

Original Article

The utility of biomarker risk prediction score in patients with chronic heart failure

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Abstract: Chronic heart failure (CHF) remains a leading cause of cardiovascular death worldwide. Current risk models allow better prognosis, however further tools for assessing risk are needed. Thus, this study was aimed to evaluate whether biomarker risk prediction score is powerful tool for risk assessment of three-year fatal and non-fatal cardiovascular events in CHF patients. Methods: A prospective study on the incidence of fatal and non-fatal cardiovascular events, as well as the frequency of occurrence of death from any cause in a cohort of 388 patients with CHF during 3 years of observation was performed. Circulating levels of NT-pro brain natriuretic peptide (NT-pro-BNP), galectin-3, high-sensitivity C-reactive protein (hs-CRP), osteoprotegerin and its soluble receptor sRANKL, osteopontin, osteonectin, adiponectin, endothelial apoptotic microparticles (EMPs) and mononuclear progenitor cells (MPCs) were measured at baseline. Results: Median follow-up of patients included in the study was 2.76 years. There were 285 cardiovascular events determined, including 43 deaths and 242 readmissions. Independent predictors of clinical outcomes in patients with CHF were NT-pro-BNP, galectin-3, hs-CRP, osteoprotegerin, CD31⁺/annexin V⁺ EMPs and EMPs/CD14⁺CD309⁺ MPCs ratio. Index of cardiovascular risk was calculated by mathematical summation of all ranks of independent predictors, which occurred in the patients included in the study. The findings showed that the average value of the index of cardiovascular risk in patients with CHF was 3.17 points (95% CI = 1.65-5.10 points). Kaplan-Meier analysis showed that patients with CHF and the magnitude of the risk of less than 4 units have an advantage in survival when compared with patients for whom obtained higher values of ranks cardiovascular risk score. Conclusion: Biomarker risk score for cumulative cardiovascular events, constructed by measurement of circulating NT-pro-BNP, galectin-3, hs-CRP, osteoprotegerin, CD31⁺/annexin V⁺ EMPs and EMPs/CD14⁺CD309⁺ MPCs ratio, reliably predicts the probability survival of patients with CHF, regardless of age, gender, state of the contractile function of the left ventricle and the number of comorbidities.

Keywords: Chronic heart failure, biomarkers, cardiovascular outcomes, predictive value

Introduction

Chronic heart failure (CHF) remains a leading cause of cardiovascular death worldwide [1]. As expected, a significant improvement in survival has occurred for patients with CHF, with an increasing array of therapeutic options sharing quite varied properties of cost, invasiveness, and impact on life expectancy [2, 3]. Contemporary risk models allow patients and physicians to achieve a better understanding of

prognosis than is possible through unstructured holistic assessment [4]. Recent clinical studies have shown that short-term and long-term prognosis among heart failure persons may be reappraised and recalculated using biological marker models demonstrated to be credible in clinical practice and useful predictable tool for physicians [5-7]. Natriuretic peptides, galectin-3 (Gal-3), high sensitive C-reactive protein (hs-CRP) were positively associated with all-cause and cardiovascular

mortality and are useful for estimating prognosis in persons with chronic stable heart failure [8-12]. A wide spectrum of biomarkers reflecting immune status, proinflammatory activation and endothelial function, was tested in predictive models for CHF patients [13-17], yet no ideal biomarkers with optimal decremented potency was found that lead to prompting of use a multi marker approach in risk modelling for heart failure persons. Although several multivariate risk scores have shown significant utility in predicting patient outcomes in acute and acutely decompensated heart failure, contemporary models, such as Seattle Heart Failure Model, substantially underestimated the absolute risk of death in ambulatory CHF patients [18].

In this regard, this study was aimed at evaluating whether biomarker risk prediction score is powerful tool for risk assessment of three-year fatal and non-fatal cardiovascular events in CHF patients.

Methods

Study population

The study population consisted of 388 consecutive patients with CHF who underwent angiography or PCI from April 2010 to June 2014, as well as were referred as post-myocardial infarction subjects within this period in our five centers participated in this investigation. All these patients were selected from 1427 patients according to our inclusion and exclusion criteria. The study protocol was approved by the Zaporozhye State Medical University Ethics committee review board. The study complied with the Declaration of Helsinki and voluntary informed written consent was obtained from all patients included in this study.

We analyzed cumulative survival related to CHF, and additionally all-cause mortality was examined. Prognosis was assessed by the composite endpoint all-cause death, CHF-related death or CHF hospitalization, censored at 3 years.

Methods for visualization of coronary arteries

Either multispiral computed tomography angiography or angiographic study has 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. When atherosclerotic lesions of the coronary arteries (CAD) were verified, patients were subjected to conventional angiographic examination provided indications for revascularization were available. CAD was considered to be diagnosed upon availability of previous angiographic examinations carried out not later than 6 months ago, providing no new cardiovascular events occurred for this period, and the procedure was available for assay. The coronary artery wall structure was measured by means of contrast spiral computed tomography angiography [19] on Somatom Volume Zoom scanner (Siemens, Erlangen, Germany) and Optima CT660 (GE, USA) After preliminary native scanning, non-ionic contrast Omnipaque (Amersham Health, Ireland) was administered for the optimal image of the coronary arteries.

Echocardiography and tissue Doppler imaging

Transthoracic B-mode echocardiography and tissue Doppler imaging were performed according to a conventional procedure on ACUSON scanner (SIEMENS, Germany) and MyLab 50 XVision ultrasound machine (ESAOTE, Italy) using phased transducer of 5 MHz. Left ventricular end-diastolic and end-systolic volumes, and ejection fraction (LVEF) were measured by modified Simpson's planimetric method [20, 21]. Peak systolic (Sm), early diastolic (Em), and late diastolic (Am) myocardial velocities were measured in the mitral annulus area, followed by calculating velocity of early diastolic left ventricular filling (E) to Am (E/Am) ratio and to Em (E/Em) ratio. Inter- and intraobserver variability coefficients for LVEF were 3.2% and 1.1% respectively.

Glomerular filtration rate measurement

Calculation of glomerular filtration rate (GFR) was calculated by CKD-EPI formula [22].

Biomarker determination

All biomarkers were determined at baseline. To measure biological marker concentrations, blood samples were drawn in the morning (at 7-8 a.m.) into cooled silicone test tubes. Samples were processed according to the recommendations of the manufacturer of the ana-

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Table 1. The characteristics of participants

	Entire patient cohort (n = 388)
Age, years	58.34±9.60
Male, n (%)	207 (53.3%)
I NYHA class, n (%)	77 (19.8%)
II NYHA class, n (%)	147 (37.9%)
III NYHA class, n (%)	83 (21.4%)
IV NYHA class, n (%)	81 (20.9%)
Hypertension, n (%)	214 (55.5%)
Dyslipidemia, n (%)	256 (66.0%)
Type two diabetes mellitus, n (%)	146 (37.6%)
Obesity, n (%)	172 (44.3%)
Adherence to smoke, n (%)	76 (19.6%)
BMI, kg/m ²	24.1 (95% CI = 21.6-28.7)
Systolic BP, mm Hg	130.90±8.41
Diastolic BP, mm Hg	77.90±5.12
Heart rate, beat per min	70.52±3.34
LVEF, %	42.80±0.76
GFR, 1.73 ml/ min/m ²	82.3 (95% CI = 68.7-102.6)
Creatinine, µmol/L	72.3 (95% CI = 58.7-92.6)
Fasting glucose, mmol/L	5.20 (95% CI = 3.3-9.7)
HbA1c, %	6.8 (95% CI = 4.1-9.5)
Hemoglobin, g/L	132.4 (95% CI = 125.5-140.1)
Total cholesterol, mmol/L	5.1 (95% CI = 3.9-6.1)
Cholesterol HDL, mmol/L	0.91 (95% CI = 0.89-1.12)
Cholesterol LDL, mmol/L	3.23 (95% CI = 3.11-4.40)
Uric acide, mmol/L	3.5 (95% CI = 25.3-40.1)
NT-pro-BNP, pg/mL	153.6 (95% CI = 644.5-2560.6)
Galectin-3, ng/mL	1.58 (95% CI = 15.90-18.65).
hs-CRP, mg/L	7.34 (95% CI = 6.77-7.95)
Osteoprotegerin, pg/mL	554.3 (95% CI = 5306.4-5782.1)
Osteopontin, ng/mL	99.5 (95% CI = 57.7-142.7)
Osteonectin, nT/mM	788.54 (95% CI = 665.12-912.30)
sRANKL, pg/mL	2206.50 (95% CI = 2057.2-2355.8)
sRANKL/osteoprotegerin ratio, unit	0.39 (95% CI = 0.22-0.45)
Adiponectin, µg/mL	10.61 (95% CI = 4.83-17.35)
MPCs with phenotype CD14 ⁺ CD309 ⁺ ×10 ⁻⁴ , %	29.18 (95% CI = 15.00-34.50)
MPCs with phenotype CD14 ⁺ CD309 ⁺ Tie2 ⁺ ×10 ⁻⁴ , %	0.67 (95% CI = 0.21-1.10)
CD31 ⁺ /annexin V ⁺ EMPs, cells/mL	0.48 (95% CI = 0.29-0.64)
EMPs/CD14 ⁺ CD309 ⁺ MPCs, units ×10	6.59 (95% CI = 4.10-8.96)

Notes: CI-95% confidence interval; NYHA-New York Heart Association; GFR-glomerular filtration rate; BMP-brain natriuretic peptide; BP-blood pressure; LVEF-left ventricular ejection fraction; BMI-body mass index, sRANKL-serum receptor activator of nuclear factor-kappa B ligand; EMPs-endothelial-derived apoptotic microparticles; MPCs-mononuclear progenitor cells; HbA1c-glycated hemoglobin, HDL-high-density lipoprotein; LDL-Low-density lipoprotein.

lytical technique used. They were centrifuged upon permanent cooling at 6,000 rpm for 3 minutes. Then, plasma was refrigerated immediately and stored at a temperature -70°C until measurement.

Circulating NT-pro-BNP level was measured by immunoelectrochemoluminescent assay using sets produced by R & D Systems (USA) on Elecsys 1010 analyzer (Roche, Mannheim, Germany). Duplicate serum concentrations of

Table 2. Treatment strategy in CHF patients enrolled in the study

	Entire patient cohort (n = 388)
ACE inhibitors or ARAs, n (%)	388 (100%)
Aspirin, n (%)	305 (78.6%)
Other antiplatelet drugs, n (%)	83 (21.4%)
Beta-adrenoblockers, n (%)	324 (83.5%)
Dihydropyridine calcium channel blockers, n (%)	63 (16.2%)
Ivabradine, n (%)	137 (35.3%)
Mineralocorticoid receptor antagonists, n (%)	152 (39.2%)
Loop diuretics, n (%)	311 (80.1%)
Statins, n (%)	294 (75.7%)
Metformin, n (%)	146 (37.6%)
Sitagliptin, n (%)	48 (12.4%)

Notes: ACE-angiotensin-converting enzyme; ARAs-angiotensin-2 receptor antagonist.

tumor necrosis factor alpha (TNF-alpha), solubilized Fas (sFas), sFas ligand, galectin-3, and adiponectine were determined with commercially available enzyme-linked immunosorbent assay kits (Bender MedSystems GmbH, Vienna, Austria).

Circulating bone-related proteins (osteopontine, osteonectin, and osteopontine) were determined in duplicate by ELISA method using kits (IBL, Immunochemie und Immunobiologie GmbH, Germany).

The high-sensitivity C-reactive protein (hs-CRP) levels were measured by using nephelometric technique on AU640 analyzer manufactured by Diagnostic Systems Group (Japan).

Concentrations of total cholesterol (TC) and cholesterol of high-density lipoproteins (HDL) were measured by fermentation method. Concentration of cholesterol of low-density lipoproteins (LDL-C) was calculated according to the Friedewald formula.

A total of 100 µl of serum samples was assayed in parallel to known standard concentrations for each biological marker. The mean intra-assay coefficients of variation were <10% of all cases.

Identifying fractions of mononuclear and endothelial progenitor cells

Mononuclear cells populations were phenotyped by flowcytometry by means of mono-

clonal antibodies labeled with FITC fluorochromes (fluorescein isothiocyanate) or double-labeled with FITC/PE (phycoerythrin) (BD Biosciences, USA) to CD45, CD34, CD14, Tie-2, and CD309 (VEGFR2) antigens as per HD-FACS (High-Definition Fluorescence Activated Cell Sorter) methodology, with red blood cells removed obligatory with lysing buffer according to gating strategy of International Society of Hematotherapy and Graft Engineering sequential (ISHAGE protocol of gating strategy) [23]. For each sample, 500,000 events were analyzed. Circulating mononuclear progenitor cells (MPCs) have been identified as CD45⁺CD34⁺ cells. Pro-angiogenic phenotype for endothelial MPCs was determined as CD14⁺

CD309 (VEGFR2)⁺Tie-2⁺ antigens, obtained when laser beam is scattered in longitudinal and transversal directions in the flowcytometer, the scattergram results were analyzed by using Boolean principles for double or triple positive events.

Endothelial-derived apoptotic microparticles determination

Endothelial-derived apoptotic microparticles were phenotyped by flow cytometry by phycoerythrin (PE)-conjugated monoclonal antibody against CD31 (BD Biosciences, USA) followed by incubation with fluorescein isothiocyanate (FITC)-conjugated annexin V (BD Biosciences, USA) per HD-FACS (High-Definition Fluorescence Activated Cell Sorter) methodology. The samples were incubated in the dark for 15 min at room temperature according to the manufacturer's instructions. The samples were then analyzed on a FC500 flow cytometer (Beckman Coulter) after 400 µL annexin-V binding buffer was added. For each sample, 500,000 events were analyzed. EMPs gate was defined by size, using 0.8 and 1.1 µm beads (Sigma, St Louis, MO, USA). CD31⁺/annexin V⁺ microparticles were defined as EMPs positively labeled for CD31 and annexin V (CD31⁺/annexin V⁺) [24, 25].

Statistical analysis

Statistical analysis of the results obtained was carried out in SPSS system for Windows,

Table 3. Univariate and multivariate Cox regression analysis

Variances	Univariate analysis			Multivariate analysis		
	OR	95% CI	P value	OR	95% CI	P value
Creatinine per 30 µmol/L	1.06	1.01-1.11	0.001	1.02	0.87-1.06	0.001
Fasting glucose per 3 mmol/L	1.04	0.96-1.09	0.002			
HbA1c per 1%	1.05	1.01-1.07	0.002			
Total cholesterol per 1 mmol/L	1.08	1.01-1.09	0.001			
Uric acid per 10 mmol/L	1.08	1.03-1.09	0.001	1.03	0.92-1.08	0.001
NT-pro-BNP per 400 pg/mL	1.97	1.25-3.06	0.001	1.37	1.08-2.10	0.001
Galectin-3 per 2.5 ng/mL	2.16	1.78-3.77	0.001	1.46	1.22-1.89	0.003
hs-CRP per 1 mg/L	1.42	1.22-1.87	0.001	1.12	1.03-1.25	0.001
Osteoprotegerin per 325 pg/mL	1.34	1.18-1.62	0.006	1.19	1.12-1.33	0.001
Osteopontin per 65 ng/mL	1.16	1.03-1.36	0.002	0.95	0.87-1.11	0.003
Osteonectin per 50 ng/mL	1.19	1.07-1.28	0.001	1.06	0.91-1.19	0.002
sRANKL per 100 pg/mL	1.08	1.02-1.15	0.001	1.02	0.86-1.07	0.001
sRANKL/osteoprotegerin per 0.15 units	1.56	1.23-1.72	0.002	1.17	1.04-1.25	0.003
Adiponectin per 3.5 µg/mL	1.05	1.01-1.09	0.006	1.03	0.89-1.07	0.001
CD14 ⁺ CD309 ⁺ MPCs per 10×10 ⁻⁴ %	1.12	1.05-1.27	0.001	1.05	1.00-1.11	0.001
CD14 ⁺ CD309 ⁺ Tie2 ⁺ MPCs per -0,2×10 ⁻⁴ %	1.15	1.03-1.29	0.006	1.06	1.01-1.09	0.001
CD31 ⁺ /annexin V ⁺ EMPs per 0.2 cells/mL	1.18	1.10-1.27	0.001	1.07	1.02-1.13	0.001
EMPs/CD14 ⁺ CD309 ⁺ MPCs per 2.5×10 units	2.14	1.18-3.55	0.001	1.19	1.12-1.27	0.001

Notes: CI -confidence interval; OR-odds ration; HbA1c-glycated hemoglobin; BNP -brain natriuretic peptide; sRANKL-serum receptor activator of nuclear factor-kappa B ligand, EMPs-endothelial-derived apoptotic microparticles; MPCs-mononuclear progenitor cells.

Table 4. Comparison of AUCs characterized biomarker models to standard model calculated for LFEV less 40%. The results of ROC curves analysis

Models	AUC	95% CI	P values
Standard Model: LVEF	0.646	0.612-0.661	-
NT-pro-BNP	0.683	0.644-0.703	0.045
Galectin-3	0.731	0.711-0.754	0.013
hs-CRP	0.656	0.634-0.687	0.068
Osteoprotegerin	0.722	0.707-0.739	0.012
sRANKL/osteoprotegerin ratio	0.734	0.723-0.752	0.001
CD14 ⁺ CD309 ⁺ Tie2 MPCs	0.785	0.755-0.794	0.001
EMPs/CD14 ⁺ CD309 ⁺ MPCs ratio	0.834	0.805-0.861	0.001

Abbreviations: AUC-area under curve, LVEF-left ventricular ejection fraction, BNP-brain natriuretic peptide, hs-CRP-high sensitive C-reactive protein, sRANKL-serum receptor activator of nuclear factor-kappa B ligand, EMPs-endothelial-derived apoptotic microparticles; MPCs-mononuclear progenitor cells.

Version 22 (SPSS Inc, Chicago, IL, USA) and GraphPad Prism for Windows, Version 5 (GraphPad Software Inc, La Jolla, CA, USA). The data were presented as mean (M) and standard deviation (± SD) or 95% confidence interval (CI); median (Me) and interquartile range (IQR), as well as numerous (n) and frequencies (%) for categorical variables. To compare the

main parameters of patients' groups (subject to the type of distribution of the parameters analyzed), two-tailed Student t-test or Mann-Whitney U-test were used. To compare categorical variables between groups, Chi2 test (χ^2) and Fisher F exact test were used. The circulating EMPs, MPCs, and NT-pro-BNP level in the blood failed to have a normal distribution, while distribution of the hs-CRP, bone-related proteins, adiponectine, total cholesterol and cholesterol fractions were normal (estimated by means of Kolmogorov-Smirnov test) and was not subjected to any mathematical transformation. The factors, which could be associated potentially with clinical outcomes,

were determined by Cox regression analysis. Receive Operation Characteristic (ROC) curves were constructed for assessment of optimal balanced cut-off points that were suitable for independent predictors of clinical outcomes. Areas under curves were compared using method provided by DeLong et al [26]. Reclassification methods (C-statistics) were

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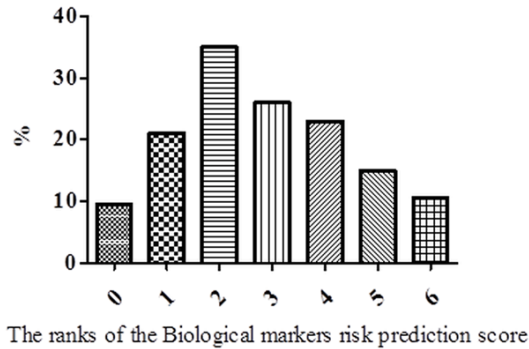


Figure 1. The distribution of various ranks of Biomarker risk prediction score in patients with ischemic CHF.

utilized for prediction performance analyses. The Kaplan-Meier curves were constructed depending categories of the Biomarker risk prediction score. A calculated difference of $P < 0.05$ was considered significant.

Results

Study patient population

The characteristics of the patients participated in the study are depicted in **Table 1**. At baseline, mean age in both sexes was 58.34 years. The prevalence of II (37.9%) and III (21.4%) NYHA class was determined. At least 55.5% of the subjects enrolled in the study were hypertensive. Likewise, cardiovascular risk factors, such as dyslipidemia, type two diabetes mellitus and obesity, were reported 66.0%; 37.6%; and 44.3% respectively. Mean left ventricular ejection fraction was decreased slightly. Regarding biomarker levels, increased Gal-3, NT-pro-BNP, hs-CRP, bone-related proteins (osteoprotegerin, osteopontin, osteonectin), sRANKL and adiponectin were found. Depletion of circulating levels of MPCs labeled as $CD14^+CD309^+$ and $CD14^+CD309^+Tie2^+$ were determined. Increased $CD31^+$ /annexin V⁺ EMPs were found.

The majority patients with CHF were treated with ACE inhibitors or ARAs, beta-adrenoblockers, I/f blocker ivabradine, mineralocorticoid receptor antagonists, and antiplatelet drugs (**Table 2**). Adding loop diuretics was done when fluid retention was observed. Dihydropyridine calcium channel blockers were added when elevated blood pressure was uncontrolled by previous treatment scheme. Metformin and/or sitagliptin were used in type two diabetes

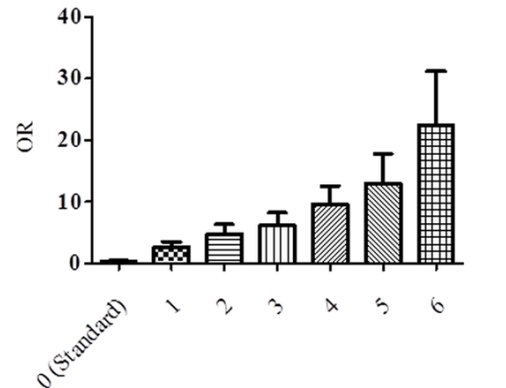


Figure 2. Stratification of CHF patients depending on the odds ratio (OR) of cumulative cardiovascular events.

patients as a component of contemporary treatment of CHF.

Clinical event determination

Median follow-up was of 2.76 years (IQR = 1.8-3.4). During follow-up, 285 cardiovascular events (including 43 fatal cases) were determined. Thirty five patients were died due to advancement of CHF, and eight cases of death were sudden, fatal myocardial infarction, and systemic thromboembolism. No other causes of death were recorded. Additionally, 206 subjects were hospitalized repetitively due to worsening CHF and also 36 subjects were readmitted in the hospital due to other cardiovascular reasons.

Biomarker predictors of cumulative cardiovascular events

The independent biomarker predictors of cumulative cardiovascular events in CHF patients obtained by multivariable Cox regression analyses were NT-pro-BNP, galectin-3, hs-CRP, osteoprotegerin, sRANKL/osteoprotegerin ratio, MPCs labeled $CD14^+CD309^+Tie2^+$, and EMPs/ $CD14^+CD309^+$ MPCs ratio (**Table 3**). ROC curves analysis have shown that there were significant difference between AUCs for independent variables and AUC for standard model (LVEF less 40%) (**Table 4**). Therefore, the best discriminate value was found for EMPs/ $CD14^+CD309^+$ MPCs ratio and $CD14^+CD309^+Tie2^+$ MPCs.

C-statistic of the model with continuous variable shown that Cox regression model contains

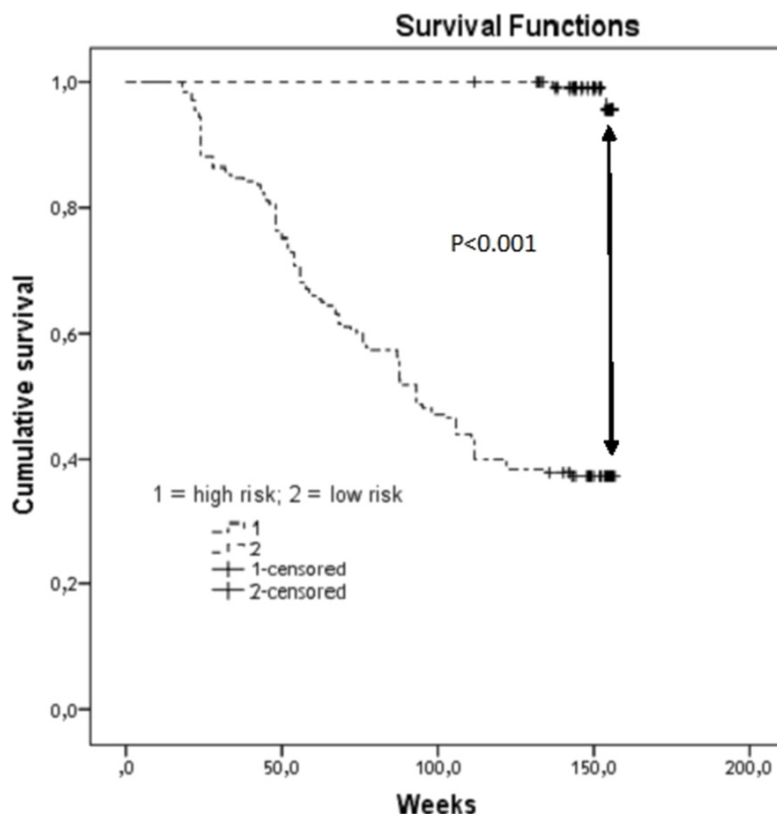


Figure 3. The Kaplan-Meier survival curves for CHF patients stratified according to low and high cumulative cardiovascular risk.

eight categorized predictors that did not differ from ABC model (C-statistic 0.81; 95% CI = 0.79-0.95; $P = 0.001$), whether C-statistic of the model with binary predictors containing sRANKL/osteoprotegerin ratio, MPCs labeled CD14⁺CD309⁺Tie2⁺, and EMPs/CD14⁺CD309⁺ MPCs ratio did distinguish from ABC model (C-statistic 1.04; 95% CI = 1.01-1.06; $P = 0.001$).

Biomarker risk prediction score for cumulative cardiovascular events

For Biomarker risk prediction score construction we enrolled six biomarkers: NT-pro-BNP, galectin-3, hs-CRP, osteoprotegerin, CD31⁺/annexin V⁺ EMPs and EMPs/CD14⁺CD309⁺ MPCs ratio. Each independent predictor was assigned the value of 1 or 0 when present or absent respectively. The sum of number of the independent predictors was ranged from 0 to 6 points, and then was used for Biomarker risk prediction score grading. The entire cohort of the CHF patients the Biomarker risk prediction score averaged 3.17 point (95% CI = 1.65-5.10

points). The distribution of the Biomarker risk prediction score in the CHF patients is shown in **Figure 1**.

The analysis of obtained results have shown that there is a significant association between rank of Biomarker risk prediction score and numerous of cumulative cardiovascular events in CHF patients ($r = 0.72$; Wald $\chi^2 = 11.9$; $P = 0.001$). Therefore, Odds ratio calculated for cumulative cardiovascular events steadily increases related with up of Biomarker risk prediction score rank per 1 point (**Figure 2**). We suggested that ranks of Biomarker risk prediction score ≤ 4 points reflect low risk of cumulative cardiovascular events in CHF patients, whether ranks ≥ 5 points of prediction score show high cardiovascular risk.

Figure 3 shows the Kaplan-Meier survival curves for CHF patients stratified according to low and high cumulative cardiovascular risk. The accumulation of clinical event determined within observation period leads to a significant divergence ($P < 0.001$) of survival curves constructed for two patient cohorts stratified depending low (≤ 4 points) and high (≥ 5 points) risk.

Discussion

The results of the present study showed that the rank of the Biomarker risk prediction score was associated with cumulative clinical outcomes in CHF patients and that score system constructed biological markers may be capable of accurately identifying patients at high-risk, irrespective of metabolic comorbidities. We included in the analysis several biological markers which reflected different aspects and faces of the pathogenesis of CHF. Thus, in addition, routinely measured biomechanical stress markers such as NT-pro-BNP, high risk pheno-

typic marker galectin-3 and the proinflammatory marker hs-CRP, we also included multi-functional markers such as osteoprotegerin and its soluble receptor sRANKL, osteopontin, osteonectin, adiponectin, CD31⁺/annexin V⁺ EMPs and MPCs with angiopoietic potency. The positive side of the multimarker approach is low dependence from demographic, metabolic comorbidities, and renal clearance that is crucial for CHF patients [27]. Earlier attempts to create new risk scores of CHF were based on isolated criteria such as clinical data or echocardiographic parameters, as well as levels of certain biomarkers, mainly natriuretic peptides and galectin-3 [7, 28]. However, this approach proved to be more successful in a population of patients with acute or acutely decompensated heart failure than in those with stable chronic heart failure [29]. In addition, for variable scores such as age, gender, metabolic conditions (obesity, type 2 diabetes), renal clearance, and anemia were already established critical for reliability of prediction [5, 6, 30]. We have tried to incorporate these data in order to minimize the influence of additional factors on the reliability prediction model to include in the biomarkers identified those that do not depend on renal clearance (MPCs and EMPs), were not associated with myocardial dysfunction (sRANKL/osteoprotegerin ratio), reflected the severity of endothelial dysfunction and coagulation (osteopontin, osteonectin). Although both biomarkers NT-pro-BNP and galectin-3 remained as the main biological indicators reflecting biomechanical/overload response and phenotypic risk of heart failure, they are influenced by age, sex, kidney function, obesity, and diabetes [8, 31]. On the other hand, there are novel biomarkers, such as ST2 protein, that are expected to overcome this limitation suitable for natriuretic peptides [32]. However, due to lack of data, surpassing ST2 protein to galectin-3 and other proinflammatory cytokines in turn of prediction of outcomes in CHF patient population is advised [33]. Moreover, results of the PRIDE study have been shown that NT-proBNP was superior to ST2 protein for primary diagnosis of acute or acutely decompensated heart failure [34]. Taken together these data are able to clearly distinguish predictive value between several biomarkers, and that it is not necessary to expect a single ideal biomarker for CHF patients. Ideally, further studies should be aimed at establishing multimarker

models that would be more powerful tools in re-stratifying patients at risk. In summary, we suggest that the Biomarker risk prediction score may reflect negative outcome for CHF but looks optimistic in terms of a reliable evaluation system as a whole, although it requires a comparison with already established systems such as the Seattle Heart Failure Model Scores. Thus, future investigations aimed at recognizing optimal combination of biomarkers incorporated in the novel predictive score are warranted.

In conclusion, we suggested that biomarker risk score for cumulative cardiovascular events, constructed by measurement of circulating NT-pro-BNP, galectin-3, hs-CRP, osteoprotegerin, CD31⁺/annexin V⁺ EMPs and EMPs/CD14⁺ CD309⁺ MPCs ratio, allows a reliable prediction for the probability survival of patients with CHF, regardless of age, gender, state of the contractile function of the left ventricle and the number of comorbidities.

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Disclosure of conflict of interest

None.

Abbreviations

BMI, body mass index; BMP, brain natriuretic peptide; CI, confidence interval; CHF, chronic heart failure; EMPs, endothelial-derived apoptotic microparticles; Gal-3, galectin-3; GFR, glomerular filtration rate; LVEF, left ventricular ejection fraction; MPCs, mononuclear progenitor cells; NYHA, New York Heart Association; OR, odds ratio; TNF, tumor necrosis factor.

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