Fast-track article

Plasma levels of tumor necrosis factor-α correlate with the six-minute walk test results in patients with mild to moderate heart failure

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Key words:
Cardiomyopathies; Exercise tolerance; Heart failure.

Background. **The plasma levels of brain natriuretic peptide, tumor necrosis factor-α, big endothelin-1 and cardiac troponins have been reported to correlate with the severity of heart failure.**

Methods. **In a single population of 80 outpatients with mild to moderate chronic heart failure the correlation between the patient's functional capacity, as evaluated at a 6-min walk test, the clinical parameters and plasma levels of brain natriuretic peptide, tumor necrosis factor-α, big endothelin-1 and cardiac troponins was evaluated.**

Results. **A significant inverse correlation was found with the patient's age ($p < 0.0001$), NYHA functional class ($p < 0.0001$), left ventricular dysfunction etiology (ischemic vs dilated cardiomyopathy, $p < 0.0005$), heart rate ($p < 0.05$), plasma levels of brain natriuretic peptide ($p < 0.05$) and of tumor necrosis factor-α ($p < 0.0005$). At multiple regression analysis a correlation was found between the 6-min walk test results and the patient's age ($p < 0.05$), NYHA functional class ($p < 0.01$), left ventricular dysfunction etiology (ischemic vs dilated cardiomyopathy, $p < 0.05$) and tumor necrosis factor-α plasma levels ($p < 0.05$).**

Conclusions. **In our patients with mild to moderate heart failure, a significant correlation was found between the results of the 6-min walk test and only the plasma concentrations of tumor necrosis factor-α among the laboratory parameters analyzed in this study.**

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Introduction

Heart failure encompasses a wide range of clinical presentations that depend not only on the degree of left ventricular dysfunction but also on many non-cardiac factors among which one of the most important is the activation of the renin-angiotensin-aldosterone and sympathetic nervous systems[1]. The pathogenetic role and the clinical correlates of other hormones, such as atrial and brain natriuretic factors[2-7] and endothelin[1,8,9], cytokines[10-12] and cardiac troponins[13-17], whose plasma concentrations have been demonstrated to be increased in patients with heart failure, are less well defined.

The aim of this study was to evaluate in a single population of outpatients with mild to moderate chronic heart failure the correlation between the patient's functional capacity, as evaluated at a 6-min walk test, which measures the distance walked in 6 min by the patient[18-20], and the plasma levels of brain natriuretic peptide, tumor necrosis factor-α, big endothelin-1 and cardiac troponins.

Methods

Study population. The main demographic and clinical characteristics are presented in table I. The study population was composed of a series of 80 consecutive outpatients, 64 males (80%), mean age 55 ± 11 years, with a history of heart failure, and with a dilated (end-diastolic volume > 70 ml/m^2) and hypokinetic (ejection fraction < 45%) left ventricle, as evaluated at two-dimensional echocardiography. The cause of left ventricular dysfunction was primary dilated cardiomyopathy in 51 patients (63.5%), coronary artery disease in 23 (29%), and valvular heart disease in 6 (7.5%). Fifty-two patients (65%) were in NYHA functional class I, 22 patients (27.5%) in class II, and 6 patients (7.5%) in class III. The mean body mass index was 25.5 ± 3.3 kg/m^2. The left ventricular end-diastolic volume was 146 ± 60 ml/m^2 and the left ventricular ejection fraction 33 ± 8%. Mitral regurgitation was absent or mild in 47 patients (58.5%) and moderate or severe in 33 (41.5%). The electrocardiographic findings and therapy are reported in table I.

Table I. Clinical characteristics of the study population.

Age (years)	55 ± 11
Male sex	64 (80%)
Etiology	
Primary dilated cardiomyopathy	51 (63.5%)
Coronary artery disease	23 (29%)
Valvular heart disease	6 (7.5%)
NYHA functional class	
I	52 (65%)
II	22 (27.5%)
III	6 (7.5%)
Arterial systolic pressure (mmHg)	129 ± 21
Heart rate (b/min)	68 ± 12
Body mass index (kg/m^2)	25.5 ± 3.3
Left ventricular ejection fraction (%)	33 ± 8
Left ventricular end-diastolic volume (ml/m^2)	146 ± 60
Mitral regurgitation	
Absent/mild	47 (58.5%)
Moderate/severe	33 (41.5%)
Left bundle branch block	32 (40%)
Cardiac rhythm	
Sinus rhythm	63 (79%)
Atrial fibrillation	9 (11%)
Pacemaker	8 (10%)
Therapy	
ACE-inhibitors	73 (91%)
Angiotensin II receptor inhibitors	7 (9%)
Beta-blockers	46 (57.5%)
Diuretics	62 (77.5%)
Digoxin	54 (67.5%)

Each patient underwent cardiological evaluation, a standard electrocardiogram, a two-dimensional echocardiogram, a 6-min walk test and a venous blood sample on the same day. The plasma levels of brain natriuretic peptide, tumor necrosis factor-α, big endothelin-1 and cardiac troponins I and T were measured using the following techniques:
- brain natriuretic peptide: a solid-phase sandwich immunoradiometric assay utilizing two monoclonal antibodies against sterically remote sites, the first being coated on the solid-phase of the beads and the second radiolabeled with iodine 125 used as a tracer (Shionoria brain natriuretic peptide, CIS Diagnostici, Vercelli, Italy); upper normal value 18 ng/l[21];
- tumor necrosis factor-α: an enzyme-amplified sensitivity immunoassay (EASIA) that is based on monoclonal antibodies against tumor necrosis factor-α specific sites; upper normal value 15 ng/l;
- big endothelin-1: an enzyme immunoassay (Biomedica, Vienna, Austria) that incorporates an immunoaffinity purified polyclonal capture antibody and a monoclonal detection antibody; upper normal value 0.95 Fmol/l;
- cardiac troponin I: a one-step enzyme immunoassay that is based on the sandwich principle (RxL Dimension analyzer, Dade Behring, Milan, Italy); upper normal value 0.4 ng/ml[22];
- cardiac troponin T: a two-site third generation chemiluminescent immunoassay (Elecsys analyzer, Roche Diagnostics, Milan, Italy); upper normal value 0.1 ng/ml[23].

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

Statistical analysis. Parameters are expressed as mean ± SD. Univariate and multiple regression analyses were performed to search for correlations between the 6-min walk test results and the other variables. At univariate analyses a simple regression method was used for the following continuous variables: age, NYHA functional class, arterial systolic pressure, ejection fraction, left ventricular end-diastolic volume, mitral regurgitation (1 = absent, 2 = mild, 3 = moderate, 4 = severe) and plasma levels of heart failure biochemical parameters. The ANOVA method was used for the following categorical variables: sex, heart failure etiology (dilated cardiomyopathy vs coronary artery disease), cardiac rhythm (sinus rhythm vs atrial fibrillation), left bundle branch block (presence vs absence) and therapy (beta-blockers vs no beta-blockers, digoxin vs no digoxin, diuretics vs no diuretics). Multiple regression analyses were performed with forward and backward stepwise selection for the most significant variables.

Results

Our patients were able to cover 559 ± 87 m in 6 min. The laboratory results are presented in table II. After dividing the patients according to their NYHA function-

Table II. Laboratory results.

Laboratory parameters	NYHA functional class			Total population
	I	II	III	
Brain natriuretic peptide (ng/l)	75.7 ± 98.97*	264.5 ± 268.7*	180.8 ± 98.6	134.2 ± 182.3
Tumor necrosis factor-α (ng/l)	17.3 ± 12.9**	24.9 ± 12.3**	53.6 ± 13.9**	21.95 ± 15.94
Big endothelin-1 (Fmol/ml)	0.70 ± 0.83	1.18 ± 1.16	1.2 ± 0.45	0.86 ± 0.94
Cardiac troponin T (ng/ml)	0.04 ± 0.18	0.035 ± 0.08	0.043 ± 0.046	0.04 ± 0.15
Cardiac troponin I (ng/ml)	0.002 ± 0.006	0.005 ± 0.009	0.002 ± 0.006	0.003 ± 0.007

* $p < 0.0005$; ** $p < 0.0001$.

al class, the plasma levels of tumor necrosis factor-α progressively increased with the decrease in functional capacity, while the sole other difference that was observed was the higher plasma concentration of brain natriuretic peptides in NYHA class II patients in comparison to that measured in NYHA class I patients. The correlations at univariate and multivariate analyses between the 6-min walk test results and the other analyzed parameters are presented in table III. A significant inverse correlation was found for: patient's age ($p < 0.0001$) (Fig. 1), NYHA functional class ($p < 0.0001$) (Fig. 2), left ventricular dysfunction etiology (ischemic vs dilated cardiomyopathy, $p < 0.0005$), heart rate ($p < 0.05$), brain natriuretic peptide plasma levels ($p < 0.05$) (Fig. 3) and tumor necrosis factor-α plasma levels ($p < 0.0005$) (Fig. 4). The correlations which persisted at multiple regression analyses were those between the 6-min walk test results and patient's age ($p < 0.05$), NYHA functional class ($p < 0.01$), left ventricular dysfunction ischemic etiology vs dilated cardiomyopathy ($p < 0.05$), and tumor necrosis factor-α plasma levels ($p < 0.05$).

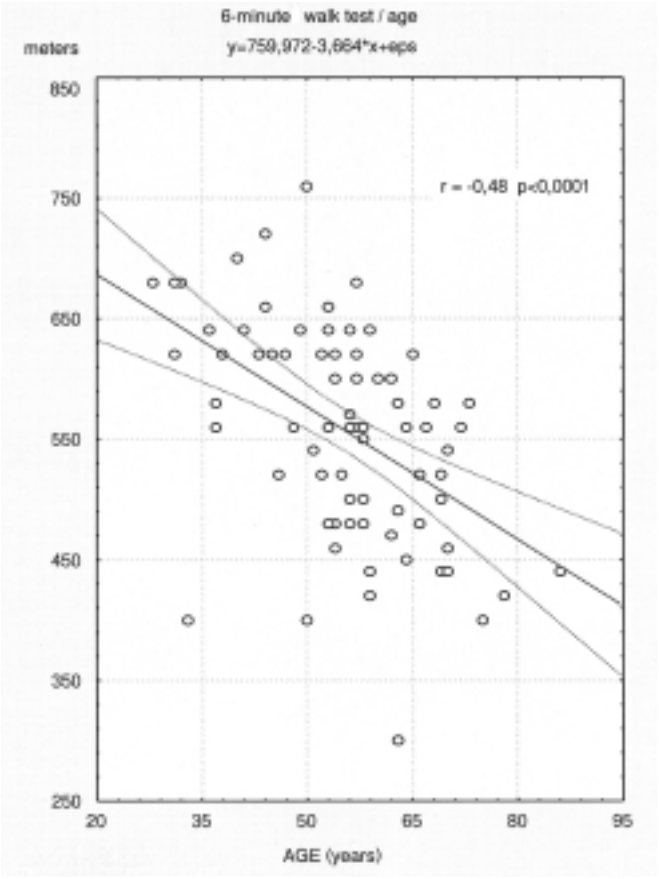

Figure 1. *Univariate analysis correlation between the 6-min walk test results and age.*

Discussion

Our results confirm the value of the plasma levels of tumor necrosis factor-α as a marker of heart failure by demonstrating, for the first time, a correlation between this biochemical parameter and a quantitative measure of functional capacity in a series of patients with mild to moderate heart failure. The plasma levels of tumor necrosis factor-α were recently reported to be elevated in patients in NYHA functional class I to III compared with control subjects and to be progressively increased in relation to a decreasing functional status of the patients[10]. Another study showed that the plasma levels of tumor necrosis factor-α were significantly higher for patients with end-stage heart failure in comparison with those with the recent-onset form of this clinical syndrome[11].

Moreover, our study demonstrates that the plasma levels of tumor necrosis factor-α appear to be better correlated with the functional capacity than any other substances that are deemed to be biochemical markers of heart failure. In fact, this parameter was the only one, among the laboratory parameters analyzed in this study, that showed an independent inverse correlation with the result of the 6-min walk test. This result seems to suggest that an increased proinflammatory status

Table III. Significant correlations between the 6-min walk test results and other variables.

	r	p
Univariate analysis		
Age	-0.48	< 0.0001
NYHA functional class	-0.60	< 0.0001
Etiology (ischemic vs dilated cardiomyopathy)	-0.40	< 0.0005
Heart rate	-0.27	< 0.05
Brain natriuretic peptide	-0.25	< 0.05
Tumor necrosis factor-α	-0.46	< 0.0005
	t	**p**
Multivariate analysis ($r^2 = 0.42$; $F = 29.7$)		
Age	-3.3	< 0.05
NYHA functional class	-5.23	< 0.01
Etiology (ischemic vs dilated cardiomyopathy)	1.99	< 0.05
Tumor necrosis factor-α	2.01	< 0.05

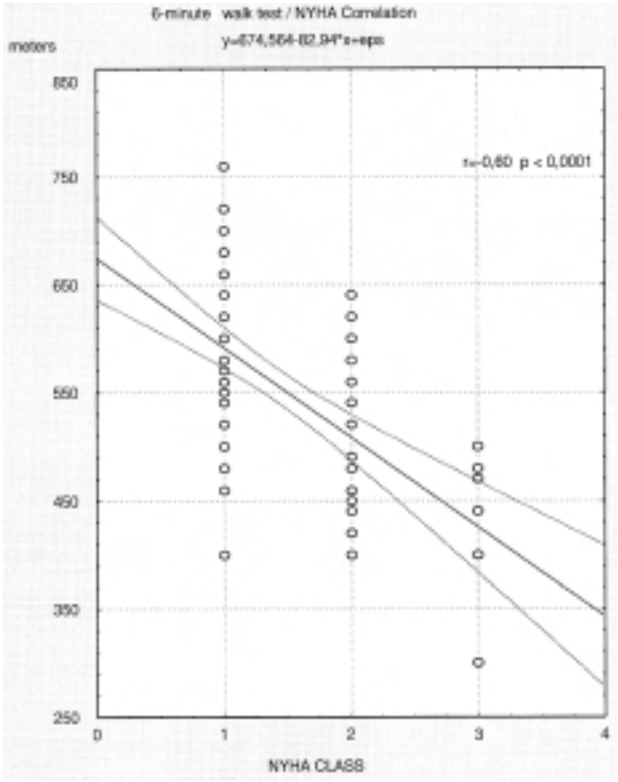

Figure 2. *Univariate analysis correlation between the 6-min walk test results and NYHA functional class.*

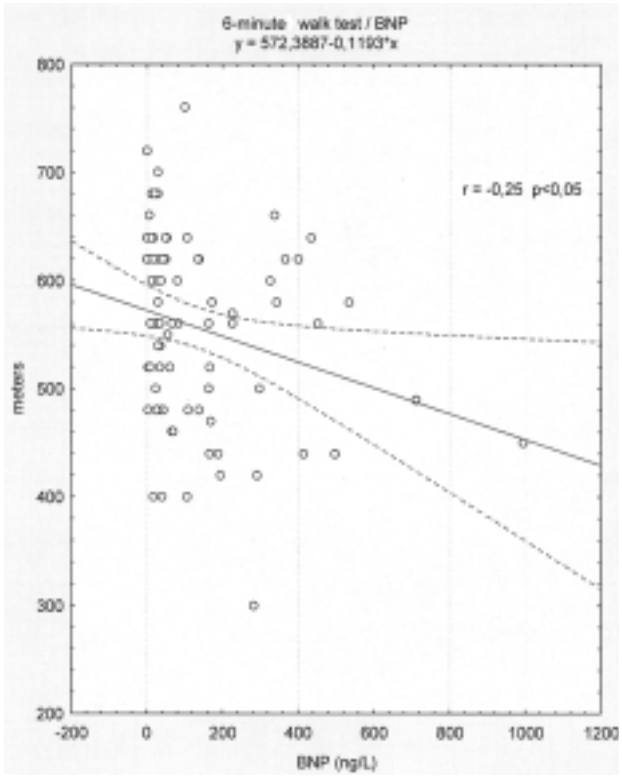

Figure 3. *Univariate analysis correlation between the 6-min walk test results and brain natriuretic peptide (BNP).*

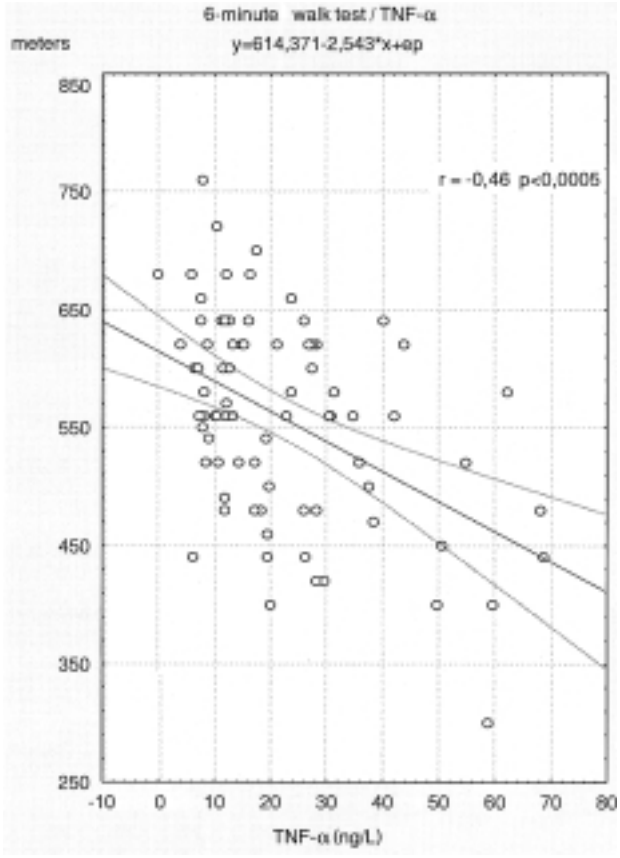

Figure 4. *Univariate analysis correlation between the 6-min walk test results and tumor necrosis factor (TNF)-α.*

may play a significant role in determining the functional capacity, even in the subgroup of patients with mild to moderate heart failure.

The correlation between the plasma levels of brain natriuretic peptide and the result of the 6-min walk test was, in our experience, disappointing. This result was somewhat surprising as the plasma concentration of brain natriuretic peptide is today considered to be the most reliable biochemical marker of heart failure to such a point that it has been proposed as a guide for the treatment of heart failure[5]. A correlation between the plasma level of brain natriuretic peptide and functional capacity, as judged on the basis of the NYHA classification, was shown in patients with moderate to severe heart failure[3]. In our experience, however, the results of the 6-min walk test correlated significantly with the plasma levels of brain natriuretic peptide at univariate analysis, but this correlation did not persist at multivariate analysis. This finding, however, is in agreement with the recently reported observation that the plasma levels of brain natriuretic peptide are of limited value in patients with systolic left ventricular dysfunction once a correct treatment of heart failure has been established and symptoms have been abolished[24]. Moreover, the plasma concentrations of big endothelin-1 and cardiac troponins did not show any correlation with the results of the 6-min walk test. Even these biochemical parameters have been shown to be increased in patients with at least a moderate degree of heart failure[8,9,13-17]. The

lack of correlation in our patients between these laboratory parameters and the results of the 6-min walk test is probably due to the high prevalence of patients in NYHA functional class I.

In conclusion, in our limited experience the plasma levels of tumor necrosis factor-α was the sole biochemical parameter, among the laboratory parameters analyzed in this study, which correlated with the results of the 6-min walk test in outpatients with mild heart failure. It will be important to verify the role of this biochemical marker in clinical practice in larger populations by using more reliable methods for the measurement of the functional capacity, such as the cardiopulmonary exercise test[25,26].

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