

# Impaired Endothelium-Dependent Forearm Vasodilation in Idiopathic Dilated Cardiomyopathy Is Related to Severe Left Ventricular Dysfunction and Elevated Serum Tumor Necrosis Factor Levels

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**Introduction and objectives.** Endothelial dysfunction has been found in patients with idiopathic dilated cardiomyopathy (IDC), but its mechanism remains unknown. Our aim was to investigate whether forearm endothelium-dependent vasoreactivity correlates with cardiac disease severity or neurohormonal activation.

**Patients and method.** We studied 23 patients with IDC and 10 healthy sex- and age-matched controls using brachial artery ultrasound to assess flow-mediated dilation (FMD) and nitroglycerin-induced vasodilation (NIV). In the IDC group, we determined plasma neurohormone and cytokine levels at the same time.

**Results.** FMD was significantly less in the IDC group compared with the control group [ $-0.06$  (2.8)% vs 4.4 (4.6)%, respectively;  $P < .01$ ], whereas NIV was similar in both groups [15.0 (6.4)% vs 14.0 (7.4)%, respectively;  $P = NS$ ]. FMD was significantly less in patients with poorer left ventricular (LV) function and more severe LV dilatation, and in those with a higher tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) level. NIV was similar in all patient subgroups. There was a significant inverse correlation between the TNF- $\alpha$  plasma level and FMD ( $r = -0.75$ ;  $P < .01$ ). No correlation was found between the plasma levels of other neurohormones and FMD.

**Conclusions.** FMD, but not NIV, was impaired in patients with IDC compared with control subjects. In patients, there were significant associations between FMD impairment and the severity of LV dilatation, the severity of LV systolic dysfunction, and the plasma TNF- $\alpha$  level. The strongest correlation was observed between TNF- $\alpha$  plasma level and FMD. These data suggest that TNF- $\alpha$  may be implicated in endothelial dysfunction in patients with IDC.

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**La disfunción endotelial periférica en la miocardiopatía dilatada idiopática se asocia con mayor disfunción ventricular y concentraciones plasmáticas elevadas de factor de necrosis tumoral**

**Introducción y objetivos.** La miocardiopatía dilatada idiopática (MCDI) se asocia con disfunción endotelial, aunque se desconoce el mecanismo que la produce. Nuestro objetivo fue estudiar si la vasodilatación dependiente del endotelio (VED) analizada en la arteria humeral se correlaciona con la severidad de la insuficiencia cardíaca o el grado de activación neurohormonal.

**Pacientes y método.** Se estudió a 23 pacientes con MCDI y a 10 sujetos sanos de edad y sexo similares. La VED y la vasodilatación secundaria a nitroglicerina (VD-NTG) se analizaron mediante eco-Doppler de la arteria humeral. También se determinaron las concentraciones de neurohormonas y citocinas en los pacientes con MCDI.

**Resultados.** En los pacientes con MCDI se observó una reducción de la VED en comparación con el grupo control ( $-0.06 \pm 2.8$  frente a  $4.4 \pm 4.6\%$ , respectivamente,  $p < 0.01$ ), mientras que la VD-NTG fue similar en ambos grupos ( $15.0 \pm 6.4$  frente a  $14.0 \pm 7.4\%$ , respectivamente;  $p = NS$ ). La VED fue significativamente menor en los pacientes con peor función ventricular y mayor dilatación ventricular, y también en los que presentaban concentraciones de factor de necrosis tumoral (TNF- $\alpha$ ) más elevadas. No se observaron diferencias significativas en cuanto a la VD-NTG entre los diferentes subgrupos. Se observó una correlación inversa significativa entre los valores plasmáticos de TNF- $\alpha$  y la VED ( $r = -0.75$ ;  $p < 0.01$ ).

**Conclusiones.** En comparación con el grupo control, los pacientes con MCDI tienen una reducción de la VED y conservan la VD-NTG. La disfunción ventricular severa, el mayor grado de dilatación ventricular y las concentraciones plasmáticas elevadas de TNF- $\alpha$  se asocian con una peor VED, pero la mayor correlación se observó en-

## ABBREVIATIONS

ED: endothelial function.  
 IDC: idiopathic dilated cardiomyopathy.  
 TNF: tumor necrosis factor.  
 HF: heart failure.  
 IL-6: interleukin-6.  
 IL-2R: interleukin-2 receptor.  
 NIV: nitroglycerin-induced vasodilation.  
 FMD: flow-mediated dilation.  
 LV: left ventricle.

tre los valores de TNF- $\alpha$  y la VED. Estos datos sugieren que el TNF- $\alpha$  puede estar implicado en la aparición de disfunción endotelial en la MCDI.

**Palabras clave:** *Miocardiopatía dilatada. Disfunción endotelial. Factor de necrosis tumoral.*

## INTRODUCTION

Endothelial dysfunction (ED) has been reported in patients with heart failure (HF) secondary to idiopathic dilated cardiomyopathy (IDC) both at the coronary and peripheral circulation.<sup>1</sup> Whether this finding is a primary phenomenon in the pathogenesis of IDC or a consequence of the HF syndrome, is an ongoing matter of controversy and indeed, the exact mechanisms leading to ED are not well established yet.<sup>2,3</sup> Nevertheless, ED is at least in part responsible for the impaired exercise capacity of patients with this syndrome and therefore, its reversion may lead to improvement in symptoms and exercise performance.<sup>4</sup> Reduced shear-stress due to decreased cardiac-output, neurohormonal imbalance and inflammatory cytokines have been all involved in the pathogenesis of ED in patients with IDC.<sup>2</sup> We sought to analyze the role of each of these factors in the impairment of ED in patients with HF secondary to IDC.

## METHODS

### Study Population

A total of 23 patients with HF secondary to IDC and 10 age and sex-matched healthy volunteers were included in the study. IDC was diagnosed by the presence of left ventricular (LV) dilation (LV end-diastolic diameter >60 mm) and systolic dysfunction (LV ejection fraction <40%) on echocardiography in the absence of coronary artery disease (normal coronary angiogram), hypertension or valvular disease. HF had

been present for a mean of 21 $\pm$ 22 months with a range of 1 to 84 months; in the 8 patients presenting with HF symptoms for less than 6 months, viral serologies did not diagnose an acute myocarditis. Exclusion criteria were history of myocardial infarction, diabetes mellitus, cancer, concomitant infections at the time of the study, renal failure, or autoimmune diseases. In addition, patients with a past or present story of alcohol intake >60 g/day were excluded. Two-dimensional echocardiography was performed in all IDC patients using a commercially available system (Sonos 5500, Agilent Technologies, Philips, Germany) to measure LV diameters and ejection fraction (Simpson's biplanar method) following the recommendations of the American Society of Echocardiography.<sup>5</sup> Normal volunteers were recruited among hospital staff and relatives with a strictly normal medical record and without vascular risk factors according to clinical anamnesis.

### Endothelial Function Studies

In all IDC patients and normal volunteers, endothelial function was studied using high-resolution ultrasound of the brachial artery as described extensively elsewhere.<sup>6</sup> In brief, a longitudinal section of the right brachial artery was scanned with a vascular probe connected to the ultrasound machine. In order to achieve a steady image throughout the whole study, the probe was fixed with a mechanical clamp, and the sample volume of the pulsed wave Doppler was placed in the middle of the arterial lumen as a reference marker. After a clear image was obtained, a baseline scan was recorded. Endothelium-dependent vasodilation was assessed by analysis of the brachial artery diameter changes in response to an increase in flow. Reactive hyperemia was achieved by the rapid release of a pneumatic pressure cuff placed around the forearm, which was inflated up to 300 mm Hg during 4.5 minutes. The pulsed wave Doppler signal of the brachial artery flow and bidimensional images were recorded 55-65 seconds after cuff release. After a 10-15 minutes rest to allow vessel recovery, a second baseline scan was obtained. To assess endothelium-independent vasodilation, 400 Hg of sublingual nitroglycerine were administered and a fourth scan was obtained 3 minutes later. All vasodilator drugs were withheld for at least 3 times their half-time period before the vascular studies.

Images were analyzed by two independent observers blinded to the results of blood assays. Arterial diameter was measured from two-dimensional echographic images at the peak of the R wave of the ECG, placing calipers from the trailing edge of the anterior wall interface to the leading edge of the posterior wall interface. Flow-mediated vasodilation (FMD) was used as an index of endothelium-dependent vasodila-

tion and was calculated as the percent change in brachial artery mean diameter after reactive hyperemia over that obtained at baseline. Reactive hyperemia was calculated as the relative ratio of maximal flow after cuff release to that measured at baseline. Vasodilation induced by sublingual nitroglycerine (NTG-VD) was used as an index of endothelium-independent vasodilation and was calculated as the percent change in brachial artery diameter after nitroglycerine administration from the second baseline scan. Using this methodology and a nested-analysis of variance, inter-observer and intraobserver variance for brachial artery diameter measurement was 0.00012 (0.02% of total variability) and 0.00075 (0.13% of total variability), respectively.

**Determinations of Cytokines and Neurohormones**

Plasma levels of cytokines, renin activity, angiotensin II, aldosterone, and norepinephrine were also determined in IDC patients after they had fasten for an overnight and rested for 1 hour in the supine position. Concentrations of interleukin-6 (IL-6), interleukin-2 receptor (IL-2R) and tumor necrosis factor (TNF- $\alpha$ ) were measured using commercial immunoenzyme assay kits (Medgenix, Fleurus, Belgium) following the manufacturer’s specifications. The average of two measurements is reported. Reference values at our laboratory of normal plasma values of IL-6, TNF- $\alpha$ , and IL-2R were obtained from tests in a population of 75 healthy control subjects. Normal values of IL-6, TNF- $\alpha$ , and IL-2R (IL-6<5 pg/mL, TNF- $\alpha$ <20 pg/mL, IL-2R<80 pmol/mL) correspond to the 98th percentile of this healthy control group. The coefficient of variability at our laboratory was 7.83% for TNF- $\alpha$  (mean value, 47 $\pm$ 3; n=16), 5.35% for IL-6 (mean value, 73 $\pm$ 4; n=15) and 4.31% for IL-2R (mean value, 88 $\pm$ 4; n=10). Norepinephrine was determined in blood samples by high-performance liquid chromatography with electrochemical detection (normal value, 253 $\pm$ 114 pg/mL). Plasma renin activity, angiotensin II and aldosterone were measured by radioimmunoassay (normal values, 1.4 $\pm$ 0.9 ng/mL/h, <15 pg/mL, and <30 ng/mL, respectively). All tests were performed at the outpatient clinic with the patient in stable condition and all patients gave their informed consent for the study.

**Statistical Analysis**

Discrete variables are presented as percentages and analyzed using the chi-square test. Continuous variables are expressed as mean value  $\pm$ SD and compared using an unpaired two-tailed *t* test. Correlation between FMD and plasma levels of cytokines and neurohormones was analyzed and expressed with the Pear-

son’s correlation coefficient *r*. Linear regression (backward method, *P* for entry =.05, *P* for elimination =.10) was used to evaluate which variables were predictive of FMD. A *P*-value <.05 was considered statistically significant.

**RESULTS**

**Patient Characteristics**

We studied a total of 23 patients with IDC (mean age, 57.2 $\pm$ 11.3; 8 [35%] women) and 10 healthy control volunteers (mean age, 49.2 $\pm$ 13.6; 5 [50%] women; *P*=NS vs IDC patients for both age and sex). Mean time from diagnosis of IDC was 21.5 $\pm$ 22.8 months (range, 1-84 months). In the 8 (34%) patients presenting with symptoms for less than 6 months, serologies were non-diagnostic for myocarditis. Fourteen (61%) patients with IDC were on NYHA functional class II and 9 (39%) on class III. Echocardiographic and laboratory assay results in IDC patients are shown in Table 1. All patients were in clinically stable condition and treated in the out-patient clinic. Medical therapy included angiotensin converting enzyme inhibitors in all of them (20 [85%] patients enalapril and 3 [15%] captopril) and beta-blockers in 8 (35%) patients (carvedilol). None of the studied patients was taking lipid lowering therapy at the time of the study or had smoked for the last 5 years.

**Endothelial Function in IDC**

Table 2 shows the results of endothelial function studies in the IDC and control groups. FMD was significantly impaired in the IDC group as compared to the control group, while NTG-VD was not different in the 2 groups. There were no differences between the 2 groups in baseline brachial artery diameter nor in reactive hyperemia.

**TABLE 1. Echocardiographic, Inflammatory, and Neurohormonal Characteristics of the Idiopathic Dilated Cardiomyopathy Patients\***

LVEDD, mm	70.5 $\pm$ 7.0
LVESD, mm	58.7 $\pm$ 7.6
Ejection Fraction LV, %	24.8 $\pm$ 6.6
Norepinephrine, pg/mL	325.4 $\pm$ 217.4
Angiotensin II, pg/mL	35.04 $\pm$ 31.9
Plasma renin activity, ng/mL/h	3.3 $\pm$ 3.8
Aldosterone, ng/mL	19.3 $\pm$ 13.5
TNF- $\alpha$ , pg/mL	32.2 $\pm$ 20.3
Interleukin-6, pg/mL	36.8 $\pm$ 84.2
Interleukin-2 receptor, pmol/mL	73.0 $\pm$ 39.3

\*LV indicates left ventricular; TNF, tumor necrosis factor.

**TABLE 2. Brachial Endothelial Function in Idiopathic Dilated Cardiomyopathy Patients and Normal Healthy Volunteers\***

	IDC (n=23)	Healthy Volunteers (n=10)	P
Baseline brachial artery diameter, mm	4.10±0.79	4.59±0.60	NS
Reactive hyperemia, %	414±163	326±152	NS
Flow-mediated dilation, %	-0.06±2.8	4.4±4.6	<.01
NTG-VD, %	15.0±6.4	14.0±7.4	NS

\*NTG-VD indicates nitroglycerine-mediated vasodilation.

**Endothelial Function and Severity of IDC**

We did not observe any correlation between time from onset of LV dysfunction or symptoms and ED nor between functional class and ED. In the IDC group, patients with larger LV end-diastolic diameter (≥70 mm) (n=13), poorer LV ejection fraction (≤25%) (n=14), and elevated plasma levels of TNF-α (>20 pg/mL) (n=16) had significantly more impaired FMD (Figure 1A); no significant differences were observed in NTG-VD between these groups (Figure 1B). Patients with elevated and normal values of TNF-α had similar baseline brachial artery diameter (4.64±0.54 vs 4.49±0.77 respectively; P=NS). Additionally, plasma levels of TNF-α were significantly and inversely correlated with FMD (r=-.75; P<.001) (Figure 2). There was also a significant but weaker correlation between

LV end-diastolic diameter and FMD (r=0.43; P=.04) and between LV ejection fraction and FMD (r=0.50; P=.01). On the other hand, there was no correlation between FMD and IL-6 or IL-2R plasma levels or neurohormonal activation (Table 3).

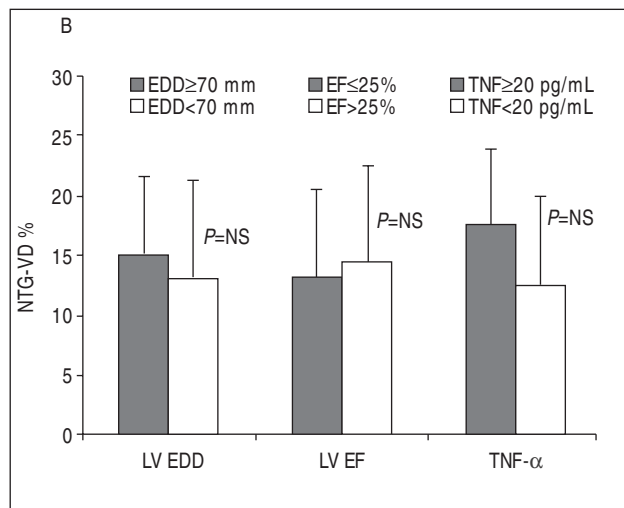
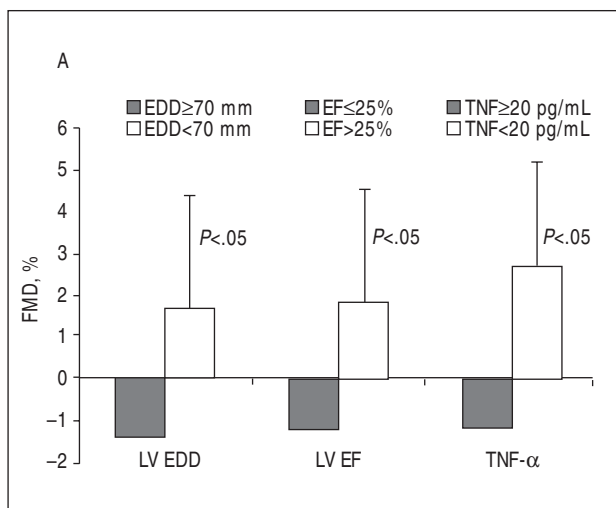
Linear regression analyses showed that both LV end-diastolic diameter and plasma levels of TNF-α, but not LV ejection fraction, were independent predictors of FMD (β=-.19; P=.027, and β=-.06; P=.013, and P>.05, respectively) in this group of IDC patients.

**DISCUSSION**

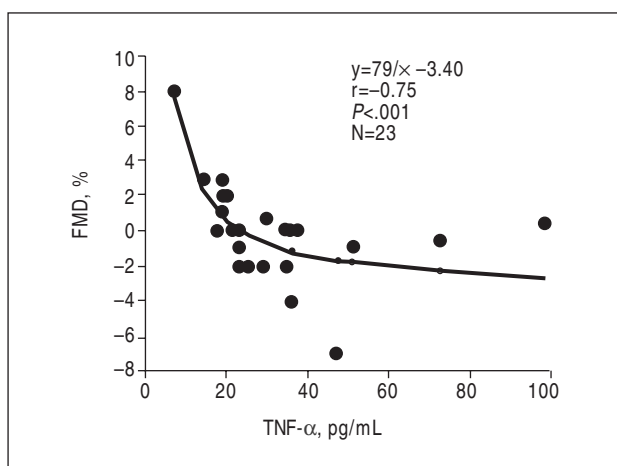
In the present study, we found an impaired FMD in patients with IDC as compared to age and sex-matched control subjects. In patients with IDC, FMD was significantly more impaired in those with higher plasma levels of TNF, poorer LV systolic function and larger LV. The strongest correlation was found between TNF-α plasma levels and FMD, being FMD worse in patients with higher TNF levels. However, both TNF-α plasma levels and LV end-diastolic diameter were determinants of FMD.

**Endothelial Dysfunction in Heart Failure**

ED with attenuated vasodilation in response to acetylcholine and reduced vasodilation in response to hyperemia has been previously demonstrated in patients with chronic heart failure. The reduction in NO release by the endothelium in the peripheral and coronary arteries translates into an increased peripheral vascular resistance, an increased impedance of the failing LV and a re-



**Figure 1A and B.** Flow-mediated dilation (FMD) (Figure 1A) and nitroglycerine-mediated vasodilation (NTG-VD) (Figure 1B) values in groups from the IDC population: in patients with left ventricular end-diastolic diameters ≥70 mm (black column) and <70 mm (white column), in patients with left ventricular ejection fraction ≤25% (black column) and >25% (white column) and in patients with TNF-α plasma levels ≥20 pg/mL (black column) and <20 pg/mL (white column). EDD indicates end-diastolic diameter; EF, ejection fraction; TNF, tumor necrosis factor.



**Figure 2.** Relationship between tumor necrosis  $\alpha$  plasma levels and forearm flow-mediated dilation (FMD) in patients with idiopathic dilated cardiomyopathy.

duced myocardial perfusion, all contributing to reduced myocardial function.<sup>1-4</sup> Additionally, ED may affect the cardiovascular system in another way by impairing peripheral perfusion and reducing exercise capacity.<sup>7</sup>

We found a marked ED at the peripheral vasculature of patients with IDC as compared to normal age and sex-matched subjects. This finding is similar to those reported before by other groups, but its relationship with inflammatory and neurohormonal activation and with LV dysfunction has been rarely and specifically addressed in patients with HF secondary to IDC.<sup>1-4,8-10</sup> TNF has been implicated in the pathogenesis of IDC and HF.<sup>11-13</sup> It has been experimentally demonstrated that TNF can induce HF in transgenic mice expressing TNF- $\alpha$ .<sup>14</sup> Also, patients with end-stage non-ischemic cardiomyopathy show a particular TNF- $\alpha$  genotype, overexpressing the TNF2 allele as compared to patients with ischemic myocardial dysfunction.<sup>15</sup>

Additionally, TNF has been implicated in the pathogenesis of ED in patients with HF. Short-term administration of TNF- $\alpha$  has been shown to severely reduce endothelium-dependent relaxation in rats.<sup>16</sup> Katz et al found a direct relationship between TNF plasma levels and forearm blood flow response to intraarterial acetylcholine, concluding that increased activation of inducible nitric oxide synthase might be responsible for those findings.<sup>17</sup> However, our results agree with those from Anker et al,<sup>18</sup> who described an inverse correlation between TNF plasma levels and peak blood flow in the limbs of patients with chronic HF.

Although both interleukins and TNF- $\alpha$  plasma levels are elevated in patients with HF, we only found correlation between TNF- $\alpha$  levels and endothelial dysfunction. IL-6 can be synthesized by vascular endothelial and other cells in response to TNF- $\alpha$ , but it does not induce vascular thrombosis or tissue injury as observed

**TABLE 3. Correlation Between Clinical Characteristics, Left Ventricular Dysfunction, Cytokines, Neurohormones, and Flow-Mediated Dilation\***

	R <sup>2</sup>	P
Time from onset of LV dysfunction	0.11	.13
NYHA functional class	0.013	.6
LV end-diastolic diameter	0.18	.04
LV Ejection fraction	0.25	.01
Mitral deceleration time	0.04	.54
IL-6	0.02	.52
IL-2R	0.009	.67
TNF- $\alpha$	0.56	0.01
Plasma renin activity	0.02	.54
Angiotensin II	0.02	.53
Aldosterone	0.10	.15
Norepinephrine	0.01	.65

\*IL-6 indicates interleukin-6; IL-2R, interleukin-2 receptor; LV, left ventricular; TNF, tumor necrosis factor.

in response to TNF- $\alpha$ .<sup>19,20</sup> This suggests that TNF- $\alpha$  may have more direct and potent deleterious effects on the endothelium, explaining why we found a correlation between peripheral ED and TNF- $\alpha$ , but not with IL-6. Although we also found a significant relationship between ED and LV systolic dysfunction and cavity dilation, the strongest correlation was observed between TNF- $\alpha$  plasma levels and FMD. These findings may suggest that the pathogenesis of ED in IDC might be multifactorial, but also support that TNF may be a key cytokine in the development of ED and in the progression of IDC and secondary HF. Additionally, previous work has shown that therapies that improve endothelial function in patients with HF such as exercise training and Beta-blockers, also reduce plasma levels of proinflammatory cytokines, including TNF- $\alpha$ .<sup>21,22</sup>

### Clinical Implications: Reverting Endothelial Dysfunction in Heart Failure

In patients with chronic HF, impaired ED can be improved by regular physical training resulting in a significant increase in exercise capacity.<sup>4</sup> Also, reduction in mortality of patients with chronic HF by angiotensin-converting enzyme and spironolactone may be in part explained by an improvement of endothelial function and subsequent improvement of perfusion and exercise capacity.<sup>23,24</sup>

Recently, it has been demonstrated that administration of two different TNF blockers can improve ED at the forearm circulation in patients with advanced HF<sup>25</sup> and in patients with rheumatoid arthritis.<sup>26</sup> Moreover, the extent of improvement of systemic endothelial function in HF patients was directly correlated with TNF plasma levels.<sup>25</sup> Although no correlation between ED reversion and survival improvement has been es-

tablished in the same group of patients, normalization of endothelial function contributes to clinical improvement by improving exercise capacity and may be in part responsible for reduction in mortality. In this sense, previous work has shown that preservation of coronary endothelial function is associated with an improvement in LVEF.<sup>27</sup>

Initial results of clinical trials with TNF antagonists in patients with advanced HF, have not shown to date a net benefit on outcome.<sup>28-30</sup> The clinical characteristics of the studied population, the doses and pharmacodynamics of the drug (etanercept and infliximab) may be in part responsible for the negative results. We studied patients with HF secondary to IDC, while previous studies included patients with different etiologies of HF. It may be possible that the effect of TNF blockers differs depending on the etiology underlying heart failure. Additionally, although TNF antagonists may have no beneficial effect on outcome, it may be helpful in correcting endothelial dysfunction and in improving exercise capacity and functional class.<sup>4,18,28-30</sup> Alternative approaches for cytokine inhibition are being evaluated and continuous research on this topic should be kept given the existing evidence pointing out their important role in the pathogenesis of heart failure.

### Study Limitations

Unfortunately, we could not determine BNP or O<sub>2</sub> consumption at the time of the study due to unavailability of these tests at our institution at that time. It would have been interesting to have these data in order to better approximate the functional status of these patients. We are aware of the small data set of our study, but we think our findings show a pathophysiologic finding that has been previously suggested using other experimental and clinical models. Finally, the low rate of beta-blockers and specially carvedilol, used in this group of patients may have influenced the presence of severe endothelial dysfunction.

### CONCLUSIONS

The present study suggests a key role of TNF in the development of ED in patients with HF secondary to IDC. The clinical implication of this finding, if confirmed in other studies, might be that TNF- $\alpha$  antagonism may revert peripheral ED as suggested by others,<sup>26,27</sup> and improve exercise tolerance. Nevertheless, more studies are still necessary to prove that ED reversion translates into a survival benefit among IDC patients.

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