

## The Origin of Proinflammatory Cytokines in Patients with Idiopathic Dilated Cardiomyopathy

Proinflammatory cytokines and their receptors are increased in the peripheral blood of patients with heart failure. We measured cytokines and their receptors in systemic artery (SA), coronary sinus (CS) and infra-renal inferior vena cava (IVC), in order to investigate their origin and influential factors. Thirty patients with idiopathic dilated cardiomyopathy were performed echocardiography at admission, and right heart catheterization after stabilization. Blood was drawn from 3 sites for measurement of tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ), interleukin-6 (IL-6) and soluble tumor necrosis factor- $\alpha$  receptor (sTNFR) I, II. TNF- $\alpha$  at CS ( $3.25 \pm 0.34$  pg/mL) was higher than those of SA ( $1.81 \pm 0.39$  pg/mL) and IVC ( $1.88 \pm 0.38$  pg/mL,  $p < 0.05$ ). IL-6 at CS ( $18.3 \pm 3.8$  pg/mL) was higher than that of SA ( $5.8 \pm 1.2$  pg/mL,  $p < 0.01$ ). The levels of sTNFR I, II showed increasing tendency in sequence of SA, IVC and CS. TNF- $\alpha$  and sTNFR I, II from all sites were proportional to worsening of functional classes at admission ( $p < 0.05$ ). E/E<sub>a</sub> by Doppler study at admission, which reflects left ventricular end-diastolic pressure (LVEDP) was positively correlated with TNF- $\alpha$  from SA ( $R = 0.71$ ,  $p < 0.01$ ), CS ( $R = 0.52$ ,  $p < 0.05$ ) and IVC ( $R = 0.46$ ,  $p < 0.05$ ). Thus, elevated LVEDP during decompensation might cause cytokine release from myocardium in patients with idiopathic dilated cardiomyopathy.

Key Words : Heart Failure, Congestive; Cytokines; Myocardial Diseases

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## INTRODUCTION

Previous studies indicated that proinflammatory cytokines such as interleukin (IL)-1, IL-6, tumor necrosis factor (TNF)- $\alpha$  and their receptors are increased in patients with both decompensated heart failure (New York Heart Association (NYHA) functional class III and IV as well as compensated (NYHA functional class II) (1-3). Furthermore, proinflammatory cytokines have been attributed as contributors to the syndrome of congestive heart failure and the underlying cardiomyopathic process of adverse left ventricular remodeling and thus, evolving into progressive left ventricular dysfunction (4-6).

The origin of proinflammatory cytokines in patients with heart failure remains unclear, hence the implication of such proinflammatory cytokines in the pathogenesis of heart failure is still controversial, which was reflected by the failure of multicenter clinical trials that used "targeted" approaches to neutralize TNF- $\alpha$  in patients with moderate to advanced heart failure (7).

Some authors suggested endotoxin may induce cytokine activation via intestinal bacterial translocation (8). However, this hypothesis failed to explain elevated cytokines in patients with compensated heart failure (9). Other studies proposed myocardium itself produce cytokines due to elevated left ventricular diastolic wall stress (10). Tissue hypoxia and free radi-

cal production was also hypothesized to cause NF- $\kappa$ B mediated cytokine production especially from skeletal muscles (11).

In order to determine the cellular source of circulating proinflammatory cytokines and their soluble receptors, we measured the proinflammatory cytokine concentrations in systemic artery, infra-renal inferior vena cava, and coronary sinus and their concentrations were compared with one another to determine the origin of its production. Furthermore, we correlated the proinflammatory cytokine levels with the clinical variables and hemodynamic parameters assessed both at admission and at recovery status.

## MATERIALS AND METHODS

### Study Subjects

We consecutively enrolled 30 patients who were admitted to Ajou University Hospital for congestive heart failure due to idiopathic dilated cardiomyopathy with various functional classes. At admission, we assessed NYHA functional classes, performed routine clinical evaluations and measured echocardiographic parameters. All patients were treated with standard medical therapy depending upon the severity of heart failure. After patients achieved hemodynamic stabilization,

cardiac catheterization was performed for blood sampling at femoral artery as a systemic artery, infra-renal inferior vena cava representative of cytokine production from skeletal muscle and coronary sinus from myocardium, and measurement of hemodynamic variables including pulmonary capillary wedge pressure, mean pulmonary arterial pressure, mean arterial pressure and cardiac index.

Patients with the following criteria were excluded; (1) any identifiable cause of dilated cardiomyopathy such as history of heavy alcohol abuse, severe hypertension, primary valvular disease or history of ischemic heart disease, (2) elevated cardiac enzyme or EKG change compatible to myocarditis, (3) history of infectious disease within recent 4 weeks or elevated acute phase reactants (e.g. ESR, C-Reactive protein), (4) contraindication for cardiac catheterization

We also selected 11 age- and gender-matched control subjects (aged 38 to 60, mean 49 yr, male: 7) who visited Ajou University Hospital for electrophysiologic study. All the control subjects had normal coronary angiogram.

All participants gave written informed consents before entering the study and the study protocol was reviewed by the ethical committee of Ajou University Hospital.

### Blood Sampling

After proper medical therapy, right heart catheterization and coronary angiography were performed. Under fluoroscopic guidance, 7Fr NIH catheter was positioned in the coronary sinus via subclavian vein. Correct catheter position was confirmed with contrast injection and oxygen saturation. Pulmonary capillary wedge pressure, mean pulmonary arterial pressure, mean arterial pressure and cardiac index were measured during blood sampling.

### Cytokine Assay

Blood samples were immediately centrifuged with 5,000

rpm for 15 min at 4°C. Then, serum was separated into aliquots and stored at -70°C for a period not exceeding 6 months. Circulating levels of cytokines and their receptors were measured using commercially available ELISA kits (Quantikine, R & D systems, Minneapolis, MN, U.S.A.). The intra-assay coefficients of variation were as follows; TNF- $\alpha$  (6.0%), sTNFR I (4.7%), sTNFR II (2.4%) and IL-6 (3.5%).

### Statistical Analysis

Statistical analysis was performed with SPSS 8.0 statistical software (SPSS, Inc. Illinois, U.S.A.). All data are presented as mean  $\pm$  SEM. Student's t-test was used for differences in continuous variables and  $\chi^2$  test was used for categorical variables. The differences in cytokine levels by NYHA functional classes were verified using ANOVA. Correlations of cytokine levels with demographic characteristics were measured by Pearson's analysis.  $p < 0.05$  was considered statistically significant.

## RESULTS

### Characteristics of Study Subjects

Among 30 patients enrolled, 21 were male and 9 were female, with age ranging from 32 to 67 yr ( $51.3 \pm 9.3$  yr). Mean left ventricular ejection fraction was  $27.7 \pm 9.5\%$ . Mean duration of symptoms was  $18.5 \pm 1.8$  days before admission (Table 1). Seven patients were classified as NYHA functional class I, 8 as II, 6 as III, and 9 as IV at admission (Table 1). At the time of cardiac catheterization after appropriate medical therapy, all patients with heart failure were compensated and their functional classes had improved (Table 2).

### Circulating Levels of Cytokines according to Site of Origin

Mean values of all the cytokines were at least fivefold higher in the patient group than those in the control group from all the three sampling sites (Fig. 1). We could not measure TNF- $\alpha$  from systemic artery in 9 patients and from inferior

Table 1. Patient characteristics at enrollment

Gender (M/F)		21/9
Age (yr)		$51.3 \pm 9.3$
NYHA Functional class	I	7 (23%)
	II	8 (27%)
	III	6 (20%)
	IV	9 (30%)
Smokers		15 (50%)
Diabetes mellitus		12 (43%)
Hypertension		11 (39%)
Symptom duration (days)		$18.5 \pm 1.8$
Hematocrit (%)		$38 \pm 4.8$
LV Ejection fraction (%)		$27.7 \pm 9.5$
LV End-diastolic Dimension (mm)		$65.7 \pm 8.7$
E/E <sub>a</sub>		$24.4 \pm 6.5$

Data are expressed mean  $\pm$  SD. LV, left ventricle; NYHA, New York Heart Association.

Table 2. Patient characteristics at cardiac catheterization

Duration from enrollment (days)		$4.8 \pm 0.5$
NYHA Functional class	I	11 (37%)
	II	19 (63%)
PCWP (mmHg)		$13.6 \pm 1.8$
MAP (mmHg)		$92.6 \pm 11.2$
MPAP (mmHg)		$23.4 \pm 4.6$
Cardiac Index (L/min/m <sup>2</sup> )		$2.2 \pm 0.1$

Data are expressed mean  $\pm$  SD. MAP, mean arterial pressure; MPAP, mean pulmonary arterial pressure; NYHA, New York Heart Association; PCWP, pulmonary capillary wedge pressure.

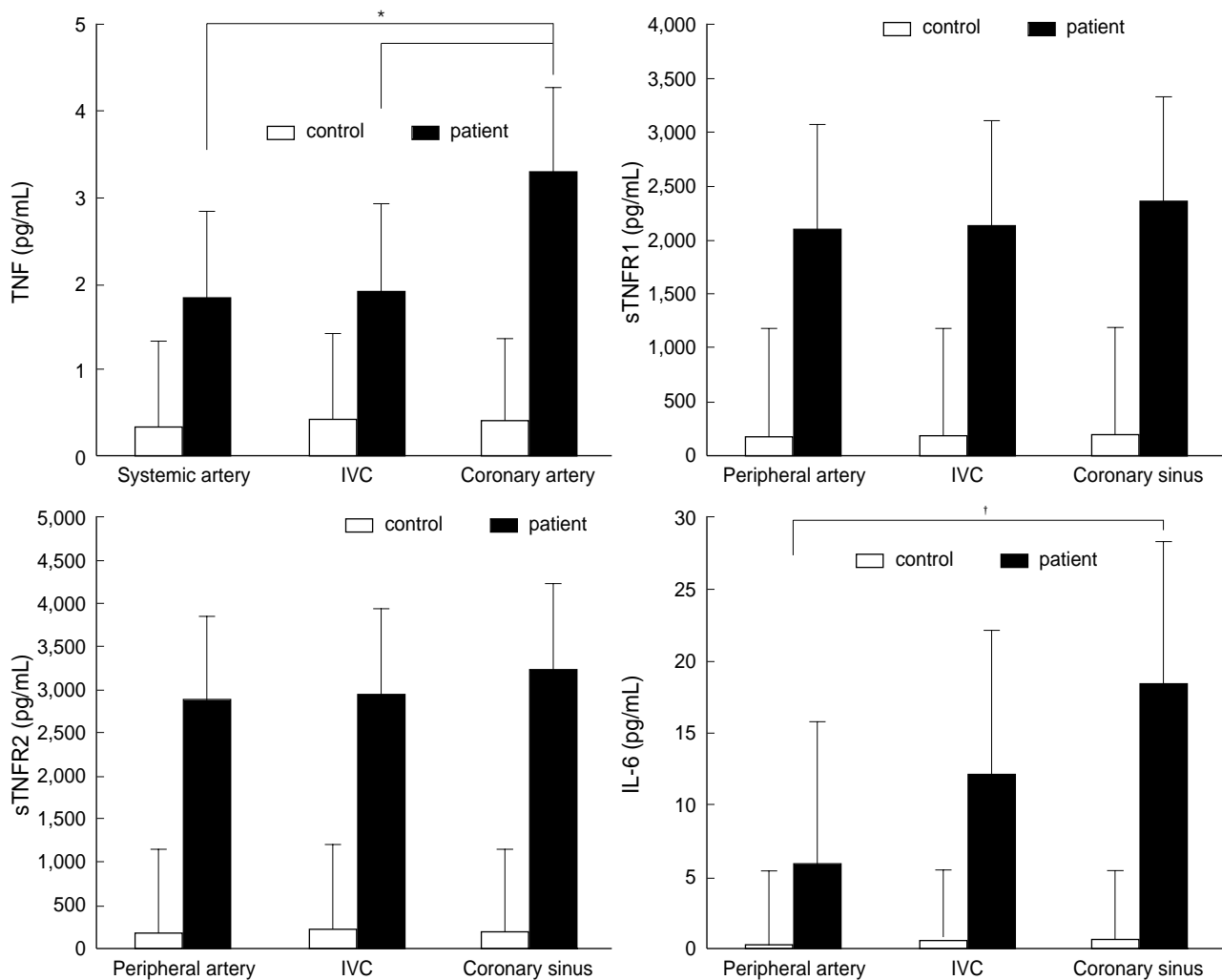


Fig. 1. Levels of proinflammatory cytokines at different sites. Data are expressed mean  $\pm$  SEM. \*:  $p < 0.05$  compared with TNF- $\alpha$  from IVC, †:  $p < 0.01$  compared with IL-6 from systemic artery, IVC: inferior vena cava.

vena cava in 8 patients, due to limited minimal detection level. In contrast, TNF- $\alpha$  at coronary sinus was measurable in all patients.

TNF- $\alpha$  of  $3.25 \pm 0.34$  pg/mL at coronary sinus was higher than that at inferior vena cava ( $1.88 \pm 0.38$  pg/mL,  $p < 0.05$ ) and systemic artery ( $1.81 \pm 0.39$  pg/mL) (Fig. 1). The levels of sTNFR I and II had a tendency to increase in order of systemic artery, inferior vena cava and coronary sinus without statistical significance, i.e., sTNFR I in systemic artery,  $2,088 \pm 212$  pg/mL; inferior vena cava,  $2,125 \pm 216$  pg/mL; coronary sinus,  $2,368 \pm 220$  pg/mL ( $p = \text{NS}$ ) and sTNFR II in systemic artery,  $2,880 \pm 306$  pg/mL; inferior vena cava,  $2,945 \pm 301$  pg/mL; coronary sinus,  $3,242 \pm 316$  pg/mL ( $p = \text{NS}$ ) (Fig. 1). However, the levels of IL-6 increased significantly in the same order (systemic artery,  $5.78 \pm 1.23$  pg/mL; inferior vena cava,  $12.1 \pm 2.7$  pg/mL; coronary sinus,  $18.3 \pm 3.84$  pg/mL,  $p < 0.01$ ) (Fig. 1). Significant differences were detected in the levels of IL-6 between coronary sinus and systemic artery ( $p < 0.01$ ), but not between coronary sinus

and inferior vena cava (Fig. 1).

Significant correlations were noted between TNF- $\alpha$  from coronary sinus, and IL-6 from coronary sinus ( $R = 0.44$ ,  $p < 0.05$ ) and inferior vena cava ( $R = 0.51$ ,  $p < 0.01$ ).

#### Circulating Levels of Cytokines and Clinical, Hemodynamic Variables

TNF- $\alpha$ , sTNFR I and II from inferior vena cava increased in relation to worsening NYHA functional classes at admission ( $R = 0.59$ ,  $0.64$  and  $0.52$ , respectively;  $p < 0.01$ ). The concentrations of IL-6 showed a tendency to increase in accordance with worsening functional status without statistical significance ( $R = 0.32$ ,  $p = 0.06$ ).

Early diastolic mitral inflow versus mitral annular velocity ratio (E/E<sub>a</sub>) at admission by Doppler study, which reflects left ventricular end diastolic pressure, was positively correlated with TNF- $\alpha$  from systemic artery ( $R = 0.71$ ,  $p < 0.01$ ), coronary sinus ( $R = 0.52$ ,  $p < 0.05$ ) and inferior vena cava ( $R =$

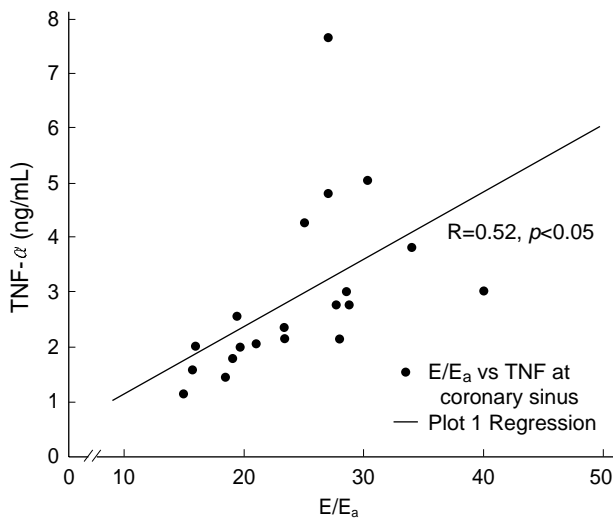


Fig. 2. Correlation between TNF- $\alpha$  at coronary sinus and E/E<sub>a</sub> by Doppler study. TNF- $\alpha$  at coronary sinus was positively correlated with E/E<sub>a</sub> by Doppler study ( $R=0.52$ ,  $p<0.05$ ).

0.46,  $p<0.05$ ) (Fig. 2). In contrast, pulmonary capillary wedge pressure measured during right catheterization did not show any significant correlation with cytokines.

## DISCUSSION

Since Levine et al. first reported that, among patients with congestive heart failure, circulating levels of TNF- $\alpha$  are most markedly increased in those with the most advanced disease and cachexia (13), many other studies have observed that cytokines are sufficient to mimic some aspects of the so-called heart failure phenotype, including progressive left ventricular dysfunction, pulmonary edema, left ventricular remodeling, fetal gene expression and cardiomyopathy (4-6). Thus, the "cytokine hypothesis" holds that heart failure progresses, at least in part, as a result of the toxic effects exerted by endogenous cytokine cascades on the heart and the peripheral circulation (14). However, the site of cytokine production in patients with congestive heart failure is still poorly understood. Anker et al. proposed that elevated plasma cytokines in heart failure resulted from a cascade of events consisting of mesenteric venous congestion, bowel wall edema, and intestinal bacterial translocation (8). But elevations in plasma cytokines have been observed in patients with functional class II and even in many patients without peripheral edema (9). Other studies proposed elevated left ventricular diastolic wall stress stimulates myocardium to produce cytokines (10). Tissue hypoxia and free radical production due to impaired vasodilator reserve was also hypothesized to cause NF- $\kappa$ B mediated cytokine production especially from skeletal muscles.

Deliargyris et al. suggested that elevated IL-6 in patients with heart failure is the result of extracardiac production (15). In contrast, Petretta et al. found no significant differences in

the levels of cytokines among coronary sinus, ascending aorta, inferior vena cava and hepatic vein (16). However, there is substantial limitation in their results because their subjects had various causes of heart failure including ischemic heart disease. Our study may be more relevant, for all the cases in the study were diagnosed as idiopathic dilated cardiomyopathy with various functional classes, and left ventricular end-diastolic pressure was measured twice; first, at admission, indirectly by Doppler study and after proper medical therapy, second, at recovery status, by cardiac catheterization.

The TNF- $\alpha$  molecule (a 157-amino acid polypeptide) exists bioactive as a membrane-bound and secreted molecule (17). The activated macrophage is the main source of TNF- $\alpha$ , although other cells releasing TNF- $\alpha$  include lymphocytes, fibroblasts, neutrophils, smooth muscle cells and mast cells (18). Furthermore, adult mammalian myocardial cells can produce TNF- $\alpha$  with extracellular stimuli such as endotoxin, hypoxia or increased mechanical stress (10).

Our data with the highest concentration of TNF- $\alpha$  at coronary sinus seem to support the concept of the myocardial cytokine production. Torre-Amione et al. demonstrated myocardial expression of TNF- $\alpha$  in failing human hearts (19). Myocardial stretch was a sufficient stimulus for the induction of TNF- $\alpha$  and mRNA biosynthesis in feline myocardium (10).

Nagueh et al. investigated E/E<sub>a</sub> by Doppler study provides the best index for the prediction of pulmonary capillary wedge pressure, irrespective of the diastolic filling pattern (12). Significant correlation between TNF- $\alpha$  and baseline E/E<sub>a</sub> was observed in the present study (Fig. 2). However, after compensation with appropriate medical therapy, no significant correlation was noted between TNF- $\alpha$  and pulmonary capillary wedge pressure gained at cardiac catheterization. Consequently, we deduced stretched myocardium may be the stimulus of increased level of TNF- $\alpha$ .

TNF- $\alpha$  also orchestrates the inflammatory cascade through regulation of transduction for proinflammatory cytokines such as IL-1 and IL-6, and nitric oxide synthase (20), which was reflected as a significant correlation between TNF- $\alpha$  and IL-6 in the present study.

TNF- $\alpha$  acts at the cellular level via both type I (p55) and type II (p75) receptors and recently, it has been suggested that both of them are present in the human myocardium. The extracellular domain fragments of both TNF receptors shed from cell surfaces can be detected as soluble forms in the blood and urine (21). At physiologic concentrations, sTNFRs may act as 'slow-release reservoir' of bioactive TNF- $\alpha$ , but at higher concentrations, as in patients with severe heart failure, they could inhibit the pathological increase of TNF- $\alpha$  activity (19).

Saraste et al. reported sTNFR II identified a subgroup of heart failure patients with increased cardiomyocyte apoptosis (22). Finally, the increased levels of sTNFR II were significantly correlated with poor short-term prognosis of heart failure patients (23).

Although our study failed to demonstrate any significant difference in the levels of sTNFR I and II from the different sites of sampling, the absence of a transmural gradient of cytokines does not rule out myocardial production of cytokines due to the diffusion barrier between interstitial space and coronary venous effluent and relatively high molecular weight compared with TNF- $\alpha$  and IL-6 (24).

Serum IL-6 was known as the most powerful independent predictor of heart failure episodes and mortality or need for heart transplantation during heart failure management (25).

Tsutamoto et al. implicated IL-6 is produced mainly in the periphery in heart failure patients (26), but their study population is different from ours in background; 60% of subject patients had history of myocardial infarction. In the setting of ischemic heart failure, both myocardial necrosis and successful reperfusion, and peripheral vascular tissue may affect to increase myocardial IL-6 production (26, 27). The mechanism for elaboration of IL-6 in idiopathic dilated cardiomyopathy has not been elucidated. TNF- $\alpha$  may be sufficient to induce IL-6 gene and protein expression in a variety of cell types, suggesting that there may be a 'cytokine cascade' in the setting of heart failure (28). We also identified a significant positive correlation between the levels of TNF- $\alpha$  and IL-6, which is similar to other study (29). On the basis of our results, we suggested that IL-6 might be produced mainly in the myocardium. But it remains controversial to determine whether the local myocardial production of IL-6 is secondary to increased TNF- $\alpha$ , or directly related to the myocardial tissue or some combination of both.

In our study, we conclude that myocardium might be the major source of proinflammatory cytokines in idiopathic dilated cardiomyopathy. It might be relevant in determining the origin for all the subjects had non-ischemic heart failure with various functional status.

Accordingly, we presumed that increased myocardial wall stress might lead to sustained expression of stretch-derived genes and increased oxidative stress as a result of subendocardial hypoperfusion, with resultant activation of families of genes which might be translated in subsequent production of cytokines.

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