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Research paper

Phoenixin plasma concentration in heart failure with reduced ejection fraction patients

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Abstract

Introduction: Heart failure (HF) nowadays in western countries is an immense problem largely due to its social impact as well as an economic burden. A widely accepted biomarker-based strategy to establish prognosis and predict re-hospitalization events in HF is lacking. Currently, besides natriuretic peptides and cardiac troponins, a variety of molecules are being studied. Phoenixin (PNX) is a neuropeptide mainly involved in the regulation of gonadotropin secretion. Recently, a significant cardioprotective effect of PNX was reported.

Aim: The aim of this study was to measure PNX plasma concentration in a group of HF with reduced ejection fraction (HFrEF) patients and to compare it to levels found in HF-negative participants.

Material and methods: A group of 74 HFrEF patients and a control group consisting of 40 participants without systolic or diastolic myocardial dysfunction were studied. Each individual underwent anthropometric measurements, laboratory testing, clinical and echocardiographic examination. To evaluate PNX plasma concentration, an immunoenzymatic assay (ELISA) was performed.

Results and discussion: PNX plasma concentration in the HFrEF group was not statistically different than in the control group. No significant correlation between PNX level and age, sex, BMI, HF etiology, diabetes or atrial fibrillation presence was found. PNX concentration correlated positively with total and LDL cholesterol blood levels in HFrEF patients. A negative correlation was found with creatinine in HFrEF, uric acid and triglycerides levels as well as AlAT activity in the control group.

Conclusions: There is no significant difference in PNX plasma concentration between HF and non-HF individuals. PNX role in cardiovascular disease requires further investigation.

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1. INTRODUCTION

Heart failure (HF) is undoubtedly a growing problem globally, both in medical and social aspects. In Poland, HF is one of the three leading causes of death among cardiovascular diseases (CVDs), next to ischemic heart disease and cerebrovascular disease.[1] CVDs, in general, remain the main cause of death in Poland.[1] According to the estimates of the Heart Failure Association of the Polish Cardiac Society presented in 2018, around 750 000 people suffered from HF in our country.[2] In the United States, HF affects about 2% of the general population, however, a significant increase in this number should be expected in the upcoming years due to the ageing of the society and advances in HF management.[3] The risk of developing HF throughout life for a 45-year-old person is estimated at 20%–45% and increases with the severity of HF risk factors, especially obesity and arterial hypertension.[3] Regarding COVID-19 pandemic, reports of myocardial damage in the course of coronavirus infection may provide the grounds for further concern. Laboratory indications of myocardial injury were reported in 20%–30% of hospitalized COVID-19 patients.[4–7] Cardiac magnetic resonance (CMR) imaging performed in a group of convalescents, 2–3 months after the onset of the disease, showed a reduced left ventricular ejection fraction (LV EF) and enlarged dimensions of the left ventricle in up to 80% of patients.[8] Whether accelerated HF development occurs in COVID-19 convalescents is yet to be investigated. According to European Society of Cardiology (ESC) guidelines, HF is divided into three categories based on LV EF:

(1) <40% – HF with reduced ejection fraction (HFrEF),
(2) 41–49% – HF with mildly reduced ejection fraction (HFmrEF),
(3) ≥ 50% – HF with preserved ejection fraction (HFpEF).[9]

Despite a mild trend towards decreased incidence in recent years, HFrEF remains a very important form of HF.

The term 'biomarker' was first introduced by the working group of the US National Institutes of Health (NIH) and was defined as 'a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention.'[10] Most commonly this term is used in reference to certain substances in blood or body fluids, which are used in a variety of diseases.[11] Natriuretic peptides (NPs) and cardiac troponins (cTn) are without a doubt biomarkers with a well-established position in HF evaluation. NPs reflect myocardial overload while cTn indicate cardiomyocyte damage. According to the ESC guidelines for the management of HF, NPs measurement, can be used as an initial diagnostic test in the evaluation of patients with symptoms indicative of HF.[9]

In recent years, numerous potential biomarkers of HF have been characterized. The ever-growing list of potential biomarkers requires some systematization. In 2008, Braunwald suggested 7 basic classes in relation to major pathophysiological processes[12]:

(1) inflammation: C-reactive protein, TNFα, IL-1, IL-6, IL-18, GDF-15, etc.;
(2) oxidative stress: myeloperoxidase, uric acid, malondialdehyde, etc.;
(3) remodeling of the extracellular matrix: soluble ST2, galectin-3, MMPs, TIMPs, etc.;
(4) myocardial damage: cTn, human fatty-acid-binding protein;
(5) neurohormones: chromogranin A, copeptin, endothelin 1, adrenomedullin, etc.;
(6) myocardial overload: natriuretic peptides;
(7) undefined: NGAL, cystatin C, miRNA.

Numerous reviews discussed potential diagnostic, prognostic or monitoring benefits of the above molecules measurement in HF patients.[12–15] Recently, multi-marker rather than single-marker evaluation strategies are gaining popularity.[16–19] On the other hand, the list of cardiovascular peptides with unknown properties is not yet complete. In 2013 Yosten et al. characterized a new, conservative neuropeptide, called phoenixin (PNX), produced mainly in the hypothalamus, the heart[20] and, to a lesser degree, in several other peripheral tissues.[21] So far, PNX was associated with hypothalamic–pituitary–gonadal (HPG) axis regulation, and other central functions, such as: thermogenesis, food intake, thirst, anxiety, memory, etc.[22] Peripherally, PNX seems to be involved in energy homeostasis. It enhances glucose-induced insulin secretion from isolated rat pancreatic islets[23] and promotes proliferation and differentiation of preadipocytes into mature white fat cells.[24] In 2018 Rocca et al. conducted a study to explore cardiovascular properties of PNX.[25] Hearts of rats fed with standard or high-fat diet were harvested and perfused retrogradely according to the Langendorff technique. The isolated hearts were subjected to 0.5 h of ischemia and 2 h of reperfusion (I/R model) with or without PNX addition. PNX was found to impair both systolic and diastolic function of the left ventricle, whereas it had no significant effect on the heart rate or coronary pressure in sham-procedure rat hearts. Ischemia enhanced heart PNX production in standard diet but not in the high-fat diet group. PNX added to perfusion solution reduced ischemia-induced hemodynamic alterations as well as infarct size via PI3K, Erk 1/2, mitoKATP, eNOS dependent pathways.[25] The hearts of the rats fed with a high-fat diet were generally resistant to cardioprotective effects of PNX. There are only a few reports of PNX plasma levels in humans in different clinical settings.[26–29] Some papers indicate that PNX plasma concentration may be associated with body mass in humans,[26–28] yet it still remains to be confirmed. So far, no research involving PNX in patients with CVDs has been published.

2. AIM

The aim of this study was to measure and compare PNX plasma concentration in a patients suffering from HFrEF and in HF-negative participants. Secondary objective was to investigate PNX concentration in prespecified subpopulations.

3. MATERIAL AND METHODS

3.1. Subjects

In total, 74 patients with HFrEF diagnosis admitted to the Cardiology Department of University Hospital in Zielona Góra, Poland, as well as 40 control patients were enrolled in this study. Adult, stable HF patients had to meet ESC guidelines diagnostic criteria,[9] while control patients were included if no prior HF signs, symptoms and echocardiographic features were found on examination. The following exclusion criteria were applied for both groups: chronic kidney disease, chronic or acute liver disease, chronic obstructive pulmonary disease, on-going infection, malignancy.

All available patient medical data was thoroughly recorded. Weight and height were measured for standard BMI (as kg/m^2) and body surface area (BSA) calculation.[30] Blood pressure was taken with a validated, automated monitor after a 5-minute rest in the supine position. The mean value out of 4 measurements on both arms was calculated. Standard transthoracic echocardiography was performed, left ventricle mass was calculated according to Devereux formula,[31] left ejection fraction was measured with Simpson's biplane method.

3.2. Laboratory analyses

Blood samples were obtained from each patient after an overnight fast from a cubital fossa vein for basic analyses according to standard local procedures. Samples for immunoenzymatic assay were collected to EDTA containing 4.9 mL tube with a separating gel. Tubes were stored on ice immediately after blood collection and centrifuged for 10 minutes at 3000 rpm at 4°C. Separated plasma was stored at –40°C for further analysis. PNX concentration was measured using a commercially available enzyme-linked immunosorbent assay (ELISA, catalog No. EK-079-01, Phoenix Pharmaceuticals Inc., Burlingame, CA, USA) according to the manufacturer's instructions. Test sensitivity is 0.07 ng/mL and 100% specificity according to data provided by the producer. Briefly, diluted plasma samples were added to microplate wells coated with antibodies against the Fc fragment of the anti-PNX primary antibody. PNX solution with a certain concentration was used to establish a standard curve. The primary antibody, as well as a biotinylated PNX solution, was added to all wells except negative control. Microplates were incubated, washed and an HRP-streptavidin solution was added. After another incubation and rinsing, TMB for HRP reaction was added. To stop the reaction hydrochloric acid was used. Absorbance at 450 nm was measured and correlated with plasma sample PNX concentration which was found to fall within the assay's linear range 0.07–2.1 ng/mL.

3.3. Statistical analysis

The obtained results were statistically analyzed using GraphPad Prism 8.0.1 software. All data is demonstrated as mean ± SD. Data normal distribution was determined by the Shapiro–Wilk test. The differences between the two groups were calculated by the Student *t*-test and Mann–Whitney *U* test respectively. The one-way ANOVA test was used to determine differences between multiple groups. Pearson's and Spearman's coefficients were calculated in the correlation analysis depending on data normality. Variance analysis involved the Chi-square test and Fisher's exact test. *P* value of less than 0.05 was accepted as significant.

4. RESULTS AND DISCUSSION

Group characteristics are presented in Table 1. The HFrEF group consisted of 74 patients with an 81% and 19% male to female ratio. The cases of HF caused by ischemic heart disease constituted half of the group while 22 patients (30%) had a past history of alcohol addiction. All patients were clinically stable and demonstrated HF symptoms, of class I (12%), class II (50%), class III (33%) and ambulatory class IV (5%) according to New York Heart Association (NYHA)

Table 1. General characteristic of the study group and control group.

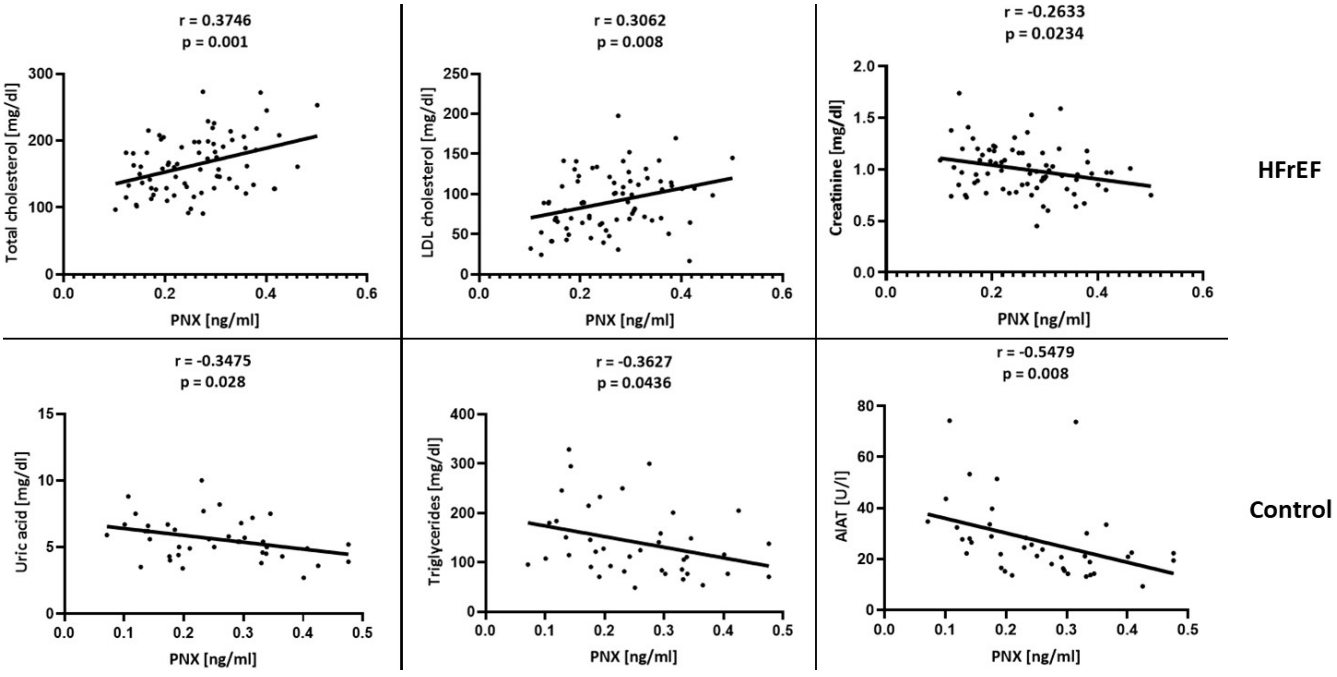
	HFrEF $n = 74$	Control $n = 40$	Significance P value
Age	61.36 ± 10.18	63.53 ± 10.09	0.3004
Male/female, %	81/19	65/35	0.0699
Hypertension, *n*(%)	48(65)	28(70)	0.6787
Diabetes/Prediabetes, *n*(%)	21(28)	13(33)	0.6722
Atrial fibrilation, *n*(%)	33(45)	2(5)	**<0.0001**
Implanted Cardioverter Defibrilator, *n*(%)	17(23)	0(0)	**<0.0001**
History of alcohol addiction, *n*(%)	22(30)	0(0)	**<0.0001**
Ischemic/non-ischemic HF etiology, *n*(%)	37(50)/37(50)		
NYHA class	2.31 ± 0.75		
SR/AF ECG rythm, %	69/31	100/0	**<0.0001**
Pharmacotherapy			
ACE inhibitor, *n*(%)	58(78)	13(33)	**<0.0001**
ARB, *n*(%)	7(10)	10(25)	**<0.0001**
Beta-blocker, *n*(%)	68(92)	23(58)	**<0.0001**
MRA, *n*(%)	59(80)	2(5)	**<0.0001**
Diuretic, *n*(%)	62(84)	12(30)	**<0.0001**
Ivabradine, *n*(%)	11(15)	0(0)	**<0.0001**
Digoxin, *n*(%)	9(12)	0(0)	**<0.0001**
CCB, *n*(%)	9(12)	10(25)	0.1130
Amiodarone, *n*(%)	13(18)	1(3)	**0.0185**
OAC, *n*(%)	35(47)	2(5)	**<0.0001**
ASA, *n*(%)	28(38)	19(48)	0.3275
P2Y12 receptor blocker, *n*(%)	13(16)	5(13)	0.5952
Insulin, *n*(%)	9(12)	1(3)	0.1615
Metformin, *n*(%)	15(20)	10(25)	0.6372
SGLT2 inhibitor, *n*(%)	2(3)	0(0)	0.5404
Statin, *n*(%)	41(55)	22(55)	>0.9999
Proton pump inhibitor, *n*(%)	16(22)	5(13)	0.3134

classification. The majority (65%) of patients had a medical history of hypertension, 28% and 45% of diabetes or prediabetes and atrial fibrillation, respectively. One in four was already implanted with a cardioverter-defibrillator device. Pharmacotherapy was generally in line with 2016 ESC guidelines[11] (Table 1). In all 40 patients in the control group, no clinical symptoms, signs or echocardiographic indices of HF were observed. Control group received anti-hypertensive drugs, used in HF treatment, less frequently than HFrEF group, with an exception of ARB. There was no significant difference in sex or age structure of the groups as well as hypertension and diabetes/prediabetes prevalence. Clinical characteristics are shown in Table 1. Echocardiographically, the two groups had significantly different left atrium area (LAA), left ventricle diastolic diameter (LVdD) and mass (LVM), as well as LV EF. Mean LV EF of 28.43% and 62.66% was observed in the HFrEF and control group, respectively. The laboratory tests results are presented in Table 2. Mean plasma PNX concentration amounted to 0.261 ± 0.091 ng/mL in HF patients and 0.254 ± 0.106 ng/mL in the control group. There was no statistically significant difference between the two values ($P = 0.6932$). PNX level was analyzed in subgroups and no relevant association was found with gender, diabetes and tobacco smoking. In the HFrEF group PNX did not depend on HF etiology (ischemic vs. non-ischemic HF), symptoms (NYHA class) or atrial fibrillation presence. In the correlation analysis performed, no association was identified between PNX plasma concentration and age, gender, BMI, BSA, WHR, blood pressure, morphology parameters, fasting glucose, sodium, potassium, CRP, BNP, HDL cholesterol concentration nor echocardiographic parameters (LAA, LVM, LVMi, LV EF, E/e'), irrespectively of

Table 2. Antropometric echocardiographic and laboratory results.

	HFrEF $n = 74$	Control $n = 40$	Significance P value
BMI, kg/m^2	29.14 ± 5.74	28.82 ± 3.99	0.7554
BSA, m^2	2.02 ± 0.26	1.96 ± 0.20	0.2730
WHR	1.01 ± 0.09	0.99 ± 0.07	0.2144
LA area, cm^2	31.07 ± 7.33	19.41 ± 3.24	**<0.0001**
LVdD, cm	6.59 ± 0.83	4.86 ± 0.48	**<0.0001**
LVM, g	306.1 ± 86.80	178.8 ± 39.81	**<0.0001**
LVM index, g/m^2	151.5 ± 33.85	91.13 ± 18.64	**<0.0001**
LV EF, %	28.43 ± 7.48	62.66 ± 6.53	**<0.0001**
E/e'	14.66 ± 5.45	9.2 ± 2.93	**<0.0001**
SBP, mmHg	130 ± 23	138 ± 15	**0.0088**
DBP, mmHg	81 ± 12	79 ± 10	0.4354
HGB, g/dL	14.38 ± 1.55	14.35 ± 1.14	0.9042
Creatinine, mg/dL	1.00 ± 0.23	0.88 ± 0.15	**0.0037**
eGFR, ml/min/m^2	80.99 ± 18.62	87.40 ± 16.97	0.0731
Total cholesterole, mg/dL	164.0 ± 43.22	179.1 ± 44.44	0.0814
Triglycerides, mg/dL	123.4 ± 60.15	141.0 ± 71.48	0.2355
LDL cholesterole, mg/dL	90.27 ± 36.58	96,94 ± 41.78	0.5375
HDL cholesterole, mg/dL	49.03 ± 18.19	56.78 ± 15.09	**0.0067**
Glucose, mg/dL	126.4 ± 56.41	111.8 ± 30.38	0.9564
CRP, mg/L	6.49 ± 11.35	2.76 ± 5.12	**0.0004**
AlAT, IU/L	38.23 ± 36.78	27.11 ± 14.75	0.1858
AspAT, IU/L	37.57 ± 30.40	25.66 ± 12.19	**0.0193**
Uric acid, mg/dL	7.33 ± 2.22	5.61 ± 1.57	**<0.0001**
Phoenixin, ng/mL	0.261 ± 0.0906	0.254 ± 0.106	0.6932

Figure 1. Phoenixin plasma concentration significant correlations with laboratory tests results.



the study group. PNX level was correlated with a total and LDL cholesterol concentration in the HFrEF group but not in the controls. A negative correlation was found between PNX and creatinine in HFrEF patients and AlAT activity, uric acid and triglycerides in control patients (Figure 1).

PNX is a novel, conservative, pluripotential polypeptide present in human blood.[27–29,32,33] Although PNX high expression in rat hearts was reported earlier[20] and a recent study by Rocca et al. in a rat model of myocardial ischemia showed the cardioprotective effect of PNX, there are not any available studies on PNX which involved patients with CVDs.[25] The report on PNX association with anxiety in obese men by Hofman et al. included patients with hypertension, diabetes and a limited number of individuals with ischemic heart disease, however this small group was not analysed in detail.[29] Even though Rocca et al. showed that ischemic damage led to significant PNX overexpression in cardiac homogenate it is yet to be clarified whether PNX is expressed by cardiomyocytes.[25] Nonetheless, these findings propelled our group to investigate blood PNX concentration in patients with advanced left ventricle dysfunction. Our HFrEF study group had a mean LV EF of 28% whereas LVEF in the age-matched control group fell within a normal range with no signs of diastolic heart failure. The two groups had similar rates of hypertension and diabetes/pre-diabetes: 65% vs. 70% and 28% vs. 33%, respectively. This is consistent with the data derived from big European registries, ESC Heart Failure Pilot Survey and the Heart Failure Long-Term Registry, presented by Balsam et al.[34] In the cited article, up to 45% of reported HFrEF cases were of non-ischemic etiology. Similarly, approximately 50% of our HFrEF group had no records of the ischemic background of myocardial dysfunction. More importantly, the majority of these patients reported excessive alcohol consumption in the past. We speculate that alcohol toxicity might be one of the leading causes of HFrEF without ischemic heart disease in Poland. Surprisingly, plasma PNX concentration did not differ statistically significantly between HFrEF and control groups. Moreover, PNX level did not correlate with HF etiology, symptoms (NYHA class), LV EF, BNP concentration nor atrial fibrillation. Our study outcomes suggest that PNX is not suitable for the role of a HF biomarker. However, recent reports of PNX cardioprotective properties,[25,35] influence on heart hemodynamics[25] as well as endothelial barrier integrity[36] suggest that further studies on PNX involvement in cardiovascular pathophysiology are required. We speculate that PNX cardioprotective activity might take place solely during the acute phase of injury, as was the case of a different neuropeptide: galanin.[37–39] On balance, further studies of plasma PNX concentration in acute heart damage, such as acute myocardial infarction, are necessary. Moreover, we have found correlation between PNX level and LDL cholesterol and triglycerides concentration in HF patients and controls, respectively. Previously PNX correlation with HDL cholesterol[26] and its stimulating on preadipocytes differentiation[24] was reported. Future studies should elaborate on PNX associations with lipids metabolism.

Our study, being the first research investigating PNX plasma concentration in HF patients, has some limitations. The study group was relatively small and heterogenous and unfortunately control group had not NPs concentration measured. We haven't conducted any prospective follow-up. Verification whether therapeutic intervention alters PNX concentration in acute HF setting would be of interest.

5. CONCLUSIONS

There is no significant difference in plasma PNX concentration between HF and non-HF patients. PNX, probably, will not be a suitable HF biomarker. The role of PNX in cardiovascular system physiology is yet to be explored.

Conflict of interest

None declared.

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Ethics

Research protocol was approved by local Ethics Committee in Regional Medical Chamber in Zielona Góra, resolution no. 01/83/2018 on day 29th of January, 2018.
Each study participant was provided with extensive data on research background, was able to ask questions and signed informed consent form, which was approved by Ethics Committee in Regional Medical Chamber in Zielona Góra.

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