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The Prognostic Value of Neuregulin-1β in Heart Failure Patients With Preserved Ejection Fraction

Aim	To determine the neuregulin-1β concentration in patients with chronic heart failure with preserved ejection fraction (HFpEF) and the association of this biomarker with the functional status of patients, echocardiographic parameters of the structural and functional condition of the heart, and the risk of unfavorable outcome.
Material and methods	This observational, prospective study included 47 patients with HFpEF; 32 (68%) of them were females. Mean age was 70 [66–77] years, EF was 57 [56; 58] %. The group of healthy volunteers consisted of 40 people; 32 (55%) of them were females; mean age was 56 [53–61] years. For all patients, the functional status was evaluated (6-min walk test, 6MWT); standard echocardiography (EchoCG) was performed; and concentrations of N-terminal pro-brain natriuretic peptide (NT-proBNP) and neuregulin-1β were measured. The follow-up period was two years. Cases of cardiovascular (CV) death and hospitalizations for decompensated chronic heart failure (CHF) were recorded.
Results	Median concentration of neuregulin-1β was 0.969 [0.348; 1.932] ng/ml in the HFpEF group, which was significantly higher than 0.379 [0.195; 0.861] ng/ml in the group of healthy volunteers (p=0.003). Significant correlations between the neuregulin-1β concentration and the distance walked in 6MWT or with EchoCG parameters of left ventricular diastolic function were not found. Mean observation time was 456 [244; 730] days. 21 outcomes were observed, including 2 CV deaths and 19 hospitalizations for CHF. Patients with high concentrations of neuregulin-1β (≥Me) had a greater frequency of hospitalizations for CHF (Log-rank, p=0.046) and a higher risk of this outcome (risk ratio, 1.30; 95% confidence interval, 1.01–1.66; p=0.037).
Conclusion	Patients with HFpEF had increased concentrations of neuregulin-1β. High levels of neuregulin-1β were associated with a higher risk of hospitalization for decompensated CHF.

Keywords	Neuregulin-1β; chronic heart failure with preserved left ventricular ejection fraction; prognosis
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Introduction

Chronic heart failure with preserved ejection fraction (HFpEF) is a socially significant unresolved problem. HFpEF is associated with a high rate of hospitalization and mortality [1]. Its incidence is steadily increasing. Despite advances in the management of HFpEF, treatment approaches are being continuously studied [2, 3].

Thus, it is necessary to further explore the key mechanisms of HFpEF to identify new therapeutic targets. According to the modern concept of HFpEF pathogenesis, subclinical systemic inflammation and endothelial dysfunction of coronary microvessels, which contribute to the development of myocardial fibrosis and impairment of cardiomyocyte (CM) relaxation, play an important role in the development of the disease [4].

Neuregulin-1β is a paracrine growth factor produced by endothelial cells of coronary microvessels. Neuregulin-1β is an ErbB receptor agonist, mainly the ErbB4 receptor, which are localized on CMs and fibroblasts. Recent studies support the anti-inflammatory and anti-fibrotic effects of neuregulin-1β and the involvement of this factor in the regulation of CM relaxation [5]. It has been shown that the neuregulin-1β/ErbB4 system is activated in patients with chronic heart failure with reduced ejection fraction (HFrEF) in the early stages of the disease and contributes to the increased resistance of CM to pathological adrenergic stimuli, oxidative stress, and apoptosis [5]. Phase II and III clinical trials show that recombinant neuregulin-1β improves myocardial contractility and leads to reverse LV remodeling [5]. Moreover, elevated plasma levels of neuregulin-1β are

associated with an unfavorable prognosis for patients with HFrEF [6]. The ongoing trials that evaluate neuregulin-1β in patients with HFpEF are limited in numbers [7].

Objective

Determine the levels of neuregulin-1β in patients with HFpEF and the association of this biomarker with functional performance of patients, echocardiographic parameters of the heart structure and function, and the risks of adverse outcomes.

Material and Methods

A prospective observational study was conducted following the STROBE (Strengthening the Reporting of Observational Studies in Epidemiology) guidelines [8].

Patients were enrolled in the University Clinical Hospital No.1 of I.M. Sechenov First Moscow State Medical University from September 2019 to June 2020. The study was carried out in accordance with the Declaration of Helsinki.

The study included 47 patients with HFpEF. The control group consisted of 40 healthy volunteers. Male and female patients from 50 to 80 years old were included in the observation groups.

Inclusion criteria: CHF symptoms (NYHA functional class (FC) II–IV); LVEF > 50%; N-terminal pro-brain natriuretic peptide (NT-proBNP) level > 300 pg/mL, NT-proBNP in atrial fibrillation (AF) 660 pg/mL; structural changes in the heart on echocardiogram: left ventricular (LV) hypertrophy with LV posterior wall thickness ≥12 mm and/or left atrial enlargement and/or increased left atrial volume index (LAVI) > 34 mL/m^2 and/or LV mass index (LVMI) > 115 g/m^2 in,ale patients and 95 g/m^2 in female patients.

Exclusion criteria: acute coronary artery disease (CAD); conduction disorders; stroke within 3 months prior to the study initiation; chronic obstructive pulmonary disease stage III–IV; severe hepatic impairment; exacerbation of chronic gastrointestinal diseases; acute renal failure; diabetes mellitus type 1; decompensated hypo- or hyperthyroidism; autoimmune diseases; chronic viral infections (including viral hepatitis B and C, HIV carrier state), bacterial and fungal infections, and cancer at the time of inclusion, or identified within 6 months after inclusion in the study.

The control group was formed to monitor neuregulin-1β levels in healthy volunteers and included 40 individuals without cardiovascular diseases (CVDs) and cardiovascular risk factors, with no specific cardiovascular complaints, documented CVDs, and signs of cardiovascular impairment (complete blood count, lipid profile, blood chemistry, electrocardiogram, echocardiogram).

Clinical data

Demographic and clinical parameters included sex, age, body weight, height, body mass index (BMI), cardiovascular risk factors, concomitant pathology, and were recorded in electronic case report forms in RedCAP. CHF was classified in patients with HFpEF using the NYHA system. Functional performance was assessed using the 6 minute walking distance (6MWD) test.

Biomarkers

Biomarker levels were determined in pre-frozen plasma samples stored at -80°C. Neuregulin-1β levels were assessed by enzyme-linked immunosorbent assay (ELISA) using a NRG-1β Duoset ELISA kit, the coefficient of variation (CV) was 6.1% within batches and 13.8 % between assays. NT-proBNP levels were determined by immunochemical analysis using an Elecsys proBNP II kit. Results were registered automatically using a Cobase E601 analyzer.

Echocardiogram

The examination was conducted on a Toshiba Apilo 500 device following the Russian clinical guidelines by a certified HCP who was not familiar with patient's clinical data. Echocardiographic parameters evaluated included areas and volumes of both atria, LV volumes and mass. All volumetric parameters were indexed to body surface area. LVEF was assessed by the Simpson's method. LV diastolic function was evaluated using pulse and tissue Doppler imaging and the calculation of the E/e' ratio. The condition of the cardiac valvular apparatus was assessed.

The rates of cardiovascular death and hospitalization for decompensated CHF were estimated two years after the inclusion.

Statistical analysis

The statistical processing of data was performed using Statistica 10.0 (Statsoft Inc., USA) and GraphPad Prism 8 (GraphPad Software Inc., USA). The quantitative variables were described using the medians (Me) and the lower and upper quartiles [Q1–Q3]. The categorical data were expressed by an absolute value and a percentage. Two groups were compared by a non-normally distributed quantitative indicator using the Mann-Whitney U-test. The direction and tightness of the correlations between the two quantitative variables were evaluated using the Spearman's rank coefficient of correlation Prognostic modeling was performed in

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the group of patients with HFpEF using the Kaplan-Meier analysis and a Cox proportional hazards regression model. Statistical significance was assessed using a log-rank test in the Kaplan-Meier analysis, and a Wald test in the Cox regression. In the Cox regression, the results were presented as a hazard ratio (HR) with a 95% confidence interval (CI). The significance of neuregulin-1β as a factor in general and as a contribution of several factors (neuregulin-1β, sex, age, NT-proBNP level) was evaluated. The differences were statistically significant with $p<0.05$.

Results

The main characteristics of patients are presented in Table 1. Female patients prevailed in the HFpEF group – 32 (68 %), the mean age was 70 [66; 77] years, and patients were overweight. LVEF was 57 [56; 58] %, LVMI was 41 [38; 45] mL/m^2, and LVMI was 110 [102; 118] g/m^2.

The group of healthy volunteers comprised 40 people, of whom 18 (45 %) were male, the mean age was 56 [53; 61] years, 5 (12.5 %) patients were smokers, mean BMI was 25.97 [24.05; 28.06] kg/m^2, and NT-proBNP level was 51 [24; 87] pg/mL.

The levels of neuregulin-1β were significantly higher in the HFpEF group than in the group of healthy volunteers – 0.969 [0.348; 1.932] ng/mL and 0.379 [0.195; 0.861] ng/mL, respectively (p=0.003) (Figure 1).

The levels of the biomarker was comparable in patients with HFpEF FC II and patients with FC III–IV (0.79 [0.32; 2.01] and 0.97 [0.55; 1.78], respectively; p=0.820). There were no correlations between neuregulin-1β and NT-proBNP (r = –0.011; p=0.98). There were no significant correlations between the levels of neuregulin-1β and 6MWD (r=0.166; p=0.275). There were no statistically significant relationship between neuregulin-1β and the parameters of LV diastolic function. There was moderate positive correlation between neuregulin-1β and LV wall thickness index (r=0.354; p = 0.015).

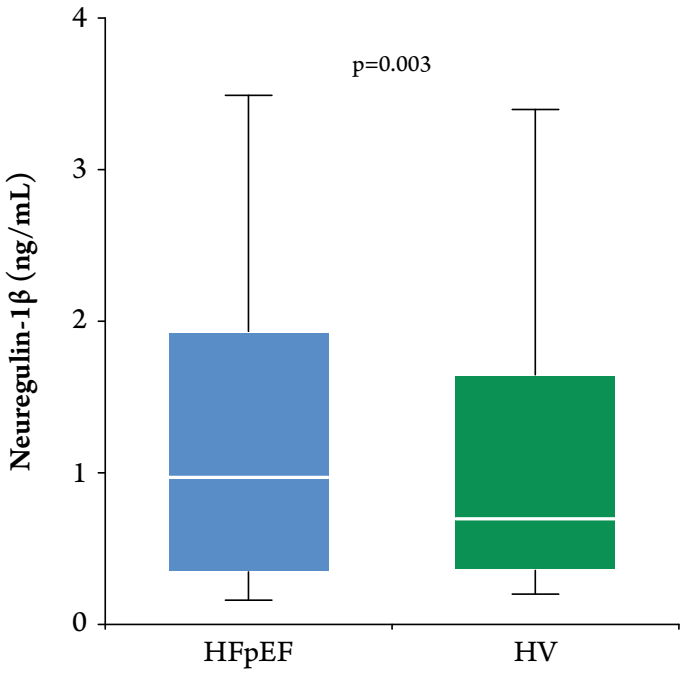
The mean follow-up period was 456 [244; 730] days. Cardiovascular death was reported in 2 patients (1 patient died of acute cerebrovascular accident, and another one dies of acute heart failure), repeat hospitalization for CHF was reported in 19 patients. The univariate Cox regression analysis showed a correlation between neuregulin-1β and the risk of repeat hospitalizations for CHF (HR 1.30; 95% CI 1.01–1.66; p=0.037). In a multivariate regression analysis with sex, age, and NT-proBNP levels included in the model, a correlation between neuregulin-1β and the risk of hospitalization for

Table 1. Clinical characteristics of patients with heart failure with preserved left ventricular ejection fraction (n = 47)

Parameter		Value
Male, n (%)		15 (31.9)
Age, years		70 [66; 77]
Smoking, n (%)		7 (31.8)
BMI, kg/m2		32.19 [29.75; 37.77]
Creatinine, μmol/L		102 [96; 110]
GFR (CKD EPI), mL/min/1.73 m2		49.74 [68.09; 40.79]
NT-proBNP, pg/mL		692 [453; 956]
CHF FC (NYHA) II, n (%)		36 (80)
CHF FC (NYHA) III–IV, n (%)		11 (20)
6MWD, m		368 [323; 405]
Comorbidity, n (%)		
HHD		46 (97.9)
DM type 2		22 (46.8)
Dyslipidemia		32 (68.0)
CKD		18 (38.53)
AF		34 (72.3)
CAD		21 (44.7)
Echocardiogram		
LVEF, %		57 [56; 58]
LAVI, mL/m^2		41 [38; 45]
LVMI, g/m^2		110 [102; 118]
LVWTI		0.46 [0.45; 0.48]
E/A		0.8 [0.6; 1.3]
E/e’		11 [10; 13]
TDI Ems, cm/s	Patients ≥ 75 years old	6 [3; 8]
	Patients < 75 years old	5 [5; 6]
TDI Eml, cm/s	Patients ≥ 75 years old	8 [6; 9]
	Patients < 75 years old	8 [7; 8]
Drug therapy		
ACE inhibitors, n (%)		26 (55.3)
ARBs, n (%)		15 (31.0)
Beta blockers, n (%)		35 (74.5)
Statins, n (%)		36 (76.6)
MCRAs, n (%)		18 (38.3)
Loop diuretics, n (%)		33 (70.2)

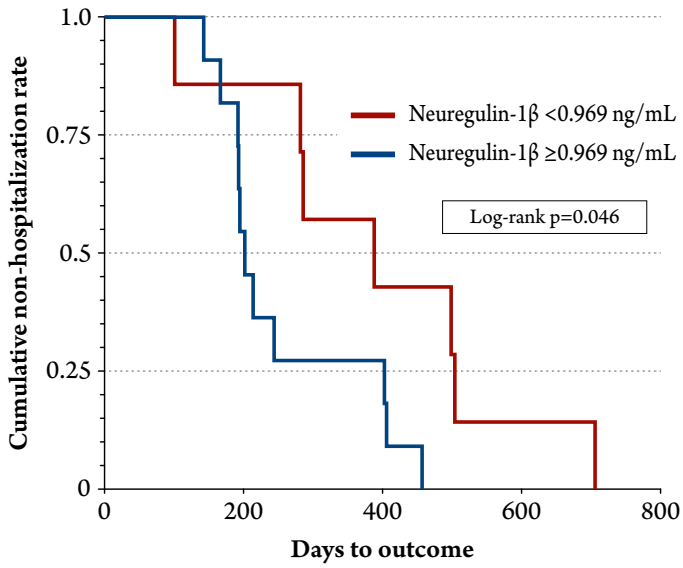
The data are expressed as the median and interquartile range (Me [Q1; Q3]), unless otherwise specified. BMI, body mass index; GFR, glomerular filtration rate calculated by the CKD-EPI formula; NT-proBNP, N-terminal pro-brain natriuretic peptide; 6MWD, 6 minute walking distance; LAVI, left atrial volume index; LVMI, left ventricular mass index; LVWTI, left ventricular wall thickness index; E/A, the ratio of transmitral early filling velocity to filling velocity during atrial contraction; TDI Ems, early diastolic mitral annular septal velocity by tissue Doppler imaging; TMD Eml, early diastolic mitral annular lateral velocity by tissue Doppler; ACE, angiotensin-converting enzyme; ARB, angiotensin II receptor blocker; MCRA, mineralocorticoid receptor antagonist.

Figure 1. Neuregulin-1β levels in the study groups



HFpEF, heart failure with preserved ejection fraction, HV, healthy volunteers.

Figure 2. Kaplan-Meier curve of the risk of repeat hospitalizations depending on the level of neuregulin-1β



Patients with HFpFE were divided into subgroups with high (HFpEF ≥Me, ≥0.969 ng/mL) and low (<Me) levels of neuregulin-1β. RR, relative risk; CI, confidence interval; HFpEF, heart failure with preserved ejection fraction.

decompensated CHF remained statistically significant (HR 1.39; 95 % CI 1.04–1.89; p=0.028). Subsequently, the HFpEF group was stratified depending on the levels of neuregulin-1β, and it was shown that patients with high levels of neuregulin-1β (≥Me, ≥0.969 ng/mL) [7] faced a statistically significantly higher risk of repeat hospitalizations for decompensated CHF (log-rank test; p=0.046; Figure 2). There was no correlation between the levels of neuregulin-1β level and the risk of death (HR 0.628; 95 % CI 0.10–1.42; p=0.397).

Discussion

This prospective observational study showed that patients with HFpEF had statistically significantly higher levels of neuregulin-1β than healthy volunteers. There were no correlations between this biomarker and the levels of NT-proBNP, functional performance, and echocardiographic indicators of LV diastolic dysfunction. High levels of neuregulin-1β were statistically significantly associated in patients with HFpEF with high risk of hospitalizations for decompensated CHF.

In our study, higher concentrations of neuregulin-1β were determined in patients with HFpEF compared to healthy volunteers. Only one study has been conducted so far to evaluate this biomarker in patients with HFpEF [7]. In this study, the authors have evaluated the levels of neuregulin-1β in patients with HFpEF and HFrEF and healthy volunteers. They have shown that the levels of neuregulin-1β were statistically significantly higher in the HFpEF group than in patients with HFrEF and lower in patients with HFpEF than in healthy volunteers [7]. It should be emphasized that in the study by Hage et al. [7], the levels of neuregulin-1β were relatively high in healthy subjects, significantly higher than in healthy volunteers in other studies [9–11].

This paper showed no correlations between neuregulin-1β levels and clinical (NYHA FC) and laboratory parameters of the severity of HFpEF (NT-proBNP) and the functional performance of patients (6MWD). Hage et al. [7] found an inverse correlation between the biomarker levels and CHF functional class. HFpEF patients with more severe CHF (NYHA FC III–IV) had lower levels of neuregulin-1β (p=0.030). We did not find any correlations between the levels of neuregulin-1β and the parameters of LV diastolic function, which is consistent with the previous findings [7].

We were first to show that higher concentrations of neuregulin-1β were associated in patients with HFpEF with a high risk of repeat hospitalizations for decompensated CHF. The effects of neuregulin-1β remained statistically significant after inclusion in the model of such parameters as sex, age, and NT-proBNP levels. Hage et al. [7] have previously shown that patients with HFpEF and CHF who had high levels of neuregulin-1β achieved the composite endpoint (all-cause death, heart transplantation) more often than patients with lower levels of neuregulin-1β (log-rank test 0.020).

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Causes of elevated neuregulin-1β in patients with HFpEF continue to be studied. The effects of inflammation on the production of neuregulin-1β and its anti-inflammatory and antifibrotic activity are discussed [5]. Given that systemic inflammation and fibrosis play a significant role in the development of HFpEF, it seems important to study the correlations between neuregulin-1β and markers of systemic inflammation, which have also demonstrated the prognostic significance in CVDs [12].

Thus, this study complements the existing concepts about the levels of neuregulin-1β in patients with HFpEF and its prognostic significance. Despite its advantages, the study has several limitations, including a small sample size, which does not allow assessing the role of some other factors potentially affecting the frequency of hospitalization for decompensated CHF, such as kidney function, comorbidities, and drug therapy. Our study was conducted before the completion of the EMPEROR-Preserved randomized clinical trial, which demonstrated the effect of empagliflozin on a decrease in the frequency of hospitalization for decompensated CHF[2]. Moreover, it cannot currently be credibly argued that the plasma levels of neuregulin-1β reflect the pathophysiological processes occurring in the myocardium.

Thus, this is a pilot study and it creates the prerequisites for further assessment of the role of neuregulin-1β in patients with HFpEF and exploration of the mechanisms of its involvement in the pathogenesis of this disease.

Conclusion

It was found in the study that patients with heart failure with preserved ejection fraction have higher levels of neuregulin-1β than healthy volunteers. Elevated levels of neuregulin-1β in these patients are significantly correlated with a high risk of hospitalization for chronic heart failure, which may indicate a significant role of the neuregulin-1β system in patients with heart failure with preserved ejection fraction. Nevertheless, further research is required in a larger patient sample.

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No conflict of interest is reported.

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