Prolonged activation of Tumor necrosis factor (TNF)-α and its soluble receptors in chronic heart failure patients both in the compensated and decompensated state. Interplay between their levels and metalloproteinase-3

Dimitrios Tziakas[1], Georgios Chalikias[1], John T. Parissis[2], Helen Hatzinikolaou[1], Dimitrios Stakos[1], Evropi Papadopoulou[3], Alexandros Kortsaris[3], Dimitrios Hatseras[1]

[1] Department of Cardiology, Democritus University of Thrace, Alexandroupolis, Greece
[2] 2nd Department of Cardiology, Amalia Fleming Hospital, Athens, Greece.
[3] Department of Biochemistry, Democritus University of Thrace, Alexandroupolis, Greece.

Correspondence: Dimitrios Tziakas, Lecturer in Cardiology, Leoforos Demokratias 116, Alexandroupolis, 68100, Greece.
Tel.: + + 30 25510 35596 (home); + + 30 25510 76205 (office). Fax: + + 30 25510 35596.
E-mail: dtziakas@hotmail.com

Accepted for publication 18 May 2004

ABSTRACT. ***Introduction.*** **Recent clinical and experimental studies indicate that upregulation of the TNF system can contribute to the progression of cardiac remodeling and heart failure decompensation, by promoting alterations in cardiomyocyte biology and extracellular matrix metabolism. Extracellular matrix turnover is regulated by the matrix metalloproteinases (MMPs), which are endogenous enzymes responsible for extracellular collagen degradation. The present study investigates the fluctuation of serum levels of TNF-α, soluble TNF receptor-1 (sTNFR1) and -2 (sTNFR2), in patients with chronic heart failure both during acute decompensation and the stable state of the syndrome. The second goal of this study was to determine if a relationship exists between serum MMPs profiles (MMP-1, MMP-2, MMP-3) and circulating TNF-α or its soluble receptors.** ***Methods.*** **Our patient group consisted of 52 patients with chronic heart failure (NYHA III-IV; mean age: 65 ± 4 years; hypertensive cardiomyopathy: 20, ischemic cardiomyopathy: 17, dilated cardiomyopathy: 10, valvular disease: 5), who were hospitalized for acute decompensation of the syndrome. Our control group consisted of 30 healthy subjects (mean age: 57 ± 6 years). Serum levels of TNF-α, sTNFR1, sTNFR2 and MMP-1,-2,-3 were measured in heart failure patients by ELISA at admission and after one month as follow-up. Values are expressed as medians and interquartile ranges. {\ital}Results. In our patient group, we observed a statistically significant increase in the levels of sTNFR1 and sTNFR2 at admission (sTNFR1: 5.15 ng/mL, 4.49-8.90 ng/mL, $P < 0.001$, sTNFR2: 13.40 ng/mL, 6.10-21.50 ng/mL, $P < 0.001$), and at one-month follow-up (sTNFR1: 5.30 ng/mL, 4.61-6.90 ng/mL, $P < 0.001$, sTNFR2: 21.80 ng/mL, 11.50-25.20 ng/mL, $P < 0.001$), compared to the control group (sTNFR1: 3.83 ng/mL, 3.70-3.95 ng/mL, sTNFR2: 4.00 ng/mL, 3.40-5.40 ng/mL). There was a statistically significant difference in the levels of sTNFR2 between admission and follow-up ($P < 0.05$). Significant correlations between serum MMP-3 and sTNFR2 levels both at admission and follow up ($r = 0.460$, $P = 0.005$ and $r = 0.338$, $P = 0.044$, respectively) were also found.** ***Conclusions.*** **Soluble TNF receptors are elevated in heart failure patients both in acute decompensation and stable phase. We have detected higher levels of soluble TNFR2 during the compensated phase of heart failure, suggesting that TNFR2 receptors appear to stabilize the cytokine and thereby prolong its half-life and biological functions. Finally, TNF system - mediated cardiac remodeling may exist through the activation of MMP-3 signaling pathways.**

Keywords: tumor necrosis factor-α, cytokine receptors, extracellular matrix, cardiac remodeling, heart failure

INTRODUCTION

Tumor necrosis factor-α (TNF-α) overexpression in human myocardium provokes changes that are relevant to the process of left ventricular (LV) remodeling such as myocyte hypertrophy [1], myocyte apoptosis [2], contractile abnormalities [3] and fetal gene expression [4]. Another important observation from past studies is that TNF-α can cause cardiac dilatation [3, 5, 6]. Changes in LV geometry that have been documented to occur with increased TNF-α levels, include alterations in myocyte size and number, alignment of myocytes and changes in the myocardial extracellular matrix (ECM) [2, 5-7].

The ECM turnover during LV emodeling is regulated by the balance between matrix metalloproteinases (MMPs, a large family of Zn^{+2} – and Ca^{+2} – dependent endogenous enzymes responsible for extracellular collagen degradation) and their tissue inhibitors (TIMPs), since TIMPS are

the naturally occurring inhibitors of MMPs (8,9). Most MMPs are secreted in an inactive or pro-MMP (zymogen) form by a variety of cell types, such as smooth muscle cells, endothelial cells, myocytes and others. The activation of the zymogen form is tightly regulated through several pathways, including other MMPs such as MMP-3 and membrane type MMP (MT-MMP).

Increased MMP zymographic activity has been reported in myocardial samples from patients with end-stage chronic heart failure [8, 9]. Furthermore, a clear relationship between MMPs and LV remodeling process has been demonstrated through the use of transgenic models [10, 11]. Cytokines such as TNF-α and interleukin-1β influence MMP activity at the transcriptional level by upregulating MMP mRNA expression [12-14]. Therefore, one molecular trigger for the induction of myocardial MMPs in LV remodeling may be increased TNF-α synthesis and release.

The biological actions of TNF-α are mediated by two distinct surface receptors, the TNFR1 and TNFR2 with molecular weights of 55-60 kDa and 70-80 kDa, respectively [15]. In the presence of mechanical stress or stimulants including TNF-α, lipopolysaccharide and phorbol esters, TNF receptors are released into the circulation by a process called "shedding" [16-19].

One of the several issues surrounding the interpretation of studies regarding circulating plasma TNF-α in chronic heart failure patients, is that since TNF-α can be synthesized locally within the myocardium, then circulating levels of TNF-α may represent "spillover" of TNF-α produced within the heart and may not accurately reflect local TNF-α activity [20]. TNF receptor plasma levels have been used as biomarkers of progression of heart failure [21, 22]. Furthermore, in other studies there was a direct relationship between levels of soluble TNF receptors and survival rates [23, 24]. Thus, the presence of TNF receptors in patients with heart failure are more likely than TNF-α, indicative of local myocardial TNF-α activation and predictors of poor prognosis.

To our knowledge, there are limited references in the current literature to how this cytokine system behaves during transition from the decompensated heat failure to the compensated state. Accordingly, the present study was undertaken to investigate the pattern of TNF-α activation and its soluble receptors (sTNFR1 and sTNFR2) in the serum of patients with acute decompensation of chronic heart failure, as well as their fluctuations after one month, as follow-up (stable phase). Furthermore, the second goal of this study was to determine if a relationship exists between serum MMPs profiles (MMP-1,MMP-2,MMP-3) and circulating TNF/TNF receptors system.

PATIENTS AND METHODS

Study population

The patient population consisted of the first, consecutive 52 patients (mean age 65 ± 4 years, 28 men) with chronic heart failure who were admitted to our Cardiology Department's Coronary Care Unit with acute decompensation of the syndrome. All patients were known, compensated heart failure patients attending the outpatient heart failure clinic of our cardiology department. Their chronic heart failure status had been determined on a prior visit to our outpatient heart failure clinic, by the presence of marked limitation or inability to carry out any physical activity without discomfort (NYHA stage III-IV), by chest radiography showing an enlarged cardiac silhouette and by a left ventricular ejection fraction of 50 percent or lower on echocardiography following Simpson's method. The cause of their heart failure was coronary artery disease in 17 patients, hypertensive cardiomyopathy in 20 patients, idiopathic dilated cardiomyopathy in 10 patients and valvular disease in five patients.

The acute decompensation of their stable heart failure was documented by the presence of recent onset dyspnea at rest, orthopnea and increased body weight on recent medical history, the presence of pulmonary rales, the presence of a third heart sound, the presence of leg edema, and chest radiography showing pulmonary venous congestion. In none of our study patients, was the acute decompensation of heart failure due to an acute coronary syndrome as determined by serial negative Troponin T measurements and with unchanged ECG serial recordings. Blood samples were taken at admission and one month after resolution of symptoms and restoration of a clinical stable phase.

This study also enrolled 30 control subjects (mean age 57 ± 6 years, 20 men). The healthy volunteers had no past history or evidence of cardiovascular disease, hypertension or diabetes mellitus. Their ECG showed normal findings and their chest X-ray gave a normal heart silhouette.

The present study did not include patients or control subjects with a history of neoplastic, hepatic, thyroid, infectious, autoimmune, peripheral atherosclerotic disease, any surgical procedure in the preceding six months or who were under treatment with anti-inflammatory and/or hypolipidemic drugs.

Patients and healthy volunteers gave written informed consent and were studied in compliance with ethical approval.

All patients were on standard medical treatment for heart failure, with combinations of medications, including ACE inhibitors, AT1 receptor blockers, nitrates, diuretics, digitalis, spironolactone and beta-adrenergic blockers.

Table 1 demonstrates the demographic, clinical and treatment characteristics of study patients and healthy controls.

Laboratory measurements

Blood samples were drawn from the peripheral vein at admission and at the one-month follow-up visit to the heart failure outpatient clinic. After centrifugation at 4 000 *g* for 10 min, serum samples were frozen and stored at – 70 °C until use. This study investigated serum levels of TNF-α, its soluble receptors sTNFR1 and sTNFR2, and three different MMPs -an interstitial collagenase MMP-1, a gelatinase MMP-2 and a stromelysin MMP-3.

An immunoenzymometric assay (ELISA), for the quantitative measurement of human tumor necrosis factor-α in serum, was performed using a commercially available kit "Biosource Europe S.A.". The sensitivity of the assay was 3 pg/mL. Intra-assay and inter-assay coefficients of variation (CVs) were < 5.2% and < 9.9%, respectively. The assay measured total TNF-α (free unbound cytokine and cytokine bound to receptors).

Serum TNFR1 levels were assessed according to the manufacturer's specifications using an ELISA kit "Human

Table 1
Demographics and medication of study population.

	Control group (n = 30)	Patients group (n = 52)
Demographics		
Age (years)	57 ± 6	65 ± 4
Male (%)	66	54
Plasma creatinine (mg/dL)	0.9 ± 0.1	1.2 ± 0.1
NYHA class III-IV	0	52
LVEF (%)	60 ± 4	35 ± 4
Etiology (%)		
Ischemic	–	32
Hypertensive	–	38
Valvular	–	10
Dilated cardiomyopathy	–	20
Medication (%)		
Nitrates	–	90
Beta-blockers	–	15
Digitalis	–	60
ACE inhibitors	–	100
AT receptor blockers	–	30
Diuretics	–	100
Spironolactone	–	45
Amiodarone	–	–
IV inotropes	–	10

sTNFR1 60 kDa ELISA- ImmunoKontact" capable of quantitative measurement of soluble, circulating - "shed" forms of TNF receptor 1. The sensitivity of the assay was < 80 pg/mL. Intra-assay and inter-assay coefficients of variation (CVs) were < 1.89% and < 8.6%, respectively. No interference was observed by tumor necrosis factor-α.

Serum TNFR2 levels were assessed according to the manufacturer's specifications using an ELISA kit "Human sTNFR2 80 kDa ELISA-ImmunoKontact" capable of quantitative measurement of soluble circulating - "shed" forms of TNF receptor 2. The sensitivity of the assay was < 0.15 ng/mL. Intra-assay and inter-assay coefficients of variation (CVs) were < 1.4% and < 2%, respectively. No interference was observed by tumor necrosis factor-α.

Sandwich enzyme-linked imunosorbent assay (ELISA) was performed for measuring concentrations of serum MMP-1, using "Quantikine R,D Systems" commercial kits capable of measuring pro - MMP-1.The sensitivity of the assay was 2.1 pg/mL. Intra-assay and inter-assay coefficients of variation (CVs) were < 6.1% and < 10.4%, respectively The antibodies did not recognize human active MMP-1 and MMP-1 bound to TIMP-1. Sandwich ELISA was also performed for measuring concentrations of serum MMP-2, using "Oncogene Research Products" commercial kits. The sensitivity of the assay was 0.1 ng/mL. Intra-assay and inter-assay coefficients of variation (CVs) were taken as < 8.3% and < 9.7%, respectively The antibodies recognized pro -MMP-2 and active MMP-2. Sandwich ELISA was performed for measuring concentrations of serum MMP-3 using "Quantikine R&D Systems" commercially available kits. The sensitivity of the assay was 9 pg/mL. Intra-assay and inter-assay coefficients of variation (CVs) were < 6.4% and < 8.6%, respectively. The antibodies recognized both active and pro -MMP-3.

Statistical analysis

TNF-α, sTNFR1 and sTNFR2 levels are expressed as median and interquartile ranges, as their values were markedly abnormally distributed. The Kolmogorov-Smirnof test was used to examine the normality of the values' distribution. The comparison of variables between the control and the patient group was performed using the non-parametric Mann-Whitney U test for unpaired data. The comparison of variables between admission and follow-up within the patient group was performed using the non-parametric Wilcoxon signed ranks test for paired data. When assessing the correlation between variables, the Spearman rank test was used first, comparing variables both at admission and at follow-up. For the serial assessment of TNF-α, MMPs and soluble cytokine receptors, admission values and the value for the follow-up period were measured and expressed as the change from admission.

The relationship between the change in sTNFR1 and sTNFR2 to that of MMP-1, MMP-2, MMP-3 and TNF-α serum levels was examined using regression analysis.

After this, a cut-off analysis was then performed. sTNFR1 and sTNFR2 serum levels were categorized into two groups: no change or decreased levels from admission, or increased TNFR1 or TNFR2 levels from admission. Next, comparison between groups was performed using the defined cut-off, which was established as the absolute change in relative soluble cytokine receptor levels, with the Mann-Whitney U test for unpaired data.

To determine any stoichiometric relationship between TNF-α and its receptor levels (sTNFR1 and TNFR2), the TNF-α/sTNFR1 ratio and TNF-α/sTNFR2 ratio were computed for each patient and a comparison was made between groups on admission and at follow-up using the non-parametric Wilcoxon signed ranks test for paired data. A value of $P < 0.05$ was considered statistically significant. All statistical procedures were performed using Systat Statistical Software (SPSS; Chicago, IL, USA).

RESULTS

Table 2 describes the fluctuation of TNF-α and its receptors in the serum of heart failure patients between admission and one-month follow-up, as well as values of these inflammatory variables in healthy controls.

We observed statistically significantly increased TNF-α levels on follow-up compared to admission ($P < 0.001$), as well as a significant increase in the levels of sTNFR2 on follow-up compared to admission ($P < 0.05$). On the other hand, no significant changes were observed in sTNFR1 levels between admission and follow-up ($P = 0.405$) *(Figure 1)*.

We found significantly increased levels of TNF-α, sTNFR1 and sTNFR2 in heart failure patients compared to healthy individuals both at admission and at the one-month follow-up ($P < 0.001$).

We observed a significant association between the levels of sTNFR2 and MMP-3, both at admission ($r = 0.460$, $P = 0.005$) and in follow-up measurements ($r = 0.338$, $P = 0.044$) *(Figures 2, 3)*. On the other hand, we observed no association between circulating TNF-α and sTNFR1 levels ($P > 0.05$) or sTNFR2 levels ($P > 0.05$) both on admission and at follow-up.

Table 2
Fluctuation of circulating TNF-α, sTNFR1 and sTNFR2 levels in heart failure patients between admission and one-month follow-up (values of various inflammatory markers in healthy controls were also included)

	TNFα controls (pg/mL)	TNF-α admission (pg/mL)	TNF-α follow-up (pg/mL)	sTNFR1 controls (ng/mL)	sTNFR1 admission (ng/mL)	sTNFR1 follow-up (ng/mL)	sTNFR2 controls (ng/mL)	sTNFR2 admission (ng/mL)	sTNFR2 follow-up (ng/mL)
Minimum	17.08	16.29	3.46	3.61	4.05	3.46	3.00	2.90	4.20
25%	18.80	21.43	49.54	3.70	4.49	4.61	3.40	6.10	10.50
Median	19.86	25.43	85.55	3.83	5.15	5.30	4.00	13.40	21.80
75%	21.50	51.38	117.22	3.95	8.90	6.90	5.40	21.50	25.20
Maximum	30.43	115.50	352.60	5.75	13.50	10.20	22.60	42.80	100.00

For the serial assessment of TNF-α, MMPs and soluble cytokine receptors, admission values and the values for the follow-up period were measured and expressed as the change from admission *(Table 3)*.

The relationships between the absolute change from baseline of TNF receptor types 1 (sTNFR1) and 2 (sTNFR2) to changes in matrix metalloproteinase-3 (MMP-3) levels, and to changes in TNF-α levels were examined by non-

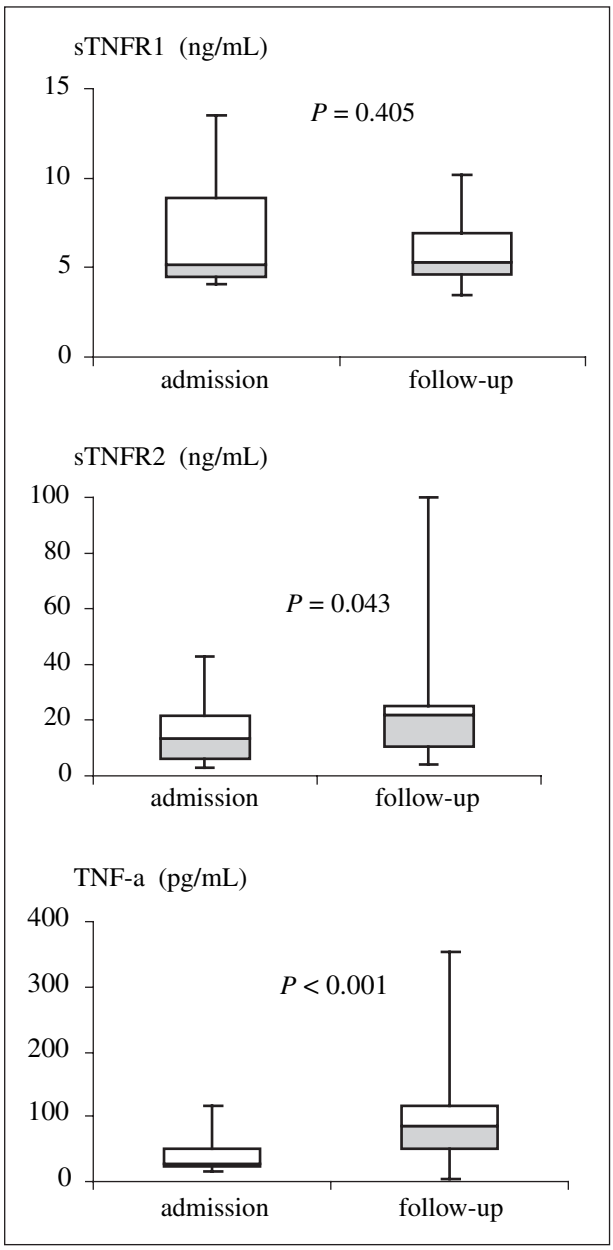

Figure 1
Comparison of TNF-α, sTNFR1 and sTNFR2 levels between admission and 30-day follow-up in heart failure patients.

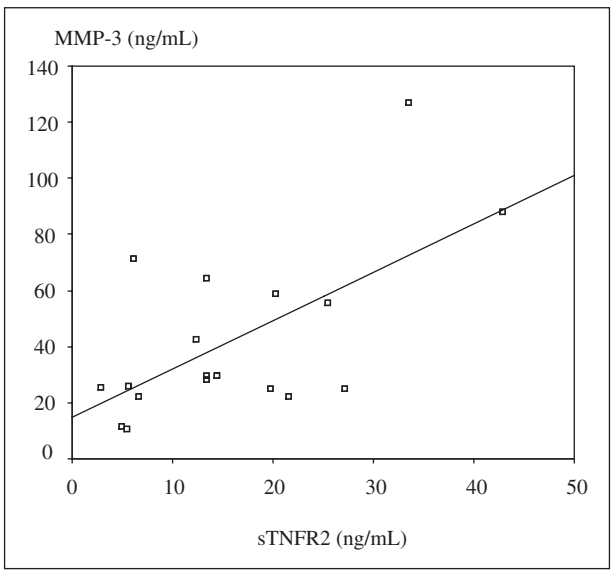

Figure 2
Correlation between MMP-3 levels and sTNFR2 levels at admission in heart failure patients ($r = 0.460$, $P = 0.005$).

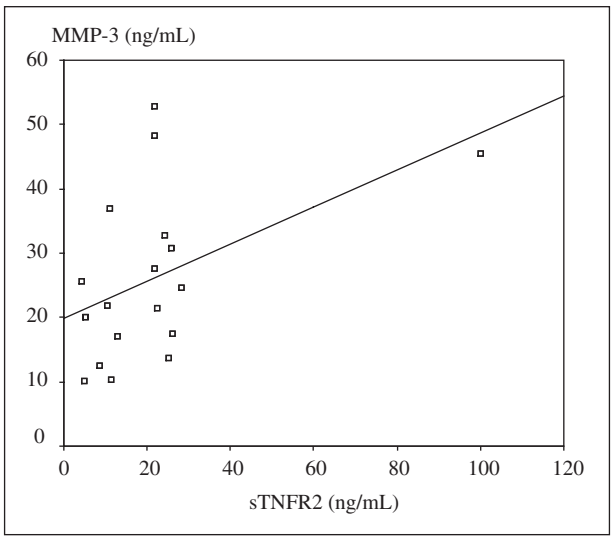

Figure 3
Correlation between MMP-3 levels and sTNFR2 levels at follow-up in heart failure patients ($r = 0.338$, $P = 0.044$).

Table 3
Change in sTNFR1, sTNFR2, TNF-α, MMP-1, MMP-2 and MMP-3 levels at one-month follow-up from admission, in heart failure patients

	Change in sTNFR1	Change in sTNFR2	Change in MMP-1	Change in MMP-2	Change in MMP-3	Change in TNF-α
Minimum	– 8.30	– 13	– 7.03	– 327.80	– 74.26	– 31.56
25%	– 2	– 0.20	– 3.96	– 113.80	– 32.38	6.71
Median	– 0.25	5	– 2.36	– 73.10	– 9.14	46.03
75%	0.70	8.40	– 0.97	58.55	– 1.02	90.98
Maximum	3	57.20	2.45	132.10	6.51	262.01

linear regression analysis. A significant quadratic relationship between sTNFR1 and MMP-3 changes ($R^2 = 0.182$, $P = 0.045$) *(Figure 4)* as well as between sTNFR1 and TNF-α changes ($R^2 = 0.254$, $P = 0.008$) *(Figure 5)* was observed. Furthermore, a significant quadratic relationship between TNFR2 and MMP-3 changes ($R^2 = 0.380$, $P = 0.001$) was also found *(Figure 6)*. On the other hand, no relationship was observed between sTNFR2 and TNF-α changes.

A cut-off point analysis was then performed between the absolute change in sTNFR1 (increased or decreased/stable) and changes in MMP-3 and TNF-α, as well as between the absolute change in sTNFR2 (increased or decreased/stable) and changes in MMP-3 and TNF-α. In those heart failure patients with elevated sTNFR2, MMP-3 levels were increased *(Figure 7)*.

The ratios TNF-α/sTNFR1 and TNF-α/sTNFR2 were computed for each patient at admission and at follow-up *(Table 4)*. We observed a significant increase of both ratios at the follow-up visit ($P < 0.001$ and $P = 0.004$, respectively) *(Figure 8)*.

DISCUSSION

Our study suggests that the TNF system is activated in severe heart failure patients (NYHA III-IV) during both the acute decompensation and compensated phase, as compared to healthy individuals. These results were in agreement with previously reported data [22, 23].

However, the activation of the TNF system is complex and difficult to interpret, since peripheral circulating levels of TNF-α may not reflect local myocardial TNF-α activity [21]. Therefore, measurement of TNF soluble receptors, which are known to be "shed" from target cells upon a stress stimulus, is necessary to assess it fully [22-25].

The present study showed that circulating TNF receptors (sTNFR1 and sTNFR2) are also increased in heart failure patients (NYHA III-IV) both during acute decompensation and at one-month follow-up, compared to healthy individuals, confirming previously reported data [26, 27].

This prolonged activation raises questions as far as their pathophysiological role in heart failure is concerned. Their presence suggests the possibility that the shedding of TNF

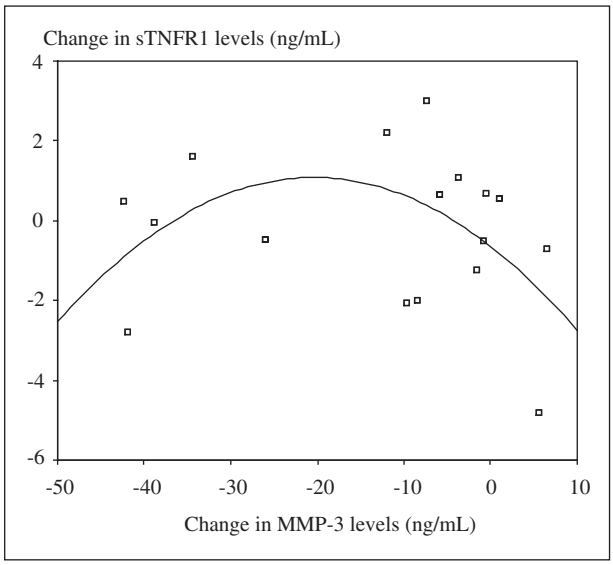

Figure 4
The relationships between the absolute change from admission of tumor necrosis factor receptor type 1 (sTNFR1) and changes in matrix metalloproteinase-3 (MMP-3) levels were examined by non-linear regression analysis. A significant quadratic relationship ($y = -0.041x^2 - 0.17x - 0.64$) between sTNFR1 and MMP-3 ($R^2 = 0.182$, $P = 0.045$) was observed.

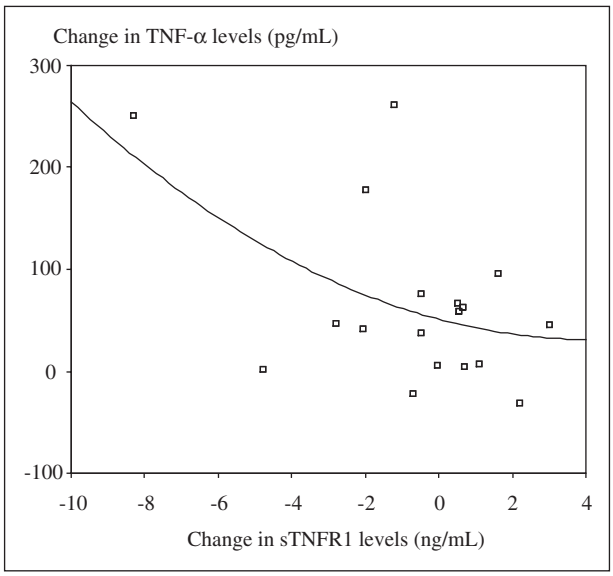

Figure 5
The relationships between the absolute change from admission of tumor necrosis factor receptor type 1 (sTNFR1) and changes in tumor necrosis factor-α levels were examined by non-linear regression analysis. A significant quadratic relationship ($y = 1.17x^2 - 9.61x + 51.01$) between sTNFR1 and TNF-α ($R^2 = 0.254$, $P = 0.008$) was observed.

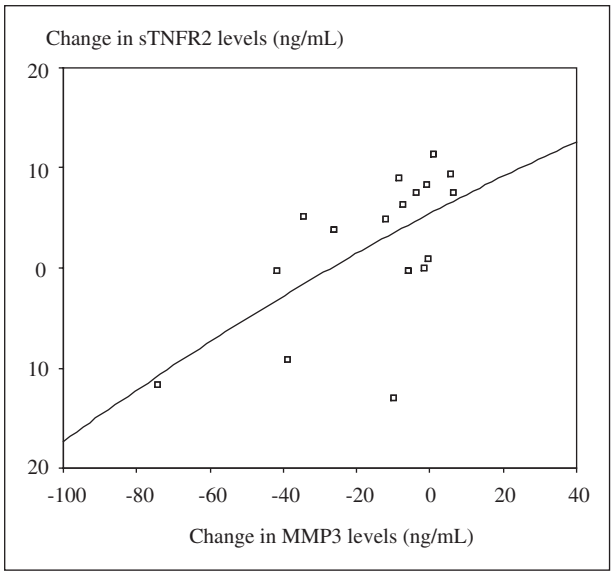

Figure 6
The relationships between the absolute change from admission of tumor necrosis factor receptor type 2 (sTNFR2) and changes in matrix metalloproteinase-3 (MMP-3) levels were examined by non-linear regression analysis. A significant quadratic relationship ($y = -0.003x^2 + 0.19x + 5.45$) between sTNFR2 and MMP-3 ($R^2 = 0.380$, $P = 0.001$) was observed.

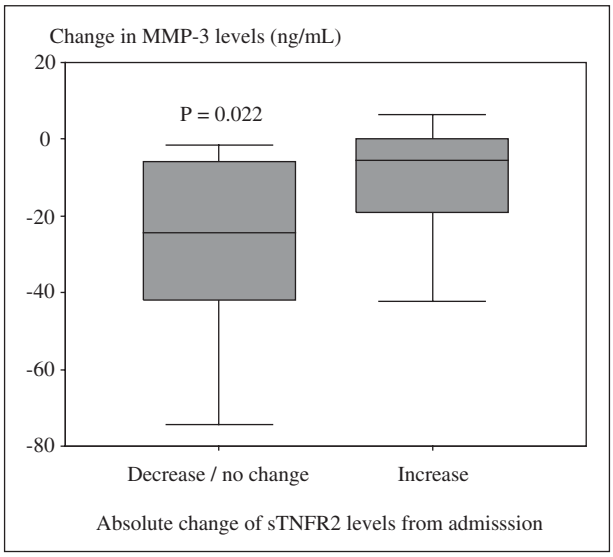

Figure 7
Changes in matrix metalloproteinase-3 (MMP-3) serum levels in heart failure patients with respect to absolute changes in soluble tumor necrosis factor receptor type 2 (sTNFR2) serum levels from admission. In patients in whom sTNFR2 plasma levels were increased from admission, increased MMP-3 levels were also observed ($P = 0.022$).

Table 4
TNF-α/sTNFR1 and TNF-α/sTNFR2 ratio in heart failure patients on admission and follow-up.

	Ratio TNFα/sTNFR1 on admission ($\times 10^{-3}$)	Ratio TNFα/sTNFR1 on follow up ($\times 10^{-3}$)	Ratio TNFα/sTNFR2 on admission ($\times 10^{-3}$)	Ratio TNFα/sTNFR2 on admission ($\times 10^{-3}$)
Minimum	1.88	0.75	0.38	0.27
25%	3.66	8.6	1.36	2.46
Median	5.48	18.04	2.88	4.17
75%	10.17	24.30	4.70	11.41
Maximum	19.36	101.91	18.49	71.96

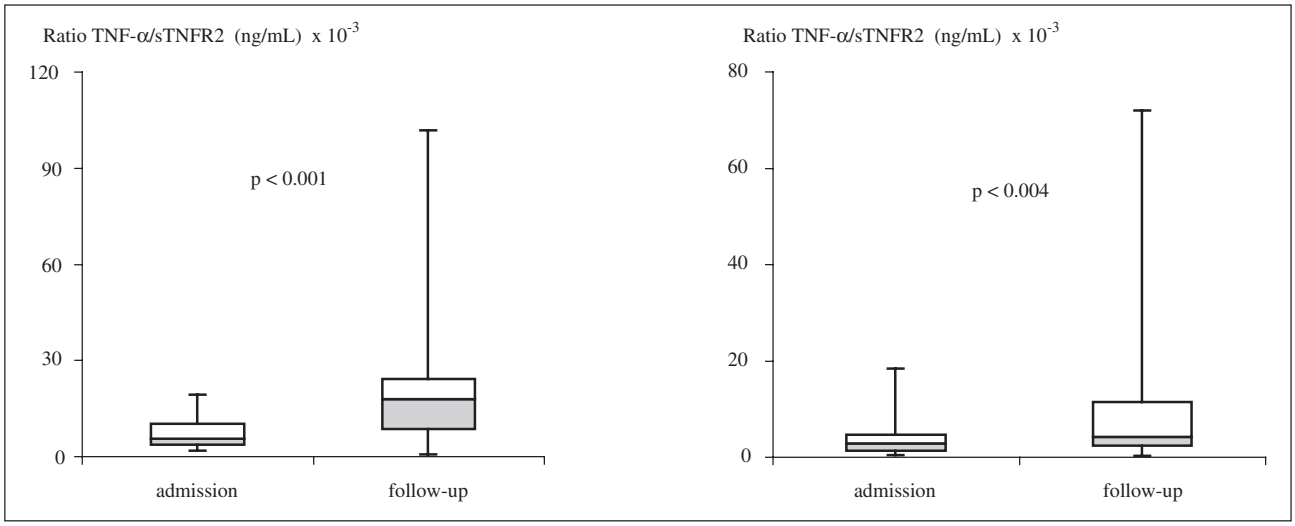

Figure 8
TNF-α/sTNFR1 ratio and TNF-α/sTNFR2 ratio profiles for heart failure patients between admission and follow-up. An increase in both the TNF-α/sTNFR1 ratio and the TNF-α/sTNFR2 ratio was observed at follow-up when admission levels were compared to follow-up levels ($P < 0.001$ and $P = 0.004$, respectively).

receptors in patients with heart failure is an adaptive response that effectively neutralizes the biological actions of TNF [27-29]. Not only does the shedding of TNF receptors reduce the number of active receptors on target cells that are necessary for TNF action, but circulating receptors can also block the actions of TNF by inhibiting the binding to the membrane receptors [28-31]. Many studies [28, 29, 31-34] suggest that the actual neutralization of TNF-α is likely only if soluble receptors circulate at extremely high levels. It will be of great interest to investigate further, the multifunctional regulation of TNF-α by its receptors.

On the other hand, some investigators have suggested that soluble TNF receptors may enhance, rather than attenuate, the biological actions of TNF. By binding to TNF, soluble TNF receptors appear to stabilize the cytokine by protecting trimeric TNF-α from monomerization and subsequent inactivation, and thereby prolonging its half life and biological functions [28, 31, 33]. Therefore, the release of soluble receptors acts to establish a circulating reservoir of TNF, which can slowly release TNF to target cell [29].

Finally, it is possible that circulating receptors act neither to enhance nor to neutralize the actions of TNF *in vivo*, but simply to provide a sensitive marker for the interaction of the cytokine with its target cells [29, 33].

Studies investigating the fluctuation of TNF-α levels during acute decompensation of heart failure patients, have reported no significant change in TNF-α concentrations between the "decompensated" state compared to the short term (5-11 days after acute decompensation) "recovery" state [35-37]. In addition, our study investigated the fluctuation of TNF-α levels and its soluble receptors between acute decompensation of heart failure patients and a longer term "recovery" state, i.e. 30 days after resolution of symptoms and restoration of clinical stability.

Our finding that both TNF-α levels and sTNFR2 levels are decreased during the acute decompensation of chronic heart failure compared to their levels at one-month follow-up (compensated phase), is more in accordance with the "immunological reservoir" hypothesis. A possible mechanism for the decreased levels of this cytokine and its receptor during the acute decompensation phase is that a stress stimulus might have led to an acute release of TNF-α from the circulating TNF-α/sTNFRs complexes with subsequent binding to its membrane bound TNF receptors, initiating its adverse effects the on the target cells and leading to the acute decompensation phenotype of chronic heart failure. The mechanism for the spontaneous release of TNF-α from the circulating TNF-α-sTNFRs complexes and the way it is sequestrated to target cells need further investigation.

It has been stated that TNF-α induces the shedding of its soluble receptors and there was indeed a positive correlation between antigenic circulating TNF-α and sTNFRs [27-29]. Accordingly, in our study we examined the association between TNF-α and soluble TNF receptors. We observed a more complex association between the change in TNF-α levels and the change in sTNFR1 levels between the acute decompensation and the stable compensated phase of heart failure.

Moreover, we observed a strong association between sTNFR2 levels and MMP-3 levels. Specifically, we observed, apart from a positive correlation between sTNFR2 and MMP-3 levels both at admission and at follow-up, that patients with increased immune activation (increased sTNFR2 levels) were characterized by persistently elevated MMP-3 levels. This finding is in accordance with the hypothesis that changes in circulating sTNF receptors can contribute to detectable changes in MMP serum levels. Furthermore, it supports the hypothesis that TNF-α-mediated LV remodeling may occur through MMP induction pathways. A number of *in vitro* studies have provided evidence that TNF-α may induce MMP over-expression through activation of various transcriptional factors (i.e. AP-1 and NF-κB), and enhanced oxidative stress. [38-41]. Oxidative stress-induced activation of MMPs may be achieved, at least partially, through the upregulation of mitogen-activated protein kinases and autocatalysis of inactive molecules by oxidative modification of their autoinhibitory domain [40, 41].

Studies in transgenic animals as well as in myocardial samples of end-stage heart failure patients, have shown that a great number of MMP species are over-expressed in failing hearts, such us MMP-1,-2,-3,-9,-13 and MT-1-MMP [9-11]. In the present study, we chose to investigate a specific portfolio of MMPs (MMP-1, MMP-2 and MMP-3) as these are the MMPs required for the complete degradation of interstitial collagen. More specifically, we examined MMP-1, a member of the interstitial collagenase family, with high affinity for the fibrillar collagens types I, II and III, MMP-2, a gelatinase that cleaves denaturated collagen as well as basement membranes, and MMP-3, a member of the stromelysins family that cleaves a wide variety of extracellular matrix components and which can activate other MMP types. Our observations suggest that from the three MMPs investigated, MMP-3 seems to be more specifically activated through sTNFR2 pathways in chronic heart failure patients. This is probably due to the fact that MMP-3 on one hand has the widest range of substrates and includes all of the fibrillar collagens as well as components of the basement membrane, while on the other hand, MMP-3 can activate other MMPs. These characteristic properties, unique among other MMP species, renders MMP-3 a major participant in the cascade required to achieve activation of other MMP types relevant to LV remodeling [8, 9].

Computing the relative stoichiometry between TNF-α levels and soluble TNF-α receptors has been used to define net TNF-α system activation [34]. In our study, we observed significantly increased ratios of TNF-α/sTNFR1 and TNF-α/sTNFR2 on follow-up, compared to admission. This is indicative of the prolonged immunological activation of chronic heart failure patients. These findings indicate that ratios between the circulating cytokine and its soluble receptors may be better markers of the complex TNF-α system activation than cytokine itself.

STUDY LIMITATIONS

We have only examined some representative members of the complex MMP cascade, and have detected in some cases, the pro-active forms of relevant molecules. On the other hand, elevated serum levels of MMPs in patients with cardiac failure may be due to multisite production (cardiac and extra-cardiac) and do not necessarily reflect their expression in the failing heart. Thus, only indirect evidence suggests that the molecules studied are associated with pathophysiological events of cardiac dysfunction or dilatation.

Finally, although statistical correlation between cytokines and MMPs does not necessarily suggest a relation to their clinical relevance, there is however increasing evidence indicating that the TNF system interacts with MMPs during the LV remodeling process.

REFERENCES

1. Yokoyama T, Nakano M, Bednarczyk JL, McIntyre BW, Entman M, Mann DL. 1997. Tumor necrosis factor alpha provokes a hypertrophic growth response in adult cardiac myocytes. *Circulation.* 95: 1247.
2. Krown KA, Page MT, Nguyen C, Zechener D, Gutierrez V, Comstock KL, Glembotski CC, Quintana PJE, Sabbadini RA. 1996. Tumor necrosis factor alpha induced apoptosis in cardiac myocytes: involvement of the sphingolipid signaling cascade in cardiac cell death. *J Clin Invest.* 98: 2854.
3. Kubota T, McTiernan CF, Frye CS, Slawson SE, Lemster BH, Koretsky AP, Demetris AJ, Feldman AM. 1997. Dilated cardiomyopathy in transgenic mice with cardiac specific overexpression of tumor necrosis factor-alpha. *Circ Res.* 81: 627.
4. Kubota T, Bounoutas GS, Miyagishima M, Kadokami T, Sanders VS, Bruton C, Robins PD, McTiernan CF, Feldman AM. 2000. Soluble tumor necrosis factor receptor abrogates myocardial inflammation but not hypertrophy in cytokine induced cardiomyopathy. *Circulation.* 101 (21): 2518.
5. Bozkurt B, Kribbs SB, Clubb FJ, Michael LH, Didenko VV, Hornsby PS, Seta Y, Oral H, Spinal FG, Mann DL. 1998. Pathophysiologically relevant concentrations of tumor necrosis factor alpha promotes progressive left ventricular dysfunction and remodeling in rats. *Circulation.* 97: 1382.
6. Li YY, Feng YQ, Kadokami T, McTiernan CF, Draviam R, Watkins SC, Feldman AM. 2000. Myocardial extracellular matrix remodeling in transgenic mice overexpressing tumor necrosis factor alpha can be modulated by anti tumor necrosis factor alpha therapy. *Proc Natl Acad Sci USA.* 97: 12746.
7. Bryant D, Becker L, Richardson J, Shelton S, Franco F, Peshock R, Thompson H, Giroir B. 1998. Cardiac failure in transgenic mice with myocardial expression of tumor necrosis factor alpha. *Circulation.* 97: 1375-81.
8. Spinale FG, Coker ML, Heung LJ, Bond BR, Gunasinghe HR, Etoh T, Goldberg AT, Zellner JL, Crumbley AJ. 2000. A matrix metalloproteinase induction/activation system exists in the human left ventricular myocardium and is upregulated in heart failure. *Circulation.*102: 1944.
9. Thomas CV, Coker ML, Zellner JL, Handy JR, Crumbley AJ 3rd, Spinale FG. 1998. Increased matrix metalloproteinase activity and selective upregulation in LV myocardium from patients with end stage dilated cardiomyopathy. *Circulation.* 97: 1708.
10. Peterson JT, Hallak H, Johnson L, Li H, O'Brien PM, Sliskovic DR, Bocan TM, Coker ML, Etoh T, Spinale FG. 2001. Matrix metalloproteinase inhibition attenuates left ventricular remodeling and dysfunction in a rat model of progressive heart failure. *Circulation.*103: 2303.
11. Spinale FG, Coker ML, Krombach SR, Mukherjec R, Hallak H, Houck WV, Clair MS, Kribbs SB, Johnson LL, Peterson JT, Zile MR. 1999. Matrix metalloproteinase inhibition during the development of congestive heart failure; effects on left ventricular dimensions and function. *Circ Res.* 85: 364.
12. Siwik DA, Chang DLH, Colucci WS. 2000. Interleukin-1β and tumor necrosis factor-α decrease collagen synthesis and increase matrix metalloproteinase activity in cardiac fibroblasts in vitro. *Circ Res.* 86: 1259.
13. Rajavashisth TB, Liao JK, Galis ZS, Tripathi S, Laufs U, Tripathi J, Chai NN, Xu XP, Shah P, Libby P. 1999. Inflammatory cytokines and oxidized low-density lipoproteins increase endothelial cell expression of membrane type-1 matrix metalloproteinase. *J Biol Chem.* 274: 11924.
14. Galis ZS, Muszynski M, Sukhova GK, Simon-Morrissey E, Unemori EN, Lark MW, Amento E, Libby P. 1994. Cytokine stimulated human vascular smooth muscle cells synthesize a complement of enzymes required for extracellular matrix digestion. *Circ Res.* 75: 181.
15. Beutler B, van Huffel C. 1994. Unraveling function in the TNF ligand and receptor families. *Science.* 264: 667.
16. Olson I, Lantz M, Nilsson E, Peetre C, Thysell H, Grubb A, Adolf G. 1989. Isolation and characterization of a tumor necrosis factor binding protein from urine. *Eur J Haematol.* 42: 270.
17. Engelmann H, Novick D, Wallach D. 1990. Two tumor necrosis factor binding proteins purified from human urine. Evidence for immunological cross-reactivity with cell surface tumor necrosis factor receptors. *J Biol Chem.* 265: 1531.
18. Brakebusch C, Nophar Y, Kemper O, Engelmann H, Wallack D. 1992. Cytoplasmic truncation of the p55 tumor necrosis factor (TNF) receptor abolishes signaling but not induced shedding of the receptor. *EMBO J.* 11: 943.
19. Joyce DA, Steer JH. 1995. Tumor necrosis factor alpha and interleukin 1 alpha stimulate late shedding of p75 TNF receptors but not p55 TNF receptors from human monocytes. *J Interf Cytokine Res.*15: 947.
20. Feldamn AM, Combes A, Wagner D, Kadakomi T, Kubota T, Li YY, McTiernan C. 2000. The role of tumor necrosis factor in the pathophysiology of heart failure. *J Am Coll Cardiol.* 35: 537.
21. Kapadia S, Dibbs Z, Kurrelmeyer K, Kalra D, Seta Y, Wang F, Bozkurt B, Oral H, Sivasubramanian N, Mann DL. 1998. The role of cytokines in the failing human heart. *Cardiol Clinics.* 16: 645.
22. Koller-Strametz J, Pacher R, Frey B, Kos T, Woloszczuk W, Stanek B. 1998. Circulating tumor necrosis factor alpha levels in chronic heart failure: relation to its soluble receptor 2, interleukin 6 and neurohormonal variables. *J Heart Lung Transplant.* 17: 356.
23. Torre Armione G, Kapadia S, Lee J, Durand TB, Bies RD, Young JB, Mann DL. 1996. Tumor necrosis factor alpha and tumor necrosis factor receptors in the failing human heart. *Circulation.* 93: 704.
24. Rauchhaus M, Doehner W, Francis DP, Daros C, Kemp M, Liebenthal C, Niebauer S, Hooper J, Volk HD, Coats AJS, Anker SD. 2000. Plasma cytokine parameters and mortality in patients with chronic heart failure. *Circulation.* 102: 3060.
25. Deswal A, Petersen NJ, Feldman AM, Young JB, White BG, Mann DL. 2001. Cytokines and cytokine receptors in advanced heart failure: an analysis of the cytokine database from the Vesnarinone trial (VEST). *Circulation.* 103: 2055.
26. Wilson EM, Gunasinghe HR, Coker ML, Sprunger P, Lee-Jackson D, Bozkurt B, Deswal A, Mann DL, Spinale FG. 2002. Plasma matrix metalloproteinase and inhibitor profiles in patients with heart failure. *J Cardiac Fail.* 6: 390.
27. Smith JJ, Shapiro L, Dolan NA. 1993. Cytokine antagonists circulate in the blood of patients with advanced congestive heart failure. *J Am Coll Cardiol.* 21: 268A (Abstract).
28. Kapadia S, Torre-Amione G, Yokohama T, Mann DL. 1995. Soluble TNF binding proteins modulate the negative inotropic properties of TNF-α in vitro. *Am J Physiol.* 268: H517.
29. Packer M. 1995. Is tumor necrosis factor an important neurohormonal mechanism in chronic heart failure ? *Circulation.* 92: 1379.
30. Pfefer K, Matsuyama T, Kundig T, Wakeham A, Kishihara K, Shahinian A, Wiegmann K, Ohashi PS, Krönke M, Mak TW. 1993. Mice deficient for the 55Kd tumor necrosis factor receptor are resistant to endotoxic shock yet succumb to L. monocytogenes infection. *Cell.* 73: 457.

31. Mohler KM, Torrance DS, Smith CA, Goodwin RG, Stremler KE, Fung VP, Madani H, Widmer MB. 1993. Soluble tumor necrosis factor receptors are effective therapeutic agents in lethal endotoxemia and function simultaneously as both TNF carriers and TNF antagonists. *J Immunol.* 151: 1548.

32. Aderka D, Engelmann H, Maor Y, Brakebusch C, Wallach D. 1992. Stabilization of the bioactivity of tumor necrosis factor by ist soluble receptors. *J Exp Med.* 175: 323.

33. Diez-Ruiz A, Tilz GP, Zangerle R, Baier-Bitterlich G, Wachter H, Fuchs D. 1995. Soluble receptors for tumor necrosis factor in clinical laboratory diagnosis. *Eur J Haematol.* 54: 1.

34. Hale KK, Smith CG, Baker SL, Vanderslice RW, Squires CH, Gleason TM, Tucker KK, Kohn T, Russel DA. 1995. Multifunctional regulation of the biological effects of TNF-α by its soluble type I and type II TNF receptors. *Cytokine.* 1: 26.

35. Milani RV, Mehra MR, Endres S, Eigler A, Lavie CJ, Ventura HO. 1996. The clinical relevance of circulating tumor necrosis factor alpha in acute decompensated chronic heart failure patients without cachexia. *Chest.* 110: 992.

36. Parissis JT, Venetsanou KF, Mentzikof DG, Ziras NG, Kefalas CG, Karas SM. 1999. Tumor necrosis factor alpha serum activity during treatment of acute decompensation of cachectic and non-cachectic patients with advanced heart failure. *Scand Cardiovasc J.* 33: 344.

37. Vanderheyden M, Kersschot E, Paulus WJ. 1998. Pro-inflammatory cytokines and endothelium-dependent vasodilation in the forearm. Serial assessment in patients with congestive heart failure. *Eur Heart J.* 19: 747.

38. Sato H, Seiki M. 1993. Regulatory mechanism of 92kDa type IV collagenase gene expression which is associated with invasiveness of tumor cells. *Oncogene.* 8: 395.

39. Yokoo T, Kitamura M. 1996. Dual regulation of IL-1 beta mediated matrix metalloproteinase 9 expression in mesangial cells by NF-kappa B and AP-1. *Am J Physiol.* 270: F123.

40. Gao CQ, Sawicki G, Suarez-Pinzon WL, Csont T, Wazniak M, Ferdinandy P, Schulz R. 2003. Matrix metalloproteinase-2 mediates cytokine induced myocardial contractile dysfunction. *Cardiovasc Res.* 57: 426.

41. Okamoto T, Akaike T, Sawa T, Miyamoto Y, van der Vliet A, Maeda H. 2001. Activation of matrix metallopoteinases by peroxinitrate induced protein S-glutathiolation via disulfide S-oxide formation. *J Biol Chem.* 276: 29596.