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ORIGINAL ARTICLE

Relationship between RANKL and neuroendocrine activation in elderly males with heart failure

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Abstract The main cytokines regulating bone remodeling are the receptor activator of nuclear factor-κB ligand (RANKL) and its decoy receptor, osteoprotegerin (OPG). Recent data have linked RANKL and OPG to cardiovascular disease as well. NT-pro-BNP and adiponectin are well-established biomarkers of heart failure reflecting neuroendocrine activation in this multi-complex disorder. The objective of this article was to investigate whether RANKL is associated with neuroendocrine activation in 75 elderly males with mild to moderate congestive heart failure (CHF) and left ventricular ejection fraction <40%. The control group consisted of 20 healthy male volunteers with matching age and body mass index (BMI). Serum RANKL (sRANKL), OPG, NT-pro-BNP, adiponectin, leptin, clinical, and echocardiography parameters were evaluated. In comparison to the control group, the CHF patients showed significantly increased sRANKL levels [126.8 (122.6) vs. 47.8 (44.4) pg/ml, $P < 0.0001$]. There was a significant relative risk of systolic CHF in elderly males associated with increased sRANKL above the calculated cut-off of 83 pg/ml [OR = 10.286 (95%CI 3.079–34.356), $P < 0.0001$; RR = 3.600 (95%CI = 1.482–8.747)]. In the CHF patients, the log-transformed values of sRANKL levels correlated positively with the log-transformed values of the serum NT-pro-BNP and adiponectin levels ($P = 0.004$, $r = 0.326$ and $P = 0.037$, $r = 0.241$, respectively), while inversely correlated with the BMI and creatinine clearance ($P = 0.015$, $r = -0.281$ and $P = 0.042$, $r = -0.236$, respectively). In multivariate regression model, sRANKL was a significant determinant of NT-pro-BNP independent of age, BMI and creatinine clearance ($P = 0.002$, $R^2 = 0.546$). In conclusion, our study suggests that in elderly males with systolic

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heart failure sRANKL was significantly associated with parameters of neuroendocrine activation such as NT-pro-BNP and adiponectin. Further studies are needed to elucidate the potential role of sRANKL in the complex pathogenesis of heart failure.

Keywords Heart failure · RANKL · OPG · Adiponectin · NT-pro-BNP

Abbreviations

BMI	Body mass index
CHF	Congestive heart failure
LVEF	Left ventricular ejection fraction
LVEDD	Left ventricular end-diastolic diameter
NT-pro-BNP	N-terminal pro-brain natriuretic peptide
NYHA	New York Heart Association
sRANKL	Serum RANKL levels

Introduction

The 5 year mortality rate for patients with congestive heart failure (CHF) ranges between 40 and 60% despite recent improvement in survival [1]. Heart failure results not only from cardiac overload or injury, but also in a complex interplay among genetic, neuroendocrine, inflammatory, and biochemical changes [2]. The discovery of biomarkers for CHF and their appropriate use have contributed to improved screening, prevention, diagnosis, and treatment of CHF. The most evaluated biomarkers for heart failure are natriuretic peptides. Measurement of N-terminal pro-brain natriuretic peptide (NT-pro-BNP) is widely used to assess the presence, severity, and prognosis of CHF [2, 3].

The receptor activator of nuclear factor-κB ligand (RANKL) and its decoy receptor, osteoprotegerin (OPG), are members of the TNF-signaling super-family and are the main cytokines regulating bone remodeling. RANKL activates osteoclasts and bone resorption, while OPG acts as a decoy receptor for RANKL inhibiting bone resorption [4, 5]. OPG and RANKL also have many pleiotropic actions such as in dendritic cell functions, lymphocyte development, and apoptosis [6–8]. Many cell types express RANKL including endothelial cells [9]. The cell-bound form of RANKL is the most common, although a soluble form created by the cleavage from truncated ecto-domain by a TNF-α converting enzyme-like protease is measured in serum [5, 10]. Interestingly, recent experimental, clinical, and epidemiological data have linked RANKL and OPG to cardiovascular diseases as well [11, 12]. OPG has been shown to reflect the severity of coronary artery disease at angiography [11] and to predict the incidence and mortality from cardiovascular diseases in the community [13]. The role of serum OPG and especially the role of serum RANKL (sRANKL) in the heart failure have been poorly studied with contradictory results [14–16].

High circulating adiponectin, a circulating hormone derived from adipose tissue, has recently attracted considerable interest because of its identification as a risk factor for CHF [2, 17]. It has been postulated that high levels of adiponectin may reflect a salvage mechanism by improving metabolism and vascular stress [17, 18]. It has been reported that in patients with CHF, adiponectin positively correlated with NT-pro-BNP [19]. The relationship between serum adiponectin with sRANKL and serum OPG levels has never been looked at in cross-sectional human studies with CHF patients.

The aim of the present study was to evaluate the relationship between sRANKL and serum OPG levels with neuroendocrine activation (serum NT-pro-BNP and adiponectin) in elderly males with stable, systolic CHF. If established, the objective was to assess the sRANKL levels related to systolic CHF.

Materials and methods

Study design

Having studied the medical history archives of the Cardiology Department, Clinical Medical Center Zvezdara Belgrade, 152 males aged over 55 years were screened by telephone, all of them with chronic heart failure due to ischemic or idiopathic dilated cardiomyopathy. A total of 75 patients were included into this cross-sectional study, all of whom fulfilled inclusion and exclusion criteria at the baseline visit. *Inclusion criteria were*: (1) duration of CHF for longer than 1 year; (2) echocardiographically assessed left ventricular ejection fraction <40%; (3) etiology of HF: ischemic or idiopathic dilated cardiomyopathy; (4) NYHA functional class II and III; (5) unchanged medication regimen within the previous 6 weeks; and (6) clinically stable condition with no clinical evidence of decompensate heart failure, such as raised jugular venous pressure, ascites, and hepatomegaly. *Exclusion criteria were*: (1) diabetes mellitus determined by either self-reported histories or evidence within hospital case notes; (2) primary lung disease including chronic obstructive pulmonary disease; (3) musculoskeletal diseases; (4) uncontrolled hypertension of more than 170/110 mmHg; (5) myocardial infarction or unstable angina within previous 3 months; (6) acute or chronic infection, inflammatory diseases such as sepsis, arthritis, or systemic connective tissue disease; (7) symptomatic peripheral vascular disease; (8) alcohol abuse; (9)

serum creatinine ≥200 μmol/l; (10) valvular cardiomyopathy or artificial heart valve; (11) malignant disease, significant liver, thyroid, suprarenal gland, or pituitary disease; (12) cardiac cachexia defined as unintentional weight loss of ≥7.5% body weight over 6 months [20]. The control group consisted of 20 healthy male volunteers aged 55 years and above taking no medication. No previous medical illness was reported (including diabetes or any other cardiovascular disease). Prior to inclusion into the study, written informed consent was obtained from all patients with CHF and healthy subjects. The study was conducted according to the principles outlined in the Declaration of Helsinki and was approved by Ethical Committee of Clinical Medical Center Zvezdara.

Clinical and cardiovascular assessment

Having obtained written consent, the medical history was reviewed. Each CHF patients was categorized according to the New York Heart Association (NYHA) criteria [21]. A physical exam was performed to assess CHF stability. The 6-min walk test was performed according to the standard protocol [22]. All patients underwent a two-dimensional Doppler echocardiography examination (GE Vivid 7). Systolic function was quantified by measurement of left ventricular ejection fraction (LVEF) using the Simpson method. Left ventricular end-diastolic diameter (LVEDD) was measured according to the ASE recommendation [23]. Grip strength was determined by Jamar dynamometer using standard protocol [24].

Laboratory analysis

Basal blood samples were taken at 8 am from an antecubital vein. Participants were asked to refrain from smoking at least 8 h prior to blood sampling, to fast from 9 pm the previous evening as well as to withhold vasoactive medication for 12 h prior to appointment. Serum samples were immediately deep frozen and kept at −70°C until assay. Serum levels of N-terminal pro B-type natriuretic peptide (NT-pro-BNP) were measured with a fully automated "sandwich" electrochemiluminescence method by using Elecsys analyzer (Roche Diagnostics, GmbH, Mannheim); result range from 5–35000 pg/ml. Total adiponectin and leptin levels in serum were measured by RIA (Linco Diagnostics, Inc., St/Charles, MO). Commercial ELISA kits (BenderMedSystems GmbH, Austria) were used to measure serum levels of RANKL and OPG. The lower limits of detection were 2.2 pg/ml for RANKL and 2.5 pg/ml for OPG. Intra- and interassay coefficients of variation were <7.8 and <7.2% for RANKL and <7.0 and <8.0% for OPG, respectively. The estimated creatinine clearance was calculated from serum creatinine values using the Cockroft–Gault formula [25].

Statistical analysis

Descriptive statistics were presented as mean values with standard deviation or median with interquartile range for numeric variables, or as absolute numbers with percentage values for categorical variables. Evaluation of normality was performed with Kolmogorov–Smirnov test. The log10-transformations were performed for NT-pro-BNP, OPG, and sRANKL which follow exponential distribution. Student *t*-test was used to calculate differences between mean values. Mann–Whitney *U*-test was used to determine differences between median values. χ^2-test tested differences in frequencies between studied groups. The Pearson coefficient was used for measuring linear correlation between variables. Finally, and as variables inter-related, multivariate regression analysis, backward method was performed to asses the independent variables that may explain NT-pro-BNP, LVEF, and LVEDD variability. The receiver operating curve (ROC) curve was generated for sRANKL levels between two studied groups. To find cut-off point, P/N ratio was used. The cluster analysis with Ward linkage was performed to evaluate obtained cut-off point for sRANKL. To validate those results we used odds ratio, relative risk, and Mantel–Haenszel statistics. A P value of <0.05 was considered to indicate statistical significance. Statistical analysis was performed using the SPSS software for Windows, version 15 (SPSS, Inc., Chicago, IL).

Results

Demographic, clinical, and biohumoral characteristics of CHF patients and healthy subjects

The demographic and clinical characteristics of CHF patients and healthy subjects are summarized in Table 1. Compared with healthy subjects, CHF patients have lower left ventricular ejection fraction, 6-min walking distance, creatinine clearance and grip strength, and greater LVEDD. No difference in body mass index (BMI) and waist/hip ratio was found between CHF patients and healthy subjects.

The biohumoral parameters are shown in Table 2. CHF patients had significantly higher serum NT-pro-BNP, OPG, RANKL, and adiponectin levels than healthy controls, but there was no significant difference in leptin levels. The more advanced the CHF was according to NYHA class, the higher the adiponectin concentration were (NYHA III vs. NYHA II, 24.1 ± 12.2 vs. 14.5 ± 6.4 μg/ml, $P = 0.003$). On the other hand, sRANKL and OPG were not significantly higher in NYHA class III compared to NYHA class II patients with CHF (data not shown).

Table 1 Demographic and clinical characteristics of CHF patients and healthy subjects

	CHF patients ($n = 75$)	Healthy subjects ($n = 20$)	P value
Age (years)	68 ± 7	67 ± 7	0.899
Duration of disease (years)	5 ± 4		
Smoking former/active, *n* (%)	18 (24)/10 (13)	3 (15)/5 (25)	0.376
Ischemic/idiopathic dilatated CMP, *n* (%)	62 (83)/13 (17)		
NYHA class II/III, *n* (%)	55 (73)/20 (27)		
Atrial fibrillation, *n* (%)	20 (27)		
Pacemaker, *n* (%)	7 (9)		
LBBB, *n* (%)	20 (27)		
History of myocardial infraction, *n* (%)	49 (65)		
Hypertension, *n* (%)	47 (63)		
Mean blood pressure (mmHg)	101 ± 12	103 ± 7	0.199
LVEF (%)	29 ± 8	65 ± 5	<0.0001***
LVEDD (mm)	66 ± 9	49 ± 4	<0.0001***
Grip strength (kg)	95 ± 20	114 ± 19	<0.0001***
6-min walking distance (m)	404 ± 83	578 ± 64	<0.0001***
Medication, *n* (%)			
ACE inhibitors	61 (81)		
Beta-blocker	57 (76)		
Statin	21 (28)		
Loop diuretics	61 (81)		
Thiazide diuretics	11 (15)		
Aldosterone antagonists	41(55)		
Digitalis	22 (29)		
Vitamin K antagonist	39 (52)		
Aspirin	48 (64)		
BMI (kg/m^2)	28 ± 5	28 ± 3	0.560
Waist/hip ratio	1.03 ± 0.04	1.01 ± 0.06	0.084
Creatinine clearance (ml/min)	63 ± 22	78 ± 14	0.001**

Data are expressed as mean ± SD or absolute number (percentage)

*** $P < 0.0001$, ** $P < 0.001$ CHF patients versus healthy subjects

ACE angiotensin-converting enzyme, *BMI* body mass index, *CMP* cardiomyopathy, *LBBB* left bundle branch block, *LVEF* left ventricular ejection fraction, *LVEDD* left ventricular end-distolic diameter, *NYHA* New York Heart Association

Table 2 Biohumoral parameters of CHF patients and healthy subjects

	CHF patients ($n = 75$)	Healthy subjects ($n = 20$)	P value	
NT-pro-BNP (pg/ml)	1868.0 ± 2803.0	67.6 ± 74.0	<0.0001***	
sRANKL (pg/ml)	126.8 ± 122.6	47.8 ± 44.4	<0.0001***	Me ± IQR
OPG (pg/ml)	79.8 ± 126.0	10.8 ± 63.5	<0.0001***	
Uric acid (μmol/l)	418.5 ± 110.3	372.7 ± 187.0	0.164	
Fasting blood glucose (mmol/l)	5.3 ± 0.6	5.6 ± 0.5	0.041*	
Total cholesterol (mmol/l)	5.5 ± 1.1	6.0 ± 0.8	0.097	
Trygliceride (mmol/l)	1.6 ± 0.6	1.6 ± 1.2	0.812	
HDL cholesterol (mmol/l)	1.3 ± 0.3	1.4 ± 0.4	0.077	Mean ± SD
LDL cholesterol (mmol/l)	3.5 ± 0.9	3.8 ± 0.8	0.307	
hsCRP (mg/l)	5.1 ± 9.3	2.8 ± 3.7	0.283	
Adiponectin (μg/ml)	17.0 ± 9.3	9.6 ± 2.3	<0.0001***	
Leptin (ng/ml)	7.7 ± 7.2	5.9 ± 3.2	0.290	

Data are expressed as median ± interquartile range (Me ± IQR) or mean ± standard deviation (Mean ± SD)

*** $P < 0.0001$, * $P < 0.05$ CHF patients versus healthy subjects

NT-pro-BNP N-terminal pro-brain natriuretic peptide, *OPG* osteoprotegerin, *sRANKL* serum receptor activator of nuclear factor κB ligand

Relations between biohumoral parameters, NT-pro-BNP, and cardiovascular parameters

Correlation analyses of sRANKL (log-transformed) (Fig. 1a) and other biohumoral parameters with NT-pro-BNP (log-transformed) and cardiovascular parameters are shown in Table 3.

sRANKL (log-transformed) (Fig. 1b) and serum OPG (log-transformed) were found to be positively correlated with serum adiponectin levels ($r = 0.241$, $P = 0.037$ and $r = 0.243$, $P = 0.036$, respectively). When adiponectin was normalized for the BMI, the same relation existed with both sRANKL and OPG levels ($r = 0.246$, $P = 0.033$ and $r = 0.257$, $P = 0.026$, respectively).

In addition, sRANKL (log-transformed), OPG (log-transformed), and adiponectin inversely correlated with grip strength ($r = -0.236$, $P = 0.041$, $r = -0.266$, $P = 0.021$, and $r = -0.319$, $P = 0.005$, respectively). sRANKL (log-transformed) and adiponectin negatively correlated with BMI ($r = -0.281$, $P = 0.015$ and $r = -0.321$, $P = 0.005$, respectively), while leptin positively correlated with BMI ($r = 0.618$, $P = 0.000$). In addition, sRANKL (log-transformed) and adiponectin inversely correlated with creatinine clearance ($r = -0.236$, $P = 0.042$ and $r = -0.257$,

Table 3 Correlation of NT-pro-BNP (log-transformed), echocardiographic parameters, and 6-min walk test with biohumoral parameters in CHF patients

	NT-pro-BNP (log scale)	LVEF	LVEDD	6-MWD
sRANKL (log scale)				
r	*0.326*	−0.130	−0.002	−0.171
P	*0.004*	0.267	0.984	0.142
OPG (log scale)				
r	*0.231*	0.114	−0.075	−0.091
P	*0.047*	0.331	0.524	0.438
Adiponectin				
r	*0.583*	*−0.364*	*0.263*	*−0.391*
P	*<0.0001*	*0.001*	*0.023*	*0.001*
Leptin				
r	−0.130	0.102	0.091	−0.073
P	0.265	0.384	0.437	0.533
Uric acid				
r	*0.462*	*−0.303*	0.214	*−0.285*
P	*<0.0001*	*0.008*	0.065	*0.013*

Significant *r* and *P* values are in italics

LVEF Left ventricular ejection fraction, *LVEDD* left ventricular end-distolic diameter, *NT-pro-BNP* N-terminal pro-brain natriuretic peptide, *OPG* osteoprotegerin, *RANKL* receptor activator of nuclear factor κB ligand, *6-MWD* 6-min walking distance

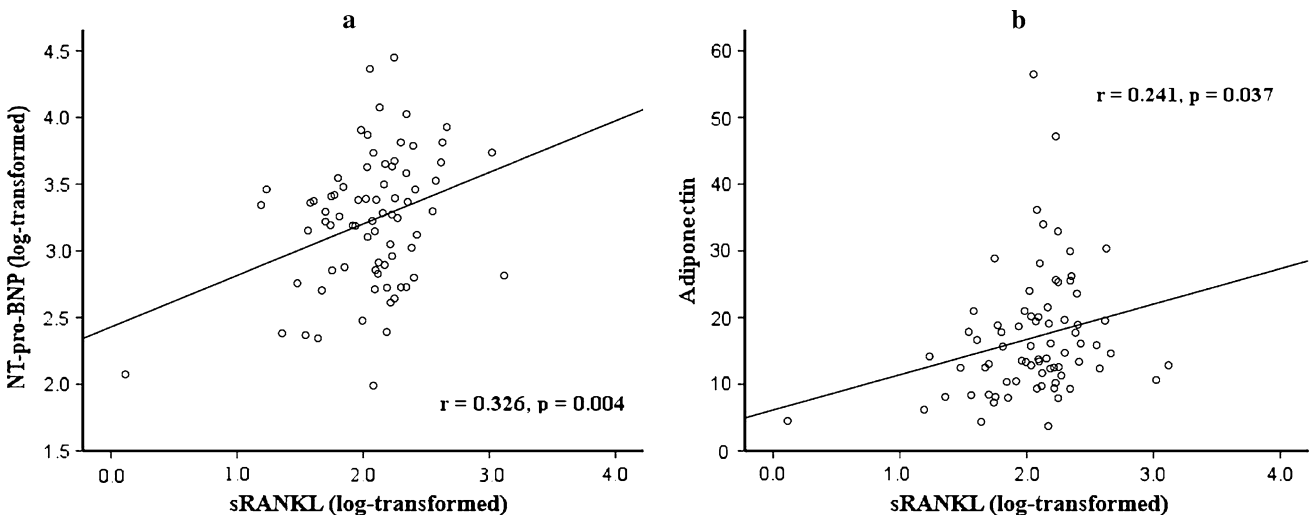

Fig. 1 Scatter plots of the association between values of sRANKL (log-transformed) and serum NT-pro-BNP (log-transformed) (**a**) and adiponectin levels (**b**) in elderly CHF males

$P = 0.026$, respectively). Serum adiponectin and leptin values were positively associated with uric acid level ($r = 0.247$, $P = 0.033$ and $r = 0.396$, $P = 0.000$, respectively).

Multivariate regression analysis

Multivariate regression analysis with backward model was used to asses the independent variables that may affect serum NT-pro-BNP, LVEF, and LVEDD (Table 4). The variables entered in the model were the following: age, BMI, sRANKL (log-transformed), serum OPG (log-transformed), adiponectin, leptin, creatinine clearance, uric acid, hsCRP, and fasting blood glucose.

ROC and cluster analysis for sRANKL

ROC analysis was performed to evaluate sensitivity and specificity of sRANKL between two studied groups. The area under the ROC curve for sRANKL levels was 0.801 (95%CI 0.702–0.900), $P < 0.0001$ (Fig. 2a). The calculated P/N ratio for sRANKL levels was 83 pg/ml (sensitivity 0.72; specificity 0.80). Cluster analysis was performed to evaluate the result of ROC. Cluster analysis of sRANKL (log-transformed) showed two clusters. First group was with a higher value of sRANKL (log-transformed) [2.2384 ± 0.23977 (95%CI 2.1754–2.3015)], while the second group was with a lower sRANKL (log-transformed) [1.5692 ± 0.31513 (95%CI 1.4641–1.6742)]. The projected cut-off point generated from distribution curves of sRANKL (log-transformed) for two clusters was 1.919 (Fig. 2b). The reproduced value of sRANKL (inverse sRANKL) was 83 pg/ml. The result of ROC and cluster analysis was concordant. To validate given results, odds ratio (OR) was used as strength of association between two studied groups regarding given cut-off point [OR = 10.286 (95%CI 3.079–34.356); $P < 0.0001$]. The relative risk for systolic CHF in males with sRANKL value more than 83 pg/ml was 3.600 (95%CI = 1.482–8.747).

Table 4 Multivariate regression analysis (backward model) with NT-pro-BNP (log-transformed), left ventricular ejection fraction and left ventricular end-diastolic diameter as dependent variables in elderly CHF males

	B	*P*	*F* (*P*)
Log NT-pro-BNP			
(Constant)	1.436		
Log RANKL	0.315	0.002	
Adiponectin	0.019	0.000	21.034 (≤0.0001)
Leptin	−0.015	0.025	
Uric acid	0.002	0.000	
$R^2 = 54.6\%$			
LVEF			
(Constant)	35.255		
Adiponectin	−0.253	0.012	
Leptin	0.242	0.073	6.088 (≤0.0001)
Uric acid	−0.024	0.008	
Log OPG	3.191	0.025	
$R^2 = 25.8\%$			
LVEDD			
(Constant)	80.495	0.017	4.965 (0.010)
Adiponectin	0.258	0.042	
Age	−0.273		
$R^2 = 12.1\%$			

B parameter estimate, *F* Fisher test

NT-pro-BNP N-terminal pro-brain natriuretic peptide, *LVEF* left ventricular ejection fraction, *LVEDD* left ventricular end-diastolic diameter

Discussion

According to our knowledge, this study for the first time showed that elevated sRANKL levels (log-transformed) were positively correlated with high circulating levels of NT-pro-BNP levels (log-transformed) in elderly males with stable systolic CHF independently of age, BMI, and renal function. In our study sRANKL levels were increased among heart failure patients compared to healthy controls. There was a significant relative risk of systolic CHF in elderly males associated with elevated sRANKL above the calculated cut-off of 83 pg/ml. Only two studies have hitherto examined the sRANKL in CHF patients. Helske et al. [16] found unaltered concentration of sRANKL in the circulation and the expression of RANKL mRNA in the heart muscle in patients with aortic stenosis independent of the presence of heart failure. Additionally, sRANKL was unrelated to the presence of heart failure with no association with NT-pro-BNP. Conversely to this, but in line with our data, Ueland et al. [15] found elevated sRANKL in heart failure due to ischemic or dilated cardiomyopathy. They also demonstrated increased expression of myocardial RANKL and suggested the mechanism for a potential role of sRANKL in the pathogenesis of heart failure. The possible underlined mechanism are interactions between infiltrating activated T cells expressing RANKL and cardiomyocytes expressing RANK, receptor for RANKL. RANKL markedly enhances matrix metalloproteinase activity in human fibroblasts which may increase collagen network destruction between cardiomyocytes leading to myocyte slippage, ventricular dilatation, and progressive dysfunction [26]. More clinical studies and experimental models are required to elucidate the association of NT-pro-BNP activation with systemic and local myocardial RANKL expression in heart failure.

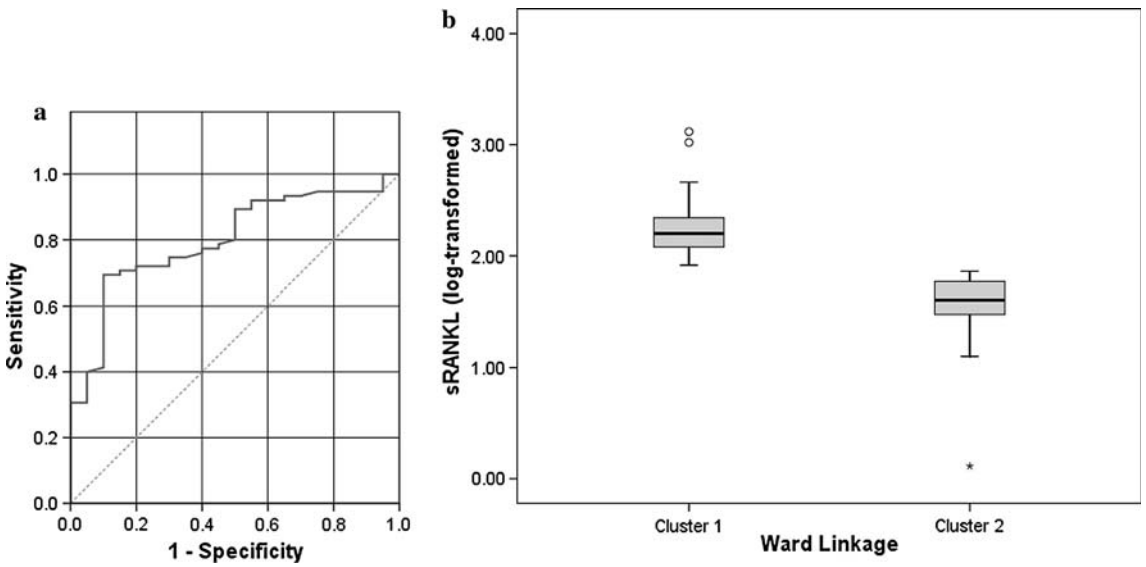

Fig. 2 Receiver operating characteristics curve (**a**) and box and whiskers cluster analysis results (**b**) of sRANKL

In line with previous studies, we observed that serum adiponectin levels were significantly higher in CHF patients than in age- and BMI-matched control subjects [27]. In addition, high adiponectin levels were related to severity of CHF in our study. We showed that increased adiponectin was determinant for left ventricular ejection fraction in CHF patients, which has not been previously demonstrated [19, 27, 28]. Similarly, this is also the first article demonstrating adiponectin as determinant of LVEDD in CHF patients. Additionally, our results showed that serum adiponectin was determinant of NT-pro-BNP (log-transformed), independent of BMI, and renal function which was concordant with previous reports [19, 27, 28]. Tsukamoto et al. [29] have recently demonstrated important evidence that BNP enhances the production of adiponectin by human adipocytes in both experimental and clinical studies including patients with CHF. Further, it seems that our study is the first to find a positive significant correlation between sRANKL and adiponectin levels in CHF patients. Adiponectin can stimulate the osteoclast RANKL pathway while inhibiting its decoy receptor, OPG [9]. Thus, adiponectin may exert its negative effect on bone metabolism by promoting the bone-resorbing RANKL pathway. In agreement with the recent study in healthy subjects, we also demonstrated a weak positive correlation between adiponectin and OPG in CHF patients [30]. Future studies are required in order to find a potential molecular link between these parameters.

In our study, serum OPG levels were higher among heart failure patients compared to healthy controls, which is concordant with the previously documented reports [14–16]. In line with recent study, weak but significant positive relationship between serum OPG and NT-pro-BNP was shown in our study [15].

In regard to leptin, the positive association of leptin with BMI in our cohort of elderly males with CHF was confirmed. In the multivariate regression model we found that serum leptin was inversely associated with NT-pro-BNP, regardless of age, BMI, and renal function. Consistent to our results, there was an inverse correlation of leptin and BNP in patients with the CHF [31], while no relationship was found in another study [32]. Furthermore, serum leptin levels had a tendency to be positively associated with LVEF in our study. In line with the previous study, it might be suggested that leptin levels decrease in parallel with progressively deteriorating cardiac function [33]. On the other hand, there was no relationship between serum leptin and sRANKL levels.

There are several limitations to this study. Firstly, the study design might limit the strength of the conclusion. The present study was a single-center and cross-sectional study analyzing a small number of subjects. The cross-sectional design of the study cannot respond to causality relations, but our study contributes to the knowledge about the strength of association and its relative contribution to the pathophysiology of the failing heart. Therefore, a study on a larger scale is warranted to confirm the complex interactions between RANKL and cardiovascular variables as well as to evaluate the potential prognostic role of RANKL and OPG. Secondly, the influence of the drugs on circulating adiponectin should be considered. Statins were prescribed in 29% of studied CHF patients. Drugs such as statins have been reported to raise the circulating adiponectin levels [34].

In conclusion, we have shown that sRANKL is significantly associated with serum NT-pro-BNP and adiponectin regardless of BMI and renal function, and that it may have a role in the pathogenesis of heart failure in elderly men. It might be assumed that the correlation of sRANKL to these well-established risk markers in patients with heart failure might qualify this molecule for further testing.

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