Correlations between clinical presentation, brain natriuretic peptide, big endothelin-1, tumor necrosis factor-α and cardiac troponins in heart failure patients

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Biochemical markers; Cardiomyopathies; Heart failure; Left ventricular insufficiency.

Background. **The term "biochemical marker" of heart failure is used to define a biochemical substance whose plasma levels correlate with the clinical and hemodynamic status and predict the prognosis of patients with heart failure. The aim of this study was to prospectively evaluate, in a single population of patients with heart failure, the correlations between the plasma levels of brain natriuretic peptide (BNP), big endothelin-1 (BET-1), tumor necrosis factor-α (TNF-α), cardiac troponin I (cTnI) and T (cTnT), the clinical presentation, and the left ventricular function.**

Methods. **The study population included a series of 120 patients (97 males, 81%, mean age 56 ± 12 years) in NYHA functional class I (49%), II (20%), III (26%), IV (5%) who were admitted to our institution or followed up as outpatients. All patients underwent cardiologic evaluation, standard electrocardiography, two-dimensional echocardiography, and venous blood sampling on the same day.**

Results. **At univariate analysis the following correlations were found to be significant: all the laboratory parameters correlated with the NYHA class (BNP r = 0.63, BET-1 r = 0.56, cTnI r = 0.25, cTnT r = 0.24, TNF-α r = 0.23); BNP (r = -0.39) and BET-1 (r = -0.27) with left ventricular ejection fraction; BNP (r = 0.37) and BET-1 (r = 0.21) with the degree of mitral insufficiency; BNP (r = -0.39), BET-1 (r = 0.25) and TNF-α (r = -0.19) with systolic blood pressure; cTnT (r = 0.34), cTnI (r = 0.33), BNP (r = 0.22) and BET-1 (r = 0.19) with heart rate; BNP with age (r = 0.33) and body mass index (r = -0.28). The plasma levels of BNP, BET-1, cTnT and cTnI were significantly higher in case of systemic or pulmonary congestion. At multiple regression analysis the following correlations were still present: BNP with the NYHA functional class (p < 0.005) and with pulmonary venous congestion (p < 0.05); BET-1 with the presence of pulmonary venous congestion (p < 0.005); TNF-α with the NYHA class (p < 0.05) and systolic blood pressure (p < 0.001); cardiac troponins with heart rate (p < 0.05).**

Conclusions. **The plasma concentrations of BNP and BET-1 showed the best and comparable correlations with parameters describing the clinical status of patients with heart failure, in particular with the presence of pulmonary venous congestion. The value of the plasma concentration of TNF-α and those of cardiac troponins were found to be limited in patients with relatively stable heart failure.**

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Introduction

The term "biochemical marker" of heart failure is commonly used to define a biochemical substance whose plasma levels correlate with the clinical and hemodynamic status and predict the prognosis of patients with heart failure[1].

Biomarkers of heart failure include hormones such as norepinephrine[2-4], atrial and brain natriuretic peptides[4,5], endothelin and its precursor big endothelin-1 (BET-1)[4,6,7], cytokines such as tumor necrosis factor-α (TNF-α) and interleukins[8,9], and cardiac troponins[10,11]. The increase in the plasma levels of each of these substances in patients with heart failure has a different significance and pathogenetic role[2-11]. The advantage of such markers in clinical practice could be the non-invasiveness and the easiness and repeatability of their laboratory determination; however, the relative degree of correlation with the severity of the clinical presentation and left ventricular dysfunction has not been investigated in detail. This relationship is important because in many studies the plasma levels of different markers are employed to monitor the results of pharmacological therapy[12-16]. Moreover, the plasma levels of brain natriuretic peptide (BNP), the biomarker most commonly used in clinical practice, have been recently proposed as a guide for the treatment of heart failure patients[12,17].

The aim of this study was to prospectively evaluate the correlation between the plasma levels of BNP, BET-1, TNF-α, cardiac troponin I (cTnI) and T (cTnT), the clinical presentation and the left ventricular function in a single population of patients with different degrees of heart failure.

Methods

The study population consisted of a series of patients who were admitted to our institution or followed up as outpatients. All patients showed at the time of enrolment, or had shown in the past, signs and/or symptoms of heart failure. In all cases, left ventricular ejection fraction was < 45%.

At the time of recruitment all patients underwent, on the same day, cardiologic evaluation, standard electrocardiography, two-dimensional echocardiography, and venous blood sampling.

The following demographic, clinical and instrumental parameters were recorded: age, sex, body mass index, NYHA functional class, systolic and diastolic blood pressure, heart rate, presence or absence of pulmonary venous congestion (as evaluated at chest X-ray and/or on the basis of the presence or otherwise of pulmonary inspiration rales), presence or absence of systemic venous congestion (jugular venous distension and/or hepatomegaly and ankle edema), cardiac rhythm (sinus rhythm, atrial fibrillation, pacemaker-led), cause of left ventricular dysfunction [coronary artery disease (history of myocardial infarction or angiographic documentation of critical stenosis of at least one major coronary artery), idiopathic or familial dilated cardiomyopathy (left ventricular systolic dysfunction without coronary artery disease or any other cardiac or systemic cause that could explain it), valvular heart disease (left ventricular end-diastolic volume and ejection fraction as measured at two-dimensional echocardiography, mitral valve regurgitation semiquantitatively evaluated at color Doppler echocardiography as absent = 1, mild = 2, moderate = 3, relevant = 4)]. All patients underwent venous blood sampling for the determination of the plasma levels of BNP, BET-1, TNF-α, cTnI and cTnT.

The plasma levels of BNP, BET-1, TNF-α, and cTnI and cTnT were measured using the following techniques:

- BNP: a solid-phase sandwich immunoradiometric assay utilizing two monoclonal antibodies against sterically remote sites, the first coated on the solid-phase of the beads and the second radiolabeled with iodine 125 used as a tracer (Shionoria BNP, CIS Biointernational, Vercelli, Italy); upper reference limit 62 ng/l[18];
- BET-1: an enzyme immunoassay (Biomedica, Vienna, Austria) that incorporates an immunoaffinity purified polyclonal capture antibody and a monoclonal detection antibody; upper reference limit 0.95 fmol/l;
- TNF-α: an enzyme-amplified sensibility immunoassay based on monoclonal antibodies against specific sites of TNF-α; upper reference limit 15 ng/l;
- cTnI: a one-step enzyme immunoassay based on the sandwich principle (RxL Dimension Analyzer, Dade Behring, Milan, Italy); upper reference limit 0.15 ng/ml[19];
- cTnT: a two-site third-generation chemiluminescent immunoassay (Elecsys Analyzer, Roche Diagnostics, Milan, Italy); upper reference limit 0.04 ng/ml[20].

All patients gave their informed consent before study entry. The investigation conforms to the principles outlined in the declaration of Helsinki.

Parameters are expressed as mean ± SD. As the distribution of the levels of biomarkers was highly skewed, the median and percentiles of these parameters were also calculated. Univariate regression analyses were performed to search for correlations between biochemical markers and clinical parameters. In univariate analyses a simple regression method was used for the following continuous variables: age, body mass index, NYHA functional class, systolic blood pressure, ejection fraction, left ventricular end-diastolic volume, mitral regurgitation (absent = 1, mild = 2, moderate = 3, severe = 4) and plasma levels of heart failure biochemical parameters. The calculation was also performed after logarithmic transformation of the levels of biochemical markers. Since the results after logarithmic transformation did not substantially differ from those obtained using simple parameters, we preferred to show the data in natural figures which are more easily interpretable. The Student's t-test was used to analyze the difference in the mean of the biochemical parameters with respect to the following categorical variables: presence or absence of signs of pulmonary venous congestion, presence or absence of signs of systemic venous congestion, sex, heart failure etiology (dilated cardiomyopathy vs coronary artery disease), cardiac rhythm (sinus rhythm vs atrial fibrillation).

Multiple regression analyses were performed with forward and backward stepwise selection for the most significant variables. Stepwise logistic regression was used for categorical variables.

Results

Population characteristics. The main demographic and clinical characteristics are presented in table I. The study population consisted of a series of 120 consecutive patients, 97 males (81%) with a mean age of 56 ± 12 years. Fifty-nine patients (49%) were in NYHA functional class I, 24 (20%) in class II, 31 (26%) in class III, and 6 (5%) in class IV. The cause of left ventricular dysfunction was idiopathic or familial dilated cardiomyopathy in 72 patients (60%), coronary artery disease in 40 (33%), and valvular heart disease in 8 (7%). Nineteen patients (16%) showed signs of pul-

Table I. Characteristics of the study population.

No. patients	120
Males	97 (81%)
Age (years)	56 ± 12
NYHA class	
I	59 (49%)
II	24 (20%)
III	31 (26%)
IV	6 (5%)
Body mass index (kg/m^2)	26 ± 3.6
Pulmonary venous congestion	19 (16%)
Systemic venous congestion	24 (20%)
Left ventricular end-diastolic volume (ml/m^2)	153 ± 60
Left ventricular ejection fraction (%)	31 ± 8
Mitral regurgitation	
Absent	23 (19%)
Mild	35 (29%)
Moderate	42 (35%)
Relevant	20 (17%)
Cardiac rhythm	
Sinus rhythm	88 (73%)
Atrial fibrillation	12 (10%)
Pacemaker	20 (17%)
Therapy	
ACE-inhibitors	106 (88%)
Angiotensin receptor blockers	15 (12%)
Beta-blockers	66 (55%)
Digoxin	81 (67%)
Spironolactone	36 (30%)

monary venous congestion whereas in 24 (20%) jugular venous distension and/or peripheral edema and hepatomegaly were present. The left ventricular end-diastolic volume was 153 ± 60 ml/m^2 and the left ventricular ejection fraction 31 ± 8%. Mitral regurgitation was absent in 23 patients, mild in 35, moderate in 42, and relevant in 20. The cardiac rhythm was normal in 88 patients, atrial fibrillation was found in 12, and it was pacemaker-led in 20.

Correlations between clinical and laboratory parameters. The laboratory results are reported in table II. Plasma levels were higher than the upper normal limit for our laboratory in 69 patients (57.5%) for BNP, in 45 (37.5%) for BET-1, in 70 (58.3%) for TNF-α and in 7 (5.8%) and 9 (5.8%) for cTnI and cTnT, respectively. At univariate analysis, the following correlations were significant (Table III): all the laboratory parameters correlated with the NYHA class (BNP r = 0.63, BET-1 r = 0.56, cTnI r = 0.25, cTnT r = 0.24, TNF-α r = 0.23) (Figs. 1 and 2); BNP (r = -0.39) and BET-1 (r = -0.27) correlated with ejection fraction (Figs. 3 and 4); BNP (r = 0.37) and BET-1 (r = 0.21) with the degree of mitral insufficiency; BNP (r = -0.39), BET-1 (r = 0.25), TNF-α (r = -0.19) correlated with systolic blood pressure; cTnT (r = 0.34), cTnI (r = 0.33), BNP (r = 0.22) and BET-1 (r = 0.19) with heart rate; BNP with age (r = 0.33) and body mass index (r = -0.28). The plasma levels of BNP, BET-1, cTnT and cTnI were significantly higher if systemic or pulmonary congestion was present (Table IV). The same correlations were present after logarithmic transformation of the levels of biochemical markers (data not shown). No difference was found when the patients were grouped on the basis of sex, cardiac rhythm and etiology. The following corre-

Table II. Plasma levels of the biochemical markers.

	Mean ± SD	Median	1st percentile	10th percentile
BNP (ng/l)	238.8 ± 336	85	12	600
TNF-α (ng/l)	35.7 ± 87.2	21.10	6.9	54.9
BET-1 (fmol/ml)	1.45 ± 2	0.80	0.29	3.07
cTnI (ng/ml)	0.28 ± 1.7	0.010	0	0.14
cTnT (ng/ml)	0.09 ± 0.6	0	0	0.03

BET-1 = big endothelin-1; BNP = brain natriuretic peptide; cTnI = cardiac troponin I; cTnT = cardiac troponin T; TNF-α = tumor necrosis factor-α.

Table III. Significant correlations at univariate analysis.

	BNP	BET-1	TNF-α	cTnI	cTnT
Age	r = 0.33, p < 0.001				
Heart rate	r = 0.22, p < 0.01	r = 0.19, p < 0.05		r = 0.33, p < 0.001	r = 0.34, p < 0.001
Body mass index	r = -0.28, p = 0.002				
LVEF	r = -0.39, p < 0.001	r = -0.27, p < 0.001			
Mitral regurgitation	r = 0.37, p < 0.001	r = 0.21, p < 0.01			
NYHA class	r = 0.63, p < 0.001	r = 0.56, p < 0.001	r = 0.23, p = 0.01	r = 0.25, p > 0.01	r = 0.24, p < 0.01
Systolic blood pressure	r = -0.3, p < 0.001	r = -0.25, p < 0.01	r = -0.19, p < 0.05		
Diastolic blood pressure	r = -0.23, p < 0.01	r = -0.17, p < 0.05			

BET-1 = big endothelin-1; BNP = brain natriuretic peptide; cTnI = cardiac troponin I; cTnT = cardiac troponin T; LVEF = left ventricular ejection fraction; TNF-α = tumor necrosis factor-α.

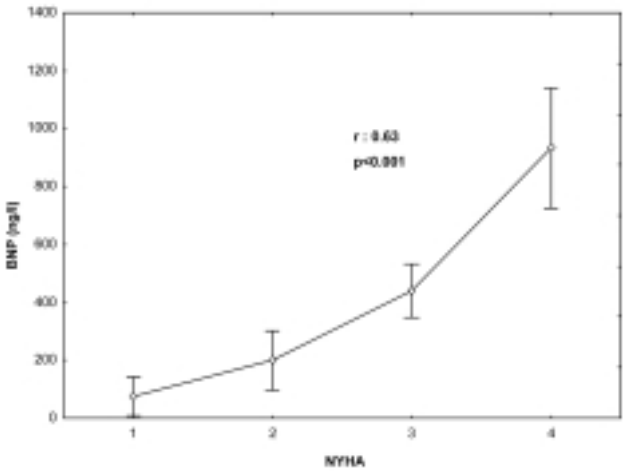

Figure 1. *Correlation between brain natriuretic peptide (BNP) plasma levels and the NYHA functional class.*

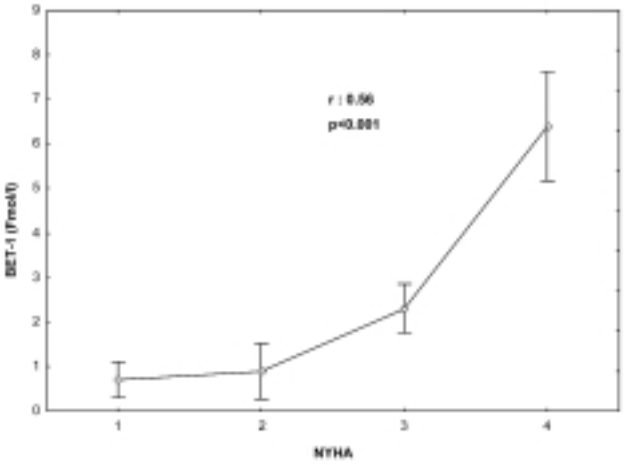

Figure 2. *Correlation between big endothelin-1 (BET-1) plasma levels and the NYHA functional class.*

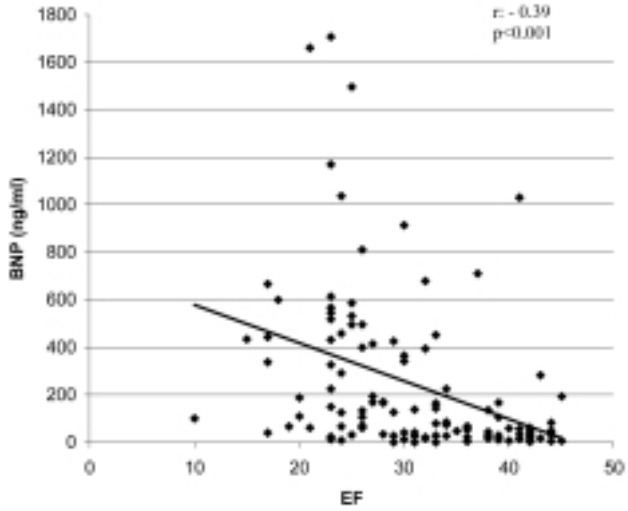

Figure 3. *Correlation between brain natriuretic peptide (BNP) plasma levels and left ventricular ejection fraction (EF).*

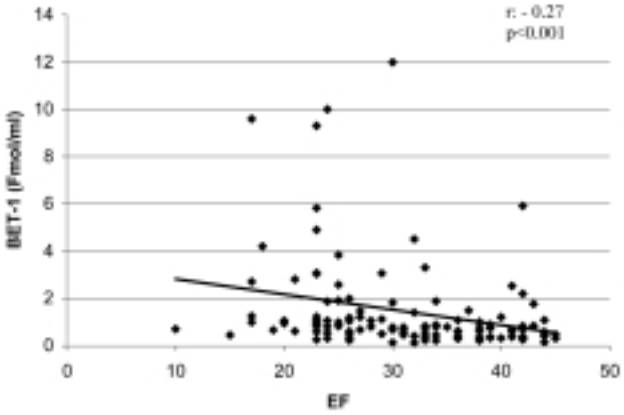

Figure 4. *Correlation between big endothelin-1 (BET-1) plasma levels and left ventricular ejection fraction (EF).*

lations were still present at multiple regression analysis (Table V): BNP with the NYHA class ($p < 0.005$) and pulmonary venous congestion ($p < 0.05$); TNF-α with the NYHA class ($p < 0.05$) and systolic blood pressure ($p < 0.001$); BET-1 with the presence of pulmonary venous congestion ($p < 0.005$); cTnI and cTnT with heart rate ($p < 0.05$).

Discussion

Correlations among biochemical markers and clinical presentation. In this study a group of biomarkers, with a different pathophysiological significance in heart failure[4], was prospectively studied in a single population. Our results demonstrate that there is a widely variable degree of correlation between the plasma concentrations of the substances we took into consideration and the clinical presentation of heart failure.

Brain natriuretic peptide. The plasma concentrations of BNP were higher than the upper reference limit in 57.5% of the patients. The value of this datum is limited by the lack of a control group matched for age and sex as the BNP plasma levels depend on both these parameters[21,22]. Moreover, in many patients creatinine levels, which can also influence plasma BNP, were not available. As compared with the other laboratory parameters evaluated in this study, BNP plasma concentration showed the highest correlations with clinical parameters. At univariate analysis, BNP levels correlated not only with severity of heart failure as determined on the basis of the NYHA functional class and the presence of signs of pulmonary and systemic congestion but also with left ventricular ejection fraction and degree of mitral regurgitation. Moreover, we also found a

Table IV. Plasma levels of biochemical markers with and without pulmonary and venous congestion.

	No	Yes	Student's t-test	p
Pulmonary congestion				
BNP	199.63 ± 330	445.05 ± 296.17	-3.01	< 0.0001
BET-1	1.11 ± 1.38	3.28 ± 3.52	-4.55	< 0.0001
TNF-α	32.24 ± 89	54.5 ± 76.35	-0.99	NS
cTnI	0.0454 ± 0.14	1.51 ± 4.02	-3.55	< 0.0001
cTnT	0.0074 ± 0.03	0.49 ± 1.37	-3.60	< 0.0001
Venous congestion				
BNP	113.68 ± 147.11	558.43 ± 414	-8.64	0.0001
BET-1	0.76 ± 0.69	3.52 ± 3.12	-7.75	0.0001
TNF-α	32.11 ± 94.17	45.9 ± 62.41	-0.72	NS
cTnI	0.04 ± 0.14	0.99 ± 3.25	-2,74	0.006
cTnT	0.002 ± 0.006	0.33 ± 1.10	-2.8	0.005

BET-1 = big endothelin-1; BNP = brain natriuretic peptide; cTnI = cardiac troponin I; cTnT = cardiac troponin T; TNF-α = tumor necrosis factor-α.

Table V. Significant correlations at multiple regression analysis.

	BNP	BET-1	TNF-α	cTnI	cTnT
NYHA class	< 0.005		< 0.05		
Pulmonary venous congestion	< 0.05	< 0.005			
Systolic blood pressure			< 0.001		
Heart rate				< 0.05	< 0.05

BET-1 = big endothelin-1; BNP = brain natriuretic peptide; cTnI = cardiac troponin I; cTnT = cardiac troponin T; TNF-α = tumor necrosis factor-α.

correlation with heart rate and systolic blood pressure. At multivariate analysis, a significant correlation persisted with the functional class and the presence of pulmonary venous congestion. Such results are consistent with the current general view that BNP plasma concentration is a powerful and reliable marker of heart failure[1,5,23]. In fact, in heart failure patients this laboratory parameter has been shown to allow the distinction between dyspnea due to heart failure and that due to pulmonary disease[24-27] and to correlate with the patient's functional capacity[28], the left ventricular function[29-32] and with the hemodynamic profile[33,34]. Moreover, BNP is a strong predictor of mortality not only due to heart failure progression[35-37] but also to sudden death[38]. Recently, BNP concentration has been proposed as a guide to heart failure treatment[12,17,37]. In two studies the clinical results were found to be superior to those observed for symptom-guided treatment if the pharmacological treatment was guided by the plasma level of BNP[12,17].

Big endothelin-1. BET-1 is a precursor of the biologically more active mature endothelin-1 and circulates in higher concentrations and for a longer time than the latter[7]. The plasma concentration of BET-1 was higher than the upper reference limit in 37% of the patients. At univariate analysis the plasma concentration of BET-1 showed the same correlations as BNP, with the sole exception of patient age. Even for BET-1 a significant correlation persisted at multivariate analysis with the presence of signs of pulmonary venous congestion. Pacher et al.[39] demonstrated in 1996 that the plasma concentration of BET-1 correlated with the NYHA functional class, with several hemodynamic parameters including right atrial pressure, pulmonary capillary pressure, and left ventricular ejection fraction. Moreover, the plasma concentration of BET-1 was found to be a strong predictor of the 1-year mortality. In another study[40] BET-1 plasma concentrations correlated with the NYHA functional class and with the presence of mitral regurgitation and atrial fibrillation; only this last correlation persisted at multivariate analysis. In a recent study, the plasma concentration of endothelin-1 was shown to rapidly decrease after intravenous treatment with diuretics and vasodilators in patients with severe heart failure[12]. The decrease in the endothelin-1 concentration occurred simultaneously with that of BNP and had a similar significance (30% endothelin-1 vs 26% BNP).

Tumor necrosis factor-α. TNF-α is a proinflammatory cytokine synthesized in robust quantities by the failing heart[9]. In our patients its plasma concentration was elevated in 58% of patients. This finding, however, is of limited value due the lack of a matched control population. Moreover, at univariate analysis the plasma concentrations of TNF-α were found to correlate only with the NYHA functional class and with systolic blood pressure. Both these correlations persisted at multivariate analysis. The correlations between TNF-α plasma

levels, however, have been investigated in less detail than those of BNP. Although the plasma levels are well known to be increased in patients with severe heart failure[41-43], a correlation has been demonstrated with the whole range of the parameters of functional capacity[44-47]. Moreover, elevated TNF-α plasma levels have been found to predict mortality in patients with advanced heart failure[43,45].

Cardiac troponin I and T. Cardiac troponins are released from damaged myocardium and are deemed to be the most reliable laboratory marker of myocardial necrosis in acute coronary artery syndromes. Their plasma levels have been reported to be increased in patients with heart failure even in the absence of acute coronary artery disease[10,11,48,49]. The plasma concentrations of cTnI and cTnT were below the upper reference limit in all but 12 patients. Five of these patients were in NYHA class IV and 5 in class III, 1 patient was in class I and 1 in class II and they were the only patients in whom only the plasma concentrations of cTnI were increased. This finding is in agreement with the results reported in the literature which demonstrate that increased plasma levels of cardiac troponins are present almost exclusively in patients with severe and/or acute heart failure[10,11,48,49]. In our experience the plasma levels of both cardiac troponins showed a weak correlation with the NYHA functional class and heart rate; the latter persisted even at multivariate analysis. This latter finding is difficult to explain and is unlikely to be of any clinical value due to the finding of normal plasma cardiac troponin levels in almost all our patients. In other studies a correlation was found between cTnI and cTnT plasma levels and the NYHA functional class[10,48,49] and left ventricular ejection fraction[11]. Moreover, these laboratory parameters have also been identified as prognostic factors in patients with heart failure[10,49,50].

Comparison of different biochemical markers of heart failure. Our results seem to suggest different significances of the various laboratory parameters we evaluated in our heart failure patients. The plasma concentration of BNP showed the most numerous and significant correlations with the parameters characterizing the presentation of heart failure; however only a few correlated independently at multivariate analysis. The type and level of correlations were similar, even if mildly less strong for the plasma level of BET-1. The most important correlation we found was that of both these laboratory parameters with the presence of pulmonary venous congestion. To date, the clinical value of BET-1 plasma levels in heart failure patients has been less deeply studied and it seems to deserve further investigation. The great advantage of BNP is the feasibility, in routine practice, of quick and simple measurements of the entire molecule or of part of it[25,27,37,51]. The value as a biochemical marker of heart failure of the plasma levels of TNF-α and of cardiac troponins appears to be limited – at least in patients with relatively stable heart failure, as the majority of those included in the present study. In particular, the plasma concentration of cardiac troponins was increased only in patients with severe heart failure. A possible role of TNF-α and cardiac troponins has been suggested as markers of progression of heart failure rather than as markers of heart failure severity or left ventricular dysfunction[52,53].

Study limitations. Almost 50% of the patients included in this study were outpatients in NYHA functional class I. The high prevalence of clinically stable and mildly symptomatic patients, despite the significant compromise of their left ventricular function, may have biased a correct evaluation of the correlations between the laboratory parameters taken into consideration and the clinical presentation of heart failure. The general weakness of the correlations we observed limits the biological relevance of our findings.

In conclusion, the plasma concentrations of BNP and BET-1 showed the best and comparable correlations with the parameters describing the clinical status of patients with heart failure, in particular with the presence of pulmonary venous congestion. The value of the plasma levels of TNF-α and of those of cardiac troponins in patients with relatively stable heart failure was found to be limited.

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