UDC 616.12-008.64-02:616.127-005.4-085

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Circulating osteonectin as a prognostic biological marker in patients with ischemic chronic heart failure

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Key words: *Osteonectin, Chronic Disease, Heart Failure, Prognosis, Survival, Hospitalization.*

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

Methods. A total of 154 patients with ischemic symptomatic moderate-to-severe CHF were enrolled in the study. Observation period was up to 3 years. ELISA methods for measurements of circulating level of osteonectin were used. Concentrations of osteonectin for cumulative survival cases due to advanced CHF were tested. Additionally, all-cause mortality, and CHF-related death were examined.

Conclusion. Increased circulating osteonectin associates with increased 3-year CHF-related death, and risk for hospitalization due to CHF.

Циркулюючий остеонектин як прогностичний біологічний маркер у пацієнтів з ішемічною серцевою недостатністю

О. О. Кремзер

З метою оцінювання прогностичного значення циркулюючого остеонектину щодо кумулятивної виживаності пацієнтів із хронічною серцевою недостатністю ішемічного генезу обстежили 154 пацієнтів із симптомами від помірної до важкої хронічної серцевої недостатності. Період спостереження становив до 3 років. Для вимірювання рівня циркулюючого остеонектину використовували методи імуноферментного аналізу. Досліджували вплив концентрацій остеонектину на загальну виживаність пацієнтів із хронічною серцевою недостатністю, смертність від усіх причин. Виявили, що збільшення концентрацій циркулюючого остеонектину в пацієнтів із хронічною серцевою недостатністю пов'язане зі збільшенням госпіталізацій і ризику трирічної смертності.

Ключові слова: *остеонектин, хронічні захворювання, серцева недостатність, прогноз, виживання, госпіталізація.*

Актуальні питання фармацевтичної і медичної науки та практики. – 2014. – № 2 (15). – С. 60–63

Циркулирующий остеонектин как прогностический биологический маркер у пациентов с хронической сердечной недостаточностью

А. А. Кремзер

С целью оценки прогностического значения циркулирующего остеонектина относительно выживания пациентов с хронической сердечной недостаточностью ишемического генеза обследовали 154 пациента с симптомами от умеренной до тяжелой хронической недостаточности. Период наблюдения составил 3 года. Для измерения уровня циркулирующего остеонектина использовали методы иммуноферментного анализа. Исследовали влияние концентраций остеонектина на общую выживаемость пациентов с хронической сердечной недостаточностью, смертность от всех причин. Установили, что увеличение концентраций циркулирующего остеонектина у пациентов с хронической сердечной недостаточностью связано с увеличением частоты госпитализаций и риска трехлетней смертности.

Ключевые слова: *остеонектин, хронические заболевания, сердечная недостаточность, прогноз, выживаемость, госпитализация.*

Актуальные вопросы фармацевтической и медицинской науки и практики. – 2014. – № 2 (15). – С. 60–63

Secreted acidic and rich in cysteine (SPARC) proteins play a pivotal key role in post-synthetic procollagen processing in heart failure myocardium and regulate cell adhesion, growth factor activity and cell cycle [4]. It has been found that SPARC family member osteonectin (OSN) causes myocardial hypertrophy, increased fibrillar collagen content, stimulates cell signaling, adhesion, survival, proliferation, and migration in several cell types [9]. OSN increases collagen deposition in response to myocardial infarction or in some types of cardiac hypertrophy can impair heart function [8]. Recent animal studies have been revealed that increased circulating OSN associates with higher incidence of mortality following myocardial infarction (MI), due to increased rates of rupture and newly heart failure over the first 14 days after MI that associate with left ventricular dysfunction and increased mortality [9, 10]. However, the roles of OSN in the ischemic chronic heart failure have not been defined.

The objective of this study was to evaluate the prognostic value of circulating OSN for survival in patients with ischemic chronic heart failure.

Methods

The study evolved 154 patients (86 males) aged 48 to 62 years with ischemic symptomatic moderate-to-severe CHF. All the patients have given their written informed consent for participation in the study. Observation period was up to 3 years. We analyzed cumulative survival related to CHF, and additionally all-cause mortality was examined.

Multispiral computed tomography angiography and/or angiographic study have been carried out to verify the ischemic nature of the disease in patients. Multispiral computed tomography/angiography has been carried out

for all the patients prior to their inclusion in the study. Transthoracic ultrasonic echocardiography was performed according to a conventional procedure on ACUSON scanner, SIEMENS, Germany, in B-mode regimen and tissue Calculation of glomerular filtration rate (GFR) was carried out using MDRD-6 formula [7].

Circulating OSN level was determined by (Bender MedSystems GmbH, Vienna, Austria). NT-pro-BNP concentration was measured by ELISA method using kits by R&D Systems (USA). Concentrations of total cholesterol (TC) and cholesterol of high-density lipoproteins (HDLP) were measured by fermentation method. Concentration of cholesterol of low-density lipoproteins (LDL-C) was calculated according to the Friedewald formula (1972).

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

Results

During a median follow-up of 2.18 years, 21 participants died and CHF-related death was defined in 18 patients. Additionally, 106 subjects were hospitalized repetitively due to advance CHF (17 cases in died cohort and 89 cases in survival cohort). *Table 1* shows a general characteristic

Table 1

General characteristic of patients participating in the study

Variables	Died subjects (n=21)	Survived subjects (n=133)
Age, years	57.20±6.70	59.50±7.30
Males, n (%)	12 (57.1%)	67 (50.3%)
Arterial hypertension, n (%)	12 (57.1%)	61 (45.9%)
Hyperlipidemia, n (%)	9 (42.8%)	52 (39.1%)
T2DM, n (%)	8 (38.1%)	45 (33.8%)
Adherence to smoking, n (%)	7 (33.3%)	24 (29.3%)
II Class NYHA	6 (28.6%)	35 (26.3%)
III Class NYHA	9 (42.8%)	65 (48.9%)
IV Class NYHA	6 (28.6%)	33 (24.8%)
BMI, kg/m^2	23.7 (95% CI=22.5–27.3)	24.2 (95% CI=22.0–27.9)
GFR, mL/min/1.73 m^2	82.1 (95% CI=69.9–93.1)	85.2 (95% CI=70.3–112.5)
HbA1c, %	6.3 (95% CI=4.4-9.0)	7.0 (95% CI=4.3-9.2)
Fasting blood glucose, mmol/L	4.80 (95% CI=3.6-8.5)	5.40 (95% CI=3.4-9.1)
Creatinine, μmol/L	70.5 (95% CI=59.6–88.3)	74.9 (95% CI=65.1–90.3)
Total cholesterol, mmol/L	5.3 (95% CI=4.6-6.0)	5.0 (95% CI=4.2-5.8)
LDL-C, mmol/L	3.60 (95% CI =3.20–4.18)	3.02 (95% CI=2.80–3.90)
HDL-C, mmol/L	0.94 (95% CI = 0.92–1.06)	0.88 (95% CI = 0.82–0.97)
NT-pro-BNP, pg /mL	1533.6 (95% CI 644.5 – 2560.6)	1031.2 (95% CI 704.8 – 1560.7)*
Systolic BP, mm Hg	129±4	135±5
Diastolic BP, mm Hg	77±5	78±5
Heart rate, beats per 1 min.	76±6	68±3
LVEF, %	42.80±0.76	55.40±0,80*
E/Am, U	16.6±0.94	16.5±1.20
E/Em, U	16.6±1.00	16.6±0.84
One-vessel lesion of CA, n (%)	5 (23.8%)	24 (18.0%)
Two-vessel lesion of CA, n (%)	8 (38.1%)	54 (40.1%)
Three- and multi-vessel lesion of CA, n (%)	8 (38.1%)	55 (41.4%)
ACEI / ARAs, n (%)	21 (100%)	133 (100%)
Acetylsalicylic acid, n (%)	19 (90.5%)	121 (91.0%)
Other antiaggregants, n (%)	2 (9.5%)	12 (9.0%)
Statins, n (%)	14 (66.7%)	80 (60.2%)
Metformin, n (%)	8 (38.1%)	45 (33.8%)
Diuretics, n (%)	18 (85.7%)	121 (91.0%)
Mineralcorticoid receptors antagonists, n(%)	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.

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, as well as differences 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. No difference was found between the two cohorts in systemic office blood pressure (BP) and heart rate (HR). Documented incidence of type 2 diabetes mellitus in patients of the two cohorts was 38.1% and 33.8% (P=0.06). Note that there was not a statistically significant change in E/Am and E/Em between the two cohorts, while decrease in the left ventricular ejection fraction value was quite anticipated in the setting in died patients. At the same time, the level of circulating NT-pro-BNP was statistically significantly higher in died patients than in survived persons. When analyzing details of pharmacotherapy, no substantial differences were found between the two cohorts with regard to administration of the majority of drugs.

Medians of circulating levels of OSN in survived and died patient cohort 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). The data suggested that OSN plasma levels were directly related to NT-pro-BNP (r = 0.648, P = 0.001), NYHA functional class of CHF (r = 0.492, P = 0.006), T2DM (r = 0.40, P = 0.006), gender (r = 0.375, P < 0.001 for male), multi-vessel lesion of coronary arteries (r = 0.362, P = 0.001), E/Am (r = 0.368, P = 0.001), E/Em (r = 0.364, P = 0.001), TC (r = 0.35, P = 0.001), age (r = 0.278, P = 0.001), smoking (r = 0.275, P = 0.001) and inversely to LVEF (r = -0.566, P = 0.001). No significant association between the levels of circulating OSN with creatinine plasma level, fasting plasma glucose, HbA1c, mean systolic and diastolic BP, premature CAD in family anamnesis, and medications for both cohorts of the patients was found.

The optimum cut-off point for OSN is determined by the relative importance of the sensitivity and specificity of the test. ROC (Receive Operation Characteristic) analysis has been shown that cut-off point of OSN concentration for cumulative survival function was 845.15 ng/mL. Area under curve was 0.918 (Std. error = 0.022; 95% CI = 0.876-0.961), sensitivity and specificity were 79.2% and 84.4% respectively. Using ROC analysis results we have been performed an assay in relationship between cumulative survivals in two patient cohorts depending on circulating OSN levels. It has been found a significantly divergence of Kaplan-Meier survival curves in patients with high (> 845.15 ng/mL) and low (<845.15 ng/mL) concentrations of OSN *(Figure 1)*. The curves divergence of events accumulation reached a statistical significance in 26 weeks of observation period (P<0.001).

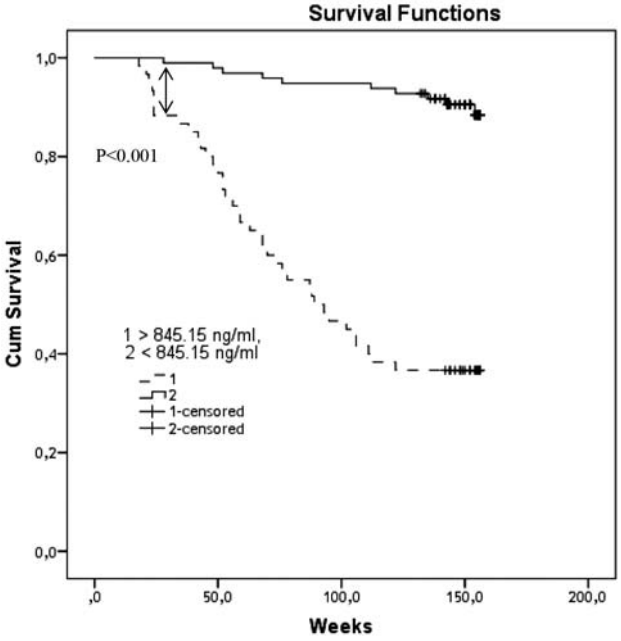

Figure 1. Results of Kaplan-Meier survival analysis: The cumulative survival in two groups patients with low (<845.15 ng/mL) and high (>845.15 ng/mL) circulating OSN.

Multivariate logistic regression was used to assess whether any combination of assays was able to better discriminate between survival and died patients. In the logistic regression analysis, the main factors independently related with cumulative mortality and CHF-related rehospitalisations were OSN, NT-pro-BNP, 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 rehospitalisation (OR = 1.92; 95% CI = 1.77 – 2.45; P<0.001) within 3 years of observation period *(table 2)*. Using a stepwise model selection method

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.

for multivariable prediction model we have been investigated the summary effect of any combinations of OSN, NT-pro-BNP, LVEF on all-cause mortality, CHF-related death, and CHF-related re-hospitalisations. We found that OSN (Model 1) and combination of OSN with NT-pro-BNP (Model 2) remained statistically significant 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 re-hospitalisations (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 3) 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. Using the ROC analysis we provided selection of possibly the most optimal predictive model based on OSN alone, NT-pro-BNP alone and its combination. As one can see, substantial difference between areas under curves, that are suitable for OSN alone, NT-pro-BNP alone and its combination were found (P<0.001 for all cases). However, sensitivity and specificity for OSN alone and OSN + NT-pro-BNP were similar (79.2% and 84.4% respectively for OSN, 79.3% and 85.1% respectively for OSN + NT-pro-BNP), whether for NT-pro-BNP alone was significantly low (74.9% and 76.1% respectively for NT-pro-BNP). Thus, reliability of the estimated models was high enough.

Discussion

SPARC in the serum of patients with CHF predominantly reflected a positive pro-inflammatory response and alterations in protein metabolism that leads to biomechanical stress [1,8]. This results in excess degradation and disruption of the cardiac extracellular matrix (ECM) network structure or accumulation of ECM proteins and formation of fibrotic lesions. 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 [6]. Recent studies have been suggested that SPARC, such as OSN, osteopontin and osteoprotegrin, presumably can play an important role in not only CHF, but in atherogenesis also [3,5]. Additionally, we currently lack data on the utility or discriminatory ability of OSN in determining the mortality from CHF. It is predisposed that increased OSN concentration would be powerful indicator of not only CHF-related events, but all-cause mortality. It was found that circulating SPARC member OSN level was really increased in CHF patient with poor short-term prognosis. Indeed, OSN concentration closely independently predicted all-cause mortality, CHF-related death, and CHF-related rehospitalisations. It has also determined that predictive value of circulating OSN was superior when compared with NT-pro-BNP, while combination of both biological markers was able to better prognostic discriminate between survival and died patients with CHF. Taken into consideration that a significant divergence of Kaplan-Meier survival curves in patients with high and low concentrations of OSN in 26 weeks of observation period was found. There exists data about age-related increasing of OSN [5], but no substantial age and gender differences of OSN among persons who died and survived was found. Because weak association of the echocardiographic score with NYHA class was previously determined, it has advocated screening all CHF patients with circulating OSN added to conventional prognostic model tools, such as NT-pro-BNP and LVEF, and probably also 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.

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Надійшла в редакцію 14.05.2014 р.