

Interleukin-4 and Cardiac Fibrosis in Patients With Heart Failure

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Interleukin-4 (IL-4) stimulates inflammatory responses, activates collagen synthesis, promotes fibrosis progression, and inhibits the production of inflammatory cytokines. We studied the relationship between the urinary IL-4 level and levels of markers of cardiac fibrosis and left ventricular volume in 98 patients with heart failure (HF). The left ventricular end-systolic volume index (LVESVI) and left ventricular end-diastolic volume index (LVEDVI) were calculated, and IL-4, tumor necrosis factor-alpha (TNF-alpha), IL-6, and aminoterminal propeptide of procollagen type III (PIIINP) levels were recorded.

Comparison of urinary IL-4 and PIIINP levels in patients and control subjects gave values of 12 (12) pg/mL and 4 (3) pg/mL ($P < .0001$), respectively, and 5 (2) ng/mL and 4 (1) ng/mL ($P < .0001$), respectively. The IL-4 level correlated with LVESVI and LVEDVI ($r = -0.22$, $P < .05$), and with PIIINP ($r = 0.24$, $P < .05$). In patients with hypertensive cardiomyopathy, there was a good correlation between IL-4 and PIIINP levels ($r = 0.7$, $P < .01$). Correlations were also observed between IL-4 and TNF-alpha ($r = 0.3$, $P < .01$) and IL-6 ($r = 0.5$, $P < .0001$).

The urinary IL-4 level correlated with cardiac fibrosis and remodeling in patients with HF. The relationship was stronger in those with hypertensive cardiomyopathy.

Key words: Interleukin-4. Heart failure. Fibrosis.

Interleucina-4 y fibrosis miocárdica en pacientes con insuficiencia cardíaca

La interleucina 4 (IL-4) estimula la respuesta inflamatoria, activa la síntesis de colágeno, promueve la progresión de fibrosis e inhibe la producción de citocinas inflamatorias. Estudiamos las concentraciones urinarias de IL-4, su relación con los marcadores de fibrosis miocárdica y con los volúmenes del ventrículo izquierdo en 98 pacientes con insuficiencia cardíaca. Calculamos el índice de volumen telesistólico (IVTS) y telediastólico (IVTD), los valores de IL-4, factor de necrosis tumoral (TNF) alfa, IL-6 y propéptido aminoterminal del procolágeno tipo III (PIIINP).

Comparamos los valores urinarios de IL-4 y PIIINP en pacientes y controles (12 ± 12 frente a 4 ± 3 pg/ml, $p < 0,0001$ y 5 ± 2 frente a 4 ± 1 ng/ml, $p < 0,0001$). La IL-4 se correlacionó con IVTS e IVTD ($r = -0,22$; $p < 0,05$) y con PIIINP ($r = 0,24$; $p < 0,05$). En la cardiopatía hipertensiva encontramos una correlación entre IL-4 y PIIINP ($r = 0,7$; $p < 0,01$). Correlacionamos la IL-4 con TNF- α y la IL-6, obteniendo $r = 0,3$, $p < 0,01$ y $r = 0,5$, $p < 0,0001$.

La IL-4 en orina se relaciona con la fibrosis miocárdica y el remodelado en la insuficiencia cardíaca. La relación es mayor en la cardiopatía hipertensiva.

Palabras clave: Interleucina-4. Insuficiencia cardíaca. Fibrosis.

INTRODUCTION

Chronic heart failure (CHF) is a functional disorder which involves activation of neurohormone and immunologic systems.^{1,2} Interleukin 4 (IL-4) is a TH2 type anti-inflammatory and profibrosis cytokine³ that stimulates and amplifies the inflammatory response by

activation of the synthesis of types I and II collagen by fibroblasts and the promotion of the progression of fibrosis. IL-4 also inhibits the proinflammatory response of tumor necrosis factor alpha (TNF- α), IL-1, and IL-6.⁴ Amino-terminal propeptide of type III procollagen (PIIINP) is an indicator of the synthesis and degradation of collagen.⁵

Studies have recently been published calculating the values of IL-4 in blood and urine and the role of its plasma concentration in various diseases.⁶ However, the relationship between its concentration in urine and parameters of cardiac function and metabolism have not yet been examined.

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Our hypothesis was that urinary concentrations of IL-4 may be associated with the myocardial metabolism of collagen, with other markers of the immunologic action and with ventricular remodeling in patients with CHF. We therefore related IL-4 concentrations in urine with the values of PIIINP, TNF- α and IL-6, as well as with the left ventricular end-systolic volume index (LVESVI) and the left ventricular end-diastolic volume index (LVEDVI).

METHODS

We examined 98 patients with CHF and 18 controls from hospital out-patient offices who had a normal electrocardiogram, Doppler echocardiographic study, blood counts and laboratory tests. The etiological diagnoses were: ischemic heart disease (46%), dilated cardiomyopathy (41%), and hypertensive cardiomyopathy (13%). Diuretics were being taken by 79%, 69% were being treated with angiotensin-converting enzyme (ACE) inhibitors, 60% with β -blockers, 49% with aldosterone antagonists, 24% with digoxin, 15% with calcium antagonists, and 16% with angiotensin II receptor antagonists (ARA-II). The study was undertaken in accordance with the codes of good general practice and the ethical norms for experimentation in human subjects established by the Declaration of Helsinki.

A Doppler echocardiographic examination was given. The echocardiographic recordings and the Doppler tracings were recorded on video tapes for later centralized analysis (Eco-Dat, Software de Medicina S.A.). The area-length method was used to calculate the LVESVI (mL/m²) and the LVEDVI (mL/m²), and to calculate the ejection fraction (EF).

The urinary samples of IL-4, IL-6 and TNF- α were measured in duplicate by a specific commercial enzyme immunoassay ELISA (R&D Systems, Minneapolis, MN). The plasma samples of PIIINP were measured in duplicate using a competitive radioimmunoanalysis assay RIA (Orion Diagnostica, Espoo, Finland).

The data for the quantitative variables are expressed as the mean (standard deviation). The normality of the variables was assessed by the Kolmogorov-Smirnov test. The Mann-Whitney U test was used to compare the values of IL-4 and PIIINP between the patients and the controls. Spearman correlation was used to relate the variables with a non-normal distribution. The threshold for statistical significance was set at $P < .05$. The calculations were performed using the statistical program SPSS 11.5 (SPSS Inc., Chicago, IL).

RESULTS

Table 1 shows the characteristics of the patients population. The mean IL-4 value for the whole group of patients was 12 (12) pg/mL. The mean values were 5 (2) ng/mL for PIIINP, 80 (50) mL/m² for the LVESVI, and 119 (59) mL/m² for the LVEDVI.

TABLE 1. Characteristics of the Patients Population*

Variables	Patients (n=98)
Sex, male, %	70
Age, mean (SD), y	65 (13)
SBP, mean (SD), mm Hg	130 (22)
Heart rate, mean (SD), b.p.m.	74 (12)
NYHA, %	
I	9
II	70
III	21
EF, mean (SD), %	36 (11)
TNF- α , mean (SD), pg/mL	3.1 (0.8)
IL-6, mean (SD), pg/mL	8.3 (6.6)
IL-4, mean (SD), pg/mL	12 (12)
PIIINP, mean (SD), ng/mL	5 (2)
LVESVI, mean (SD), mL/m ²	80 (50)
LVEDVI, mean (SD), mL/m ²	119 (59)

*EF indicates ejection fraction; IL-4, interleukin 4 in urine; IL-6, interleukin 6 in urine; LVEDVI, left ventricular end-diastolic volume index; LVESVI, left ventricular end-systolic volume index; NYHA, New York Heart Association; SBP, systolic blood pressure; PIIINP, amino-terminal propeptide of type III procollagen; TNF- α , tumor necrosis factor alpha in urine. The data are expressed as the mean (standard deviation).

Comparison of the IL-4 values between the patients and controls showed a significantly greater difference in the CHF patients (12 [12] vs 4 [3] pg/mL; $P < .0001$). Comparison of the PIIINP values between the patients and controls showed higher levels in the patient group (5 [2] vs 4 [1] ng/mL; $P < .0001$).

We also divided the patients into 3 etiological groups to compare the IL-4 and PIIINP values and the LVESVI and LVEDVI values (Table 2).

In the patient group, IL-4 correlated with LVESVI and LVEDVI ($r = -0.22$; $P < .05$, for both) (Figure 1) and with PIIINP ($r = 0.24$; $P < .05$). In the group with a hypertensive etiology we found a correlation between IL-4 and PIIINP, $r = 0.7$ and $P < .01$ (Figure 2). Correlations were also found between IL-4 and the urinary concentrations of TNF- α and IL-6 (Figure 3), with $r = 0.3$, $P < .01$ and $r = 0.5$, $P < .0001$, respectively.

DISCUSSION

Cytokines play an important role in the induced immunologic inflammatory response. They are secreted in response to various inducing stimuli, including those caused by microorganisms as well as mechanical stimuli, such as volume or pressure overload.⁷

IL-4 is an anti-inflammatory cytokine that inhibits production of proinflammatory cytokines and promotes progression of fibrosis, as it activates the synthesis of types I⁸ and III⁹ collagen. Inflammatory fibrosis is a typical feature of myocarditis and CHF and it is characterized by overproduction and deposition of collagen by the fibroblasts. PIIINP is a marker of fibrosis and its serum concentrations reflect the process of collagen synthesis and degradation.⁵

TABLE 2. Characteristics of the Patients in the 3 Etiologic Groups and the Control Group*

Variables	Control Group (n=18)	Ischemic Heart Disease (n=45)	Dilated Cardiomyopathy (n=40)	Hypertensive Cardiomyopathy (n=13)	P
LVESVI, mean (SD), mL/m ²	25 (7)	70 (30)	95 (50)	<.001	
LVEDVI, mean (SD), mL/m ²	69 (13)	106 (35)	137 (57)	88 (33)	<.001
IL-4, mean (SD), pg/mL	4 (3)	13.4 (12.3)	8.8 (8.7)	18.1 (15.9)	.1
PIIINP, mean (SD), ng/mL	4 (1)	4.9 (1.5)	4.9 (2.2)	5.5 (1.9)	<.05

*IL-4 indicates interleukin 4 in urine; LVEDVI, left ventricular end-diastolic volume index; LVESVI, left ventricular end-systolic volume index; PIIINP, amino-terminal propeptide of type III procollagen.

Figure 1. Correlation between IL-4 and LVESVI for the whole group of patients with chronic heart failure. IL-4 indicates interleukin 4; LVESVI, left ventricular end-systolic volume index.

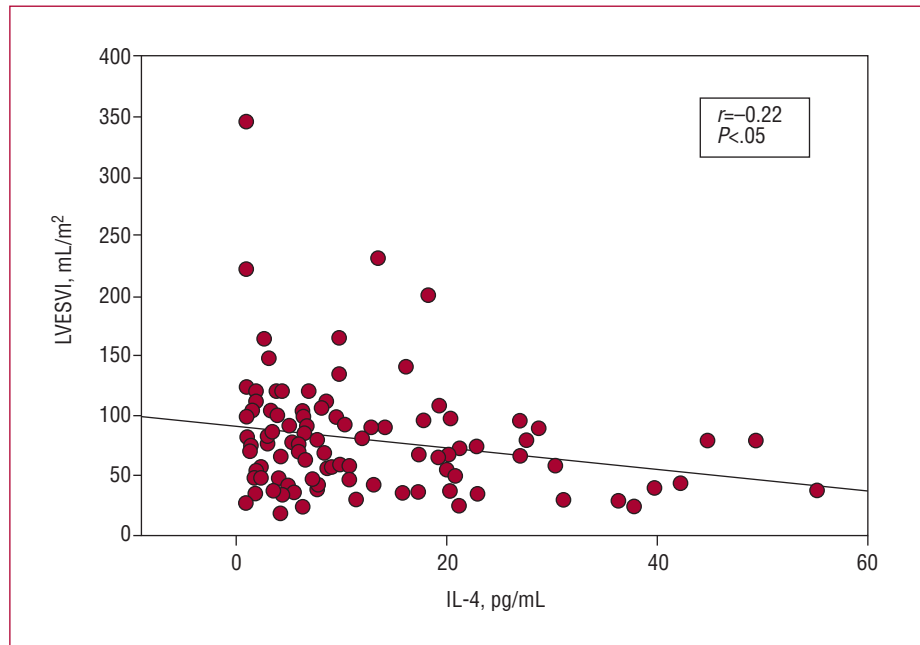
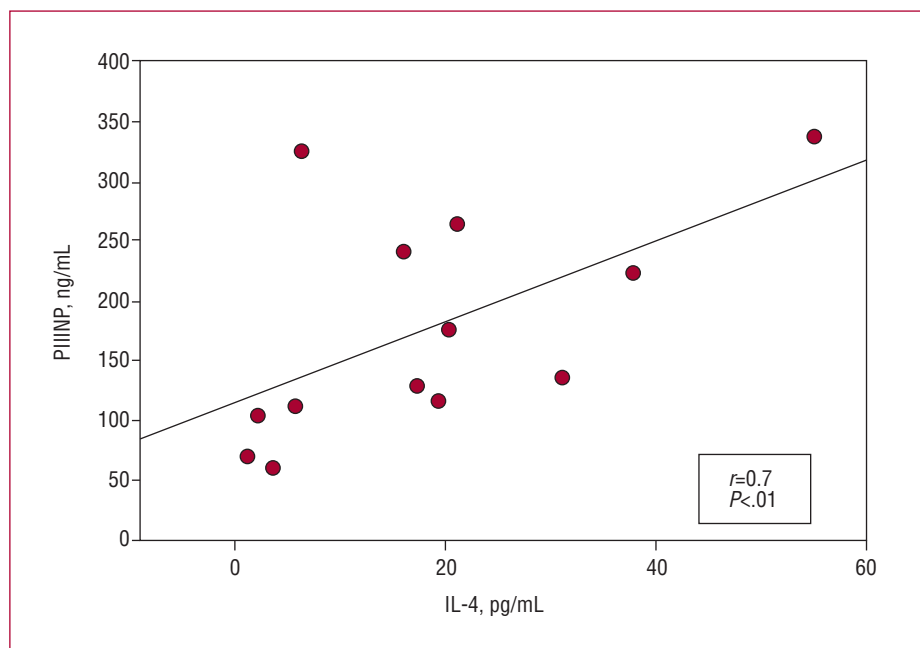


Figure 2. Correlation between IL-4 and PIIINP for the group of patients with hypertensive cardiomyopathy. IL-4 indicates interleukin 4; PIIINP, amino-terminal propeptide of type III procollagen.



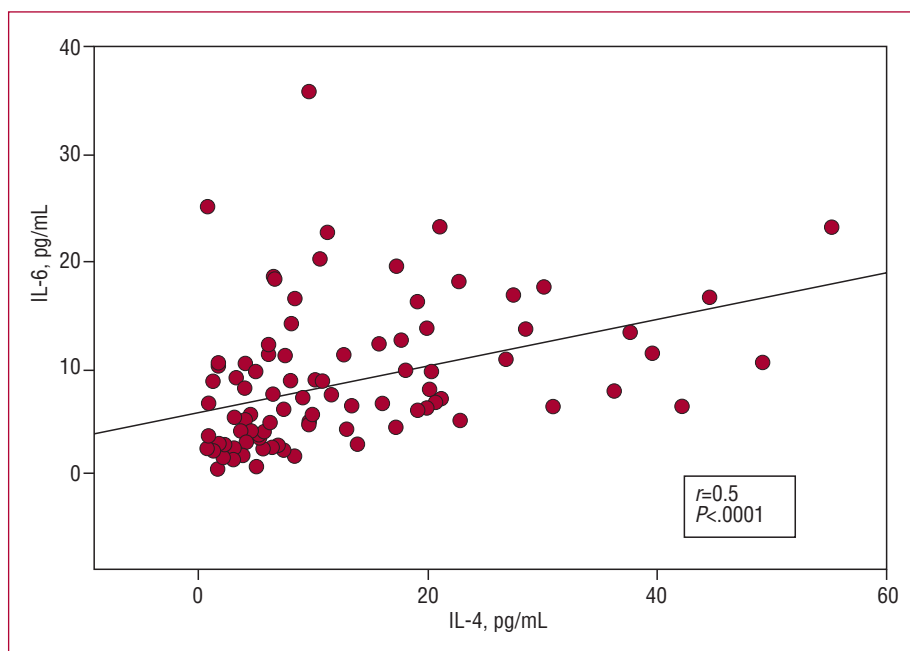


Figure 3. Correlation between urinary IL-4 and IL-6 for the whole group of patients with chronic heart failure.

Our study group found that the patients with CHF had higher IL-4 and PIIINP values than the controls. We also found that the patients with hypertensive cardiomyopathy had higher concentrations of IL-4 and PIIINP. This latter finding has also been reported with markers of oxidative stress, a process linked to worsening of patients with CHF.¹⁰

The IL-4 correlated significantly with PIIINP in the whole group of CHF patients. This correlation was even more evident in the subgroup with hypertensive cardiomyopathy, probably related with the great profibrotic activity in these patients.¹¹ In addition, we also found a negative correlation between IL-4, an anti-inflammatory cytokine, and LVESVI and LVEDVI, probably due to its anti-inflammatory action (inhibition of TNF- α , IL-6, and IL-1).⁴

Furthermore, our studies showed that IL-4 (anti-inflammatory) was highly and significantly correlated with other proinflammatory cytokines, which emphasizes the involvement of this protein in the development of the immunologic cascade that accompanies CHF.

In conclusion, this study demonstrates the involvement of IL-4 in the metabolism of myocardial collagen (PIINP) in patients with CHF. This involvement was even more obvious in the patients with hypertensive cardiomyopathy. The urinary concentrations of IL-4 were correlated with those of TNF- α and IL-6.

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