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Interleukin-6 Spillover in the Peripheral Circulation Increases With the Severity of Heart Failure, and the High Plasma Level of Interleukin-6 Is an Important Prognostic Predictor in Patients With Congestive Heart Failure

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Objectives. **We 1) evaluated whether interleukin-6 (IL-6) is produced in the peripheral circulation in patients with congestive heart failure (CHF), 2) estimated the factors for increased IL-6, and 3) clarified the prognostic role of high plasma levels of IL-6 in patients with CHF.**

Background. **Although plasma levels of IL-6 have been reported to increase in patients with CHF, and production of IL-6 in endothelial cells and vascular smooth muscle cells has been postulated from in vitro studies, the origin of the increase of IL-6 in CHF remains unknown. Moreover, the prognostic value of a high plasma level of IL-6, independent of classic neurohumoral factors, remains to be elucidated.**

Methods. **A comparison was made of the plasma levels of IL-6 between the femoral artery and the femoral vein in 13 normal subjects and in 80 patients with CHF. In another study, we measured plasma IL-6 in 100 patients with CHF and follow-up data.**

Results. **Plasma IL-6 levels increased significantly from the femoral artery to the femoral vein in normal subjects and in patients with CHF. Arteriovenous IL-6 spillover in the leg increased with the severity of CHF. Among the hemodynamic variables and the various neurohumoral factors, the plasma norepinephrine (NE) level showed an independent and significant positive relation with the plasma IL-6 level in patients with CHF. Moreover, treatment with beta-adrenergic blocking agents showed an independent and significant negative relation with plasma IL-6 levels. In 100 patients, plasma IL-6 ($p < 0.0001$), NE ($p = 0.0004$) and left ventricular ejection fraction (0.015) were significant independent prognostic predictors by Cox proportional hazards analysis.**

Conclusions. **Our findings indicate that the IL-6 spillover in the peripheral circulation increases with the severity of CHF and that the increase in plasma IL-6 is mainly associated with the activation of the sympathetic nervous system. High plasma levels of IL-6 can provide prognostic information in patients with CHF, independent of left ventricular ejection fraction and plasma NE, suggesting an important role for IL-6 in the pathophysiology of CHF.**

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Hemodynamic abnormality as well as overexpression of neurohormonal factors has been considered to play an important role in the progression of congestive heart failure (CHF). Increased levels of various vasoconstrictor neurohumoral factors have been found in patients with chronic CHF (1–6), and high plasma levels of norepinephrine (NE), renin and endothelin-1 (ET-1) have been reported to be significant prognostic predictors (4,7,8), suggesting an important role for vasoconstrictors in the pathogenesis of CHF. High levels of vasodilator neurohumoral factors have also been found in patients with CHF, and a high plasma level of cardiac natriuretic peptides has been reported to be a significant prognostic predictor (7,9–11). In addition, recent studies have indicated the potential role of the immune system in the pathophysiology of CHF (12–14). However, whether cytokines play a significant role in the pathophysiology or prognosis of CHF, or both, independent of classic neurohumoral factors, remains to be elucidated.

Interleukin-6 (IL-6) is a multifunctional proinflammatory and vasodepressor cytokine that mediates both immune and inflammatory responses (15). The recent interest in studying IL-6 in CHF has been prompted by the observation that IL-6, like tumor necrosis factor (TNF)-alpha, can produce not only myocardial dysfunction (16) but also abnormal endothelium-

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Abbreviations and Acronyms

AMP = adenosine monophosphate
ANP = atrial natriuretic peptide
CHF = congestive heart failure
ET-1 = endothelin-1
IL-6 = interleukin-6
NE = norepinephrine
TNF = tumor necrosis factor

dependent vasodilation (17) and muscle wasting (18,19). Although elevated levels of IL-6 have been recently identified in patients with CHF (20,21), the origin of the increase remains unknown. IL-6 is produced not only in leukocytes (15,22,23) but also in endothelial cells (24) and vascular smooth muscle cells (25) in vitro, and its secretion from the endothelium was increased by ET-1 (26), a potent vasoconstrictor (27), suggesting that the local production of IL-6 regulates vascular resistance or permeability, or both, in patients with CHF. We hypothesized that local IL-6 production from the peripheral vascular beds increases with the severity of CHF and is related to muscle wasting in patients with severe CHF. In addition, IL-6 and the IL-6 receptor complex, including gp-130, play an important role in ventricular hypertrophy (28) and left ventricular remodeling, and the high concentration of IL-6 may exert endocrine effects such as a decrease of left ventricular contractility through nitric oxide production (16), indicating that patients with CHF with high plasma levels of IL-6 may have a poor prognosis. Therefore, the present study was performed 1) to determine the difference in arterial and venous IL-6 levels; 2) to evaluate the factors for increased IL-6; and 3) to clarify the prognostic role of high plasma levels of IL-6 in patients with CHF.

Methods

Study 1. *Patients.* We studied 80 patients with CHF who were without renal failure, infection, malignancy or collagen disease and who underwent cardiac catheterization for clinical indications. We also selected 13 age-matched normal subjects who were admitted complaining of chest pain, which proved to be normal by coronary angiography. Written informed consent was obtained from all patients before participation in the study, and the protocol was approved by the Human Investigations Committee of our institution. The subjects included 60 men and 20 women (age range 27 to 79 years, mean 59). Forty-eight patients had a myocardial infarction more than 3 months before the study, and 32 had dilated cardiomyopathy. Fifty-six patients were classified according to the standards of the New York Heart Association as functional class II, 14 patients as class III and 10 patients as class IV. At the beginning of the study, 63 patients were treated with diuretic agents, 41 with angiotensin-converting enzyme inhibitors, 40 with digitalis, 60 with vasodilators, 15 with beta-blockers and 5 with docarpamine, an orally active dopamine prodrug (29). All drug treatments were discontinued at least 12 h before the study.

Protocol. All patients were premedicated with an oral dose of diazepam (5 mg) and left to rest in bed in the supine position for at least 20 min. Right-sided cardiac catheterization was performed using a 7F Swan-Ganz catheter. The heart rate was monitored by electrocardiography. Blood samples for measuring plasma IL-6 and TNF-alpha were collected simultaneously from the right femoral artery and vein. Blood samples for measuring the plasma levels of NE, epinephrine, angiotensin II and ET-1 were also drawn from the right femoral artery. A Swan-Ganz catheter was inserted through the right femoral vein into the main pulmonary artery, where the pressure was measured. All blood sampling was done within 5 min before the administration of contrast medium and heparin. Cardiac output was determined by the thermodilution method immediately after blood collection. Left ventriculography was performed with a contrast medium or radioisotope before or within 1 week of the hemodynamic measurements and blood sampling.

Measurement of IL-6 and TNF-alpha. Blood for the measurement of plasma levels of IL-6 and TNF-alpha was transferred to a chilled tube containing EDTA (1 mg/ml) and aprotinin (500 kallikrein inactivator U/ml), and then centrifuged at 3,000 rpm for 15 min at 4°C. The plasma thus obtained was stored at −30°C until it was assayed. Both IL-6 and TNF-alpha measurements were performed using a commercially available immunoassay (Quantikine HS, R&D Systems). The intraassay coefficients of variation for IL-6 and TNF-alpha are 3.6% and 6.1%, respectively; the intraassay coefficients of variation for IL-6 and TNF-alpha are 3.8% and 7.8%, respectively. The minimal detectable values of TNF-alpha and IL-6 were 0.12 and 0.094 pg/ml, respectively. The assay system for IL-6 has no cross reactivity for other cytokines, including TNF-alpha, and the assay system for TNF-alpha has no cross reactivity for other cytokines, including IL-6.

Measurement of immunoreactive ET-1 and other vasoconstrictor factors. Blood for the measurement of plasma ET-1 levels was transferred to a chilled tube containing EDTA (1 mg/ml) and aprotinin (500 kallikrein inactivator U/ml) and then centrifuged at 3,000 rpm for 15 min at 4°C. The plasma thus obtained was stored at −30°C until it was assayed. Extraction of ET-1 was performed by mixing 2 ml of plasma with 0.4 ml of 60% methanol containing 0.25 mg/ml of Preparative C18 (Waters Chromatography Division). The recovery rate was calculated to be 72 ± 3.9% (n = 5) by iodine-125 ET-1 with this method. The plasma ET-1 levels was determined by using an antibody directed against synthetic ET-1 (Peninsula Laboratories, Inc.) and iodine-125 ET-1 (Amersham Japan, Tokyo, Japan), as previously reported (6). This antibody showed 100% cross reactivity with ET-1, 7% with ET-2, 7% with ET-3 and 17% with human big ET-1. However, it did not cross react with angiotensin I or II, vasopressin or human cardiac natriuretic peptides. The minimal detectable level of ET-1 was 0.5 pg/tube; the interassay

Table 1. Hemodynamic Data

	HR (beats/min)	LVEF (%)	RAP (mm Hg)	MPAP (mm Hg)	PCWP (mm Hg)	CI (liters/min per m^2)	MBP (mm Hg)	SVR (dynes·s·cm^{-5})
Normal (n = 13)	71.9 ± 3.5	64 ± 3.2	1.8 ± 0.5	11.5 ± 0.9	5.2 ± 0.8	3.0 ± 0.1	86 ± 2.3	1,544 ± 103
Mild CHF (n = 56)	70.7 ± 1.7	39 ± 1.3*	2.8 ± 0.4	18.7 ± 0.9†	10.9 ± 0.6†	2.8 ± 0.1	88 ± 1.9	1,605 ± 53
Severe CHF (n = 24)	87.6 ± 3.0‡	32 ± 2.0‡	6.6 ± 1.0§	28.7 ± 2.5§	18.0 ± 1.8§	2.6 ± 0.1	88 ± 2.8	1,665 ± 78

*$p < 0.001$, †$p < 0.05$, versus normal subjects by analysis of variance with Scheffé F test. ‡$p < 0.05$, §$p < 0.01$ versus normal subjects and patients with mild congestive heart failure (CHF) (functional class II) by analysis of variance with Scheffé F test. Data presented are mean value ± SEM. CI = cardiac index; HR = heart rate; LVEF = left ventricular ejection fraction; MBP = mean arterial blood pressure; MPAP = mean pulmonary artery pressure; PCWP = pulmonary capillary wedge pressure; RAP = right atrial pressure; SVR = systemic vascular resistance; Severe CHF = patients with severe congestive heart failure (functional class III or IV).

coefficient of variation was 13% (n = 10); and the intraassay coefficient of variation was 11% (n = 9).

Blood for measurement of the plasma levels of NE, epinephrine and angiotensin II was transferred to a chilled tube containing EDTA (1 mg/ml) and centrifuged at 3,000 rpm for 15 min at 4°C, and the plasma thus obtained was stored at −30°C until assay. Plasma NE and epinephrine concentrations were measured by high performance liquid chromatography. Plasma angiotensin II levels were measured by a radioimmunoassay using a specific antibody directed against synthetic angiotensin II (Special Research Laboratory, Tokyo, Japan), as previously reported (6).

Calculations. The mean blood pressure and systemic vascular resistance were calculated from standard formulas. The index of the amount of IL-6 spillover in the peripheral circulation was determined with the following formula (30): (IL-6 level at site of femoral vein − IL-6 level in femoral artery) × Cardiac output × (1 − Hematocrit/100) (ng/min).

Study 2. *Patients.* The group consisted of 100 patients with chronic CHF (75 men and 25 women; age range 17 to 82 years, mean 59) admitted to the hospital at our institution. The cause of heart failure was dilated cardiomyopathy in 41 patients, ischemic heart disease (old myocardial infarction >3 months after the attack) in 53 patients and hypertensive heart disease in 6 patients. The mean left ventricular ejection fraction was 39% by left ventriculography with a radionuclide or contrast medium. Patients who had an infection, chronic inflammatory disease, malignancy or renal failure were excluded. Forty-five patients were in functional class II heart failure, 35 in class III and 20 in class IV. All patients were clinically stable with constant doses of diuretic agents; 75 patients were treated with digoxin, 68 with angiotensin-converting enzyme inhibitors, 50 with vasodilators and 15 with beta-blockers. All drugs were administered for at least 4 weeks (most drugs >3 months).

Protocol. Blood samples for the measurement of plasma atrial natriuretic peptide (ANP), NE and IL-6 were collected from the peripheral vein after at least 30 min of bed rest with the patients in the supine position. Left ventriculography was performed with a contrast medium or radioisotope before or within 1 week of the blood sampling. Thirty-one patients had a cardiac death during the follow-up period (after 0.5 to 24 months). All surviving patients were followed up for >3 months (mean 28 months, range 3 to 54).

Measurements of NE, IL-6 and ANP. Measurements of NE and IL-6 were obtained as in study 1. Samples for the assay of the plasma ANP concentrations were transferred to chilled disposable tubes containing aprotinin (500 kallikrein inactivator U/ml). The blood samples were immediately placed on ice and centrifuged at 4°C. Plasma ANP concentrations were measured with a specific immunoradiometric assay for alpha-human ANP using a commercial kit (Shionoria, Japan), as previously reported (11). Briefly, this assay used two monoclonal antibodies against alpha-human ANP, one recognizing a carboxyterminal sequence and the other the ring structure of ANP, and measured alpha-human ANP by sandwiching it between the two antibodies without plasma extraction. The minimal detectable quantity of alpha-human ANP was 5 pg/ml. The intraassay and interassay coefficients of variation were 5.1% and 5.8%, respectively. This assay system did not cross react with angiotensin I or II, vasopressin or human brain natriuretic peptide.

Statistical analysis. All results were expressed as the mean value ± SEM. Comparison of the plasma levels of IL-6 and TNF-alpha at the femoral artery and femoral vein was done using the paired Student *t* test. Comparisons between multiple groups were determined by one-way analysis of variance with the Scheffé F test. Univariate and stepwise multivariate linear regression analyses were used to detect independent predictors of plasma IL-6 and IL-6 spillover in the peripheral circulation among 22 variables. Linear regression analysis was used to determine the relations between continuous variables. Kaplan-Meier analysis was performed on the cumulative rates of survival in patients with CHF stratified into two groups on the basis of median plasma levels of IL-6, and the differences between survival curves were analyzed by the log-rank test. To determine whether the plasma IL-6 concentration seen in our patients with CHF was an independent prognostic factor or only reflected the importance of other factors, eight variables, as listed in Table 1, were entered into a Cox proportional hazards analysis (univariate and stepwise multivariate analyses). A p value <0.05 was considered to indicate statistical significance.

Table 2. Neurohumoral and Cytokine Data

					TNF-Alpha		IL-6	
	NE (pg/ml)	Epi (pg/ml)	Ang II (pg/ml)	ET-1 (pg/ml)	FA (pg/ml)	FV (pg/ml)	FA (pg/ml)	FV (pg/ml)
Normal (n = 13)	209 ± 27	32 ± 5	10 ± 3	2.1 ± 0.2	2.8 ± 0.4	2.7 ± 0.4	1.0 ± 0.2	1.2 ± 0.2
Mild CHF (n = 56)	329 ± 38	28 ± 3.3	18 ± 2.4	2.3 ± 0.1	4.2 ± 0.3	4.3 ± 0.3	2.6 ± 0.2	2.9 ± 0.3
Severe CHF (n = 24)	931 ± 180*	78 ± 17*	27 ± 7	3.9 ± 0.5*	5.8 ± 0.8†	5.4 ± 0.7†	18.3 ± 7.8‡	20.9 ± 8.5‡

*$p < 0.01$, †$p < 0.05$ versus normal subjects and patients with mild congestive heart failure (CHF) (functional class II) by analysis of variance with Scheffé F test. ‡$p < 0.01$ versus patients with mild congestive heart failure by analysis of variance with Scheffé F test. Data presented are mean value ± SEM. Ang = angiotensin II; Epi = epinephrine; ET-1 = endothelin-1; FA = femoral artery; FV = femoral vein; IL-6 = interleukin-6; NE = norepinephrine; TNF-Alpha = tumor necrosis factor-alpha.

Results

Hemodynamic data (Table 1). The left ventricular ejection fraction was significantly lower in patients with severe CHF than in patients with mild CHF. In contrast, the mean pulmonary artery pressure and pulmonary capillary wedge pressure increased with the severity of CHF. Systemic vascular resistance was slightly higher in patients with severe CHF than in patients with mild CHF.

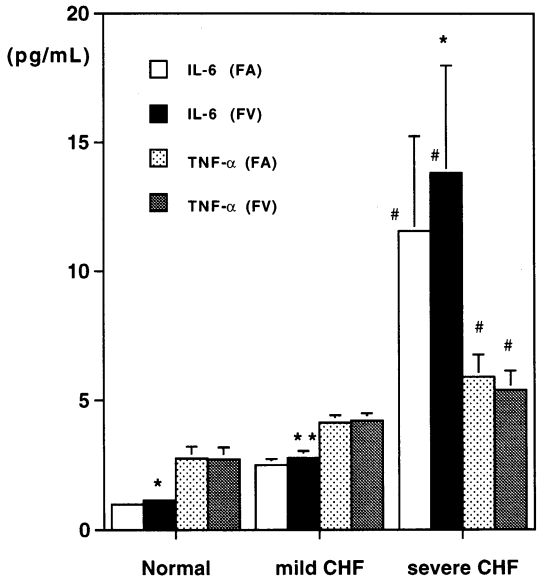
Neurohumoral and cytokine data (Table 2). Plasma levels of NE, epinephrine and ET-1 were significantly higher in patients with severe CHF than in patients with mild CHF. Plasma levels of TNF-alpha were significantly higher both in the femoral artery and in the femoral vein in patients with severe CHF than in patients with mild CHF. Plasma levels of IL-6 were also significantly higher both in the femoral artery and in the femoral vein in patients with severe CHF than in patients with mild CHF.

Plasma IL-6 concentrations and IL-6 spillover in the peripheral circulation. Figure 1 shows the plasma levels of TNF-alpha and IL-6 at the femoral artery and femoral vein in age-matched normal subjects and patients with CHF. There was no difference in plasma TNF-alpha levels between the femoral artery and the femoral vein both in normal subjects and in patients with CHF. In contrast, plasma IL-6 concentrations increased significantly from the femoral artery to the femoral vein in normal subjects and in patients with CHF. IL-6 spillover in the leg was markedly increased in patients with severe CHF compared with normal subjects and patients with mild CHF (Fig. 2). Both plasma IL-6 levels and IL-6 spillover in the leg were significantly higher in female patients than in male patients (Fig. 3).

Relation between plasma IL-6 concentrations and 22 variables, including hemodynamic data, clinical variables and neurohumoral factors. On univariate analysis, there were significant correlations between the plasma IL-6 concentration and hemodynamic data such as heart rate, right atrial pressure, mean pulmonary artery pressure, pulmonary capillary wedge pressure and left ventricular ejection fraction. There were also significant positive correlations between the plasma IL-6 level and other biochemical factors such as NE (Fig. 4), epinephrine, ET-1, TNF-alpha and angiotensin-II levels. In addition, plasma IL-6 levels were significantly higher in female patients and in patients with severe symptoms or those treated with docarpamine (Table 3). On multivariate analysis, plasma levels of NE and epinephrine showed independent and significant positive relations with the plasma IL-6 level, and treatment with beta-blockers showed independent and significant negative relations with plasma IL-6 levels among 22 variables (Table 3). Moreover, female gender was also an independent significant predictor of high plasma levels of IL-6 in patients with CHF (Table 3).

Relation between plasma IL-6 spillover in the peripheral circulation and 22 variables, including hemodynamic data, clinical variables and neurohumoral factors. On univariate analysis, there were significant correlations between the

Figure 1. Plasma levels of IL-6 and TNF-alpha in the femoral artery (FA) and femoral vein (FV) in normal subjects and in patients with CHF. *$p < 0.01$ and **$p < 0.001$ compared with the value of IL-6 in the femoral artery. #$p < 0.01$ compared with mild CHF (analysis of variance with the Scheffé F test).



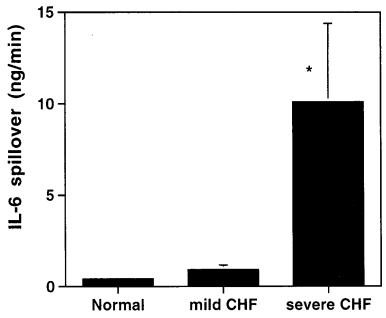


Figure 2. Interleukin-6 spillover in the peripheral circulation in normal subjects and in patients with CHF. *$p < 0.05$ compared with the value for normal subjects and those with mild CHF (analysis of variance with the Scheffé F test).

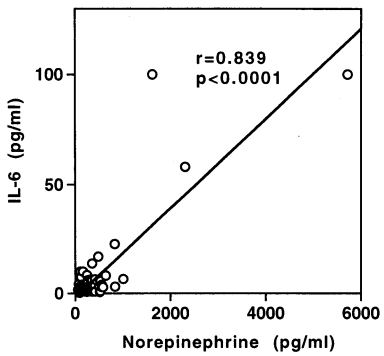


Figure 4. Relation between the plasma levels of NE and IL-6 concentrations in patients with CHF.

plasma IL-6 spillover in the peripheral circulation and hemodynamic data such as heart rate, right atrial pressure, mean pulmonary artery pressure, pulmonary capillary wedge pressure and left ventricular ejection fraction, as well as other biochemical factors such as NE, epinephrine, ET-1, TNF-alpha and angiotensin-II levels. In addition, plasma IL-6 spillover in the peripheral circulation was significantly higher in female patients and in patients with severe symptoms or those treated with docarpamine (Table 4). On multivariate analysis, only high plasma NE levels and treatment with docarpamine showed independent and significant positive relations with the plasma IL-6 spillover in the peripheral circulation (Table 4).

Relation between plasma IL-6 spillover in the peripheral circulation and body mass index. Although there was no correlation between plasma IL-6 levels in the femoral artery or vein and body mass index, there was a significant negative correlation between plasma IL-6 spillover in the peripheral circulation and body mass index in patients with CHF ($r = -0.24$, $p = 0.034$).

High plasma levels of IL-6 as a predictor of mortality in patients with CHF. Eight variables, including functional class, ejection fraction, neurohumoral variables and plasma IL-6 levels, were analyzed using univariate and stepwise multivariate Cox proportional hazards regression analyses in study 2 groups (Table 5). On univariate analyses, high plasma levels of NE, ANP and IL-6, functional class, ejection fraction and age were significant predictors of mortality. According to stepwise multivariate analyses, only high plasma levels of IL-6 ($p < 0.0001$) and NE ($p = 0.0004$) and a low left ventricular ejection fraction were significant independent predictors (Table 5). An elevated level of plasma IL-6 was a prognostic predictor by Kaplan-Meier analysis (Fig. 5).

Figure 3. Gender difference of plasma IL-6 level and IL-6 spillover in the peripheral circulation in patients with CHF. *$p < 0.001$ compared with the value for male patients.

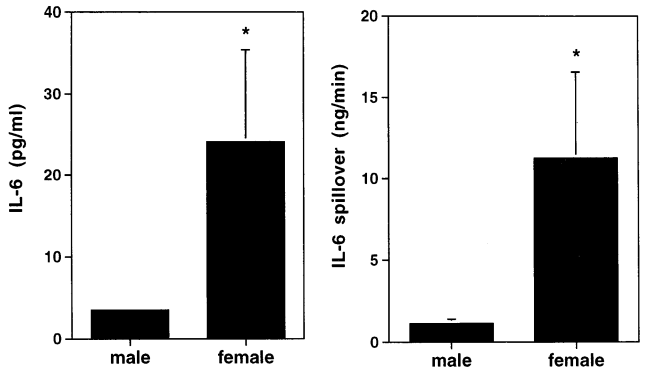


Table 3. Univariate and Multivariate Linear Model of Plasma Interleukin-6 in Femoral Vein of Patients With Congestive Heart Failure

Variable	Univariate Corr Coeff	p Value	Multivariate Beta-Coeff (SE)	p Value
Age	0.185	0.095		NS
Gender (M = 0; F = 1)	0.351	0.0012	4.06 (1.77)	0.024
Functional class	0.388	0.0003		NS
Diuretic drugs*	−0.04	0.732		NS
Digitalis*	0.14	0.208		NS
ACE inhibitors*	−0.125	0.263		NS
Vasodilators*	−0.20	0.071		NS
Beta-blockers*	−0.08	0.498	−6.16 (2.54)	0.017
Docarpamine*	0.479	< 0.0001		NS
HR	0.343	0.0016		NS
MBP	−0.09	0.411		NS
CI	0.122	0.275		NS
RAP	0.325	0.0029		NS
MPAP	0.231	0.036		NS
PCWP	0.295	< 0.0001		NS
LVEF	−0.323	0.0031		NS
NE	0.839	< 0.0001	0.015 (0.001)	< 0.0001
Epi	0.676	< 0.0001	0.103 (0.011)	< 0.0001
ET-1	0.576	< 0.0001		NS
TNF-alpha	0.381	0.0004		NS
Ang II	0.376	0.0005		NS
WBC	0.151	0.177		NS

*Treatment = 1; no treatment = 0. ACE = angiotensin-converting enzyme; Coeff = coefficient; Corr = correlation; F = female; M = male; WBC = white blood cells; other abbreviations as in Tables 1 and 2.

Table 4. Univariate and Multivariate Linear Model of Interleukin-6 Spillover in Peripheral Circulation of Patients With Congestive Heart Failure

Variable	Univariate Corr Coeff	p Value	Multivariate Beta Coeff (SE)	p Value
Age	0.121	0.28		NS
Gender (m = 0; F = 1)	0.329	0.0025		NS
Functional class	0.320	0.0034		NS
Diuretic drugs*	−0.03	0.817		NS
Digitalis*	0.08	0.44		NS
ACE inhibitors*	−0.09	0.408		NS
Vasodilators*	−0.163	0.143		NS
Beta-blockers*	−0.06	0.603		NS
Docarpamine*	0.677	< 0.0001	14.38 (2.8)	< 0.0001
HR	0.256	0.02		NS
MBP	−0.121	0.075		NS
CI	0.165	0.138		NS
RAP	0.261	0.017		NS
MPAP	0.252	0.022		NS
PCWP	0.262	0.017		NS
LVEF	−0.242	0.0288		NS
NE	0.875	< 0.0001	0.0013 (0.001)	< 0.0001
Epi	0.348	< 0.0001		NS
ET-1	0.557	< 0.0001		NS
TNF-alpha	0.510	< 0.0001		NS
Ang II	0.338	0.0019		NS
WBC	0.124	0.267		NS

*Treatment = 1; no treatment = 0. Abbreviations as in Tables 1 to 3.

Discussion

We detected IL-6 spillover in the peripheral circulation of normal subjects and demonstrated that it increased in relation to the severity of CHF, suggesting that part of the source of the increased plasma IL-6 was the peripheral circulation in patients with CHF. In contrast, there was no difference in plasma TNF-alpha levels between the femoral artery and vein in patients with CHF. In the present study, both plasma IL-6 and TNF-alpha concentrations were increased in patients with severe CHF, consistent with other studies (20,21). The origin of the increase is thought to be mainly leukocytes, including macrophages and lymphocytes (22,23). Because IL-6 was produced not only by leukocytes but also by endothelial cells and vascular smooth muscle cells in vitro (24,25), our findings suggest that IL-6 is produced by the vascular tissue in patients with severe CHF. We cannot rule out that the arteriovenous difference in IL-6 is derived from the leukocytes, which adhere to endothelial cells, but there was no arteriovenous difference in TNF-alpha, suggesting that the main source of IL-6 spillover in the peripheral circulation is the vascular endothelial cells or smooth muscle cells. Although the pathophysiologic role of IL-6 production in the peripheral circulation remains unknown, local IL-6 production may contribute to abnormalities of endothelium-dependent vasodilation, vascular resistance, increased vascular permeability (31) or muscle wasting (18,19) in patients with severe CHF. Indeed, there was a significant negative correlation between IL-6 spillover in the peripheral circulation and body mass index. Muscles of IL-6–producing transgenic mice suffer from atrophy and IL-6 receptor antibody inhibits muscle atrophy in this model (19); thus, the increase in IL-6 production in the peripheral circulation may contribute to muscle wasting in patients with CHF.

Table 5. Univariate and Multivariate Predictors of Mortality in 100 Patients With Chronic Heart Failure

Variable	Univariate Chi-Square	p Value	Multivariate Chi-Square	p Value
NE	32.9	< 0.0001	12.55	0.0004
ANP	27.5	< 0.0001	2.78	0.095
IL-6	18.8	< 0.0001	19.01	< 0.0001
Functional class	8.93	0.0028	1.55	0.213
LVEF	6.88	0.0087	5.92	0.015
Age	5.03	0.025	0.151	0.697
Gender	1.19	0.275	2.63	0.105
Etiology (DCM, non-DCM)	0.012	0.913	0.90	0.764

ANP = atrial natriuretic peptide; DCM = dilated cardiomyopathy; other abbreviations as in Tables 1 and 2.

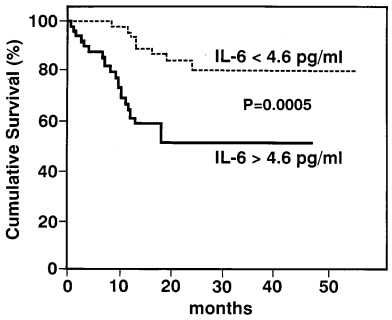


Figure 5. Kaplan-Meier analysis of cumulative rates of survival in patients with CHF stratified into two groups on the basis of median plasma concentration (4.6 pg/ml) of IL-6.

Why are the plasma IL-6 levels increased in patients with CHF? Because both TNF-alpha and ET-1 are increased in CHF (5,6,12,21) and stimulate IL-6 production in the endothelium in vitro (26,32), we hypothesized that IL-6 spillover is regulated by the local plasma levels of TNF-alpha and ET-1. Although there was a significant positive correlation between the plasma levels of IL-6 and TNF-alpha and ET-1 on univariate analysis, no significant relation was obtained between the plasma level of IL-6 and the plasma levels of TNF-alpha and ET-1 when the plasma NE level was included by multivariate analysis. Interleukin-6 has been reported to be produced in various cells, including lymphocytes, monocytes, granulocytes, mast cells and endothelial cells. Although the mechanism of the increase of IL-6 production has not been fully elucidated, IL-6 secretion is increased through an elevation of intracellular cyclic adenosine monophosphate (AMP) levels (33,34), suggesting that the main mechanism for regulation of the increase of IL-6 spillover in the peripheral vascular circulation is activity of the sympathetic nervous system involving probably an

increase in intracellular cyclic AMP. Docarpamine, an orally active dopamine prodrug, is also known to increase intracellular cyclic AMP through the dopamine receptor, and the finding that treatment with docarpamine is a significant predictor of IL-6 spillover independent of plasma NE level supports our hypothesis. Because IL-6 inhibits vascular constriction by phenylephrine in vitro (35), an increase in its IL-6 production in the peripheral vascular tissue may counteract the excessive vasoconstriction through the alpha receptor by stimulation of the sympathetic nervous system in patients with CHF. The local increase of IL-6 production in the vascular smooth muscle cells also increases inducible nitric oxide synthase and may cause a compensatory vasodilation against various activated vasoconstrictors such as ET-1, angiotensin II and NE in patients with CHF.

The increase in the plasma levels of IL-6, which is derived from various cells including leukocytes (lymphocytes, macrophages and granulocytes), is also regulated by plasma levels of catecholamines such as NE and epinephrine. In the present study, interestingly, treatment with beta-blockers showed a negative relation to plasma IL-6 levels in patients with CHF. These findings indicate that the increase in IL-6 production in not only vascular tissue but also in other cells such as lymphocytes and macrophages is mainly associated with activation of the sympathetic nervous system in patients with CHF. Moreover, female gender also shows an independent significant relation to high plasma levels of IL-6 in patients with CHF. Although various neurohumoral factors including cytokine concentrations have been reported to increase in CHF, to our knowledge, there has been no report with regard to a gender difference independent of severity of CHF or hemodynamic abnormalities. In the present study, plasma IL-6 levels were significantly higher in female patients (most were postmenopause). Because it is well known that estrogen loss causes an increase in IL-6 activity and contributes to the increased bone resorption in postmenopausal osteoporosis (36), the decrease of estrogen may increase plasma IL-6 levels in postmenopausal women with CHF. Indeed, recently, plasma levels of IL-6 were reported to correlate with postmenopausal status in normal subjects (37).

Plasma IL-6 levels as an independent prognostic predictor in patients with CHF. Although concentrations of various cytokines have been reported to increase in patients with CHF, it is not clear whether these activations are associated with the progression of CHF independent of activated classic neurohumoral factors or low left ventricular ejection fraction. In the present study, eight variables, including functional class, ejection fraction, neurohumoral variables and plasma IL-6 levels, were analyzed using univariate and stepwise multivariate Cox proportional hazards regression analyses. As previously reported, high plasma levels of NE and ANP and low left ventricular ejection fraction were prognostic factors in CHF by univariate analysis. According to stepwise multivariate analyses, a high plasma level of IL-6 was a significant independent predictor, suggesting an important role for IL-6 in the pathophysiology of CHF. The prognostic role of IL-6 is not reflected by the etiology of CHF (dilated cardiomyopathy or nondilated cardiomyopathy), also suggesting that a high plasma level of IL-6 is a significant prognostic predictor independent of etiology in patients with chronic CHF. A recent report that IL-6 production is detected in cardiac myocytes and increased by catecholamine (38) suggests that the increase in plasma IL-6 is partly of ventricular origin, and IL-6 modifies the ventricular function through increase of nitric oxide synthase (16). Indeed, in the present study, there was a negative correlation between the plasma IL-6 level and left ventricular ejection fraction by univariate analysis, but the relation was no longer significant when the plasma catecholamine level was included by multivariate analysis, indicating that ventricular IL-6 is a more important regulator of ventricular dysfunction than plasma IL-6, because IL-6 acts in an autocrine and a paracrine manner.

Study limitations. In the present study, blood samples were collected from the femoral artery and vein to confirm the spillover or extraction of IL-6 in the peripheral circulation. We previously used the same methods to evaluate the extraction of ANP and the production of cyclic guanosine monophosphate in the peripheral circulation (30). However, the calculated values for IL-6 spillover may be underestimated because IL-6 acts in an autocrine and paracrine manner. The pathophysiologic role of the production of IL-6 in the peripheral circulation of patients with CHF remains unknown and we did not evaluate endothelium-dependent vasodilation in the present study; therefore, further studies using IL-6 receptor antagonists are need to clarify our hypothesis.

Conclusions. A partial source of circulating IL-6 is the peripheral vascular bed in normal subjects and in patients with CHF. Although the physiologic and pathophysiologic role of IL-6 spillover in the legs remains unclear, IL-6 spillover in the peripheral circulation increases with the severity of CHF and is mainly associated with the activation of the sympathetic nervous system. A high plasma level of IL-6 is a prognostic predictor in patients with CHF independent of left ventricular ejection fraction and plasma NE, suggesting an important role for IL-6 in the pathophysiology of CHF.

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