Predictive Value of Circulating SPARC-Related protein Osteonectin in Patients with Symptomatic Moderate-to-Severe Ischemic-Induced Chronic Heart Failure

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Abstract: *Aim:* To evaluate the prognostic value of circulating osteonectin for cumulative survival and hospitalization in patients with ischemic chronic heart failure (CHF).

Methods: One hundred fifty four patients with ischemic symptomatic moderate-to-severe CHF were prospectively enrolled at discharge from the hospital. Observation period was up to 3 years (156 weeks). Blood samples for hematology, chemistry, and biomarker measurements were collected at baseline prior to study entry. ELISA method for measurement of circulating osteonectin (OSN) was used.

Results: During a median follow-up of 2.18 years we identified 21 deaths and 106 readmissions. Medians of circulating levels of OSN in survivors patient and subjects who died were 670.96 ng/mL (95% confidence interval [CI] = 636.53-705.35 ng/mL) and 907.84 ng/mL (95% CI = 878.02-937.60 ng/mL). Receive Operation Characteristic curve analysis has shown the best balanced cutoff point of OSN concentration for cumulative survival equal 845.15 ng/mL. A significantly divergence of Kaplan-Meier survival curves constructed for patients with high (> 845.15 ng/mL) and low (<845.15 ng/mL) concentrations of OSN was found. Circulating OSN independently predicted all-cause mortality (OR = 1.23; 95% CI = 1.10–1.36; P < 0.001), CHF-related death (OR = 1.46; 95% CI 1.22–1.80; P < 0.001), and also CHF-related readmission (OR = 1.92; 95% CI = 1.77 – 2.45; P<0.001) within 3 year of follow-up period.

Conclusion: Increased circulating SPARC family member OSN associates with increased 3-year CHF-related death, all-cause mortality, and risk for readmission due to CHF.

Keywords: Osteonectin, Chronic heart failure, Prognosis, Survival, Readmission.

1. INTRODUCTION

Matrix cellular proteins, such as secreted protein acidic and rich in cysteine (SPARC), play a key role in post-synthetic procollagen processing in myocardium and regulate cell adhesion, growth factor activity and cell cycle in heart failure [1]. It has found SPARC family member osteonectin (OSN) causes myocardial hypertrophy, increased fibrillar collagen content, stimulates cell signaling, adhesion, survival, proliferation, and migration in several cell types, as well as mediates calcification of the vascular wall, coagulation, and endothelial dysfunction [2]. Moreover, OSN induces increased collagen deposition in response to acute myocardial infarction (MI) and as result cardiac hypertrophy and impaired cardiac function [3]. Recent animal studies have revealed that increased circulating OSN closely associates with higher incidence of mortality following MI [4, 5]. Therefore, increased rates of rupture and newly chronic heart failure (CHF) over the first 14 days after MI may relate to elevated OSN [4, 5]. However, the causative role of OSN in the CHF has not defined. The objective of this study was to evaluate the prognostic value of circulating OSN for survival and readmission in patients with ischemic CHF.

2. METHODS

The study prospectively evolved 154 patients (86 male, 68 females) aged 48 to 62 years with ischemic symptomatic CHF with II-IV NYHA (New York Heart Association) class. Chronic heart failure (CHF) was diagnosed according to current European Society of Cardiology clinical guidelines [6]. All the patients have given their written informed consent for participation in the study. The following are exclusion criteria: Q-wave and non-Q-wave MI within 3 months before study entry; severe kidney and liver diseases that may affect clinical outcomes; malignancy; creatinin plasma level above 440 μmol/L; estimated GFR < 35 ml/min/м^2; brain injury within 3 months before the enrollment; body mass index above 30 kg/m^2; pulmonary edema; tachyarrhythmia; valvular heart disease; thyrotoxicosis; ischemic stroke; intracranial hemorrhage; acute infections; surgery; trauma; all the ischemic events within 3 previous months; inflammations within a previous month; neoplasm; pregnancy; implanted

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pacemaker, any disorder that may discontinue patient's participation in the study according to investigators; and patient's refusal to participate in the study or to give his consent for it. Follow-up period was up to 3 years (156 weeks). We also analyzed cumulative survival related to CHF, and therefore all-cause mortality was examined additionally.

2.1. Methods for Visualization of Coronary Arteries

Multispiral computed tomography angiography and/or angiographic study have been performed to verify the ischemic nature of CHF. Angiographic procedure was used when ischemic sings were presented at baseline and no myocardial infarction was found previously. Multispiral computed tomography angiography was performed when no ischemic sings / previously documented old myocardial infarction at baseline were detected, but signs and symptoms of CHF were presented. Multispiral computed tomography angiography has performed for all the patients prior to the study entry. The coronary artery wall structure was investigated by contrast-enhanced spiral computed tomography angiography [7] on Somatom Volum Zoom scanner (Siemens, Erlangen, Germany) with two detector rows. After preliminary native scanning, non-ionic contrast "Omnipaque" (Amersham Health, Ireland) was administered for the optimal image of the coronary arteries. To reconstruct the image, 0.6-mm-width axial tomographic slices were used. All patients with exiting atherosclerotic lesions of the coronary arteries were subjected to conventional angiographic examination. Coronary artery disease (CAD) was considered to be diagnosed upon availability of previous angiographic examinations carried out not later than 6 months ago provided no new cardiovascular events occurred for this period, and the procedure are available for assay.

2.2. Assessment of Hemodynamics

Transthoracic ultrasonic echocardiography was performed according to a conventional procedure on scanner equipped ACUSON (SIEMENS, Germany) in two-dimensional mode and Tissue Doppler Imaging echocardiography regimen from parasternal, subcostal, and apical positions over the short and long axis with probe P of 2.5-5 MHz. Left ventricular end-diastolic and end-systolic volumes were measured by modified Simpson's method. Left ventricular ejection fraction (LVEF) was assessed in compliance with the requirements of American Society of Echocardiography [8]. Tissue Doppler echocardiography was performed in 4-, 3- and 2-chamber views in each of 16 segments of the left ventricle and in 4 spots of the mitral annulus: at the base of posterior septal, lateral, inferior, and anterior left ventricular walls [9]. Peak systolic (Sm), early diastolic (Em), and late diastolic (Am) myocardial velocities were measured in the mitral annulus area.

2.3. Calculation of Glomerular Filtration Rate

Glomerular filtration rate (GFR) was calculated using CKD-EPI formula [10].

2.4. Measurement of Biomarkers

Blood samples were collected at baseline in the morning (at 7-8 a.m.) into cooled silicone test tubes, when patients were discharged from the hospital with stable clinical status. Samples were processed according to the recommendations of the manufacturer of the analytical technique used. They were centrifuged upon permanent cooling at 6,000 rpm for 3 minutes. Then plasma samples were stored at a temperature -70^{0}C. Circulating OSN level was determined by ELISA method (Bender MedSystems GmbH, Vienna, Austria). N-terminal pro-brain natriuretic peptide (NT-pro-BNP) concentration was measured by immunoelectro-chemoluminescent assay using kit produced by R&D Systems (USA) on Elecsys 1010 analyzer (Roche, Mannheim, Germany). Concentrations of total cholesterol (TC) and cholesterol of high-density lipoproteins (HDL) were measured by direct fermentation method.

2.5. Ethical Principles

The investigators followed strictly all the requirements to clinical trials in conformity with the World Medical Association (WMA) Declaration of Helsinki, 1964, Good Clinical Practice provided by International Conference on Harmonization (GCP-ICH), Council of Europe Convention for the Protection of. Human Rights and Dignity of the Human Being in view of using achievements in biology and medicine, Convention on Human Rights and Biomedicine, including Additional Protocol to the Convention on Human Rights and Biomedicine, concerning Biomedical Research, and legislation of Ukraine.

2.6. Statistical Analysis

Statistical analysis of the results obtained was performed in SPSS system for Windows, Version 20 (SPSS Inc, Chicago, IL, USA). The data were presented as mean (M) and standard error (±SE) or 95% confidence interval (CI); median (Me) and

interquartile range (IQR). A design of the study (non-randomized prospective open cohort study) with two-tailed hypothesis requires the alpha level 0.05 and beta-level 0.20. The sample size was computed on http://www.sealedenvelope.com/power/binary-superiority/ taken into consideration that the level of significance is 5%, $Z_{\alpha/2}$ is 1.96. The result shows that the total sample size required is 139 patients. Adjustment for non-compliance/cross-over and lost for follow-up patients (10%) the total sample size was calculated 154 subjects. The hypothesis of normal distribution of the parameters analyzed was checked by means of Shapiro–Wilk test and Kolmogorov-Smirnov test. To compare the main parameters of patients' groups (subject to the type of distribution of the parameters analyzed), two-tailed Student t-test or Shapiro–Wilk U-test were used. To compare categorical variables between groups, Chi2 test ($\chi 2$) and Fisher F exact test were used. The circulating OSN and NT-pro-BNP level in the blood failed to have a normal distribution, while distribution of the total cholesterol and cholesterol fractions had a normal character (estimated by means of Kolmogorov-Smirnov test) and was not subjected to any mathematical transformation. Receive Operation Characteristic (ROC) analysis was carried out to identify the optimal cutoff points of the OSN concentration with predicted value. The difference between the areas under ROC curves was calculated with the method of DeLong al. (1988) [11] and reclassification measures (index discrimination improvement - IDI) [12]. Odds ratio (OR) and 95% confidence interval (95% CI) were calculated for all the independent predictors of survival of the patients. A calculated difference of $P<0.05$ was considered significant.

3 RESULTS

3.1. General Characteristics of Study Patient Population

A median follow-up was 2.18 years. We identified 21 died cases included 18 CHF-related deaths. Additionally, 106 subjects were readmitted in the hospital due to advance CHF (17 cases in died cohort and 89 cases in survivors cohort). Table **1** shows a general characteristic of the patients included in the study. As one can see from Table **1**, no substantial age and gender differences were found among persons who died and survived. No any differences were determined between both cohorts in body mass index (BMI), glomerular filtration rate (GFR), HbA1c, fasting blood glucose level, blood creatinine level, total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C) and high-density lipoprotein cholesterol (HDL-C), numerous of coronary vessels damaged. Therefore, no difference was found between both cohorts in systemic office blood pressure (BP) and heart rate (HR). Incidences of type 2 diabetes mellitus among patients enrolled in both cohorts was 38.1% and 33.8% (P=0.06). Noted that there was not a statistically significant change in E/Am and E/Em between both cohorts, while decreased left ventricular ejection fraction value was detected in died patients. At the same time, the level of circulating NT-pro-BNP was statistically significantly higher in died patients than in survivor's persons. When analyzing details of pharmacotherapy, no substantial differences were found between two cohorts with regard to administration of the majority of drugs

3.2. Circulating OSN Level in Survived and Died Patients

Means of circulating level of OSN in survivors and died patients were 670.96 ng/mL (95% confidence interval [CI] = 636.53-705.35 ng/mL) and 907.84 ng/mL (95% CI = 878.02-937.60 ng/mL) ($P<0.001$).

3.3. The Predictive Value of OSN Concentration in Study Patient Population

Multivariate logistic regression was used to assess whether any combination of assays was able to better discriminate between survivors and died patients. In the logistic regression analysis, the main factors independently related with cumulative mortality and CHF-related readmissions were some biomarkers (OSN alone, NT-pro-BNP alone), LVEF, T2DM, and three- and multi-vessel lesion. Circulating OSN independently predicted all-cause mortality (OR = 1.23; 95% CI = 1.10–1.36; $P < 0.001$), CHF-related death (OR = 1.46; 95% CI 1.22–1.80; $P < 0.001$), and also CHF-related readmissions (OR = 1.92; 95% CI = 1.77 – 2.45; $P<0.001$) up to 3 years (Table **2**). NT-pro-BNP and LVEF remained statistically significant for all categories: all-cause mortality, CHF-related death, and CHF-related readmissions, whereas T2DM and three- and multi-vessel lesions for all variables did not.

Using a stepwise model selection method for multivariable prediction model we have investigated the summary effect of any combinations of OSN, NT-pro-BNP, LVEF on all-cause mortality, CHF-related death, and CHF-related readmissions. We found that OSN alone (Model 1) and combination of OSN with NT-pro-BNP (Model 2) remained statistically significant

Table 1: General characteristic of patients participating in the study

Variables	All Patients (n=154)	Subjects Who Died (n=21)	Subjects Who Survived (n=133)
Age, years	58.50±6.10	57.20±6.70	59.50±7.30
Males, n (%)	79 (51.3%)	12 (57.1%)	67 (50.3%)
Arterial hypertension, n (%)	73 (47.4%)	12 (57.1%)	61 (45.9%)
Hyperlipidemia, n (%)	61 (39.6%)	9 (42.8%)	52 (39.1%)
T2DM, n (%)	53 (34.4%)	8 (38.1%)	45 (33.8%)
Adherence to smoking, n (%)	31 (20.1%)	7 (33.3%)	24 (29.3%)
II Class NYHA	41 (26.6%)	6 (28.6%)	35 (26.3%)
III Class NYHA	74 (48.1%)	9 (42.8%)	65 (48.9%)
IV Class NYHA	39 (25.3%)	6 (28.6%)	33 (24.8%)
BMI, kg/m^2	23.9 (95% CI=22.8–26.1)	23.7 (95% CI=22.5–27.3)	24.2 (95% CI=22.0–27.9)
GFR, mL/min/1.73 m^2	83.4 (95% CI=70.2–91.3)	82.1 (95% CI=69.9–93.1)	85.2 (95% CI=70.3–112.5)
HbA1c, %	6.5(95% CI=4.7-8.6)	6.3 (95% CI=4.4-9.0)	7.0 (95% CI=4.3-9.2)
Fasting blood glucose, mmol/L	4.95 (95% CI=3.8-8.0)	4.80 (95% CI=3.6-8.5)	5.40 (95% CI=3.4-9.1)
Creatinine, μmol/L	72.6 (95% CI=61.3–82.5)	70.5 (95% CI=59.6–88.3)	74.9 (95% CI=65.1–90.3)
Total cholesterol, mmol/L	5.1 (95% CI=4.7-5.6)	5.3 (95% CI=4.6-6.0)	5.0 (95% CI=4.2-5.8)
LDL-C, mmol/L	3.35 (95% CI =3.16–4.02)	3.60 (95% CI =3.20–4.18)	3.02 (95% CI=2.80–3.90)
HDL-C, mmol/L	0.92 (95% CI = 0.90–1.02)	0.94 (95% CI = 0.92–1.06)	0.88 (95% CI = 0.82–0.97)
NT-pro-BNP, pg /mL	1266.1 (95% CI 811.5 – 2220.7)	1533.6 (95% CI 644.5 – 2560.6)	1031.2 (95% CI 704.8 – 1560.7)*
Osteonectin, ng/mL	788.54 (95% CI = 665.12-912.30)	907.84 (95% CI = 878.02-937.60)	670.96 (95% = 636.53-705.35)*
Systolic BP, mm Hg	131±6	129±4	135±5
Diastolic BP, mm Hg	77±4	77±5	78±5
Heart rate, beats per 1 min.	71±5	76±6	68±3
LVEF, %	47.60±0.82	42.80±0.76	55.40±0,80*
E/Am, U	16.6±0.72	16.6±0.94	16.5±1.20
E/Em, U	16.6±0.90	16.6±1.00	16.6±0.84
One-vessel lesion of CA, n (%)	29 (18.8%)	5 (23.8%)	24 (18.0%)
Two-vessel lesion of CA, n (%)	64 (41.6%)	8 (38.1%)	54 (40.6%)
Three- and multi-vessel lesion of CA, n (%)	63 (40.9%)	8 (38.1%)	55 (41.4%)
ACEI / ARAs, n (%)	154 (100%)	21 (100%)	133 (100%)
Acetylsalicylic acid, n (%)	130 (84.4%)	19 (90.5%)	121 (91.0%)
Other antiaggregants, n (%)	14 (9.1%)	2 (9.5%)	12 (9.0%)
Statins, n (%)	94 (61.0%)	14 (66.7%)	80 (60.2%)
Metformin, n (%)	53 (34.4%)	8 (38.1%)	45 (33.8%)
Diuretics, n (%)	139 (90.3%)	18 (85.7%)	121 (91.0%)
Mineralcorticoid receptor antagonist eplerenon, n(%)	79 (51.3%)	9 (42.9%)	70 (52.6%)

Note: * - statistically differences between parameters in the two groups (P<0.05); CI – confidence interval; CAD – coronary artery disease, T2DM – type two diabetes mellitus, GFR - Glomerular filtration rate, *HDL-C - high-density lipoprotein cholesterol,* LDL-C - *Low-density lipoprotein cholesterol, CA* - coronary arteries, *BP – blood pressure,* BMI - Body mass index, NYHA - New York Heart Association, BNP – brain natriuretic peptide, LVEF - Left ventricular ejection fraction, U – unit, Em - early diastolic myocardial velocity, Am - late diastolic myocardial velocity, E – peak velocity of early diastolic left ventricular filling, ACEI – angiotensin-converting enzyme inhibitor, ARAs – angiotensin-2 receptors antagonists

Table 2: Independent Variables Related to 3-Years All-Cause Mortality, CHF-Related Death, and CHF-Related Rehospitalisation, Obtained By Logistic Regression Analysis

Variables	All-Cause Mortality			CHF-Related Death			CHF-Related Rehospitalisation		
	OR	95% CI	P	OR	95% CI	P	OR	95% CI	P
OSN	1.23	1.10–1.36	0.006	1.46	1.22–1.80	0.004	1.92	1.77 – 2.45	0.001
NT-pro-BNP	1.09	1.02–1.16	0.002	1.42	1.22–1.73	0.006	1.44	1.28–1.67	0.002
LVEF	1.06	1.01–1.12	0.001	1.15	1.12–1.18	0.014	1.22	1.07–1.45	0.016
T2DM	1.05	1.01–1.11	0.001	1.03	0.93–1.10	0.32	1.04	0.97–1.06	0.42
Three- and multi-vessel lesion of coronary arteries	1.02	0.88–1.09	0.56	1.01	0.92–1.07	0.27	1.14	1.03–1.26	0.012

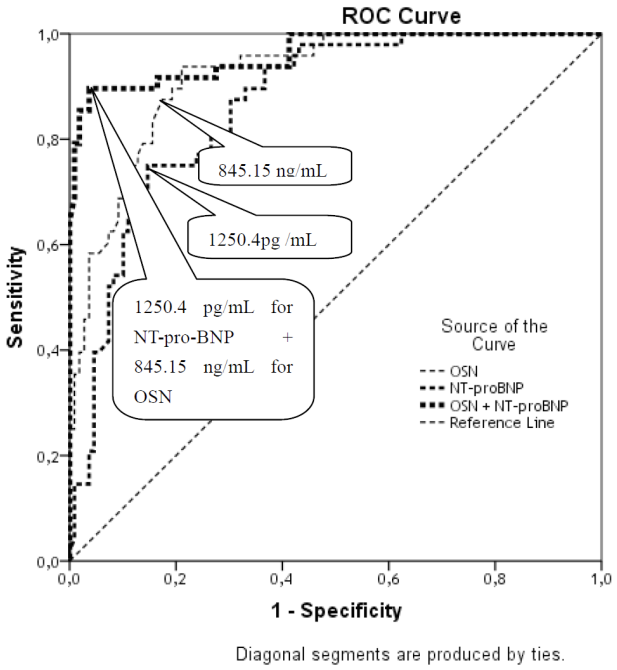
Note: OR – odds ratio, CI – confidence interval; LVEF – left ventricular ejection fraction; BNP – brain natriuretic peptide; T2DM – type two diabetes mellitus.

predictors for all-cause mortality (B-coefficient=1.14, p= 0.001, and B-coefficient=1,14, p=0.001 respectively), CHF-related death (B-coefficient=2.24, p= 0.003, and B-coefficient=2,76, p=0.008 respectively), and CHF-related readmissions (B-coefficient=2.06, p=0.003, and B-coefficient=2,11, p=0.004 respectively), whereas combination of OSN with both NT-pro-BNP and LVEF (Model 4) did not (B-coefficient= 0.014, p= 0.543, and B-coefficient=0,016, p=0.528, and B-coefficient=0,012, p=0.448 respectively). NT-pro-BNP alone (Model 3) predicted CHF-related readmissions (B-coefficient=1.88, p= 0.001) only, whereas all-cause mortality (B-coefficient=0,025, p=0.68) and CHF-related death (B-coefficient=0,036, p=0.62) did not. In fact, a stepwise model selection method demonstrated that LVEF, T2DM and three- and multi-vessel lesions of coronary arteries added to combination of OSN and NT-pro-BNP do not offer any additional information to discriminate between survivors and died patients with CHF (B-coefficient of 0.012, 0.067, and 0.023 respectively; p-values of 0.277, 0.300 and 0.522 respectively).

The optimal balanced cut-off point for OSN is determined by the relative importance of the sensitivity and specificity. ROC (Receive Operation Characteristic) curve analysis has shown that the best balanced cut-off point of OSN concentration reflected superiority in cumulative survival was 845.15 ng/mL (Figure **1**). Area under ROC Curve was 0.918 (Std. error = 0.022; 95% CI = 0.876-0.961), sensitivity and specificity were 79.2% and 84.4% respectively. Derived from ROC curve, a NT-pro-BNP cut-off of 1250.4 pg /mL showed the best balanced sensitivity and specificity for predicting mortality and hospital readmission (72.3% sensitivity and 81.6% specificity). Using cut-off points for OSN and NT-pro-BNP over mentioned above we found that the discriminative ability for both biomarkers showed a small trend to increase only. Combination of both biomarkers leads increased sensitivity up 88.6% and specificity up 92.4%. Discrimination Model was excellent for OSN alone, NT-pro-BNP alone, and their combination. For determination of difference between the ROC curves we used DeLong method and reclassification method (index discrimination improvement - IDI). Using DeLong method we found a statistical difference between areas under curves, that are suitable for OSN alone, NT-pro-BNP alone and its combination (P<0.001 for all cases). IDI after the inclusion of NT-pro-BNP into the model was calculated. However, we found that adding of NT-pro-BNP to standard model constructed on OSN does not improve its discrimination value (AUC 0.61 vs. 0.918, p=0.22; and IDI=0.035, p=0.42). There was significantly difference between discriminates calculated for OSN alone and NT-pro-BNP alone (IDI = 0.04; P<0.01). Therefore we found a significantly divergence of Kaplan-Meier survival curves constructed for patients with high (> 845.15 ng/mL) and low (<845.15 ng/mL) concentrations of OSN (Figure **2**). The curve's divergence affected accumulation of clinical events have been reached a statistical significance after 26 weeks of follow-up (P<0.001).

4. DISCUSSION

It is well known that extracellular matrix (ECM) proteins may modulate cell–matrix interactions and cell function, and they do not seem to have a direct structural role and mediate left ventricular remodeling. Several members of the matricellular protein family, like OSN, are up-regulated in CHF. In fact, OSN (also known as Secreted Protein Acidic and Rich in Cysteine - SPARC) is synthesized by wide spectrum cells, such as osteoblasts, fibroblasts, and activated macrophages



Area Under the Curve					
Test Result Variables	Area	Std. Error[a]	Asymptotic Sig.[b]	Asymptotic 95% Confidence Interval	
				Lower Bound	Upper Bound
OSN	0.918	0.022	0.001	0.876	0.961
NT-pro-BNP	0.865	0.029	0.001	0.808	0.922
OSN + NT-pro-BNP	0.961	0.016	0.001	0.929	0.993
The test result variables: OSN, NT-pro-BNP, OSN + NT-pro-BNP have at least one tie between the positive actual state group and the negative actual state group. Statistics may be biased.					
a. Under the nonparametric assumption					
b. Null hypothesis: true area = 0.5					

Figure 1: Results of the Receive Operation Characteristic analysis: The graphical plot illustrates the performance of discriminations for OSN alone, NT-pro-BNP alone and its combination of both biological markers for cumulative survival in CHF patients.

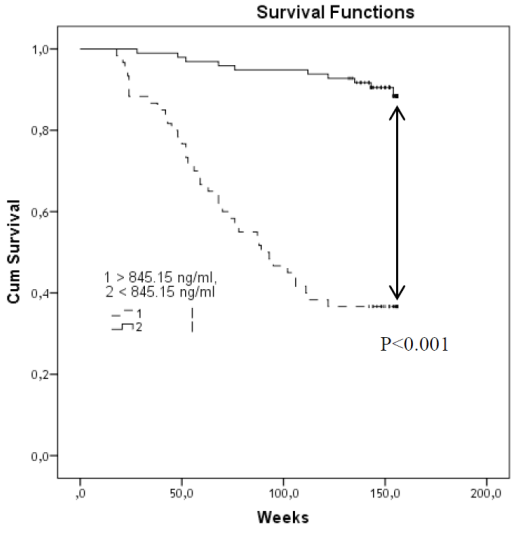


Figure 2: Results of Kaplan-Meier survival analysis: The cumulative survival in two group's patients with low (<845.15 ng/mL) and high (>845.15 ng/mL) circulating OSN.

at sites of wound repair and platelet degranulation [3]. Elevated OSN was found in the serum of patients with CHF and it predominantly reflected a positive pro-inflammatory response and alterations in protein metabolism leaded to biomechanical stress [2-4]. OSN may regulate the proliferation of some cells, especially endothelial cells; mediate by its ability to bind to cytokines and circulating growth factors [5]. As a result in excess degradation and disruption of the cardiac ECM network structure thereby OSN over expression is formation of fibrotic lesions. Because myocardial fibrosis is also a well-known cause of diastolic dysfunction and CHF, remodeling of ECM is considered a key aspect of myocardial response to biomechanical stress and advanced heart failure [13]. Recent studies have suggested that SPARC proteins, such as OSN, osteopontin and osteoprotegrin, are able presumably to play an important role in atherogenesis too [14, 15]. The animal models give arised evidences regarding osteonectin level directly correlates with increased mortality in animals with MI [16]. Probably, this effect may be related to OSN inhibition ability of mitogenesis of vascular endothelial growth factor on microvascular endothelial cells [17]. Because OSN may induce deadhesion, through loss of actin fibers and focal adhesion plaques, cell migration and cell infiltration of vulnerable zone of plaque may facilitate. Thereby OSN regulates MMP activity involved in regulating matrix rearrangement during remodeling of the vessels too [17, 18]. Both these processes are considered pivotal mechanism for triggering plaque instability as well as heart wall rupture [19]. Thus, OSN is considered a multifunctional matrix protein with powerful ability to inhibition of tissue response to injury, and, probably, mediation of low-intensity inflammation. In fact, the predictive role of OSN in cardiovascular diseases is uncertain. Taken together the all data mentioned above clarify that OSN may be considered biological marker with high predictive value for CHF evolution, especially for patients with ischemic causes of myocardial dysfunction. Currently there are not consisted data on the utility or discriminatory ability of OSN in determining of the mortality related to CHF. It is suggested that increased OSN might be powerful indicator of CHF-related events and all-cause mortality rate. We found that circulating OSN level was significantly increased among CHF patients with poor short-term prognosis. Indeed, OSN concentration independently predicted all-cause mortality, CHF-related death, and CHF-related readmissions. On the one hand, OSN is secreted by activated macrophages due to pro-inflammatory activation and leads to profound extracellular matrix reposition, exaggerated left ventricular remodeling, endothelial dysfunction, vascular calcification, and coagulation [1, 3]. On the other hand, given results of recent investigations, absence of tissue over-expression of OSN associates with increased cardiac rupture and dysfunction after acute myocardial infarction due to worsening post-synthetic procollagen processing [20, 21]. There are predispositions that these mechanisms, which are involved in reparation of heart and vessels, are under control of mineralcorticoid receptors. It has been postulated that beneficial effect of aldosterone receptor antagonist eplerenone in CHF was associated with normalization of matricellular protein expression, such as OSN [22]. Decreased OSN expression improves cardiac structural and functional parameters, delaying the progression of heart failure that was found in small animal study [22].Thus, role of OSN in cardiac outcomes is probably controversial. We have enrolled in our study patients with ischemic CHF treated with eplerenone too. At least 42.9% and 52.6% patients of the both cohorts (died persons and survivors subjects) are given mineralcorticoid receptor antagonist eplerenone. Despite similar co-administration of eplerenone with standard heart failure therapy, no significant effect of medication for survival rate of the patients with CHF was found. Probably, collagen deposition as functions in the extracellular processing that is controlled by OSN is a key protective event for acute myocardial infarction, whereas for advance CHF similar process is considered potential harm and prognostically negative [2, 3, 5]. Obviously, structural changes of the extracellular matrix are significantly modulated by OSN associates several signaling pathways, which are differed on their ability to induce repair changes on the several stages on cardiovascular continuum [23, 24]. It may a have important value for risk re-classification of the patients with ischemic CHF. We also determined that predictive value of circulating OSN was superior when compared with NT-pro-BNP alone, while combination of both biological markers was able to better prognostic discriminate between survived and died patients with ischemic CHF. Taken into consideration that a significant divergence of Kaplan-Meier survival curves constructed for patients with high (> 845.15 ng/mL) and low (<845.15 ng/mL) concentrations of OSN was found after 26 weeks of follow-up period. The convergence of Kaplan-Meier survival curves at the study end was not found. There are data about age-related increasing of OSN [15], but

we found no substantial age and gender differences of OSN among persons who died and survived. Because weak association of the echocardiographic score with NYHA class was previously determined, we advocate screening all CHF patients with circulating OSN added to conventional prognostic model tools, such as NT-pro-BNP and LVEF, and probably to assist with the optimum timing of other drugs interventions to be improving prognosis. In fact, long term prospective studies are required to provide robust evidence of the prognostic role of combination OSN and NT-pro-BNP in the associated mortality from CHF. However, some limitation of our study, such as small size, absence of randomization, and, probably, conventional treatment of CHF without biological markers-guided control, may limit real-time prognostic value of OSN leading increased statistical power. In this study, OSN level was measured at baseline after discharge of the patients from the hospital with stable coronary artery disease and without clinical signs and symptoms of acute decompensated CHF. It can introduce some limitations to the interpretation of the results of the investigation, especially around OSN level in CHF subjects independently before readmission. New studies are required for determination whether OSN may predict cardiovascular outcomes in ischemic CHF patients. Therefore, understanding the mechanisms that contribute to the cardiac remodeling may help to design new studies aimed at determination of OSN role in CHF.

CONCLUSION

We suggest that increased circulating SPARC family member OSN closely associates with increased three-year CHF-related death, all-cause mortality, and risk for readmission due to CHF.

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LIMITATIONS OF THE STUDY

This study has some limitations. We believed that a greater cohort would be desirable to improve the power of the study. Therefore, serial measurements of OSN might be desirable for determination of the biomarker level in patients at readmission and after discharge from the hospital. Dynamic of OSN may have powerful value for prediction of cardiovascular outcomes and it is able be considered a part of guided-therapy of CHF in perspective. We also relied on clinical data to rule out infection and other inflammatory diseases before sampling, but we couldn't exclude that some patients had unrecognized the conditions responsible for the elevated OSN levels observed. We supposed to mean that these limitations would not have a significant influence to study data interpretation.

CONFLICT OF INTERESTS

Not declared

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