

Circulating Erythropoietin Levels and Prognosis in Patients With Congestive Heart Failure

Comparison With Neurohormonal and Inflammatory Markers

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Background: Considerable morbidity and mortality are still associated with congestive heart failure (CHF) syndromes, despite improvement in therapy. Activation of neurohormonal, inflammatory, and oxidative mechanisms has been shown to contribute to the significant morbidity and mortality. Erythropoietin (EPO) is a cytokine known to regulate erythroid proliferation, attenuate apoptosis and oxidative stress, and promote angiogenesis. We prospectively evaluated the predictive value of baseline EPO, N-terminal pro-B-type natriuretic peptide, and C-reactive protein levels in patients with clinically controlled chronic CHF.

Methods: One hundred eighty-eight outpatients from a CHF clinic had baseline assessment of EPO, N-terminal pro-B-type natriuretic peptide, and C-reactive protein levels and a complete clinical data profile. These patients were followed up for 24 months for any hospitalization due to CHF or mortality.

Results: Circulating EPO levels were higher in CHF pa-

tients and increased in subjects with higher New York Heart Association scores. Levels of EPO (at a cutoff of 23 mU/mL) and N-terminal pro-B-type natriuretic peptide (cutoff at the median of 1556 pg/mL) were found to be strong predictors of mortality and CHF hospitalization, whereas C-reactive protein levels (cutoff of 10 mg/L) predicted CHF hospitalizations but not mortality. Left ventricular ejection fraction was found to be a predictor of mortality but not of CHF hospitalizations. Serum levels of EPO were significantly correlated with N-terminal pro-B-type natriuretic peptide and C-reactive protein levels but not with left ventricular ejection fraction.

Conclusion: If confirmed in large-scale clinical studies, determination of circulating EPO levels may aid in predicting morbidity and mortality in patients with clinically controlled congestive CHF.

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CONGESTIVE HEART FAILURE (CHF) is a syndrome with an adverse prognosis in which the short-term (1-year) mortality rates in population-based studies have been reported to be in the range of 30% to 40%.¹⁻³

Although several prognostic factors have been identified among clinical trial enrollees, no marker has been established as an optimal surrogate to allow for accurate prediction of morbidity and mortality. As activation of neurohormonal and inflammatory mechanisms is operative in patients with CHF, there has been growing interest in finding respective humoral markers that will predict outcomes in these patients.⁴ In this respect, B-type natriuretic peptide (BNP) and the N-terminal pro-BNP (NT-proBNP) are recent, particularly attractive candidates that may provide considerable knowledge as to

the status of clinical control of CHF and prediction of future deterioration.^{5,6}

Patients with CHF often exhibit anemia that correlates with the severity of the disease such that patients with higher New York Heart Association (NYHA) scores have more profound reduction of their hemoglobin levels.^{7,8} Moreover, it has been shown that anemia is associated with a worse prognosis, even when it is relatively mild.^{9,10}

Erythropoietin (EPO) is a principal regulator of erythropoiesis, supporting survival and proliferation of erythroid progenitors.¹¹ This cytokine is up-regulated in response to hypoxia, after which the kidney produces EPO to overcome the need for reduced oxygen concentrations. However, mechanisms of potential benefit from EPO are probably more diverse than previously thought and include antiapoptotic effects, attenuation of oxidative stress, and promotion of angiogenesis.¹¹ Several

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small-scale clinical trials have demonstrated that administration of EPO to patients with CHF is associated with improvement in NYHA scores, left ventricular ejection fraction (LVEF), exercise capacity, and quality of life.¹²⁻¹⁴ However, it cannot be determined which of the mechanisms of EPO was responsible for the apparently favorable effects. Small studies^{15,16} have reported high levels of circulating EPO in patients with CHF (hereafter referred to as CHF patients), yet the prognostic value of this finding has not been tested.

In the present study, we studied the predictive value of baseline EPO levels in CHF patients on outcome and compared it with markers of neurohormonal and inflammatory activation that have been reported to correlate with prognosis.

METHODS

PATIENT SELECTION

We recruited 188 consecutive patients with advanced CHF attending the outpatient CHF clinic of the Tel Aviv Sourasky Medical Center, Tel Aviv, Israel, from June 1, 2000, through February 28, 2001. We included patients with chronic CHF in NYHA functional classes II to IV. Exclusion criteria were age younger than 18 years, pregnancy, therapeutic use of epoetin (erythropoietin), or known concurrent malignancy. The local research ethics committee approved the study protocol, and all patients gave written informed consent.

At baseline, patients underwent full medical history, clinical examination, and assignment of NYHA class. Patients were followed up every 1 to 3 months or more frequently as required. Baseline serum samples were drawn at the initial visit and frozen at -80°C until performance of the laboratory assays. Healthy control patients ($n=30$) were recruited from the hospital staff, and blood samples were drawn and preserved in a similar manner. The control group did not differ significantly from the CHF patients with regard to age (mean \pm SD age, 68 ± 16 years) and sex (80% men).

FOLLOW-UP

The study end points were all-cause mortality, hospitalization due to CHF, or the combined occurrence of each (CHF hospitalization or mortality). Patients were followed up for a minimum of 24 months. No patients were unavailable for follow-up.

EPO SERUM LEVEL DETERMINATION

Serum levels of EPO were determined by means of an enzyme-linked immunosorbent assay kit according to the manufacturer's instruction (Quantikine IVD human erythropoietin kit; R&D Systems, Minneapolis, Minn). Detection concentrations derived by standard curve were in the range of 2.5 to 200.0 mU/mL. The correlation coefficient of the fitted standard curve was 0.99 in all plates. Assays were always performed in duplicates.

DETERMINATION OF NT-proBNP LEVELS

Measurement of serum NT-proBNP levels was performed by means of automated immunoassay (Elecsys proBNP test; Roche Diagnostics, Mannheim, Germany). The test principle includes using 2 polyclonal antibodies directed against NT-proBNP: epitope 1 consisting of amino acid sequence 1-21 and epitope 2 consisting of amino acid sequence 39-50. The re-

Table 1. Clinical Characteristics of 188 Patients With Chronic CHF*

Characteristic	Data
Age, mean \pm SD, y	71.4 \pm 11.8
Men	145 (77.1)
NYHA score, mean \pm SD	2.8 \pm 0.59
LVEF, mean \pm SD	38 \pm 13.8
Hyperlipidemia	106 (56.4)
Anemia	64 (34.0)
Renal failure	78 (41.5)
Smoking	71 (37.8)
Hypertension	110 (58.5)
Diabetes mellitus	64 (34.0)
Ischemic heart disease	137 (72.9)
Diastolic heart failure	53 (28.2)
Chronic atrial fibrillation	58 (31.0)
Transient ischemic attacks/cerebrovascular accidents	23 (12.2)
Percutaneous transluminal coronary angioplasty or coronary artery bypass surgery	97 (51.6)
Use of medication	
ACE inhibitors/ARBs	146 (77.7)
Spironolactone	79 (42.0)
β -Blockers	130 (69.1)
Digoxin	45 (23.9)
Diuretics	135 (71.8)

Abbreviations: ACE, angiotensin-converting enzyme; ARBs, angiotensin receptor blockers; CHF, congestive heart failure; LVEF, left ventricular ejection fraction; NYHA, New York Heart Association.

*Unless otherwise indicated, data are expressed as number (percentage) of patients.

sults are calibrated against a synthetic NT-proBNP (amino acid 1-76). Results range from 5 to 35 000 pg/mL.

HIGH-SENSITIVITY C-REACTIVE PROTEIN CONCENTRATIONS

The assay for C-reactive protein (CRP) level was conducted according to manufacturer's instructions (Dade Behring Inc, Deerfield, Ill). Briefly, the principle of the method includes using polystyrene particles coated with monoclonal antibodies to CRP. These particles agglutinate with CRP. The CRP level was determined according to the intensity of the scattered light in the nephelometer, compared with standards of a known concentration.

STATISTICAL ANALYSIS

We compared the EPO levels in relation to NYHA class using the 1-way analysis of variance (ANOVA) test. We calculated Kaplan-Meier survival curves with the data dichotomized at the median values of the 75 percentile for NT-proBNP and EPO levels.

Because EPO, proBNP, and CRP levels were not normally distributed, we performed correlations between the logarithm concentrations using the Spearman rank correlation test. Logarithm-transformed data were presented for the correlation due to the skewed nature of the values obtained.

To compare the predictive value of NT-proBNP, EPO, and CRP levels and LVEF, we performed receiver operating characteristic analysis and calculated the areas under the curves. To identify predictors of death, we used Cox proportional hazards analysis, and then tested variables achieving $P < .10$ on univariate analysis in a stepwise (forward) multiple Cox regression survival model to determine the independent predictors of the primary and secondary end points. The following vari-

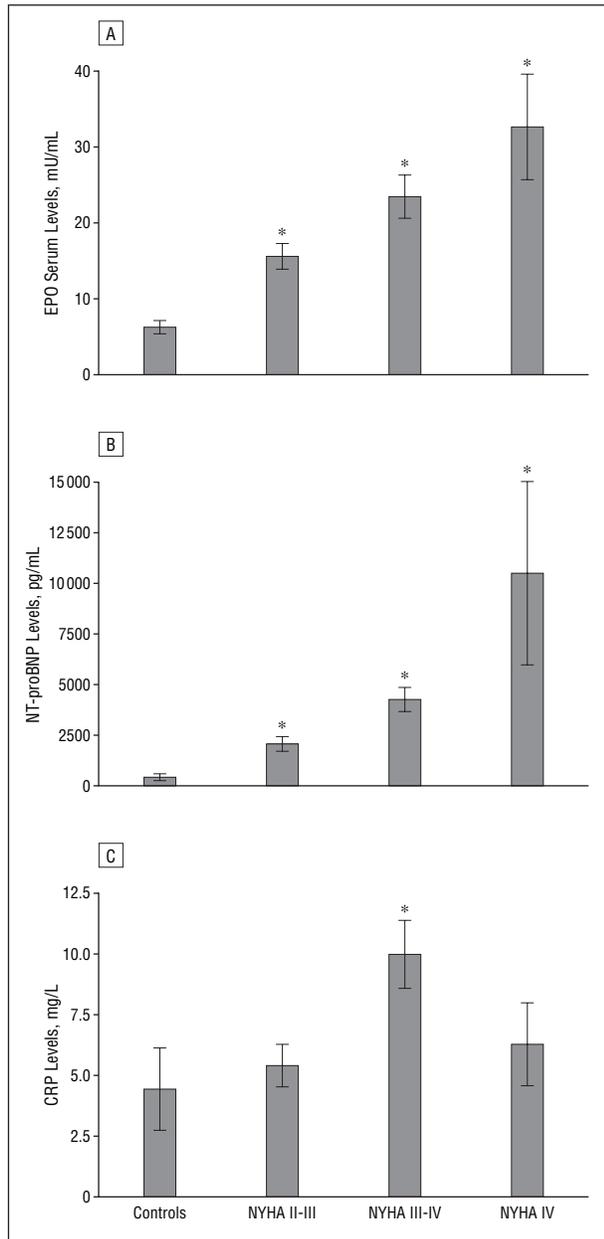


Figure 1. Mean circulating levels of erythropoietin (EPO) (A), N-terminal pro-B-type natriuretic peptide (NT-proBNP) (B), and C-reactive protein (CRP) (C) according to New York Heart Association (NYHA) score in healthy control subjects and patients with congestive heart failure. Limit lines indicate standard deviation; asterisk indicates $P < .05$. Ranges of NYHA scores are described in the "Results" section.

ables were entered into the model: age, sex, NYHA class, LVEF, and levels of serum creatinine, hemoglobin, EPO, CRP, and NT-proBNP. Statistical significance was considered achieved at $P < .05$. Unless otherwise indicated, means are expressed as mean \pm SD.

RESULTS

Baseline clinical variables and use of medications at the time of enrollment are provided in **Table 1**. Of the total CHF cohort, 87 (46.3%) were in NYHA classes II to III (includes class II and the range between classes II and III), 90 (47.9%) were in NYHA classes III to IV (in-

Table 2. Median Levels of EPO, NT-proBNP, and CRP in CHF Patients According to the Prespecified Clinical End Points*

End Point	EPO Level, mU/mL	NT-proBNP Level, pg/mL	CRP Level, mg/L
Dead	19.6 (11.6-36.2)	4333 (2149-9000)	4.0 (2.2-12.2)
Alive	12.8 (7.7-21)	1260 (664-2917)	4.0 (1.7-8.3)
<i>P</i> value	.006	<.001	.52
Hospitalized with CHF	20.2 (9.7-31)	2863 (1266-5536)	5.8 (2.5-12.5)
Not hospitalized	13.7 (7.7-20.4)	1327 (801-3468)	3.6 (1.6-7.5)
<i>P</i> value	.01	.005	.03
Dead or hospitalized	18.3 (10-31.4)	2899 (1266-5833)	4.5 (1.7-12.2)
Alive, not hospitalized	13.2 (7.7-20)	1247 (696-3183)	3.9 (1.8-7.3)
<i>P</i> value	.01	<.001	.24

Abbreviations: CHF, congestive heart failure; CRP, C-reactive protein; EPO, erythropoietin; NT-proBNP, N-terminal pro-B-type natriuretic peptide.

*Unless otherwise indicated, data are expressed as median (interquartile range).

cludes class III and the range between classes III and IV), and 11 (5.9%) were in NYHA class IV.

Patients with the more severe forms of CHF exhibited significantly elevated levels of EPO. Although mean EPO concentrations in healthy patients were 6.5 ± 0.8 mU/mL, patients with NYHA classes II to III, III to IV, and IV had levels of 15.9 ± 1.6 , 26.2 ± 2.8 , and 32.9 ± 6.9 mU/mL, respectively ($P < .05$, by means of ANOVA) (**Figure 1A**). A similar trend demonstrating elevated levels of NT-proBNP with rise in the NYHA class was also evident and significant (**Figure 1B**). With respect to CRP levels, although higher levels were observed in higher NYHA classes, the relationship was less statistically robust (**Figure 1C**).

Of the total 188 patients, 38 (20.2%) died during the 2-year follow-up, whereas 43 were hospitalized for CHF deterioration (22.8%) and 67 (35.6%) reached the end point of mortality or CHF hospitalization within the follow-up period. **Table 2** demonstrates the levels of EPO, NT-proBNP, and CRP according to the different end points predefined.

At a cutoff level of EPO above the 75th percentile (concentrations of 23 mU/mL), patients with values above this threshold exhibited significantly poorer prognosis in terms of all-cause mortality ($P = .005$) (**Figure 2**). The predictive power of EPO for detection of subsequent CHF hospitalizations or the combined end point of mortality or CHF hospitalizations during a 2-year follow-up was even more pronounced ($P = .003$ and $P < .001$, respectively).

Levels of NT-proBNP were also found to be a good predictor of mortality, CHF hospitalizations, and the combined end points.

After testing by univariate analysis and selecting variables achieving $P < .10$, the Cox proportional hazards test confirmed the independent predictive value of levels of EPO, hemoglobin, and NT-proBNP in assessment of mortality and CHF hospitalizations (**Table 3**).

Kaplan-Meier curves calculated for CRP levels showed that serum levels greater than 10 mg/L were able to predict subsequent CHF hospitalizations ($P = .02$) but not

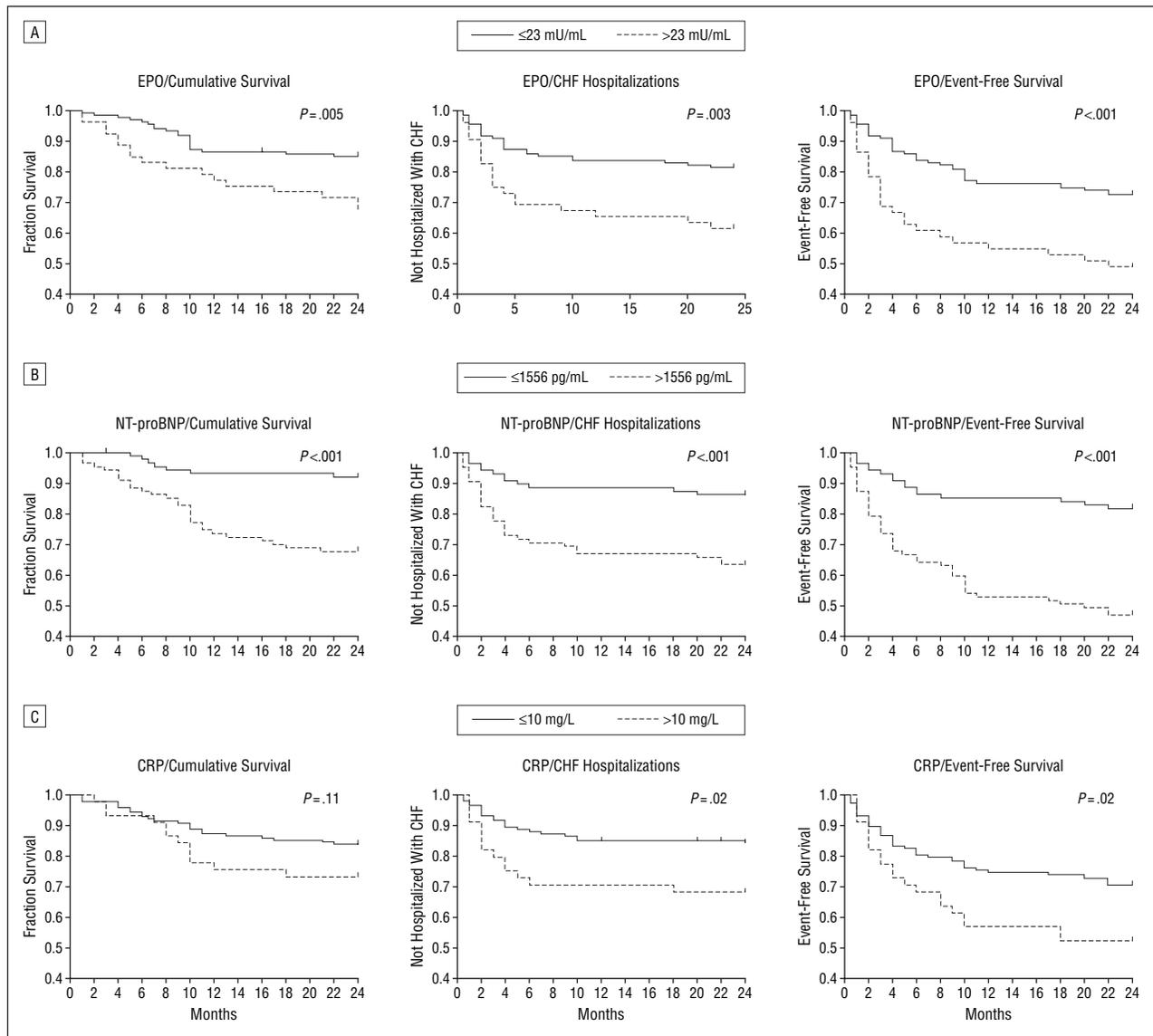


Figure 2. Kaplan-Meier curves showing the relation of baseline circulating levels of erythropoietin (EPO) (A), N-terminal pro-B-type natriuretic peptide (NT-proBNP) (B), and C-reactive protein (CRP) (C) to the prespecified end points of the study, ie, mortality, congestive heart failure (CHF) hospitalizations, or these combined end points.

mortality ($P=.11$). Analysis of survival curves for baseline LVEF demonstrated that a cutoff of 40% was a good predictor of mortality (hazards ratio, 3.3; $P=.003$) but not of CHF hospitalizations ($P=.65$).

A significant correlation was found between serum EPO and NT-proBNP levels ($P=0.4$; $P<.001$) (Figure 3A) and between serum EPO and CRP levels ($P=0.28$; $P<.001$). Levels of NT-proBNP and CRP also correlated, yet no association was found between LVEF and EPO serum levels (Figure 3D). No correlation was found between circulating EPO levels and hemoglobin or creatinine levels in the CHF patients, nor was there an association between EPO levels and age.

COMMENT

We confirmed the findings of elevated EPO serum levels in CHF patients.^{15,16} Moreover, we have found that the se-

verity of CHF as estimated by the NYHA score correlated with the elevation of serum EPO. Next, we assessed the prognostic value of circulating EPO, NT-proBNP, and CRP levels in the study cohort. We have found that NT-proBNP level was indeed a strong and independent predictor of mortality and CHF hospitalizations during the 2-year surveillance period. This finding is in accord with previous observations,¹⁷ although to our knowledge this is the largest cohort of prospectively studied outpatients with CHF described to date with regard to NT-proBNP level. We have found that serum EPO levels set at a cutoff of 23 U/mL were able to discriminate patients who are likely to be hospitalized for CHF or die during the subsequent 2 years. When these prognostic data were compared with the power prediction of CRP level, we found that the latter was a poor predictor of mortality in CHF patients, whereas its ability to predict CHF hospitalization was reasonable. Recently, van der Meer et al¹⁸ studied a cohort of

74 patients with CHF and showed that a similar EPO threshold was accurate in predicting mortality. Our study extends and supports these findings by showing its validity in a larger population and expanding the relative prognostic value to mortality and to an acceptable end point of quality of life, ie, hospitalizations due to CHF exacerbation.

Table 3. Independent Predictors of Mortality and CHF Hospitalizations Calculated by Means of the Cox Proportional Hazards Model

Variable	χ^2	P Value
All-cause mortality		
LVEF, %	3.3	.04
Hemoglobin level, mg/dL	6.2	.003
EPO level, mU/mL	5.1	.02
NT-proBNP level, pg/mL	13.6	<.001
CHF hospitalizations		
NYHA class	4.1	.01
Hemoglobin level, mg/dL	8.8	.001
EPO level, mU/mL	6.6	.003
NT-proBNP level, pg/mL	11.2	<.001

Abbreviations: CHF, congestive heart failure; EPO, erythropoietin; LVEF, left ventricular ejection fraction; NT-proBNP, N-terminal pro-B-type natriuretic peptide; NYHA, New York Heart Association.

In this context, it should be emphasized that the CHF cohort in our study was relatively homogeneous, consisting of patients with advanced CHF. Thus, conclusions as to the predictive value of EPO level cannot be drawn with regard to patients with milder forms of CHF or those presenting with acute exacerbations.

An additional potentially confounding issue is the reference group for EPO level threshold determination that consisted of healthy subjects. This would obviously tend to overestimate the results, yet it was imposed by the lack of previous comparative data as to this comparison at the time the study was designed.

Erythropoietin is a glycoprotein produced primarily in the kidneys that stimulates the proliferation and differentiation of committed erythroid progenitors in the bone marrow.¹¹ Recent interest in EPO has increased due to the understanding of additional mechanisms of action and other potential implementations of this pleiotropic cytokine.¹¹ Erythropoietin has been reported to have antiapoptotic and antioxidant properties, and recent findings suggest that it promotes endothelial precursor cell mobilization, thereby potentially contributing to tissue angiogenesis and vasculogenesis. In view of these diverse properties, it may be assumed that EPO could represent a form of a stress hormone that is up-regulated in CHF patients as their clinical status deteriorates. Pa-

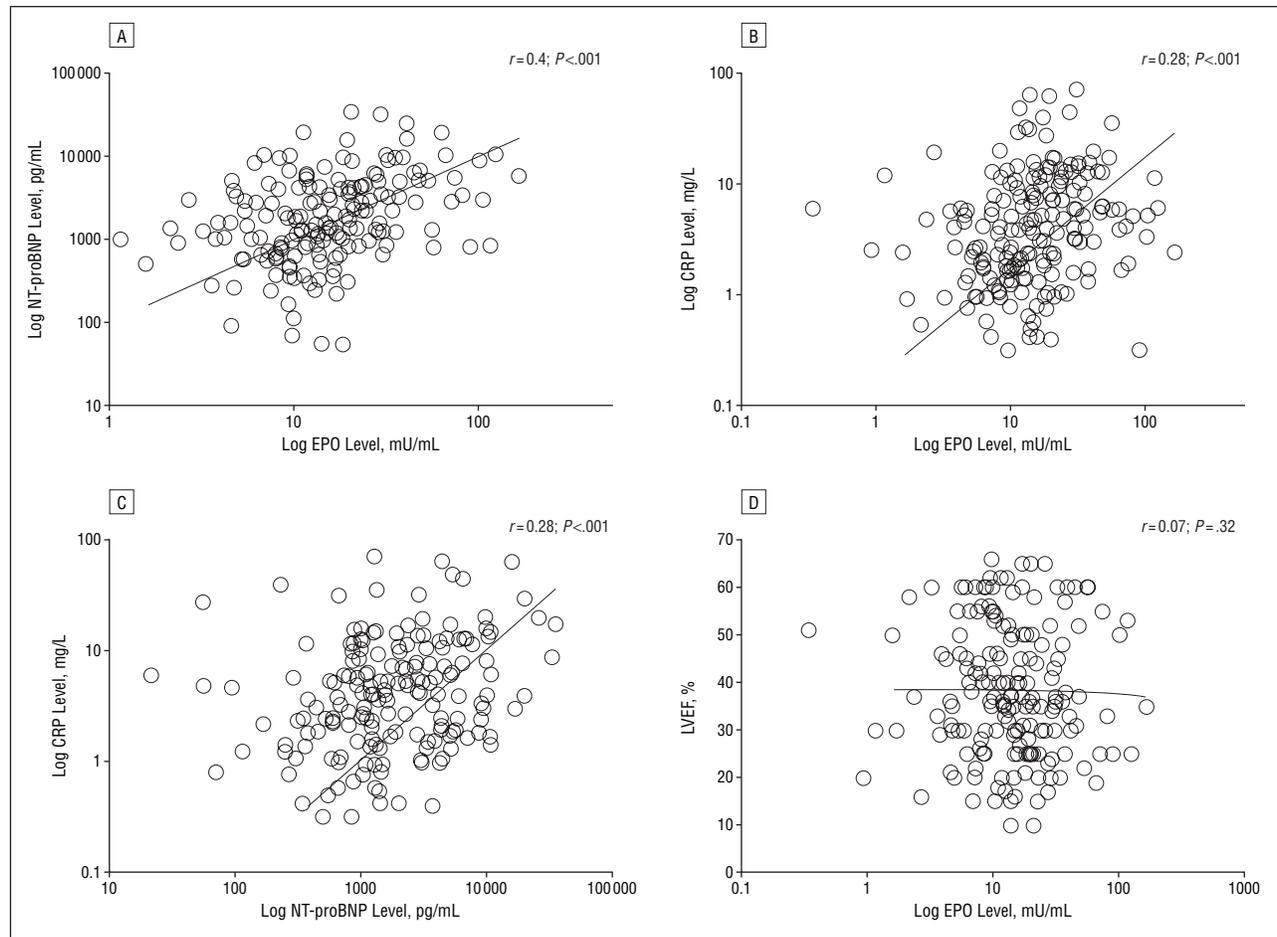


Figure 3. Correlation of erythropoietin (EPO) levels to levels of N-terminal pro-B-type natriuretic peptide (NT-proBNP) (A) and C-reactive protein (CRP) (B), NT-proBNP levels to CRP level (C), and EPO level to left ventricular ejection fraction (LVEF) (D). Values were logarithm-transformed (log) owing to their widely skewed distribution.

tients with CHF by definition have reduced tissue perfusion, resulting in local hypoxic environments. Erythropoietin was shown to be up-regulated due to hypoxia, and the lack of sufficient oxygen tension could be a major trigger for higher levels of EPO in the CHF patients. However, it is likely that additional stimuli that result from tissue hypoperfusion can result in up-regulation of EPO in patients with CHF, including the formation of free radicals due to enhanced oxidative stress. Elevation of the EPO level may not only reflect tissue stress, but could also potentially contribute to the worsening outcome. This effect could be mediated by increased blood pressure, increased platelet aggregability, increased blood viscosity, activation of endothelium by up-regulation of adhesion molecules, and other potentially disadvantageous mechanisms.^{11,12}

We have found that there was a significant correlation among serum EPO, NT-proBNP, and CRP levels. Collectively, these findings suggest that activation hormonal and inflammatory processes are reactive to the progression toward more advanced CHF.

Serum EPO level was found to be an independent predictor of mortality and hospitalization in CHF patients and is more accurate than the LVEF. Accordingly, no correlation was found between EPO levels and LVEF, in contrast to the good relation between EPO levels and the NYHA class, suggesting that this humoral factor may be more applicable for assessment of patient prognosis and therapy monitoring in these patients.

The NT-proBNP levels were also found to be of considerable accuracy in the prediction of mortality and morbidity in this study. Determination of circulating EPO levels could prove helpful in assessing prognosis, as its source of production (kidney rather than the heart) minimizes short-term effects that may arise due to rapid change in volume status that could occur in CHF patients.

Elevated levels of CRP have been demonstrated in patients with CHF^{17,19} and shown to predict readmission in patients hospitalized with acute deterioration.²⁰ Furthermore, activation of the immune system may play a role in CHF by influencing the renin-angiotensin-aldosterone and sympathetic nervous systems.²¹⁻²³ Herein, we tested for the first time a cohort of outpatients with controlled CHF and found that CRP levels predicted the rate of hospitalizations with no statistically significant effect on mortality. The CRP levels correlated with EPO and NT-proBNP concentrations, which suggests that activation of neurohumoral and inflammatory mechanisms are associated and may be sampled intercurrently for the purpose of determining individual risk and prognosis in CHF patients.

In conclusion, we demonstrate for the first time, to our knowledge, that serum levels of EPO in CHF patients are good predictors of morbidity and mortality. If this finding is confirmed in larger-scale studies, measurement of EPO levels could represent an additional test by which to assess prognosis in these patients.

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