Int Cardiovasc Res J.2015;**9**(4):203-209. doi: 10.17795/icrj-9(4)203

International Cardiovascular Research Journal
www.ircrj.com

Circulating Osteonectin as a Predictive Biomarker in Patients with Ischemic Symptomatic Chronic Heart Failure

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ARTICLE INFO

Article Type:
Research Article

Article History:
Received: 31 May 2014
Revised: 06 Jan 2015
Accepted: 22 Apr 2015

Keywords:
Osteonectin
Heart Failure
Prognosis
Survival
Hospitalization

ABSTRACT

***Background*:** Recently, some studies have revealed Osteonectin's (OSN) promising role as a marker in cardiovascular diseases.

***Objectives*:** This study aimed to evaluate the prognostic value of circulating OSN for cumulative survival and hospitalization in patients with ischemic Chronic Heart Failure (CHF).

***Patients and Methods*:** This open cohort prospective study was conducted on 154 patients with ischemic symptomatic moderate-to-severe CHF at discharge from hospital. The observation period was up to 3 years (156 weeks). Blood samples for biomarker measurements were collected at baseline. ELISA method was used for measurement of OSN circulating level. Then, Receiver Operating Characteristic (ROC) curve analysis was carried out to identify the optimal cut-off points of the OSN concentration with predicted values. Odds ratios were also calculated for all the independent predictors of patients' survival. Kaplan-Meier survival curves were also structured for both cohorts with low and high OSN levels.

***Results*:** During a median follow-up of 2.18 years, 21 participants died and 106 subjects were hospitalized repetitively. The median of circulating OSN levels 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) in the survived and dead patients cohorts, respectively. Besides, ROC curve analysis showed that optimal cut-off point of OSN for cumulative survival function was 845.15 ng/mL. The results also revealed significant divergence of Kaplan-Meier survival curves in the patients with high (> 845.15 ng/mL) and low (< 845.15 ng/mL) concentrations of OSN.

***Conclusions*:** Increased circulating OSN levels were associated with increased 3-year CHF-related death, all-cause mortality, and risk of recurrent hospitalization due to CHF.

►*Implication for health policy/practice/research/medical education:*
This is the first pilot study evaluating the prognostic role of osteonectin in CHF patients with an ischemic etiology. This study provides original and significant preliminary information in the crowded field of CHF-related biomarkers.

1. Background

Extra Cellular Matrix (ECM) proteins, such as Secreted Protein Acidic and Rich in Cysteine (SPARC), play a key role in post-synthetic procollagen processing in heart failure myocardium and regulate cell adhesion, growth factor activity, and cell cycle (1). It has been found that Osteonectin (OSN), a SPARC family member, causes myocardial hypertrophy, increases fibrillar collagen content, stimulates cell signaling, adhesion, survival, proliferation, and migration in several cell types, and mediates calcification of the vascular wall, coagulation, and endothelial dysfunction (2).

Moreover, OSN increases collagen deposition in response

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to Myocardial Infarction (MI) or can impair heart function in some types of cardiac hypertrophy (3). Recent animal studies have revealed that increased circulating OSN level was associated with higher incidence of mortality following MI due to increased rates of rupture and new heart failure over the first 14 days after MI that is accompanied by left ventricular dysfunction and increased mortality in short- and long-run (4, 5). However, the role of OSN in ischemic Chronic Heart Failure (CHF) has not been defined yet.

2. Objectives

The present study aims to evaluate the prognostic value of circulating OSN for survival and hospitalization in patients with ischemic CHF.

3. Patients and Methods

This prospective study was conducted on 154 patients (86 males and 68 females) aged 48 to 62 years suffering from ischemic symptomatic CHF with New York Heart Association (NYHA) class II-IV. CHF was diagnosed according to the current European Society of Cardiology clinical guidelines (6). All the patients signed written informed consents for participation in the study. The exclusion criteria of the study were Q-wave and non-Q-wave MI within 3 months before entering the study, severe kidney and liver diseases that might affect the clinical outcomes, malignancy, creatinine plasma level > 440 μmol/L, estimated Glomerular Filtration Rate (GFR) < 35 mL/min/м2, brain injury within 3 months before enrollment, Body Mass Index (BMI) > 30 kg/m2, acute pulmonary edema, implanted pacemaker, valvular heart disease, ischemic stroke, intracranial hemorrhage, thyrotoxicosis, acute infections, surgery, trauma, neoplasm, and pregnancy. The observation period was up to 3 years (156 weeks). We also analyzed cumulative survival related to CHF and all-cause mortality.

3.1. Methods for Visualization of Coronary Arteries

Multispiral computed tomography angiography was performed prior to the study when no ischemic signs of or previously documented old MI were detected at baseline, but CHF was present. In addition, conventional angiographic examination was provided when atherosclerotic lesions were determined. Coronary Artery Disease (CAD) was considered to be diagnosed upon availability of previous angiographic examinations or MI. All angiography procedures were performed prior to the study. The structure of coronary artery wall was examined by contrast spiral computed tomography angiography (7) on Somatom Volume 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.

3.2. Assessment of Hemodynamics

Transthoracic B-mode echocardiography and Tissue Doppler Imaging (TDI) were performed using ACUSON ultrasound scanner (SIEMENS, Germany). Additionally, left ventricular end-diastolic and end-systolic volumes were measured by modified Simpson's method (8). TDI was carried out in 4-3-, and 2-chamber views in each of the 16 segments of the left ventricle (9). Peak E-wave velocity and TDI-derived velocities in systole and diastole were measured, as well.

3.3. Calculation of Glomerular Filtration Rate

GFR was calculated using MDRD-6 formula (10).

3.4. Measurement of Biomarkers

Blood samples were collected in cooled silicone test tubes at baseline in the morning (at 7 - 8 A.M.) when the patients were discharged from the hospital with stable clinical status. The samples were processed according to the recommendations of the manufacturer of the used analytical technique. They were then centrifuged upon permanent cooling at 6000 rpm for 3 minutes. After that, the plasma samples were stored at ≤ -200 °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 also measured by immune electro chemoluminescent assay using kits by R&D Systems (USA) on Elecsys 1010 analyzer (Roche, Mannheim, Germany). In addition, the concentrations of Total Cholesterol (TC) and High-Density Lipoprotein-Cholesterol (HDL-C) were measured by enzymatic method. The concentration of Low-Density Lipoprotein-Cholesterol (LDL-C) was also calculated according to Friedewald formula (1972).

3.5. Ethical Principles

The investigators strictly followed all the requirements for clinical trials in conformity to 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, and Convention on Human Rights and Biomedicine, including Additional Protocol to the Convention on Human Rights and Biomedicine concerning Biomedical Research and legislation of Ukraine.

3.6. Statistical Analysis

The study data were statistically analyzed using the SPSS statistical software 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. In this non-randomized prospective open cohort study, the sample size was computed using power sample size calculator (11). Our sample size (17 cases in the dead cohort and 89 cases in the survived cohort) allowed us to detect a large effect size (> 0.80) for systemic inflammatory marker, OSN, with a power of 80% and a two-sided type I error of 5%. Normal distribution of the data was checked by means of Shapiro-Wilk and Kolmogorov-Smirnov tests. Accordingly, circulating OSN and NT-pro-BNP levels did not follow normal distribution, while distribution of TC and cholesterol fractions was normal and was not subjected to any mathematical transformation. In order to compare the main parameters of the patients group, two-tailed Student

t-test or Mann Whitney test was used. Besides, chi-square test or Fisher's exact test was employed to compare categorical variables between the study groups. Receiver Operating Characteristic (ROC) curve analysis was carried out to identify the optimal cutoff points of the OSN concentration with predicted values. The difference between the areas under the ROC curves was calculated using the method proposed by De Long et al. (1988) (12) and reclassification measures, such as Index Discrimination Improvement (IDI) (13). Odds Ratios (ORs) and 95% CI were also calculated for all the independent predictors of patients' survival. $P < 0.05$ was considered as statistically significant.

4. Results

4.1. Clinical Event Determination

During a median follow-up of 2.18 years, 21 participants died and CHF-related death was detected in 18 patients. Additionally, there were 106 cases of re-admission in the hospital due to advanced CHF in both cohorts (17 cases in the dead patients cohort and 89 cases in the survived patients cohort).

4.2. General Characteristics of the Study Population

The results showed no significant age and gender differences between the two cohorts (Table 1). Also, no significant differences were found between the two cohorts regarding BMI, GFR, HbA1c, fasting blood glucose level, blood creatinine level, TC, LDL-C, HDL-C, and number of damaged coronary vessels. The patients in both cohorts were also similar with respect to systemic office Blood Pressure (BP) and Heart Rate (HR). Besides, no significant differences were observed between the cohorts concerning

Table 1. General Characteristics of the Study Patients Survived Subjects

Variables	All Patients (N = 154)	Dead Subjects (N = 21)	Survived Subjects (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)
Hyper lipidemia, 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)
NYHA class II	41(26.6%)	6(28.6)	35(26.3)
NYHA class III	74(48.1)	9(42.8)	65(48.9)
NYHA class IV	39(25.3)	6(28.6)	33(24.8)
BMI, kg/m2	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/1.73m2	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, mmHg	131 ± 6	129 ± 4	135 ± 5
Diastolic BP, mmHg	77 ± 4	77 ± 5	78 ± 5
Heart rate, beats per 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)
Acetylsalicylicacid, n (%)	130(84.4)	19(90.5)	121(91.0)
Other antiplatelet drugs, 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)
Mineral ocorticoid receptor antagonist eplerenone, n (%)	79(51.3)	9(42.9)	70(52.6)

Abbreviations: 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

* Statistically significant differences between the two groups ($P < 0.05$)

comorbidities and traditional cardiovascular risk factors. Type 2 Diabetes Mellitus (T2DM) was identified in 38.1% of the dead cases and 33.8% of the survived ones (P = 0.06). However, no statistically significant difference was found between the two cohorts regarding E/Am and E/Em. Nevertheless, two-dimensional echocardiography revealed a significant difference between the dead and survived cohorts with regard to Left Ventricular Ejection Fraction (LVEF). At the same time, the level of circulating NT-pro-BNP was significantly higher in the dead patients compared to the survived ones. Yet, no significant differences were found between the two cohorts regarding concomitant medications.

4.3. Circulating OSN Levels in the Survived and Dead Patients

The median level of circulating OSN was 670.96 ng/mL (95% CI = 636.53 - 705.35 ng/mL) and 907.84 ng/mL (95% CI = 878.02 - 937.60 ng/mL) in the survived and dead patients, respectively (P < 0.001).

4.4. The Predictive Value of OSN Concentration in the Study Population

Multivariate logistic regression analysis was used to assess whether any combination of assays was able to better discriminate between survived and dead patients. According to the results, the main factors independently related to cumulative mortality and CHF-related readmission were some biomarkers (OSN alone and NT-pro-BNP alone), LVEF, T2DM, and three- and multi-vessel lesions. 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 CHF-related readmission (OR = 1.92; 95% CI: 1.77 – 2.45; P < 0.001) within the 3-year observation period (Table 2). However, NT-pro-BNP and LVEF remained significant independent predictors of all-cause mortality, CHF-related death, and CHF-related readmission. On stepwise model selection method, OSN alone (Model 1) and OSN in combination with NT-pro-BNP (Model 2) remained significant independent predictors of all-cause mortality (B-coefficient = 1.14, P = 0.001 and B-coefficient = 1.04, 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 readmission (B-coefficient = 2.06, P = 0.003 and B-coefficient = 2.11,P = 0.004, respectively). On the contrary, OSN in combination with both NT-pro- BNP and LVEF (Model 4) did not predict all-cause mortality (B-coefficient = 0.014, P = 0.543), CHF-related death (B-coefficient = 0.016, P = 0.528), and CHF-related readmission (B-coefficient = 0.012, P = 0.448). On the other hand, CHF-related readmission (B-coefficient = 1.88, P = 0.001) was predicted by circulating NT-pro-BNP alone (Model 3). Nonetheless, all-cause mortality (B-coefficient = 0.025, P = 0.68) and CHF-related death (B-coefficient = 0.036, P = 0.62) were not predicted by NT-pro-BNP alone.

The results of 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 did not offer any additional information to discriminate between survived and dead patients with CHF (B-coefficients = 0.012, 0.067, and 0.023, respectively; P values = 0.277, 0.300, and 0.522, respectively).

According to ROC curve analysis, the cut-off point of OSN concentration for cumulative survival function was 845.15 ng/mL (sensitivity = 79.2% and specificity = 84.4%) (Figure 1). Besides, the area under the curve was 0.918 (SE = 0.022; 95% CI: 0.876 - 0.961). Derived from ROC curve analysis, 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 the above-mentioned cut-off points

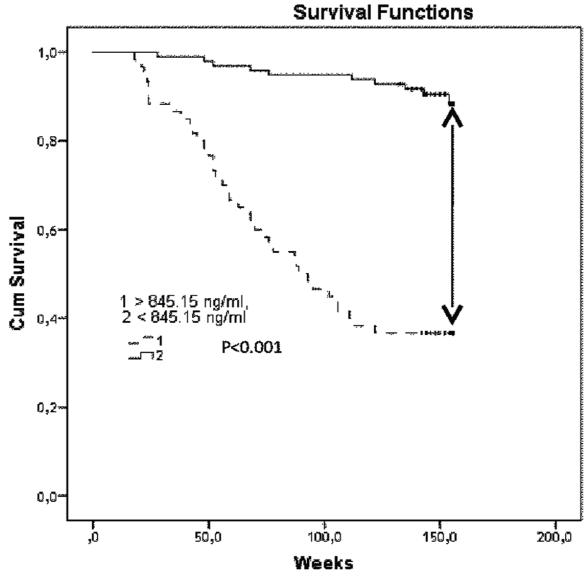


Figure 1. The Results of ROC Curve Analysis. The Graphical Plot Illustrates Discriminations for OSN Alone, NT-pro-BNP Alone, and the Combination of Both Biological Markers for Cumulative Survival in CHF Patients.

Table 2. Independent Variables Related to 3-Year All-Cause Mortality, CHF-Related Death, and CHF-Related Re-hospitalization Obtained by Logistic Regression Analysis

Variables	All-Cause Mortality			CHF-Related Death			CHF-Related Re-Hospitalization		
	OR	95% CI	P value	OR	95% CI	P value	OR	95% CI	P value
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	1.12 – 1.18	0.014	1.22	1.07 – 1.45	0.016	0.001	1.15
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

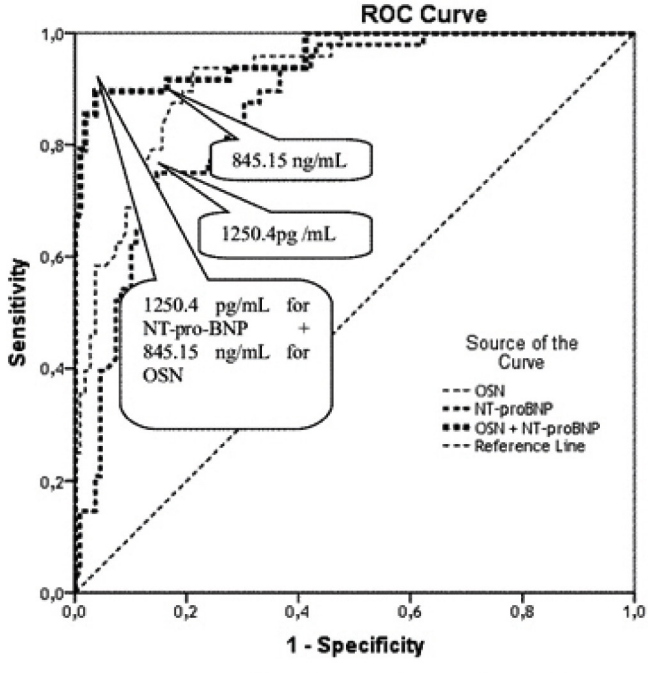
Abbreviations: OR, odds ratio; CI, confidence interval; LVEF, left ventricular ejection fraction; BNP, brain natriuretic peptide; T2DM, type two diabetes mellitus

for OSN and NT-pro BNP, we found similar discrimination values for both biomarkers. Combination of both biomarkers increased sensitivity to 88.6% and specificity to 92.4%. Model discrimination was excellent for OSN alone, NT-pro-BNP alone, and combination of both. DeLong method and categorical reclassification measures of IDI were used for determination of differences between the ROC curves. The results of DeLong method indicated a significant difference between the areas under the curves that was suitable for OSN alone, NT-pro-BNP alone, and their combination ($P < 0.001$ for all cases). Then, integrated discrimination improvement after the inclusion of NT-pro-BNP in to the model was calculated. Accordingly, inclusion of NT-pro-BNP did not significantly improve model discrimination based on OSN alone (AU: 0.61 vs. 0.918, $P = 0.22$; IDI = 0.035, $P = 0.42$). The discrimination value for OSN alone was superior to NT-pro-BNP alone (IDI = 0.04; $P < 0.01$).

Moreover, the results demonstrated a significant divergence of Kaplan-Meier survival curves in the patients with high (> 845.15 ng/mL) and low (< 845.15 ng/mL) concentrations of OSN (Figure 2). The curves' divergence of events accumulation reached statistical significance in the 26th week of the observation period ($P < 0.001$).

5. Discussion

It is well known that ECM proteins may modulate cell–matrix interactions and cell functions, do not have a direct structural role, and mediate left ventricular remodeling. Several members of the ECM proteins family, such as OSN, are up-regulated in CHF. OSN (also known as SPARC) is synthesized by wide spectrum cells, such as osteoblasts, fibroblasts, and activated macrophages, at sites of wound repair and platelet degranulation (3). It has been found that the sera of patients with CHF predominantly reflected a positive pro-inflammatory response and alterations in protein metabolism, leading to biomechanical stress (2, 3, 5). OSN also regulates the proliferation of some cells, especially endothelial cells, mediated by its ability to bind to cytokines and growth factors (4). As a result, in excessive degradation and disruption of the cardiac ECM network structure, OSN over expression might mediate fibrotic lesions formation. Because myocardial fibrosis is also a well-known cause of diastolic dysfunction and CHF, remodeling of ECM is considered as a key aspect of myocardial response to biomechanical stress and advanced heart failure (14). Recent studies have suggested that SPARCs, such as OSN, osteopontin, and osteoprotegrin,



Diagonal segments are produced by ties.

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 2. The Results of Kaplan-Meier Survival Analysis. The Cumulative Survival in Two Groups of Patients with Low (< 845.15 ng/mL) and High (> 845.15 ng/mL) Circulating OSN Levels.

can presumably play an important role in CHF, MI, and atherogenesis (15-17). Animal models have also provided evidence that OSN levels were directly correlated to increased mortality post-MI due to increased rupture rate (17). This effect might be associated with the fact that OSN may inhibit mitogenesis of vascular endothelial growth factor on microvascular endothelial cells (18, 19). Because OSN may induce adhesion, it may facilitate cell migration and cell infiltration of plaque's vulnerable zone through loss of actin fibers and focal adhesion plaques. Therefore, OSN also regulates Matrix Metalloproteinase (MMP) activity involved in regulating matrix rearrangement during remodeling of the vessels (18, 19). Both of these processes are considered pivotal mechanisms for triggering plaque instability as well as heart wall rupture (20). Thus, OSN is a multifunctional ECM protein with powerful ability to inhibit tissue response to injury and, probably, mediate low-intensity inflammation. Yet, the predictive role of OSN in cardiovascular diseases is uncertain. Taken together, what was mentioned above clarified that OSN may be considered as a biological marker with high predictive value for CHF evolution, especially for patients with ischemic causes of myocardial dysfunction. Currently, data regarding the role of OSN in determining CHF mortality are not available. It has been proposed that increased OSN concentrations would be a powerful indicator of not only CHF-related events, but also all-cause mortality. The findings of the current study revealed that circulating OSN levels were increased in the CHF patients with poor short-term prognosis. Indeed, OSN concentration independently predicted all-cause mortality, CHF-related death, and CHF-related readmission. On one hand, OSN is secreted by activated macrophages due to pro-inflammatory activation and leads to profound ECM reposition, exaggerated left ventricular remodeling, endothelial dysfunction, vascular calcification, and procoagulation (1, 3). On the other hand, given the results of the recent investigations, absence of OSN tissue over-expression is associated with increased cardiac rupture and dysfunction after acute myocardial infarction due to worsening post-synthetic procollagen processing (5, 21). It has been suggested that the mechanisms, which are involved in reparation processes of heart and vessels, are under control of mineralocorticoid receptors. It has also been postulated that the beneficial effects of aldosterone receptor antagonist eplerenone on CHF are associated with normalization of expression of ECM proteins, such as OSN (22). The results of an animal study disclosed that decreased expression of OSN improved cardiac structural and functional parameters, delaying the progression of heart failure (22). Thus, the role of OSN in cardiac outcomes is probably controversial.

The CHF patients treated with eplerenone were also enrolled into the present study. Based on the results, 42.9% of the dead patients and 52.6% of the survived ones were given mineralocorticoid receptor antagonist, eplerenone. Despite similar co-administration of eplerenone with standard heart failure therapy, the medication had no significant effects on the CHF patients' survival rate. OSN might control extracellular collagen deposition and demonstrate protective effects on acute MI. However, the predictive role of OSN in CHF has not been determined yet (3-5). Obviously, structural changes of ECM are significantly modulated by several OSN-associated signaling pathways, which are different in their ability to induce repair changes in the cardiovascular continuum (23, 24). This may be of great value for risk reclassification of patients with CHF. The present study findings also determined that predictive value of circulating OSN level was superior compared to NT-pro-BNP alone, but the combination of both biological markers was able to better discriminate between the survived and dead patients with ischemic CHF. Furthermore, a significant divergence of Kaplan-Meier survival curves was observed in the patients with high (> 845.15 ng/mL) and low (< 845.15 ng/mL) concentrations of OSN in the 26th week of the observation period. Additionally, convergence of Kaplan-Meier survival curves was not found at the end of the study. There exists evidence about age-related increase of OSN (16), but our study showed no age and gender differences between the dead and survived patients regarding OSN levels. Because previous studies reported a weak association between echocardiographic score and NYHA class, we believe that circulating OSN level added to conventional prognostic models, such as NT-pro-BNP and LVEF, might assist optimum timing of other drug interventions to improve prognosis. Yet, long-term prospective studies are required to provide robust evidence of the prognostic role of combination of OSN and NT-pro-BNP in CHF-associated mortality. Some limitations of our study, such as small sample size, absence of randomization, and conventional treatment of CHF without biological markers-guided control, might have limited real-time prognostic value of OSN. In this study, OSN level was measured at baseline after the patients' discharge from the hospital with stable coronary artery disease and without clinical signs and symptoms of acute decompensated CHF. This might have introduced some limitations to interpretation of the results of the investigation, especially about OSN level in CHF subjects before readmission. Hence, future studies are required to determine whether OSN may predict cardiovascular outcomes in ischemic CHF patients. Understanding the mechanisms that contribute to cardiac remodeling may help design new studies aiming at determination of the role of OSN in CHF.

In conclusion, increased circulating level of OSN was closely associated with increased 3-year CHF-related death, all-cause mortality, and risk of recurrent hospitalization due to CHF. Using the combination of OSN and NT-pro-BNP showed the best-balanced sensitivity and specificity for predicting mortality and hospital readmission in the ischemic CHF subjects.

Acknowledgements

The authors would like to thank all the patients for their participation in the investigation, staff of the Regional Zaporozhye Hospital (Ukraine), doctors, nurses, and administrative staff in City hospital #6 (Zaporozhye, Ukraine), general practices, and site-managed organizations that assisted with the study.

Authors' Contribution

All the authors had equal role in the study design, collection, analysis, and interpretation of data, writing of the manuscript, and the decision to submit the manuscript for publication.

Financial disclosure

This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

Funding/Support

There is no funding/support.

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