Amino-Terminal Pro–C-Type Natriuretic Peptide in Heart Failure

Sue P. Wright, Tim C.R. Prickett, Robert N. Doughty, Chris Frampton, Greg D. Gamble, Tim G. Yandle, Norman Sharpe, Mark Richards

Abstract—The levels and pathophysiological role of amino terminal C-type natriuretic peptide in heart failure are unknown. The potential of plasma amino-terminal C-type natriuretic peptide (N-CNP) as a marker of cardiac function was investigated in symptomatic patients. In 305 patients with recent-onset dyspnea and/or peripheral edema, presenting to primary care, assay of plasma amino-terminal C-type natriuretic peptide and other plasma vasoactive hormones was conducted together with echocardiography. Heart failure was diagnosed in 77 (of the 305) patients by rigorous application of predefined criteria. Plasma amino-terminal C-type natriuretic peptide concentrations were elevated in patients with heart failure, and by univariate analysis were related to age, renal function, and other hormones. On multivariate analysis, tertile of plasma N-CNP interacted with tertile of plasma amino-terminal B-type natriuretic peptide to predict heart failure independent of age, gender, renal function, or echocardiographic left ventricular fractional shortening. N-CNP showed significant relations to concurrent plasma CNP, atrial natriuretic peptide (ANP), N-ANP, B-type (or brain) natriuretic peptide (BNP), N-BNP, endothelin-1, and adrenomedullin but not to echocardiographic indicators of left ventricular systolic function. Plasma amino-terminal C-type natriuretic peptide is elevated in heart failure and is related to other plasma hormones in heart failure. These findings suggest a possible compensatory response from the peripheral vasculature to heart failure by an endothelium-based vasodilator peptide and mandate further exploration of the role of C-type natriuretic peptide in this condition. (Hypertension. 2004;43:1-7.)

Key Words: natriuretic peptides ■ heart failure ■ echocardiography ■ hormones

C-type natriuretic peptide (CNP) shares structural and (some) bioactive properties with the cardiac natriuretic peptides, atrial natriuretic peptide (ANP) and B-type (or brain) natriuretic peptide (BNP). Unlike ANP and BNP, predominantly cardiac hormones, CNP is a vascular endothelial product. It has also been isolated from the kidney and central nervous system.1–3 It has a potential paracrine role in vascular remodeling and the modulation of vascular tone.4,5 In common with ANP and BNP, CNP is synthesized as a precursor and cleaved into a biologically active carboxy terminal portion and an amino-terminal peptide. The 103–amino acid propeptide is cleaved either between residues 50 and 51 or 81 and 82 to produce one of two biologically active peptides, carboxy terminal proCNP (51–103) or carboxy terminal proBNP (82–103), respectively, and an amino-terminal congener, N-terminal proCNP. In contrast to ANP and BNP, in humans, circulating levels of carboxy terminal CNP are so low that this poses a challenge to immunoassay technologies that are operating at the limits of detection. The greater size and presumed longer plasma half-life of amino-terminal proCNP (N-CNP) offers a potentially more reliable method of indirectly assessing CNP production through accurate measurement of plasma N-CNP concentration. This also offers the opportunity to compare and contrast the performance of plasma N-CNP with that of BNP and amino-terminal proBNP (N-BNP) as markers of cardiac function.

The roles of ANP and BNP as compensatory peptides and markers of both function and prognosis in heart failure have been well described.4–8 CNP has potent indirect cardiovascular actions including reducing cardiac filling pressures secondary to vasorelaxation and decreased venous return,9 but when infused into healthy subjects, at least at doses calculated to restrict increments in plasma CNP to within the pathophysiological range, CNP does not have diuretic or natriuretic effects.10–12 The levels and potential role of CNP in heart failure are unknown. At least three reports suggest that plasma carboxy terminal CNP concentrations are not elevated in congestive heart failure.13–15 This is surprising, considering that cardiac tissue levels have been reported as increased16 and recently cardiac generation of CNP has been reported together with a significant positive correlation between plasma coronary sinus concentrations of CNP and concurrent pulmonary capillary wedge pressure measurements in patients with heart failure.17 In addition, tissue and...
plasma agents that are activated in heart failure, including cytokines and BNP, have been shown to enhance cellular CNP production in vitro.\cite{18,19} Finally, infusion of ANP and/or BNP results in a rise in concurrent levels of endogenous plasma CNP.\cite{20} As both plasma ANP and BNP are clearly elevated in human heart failure, the apparent absence of increased circulating levels of plasma CNP in this condition is yet more unexpected and raises the possibility that current ability to measure plasma CNP may not be sufficiently robust or that previous reports have had insufficient sample size to detect what may be small elevations of this peptide as it spills over from endothelial cells into the vascular lumen. To date, studies of CNP in heart failure have been limited by relatively small numbers and classification of heart failure, primarily on the basis of New York Heart Association class. The current report is the first to document the relation of plasma N-CNP with other neurohormones and clinical together with echocardiographic variables in a substantial cohort in which heart failure was rigorously and prospectively defined. We tested the hypotheses that plasma amino-terminal CNP (a) is elevated in heart failure along with other neurohormonal factors known to be activated in this condition and (b) is an independent marker of the presence of this condition.

**Methods**

**Patients**

Patients in this study were participants in the Natriuretic Peptides in the Community study, a prospective, randomized trial of the diagnostic efficacy of N-BNP for the diagnosis of suspected new-onset heart failure in primary care.\cite{21} Patients presenting in primary care with dyspnea and/or edema were referred to the study by their general practitioner. Each patient was seen for a study visit in which blood was collected for neurohormonal analysis, and further cardiologic assessment including ECG, chest radiography, and echocardiography (ATL HDI 5000) was performed. Blood samples were collected from seated patients without resting or other special patient preparation. Blood was collected into standard blood collection tubes containing EDTA (7.5 mg/mL, Vacutainer, Becton Dickinson), centrifuged within 2 hours (3000 rpm for 10 minutes); plasma was stored at −80°C.

**Definition of Heart Failure**

The definitive reference test for the purposes of this study was provided by a panel of three experts who reviewed each clinical presentation and all investigations (excluding natriuretic peptide results) and decided whether individual patients met the case definition criteria for heart failure, using the European Society of Cardiology guidelines.\cite{22} To meet the case definition of heart failure, patients had to have appropriate symptoms and clinical signs of heart failure, primarily on the basis of New York Heart Association class. The current report is the first to document the relation of plasma N-CNP with other neurohormones and clinical together with echocardiographic variables in a substantial cohort in which heart failure was rigorously and prospectively defined. We tested the hypotheses that plasma amino-terminal CNP (a) is elevated in heart failure along with other neurohormonal factors known to be activated in this condition and (b) is an independent marker of the presence of this condition.

**Radioimmunoassays**

N-CNP, carboxy terminal CNP, and N-BNP were measured in all patients. BNP, ANP, N-ANP, adrenomedullin, and endothelin were also measured. Subsequent analyses were performed by comparing patients with and without heart failure as identified by the panel gold standard.

N-terminal proCNP1–15 was assayed as previously described,\cite{28} except a more sensitive primary rabbit antiserum (J39) was used and peptide standards were made from synthetic human proCNP1–19 (Chiron Technologies Pty Ltd). Briefly, 3 mL of EDTA plasma was extracted on Sep-Pak C\textsubscript{18} cartridges and reconstituted in 700 μL of assay buffer. The extract (200 μL) plus 100 μL antisera (J39) was incubated for 24 hours at 4°C before addition of 100 μL iodinated proCNP1–15-Tyr,\cite{16} prepared by the chloramine T method and purified by HPLC. The assay was incubated for a further 24 hours at 4°C when bound and free labeled peptides were separated by a solid-phase second antibody method. The assay detection limit was 0.5 pmol/L in plasma. The N-terminal proCNP1–15 assay within and between assay coefficients of variation were 6.0% and 7.9%, respectively, at 19 pmol. Normal values for people 50 to 80 years of age were derived from 101 subjects recruited through random selection from the local electoral role (23 were 50 to 59 years of age; 34 were 60 to 69 years of age, and 44 were 70 to 80 years of age, including 42 men and 59 women). Control subjects were free of any history of cardiovascular disease or abnormal findings on physical examination.

Previously described radioimmunoassays were used for BNP, N-BNP, ANP, N-ANP, CNP, adrenomedullin, and endothelin.\cite{8,25–29}

**Renal Function**

Renal function was assessed by calculation of creatinine clearance, using the Cockcroft-Gault formula incorporating plasma creatinine, weight, age, and gender.

**Statistical Methods**

Plasma concentrations of neurohormones are expressed as medians (interquartile range). Nonparametric variables were compared using the Wilcoxon rank sum test. Other continuous variables are expressed as mean (standard deviation) and were compared by means of the Student t test. Categorical variables were compared with the Fisher exact test. In univariate analyses, Spearman rank coefficient was used to determine correlations between variables, presented as r values. Modeling to investigate independent determinants of N-CNP was performed by means of multiple linear regression. Multiple logistic regression with stepwise selection was used to test tertile of plasma N-CNP and other variables for potential independent association with heart failure.

**Results**

**Patient Characteristics**

Three hundred five patients with a mean age of 72 years (range, 40 to 95 years), 65% women, were included in the study (Table 1). Forty-nine percent presented with dyspnea alone, 12% with edema alone, and 39% had both symptoms at presentation. The majority fell into New York Heart Association symptomatic class II. Overall, patients had impaired creatinine clearance with a mean of 66.7 mL/min (normal, 90 to 120 mL/min).

Seventy-seven (77) of 305 patients (25%) were allocated a diagnosis of heart failure by the panel. The underlying cause of heart failure was ischemic heart disease in 27 (35%), multifactorial (with contributions from 2 or more of ischemic heart disease, diabetes, hypertension, and/or atrial fibrillation) in 23 (30%), valvular heart disease in 5 (6.5%), dilated cardiomyopathy in 5 (6.5%), isolated diastolic heart failure in 11 (14%), and unknown in 6 (8%).

Common conditions leading to presentation among the remaining 228 patients included respiratory disease (obstructive airways disease, infections and malignancies), myocardial ischemia with exertional dyspnea, and obesity. Less common causes included anxiety with hyperventilation, thoracic deformity, and chronic pulmonary venous thromboembolic disease.
Although hypertension was similarly frequent in both heart failure (48%) and non–heart failure (53%) groups, the prevalence of diabetes (28% versus 10%; \( P < 0.0001 \)), previous myocardial infarction (34% versus 8%; \( P < 0.0001 \)), and rates of prescription of some cardiovascular drugs including loop diuretics (38% versus 18%; \( P < 0.0001 \)), ACE inhibitors (47% versus 20%; \( P < 0.0001 \)), and (to a lesser degree) \( \beta \)-blockers (30% versus 23%; \( P = 0.201 \)) were higher in the patients with heart failure.

### Neurohormones

Median plasma N-CNP in normal control subjects at 18.47 (interquartile range, 15.75 to 20.90) pmol/L was significantly lower than levels recorded in the non–heart failure symptomatic group (median, 24.7 pmol/L; range, 21.0 to 29.5; \( P < 0.001 \)), which in turn were lower than in the patients with heart failure (median, 32.4 pmol/L; range, 25.4 to 39.4; \( P < 0.001 \) for all intergroup comparisons). The significant intergroup differences were independent of age and gender effects.

N-CNP was modestly related to age within both symptomatic and control subjects (\( r = 0.36 \) and 0.38 respectively; \( P < 0.0001 \) for both). Within the total patient group (n=305), N-CNP was related to both serum creatinine and creatinine clearance (\( r = 0.64 \) and \( r = 0.49 \), respectively; \( P < 0.0001 \) for both).

N-CNP levels in normal men (median, 20.7 pmol/L; range, 12.3 to 31.5) were higher than in normal women (median, 17.6 pmol/L; range, 11.2 to 29.1; \( P < 0.001 \)). In the symptomatic group overall (n=305), median (interquartile range) plasma N-CNP was also higher in men (29.0 [23.9 to 35.5]) than in women (24.3 [20.5 to 29.9]; \( P < 0.001 \)), though no significant intergender difference was found when the heart failure group was analyzed separately (33.7 [25.8 to 39.4] versus 31.6 [23.3 to 40.8]; not significantly different).

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Plasma N-CNP was related to concurrent CNP (r=0.52; P<0.0001) and was significantly but less strongly correlated to all the other hormones measured (r=0.29 to 0.45; all P<0.001, Figure 2).

In contrast to N-BNP (r=0.49; P<0.001), N-CNP showed no significant relation with LVEF (r=0.05; NS) but was weakly related to left ventricular mass (r=0.18; P<0.05).

All other hormones measured in this study were also significantly elevated in heart failure (Table 1). Most notably, N-BNP was increased almost 5-fold in subjects with heart failure when compared with subjects without heart failure (Figure 1). Patients diagnosed with heart failure also had significant impairment of cardiac function on echocardiographic (Figure 1). Patients diagnosed with heart failure also had significant impairment of cardiac function on echocardiographic assessment (Table 1).

In the symptomatic subjects (n=305), multivariate analysis by linear regression including age, gender, creatinine clearance, plasma N-BNP, echocardiographic fractional shortening, and presence or absence of heart failure revealed that with the exceptions of creatinine clearance (P=0.076) and plasma N-BNP (P=0.124), these variables were all independently predictive of increasing plasma N-CNP (P≤0.001 for all).

N-CNP, CNP, and N-BNP were assessed for their individual ability to predict the presence or absence of heart failure by receiver operator analysis. CNP and N-CNP gave strikingly similar test performance values (areas under the curve, 0.68 and 0.70, respectively, with associated sensitivities and specificities between 63% and 66%, positive predictive values 37% and 39%, negative predictive values 66% for both, and overall accuracy, 65% for both) and were both clearly weaker than plasma N-BNP with corresponding performance values of AUC=0.85; sensitivity, 80%; specificity, 81%; positive predictive value, 58%; negative predictive value, 93%; and overall accuracy, 81%. Results for the other cardiac natriuretic peptides (BNP, ANP, and N-ANP) were similar to N-BNP (AUCs 0.83 to 0.84). Next ranked was plasma endothelin-1 (AUC=0.75) followed by N-CNP and CNP and finally adrenomedullin (AUC=0.59).

By univariate analysis, gender, left ventricular fractional shortening, creatinine clearance, N-CNP (but not CNP) divided by tertiles, N-BNP divided by tertiles, and an interaction for N-CNP tertile by N-BNP tertile were significantly associated with increased risk of a final diagnosis of heart failure (Table 2). After stepwise multiple logistic regression tertile of N-BNP, the interaction between N-CNP and N-BNP tertiles and fractional shortening remained independently predictive of risk of heart failure (Table 3).

The risk of heart failure rose from 12% to 18% and then 44% in first, second, and third tertiles of plasma N-CNP, respectively. For tertiles of plasma N-BNP, the corresponding figures were 5%, 12%, and 58%. In those patients falling within both the lowest tertile of N-CNP and lowest tertile of N-BNP, none had heart failure. Conversely, in those falling in the upper tertile for both amino-terminal peptides, the individual risk of heart failure was 65%, and 47% of all patients with heart failure fell into this subgroup (Figure 3).

Discussion

This is the first report describing the relation of plasma concentrations of amino-terminal N-CNP to concurrent CNP, ANP, N-ANP, BNP, N-BNP, endothelin-1, and adrenomedullin, and echocardiographic assessments of cardiac function in a cohort of 305 generally elderly patients, including 77 with a clinical diagnosis of heart failure by a rigorously standardized definition. Both plasma N-CNP and CNP were significantly increased in heart failure and exhibited weak to moderate positive correlations with concurrent plasma levels of ANP, BNP, their amino-terminal congeners, endothelin-1, and adrenomedullin. In contrast to current and previous reports indicating that ANP and BNP are related to echocardiographic indicators of cardiac function, no strong relations between N-CNP and echocardiographic variables were observed.

Independent predictors of plasma N-CNP included gender, age, ventricular fractional shortening, and the presence of heart failure.

Like ANP and BNP, N-CNP rises with age, but in contrast to the cardiac peptides, plasma N-CNP may be higher in men than in women. This pattern held true in both the symptomatic cohort overall and in the age- and gender-matched healthy subjects. The underlying reasons for this gender difference are as yet unknown. Levels of N-CNP are also inversely related to renal function by univariate analysis, although this relation to creatinine clearance was not independent of other predictors of elevated N-CNP in multivariate analysis. Tertiles of plasma N-CNP and N-BNP interacted to predict the diagnosis of heart failure independent of age, gender, and renal function. Notably, even in the symptomatic patients without heart failure, N-CNP levels exceeded those in asymptomatic age- and gender-matched normal control subjects. The reasons for this are not certain, but it is
possible the high burden of cardiovascular disease within the non–heart failure group produced increased N-CNP at an intermediate level between truly healthy subjects and those with symptomatic heart failure.

Plasma N-CNP is clearly not a useful indicator of cardiac function as assessed by left ventricular imaging, nor is it a particularly powerful univariate indicator of the presence of heart failure in comparison with ANP, BNP, or the amino-terminal congeners of these two cardiac natriuretic peptides. However, it does add additional information beyond that offered by established predictors of the presence of heart failure, as reflected in the significant interaction seen between N-BNP and N-CNP in multivariate analysis, and the additional risk of the presence of heart failure conferred by the presence of upper tertile N-CNP together with upper tertile N-BNP.

In contrast to previous reports, we also observed a significant increment in carboxy terminal CNP concentrations in heart failure. This probably reflects the larger cohort used in the current study. Notably, the mean levels and the absolute increment seen in heart failure versus subjects without heart failure was small relative to that seen for N-CNP, presumably reflecting the longer half-life and relatively delayed metabolism of N-CNP. Our findings are consistent with previous reports of augmented cardiac tissue levels and cardiac secretion of CNP in heart failure. They are also consistent with reported interactions between circulating ANP, BNP, and CNP, in which elevation of one tends to lead to the concurrent augmentation of circulating levels of the other two, presumably by competition for clearance pathways. Experimental reports that cytokines and BNP augment cellular production of CNP in vitro would also be consistent with augmented levels of N-CNP and CNP in human heart failure. Further studies will be required to better define the relative contributions of altered secretion versus clearance.

**Figure 2.** Scatter plots of plasma amino-terminal pro-C type natriuretic peptide (N-CNP) concentrations plotted against concurrent plasma concentrations of C-type natriuretic peptide (CNP), amino-terminal pro-atrial natriuretic peptide (N-AANP), amino-terminal pro-B-type natriuretic peptide (N-BNP), endothelin-1 (ET) and adrenomedullin (ADM) in 284 to 291 patients (including 70 with subsequently confirmed heart failure). Scales are logarithmic.
Figure 3. Distribution of 70 patients with heart failure (HF) by concurrent tertiles of plasma amino-terminal pro-C type natriuretic peptide (N-CNP) and amino-terminal pro-B type natriuretic peptide (N-BNP). 1, 2, and 3, Tertile of plasma N-CNP. First, second, and third tertiles of N-BNP are indicated by diagonally hatched, dotted, and filled columns, respectively. Numerals above columns indicate absolute number (out of 70) located in each cell. No patients with HF fell in the first tertile for both N-CNP and N-BNP. Forty-seven percent of patients with HF (33/70) had plasma peptide concentrations within the top tertile for both N-CNP and N-BNP.

whether regional or systemic) of N-CNP underlying elevated plasma peptide concentrations in heart failure.

Perspectives

In community-recruited, symptomatic (and generally elderly) patients, plasma N-CNP and CNP were elevated in heart failure and related to age, renal function, and other circulating peptides. Plasma N-CNP and N-BNP interacted statistically to independently predict heart failure in multivariate analysis. Assuming cosecretion of N-CNP and CNP, the data suggest the possibility of upregulation and/or impaired clearance of this local endothelium-based vasodilator in heart failure that could be interpreted as a beneficial compensatory response opposed to the increased peripheral vascular resistance characteristic of decompensated cardiac failure. Cardiac tissue levels of CNP and expression of its specific receptor (NPR-B) are enhanced in heart failure, and in view of reports of direct cardiac effects, it is possible that increased tissue and circulating levels of CNP exert direct positive effects on cardiac contractile function. Furthermore, CNP inhibits vascular angiotensin-converting enzyme activity, which may be a further beneficial effect in heart failure. Further measurements of plasma and tissue levels of N-CNP in experimental and clinical settings should further elucidate the role of CNP in the pathophysiology of heart failure.

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References


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