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# High Levels of Plasma Brain Natriuretic Peptide and Interleukin-6 After Optimized Treatment for Heart Failure Are Independent Risk Factors for Morbidity and Mortality in Patients With Congestive Heart Failure

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**OBJECTIVES** The aim of this study was to evaluate whether repetitive measurements of plasma levels of neurohumoral factors and cytokines before and after additional treatment are useful for predicting mortality in patients with congestive heart failure (CHF).

**BACKGROUND** Neurohumoral and immune activation play an important role in the pathophysiology of CHF. However, the effects of serial changes in these factors on the prognostic value remain unknown.

**METHODS** We measured plasma levels of neurohumoral factors and cytokines and left ventricular ejection fraction (LVEF) before and three months after optimized treatment for CHF in 102 consecutive patients with severe CHF (New York Heart Association class III to IV) on admission to our hospital. Physicians who were blind to the plasma neurohumoral factors until study completion treated patients using standard drugs. Patients were monitored for a mean follow-up period of 807 days.

**RESULTS** Plasma levels of neurohumoral factors, cytokines and LVEF were significantly improved three months after optimized treatment. Cardiac death occurred in 26 patients. Among 19 variables including LVEF, only a high level of brain natriuretic peptide (BNP) and interleukin-6 (IL-6) at three months after optimized treatment showed significant independent relationships by Cox proportional hazard analysis with a high mortality for patients with CHF.

**CONCLUSIONS** These findings indicate that high plasma BNP and IL-6 levels three months after optimized treatment are independent risk factors for mortality in patients with CHF, suggesting that sustained high plasma levels of BNP and IL-6 after additional standard treatment were independent risk factors for mortality in patients with CHF despite improvements in LVEF and symptoms. (J Am Coll Cardiol 2000;36:1587–93) © 2000 by the American College of Cardiology

Increased levels of various neurohumoral factors have been found in patients with chronic congestive heart failure (CHF) (1–8), and high plasma levels of norepinephrine (NE), endothelin-1 (ET-1), atrial natriuretic peptide (ANP) and brain natriuretic peptide (BNP) have been reported to be significant prognostic predictors (9–15), suggesting an important role of neurohumoral activation in the pathogenesis of CHF. Moreover, recent studies have indicated the potential role of the immune system in the pathophysiology of CHF (16–19). Plasma levels of interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF-alpha) increased in patients with CHF (20–22) and have been reported to be significant prognostic predictors in patients with CHF (22).

In previous reports, the neurohumoral factors and cytokines were obtained by only one point sampling, when the condition of patients was relatively stable, to evaluate the prognostic value (2,10–16,22). Although there are several reports estimating the effect of cardiovascular drugs on neurohumoral factors and cytokines over two time points (7,8,23–25), there is no report indicating whether repetitive measurements of these factors before and after additional treatment for CHF are useful for predicting mortality.

The results of recent clinical trials evaluating the drug effect on mortality in CHF strongly suggested the important role of the activation of neurohumoral factors and cytokines, but there was no report about the relationship between changes in neurohumoral factors and prognosis after standard therapy. Therefore, in this study, we evaluated whether repetitive measurements of plasma levels of neurohumoral factors and cytokines before and after optimized treatment for CHF are useful for predicting morbidity and mortality for patients with CHF.

## METHODS

**Patients.** One hundred twelve consecutive patients with chronic severe CHF (New York Heart Association

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**Abbreviations and Acronyms**

| | | |
|---|---|---|
| ACE | = | angiotensin-converting enzyme |
| ANP | = | atrial natriuretic peptide |
| BNP | = | brain natriuretic peptide |
| CHF | = | congestive heart failure |
| ET-1 | = | endothelin-1 |
| IL-6 | = | interleukin-6 |
| LVEF | = | left ventricular ejection fraction |
| MI | = | myocardial infarction |
| NE | = | norepinephrine |
| NYHA | = | New York Heart Association |
| TNF-alpha | = | tumor necrosis factor-alpha |

[NYHA] functional class III to IV) who were hospitalized in our institution from January 1995 to January 1999 were entered into this study. Informed consent was obtained from all patients for participation in the study according to a protocol approved by the Committee on Human Investigation at our institution. The 70 men and 42 women ranged in age from 16 to 83 years (mean age 63.9 ± 1.4 years). On echocardiography, all patients showed left ventricular ejection fraction (LVEF) measured by the biplane disc summation method (Simpson's rule) (26) <45% (mean LVEF 23.2 ± 0.9%) before optimized treatment for CHF. The cause of CHF was dilated cardiomyopathy in 54 patients, ischemic heart disease for more than three months since myocardial infarction (MI) in 39 patients, hypertensive heart disease in 14 patients and regurgitative valvular heart disease in 5 patients. Patients with acute MI, renal failure, infection, chronic inflammatory disease and malignancy were excluded. Sixty-one patients were in NYHA functional class III, and 51 patients were in class IV. Thirty-eight patients had been treated previously with diuretics, 28 with angiotensin-converting enzyme (ACE) inhibitors, 26 with digitalis, 16 with beta-adrenergic blocking agents, 13 with nitrates and 10 with a calcium antagonist (amlodipine).

**Study protocol.** This is a prospective study; study protocol is shown in Figure 1. At entry, after at least 30 min of bed rest in a supine position, blood samples were drawn from the antecubital vein, and, at the same time, LVEF was measured by echocardiography. After all patients had been treated using optimal oral drugs for three months, plasma levels of neurohumoral factors and cytokines and LVEF were measured again by the same sonographer before and three months after additional treatment of CHF in all patients. Physicians who were blind to the plasma levels of neurohumoral factors and cytokines until study completion independently selected the additional drugs and managed patients by standard regimens, such as improvement of symptoms, physical examination and pulmonary congestion on chest X-ray. Stable clinical condition was defined as stable body weight and the absence of signs of pulmonary or peripheral congestion (27). If digitalis was titrated, the serum concentration was between 0.5 and 1.0 ng/ml. Diuretics were given in flexible dosages on the basis of body weight and daily diuresis. Angiotensin-converting enzyme inhibitors and beta-blockers were gradually increased to the maximal dosage possible. The maximal dosage for enalapril was 20 mg. The maximal dosage for carvedilol and metprolol were 20 mg and 60 mg/day, respectively. All surviving patients were monitored for >120 days, for a mean follow-up period of 807 ± 42.3 days (range 120 to 1,568 days). The end points, which were judged independently by researchers, were cardiac death (worsening CHF, lethal MI or sudden death) or hospitalization for worsening CHF or MI.

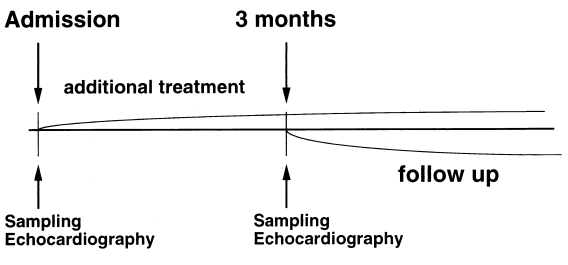


**Figure 1.** Study protocol.

**Measurements of plasma levels of neurohumoral factors and cytokines.** Samples for the assay of plasma ANP, BNP, ET-1, IL-6 and TNF-alpha levels were transferred to chilled disposable tubes containing aprotinin (500 kallikrein inactivator U/ml) and ethylenediaminetetraacetic acid (1 mg/ml). Samples for the assay of plasma NE levels were transferred to chilled disposable tubes containing ethylenediaminetetraacetic acid (1 mg/ml). The blood samples were immediately placed on ice and centrifuged at 4°C, and aliquots of plasma were immediately stored at −30°C until assay.

Plasma ANP levels were measured with a specific immunoradiometric assay for human alpha-ANP using a commercial kit (Shionoria ANP kit, Osaka, Japan) as reported previously (13). Plasma BNP levels were measured with a commercially available specific immunoradiometric assay kit for human BNP (Shionoria BNP kit, Osaka, Japan) as reported previously (13). Plasma ET-1 levels were measured by radioimmunoassay as described (7). Plasma NE levels were measured by high performance liquid chromatography.

Both plasma levels of IL-6 and TNF-alpha measurements were performed using a commercially available immunoassay kit (Quantikine HS, R&D Systems, Minnesota). The intraassay coefficients of variation for IL-6 and TNF-alpha were 3.6% and 6.1%, respectively; the intraassay coefficients of variation for IL-6 and TNF-alpha were 3.8% and 7.8%, respectively. The minimal detectable values of TNF-alpha and IL-6 were 0.12 and 0.0094 pg/ml, respectively. The assay system for IL-6 had no cross reactivity for other cytokines, including TNF-alpha, and the assay system for TNF-alpha had no cross reactivity for other cytokines, including IL-6, as we previously reported (22).

**Statistical analysis.** All results were expressed as the mean ± SEM. Comparison of the LVEF and plasma levels of ANP, BNP, NE, ET-1, IL-6 and TNF-alpha before and three months after optimized treatment were made by

**Table 1.** Patient Characteristics in 102 Patients With CHF—Survivors and Nonsurvivors or Patients With Cardiac Event and Without Cardiac Event

| Characteristic | All Patients (n = 102) | Survivors (n = 76) | Nonsurvivors (n = 26) | Cardiac Event (−) (n = 55) | Cardiac Event (+) (n = 47) |
|---|---|---|---|---|---|
| Age (yr) | 63.6 ± 1.5 | 62.7 ± 1.7 | 66.3 ± 3.3 | 62.0 ± 2.1 | 65.6 ± 2.2 |
| Gender | | | | | |
| Male | 65 | 48 (63%) | 17 (65%) | 36 (65%) | 29 (62%) |
| Female | 37 | 28 (37%) | 9 (35%) | 19 (35%) | 18 (38%) |
| LVEF (%) | 23.0 ± 0.9 | 23.5 ± 1.0 | 21.6 ± 1.9 | 23.7 ± 1.3 | 22.2 ± 1.3 |
| NYHA class | | | | | |
| III | 57 | 45 (59%) | 12 (46%) | 29 (53%) | 28 (60%) |
| IV | 45 | 31 (41%) | 14 (54%) | 26 (47%) | 19 (40%) |
| Etiology | | | | | |
| IHD | 35 | 21 (28%) | 14 (54%)* | 13 (24%) | 22 (47%)* |
| Non-IHD | 67 | 55 (72%) | 12 (46%) | 42 (76%) | 25 (53%) |

*$p < 0.01$ vs. survivors or cardiac event (−).

ACE = angiotensin-converting enzyme; Cardiac Event = cardiac death (worsening CHF, lethal myocardial infarction or sudden death) and hospitalization for worsening CHF or myocardial infarction; CHF = congestive heart failure; IHD = ischemic heart disease; LVEF = left ventricular ejection fraction; NYHA = New York Heart Association.

Student paired *t* test. To determine whether repetitive measurements of plasma levels of neurohumoral factors and cytokines before and after additional treatment for CHF are useful for predicting mortality or morbidity and mortality in CHF patients, 19 variables, as listed below, were entered into a Cox proportional hazard analysis. Kaplan-Meier analysis was performed on the cumulative survival and cardiac event-free rates in patients with CHF stratified into two groups based on median plasma BNP and IL-6 levels; the differences between survival and cardiac event-free rate curves were analyzed by log-rank test. The sensitivity and specificity of plasma BNP and IL-6 values for predicting mortality or morbidity and mortality were determined, and receiver operating characteristic curves were constructed by plotting sensitivity against (1- specificity). The area under the curves were calculated and analyzed by one-tailed test. The optimal compromise between sensitivity and specificity was determined graphically. A value of $p < 0.05$ was considered significant.

## RESULTS

**Patient characteristics.** There were 112 patients enrolled in this study; however, seven patients died within three months during optimized treatment (six died of worsening CHF and one died of cerebral infarction), and three patients dropped out of the study. As a result, 102 patients could be followed-up from three months after optimized treatment. Cardiac death occurred in 26 patients during the follow-up period; the causes of death were: worsening CHF in 18 patients, sudden death in 6 patients and lethal MI in 2 patients. Twenty-one patients were hospitalized during the follow-up period; the causes of hospitalization were: worsening CHF in 19 patients and recurrent MI in 2 patients.

Patient characteristics are shown in Table 1.

There was no difference between survivors and nonsurvivors in age, gender, NYHA functional class and LVEF before optimized treatment. The number of patients with ischemic heart disease among nonsurvivors was greater than that among survivors. Otherwise, there was no difference between patients with and without cardiac event in age, gender, NYHA functional class and LVEF before optimized treatment. The number of patients with ischemic heart disease among those with a cardiac event was greater than that among patients without a cardiac event.

Total treatments for CHF and optimized treatments for patients with CHF are shown in Table 2. There was no difference between survivors and nonsurvivors treated with diuretics, digitalis, ACE inhibitors, beta-blockers and calcium antagonists, and there was no difference between patients with a cardiac event and those without a cardiac event treated with these agents. The number of patients treated with nitrates was greater among survivors than it was among nonsurvivors and among those with a cardiac event compared with those without a cardiac event. This was most

**Table 2.** Optimal Treatments for CHF in 102 Patients With CHF During Three Months

| | All Patients Before/3 Months | Survivors (n = 76) | Nonsurvivors (n = 26) | Cardiac Event (−) (n = 55) | Cardiac Event (+) (n = 47) |
|---|---|---|---|---|---|
| Diuretics | 35/79 | 56 (74%) | 23 (88%) | 40 (73%) | 39 (83%) |
| ACE inhibitors | 25/71 | 54 (71%) | 17 (65%) | 42 (76%) | 29 (62%) |
| Digitalis | 23/55 | 41 (54%) | 14 (54%) | 32 (58%) | 23 (49%) |
| β-blockers | 15/29 | 24 (32%) | 5 (19%) | 17 (31%) | 12 (26%) |
| Nitrates | 13/41 | 24 (31%) | 17 (65%)* | 16 (29%) | 25 (53%)* |
| Calcium antagonist (amlodipine) | 10/22 | 16 (21%) | 6 (23%) | 12 (22%) | 10 (21%) |

*$p < 0.05$ vs. survivors or cardiac events (−). Abbreviations as in Table 1.

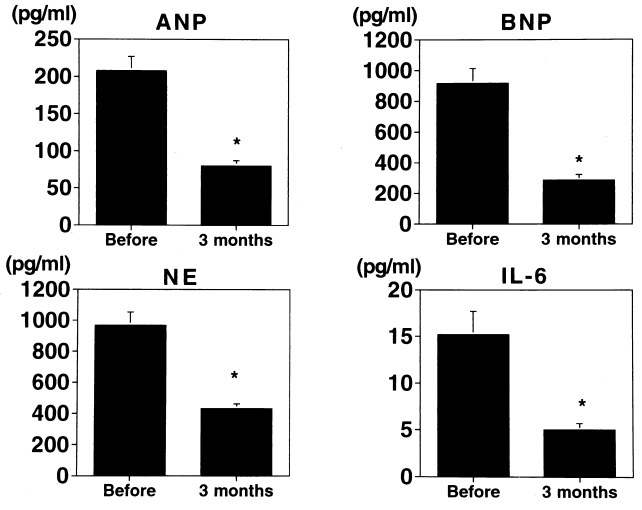


**Figure 2.** Comparison of plasma levels of neurohumoral factors and cytokines before and three months after optimized additional treatment for heart failure in patients with congestive heart failure. *$p < 0.0001$ vs. before additional treatment for congestive heart failure. ANP = atrial natriuretic peptide; BNP = brain natriuretic peptide; NE = norepinephrine; IL-6 = interleukin-6.

likely because the number of patients with ischemic heart disease among nonsurvivors or those with a cardiac event was greater than that among survivors or those without a cardiac event.

**Plasma levels of neurohumoral factors and LVEF before and at three months after additional treatment for CHF.** New York Heart Association classification, LVEF and the plasma levels of ANP, BNP, NE, ET-1, IL-6 at three months after optimized treatment were significantly improved compared with those before optimized treatment (NYHA; 3.4 ± 0.05 vs. 2.2 ± 0.05, LVEF; 23 ± 0.9 vs. 32.7 ± 1.3%, ANP; 208 ± 19 vs. 80 ± 7.2 pg/ml, BNP; 917 ± 96 vs. 285 ± 37 pg/ml, NE; 968 ± 84 vs. 431 ± 32 pg/ml, ET-1; 4.58 ± 0.23 vs. 2.69 ± 0.1 pg/ml, IL-6; 15.2 ± 2.5 vs. 5.0 ± 0.7 pg/ml, $p < 0.0001$) as shown in Figure 2. There was no difference in plasma levels of TNF-alpha before and three months after optimized treatment (6.8 ± 0.5 vs. 7.0 ± 0.6 pg/ml).

**Univariate and multivariate predictors of morbidity and mortality.** Nineteen variables, including clinical factors, neurohumoral factors, cytokines and LVEF before and three months after optimized treatment were analyzed using univariate and stepwise multivariate Cox proportional hazard regression analysis for mortality, as shown in Table 3. By univariate analysis, 12 clinical and neurohumoral factors were significant predictors of mortality. According to stepwise multivariate analysis, only high levels of plasma BNP and IL-6 at three months after optimized treatment were significant independent predictors. In this study, the relative risk ratio of BNP at three months after optimized treatment was 1.001 (95% confidence interval, 1.001 to 1.002) and that of IL-6 at three months after optimized treatment was 1.075 (95% confidence interval, 1.036 to 1.117).

Furthermore, the same factors were analyzed using univariate and stepwise multivariate Cox proportional hazard regression analysis for morbidity and mortality as shown in Table 4. By univariate analysis, seven clinical and neurohumoral factors were significant predictors of morbidity and mortality. According to stepwise multivariate analysis, only high levels of plasma BNP and IL-6 at three months after optimized treatment were significant independent predictors. In this study, the relative risk ratio of BNP at three months after optimized treatment was 1.001 (95% confidence interval, 1.001 to 1.002), and that of IL-6 at three

**Table 3.** Univariate and Multivariate Predictors of Mortality of 102 Patients With CHF According to Survival

| Variables | Univariate chi-square | p | Multivariate chi-square | p |
|---|---|---|---|---|
| Age (yr) | 1.06 | NS | 0.24 | 0.63 |
| Gender (male = 1) | 0.003 | NS | 0.23 | 0.63 |
| IHD (yes = 1) | 4.11 | 0.0427 | 1.06 | 0.30 |
| NYHA (before) | 1.34 | NS | 2.25 | 0.13 |
| NYHA (3 months) | 17.4 | 0.0006 | 3.46 | 0.33 |
| LVEF (before) | 0.51 | NS | 1.54 | 0.22 |
| LVEF (3 months) | 6.45 | 0.0111 | 0.0001 | 0.99 |
| ANP (before) | 0.63 | NS | 1.06 | 0.30 |
| ANP (3 months) | 15.6 | < 0.0001 | 0.005 | 0.95 |
| BNP (before) | 5.79 | 0.0161 | 2.61 | 0.11 |
| BNP (3 months) | 40.7 | < 0.0001 | 29.1 | < 0.0001 |
| NE (before) | 1.49 | NS | 0.17 | 0.68 |
| NE (3 months) | 12.3 | 0.0005 | 1.10 | 0.29 |
| ET-1 (before) | 0.42 | NS | 0.42 | 0.52 |
| ET-1 (3 months) | 4.43 | 0.0354 | 0.34 | 0.56 |
| IL-6 (before) | 4.08 | 0.0435 | 2.40 | 0.12 |
| IL-6 (3 months) | 18.5 | < 0.0001 | 14.3 | 0.0002 |
| TNF-alpha (before) | 4.09 | 0.043 | 0.05 | 0.82 |
| TNF-alpha (3 months) | 6.04 | 0.014 | 1.79 | 0.18 |

ANP = atrial natriuretic peptide; BNP = brain natriuretic peptide; ET-1 = endothelin-1; IL-6 = interleukin-6; NE = norepinephrine; TNF-alpha = tumor necrosis factor-alpha; other abbreviations as in Table 1.

**Table 4.** Univariate and Multivariate Predictors of Morbidity and Mortality of 102 Patients With CHF According to Cardiac Event-Free

| Variables | Univariate Chi-Square | p | Multivariate Chi-Square | p |
|---|---|---|---|---|
| Age (yr) | 1.44 | NS | 0.01 | 0.93 |
| Gender (male = 1) | 0.15 | NS | 0.39 | 0.53 |
| IHD (yes = 1) | 3.70 | NS | 0.89 | 0.35 |
| NYHA (before) | 0.008 | NS | 0.13 | 0.71 |
| NYHA (3 months) | 5.91 | NS | 0.70 | 0.87 |
| LVEF (before) | 0.15 | NS | 1.41 | 0.24 |
| LVEF (3 months) | 12.0 | 0.0005 | 1.81 | 0.18 |
| ANP (before) | 1.02 | NS | 1.17 | 0.28 |
| ANP (3 months) | 23.7 | < 0.0001 | 1.84 | 0.17 |
| BNP (before) | 3.65 | NS | 1.62 | 0.20 |
| BNP (3 months) | 34.6 | < 0.0001 | 28.2 | < 0.0001 |
| NE (before) | 0.23 | NS | 1.00 | 0.32 |
| NE (3 months) | 7.23 | 0.0072 | 0.83 | 0.36 |
| ET-1 (before) | 0.004 | NS | 0.54 | 0.46 |
| ET-1 (3 months) | 4.70 | 0.0303 | 0.25 | 0.62 |
| IL-6 (before) | 0.32 | NS | 0.11 | 0.74 |
| IL-6 (3 months) | 8.14 | 0.0043 | 7.60 | 0.0059 |
| TNF-alpha (before) | 3.46 | NS | 0.26 | 0.61 |
| TNF-alpha (3 months) | 5.77 | 0.0163 | 1.74 | 0.19 |

Abbreviations as in Table 3.

months after optimized treatment was 1.049 (95% confidence interval, 1.014 to 1.085).

**Kaplan-Meier lifetime analysis.** The patients were stratified into two groups based on median plasma level of BNP (170 pg/ml) and IL-6 (3.0 pg/ml) three months after optimized treatment, and cumulative survival curves were constructed according to Kaplan-Meier survival methods. Survival rates, as elevated by Kaplan-Meier analysis, were significantly 3.4 times higher in patients with plasma BNP three months after optimized treatment of <170 pg/ml (p = 0.0025) and were significantly 2.5 times higher in patients with plasma IL-6 three months after optimized treatment of <3.0 pg/ml (p = 0.0133) as shown in Figure 3A.

Otherwise, the cardiac event-free curve, as elevated by Kaplan-Meier analysis, was significantly 5.6 times higher in patients with plasma BNP three months after optimized treatment of <170 pg/ml (p < 0.0001), and they were significantly 1.9 times higher in patients with plasma IL-6 three months after optimized treatment of <3.0 pg/ml (p = 0.016) as shown in Figure 3B.

**Comparison of the usefulness of BNP and IL-6 as a predictor of mortality or morbidity and mortality.** Receiver operating characteristic curves showed a greater area under the curve for the plasma BNP level than for the plasma IL-6 level, confirming a significantly better prognostic value of the former in predicting mortality (p < 0.05) or morbidity and mortality (p < 0.001) (Fig. 4, A and B). Optimal peptide level was determined as the peptide level at the point closest to that of perfect separation on each of the receiver operating characteristic curves; the plasma BNP level was 240 pg/ml, and plasma IL-6 level was 3.0 pg/ml as both mortality, and morbidity and mortality.

The sensitivity and specificity of mortality or morbidity and mortality of the patients with a plasma level of BNP >240 pg/ml were 73.1% and 73.7% or 70.2% and 89.1%, respectively. The sensitivity and specificity of mortality or

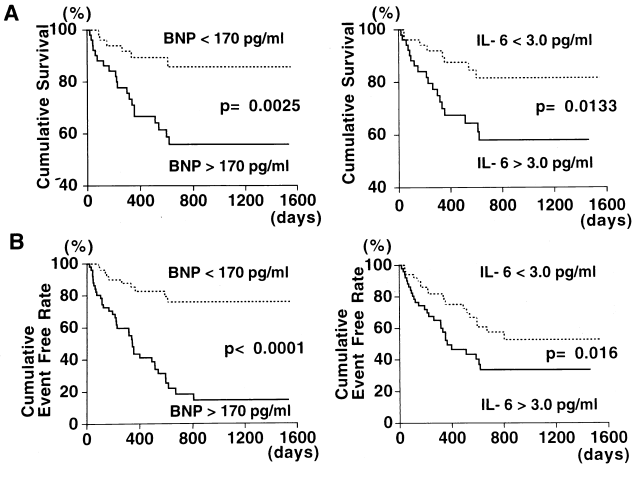


**Figure 3.** Kaplan-Meier survival (**A**) and cardiac event-free rate (**B**) plots for 102 patients with congestive heart failure subdivided into two groups according to the median level of BNP (170 pg/ml) and IL-6 (3.0 pg/ml) three months after additional treatment for congestive heart failure. BNP = brain natriuretic peptide; IL-6 = interleukin-6.

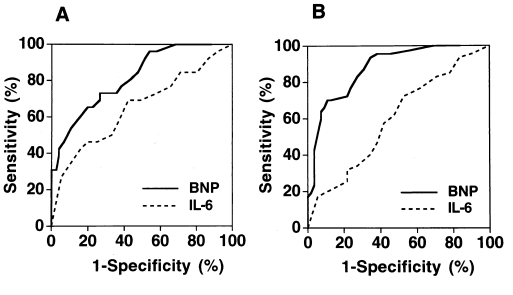


**Figure 4.** Plot showing receiver operating characteristic curves for prediction of mortality (**A**) and morbidity and mortality (**B**) for plasma level of BNP and IL-6. BNP = brain natriuretic peptide; IL-6 = interleukin-6.

morbidity and mortality of patients with a plasma level of IL-6 >3.0 pg/ml were 69.2% and 56.6% or 59.6% and 56.4%, respectively. However, the sensitivity and specificity of mortality or morbidity and mortality of patients with a plasma level of BNP >240 pg/ml and IL-6 >3.0 pg/ml were 92.3% and 79.1% or 81.8% and 89.7%, respectively.

## DISCUSSION

This study reports for the first time that only high levels of the plasma BNP and IL-6 three months after optimized treatment were found to be independent and important prognostic predictors in patients with CHF, despite improvements of LVEF and symptoms. This suggests that plasma levels of BNP and IL-6 are useful for assessment of the treatment of individual patients with CHF.

**Usefulness of repetitive measurements of neurohumoral factors and cytokines in patients with CHF.** In previous reports of prognostic predictors in patients with CHF, neurohumoral factors or cytokines were used with only 1 point sampling (2,8,10–16,22). There were several reports estimating prognostic predictors in patients with CHF using noninvasive repetitive measurements by echocardiography (27,28); however, to our knowledge, there is no report estimating prognostic predictors in patients with CHF using repetitive measurements of neurohumoral factors and cytokines.

In this study, on stepwise multivariate analysis, only high levels of the plasma BNP and IL-6 three months after optimized treatment were significant independent predictors. These results suggested titration of the drug and the doses needed to decrease plasma BNP and IL-6 levels in CHF, which may be important for a beneficial outcome in patients with CHF, and repetitive measurements of plasma BNP and IL-6 levels may be useful as an index for CHF treatment. In other words, if plasma levels of BNP and IL-6 remain high three months after a patient has received full doses of ACE inhibitors and beta-blockers, these patients may be a high-risk group that requires intensive care.

Motwani et al. (29) reported that the serial change of BNP is more useful than ANP for predicting the effect of ACE inhibitors on ventricular remodeling in patients with acute MI. Angiotensin-converting enzyme inhibitors reduce the mortality of patients with CHF partly due to the prevention of left ventricular remodeling, and plasma BNP is thought to be a useful marker of left ventricular remodeling after MI (30). These previous studies may support our findings—that optimized therapy to decrease plasma BNP reduces the mortality and morbidity of patients with CHF who have ischemic heart disease.

**Why are plasma levels of BNP and IL-6 at three months after optimized treatment for CHF independent important prognostic predictors for morbidity and mortality in patients with CHF?** We previously reported that independent predictors of BNP, mainly from the ventricle, were left ventricular end-diastolic pressure, ET-1 and NE by multivariate analysis among hemodynamic parameters, ET-1 and NE, suggesting that ET-1 and NE, which stimulate myocardial damage, increase the secretion of BNP directly or indirectly (24). Therefore, plasma levels of BNP imply the degree of left ventricular damage or dysfunction more than that of ANP, and BNP is a more important prognostic predictor than NE, ET-1 and ANP for patients with CHF.

Interleukin-6 is produced mainly in leukocytes, whereas partly in cardiac myocytes and increased by catecholamine (31,32). Interleukin-6 can modify the ventricular function through increase of nitrate oxide synthase (19), suggesting that IL-6 also plays an important role in ventricular dysfunction in patients with CHF. In this study, receiver operating characteristic curves confirmed the significantly better prognostic value of BNP in predicting mortality, and morbidity and mortality. However, high plasma BNP levels, in addition to high IL-6 levels, might be better predictors of mortality, and morbidity and mortality.

In this study, there was no difference in plasma levels of TNF-alpha before and three months after optimized treatment. A few studies have prospectively shown that treatment for CHF significantly affected TNF-alpha. Mohler et al. (23) reported that IL-6 levels were significantly decreased after 26 weeks in patients treated with amlodipine compared with placebo; however, TNF-alpha levels did not change significantly over a 26-week period in patients with CHF treated with amlodipine. Recently, we reported that plasma levels of TNF-alpha and IL-6 significantly decreased after 14 weeks in patients with CHF treated with angiotensin II type 1 receptor antagonist (candesartan cilexetil) compared with the values determined before treatment (33). These differences may be explained by differences in the effects of the drugs; however, future studies with more cases are needed to clarify these findings.

**Study limitations.** In this study, all patients were treated using additional drugs such as digitalis, diuretics, vasodilators, ACE inhibitors and beta-blockers for three months, as judged independently by physicians. Due to side effects, such as hypotension, dry cough or worsening renal dysfunction, not all patients could be treated with ACE inhibitors, and beta-blockers were not always given to patients who showed improvement in CHF after treatments such as diuretics or ACE inhibitors. Otherwise, optimized treatments were not randomized; therefore, it may be difficult to evaluate the effects of specific drugs for CHF on morbidity and mortality. If we evaluated drug treatments three months after optimized treatment in addition to the other 19 variables on Cox multivariate analysis, none of the drugs would have been independent predictors of mortality and morbidity and mortality in this study. Therefore, our findings indicate that decreased plasma BNP and IL-6 are more closely associated with decreased mortality rather than improvement of symptoms and LVEF after three months, independent of the treatment. This suggests that it would be cost-effective therapy to monitor plasma BNP during treatment of CHF since CHF patients with low plasma BNP have a good prognosis independent of the therapy

administered (15). However, future large and multicenter studies are needed to investigate our hypothesis.

**Conclusions.** High plasma BNP and IL-6 levels three months after optimized treatment for CHF are independent risk factors for morbidity and mortality of patients with CHF; moreover, plasma BNP and IL-6 levels three months after optimized treatment are more useful predictors than the levels of other neurohumoral factors and cytokines such as NE, ET-1, ANP and TNF-alpha and LVEF. These findings suggest that the repetitive measurements of plasma BNP and IL-6 levels were useful for estimating the effects of treatment for individual patients with CHF. The assessment of plasma levels of BNP and IL-6 is simple and cost-effective and can be repeated and, thus, may be a useful addition to the standard clinical evaluation of patients with severe CHF.

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