. Regarding sTNF-RI, a statistical trend was found in the dilated cardiomyopathy group when we compared its plasma values to PIIINP. We should take into account that both receptors are transmembrane glycoproteins that have similarities in each of their extracellular domains, but which are distinguishable immunologically.<sup>28</sup>

Multiple regression analysis was also done to test how variables such as age, sex and function parameters influence the relationship between the immune factors and PIIINP serum concentrations as a matrix turnover marker in heart failure. The best model was found when sTNF-RII was associated with PIIINP values, and no additional significant explanatory power was found when TNF $\alpha$ , sTNF-RI, IL-6, IL-10, age, sex, LVEF, E/A, and diabetes were added as independent variables in the model with PIIINP as the dependent variable. This fact suggests, once again, that sTNF-RII has direct involvement in extracellular matrix remodeling in heart failure.

Furthermore, IL-6 values also have a direct relation with PIIINP in both myocardopathies, but reach significance in the dilated cardiomyopathy group only, although a strong statistical trend was found in the ischemic group. This result is in keeping with the results published by Puhakka et al.<sup>29</sup>

Finally, LVEF decreases when the values of IL-6, sTNF-RI, and sTNF-RII increase in the ischemic group. It is clear that when systolic dysfunction is present proinflammatory cytokine expression increases, which shows its involvement in this problem.

This fact could indicate the prognostic relevance of cytokine values in this cardiomyopathy. This also implies that there could be an opportunity to improve ventricular function and prognosis in these patients through a therapeutic approach aimed at decreasing the functional activity of these cytokines.<sup>30</sup>

## Study Limitations

It has been reported that assessing ventricular function is more precise when done via magnetic resonance imaging.<sup>31</sup> In our study, LV volumes were measured echocardiographically, which could have introduced more variation during image capture. Nevertheless, the fact that all the examinations were done by a single cardiologist in one center supports the reliability of our results.

A common limitation, in this type of study, is that the patients receive conventional therapy for their disease and it is known that several drugs can reduce plasma concentrations of proinflammatory cytokines and their receptors. However, this study confirms that a high degree of immune activation persists in heart failure patients even during standard therapy.

All the correlations we obtained have been presented in the results section. Some of them are only weakly significant. In this regard, although the immune system's involvement in the process seems beyond question, the degree of correlation leaves room for doubt, given the level of significance.

As already mentioned in the Discussion, we took the ventricular volumes of our patients as a surrogate for the change in LV volume occurring during the ventricular remodeling process. We believe that the strong increase seen in LVESVI and LVEDVI compared to normal values suggests that the relationship of these parameters with cytokine concentrations has the same physiopathological and statistical meaning as that presented by the increase in volume measured at the time of evolution.

## CONCLUSIONS

Proinflammatory cytokines interact with the extracellular matrix in heart failure.

The involvement of the immune system differs depending on the etiology. In general, proinflammatory cytokines, TNF receptors and also PIIINP values are higher in ischemic patients than in those with dilated cardiomyopathy. There was a stronger increase in LV volume in the ischemic cardiomyopathy group. The results of the multiple regression analysis showed that sTNF-RII is an independent prognostic factor for ventricular remodeling, measured biochemically and geometrically, in both ischemic and dilated etiologies.

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