ONLINE FIRST

CARDIOLOGY JOURNAL

ISSN: 1897-5593

e-ISSN: 1898-018X

Endoplasmic reticulum stress and expression of nitric oxide synthases in heart failure with preserved and with reduced ejection fraction — pilot study

Authors: Karol Momot, Małgorzata Wojciechowska, Kamil Krauz, Katarzyna Czarzasta, Liana Puchalska, Maciej Zarębiński, Agnieszka Cudnoch-Jędrzejewska

DOI: 10.5603/cj.97962

Article type: Original Article

Submitted: 2023-10-25

Accepted: 2024-07-23

Published online: 2024-10-03

ORIGINAL ARTICLE

Endoplasmic reticulum stress and expression of nitric oxide synthases in heart failure with preserved and with reduced ejection fraction — pilot study

Running title: GRP78 and Nitric Oxide Synthases Levels in HFpEF and HFrEF

Karol Momot[1] https://orcid.org/0000-0001-8659-2948, Małgorzata Wojciechowska[1], Kamil Krauz[1], Katarzyna Czarzasta[1], Liana Puchalska[1], Maciej Zarębiński[2], Agnieszka Cudnoch-Jędrzejewska[1]

[1]Department of Experimental and Clinical Physiology, Laboratory of Centre for Preclinical Research, Medical University of Warsaw, Poland

[2]Department of Invasive Cardiology, Independent Public Specialist Western Hospital John Paul II Lazarski University, Grodzisk Mazowiecki, Poland

DOI: 10.5603/cj.97962

Date submitted: 25.10.2023

Date accepted: 23.07.2024

Early publication date: 03.10.2024

Address for correspondence:

Małgorzata Wojciechowska MD PhD,

Laboratory of Centre for Preclinical Research, Medical University of Warsaw,

Department of Experimental and Clinical Physiology, 02–097 Warsaw, Poland; tel: (0-22) 572 07 08, e mail: malgorzata.wojciechowska2@wum.edu.pl

Keywords: nitrosative stress, oxidative stress, endoplasmic reticulum stress, heart failure with preserved ejection fraction, heart failure with reduced ejection fraction

ABSTRACT

Background: Unfolded Protein Response (UPR), endoplasmic reticulum (ER) stress, and inducible nitric oxide synthase (iNOS) overexpression have been found to influence heart failure with preserved ejection fraction (HFpEF) pathogenesis. Their importance in heart failure with reduced ejection fraction (HFrEF) is not entirely established; there is little data involving a detailed comparison between HFpEF and HFrEF from this perspective. This pilot study aimed to compare circulating levels of Glucose-regulated protein 78kDa (GRP78) (ER — stress marker) and all NOS isoforms between both HFpEF and HFrEF and to analyze the correlation between these markers and the clinical characteristics of the patients.

Methods: Forty-two patients with HFpEF and thirty-eight with HFrEF were involved in this study. Clinical characteristics and echocardiographic data were obtained. Basic laboratory tests were performed and ELISA tests for iNOS, endothelial NOS (eNOS), neuronal NOS (nNOS), and GRP78.

Results: Patients with HFpEF had lower circulating levels of GRP78 and higher iNOS concentrations when compared to HFrEF patients ($P = 0.023$, $P < 0.0001$, accordingly). The subgroup of the HFpEF population with eGFR < 60 mL/min/1.73m2 had higher nNOS and eNOS levels than HFpEF patients with normal GFR ($P = 0.049$, $P = 0.035$, respectively). In the HFrEF subgroup, patients with coexistent diabetes mellitus had elevated concentrations of nNOS compared to the subpopulation without diabetes mellitus ($P = 0.041$). There was a positive correlation between eNOS and nNOS concentrations ($\rho = 0.86$, $P < 0.0001$).

Conclusions: In HFpEF, there is a more intensified iNOS overexpression, while in HFrEF, ER stress is more prominent.

Introduction

Heart failure (HF) is a clinical syndrome characterized by symptoms and signs arising from structural and functional changes in the heart, which results in elevated cardiac pressures and abnormal cardiac output. The cardinal symptoms of HF include shortness of breath, fatigue, swelling (edema), and impaired exercise tolerance. Considering the left ventricular ejection fraction (LVEF), HF can be divided into three types: heart failure with preserved ejection fraction (HFpEF; LVEF ≥ 50%), heart failure with mildly reduced ejection fraction (HFmrEF;

LVEF: 41 — 49%) and heart failure with reduced ejection fraction (HFrEF; LVEF ≤ 40%) [1]. The estimated occurrence of HF among adults from developed countries ranges from 1 to 3%. Approximately 50% of them have HFrEF. However, the population diagnosed with HFpEF is rising [2].

There are several differences and similarities between HFpEF and HFrEF concerning their pathogenesis, progression, and abnormalities at the molecular level [3]. Both types are associated with systemic and cardiac inflammation, endothelial dysfunction, cardiac injury [3–5]. However, even within their similarities, differences can be observed. For example, inflammatory response in HFrEF results rather from cardiomyocyte damage, inflammation in HFpEF arises from extra-cardiac metabolic and inflammatory risk factors. In HFpEF, endothelial dysfunction mainly precedes its progression, whereas in HFrEF, endothelial dysfunction may rather be a late-stage consequence. Furthermore, there are differences in etiology between these types. For instance, a history of myocardial infarction and ischemic heart disease is more common in HFrEF [3], while obesity, kidney disease, and old age are more likely to contribute to the development of HFpEF [6].

Schiattarella et al., using preclinical models of HFpEF and human myocardial samples, presented the important role of meta-inflammation, inducible nitric oxide synthase (iNOS), nitrosative stress, and alterations in the unfolded protein response (UPR) in the pathogenesis of the disease [7]. On the contrary, data about iNOS expression and UPR in HFrEF pathophysiology are currently very limited.

UPR plays a crucial role in maintaining endoplasmic reticulum (ER) homeostasis. When the protein-folding capacity in the ER is impaired, it results in the accumulation of unfolded and

misfolded proteins. This disruption in ER homeostasis is known as ER stress. Glucose-regulated protein 78 kDa (GRP78) is involved in this process. This protein functions as a quality control system [8] by monitoring the proteins' folding process and ensuring they are transported only when properly folded. Induction of this protein causes a reduction in ER stress and has cardioprotective effects [8, 9]. Overexpression of GRP78 reduces ER stress and cardiac damage by inducing UPR. A study conducted on muscle cell lines found that GRP94, similar to GRP78, reduced cardiomyocyte necrosis in ischemia conditions [10].

The enzyme nitric oxide synthase (NOS) produces nitric oxide (NO) from the amino acid L-arginine. This enzyme exists in three distinct isoforms: neuronal (nNOS), endothelial (eNOS), and inducible (iNOS) [11]. The constitutive expression of nNOS was found in neurons and endothelial cells and has a role in regulating blood pressure. nNOS is also the source of myocardial NO, which takes part in cardiac relaxation and contraction [12], and both of these functions are disrupted in HF. eNOS is constitutively expressed in every endothelial cell, including heart vessels. eNOS is a dimer consisting of two identical monomers. In a coupled state (dimer), eNOS typically produces NO. Uncoupled eNOS (monomer) shifts to produce dangerous cytotoxic superoxide anions instead of NO, which causes oxidative stress and endothelial dysfunction, leading to cardiovascular diseases, including HF [13, 14]. Finally, recently popular- iNOS is usually expressed in the human heart. However, this process in cardiac tissue can be intensified after relevant triggers such as inflammation, hypoxia, or excessive oxidative stress. All of the mentioned triggers are present in cardiovascular pathologies [15, 16]. iNOS-derived NO causes S-nitrosylation of proteins and, in this way, disrupts their activity. The role of other abnormalities related to NO metabolism is broadly explained in cardiovascular pathologies [17]. This pilot, cross-sectional study provides a novel approach to HFrEF and HFpEF. It compares the circulating serum levels of GRP78 (a

marker of endoplasmic reticulum stress) and all nitric oxide synthases between HFpEF and HFrEF, aiming to identify potential differences or similarities in the pathogenesis of these conditions. Herein, the correlation between these markers and the clinical and echocardiographic characteristics of the patients is analyzed.

Methods

The study was conducted according to the principles outlined in the Declaration of Helsinki, reported according to the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) Statement, and received approval from the Local Ethics Committee (LU no. 02/11/22). Before recruitment, all participating patients were asked to sign a written informed consent form.

Study Population

Forty-two patients with HFpEF and thirty-eight with HFrEF, identified from their hospital medical records, were invited to this pilot, cross-sectional study conducted at a single tertiary cardiac center. The patients with mid-range EF (HFmrEF) were not included to avoid bias in the characterization of HFpEF and HFrEF. All enrolled patients were diagnosed with HF at least 3 months prior and were treated according to the current guidelines. Exclusion criteria were refusal to participate in the study, an active neoplastic process, and an active inflammatory process. Patients with a myocardial infarction, exacerbation of HF, or cardiac surgery within the prior 3 months were not included.

Serum biomarker assessment

Venous blood samples from the cephalic vein were collected in citrate tubes and then centrifuged. Samples with obtained serum were promptly frozen at –80°C and preserved for a maximum duration of four months prior to the biochemical assessment. Serum concentrations of markers were analyzed using ELISA kits: iNOS [NBP2–80255, Novusbio], eNOS [NBP2–

80134, Novusbio], nNOS [NBP2–80252, Novusbio], GRP78 [NBP2–82201, Novusbio]. Each ELISA test was conducted following the manufacturer's instructions. The assessment also included evaluating NT–proBNP and the concentration of serum creatinine. Estimated GFR (eGFR) was calculated by an equation developed by the Chronic Kidney Disease Epidemiology Collaboration (EPI-CKD). Renal dysfunction was defined by an eGFR below 60 mL/min/1.73m^2.

Transthoracic echocardiography examination

Echocardiography was conducted following the European Society of Cardiology guidelines, using a Philips Epiq Ultrasound machine by a single experienced sonographer, to reduce the risk of bias in measurements. LVEF was measured using Simpson's method by obtaining LV volume in systole and diastole through apical four- and two-chamber views. Other echocardiographic characteristics were evaluated: peak early diastolic transmitral flow velocity (E), peak late diastolic transmitral flow velocity (A), left ventricular end-diastolic dimension (LVEDD), intraventricular septal thickness (IVS), posterior wall thickness (PW), relative wall thickness (RWT), left ventricular mass index (LVMI), left atrial (LA) volume, LA volume index (LAVI) and the inferior vena cava (IVC) collapsibility (IVCC). Tissue Doppler imaging (TDI) was performed (both the septal and lateral aspects of the mitral annulus): peak systolic mitral annular tissue velocity (s’), peak early diastolic mitral annular tissue velocity (e’), peak late diastolic mitral annular tissue velocity (a’). Based on the measurements, the E/e' ratio was assessed.

Statistical analysis

Statistical analysis was carried out with the Statistica software, version 13.3. Quantitative variables are presented as means with standard deviation (SD) or medians with interquartile range (IQR). The T-test was performed with data following a normal distribution, while the Mann-Whitney U test was done with data that did not conform to a normal distribution. These

tests were used to compare quantitative variables between the groups. Categorical variables are presented as numbers with percentages (%), and their comparison was conducted using the $\chi 2$ test. The normality of the data was assessed using the Shapiro-Wilk test, while the equality of variances was evaluated using the Levene test. The correlation between the quantitative variables was assessed using Spearman's rank correlation coefficient, and Spearman's rho is labeled as ρ. Statistical significance was determined when the P-value was less than 0.05. The figures were created with Past4 and RStudio.

Results

Table 1 presents the baseline characteristics of the study population. As shown in this table, patients with HFpEF were older than those with HFrEF (mean age: 73 *vs*. 68, P = 0.020). In this study, it was more common for individuals with HFpEF to be female (57% *vs*. 21%, P = 0.001). Most of the subjects from both groups presented NYHA II class symptoms. In the HFrEF group, there were more cases of NYHA III manifestations (7% vs. 21%, P = 0.071). As shown in Table 2, all of the LV dimensions were greater in the HFrEF group (LVEDD: 4.88 *vs.* 5.86, cm, P < 0.001; IVS: 1.11 *vs.* 1.19, cm, P = 0.210; PW: 1.14 *vs.* 1.33, cm, P = 0.002). LVMI and LAVI were greater in the HFrEF group than in the HFpEF (106.47 *vs.* 154.79, g/m^2, P < 0.001 and 39.37 *vs.* 49.68, ml/m^2, P = 0.014; accordingly).

Figure 1 shows the differences between HFpEF and HFrEF groups in blood serum concentrations of nitric oxide synthases and GRP78. Patients with HFpEF presented lower GRP78 serum concentrations than those with HFrEF [0.44 (0.33) *vs.* 0.84 (0.75), respectively, ng/ml, P = 0.008]. The HFpEF group had greater iNOS serum concentrations than the HFrEF group [605.13 (399.13) *vs.* 402.49 (266.06), respectively, pg/ml, P < 0.0001]. The serum concentrations of nNOS and eNOS did not differ significantly between groups.

A subdomain analysis revealed that patients with HFrEF with the coexistence of DM2 presented significantly higher serum concentrations of nNOS than those without DM2 [1.06 (0.68–1.77) *vs*. 0.55 (0.35–1.23), ng/mL, P = 0.041].

In the HFpEF group, patients with renal dysfunction, when compared to those without decreased eGFR, had significantly greater blood serum concentrations of nNOS [1.13 (0.94–1.24) *vs.* 0.69 (0.56–1.12), ng/mL, P = 0.049] and eNOS [424.63 (358.05–498.72) *vs*. 313.96 (233.86–380.40), pg/mL, P = 0.035].

Other comorbidities, including CAD, COPD, or hypertension, were not found to be related to changes in serum concentrations of GRP78 and all NOS.

Age-related significance in GFR decrease in the population with confirmed HF regardless of LVEF is presented in Figure 2A ($\rho = - 0.34$, P = 0.0022). The concentration of both eNOS (Figure 2B) and nNOS (Figure 2C) were negatively correlated with GFR regardless of the group ($\rho = –0.39$, p = 0.00034 and $\rho = –0.35$, p = 0.0014 respectively). The correlation comparing eNOS and nNOS concentrations, regardless of the group, was positive ($\rho = 0.86$, P < 2.2e-16) (figure 2D). Among the general population, iNOS concentration was negatively related to LVMI (Figure 2E) and GRP 78 (Figure 2F) ($\rho = –0.3$, P = 0.0064 and $\rho = –0.25$, P = 0.036 respectively). In the HFpEF group, eNOS concentration was positively correlated with GRP78 concentration ($\rho = 0.33$, P = 0.046) (Figure 2G). Concentrations of iNOS, eNOS, and nNOS were not influenced by age, BMI, or NT-proBNP concentration and other echocardiography findings. No linear and rank correlations, apart from the ones listed above, were found.

Discussion

The current results revealed that HFpEF is associated with significantly higher iNOS overexpression than in the HFrEF (figure 1D), suggesting its essential role in the pathogenesis

of HFpEF but not in HFrEF. According to available research, there are currently no studies comparing circulating serum iNOS between those types of HF.

Low levels of iNOS are expressed in normal, healthy heart tissue. However, during cell stress, especially chronic inflammation, iNOS becomes activated, and large quantities of NO are generated [15]. Induction of iNOS is also present in conditions characterized by reactive oxygen species overproduction [18], such as metabolic diseases, and kidney disorders [19, 20].

Even though NO plays a crucial role in regulating vascular tone as well as in inhibiting platelet aggregation and adhesion, significantly excessive NO levels can exacerbate inflammation and cytotoxic injury [21]. Furthermore, iNOS-induced nitrosative stress contributes to the progression of HFpEF, and inhibiting the synthesis or activity of iNOS improves the HFpEF phenotype in an animal mouse model [7]. iNOS gene knockout improves cardiac diastolic function in this HFpEF model. Furthermore, iNOS overexpression leads to cardiac nitrosative stress by upregulating Akt S-nitrosylation in cardiomyocytes [22]. While the significance of iNOS in HF development appears pivotal in animal models, no investigations have assessed iNOS levels in the blood of human individuals with either HFpEF or HFrEF.

The present research revealed higher concentrations of GRP78 in patients with HFrEF than those with HFpEF (figure 1A), which is consistent with the conclusions of Zhao et al. [23]. According to conjecture herein, lower concentrations of GRP78 in HFpEF may be attributed to a disrupted and downregulated UPR. In the present study, serum concentration of GRP78 was not associated with other comorbidities such as CAD, hypertension, and especially DM2,

contrary to other studies [24]. GRP78 concentrations were negatively correlated with LVEF, which confirms the differences in pathogenesis in HFpEF and HFrEF. Additionally, the concentration of GRP78 was significantly lower in the elderly patients.

The ER stress leads to upregulating GRP78 expression, which is crucial for maintaining the ER homeostasis [25]. The ER stress can be advantageous under specific circumstances; however, it can result in cell death via apoptosis when it becomes severe and persists for an extended duration. The ER stress is present during cardiac hypoxia and acute or chronic inflammation, which are also closely linked to HF pathogenesis [26]. The disruption of ER homeostasis results in HF, aggravating ER stress even more [27]. GRP78 is a protein that inhibits apoptotic signaling and protects cells from apoptosis caused by ER stress [28].
In the current research, serum concentrations of nNOS did not differ between the HFrEF and HFpEF groups (Figure 1B). Although Dumy et al. pointed out overexpression in myocardial nNOS in HFrEF patients [29]. Similarly to eNOS, the concentration of nNOS was significantly greater in the HFpEF subpopulation with renal dysfunction. In the HFrEF group, serum concentration of nNOS was substantially higher among patients with DM2 compared to individuals without this disease. Surprisingly, a recent study showed that elevated expression of nNOS in cardiac tissue acted as a protective factor against cardiac hypertrophy and HF [30]. Also, nNOS gene knockout in mice raised oxidative stress in the heart [28], suggesting its protective role in oxidative stress.

In this study, the serum concentrations of eNOS did not differ between the two groups (figure 1C). At this moment, only one study evaluating eNOS concentrations in HF patients was conducted. Results revealed that in left ventricular tissue samples from humans, eNOS levels were significantly higher in HFpEF, but they compared it to samples from healthy donors, not

from donors with HFrEF [31]. Nevertheless, the present study found that eNOS overexpression was more significant in the HFpEF subpopulation with decreased GFR. It has been shown that eNOS can be involved in renal vascular damage by inducing nodular glomerulosclerosis, which ultimately declines GFR [32].

Recent findings on HFpEF pathophysiology, regarding intensified pro-inflammatory state, elevated iNOS activity, and also nitrosative stress [3], showed why previous attempts of treatment based on elevating NO levels (for example, phosphodiesterase-5 inhibition) were unsuccessful in HFpEF management. Future studies aiming to find HFpEF treatment should focus on inhibiting nitrosative stress, ER stress, and S-nitrosylation. The research for any future treatments for HFpEF should concentrate on decreasing iNOS activity specifically, not increasing it [33]. A recent study showed that both pharmacological inhibition of iNOS with L-NIL administration and iNOS gene knockout improved the phenotype of HFpEF in mice, mitigating mitochondrial dysfunction, oxidative stress, and S-nitrosylation [22]. GW274150 and GW273629 are two highly selective iNOS inhibitors that have the potential to be promising therapeutic agents. They were discovered about 20 years ago, and their role was studied in human breast tumors or renal ischemia/reperfusion injury [34–36]. However, GW274150 and GW273629 were not tested for HFpEF management in any animal model of this disease. iNOS expression is not limited to the cardiovascular system. Therefore, its inhibition may potentially cause serious adverse events. Further studies regarding the safety and efficacy of iNOS inhibitors are needed.

Despite the fact that all NOS enzymes are intracellular, cell death leads to their release into the bloodstream. As a result, they become detectable in ELISA tests, and it has been found that plasma levels of certain NOS enzymes can be elevated in depression following a stroke [37].

Limitations of the study

The study has some limitations. Firstly, the current investigation is only theoretical, and further exploration of specific approaches is necessary to discover therapeutic benefits in the future. The measurements of selected markers were related to plasma rather than cardiac tissue, which may reduce their sensitivity and specificity. However, it is worth emphasizing that the results remain statistically significant even after accounting for comorbidities. Although these markers may originate from different compartments, nitrosative stress is known to affect the entire organism, including the heart. This systemic impact can contribute to conditions like HFpEF, which is characterized as a clinical syndrome rather than a distinct disease. Secondly, in the HFrEF group, there were significantly more cases of patients with CAD. This is not surprising because, as previously mentioned, this factor is fundamental to HFrEF etiology. The HFrEF patients also had more implanted ICD/pacemakers, which can be explained by current guidelines about primary prevention of sudden cardiac death in individuals with LVEF below 35%.

Conclusions

The present study reveals that nitrosative stress, as assessed by iNOS levels, appears to be more pronounced in HFpEF, whereas endoplasmic reticulum stress, measured by GRP78 concentration, seems to be more intensified in HFrEF. While these observations may not offer definitive insights into the pathophysiology of heart failure, they do serve as a valuable starting point for future research and also hold promise for potential future applications in differential diagnostics.

Funding: This study was financed by a research grant from the Ministry of Education and Science of Poland (SKN/SP/534125/2022).

Conflict of interest: None declared.

Table 1. Baseline characteristics of patients

Characteristics	HFpEF (N = 42)	HFrEF (N = 38)	*P*-value
Female gender, n (%)	24 (57.14%)	8 (21.05%)	0.001
Age, years, mean (SD)	73 (7.14)	68.26 (9.74)	0.020
BMI, kg/m^2, median (IQR)	29.49 (25.77–32.02)	28.41 (24.54–30.49)	0.423
BMI > 30 kg/m^2, n (%)	20 (47.62%)	13 (34.21%)	0.223
NT-proBNP, pg/ml, mean, (IQR)	723 (430.5–1479.5)	1048.50 (592.25–2050.00)	0.136
NYHA II, n (%)	39 (92.86%)	30 (78.95%)	0.071
NYHA III, n (%)	3 (7.14%)	8 (21.05%)	0.071
H2FPEF score, median (IQR)	5 (3–8)	5 (3–6)	0.034
HFA-PEFF score, median (IQR)	6 (5–6)	6 (6–6)	0.818
Comorbidities			
Diabetes Mellitus, n (%)	13 (30.95%)	20 (52.63%)	0.051
Hypertension, n (%)	31 (73.81%)	30 (78.95%)	0.590
History of chronic kidney disease, n (%)	10 (23.81%)	8 (21.05%)	0.768
eGFR at the time of enrollment, ml/min/1,73 m^2, mean (SD)	73.81 (26.55)	67.66 (24.62)	0.287
Renal dysfunction, n (%)	10 (23.81%)	16 (42.11%)	0.081

Coronary Artery Disease, n (%)	21 (50.00%)	26 (68.42%)	0.049
History of MI, n (%)	16 (38.10%)	20 (52.63%)	0.192
Thyroid disease, n (%)	8 (19.05%)	10 (26.32%)	0.437
COPD, n (%)	4 (9.52%)	3 (7.89%)	0.797
Present AF at the time of enrollment, n (%)	20 (47.62%)	16 (42.11%)	0.168
Smoking, n (%)	3 (7.14%)	5 (13.16%)	0.370
Moderate valve disease, n (%)	8 (19.05%)	9 (23.68%)	0.700
Severe valve disease, n (%)	1 (2.38%)	4 (10.53%)	0.133
History of valve surgery, n (%)	2 (4.76%)	7 (18.42%)	0.054
Cardiomyopathy, n (%)	1 (2.38%)	13 (34.21%)	< 0.001
Implanted ICD/pacemaker, n (%)	14 (33.33%)	27 (71.05%)	< 0.001
Documented VF/VT, n (%)	2 (4.76%)	11 (28.95%)	0.003
Medications			
Statin	24 (57.14%)	29 (76.32%)	0.070
ACEI/ARB	36 (85.71%)	30 (78.95%)	0.426
NOAC	30 (71.43%)	21 (55.26%)	0.133

ACEI, AF — Atrial Fibrillation; ARB; BMI — Body Mass Index; CABG — Coronary Artery Bypass Grafting; COPD — Chronic Obstructive Pulmonary Disease; eGFR — estimated Glomerular Filtration Rate; ICD — Implantable Cardioverter-Defibrillator; IQR —

Interquartile Range; NYHA — New York Heart Association; NOAC, SD — Standard Deviation; VF — Ventricular Fibrillation; VT — Ventricular Tachycardia

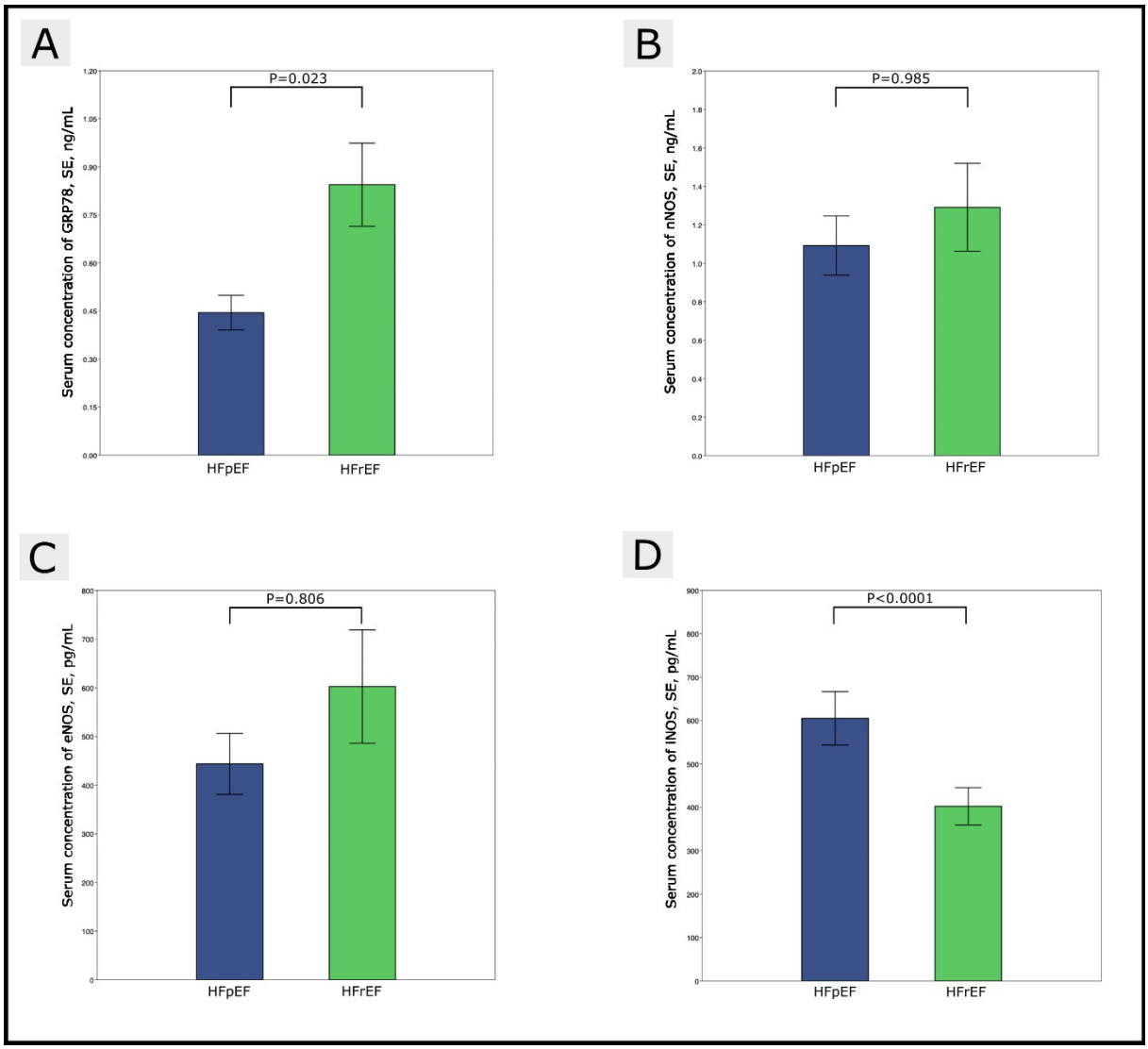
Table 2. Echocardiographic characteristics

	HFpEF (N = 42)	HFrEF (N = 38)	*P*-value
LVEF (%), median (IQR)	55 (51–62)	36 (31–39)	< 0.001
E, cm/s, mean (SD)	84.79 (23.21)	76.58 (36.01)	0.790
A, cm/s, mean (SD)	70.95 (21.83)	56.23 (24.52)	0.041
E/A, ratio, mean (SD)	1.12 (0.85–1.37)	1.33 (0.96–2.20)	0.234
E/e', ratio, median (IQR)	11 (10–16)	13 (9–21)	0.484
TDI septal			
s', cm/s, median (IQR)	6 (5–7)	5 (4–6)	< 0.001
e' cm/s, median (IQR)	6 (5–8)	5 (4–6)	0.004
a' cm/s, median (IQR)	7 (5–7.5)	6 (4–6)	0.015
TDI lateral			
s' cm/s, median (IQR)	7 (6–8)	5 (4–6)	< 0.001
e' cm/s, median (IQR)	8 (6–9)	6 (5–8)	0.016
a' cm/s, median (IQR)	7 (5–8.5)	4 (3–7)	0.004
RWT, mean (SD)	0.48 (0.13)	0.46 (0.09)	0.509
LVMI, g/m^2, median (IQR)	106.47 (85.77–131.58)	154.79 (132.24–187.56)	< 0.001
LAVI, ml/m^2, median (IQR)	39.37 (32.11–50.05)	49.68 (38.28–	0.014

		58.94)	
IVCC (%), median (IQR)	44.44 (35.49–50.00)	35.71 (26.67–40.00)	0.031

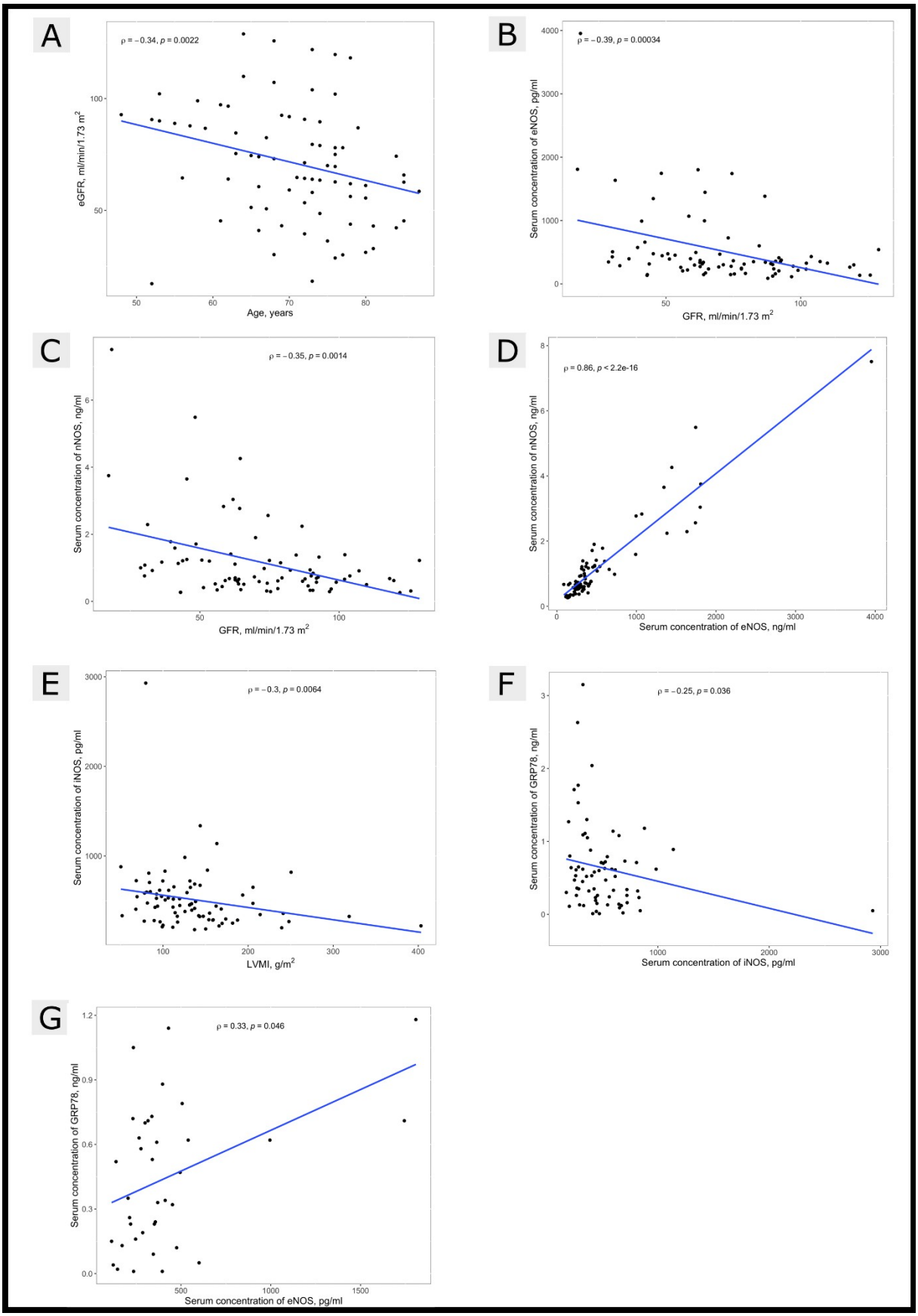
A — peak late diastolic transmitral flow velocity; a' — peak late diastolic mitral annular tissue velocity; E — peak early diastolic transmitral flow velocity; E/e' — the ratio of mitral peak velocity of early filling to early diastolic mitral annular velocity; e' — peak early diastolic mitral annular tissue velocity; IQR — Interquartile Range; IVCC — inferior vena cava collapsibility; LAVI — left atrial volume index; LVEF — left ventricular ejection fraction; LVMI — left ventricular mass index; RWT — relative wall thickness; s', peak systolic mitral annular tissue velocity; TDI — Tissue Doppler imaging

Figure 1. Comparison of serum concentrations of glucose-regulated protein 78 (**A**), neuronal nitric oxide synthase (**B**), endothelial nitric oxide synthase (**C**), inducible nitric oxide synthase (**D**)



eNOS — endothelial nitric oxide synthase; GRP78 — glucose-regulated protein 78; HFpEF — heart failure with preserved ejection fraction; HFrEF — heart failure with reduced ejection fraction; iNOS — inducible nitric oxide synthase; nNOS — neuronal nitric oxide synthase; SE — standard error

Figure 2. Rank correlations between quantitative variables in the general population (**A–F**) and in a population with Heart Failure with Preserved Ejection Fraction (**G**)


A
ρ = −0.34, p = 0.0022
eGFR, ml/min/1.73 m2
Age, years
B
ρ = −0.39, p = 0.00034
Serum concentration of eNOS, pg/ml
GFR, ml/min/1.73 m2
C
ρ = −0.35, p = 0.0014
Serum concentration of nNOS, ng/ml
GFR, ml/min/1.73 m2
D
ρ = 0.86, p < 2.2e-16
Serum concentration of nNOS, ng/ml
Serum concentration of eNOS, ng/ml
E
ρ = −0.3, p = 0.0064
Serum concentration of iNOS, pg/ml
LVMI, g/m2
F
ρ = −0.25, p = 0.036
Serum concentration of GRP78, ng/ml
Serum concentration of iNOS, pg/ml
G
ρ = 0.33, p = 0.046
Serum concentration of GRP78, ng/ml
Serum concentration of eNOS, pg/ml

eGFR — estimated glomerular filtration rate; eNOS — endothelial nitric oxide synthase; GRP78 — glucose-regulated protein 78; HFpEF — heart failure with preserved ejection fraction; iNOS — inducible nitric oxide synthase; LVMI — left ventricular mass index; nNOS — neuronal nitric oxide synthase

References

1. Corrigendum to: 2021 ESC Guidelines for the diagnosis and treatment of acute and chronic heart failure: Developed by the Task Force for the diagnosis and treatment of acute and chronic heart failure of the European Society of Cardiology (ESC) With the special contribution of the Heart Failure Association (HFA) of the ESC. Eur Heart J . 2021; 42(48): 4901, doi: 10.1093/eurheartj/ehab670, indexed in Pubmed: 34649282.
2. Savarese G, Becher PM, Lund LH, et al. Global burden of heart failure: a comprehensive and updated review of epidemiology. Cardiovasc Res. 2023; 118(17): 3272–3287, doi: 10.1093/cvr/cvac013, indexed in Pubmed: 35150240.
3. Simmonds SJ, Cuijpers I, Heymans S, et al. Cellular and molecular differences between HFpEF and HFrEF: A step ahead in an improved pathological understanding. Cells. 2020; 9(1), doi: 10.3390/cells9010242, indexed in Pubmed: 31963679.
4. Boulet J, Sridhar VS, Bouabdallaoui N, et al. Inflammation in heart failure: pathophysiology and therapeutic strategies. Inflamm Res. 2024; 73(5): 709–723, doi: 10.1007/s00011-023-01845-6, indexed in Pubmed: 38546848.
5. Alcaide P, Kallikourdis M, Emig R, et al. Myocardial Inflammation in Heart Failure With Reduced and Preserved Ejection Fraction. Circ Res. 2024; 134(12): 1752–1766, doi: 10.1161/CIRCRESAHA.124.323659, indexed in Pubmed: 38843295.
6. Glezeva N, Baugh JA. Role of inflammation in the pathogenesis of heart failure with preserved ejection fraction and its potential as a therapeutic target. Heart Fail Rev. 2014; 19(5): 681–694, doi: 10.1007/s10741-013-9405-8, indexed in Pubmed: 24005868.
7. Schiattarella GG, Altamirano F, Tong D, et al. Nitrosative stress drives heart failure with preserved ejection fraction. Nature. 2019; 568(7752): 351–356, doi: 10.1038/s41586-019-1100-z, indexed in Pubmed: 30971818.
8. Sabirli R, Koseler A, Mansur N, et al. Predictive value of endoplasmic reticulum stress markers in low ejection fractional heart failure. In Vivo. 2019; 33(5): 1581–1592, doi: 10.21873/invivo.11640, indexed in Pubmed: 31471408.
9. Minamino T, Komuro I, Kitakaze M. Endoplasmic reticulum stress as a therapeutic target in cardiovascular disease. Circ Res. 2010; 107(9): 1071–1082, doi: 10.1161/CIRCRESAHA.110.227819, indexed in Pubmed: 21030724.
10. Vitadello M, Penzo D, Petronilli V, et al. Overexpression of the stress protein Grp94 reduces cardiomyocyte necrosis due to calcium overload and simulated ischemia. FASEB J. 2003; 17(8): 923–925, doi: 10.1096/fj.02-0644fje, indexed in Pubmed: 12670879.
11. Alderton WK, Cooper CE, Knowles RG. Nitric oxide synthases: structure, function and inhibition. Biochem J. 2001; 357(Pt 3): 593–615, doi: 10.1042/0264-6021:3570593, indexed in Pubmed: 11463332.

12. Zhang YH, Jin CZi, Jang JiH, et al. Molecular mechanisms of neuronal nitric oxide synthase in cardiac function and pathophysiology. J Physiol. 2014; 592(15): 3189–3200, doi: 10.1113/jphysiol.2013.270306, indexed in Pubmed: 24756636.
13. Tran N, Garcia T, Aniqa M, et al. Endothelial Nitric Oxide Synthase (eNOS) and the Cardiovascular system: in physiology and in disease states. Am J Biomed Sci Res. 2022; 15(2): 153–177, indexed in Pubmed: 35072089.
14. Kourosh-Arami M, Hosseini N, Mohsenzadegan M, et al. Neurophysiologic implications of neuronal nitric oxide synthase. Rev Neurosci. 2020; 31(6): 617–636, doi: 10.1515/revneuro-2019-0111, indexed in Pubmed: 32739909.
15. Shah AM. Inducible nitric oxide synthase and cardiovascular disease. Cardiovasc Res. 2000; 45(1): 148–155, doi: 10.1016/s0008-6363(99)00316-8, indexed in Pubmed: 10728328.
16. Aljada A, Dandona P. Nitric oxide synthase. Methods Mol Biol. 1998; 108: 191–198, doi: 10.1385/0-89603-472-0:191, indexed in Pubmed: 9921529.
17. Pérez-Torres I, Manzano-Pech L, Rubio-Ruíz ME, et al. Nitrosative stress and its association with cardiometabolic disorders. Molecules. 2020; 25(11), doi: 10.3390/molecules25112555, indexed in Pubmed: 32486343.
18. Aliev G, Bodin P, Burnstock G. Free radical generators cause changes in endothelial and inducible nitric oxide synthases and endothelin-1 immunoreactivity in endothelial cells from hyperlipidemic rabbits. Mol Genet Metab. 1998; 63(3): 191–197, doi: 10.1006/mgme.1997.2664, indexed in Pubmed: 9608541.
19. Al-Khlaiwi T, Habib SS, Al-Khliwi H, et al. Relationship of serum inducible and endothelial nitric oxide synthase with exercise in healthy adult males and patients with type 2 diabetes mellitus. Eur Rev Med Pharmacol Sci. 2023; 27(10): 4619–4625, doi: 10.26355/eurrev_202305_32471, indexed in Pubmed: 37259745.
20. Zamora R, Vodovotz Y, Billiar TR. Inducible nitric oxide synthase and inflammatory diseases. Mol Med. 2000; 6(5): 347–373, indexed in Pubmed: 10952018.
21. Yasukawa T, Tokunaga E, Ota H, et al. S-nitrosylation-dependent inactivation of Akt/protein kinase B in insulin resistance. J Biol Chem. 2005; 280(9): 7511–7518, doi: 10.1074/jbc.M411871200, indexed in Pubmed: 15632167.
22. Guo Y, Wen J, He An, et al. iNOS contributes to heart failure with preserved ejection fraction through mitochondrial dysfunction and Akt S-nitrosylation. J Adv Res. 2023; 43: 175–186, doi: 10.1016/j.jare.2022.03.003, indexed in Pubmed: 36585107.
23. Zhao X, Zhang DQ, Song R, et al. The clinical significance of circulating glucose-regulated protein 78, Caspase-3, and C/EBP homologous protein levels in patients with heart failure. Heliyon. 2023; 9(2): e13436, doi: 10.1016/j.heliyon.2023.e13436, indexed in Pubmed: 36820047.
24. Ma N, Xu N, Yin D, et al. Levels of circulating GRP78 and CHOP in endoplasmic reticulum stress pathways in Chinese type 2 diabetic kidney disease patients. Medicine (Baltimore). 2021; 100(33): e26879, doi: 10.1097/MD.0000000000026879, indexed in Pubmed: 34414940.
25. Ni M, Lee AS. ER chaperones in mammalian development and human diseases. FEBS Lett. 2007; 581(19): 3641–3651, doi: 10.1016/j.febslet.2007.04.045, indexed in Pubmed: 17481612.
26. Dickhout JG, Carlisle RE, Austin RC. Interrelationship between cardiac hypertrophy, heart failure, and chronic kidney disease: endoplasmic reticulum stress as a mediator of pathogenesis. Circ Res. 2011; 108(5): 629–642, doi: 10.1161/CIRCRESAHA.110.226803, indexed in Pubmed: 21372294.

27. Ren J, Bi Y, Sowers JR, et al. Endoplasmic reticulum stress and unfolded protein response in cardiovascular diseases. Nat Rev Cardiol. 2021; 18(7): 499–521, doi: 10.1038/s41569-021-00511-w, indexed in Pubmed: 33619348.
28. Suyama K, Watanabe M, Sakabe K, et al. Overexpression of GRP78 protects glial cells from endoplasmic reticulum stress. Neurosci Lett. 2011; 504(3): 271–276, doi: 10.1016/j.neulet.2011.09.045, indexed in Pubmed: 21970967.
29. Damy T, Ratajczak P, Shah AM, et al. Increased neuronal nitric oxide synthase-derived NO production in the failing human heart. Lancet. 2004; 363(9418): 1365–1367, doi: 10.1016/S0140-6736(04)16048-0, indexed in Pubmed: 15110495.
30. Wu QQ, Xiao Y, Duan MX, et al. Aucubin protects against pressure overload-induced cardiac remodelling via the β -adrenoceptor-neuronal NOS cascades. Br J Pharmacol. 2018; 175(9): 1548–1566, doi: 10.1111/bph.14164, indexed in Pubmed: 29447430.
31. Kolijn D, Pabel S, Tian Y, et al. Empagliflozin improves endothelial and cardiomyocyte function in human heart failure with preserved ejection fraction via reduced pro-inflammatory-oxidative pathways and protein kinase Gα oxidation. Cardiovasc Res. 2021; 117(2): 495–507, doi: 10.1093/cvr/cvaa123, indexed in Pubmed: 32396609.
32. Balakumar P, Chakkarwar VA, Krishan P, et al. Vascular endothelial dysfunction: a tug of war in diabetic nephropathy? Biomed Pharmacother. 2009; 63(3): 171–179, doi: 10.1016/j.biopha.2008.08.008, indexed in Pubmed: 18823739.
33. Tona F, Montisci R, Iop L, et al. Role of coronary microvascular dysfunction in heart failure with preserved ejection fraction. Rev Cardiovasc Med. 2021; 22(1): 97–104, doi: 10.31083/j.rcm.2021.01.277, indexed in Pubmed: 33792251.
34. Fahey JM, Girotti AW. Nitric oxide-mediated resistance to photodynamic therapy in a human breast tumor xenograft model: Improved outcome with NOS2 inhibitors. Nitric Oxide. 2017; 62: 52–61, doi: 10.1016/j.niox.2016.12.003, indexed in Pubmed: 28007662.
35. Chatterjee PK, Patel NSA, Sivarajah A, et al. GW274150, a potent and highly selective inhibitor of iNOS, reduces experimental renal ischemia/reperfusion injury. Kidney Int. 2003; 63(3): 853–865, doi: 10.1046/j.1523-1755.2003.00802.x, indexed in Pubmed: 12631066.
36. Alderton WK, Angell ADR, Craig C, et al. GW274150 and GW273629 are potent and highly selective inhibitors of inducible nitric oxide synthase in vitro and in vivo. Br J Pharmacol. 2005; 145(3): 301–312, doi: 10.1038/sj.bjp.0706168, indexed in Pubmed: 15778742.
37. Wang X, Fang C, Liu X, et al. High Serum Levels of iNOS and MIP-1α are Associated with Post-Stroke Depression. Neuropsychiatr Dis Treat. 2021; 17: 2481–2487, doi: 10.2147/NDT.S320072, indexed in Pubmed: 34349514.