Clinical correlates of elevated plasma natriuretic peptides and Big endothelin-1 in a population of ambulatory patients with heart failure. A substudy of the Italian Network on Congestive Heart Failure (IN-CHF) registry

Serge Masson, Marco Gorini*, Monica Salio, Donata Lucci*, Roberto Latini, Aldo P. Maggioni*, on behalf of the IN-CHF Investigators

*Department of Cardiovascular Research, Istituto di Ricerche Farmacologiche Mario Negri , Milan, *ANMCO Research Center, Florence, Italy*

Key words:
Heart failure; Natriuretic peptides; Hormones; Atrial natriuretic peptide; Brain natriuretic peptide; Big endothelin-1.

Background. **Activation of neuroendocrine factors plays a major role in the pathophysiology and progression of heart failure. The aim of the present study was 1) to assess the clinical correlates of elevated plasma natriuretic peptides [atrial natriuretic peptide (ANP) and brain natriuretic peptide (BNP)] and Big endothelin-1 in a population of 180 ambulatory patients from the Italian registry of heart failure (Italian Network on Congestive Heart Failure, IN-CHF) in 22 clinical centers, 2) to assess the within-patient variability of plasma BNP concentration, and 3) to evaluate the analytical agreement for BNP determination between a core laboratory and local sites.**

Methods. **ANP and BNP were measured with specific immunoradiometric methods, Big endothelin-1 with an enzyme immunoassay.**

Results. **Elevated BNP was associated with severe mitral valve regurgitation (odds ratio 8.546, 95% confidence interval 1.879-38.510, p = 0.0052); high circulating concentrations of ANP and BNP were found in older patients, and in patients with higher NYHA functional class or reduced left ventricular ejection fraction. Elevated plasma concentration of Big endothelin-1 was a strong and independent predictor of atrial fibrillation (odds ratio 4.001, 95% confidence interval 1.531-10.454, p = 0.0047). Plasma concentration of BNP was reasonably stable at 3-month interval in patients with mild-to-moderate heart failure (mean between-visit difference -1.5 ± 45 pg/ml, n = 96). There was a satisfactory analytical agreement between the central laboratory and sites, over a broad range of concentrations (2-1133 pg/ml, n = 283) with a slope for the best line fitted by linear regression of 1.09 (r^2 = 0.96).**

Conclusions. **BNP assay may become an appropriate tool for routine clinical practice in patients with congestive heart failure.**

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Address:

Dr. Aldo P. Maggioni

Centro Studi ANMCO
Via La Marmora, 34
50121 Firenze
E-mail:
centro_studi@anmco.it

Introduction

Our understanding of the pathophysiology of heart failure and its pharmacological therapy has evolved rapidly in the past 20 years. Advances in experimental and clinical knowledge have allowed for the integration of the first concepts of hemodynamic disorders into a more complex model that attempts to explain the progression from an initial cardiac lesion to ventricular dysfunction and, eventually, heart failure[1]. More recently, it has been hypothesized that the activation of various neuroendocrine factors plays a major role in the initial compensatory mechanisms triggered by hemodynamic alterations (reduction in cardiac output and arterial filling pressure, atrial overload) associated with heart failure[2].

As a direct consequence of neuroendocrine activation, circulating levels of these factors may be used as tools to improve our knowledge of the pathophysiology and clinical aspects of heart failure. Ideally, such biohumoral factors should help to gain further information on the diagnosis and prognosis of the disease, monitoring treatment efficacy, and possibly be used as therapeutic targets. Brain natriuretic peptide (BNP), a member of the natriuretic peptide family,

has recently emerged as a reliable marker of heart failure[3]. This hormone is secreted mainly by ventricular myocytes in response to chamber wall stretching and promotes salt and water excretion, and vasorelaxation through its interaction with specific receptors[4]. Several studies have contributed collectively to establish the diagnostic and prognostic value of BNP in selected populations of patients with heart failure[5,6]. It has also been suggested that plasma BNP measurement could be used as a screening test to identify patients at risk of heart failure in primary care and in the general population, as a part of health care programs[6]. The diffusion of plasma BNP measurement in clinical routine and primary care will depend, in part, on the availability of simple, reliable and cost-effective analytical methods.

The aim of the present study was therefore three-fold: a) to assess the clinical correlates of elevated plasma natriuretic peptides (atrial natriuretic peptide-ANP and BNP) and Big endothelin-1 (Big ET-1) in a population of ambulatory patients with heart failure selected from a national registry, b) to evaluate the time-dependent and within-patient variability of plasma BNP concentration, and c) to evaluate the analytical agreement for BNP determination between a centralized laboratory and local sites.

Methods

Protocol design. One hundred and eighty ambulatory patients with heart failure were selected from a national registry (Italian Network on Congestive Heart Failure, IN-CHF) in a subset of 22 clinical centers, nationwide (see Appendix). Clinical data were collected during the clinical visits using *ad hoc* software specifically prepared by the Research Center of the Italian Association of Hospital Cardiologists (ANMCO). Cardiologists participating in the IN-CHF registry were specifically trained to collect data using the software. Diagnosis of congestive heart failure was made by the cardiologist responsible according to the recommendations reported in the guidelines of the European Society of Cardiology for the diagnosis and management of congestive heart failure[7]. In each participating center, the presence of mitral regurgitation was estimated semiquantitatively by color Doppler according to three grades of severity (mild, moderate and severe)[8].

Blood collection for neurohormone determination was performed at baseline and again after a period of 3 months. At each visit, plasma was divided into two aliquots to compare plasma BNP concentrations assayed in a central laboratory and in local sites, and to measure ANP and Big ET-1.

Blood sampling and assays. A 7 ml sample of venous blood was collected in pre-chilled vacutainers containing EDTA-K^+ and aprotinin (500 kallikrein inhibitor units/ml, an inhibitor of natriuretic peptide degradation) with patients lying supine or in a sitting position for 30 min. Blood was immediately centrifuged at 2500 rpm for 15 min at 4 C, plasma divided into two parts and stored either at -70 C or at -20 C for a maximum of 2 months, before long-term storage at -70 C.

ANP and BNP were measured with nonextraction immunoradiometric assays (IRMA) from Shionogi (Shionoria[fi] ANP and BNP), according to the manufacturer s recommendations[9]. The ANP IRMA has a cross-reaction for hBNP of < 0.01%, while the BNP IRMA shows a cross-reaction for hANP of < 1.10-5%. Intraassay precision for ANP, evaluated in the core laboratory on a sample with a mean concentration of 68 pg/ml, is 2.3% (coefficient of variation-CV, n = 10), and interassay precision is 6.8% (CV, n = 5). Intraassay precision for BNP evaluated on a sample with a mean concentration of 28 pg/ml is 6.8% (CV, n = 8), and interassay precision is 7.5% (CV, n = 5). Big-ET-1 (1-38) was measured with a direct enzyme immunoassay (Biomedica, Austria, catalogue number BI-20072). Cross reactivity for endothelin (ET-1, ET-2 or ET-3) is < 1%, intraassay precision evaluated on a sample with a mean concentration of 0.93 fmol/ml is 9.1% (CV, n = 5), and interassay precision is 9.7% (CV, n = 12). Upper limits of normal ranges for ANP, BNP and Big ET-1 were 43 pg/ml, 19 pg/ml and 0.70 fmol/ml, respectively.

Statistical analysis. Univariate and multivariate analyses were performed to evaluate the association between neurohormonal and clinical variables. The χ^2 statistics was used to test the significance of differences among groups of patients. All p values are two-tailed. The adjusted analysis was performed utilizing logistic regression models. All variables were included in the model, irrespective of their significance at univariate analysis. The results are presented in terms of odds ratios and their 95% confidence intervals (CI). Variables included in the multivariate model were: age (as a continuous variable), gender (males vs females), etiology (coronary heart disease vs non-coronary heart disease), NYHA functional class (III-IV vs I-II), ejection fraction (as a continuous variable), mitral regurgitation (severe vs moderate vs none), ACE-inhibitor treatment (yes vs no), beta-blocker treatment (yes vs no), diuretic treatment (yes vs no), and digitalis treatment (yes vs no). A p value of < 0.01 was considered statistically significant.

Within-subject variability of plasma BNP concentrations is illustrated according to the representation of Bland and Altman[10]. The within-subject variability of plasma BNP was evaluated by calculating the repeatability of measurements, that is 2.77 s_w where s_w is the within-subject standard deviation of repeated measurements computed according to the formula: $s_w = 1/2n \Sigma d^2_i$ where n is the number of subjects and d_i the difference between two observations[11].

Results

Study population. The main clinical characteristics of the population selected for this study are presented in table I. The patients were mainly males (74%), aged 63 – 12 years, in NYHA functional class I or II (75%) and left ventricular ejection fraction of 0.37 – 0.12. This distribution was similar to the clinical characteristics of the overall population included in the IN-CHF registry at the time of the study. Heart failure was predominantly of dilatative etiology (45%), followed by ischemic (32%) and hypertensive (12%) causes. Symptom duration of heart failure was longer than 6 months for 76.1% of the subjects. Seventy percent of the patients had mitral valve regurgitation, which was severe in 11% of the cases. Chronic atrial fibrillation was present in 33% of the patients.

Neurohormones at baseline. The distribution of ANP, BNP and Big ET-1 plasma levels at baseline is presented in table I. The circulating concentrations were below the upper limit of normal values for 70 patients (40%), 34 patients (19%) and 106 patients (60%) for ANP, BNP and Big ET-1, respectively. Univariate and multivariate analyses were performed by dividing the population according to a cut-off point set at the second tertile value of the concentrations, that were 92 pg/ml, 121 pg/ml and 0.78 fmol/ml, for ANP, BNP and Big ET-1, respectively. By univariate analysis (Table II), ANP was

Table I. Characteristics of the study population and baseline levels of ANP, BNP and Big ET-1.

Variables	Patients (n=,%)	ANP (pg/ml)	BNP (pg/ml)	Big ET-1 (fmol/ml)
Population	180 (100)	91 – 7 (175)	146 – 15 (180)	0.84 – 0.04 (175)
Age				
< 70 years	123 (68)	80 – 8 (119)	115 – 16 (123)	0.79 – 0.04 (119)
70 years	57 (32)	113 – 15 (56)	217 – 31 (57)	0.95 – 0.09 (56)
Gender				
Males	133 (74)	91 – 9 (129)	154 – 15 (133)	0.84 – 0.04 (129)
Females	47 (26)	91 – 14 (46)	129 – 22 (47)	0.86 – 0.11 (46)
NYHA class				
I-II	135 (75)	79 – 8 (130)	115 – 15 (135)	0.76 – 0.04 (130)
III-IV	45 (25)	125 – 16 (45)	245 – 37 (45)	1.08 – 0.12 (45)
LVEF				
< 30%	47 (26)	122 – 17 (46)	207 – 36 (47)	0.95 – 0.12 (46)
30-40%	80 (45)	85 – 10 (77)	138 – 21 (80)	0.82 – 0.05 (77)
> 40%	53 (29)	71 – 12 (52)	110 – 20 (53)	0.79 – 0.06 (52)
Etiology				
Dilatative	80 (45)	77 – 9 (78)	109 – 20 (80)	0.74 – 0.05 (78)
Ischemic	58 (32)	115 – 16 (55)	205 – 32 (58)	0.88 – 0.07 (55)
Hypertensive	22 (12)	92 – 26 (22)	128 – 31 (22)	0.88 – 0.12 (22)
Valvular	15 (8)	77 – 16 (15)	182 – 45 (15)	1.19 – 0.29 (15)
Others	5 (3)	79 – 25 (5)	89 – 42 (5)	0.88 – 0.14 (5)
Mitral regurgitation				
No	45 (25)	77 – 15 (45)	120 – 34 (45)	0.79 – 0.12 (45)
Grade 1-2	106 (59)	87 – 8 (103)	141 – 17 (106)	0.81 – 0.04 (103)
Grade 3-4	19 (11)	143 – 35 (17)	229 – 61 (19)	1.10 – 0.16 (17)
Missing	10 (5)	104 – 26 (10)	194 – 55 (10)	0.98 – 0.18 (10)
Atrial fibrillation				
Yes	33 (18)	84 – 13 (31)	144 – 23 (33)	1.06 – 0.15 (31)
No	147 (82)	92 – 8 (144)	149 – 17 (147)	0.80 – 0.04 (144)
ACE-inhibitor therapy				
Yes	154 (86)	93 – 8 (149)	146 – 16 (154)	0.85 – 0.05 (149)
No	26 (14)	76 – 16 (26)	156 – 45 (26)	0.79 – 0.09 (26)
β-blocker therapy				
Yes	68 (38)	77 – 8 (68)	107 – 16 (68)	0.74 – 0.04 (68)
No	112 (62)	99 – 11 (107)	173 – 21 (112)	0.91 – 0.06 (107)
Diuretic therapy				
Yes	143 (79)	96 – 9 (138)	155 – 16 (143)	0.88 – 0.05 (138)
No	37 (21)	71 – 11 (37)	121 – 36 (37)	0.70 – 0.05 (37)
Digitalis therapy				
Yes	108 (60)	100 – 10 (104)	170 – 20 (108)	0.90 – 0.06 (104)
No	72 (40)	81 – 9 (71)	115 – 22 (72)	0.75 – 0.05 (71)

Data are mean – SE. ACE = angiotensin-converting enzyme; ANP = atrial natriuretic peptide; BNP = brain natriuretic peptide; Big ET-1 = precursor of endothelin-1 (1-38); LVEF = left ventricular ejection fraction; NYHA = New York Heart Association.

shown to be elevated in older patients (p= 0.004) and in the patients with more severe heart failure (NYHA functional classes III-IV, p = 0.003). Similarly, BNP was significantly higher in patients over 70 years (p = 0.001), in patients with NYHA functional class III-IV (p= 0.001), with a depressed left ventricular ejection fraction (p = 0.008), and with mitral regurgitation (p = 0.002). Finally, Big ET-1 was significantly higher in patients with more severe heart failure (NHYA functional classes III-IV, p = 0.009), with mitral regurgitation (p = 0.005) or with atrial fibrillation (p = 0.001). By univariate analysis, gender, etiology, and treatment with ACE-inhibitors, beta-blockers, diuretics or digitalis were not found as determinants of elevated ANP, BNP or Big ET-1.

To identify the independent predictors of elevated circulating neurohormones, we performed multivariate analyses according to a model of logistic regression (Table II). Severe mitral regurgitation was the strongest independent predictor of elevated plasma BNP and Big ET-1, with odds ratios of 8.546 (95% CI 1.897-38.510) and 5.722 (95% CI 1.473-22.228), respectively. Patients in NYHA functional class III and IV had a 3-fold higher likelihood of having elevated plasma BNP, whereas a 1-year increase in age or a 1-unit decrease in ejection fraction was associated with a 7-8% increase in the probability of having elevated (higher tertile) plasma ANP or BNP. Interestingly, the levels of plasma Big ET-1 were significantly elevated in patients with atrial fibrillation (odds ratio 4.001, p = 0.0047), while ANP or BNP levels were not. The other variables included in the statistical model (ischemic etiology, gender, therapy with ACE-inhibitors, beta-blockers, diuretics or digitalis) were not associated with elevated ANP, BNP or Big ET-1.

Within-patient variability of plasma brain natriuretic peptide. Figure 1 shows the within-patient variability of plasma BNP over a 3-month interval, according to the representation of Bland and Altman[10]. Plasma was available at both time points for 156 patients. Within-patient variability of plasma BNP was low when considering patients with a mean BNP concentration of up to 100 pg/ml where the between-visit difference was -1.5 – 45 pg/ml (mean – 2 SD, n = 96). The variability increased rapidly among patients with an average BNP above 100 pg/ml, giving a between-visit difference 8.1 – 289 pg/ml (mean – 2 SD, n = 60). Overall, the repeatability was 154 pg/ml, implying that the difference between two plasma BNP measurements for the same subject was < 154 pg/ml for 171 (95%) pairs of observations. For the subgroup of patients with an average plasma BNP concentration of < 100 pg/ml, repeatability was 44 pg/ml. When considering the population with plasma BNP > 100 pg/ml, repeatability rose to 244 pg/ml.

Table II. Univariate and mutivariate analysis of plasma ANP, BNP and Big ET-1 by risk factors.

Variable	Univariate	Multivariate		
	p	OR	95% CI	p
ANP				
Age	0.004	1.067	1.025-1.110	0.0015
Gender	0.783	0.924	0.392-2.177	0.8557
NYHA class	0.003	1.866	0.817-4.264	0.1389
LVEF	0.028	0.946	0.912-0.981	0.0027
Ischemic etiology	0.192	0.935	0.417-2.094	0.8695
Mitral regurgitation	0.026			
Severe		5.013	1.242-20.237	0.0236
Moderate		2.180	0.826-5.752	0.1153
Atrial fibrillation	0.908	0.829	0.309-2.221	0.7093
ACE-inhibitor therapy	0.781	0.881	0.300-2.585	0.8175
β-blocker therapy	0.244	0.987	0.444-2.193	0.9750
Diuretic therapy	0.199	1.092	0.413-2.887	0.8585
Digitalis therapy	0.070	1.914	0.856-4.282	0.1140
BNP				
Age	0.001	1.082	1.037-1.129	0.0003
Gender	0.631	1.138	0.462-2.801	0.7784
NYHA class	0.001	2.628	1.105-6.254	0.0289
LVEF	0.008	0.939	0.893-0.967	0.0003
Ischemic etiology	0.055	1.298	0.555-3.039	0.5473
Mitral regurgitation	0.002			
Severe		8.546	1.897-38.510	0.0052
Moderate		3.491	1.128-10.799	0.0300
Atrial fibrillation	0.220	1.490	0.542-4.097	0.4393
ACE-inhibitor therapy	0.294	0.356	0.113-1.124	0.0782
β-blocker therapy	0.128	0.974	0.411-2.308	0.9515
Diuretic therapy	0.192	0.854	0.303-2.405	0.7646
Digitalis therapy	0.024	2.618	1.090-6.289	0.0314
Big ET-1				
Age	0.027	1.049	1.009-1.090	0.015
Gender	0.649	0.732	0.310-1.728	0.4766
NYHA class	0.009	1.728	0.764-3.909	0.1893
LVEF	0.644	0.991	0.960-1.023	0.5762
Ischemic etiology	0.192	0.538	0.699-3.388	0.2849
Mitral regurgitation	0.005			
Severe		5.722	1.473-22.228	0.0118
Moderate		2.150	0.793-5.831	0.1327
Atrial fibrillation	0.001	4.001	1.531-10.454	0.0047
ACE-inhibitor therapy	0.863	1.334	0.460-3.869	0.5956
β-blocker therapy	0.244	1.055	0.476-2.338	0.8949
Diuretic therapy	0.373	0.863	0.332-2.243	0.7617
Digitalis therapy	0.138	1.408	0.638-3.108	0.3974

Abbreviations as in table I. Odds ratio (OR) with 95% confidence interval (CI) for having elevated plasma biohumoral factor concentration at baseline was calculated according to a model of multiple logistic regression including the variables presented in the table. The cut-off points were set at the value of the second tertile of plasma concentration, i.e. 92, 121 pg/ml and 0.78 fmol/ml for ANP, BNP and Big ET-1, respectively.

Analytical agreement. As part of a program of quality control, plasma BNP was assayed both in a central laboratory and in the clinical chemistry laboratories of each of the 22 participating hospitals. Figure 2 illustrates the relationship between these determinations (n = 283 pairs) over a broad range of concentrations (2-1133 pg/ml). The slope of the best line fitted by linear regression analysis between the two variables was 1.09 and the coefficient of correlation r^2 was 0.96.

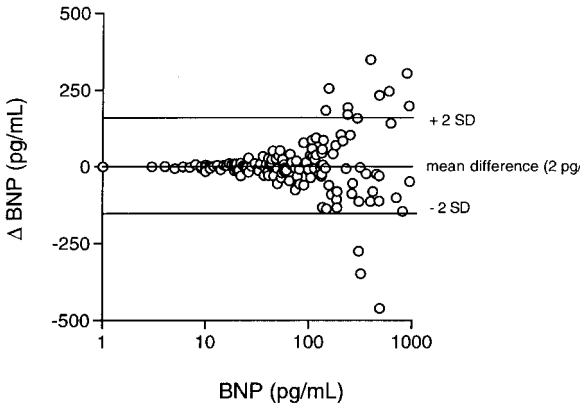

Figure 1. *Within-patient variability of plasma brain natriuretic peptide (BNP) concentration in patients with heart failure. For each patient, plasma BNP was drawn at baseline and 3 months later, and assayed with an immunoradiometric method in a single batch of determination. The figure illustrates the within-patient and between-visit variability of plasma BNP concentration according to the representation of Bland and Altman, 156 pairs of determinations.*

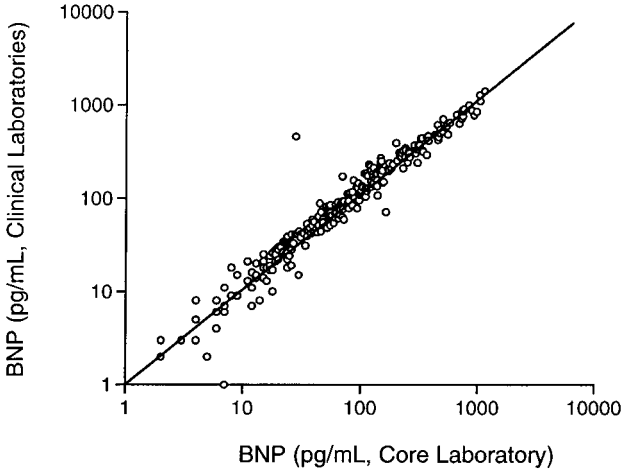

Figure 2. *Analytical agreement for plasma brain natriuretic peptide (BNP) determination between a central core laboratory and local sites. Plasma BNP was assayed in 22 sites with an immunoradiometric method. As a quality control, the same plasma was also assayed in parallel in a central laboratory. The figure illustrates the analytical agreement between these two determinations for 283 pairs of samples. The slope of the best line fitted by linear regression analysis between the two variables was 1.09 and the coefficient of correlation r^2 was 0.96. Please note the log-log scale.*

Discussion

The main findings of the present study are that a) plasma BNP concentration determined locally in 22 laboratories with the same immunometric method, compared fairly well with a central measurement performed by a core laboratory, b) plasma concentration of BNP was fairly stable over a 3-month interval in patients with mild-to-moderate heart failure, c) elevated plasma BNP was associated with moderate and severe mitral valve regurgitation, while high circulating concentrations of ANP and BNP were encountered in the older patients, and in patients with worse NYHA functional class or reduced left ventricular ejection fraction, d) elevated plasma concentrations of Big ET-1 were strongly and independently associated with atrial fibrillation as a dominant rhythm.

The present study was not designed to assess the prognostic value of these neurohormones.

The diffusion of hormonal assays in clinical routine and general practice as a simple and cost-efficient means of monitoring cardiac function or the progress of a pharmacological therapy will depend on the clinical usefulness and availability of reliable analytical methods. BNP fulfils some requisites as a marker of cardiac function in patients with heart failure inasmuch as numerous studies have documented its relation to the severity of heart disease[12-15], its diagnostic and prognostic value[5,6], the possibility of monitoring the efficacy of a drug therapy[16], and in view of its relative stability in biological fluid[17-19]. Here, we have extended these data and verified that plasma BNP levels remained stable in patients with mild-to-moderate heart failure (and stable drug therapy) over 3 months, and the analytical agreement for BNP determination between a centralized laboratory and local sites was satisfactory. This identifies BNP as a possible marker of cardiac function to be selected in general population studies and health screening programs of heart failure. The analytical method can yield reliable results even when carried out in clinical laboratories routinely performing radioimmunoassays, but without specific experience in this assay. These features confirm that BNP could be a good candidate as a fast-response indicator for the titration of tailored therapy of chronic heart failure[20].

Big endothelin-1 and atrial fibrillation. ET-1 is synthesized by cardiac myocytes and vascular endothelial cells and increases the contractility of cardiac muscles and vascular smooth muscles in an autocrine-paracrine manner. It also provokes myocardial hypertrophy and cardiomyocyte injury[21]. Plasma ET-1 levels are elevated in both humans and experimental animal models of heart failure[22-24]. The predominant molecular form of immunoreactive endothelin in the plasma of patients with heart failure is however Big ET-1, the precursor of endothelin that is cleaved by the endothelin-converting enzyme[25]. Big ET-1 is as potent as ET-1 in increasing arterial blood pressure and in evoking coronary constriction in humans[26]. Plasma levels of Big ET-1 correlate with the severity of left ventricular dysfunction and clinical symptoms of heart failure[27], and has been found to be a good prognostic indicator of heart failure[28,29]. In the present study, in spite of the fact that most patients (60%) were within the normal range for Big ET-1, we found that this factor was associated with symptoms of heart failure ($p = 0.009$ for NYHA functional class by univariate analysis). However, the clinical event that was more strongly associated by multivariate analysis with elevated plasma levels of Big ET-1 was the presence of atrial fibrillation ($p = 0.001$). A similar elevation of endothelin isoforms has been observed in the plasma of patients with heart failure and atrial fibrillation as compared to patients in sinus

rhythm[30]. The reason why circulating endothelin is elevated in patients with atrial fibrillation is unknown at present. By multivariate analysis, we did not observe any significant elevation of plasma natriuretic peptides (ANP or BNP) among patients with atrial fibrillation, compared to those in sinus rhythm (Table II). Data regarding natriuretic peptides in patients with heart failure and atrial fibrillation are contrasting: for instance, the N-terminal fragment proatrial natriuretic factor has been reported to be either unchanged[30] or elevated in patients with atrial fibrillation[31], compared to a group of patients in sinus rhythm. The apparent discrepancy may be related to differences in the duration of atrial fibrillation since longer duration is predictive of lower plasma ANP[32]. Data about the duration of atrial fibrillation are not available in the IN-CHF registry.

Chronic mitral regurgitation. Natriuretic peptides secretion by atrial and ventricular myocytes is regulated by hemodynamic changes (wall stress on cardiac chambers) and neurohormonal factors including alpha-adrenergic stimulation, endothelin and angiotensin II[4]. In the present study, we found that severe mitral regurgitation was associated with elevated BNP by multivariate analysis (and less strongly to ANP and Big ET-1 plasma levels). Our data confirm the few original observations made in experimental models of mitral regurgitation[33] and in patients with heart diseases of different etiologies[34]. However, the significance of these findings, and in particular whether elevated BNP is a marker of the degree or a consequence of mitral regurgitation remains to be investigated[35,36]. Stretch of atrial myocytes induces not only ANP, but also BNP secretion.

Natriuretic peptides and aging. In our study, plasma ANP and BNP strongly and independently increased with advancing age ($p = 0.0015$ and 0.0003 by multivariate analysis). Accordingly, plasma ANP levels are known to be higher in healthy, elderly subjects than in younger subjects[37,38]. The difference may be related to impaired capacity of synthesis in response to hemodynamic (atrial stretch) or endocrine stimuli, or to a reduced clearance capacity in the elderly.

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Appendix

Centers participating in the study. Bergamo, Ospedali Riuniti (M. De Tommasi, M.G. Valsecchi, F. D Adda, L. Gualandris); Cagliari, Ospedale San Michele Brotzu (A. Sanna, M. Porcu, S. Salis, L. Pistis, G. Melis, S. Ledda); Como, Ospedale Sant Anna (G. Ferrari, M. Landro, R. Belluschi, A. Pagani); Cosenza, Ospedale Dell Annunziata (F. Plastina, G. Misuraca, O. Serafini, R. Caporale, G. Greco), Cuneo, Ospedale Santa Croce (E. Uslenghi, U. Milanese, G. Ugliengo, A. Bramardi), Desio, Ospedale Provinciale (S. Gramenzi, G. Foti, P. Mocarelli, S. Signorini); Francavilla Fontana, Ospedale Civile Dario Camberlingo (V. Cito, F. Cocco, G. Lupo, J. Bottari); La Spezia, Ospedale Civile Sant Andrea (A. Sciarra); Milano, Ospedale Luigi Sacco (A. Malliani, S. Muzzupappa, M. Turiel, M. Bevilacqua, T. Vago, G. Baldi); Monfalcone, Ospedale Civile (T. Morgera, E. Barducci, C. Rieppi); Napoli, Azienda Ospedaliera Vincenzo Monaldi (P. Sensale, O. Maiolica, V. Rullo, L. Romano); Padova, Azienda Ospedaliera (S. Dalla Volta, L. Cacciavillani, G.M. Boffa, M. Plebani); Palermo, Ospedale G.F. Ingrassia (P. Di Pasquale, F. Clemenza); Palermo, Presidio Ospedaliero Villa Sofia (A. Battaglia, F. Ingrill , G. Gallo); Pavia, IRCCS Policlinico San Matteo (C. Campana, R. Sebastiani, A. Giusti, M. Autelli); Roma, Ospedale Santo Spirito (V. Ceci, N. Aspromonte, N. Federici); Sarzana, Ospedale San Bartolomeo (D. Bertoli, L. Magliani); Spoleto, Ospedale San Matteo degli Infermi (G. Maragoni, G. Bardelli, G. Comastri, C. Gaggi); Treviso, Presidio Ospedaliero Ca Foncello (P. Stritoni, G. Renosto, L. Caberlotto); Tricase, Ospedale Cardinale Panico (A. Galati, R. Mangia, M. Rizzello); Udine, Azienda Ospedaliera S. Maria della Misericordia (P. Fioretti, M.C. Albanese, C. Fresco, U.P. Guerra, C. Toso); Valeggio sul Mincio, Centro Ospedaliero Clinicizzato (G. Perini, C. Bonadiman); Varese, Ospedale di Circolo (S. Repetto, F. Morandi, S. Provasoli, L. Giovanella).

References

1. Mann DL. Mechanisms and models in heart failure. A combinatorial approach. Circulation 1999; 100: 999-1008.
2. Schrier RW, Abraham WT. Hormones and hemodynamics in heart failure. N Engl J Med 1999; 341: 577-85.
3. Grantham JA, Burnett JC Jr. BNP: increasing importance in the pathophysiology and diagnosis of congestive heart failure. Circulation 1997; 96: 388-90.
4. Thibault G, Amiri F, Garcia R. Regulation of natriuretic peptide secretion by the heart. Annu Rev Physiol 1999; 61: 193-217.
5. Tsutamoto T, Wada A, Maeda K, et al. Attenuation of compensation of endogenous cardiac natriuretic peptide system in chronic heart failure. Prognostic role of plasma brain natriuretic peptide concentration in patients with chronic symptomatic left ventricular dysfunction. Circulation 1997; 96: 509-16.
6. Cowie MR, Struthers AD, Wood DA, Coats AJS, Thompson SG, Poole-Wilson PA. Value of natriuretic peptides in assessment of patients with possible new heart failure in primary care. Lancet 1997; 350: 1347-51.
7. Anonymous. Guidelines for the diagnosis of heart failure. The task force on heart failure of the European Society of Cardiology. Eur Heart J 1995; 16: 741-51.
8. Dall Aglio V, D Angelo G, Moro E, et al. Interobserver and echo-angio variability of two-dimensional colour Doppler evaluation of aortic and mitral regurgitation. Eur Heart J 1989; 10: 334-40.

9. Clerico A, Iervasi G, Del Chicca MG, et al. Analytical performance and clinical usefulness of a commercially available IRMA kit for measuring atrial natriuretic peptide in patients with heart failure. Clin Chem 1996; 42: 1627-33.
10. Bland JM, Altman DG. Statistical methods for assessing agreement between two methods of clinical measurement. Lancet 1986; i: 307-10.
11. Bland JM, Altman DG. Measurement error. BMJ 1996; 313: 744.
12. Mulcoyama M, Nakao K, Hosoda K, et al. Brain natriuretic peptide as a novel cardiac hormone in humans. Evidence for an exquisite dual natriuretic peptide system, atrial natriuretic peptide and brain natriuretic peptide. J Clin Invest 1991; 98: 1402-12.
13. Yoshimura M, Yasue H, Okumura K, et al. Different secretion patterns of atrial natriuretic peptide and brain natriuretic peptide in patients with congestive heart failure. Circulation 1993; 87: 464-9.
14. Wei CM, Heublein DM, Perrella MA, et al. Natriuretic peptide system in human heart failure. Circulation 1993; 88: 1004-9.
15. Clerico A, Iervasi G, Del Chicca MG, et al. Circulating levels of cardiac natriuretic peptides (ANP and BNP) measured by highly sensitive and specific immunoradiometric assays in normal subjects and in patients with different degrees of heart failure. J Endocrinol Invest 1998; 21: 170-9.
16. McKelvie RS, Yusuf S, Pericak D, et al. Comparison of candesartan, enalapril, and their combination in congestive heart failure. Randomized Evaluation of Strategies for Left Ventricular Dysfunction (RESOLVD) Pilot Study. The RESOLVD Pilot Study Investigators. Circulation 1999; 100: 1056-64.
17. Tsuji T, Imagawa K, Masuda H, et al. Stabilization of human brain natriuretic peptide in blood samples. Clin Chem 1994; 40: 672-3.
18. Murdoch DR, Byrne J, Morton JJ, et al. Brain natriuretic peptide is stable in whole blood and can be measured using a simple rapid assay: implications for clinical practice. Heart 1997; 78: 594-7.
19. Buckely MG, Marcus NJ, Yacoub MH, Singer DRJ. Prolonged stability of brain natriuretic peptide: importance for non-invasive assessment of cardiac function in clinical practice. Clin Sci 1998; 95: 235-9.
20. Murdoch DR, McDonagh TA, Byrne J, et al. Titration of vasodilator therapy in chronic heart failure according to plasma brain natriuretic peptide concentration: randomized comparison of the hemodynamic and neuroendocrine effects of tailored versus empirical therapy. Am Heart J 1999; 138: 1126-32.
21. Miyauch T, Masaki T. Pathophysiology of endothelin in the cardiovascular system. Annu Rev Physiol 1999; 61: 391-415.
22. Hiroe M, Hirata Y, Fujita N, Umezawa S, Ito H. Plasma endothelin-1 levels in idiopathic dilated cardiomyopathy. Am J Cardiol 1991; 68: 1114-5.
23. McMurray JJV, Ray SG, Abdullah IA, Dargie HJ, Morton JJ. Plasma endothelin in chronic heart failure. Circulation 1992; 85: 1374-9.
24. Margulies KB, Hildebrand FL Jr, Lerlan A, Perrella MA, Burnett JC Jr. Increased endothelin-1 in experimental heart failure. Circulation 1990; 82: 2226-30.
25. Wei CM, Lerman A, Rodeheffer RJ, et al. Endothelin in human congestive heart failure. Circulation 1994; 89: 1580-6.
26. Pernow J, Kaijser L, Lundberg JM, Ahlborg G. Comparable potent coronary constrictor effects of endothelin-1 and big endothelin-1 in humans. Circulation 1996; 94: 2077-82.
27. Zierhut W, Zimmer HG, Gerdes AM. Effect of angiotensin converting enzyme inhibition on pressure-induced left ventricular hypertrophy in rats. Circ Res 1991; 69: 609-17.
28. Hulsmann M, Stanek B, Frey B, et al. Value of cardiopulmonary exercise testing and big endothelin plasma levels to predict short-term prognosis of patients with chronic heart failure. J Am Coll Cardiol 1998; 32: 1695-700.
29. Pacher R, Stanek B, Hulsmann M, et al. Prognostic impact of big endothelin-1 plasma concentrations compared with invasive hemodynamic evaluation in severe heart failure. J Am Coll Cardiol 1996; 27: 633-41.
30. Tuinenburg AE, Van Veldhuisen DJ, Boomsma F, Van Den Berg MP, De Kam PJ, Crijns HJGM. Comparison of plasma neurohormones in congestive heart failure patients with atrial fibrillation versus patients with sinus rhythm. Am J Cardiol 1998; 81: 1207-10.
31. Dickstein K, Larsen AI, Bonarjee V, Thoresen M, Aarsland T, Hall C. Plasma proatrial natriuretic factor is predictive of clinical status in patients with congestive heart failure. Am J Cardiol 1995; 76: 679-83.
32. Van Den Berg MP, Crijns HJGM, Van Veldhuisen DJ, Van Gelder IC, De Kam PJ, Lie KI. Atrial natriuretic peptide in patients with heart failure and chronic atrial fibrillation: role of duration of atrial fibrillation. Am Heart J 1998; 135: 242-4.
33. Asano K, Masuda K, Okumura M, Kadosawa T, Fujinaga T. Plasma atrial and brain natriuretic peptide levels in dogs with congestive heart failure. J Vet Med Sci 1999; 61: 523-9.
34. La Vecchia L, Fortunato A, Varotto L, et al. Hemodynamic correlates of atrial natriuretic peptide concentration in unselected patients with heart disease of different etiologies. G Ital Cardiol 1998; 28: 1363-71.
35. Brookes CI, Kemp MW, Hooper J, Oldershaw PJ, Moat NE. Plasma brain natriuretic peptide concentrations in patients with chronic mitral regurgitation. J Heart Valve Dis 1997; 6: 608-12.
36. Sarano ME, Rossi A, Dujardin KS, Burnett JC Jr, Seward JB. Natriuretic peptides in patients with mitral regurgitation: markers of degree or consequences of the regurgitation? (abstr) J Am Coll Cardiol 1998; 31: 424A.
37. Davis KM, Fish LC, Minaker KL, Elahi D. Atrial natriuretic peptide levels in the elderly: differentiating normal aging changes from disease. J Gerontol 1996; 51: M95-M101.
38. Jensen KT, Carstens J, Ivarsen P, Pedersen EB. A new, fast, and reliable radioimmunoassay of brain natriuretic peptide in human plasma. Reference values in healthy subjects and in patients with different diseases. Scand J Clin Lab Invest 1997; 57: 529-40.