Comparative study of galectin-3, brain natriuretic peptide, cystantine C and high sensitivity troponin determinations in the diagnosis of heart failure in elderly italian males

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ABSTRACT

Introduction: heart failure (HF) has been defined a modern pandemic. The complex array of physiologic, psychological, social and health care issues makes it a challenging chronic disease to diagnose and manage. Our study is aimed to evaluate a multi-markers approach in diagnosis of HF in aged males.
Methods: 68 Italian males aged >65 years have been enrolled; 25 were patients with heart failure (HF) and 43 were healthy controls. In all the subjects, measurements of high sensitivity troponin I (TNI), galectin-3 (GAL), cystatin C (CYS) and brain natriuretic peptide (BNP) were performed using routine methods.
Results: in patients with HF, mean concentrations of TNI, GAL, CYS and BNP were significantly higher ($p<0.001$) than values observed in controls subjects. Only BNP and GAL showed a correlation with NHYA stage of HF. In this study, GAL and BNP demonstrated better diagnostic perfomamces to differentiate of HF patients from controls subjects.
Conclusions: our study showed the usefulness of a strategy involving multiple biomarkers determination in laboratory diagnosis of HF in elderly males.

INTRODUCTION

Heart failure (HF) is a major public health problem worldwide, entailing high morbidity and mortality as well as high costs (1). This chronic syndrome associates with a low functional status and quality of life. Most patients with HF are elderly, constituting up to 80% of patients suffering from this disease with both incidence and prevalence of the condition increasing with age (2). In patients with HF, age is associated with an increased risk of cardiovascular events and mortality during short- and long-term follow-up. Elderly patients with HF often present with complex co-morbidities (hypertension, atrial fibrillation, peripheral vascular disease and coronary artery disease, valvular disease and kidney failure or anemia) and polypharmacy (3,4). The pathophysiological concept of HF has changed during the last decade with increased understanding of a multiorgan neurohormonal response, low-grade myocardial damage as well as activation of immunological and inflammatory systems. As a consequence, a number of biomarkers for diagnosis and prognosis in HF have emerged (5-7).
Several studies have assessed the relevance of measuring troponin I (TNI) in patient with stable-chronic HF in order to establish the prognostic potential and future cardiovascular events (8). Addition of the brain natriuretic peptide (BNP) may improve the prognostic potential in patients with chronic HF as demonstrated by Tsutamoto et al, who showed that elevations of both BNP and TNI concentrations carry the highest risk of mortality (9).
Inflammatory and fibrotic processes are central to cardiac remodeling and the development of HF.

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Galectin-3 (GAL), secreted by activated macrophages, causes cardiac fibrosis via proliferation of cardiac fibroblasts and deposition and irreversible cross-linking of collagen I in myocytes. Recent examinations of GAL in the context of HF, have revealed the potential clinical value of this biomarker as a prognostic indicator (10-12). BNP plasma concentrations fluctuate depending on the severity of the disease. BNP increases particularly when there is an abnormal dilatation of the cardiac wall chamber, increased fluid volume or reduced elimination of peptides such as in kidney failure. Usually, BNP concentration <100 pg/mL allows to exclude HF. BNP levels in the acute HF can predict the risk of death or readmission within 30 days (13,14). In patients with chronic HF, BNP can predict future cardiac events and hospitalizations. Results from the Val-HeFT trial reported by Masson et al. (15) demonstrated that BNP and NT-pro BNP were the strongest predictors of mortality and hospitalization for HF (16).

Cystatin C (CYS) is a small protein molecule (approximately 13kDa) produced by virtually all nucleated cells, at a constant rate, freely filtered from the glomerular membrane and then completely reabsorbed without secretion by the proximal tubular cells. These characteristics made this protein an almost perfect candidate for estimating renal function being renal function a strong predictor of adverse events in HF (17,18).

The aim of the present study is to evaluate if combined measurement of TNI, GAL, BNP and CYS in the diagnosis of HF, could provide useful information in an elderly population with symptoms associated with HF in primary healthcare setting.

METHODS

Subjects

68 Italian males aged >65 years have been enrolled: twenty-five patients with a previous diagnosis of HF, aged between 65 and 83 years (median 72 years), and 43 healthy controls, aged between 65 and 81 years (median 69 years). 18 subjects of the control group were repeat blood donors and 25 were subjects recruited during a medical examination for non-competitive sports activities.

The study protocol was approved by the Ethics Committee of the Local Health Authority; all patients provided written informed consent to be included in the study. The study was performed in accordance to the Helsinki declaration as revised in 1996.

Laboratory methods

At the initial subject examination, blood samples were collected for routine analysis. Several aliquots of EDTA plasma samples were stored at -80 °C for later determination of TNI, GAL CYS and BNP. TNI and GAL plasma concentrations were measured using immunoassays applied to ARCHITECT I2000SR analyzer (Abbott Laboratories, Abbott Park, IL, USA). CYS and BNP plasma concentrations were measured using a ST-AIA-PACK-CYS and BNP immunoassays applied to AIA 2000 analyzer (TOSOH Corporation Tokyo Japan). In the present study cutoff value have been set as following: TNI, 39 ng/L; GAL, 16 ng/mL (19); CYS, 1.00 mg/L; BNP, 100 pg/mL (20,21).

Heart failure diagnosis

These subjects underwent an echocardiographic test with a portable CX-30 equipment. For the study of the left ventricular systolic function, the ejection fraction (EF) was calculated using the Simpson biplane method. The diagnosis of left ventricular systolic dysfunction requires the presence of an EF <50% (22-24). The study of diastolic function of the left ventricle was performed by evaluating the mitral inflow from the A4C projection.

Statistical analysis

Data were analyzed using MedCalc Ver.8.0.0 (Medcalc SW bvda Ostend, Belgium). Concentration of TNI, GAL. CYS and BNP are reported as median, I and III quartiles (IQ range). Differences between groups were analyzed using the independent-sample Mann-Whitney test for independent samples. Correlation analysis was performed using Spearman's rank correlation. Receiver operating characteristic (ROC) curve and area under the ROC curve (AUC) were calculated and compared by using the Hanley-McNeil test (25,26). P <0.05 was considered statistically significant. Moreover, sensitivity (SE), specificity (SP), positive predictive value (PPV), negative predictive value (NPV) and correctly classified incidence (CCI) were calculated.

RESULTS

Among patients with HF, 16 (64%) had an EF <50% and were classified as left ventricular systolic dysfunction patients, while 9 (36%) had an EF >50% and were classified as HF with preserved left ventricular function. In patients with HF, median TNI concentration was 34.5 ng/L (IQ range 27.6 - 43.7), significantly higher (p<0.001) than values observed among control subjects (median 3.5 ng/L; IQ range 2.25 - 7.55). Furthermore, for all biomarkers considered in our study, the concentrations observed in HF patients were significantly higher than those measured in control subjects. These data are reported in table 1.

In figure 1, ROC curves are shown; for TNI AUC is 0.98 [confidence interval (CI95%) 0.87 - 0.98], for GAL 0.93 (CI95% 0.85 - 0.98), for CYS 0.95 (CI95% 0.89 - 0.99), for BNP 0.98 (CI95% 0.91 - 0.99). We did not observe any significant differences between AUCs: CYS versus BNP (p=0.59), GAL versus BNP (p=0.76), TNI versus BNP (p=0.56), GAL versus CYS (p=0.41), TNI versus CYS (p=0.91), TNI versus GAL (p=0.38).

Among patients with HF, 7 (28%), showed TNI concentration higher than the cutoff value, and in none of

Table 1
Characteristics of heart failure patients and healthy controls

Variable	Heart Failure Patients (n=25)	Healthy Controls (n=43)	P
Age, median (min-max)	72 years (65-83)	69 years (65-81)	
Etiology, n (%)			
Ischemic	10 (40%)		
Valvular	3 (12%)		
Primitive	4 (16%)		
Hypertensive	8 (32%)		
NHYA class, n (%)			
I	3 (12%)		
II	13 (52%)		
III	9 (36%)		
IV	0 (0%)		
EF, mean (SD)	42% (9)		
<50%, n (%)	16 (64%)		
>50%, n (%)	9 (36%)		
Comorbidities, n (%)			
Diabetes	6 (24%)		
COPD	3 (12%)		
CKD	10 (40%)		
HF symptoms	23 (92%)		
Treatment, n (%)			
ACEI	9 (36%)		
BB	14 (56%)		
MRA	9 (36%)		
DG	6 (24%)		
DIU	15 (60%)		
ARB	6 (24%)		
ICD	1 (4%)		
CRT	1 (4%)		
BNP pg/mL, median (IQ range)	65.0 (43.0-146.0)	23.0 (16.5-27.0)	<0.001
CYS mg/L, median (IQ range)	1.51 (1.07-2.01)	0.80 (0.65-0.85)	0.04
GAL ng/mL, median (IQ range)	20.6 (17.4-29.1)	10.4 (9.25-11.85)	0.008
TNI ng/L, median (IQ range)	34.5 (27.6-43.7)	3.5 (2.25-7.55)	<0.001

NHYA, New York Heart Association; EF, ejection fraction; COPD, chronic obstructive pulmonary disease; CKD, chronic kidney disease;ACEI, angiotensin converting enzyme inhibitors; BB, beta blockers; MRA, mineralocorticoid receptors antagonists; DG, digitalis glycosides; DIU, diuretics; ARB, angiotensin receptors blockers; ICD, implantable cardioverter defibrillator; CRT, cardiac resynchronization therapy; BNP, brain natriuretic peptide; CYS, cystatin C; GAL, galectin 3; TNI, troponin I; IQ range, interquartile range.

these patients TNI concentration was below the limit of detection. No correlation has been observed between TNI concentration and NYHA class of patients. None of the control subjects showed TNI values higher than cutoff, and in 9 (21%) TNI concentrations were below the limit of detection. In all the HF patients we observed GAL concentrations higher than the cutoff, and a weak correlation between GAL values and NYHA class (p=0.04, r=0.31). In the control group we observed only 2 (4%) subjects with GAL values above the cutoff value. Among patients with HF, in 19 (76%) we found CYS concentration higher than the cutoff value, and we did not find any correlation between CYS concentration and NYHA class of patients. Among patients with HF, 13 (52%) showed BNP concentrations above the cutoff value, and a weak correlation between BNP concentration and NYHA class of patients (p=0.03, r=0.35) was observed. BNP was normal in controls subjects.

As reported in Figure 2, among the 43 healthy controls, 39 (91%) presented all the biomarkers below the cutoff values and 4 (9%) presented elevation of a

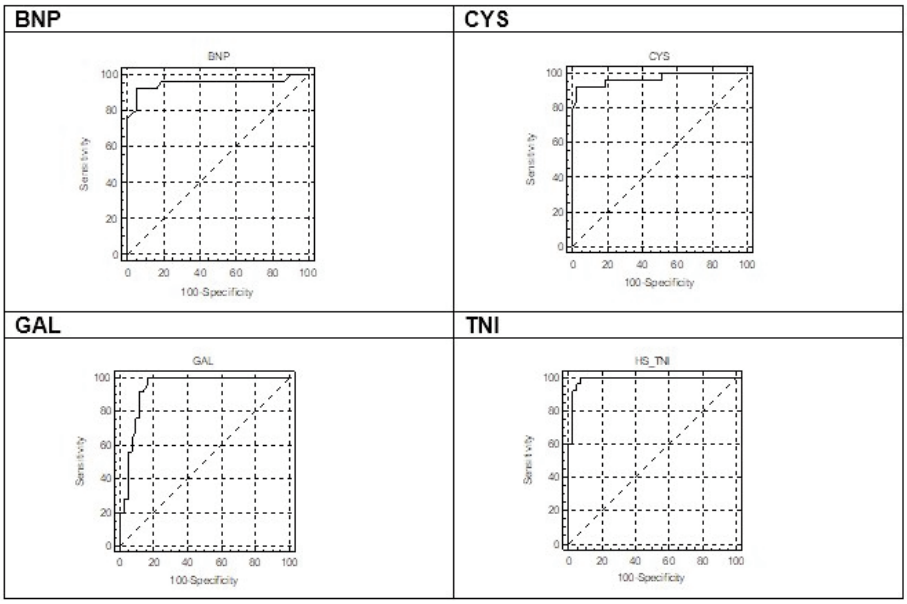

Figure 1
ROC curves of high sensitivity troponin I (TNI), Galectin 3 (GAL), Cystatin (CYS) and Brain natriuretic peptide (BNP) in differentiating heart failure patients (HF) from control subjects.

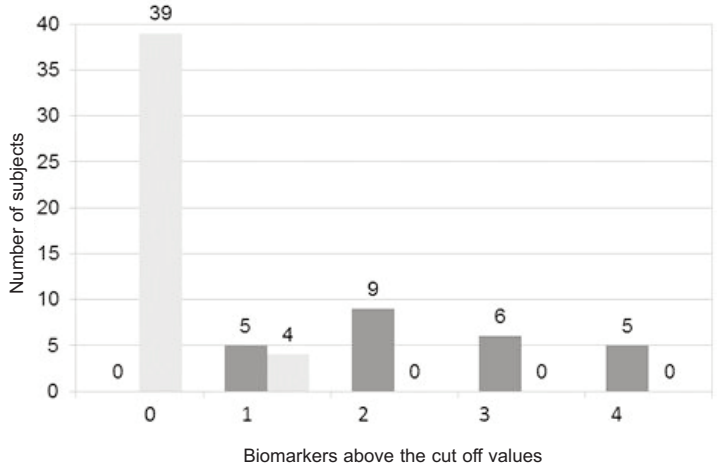

Figure 2
Number of subjects with elevated biomarkers plasma concentration in healthy controls (pale grey bars) and heart failure patients (dark grey bars).

single biomarker (in two cases it was the GAL and in the other two, it was CYS and TNI respectively). In contrast, among the 25 patients with HF, no one had all the biomarkers below the cutoff values, 5 (20%) had one biomarker , 9 (36%) had two, 6 (24%) had three and 5 (20%) had all the four biomarkers considered in this study above the cutoff values.

Table 2 shows the prevalence of HF patients with raised biomarkers concentration in relation to NYHA class.

The diagnostic performances of the biomarkers in differentiation of previously classified HF patients from healthy controls are shown in Table 3.

DISCUSSION

The pandemic trend in the last decade and demographic projections for the near future, classify HF as the cardiovascular disease involving the greatest financial burden: it currently uses up to 1-2% of the

Table 2
Prevalence of patients with raised concentration of biomarkers

NHYA class	BNP	CYS	GAL	TNI
NHYA I (n= 3)	1 (33%)	3 (100%)	3 (100%)	1 (33%)
NHYA I (n= 13)	6 (46%)	9 (69%)	13 (100%)	10 (77%)
NHYA I (n= 9)	6 (67%)	7 (78%)	9 (100%)	6 (67%)

The first column reports NHYA classification and the number of patients assigned to each class; columns 2 to 5 show the number and the percentage of patients with increased concentration of the biomarker.
NHYA, New York heart association; BNP, brain natriuretic peptide; CYS, cystatin C; GAL, galectin 3; TNI, troponin I.

Table 3
Diagnostic characteristics of the biomarkers

	BNP	CYS	GAL	TNI
Cutoff value	< 100 pg/mL	<1 mg/L	<18 ng/mL	<39 ng/L
SE	0.72	0.76	1.00	0.28
SP	1.00	0.98	0.95	1.00
PPV	1.00	0.98	0.93	1.00
NPV	0.78	0.88	1.00	0.70
CCI	0.93	0.91	0.91	0.74

SE: sensitivity; SP: cpecificity; PPV: predictive positive value; NPV: negative predictive value; CCI: correctly classified incidence.
BNP, brain natriuretic peptide; CYS, cystatin C; GAL, galectin 3; TNI, troponin I.

health budgets of countries comparable to Italy. Available data on HF epidemiology in the developed countries, show that HF represents a disease of the elderly (27,28). HF is known to be a proteiform syndrome; in the elderly particularly, symptoms and signs may be atypical and can be simulated or disguised by co-morbidities such as respiratory disease, kidney function impairment, diabetes, cognitive decline, obesity, venous insufficiency. Clinical evaluation is typically unreliable for an early diagnosis of HF. With regard to prognosis and risk stratification, a variety of risk models have been developed to predict the mortality risk of general HF patients. However, less conclusive evidence is available for the elderly (27,28).

Myocardial stretch leads to production of pro-BNP that is later broken down into BNP and NT-pro-BNP. Higher concentration of BNP in the blood of a patient who presents to the emergency room is associated with greater probability of a diagnosis of HF. Higher BNP concentration on admission to the hospital is also associated with greater in-hospital mortality. BNP levels are inversely associated with obesity and may also be influenced by the presence of kidney disease (14-16).

Cardiomyocyte necrosis releases troponin I or T (cardiac isomers of proteins from troponin-tropomyosin complex) into the circulation of an individual and they are typically useful in detection of a myocardial ischemia. Troponin T and I, measured with high sensitive assays show elevated values in patients with severe HF and therefore have been studied for the prediction of the onset of HF as well as for prognosis in patients with already established HF (14-16).

Renal function is a strong predictor of adverse events in HF. Current renal function measurements are imperfect, and CYS is considered a marker of glomerular filtration rate with better performances than creatinine [14-16].

GAL is a biomarker with a relevant role in the development and regulation of cardiac fibrosis and remodeling. In patients with acute decompensated HF, GAL levels seems to be predictive of mortality on short-term follow up. In fact, several authors have also suggested the superiority of GAL and the enhanced predictive power for mortality when used in associations

with BNP, in patients with both preserved and reduced left ventricular ejection fraction (14-16).

In summary, there are several biomarkers available demonstrating diagnostic, prognostic or predictive value in HF. Demonstration of the validity and the clinical utility of any specific biomarker across different sets of patients is essential before their introduction in routine clinical practice. Biomarkers may be able to reflect pathophysiologic process of heart disease, and also can provide meaningful information about prognosis and to assist the process of clinical decision making without duplicating any information that is already clinically available. Our data show that BNP, TNI and CYS have the best PPV and GAL has the best NPV in this group of subjects when compared to other biomarkers. In our opinion, the results obtained in this study could provide useful information about the possible use of GAL in the diagnosis of HF among the elderly patients. It has previously suggested that GAL has a lower or a similar diagnostic accuracy when compared to natriuretic peptides (29,30). However, the accuracy of GAL could be greater among elderly, because it is able to more accurately reflect the pathophysiological mechanisms underlying HF in the older age. Moreover, GAL could be directly involved in the genesis of HF promoting inflammation and cardiac fibrosis. Finally, this association could be even more evident in patients with HF and preserved EF, where GAL has also shown a stronger prognostic value (31,32). As matter of fact, in our series of 25 patients with HF, 9 (36%) had a preserved left ventricular function. The results obtained in this study show that the considered biomarkers have a good diagnostic power since they seem able to reliably discriminate patients with HF from healthy subjects. However, they show only a modest correlation with the severity of HF; this could be due to the fact that in the group of patients enrolled in this study, there were no patients in class IV NYHA. Other results difficult to interpret are that we found more elevated biomarkers in class II, than in class III.

Our study presents some limitations; in particular, the number of patients might not have been large enough to enable us to show the independent predictive power of some biomarkers (TNI for instance). The presence in the population of patients with symptoms of HF may have driven the superiority of BNP. Our study is obviously not a randomized prospective study and thus our conclusions should be seen as hypothesis generating results. Even with the limitations mentioned above, on the basis of the data obtained, we believe that in the diagnosis of HF in elderly patients, an approach based on the simultaneous measurement of multiple biomarkers, could be useful. We used a combination of biomarkers, that are considered able to explore different aspects related to HF. In fact, BNP is considered a cardiac function index, GAL an index of myocardial fibrosis/reshaping, TNI an indicator of myocardiocyte injury, CYS an excellent indicator of renal function (14-16), four factors of great importance in the physiopathology of HF. Really, this group of biomarkers showed a good performance in identifying patients with HF compared to healthy control. We therefore believe that a diagnostic approach based on the use of multiple biomarkers may be of relevant value in the diagnosis of HF in elderly patients.

CONFLICT OF INTERESTS

None.

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