The Pattern of Ventricular Remodeling Influences the Level of Oxidative Stress in Heart Failure Patients

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Oxidative stress (OS) is increased in chronic diseases, including cardiovascular (CV), but there are few data on its effects on the heart and vessels. The isoprostanes (IsoP) are bioactive compounds, with 8-iso-PGF$_{2\alpha}$ being the most representative in vivo marker of OS. They correlate with the severity of heart failure (HF), but because data regarding OS levels in different types of HF are scarce, our study was aimed to evaluate it by assessing the urinary levels of 8-iso-PGF$_{2\alpha}$ and its correlations with various biomarkers and parameters. Our prospective study included 53 consecutive patients with HF secondary to ischemic heart disease or dilative cardiomyopathy, divided according to the type of HF (acute, chronic decompensated or chronic compensated HF). The control group included 13 hypertensive patients, effectively treated. They underwent clinical, laboratory - serum NT-proBNP, creatinine, uric acid, lipids, C reactive protein (CRP) and urinary 8-iso-PGF$_{2\alpha}$ and echocardiographic assessment. HF patients, regardless the type of HF, had higher 8-iso-PGF$_{2\alpha}$ than controls (267.32pg/µmol vs. 19.82pg/µmol, p<0.001). The IsoP level was directly correlated with ejection fraction (EF) (r=-0.31, p=0.01) and NT-proBNP level (r=0.29, p=0.019). The relative wall thickness (RWT) was negatively correlated with IsoP (r=-0.55, p<0.001). Also 8-iso-PGF$_{2\alpha}$ was higher by 213.59pg/µmol in the eccentric left ventricular (LV) hypertrophy subgroup comparing with the concentric subgroup (p=0.014), and the subgroups with severe mitral regurgitation (MR) and moderate/severe pulmonary hypertension (PAH) had the highest 8-iso-PGF$_{2\alpha}$ levels. Male sex, severe MR, moderate/severe PAH, high LV mass and low RWT values were predictive for high OS level in HF patients.Eccentric cardiac remodeling, MR severity and PAH severity are independent predictors of OS in HF patients.

Keywords: oxidative stress, heart failure, isoprostanes, 8-iso-PGF$_{2\alpha}$ ventricular remodeling, relative wall thickness.

OS– the imbalance between oxidants i.e. reactive oxygen species (ROS) and antioxidants – is increased in chronic diseases [1, 2] and particularly in cardiovascular diseases (CVD) [3].

Although the ROS play a well-studied role in CVD pathophysiology – first in atherosclerosis, starting with lipid oxidation [3] and increased vascular production caused by oscillatory shear [4], and later in heart failure (HF) progression – what is still missing is a thorough understanding of the effects of increased ROS levels on cardiac and vascular tissues.

Recent data has proven a strong connection between OS and the renin-angiotensin-aldosterone system (RAAS) in CV system [5], kidney and brain [6] mediated by the endothelial mineralocorticoid receptor, allowing a deeper understanting of the upregulation of OS by aldosterone, ultimately leading to myocardial dysfunction.

In vivo evaluation of OS is further hindered by differences in the choice of the biomarkers, tissues used, techniques and available commercial kits. One of the most accurate method to evaluate OS measures the F2-isoprostanes - prostaglandin-like compounds produced *in vivo* by ciclooxygenase-independent arachidonic acid oxidation (fig. 1)[7, 8].

8-iso-PGF$_{2\alpha}$ was reported to be the most accurate *in vivo* OS marker; measuring its urinary level is simple and accurate because it lacks the fatty component, thus the influence of self-oxidation is minimal.

IsoP are not only diagnostic markers but also active molecules with a large spectrum of biological effects: they promote lipid oxidation and local inflammation in atherosclerosis [3], modulate the fibroblasts and promote collagen production and storage in the myocardial interstitial space [9, 10].

Various biomarkers – e.g. endotelin-1, cytokines, TNF-α, C-reactive protein (CRP) – have proven the increased level of inflammation and endothelial dysfunction. Aldosterone antagonists and statins used in therapy have proven the decrease of inflammation and have shown the correlations between the RAAS, OS, inflammation, myocardial dysfunction and progression of HF [11-13].

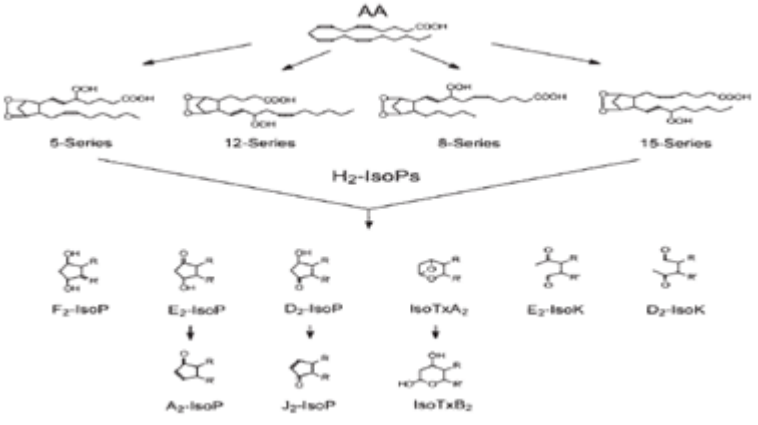

Fig. 1. Mechanims of IsoP formation from the free radical–catalyzed peroxidation of arachidonate [7]

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Besides, patients with compensated HF have increased levels of inflammation markers, OS and extracellular matrix degradation that correlate with plasma aldosterone level [14]. To the best of our knowledge there is no published data regarding OS levels in patients with different types of HF – acute (de novo) or decompensated. Therefore our study was aimed to evaluate the level of OS in different types of HF by assessing the urinary levels of 8-iso-$PGF_{2\alpha}$ and its correlation with various biomarkers.

Experimental part

We performed a prospective study that included 53 consecutive patients admitted to the Cardiology Department of the Emergency Clinical County Hospital in Cluj-Napoca with a diagnosis of HF secondary to ischaemic heart disease or dilated cardiomyopathy. We excluded patients with significant valvular diseases - except for those secondary to left ventricular (LV) and/or mitral annular dilatation – acute coronary syndromes, diabetes mellitus, chronic kidney disease - Creatinine clearance (CLcr) <35 mL/min- uncontrolled hypertension and known current malignancies. The control group consisted of 13 patients with controlled hypertension (beta-blocker and/or ACE-inhibitor) with no other CVD.

All patients were informed on study procedures and signed the informed consent. The study was approved by the Ethics Committee of the Iuliu Haieganu University of Medicine and Pharmacy Cluj-Napoca (No. 377/November 3, 2014).

A blood sample and a morning urine sample was collected from each patient. The laboratory markers assessed were: NT-proBNP, serum creatinin, uric acid, total cholesterol, LDL-cholesterol, HDL-cholesterol, triglycerides and CRP. CLcr was calculated according to the Cockroft-Gault formula, using serum creatinin, age, weight and gender [15].

Urine samples were stored in Ependorf vials at -80°C after adding 10µL of hydroxytoluen butilate per each mL of urine as an antioxidant. The 8-*iso*-$PGF_{2\alpha}$ was assessed by immunoenzymatic method using a commercially available kit from Cayman Chemical Company, Ann Arbor, Michigan, USA with IC_{50} = 10pg/mL and a detection threshold of 2.7pg/mL. 8-*iso*-$PGF_{2\alpha}$ evels were adjusted to the urine creatinine level assessed from the same urine sample using the reaction between urinary creatinine and alkaline picrate (intratest and intertest variability coefficients of 2 and 4%, respectively).

Echocardiography was performed for each patient using a 2-5 MHz transducer on a Siemens Acuson X300 ultrasound machine to evaluate LV structural and performace parameters.

Statistical analysis

Data analysis was performed using R software version 3.2.3 [14]. Demographic, clinical and laboratory characteristics were analyzed for all patients and also stratified for HF type and EF. Data distribution was evaluated using the Shapiro Wilk test. Normal distribution variables were presented as average ± standard deviation while the other as median and interquartile range (IQR). Group comparison was done using the chi-square or Fisher test for categorial variables, Student test for continuous variables with normal distribution and Mann-Whitney U test for ordinal and continuous variables without normal distribution. Comparisons between ≥ 3 independent groups (for quantitative variables without normal distribution) were done using the Kruskal Wallis test, followed by non-parametric post-hoc test. Correlations between quantitative variables were analyzed using Spearman's test (when at least one variable didn't have normal distribution) and Pearson's in all other cases.Univariate linear and multiple regression was performed for the relationship between 8-iso-$PGF_{2\alpha}$ and independent variables- gender, HF presence and echocardiographic parameters (RWT, LV mass, moderate/severe MR and moderate/severe PAH). A two - sided p value ≤ 0.05 was deemed statistically significant.

Results and discussions

Table 1 shows patient demographics. HF patients were older, more frequently males and with longer QRS duration compared to the control group ($p<0.05$ all). Uric acid and NT-proBNP levels were significantly higher while total cholesterol, LDL-cholesterol, HDL-cholesterol and CLcr were significantly lower compared to the control group ($p<0.05$ all). We found no significant differences in triglyceride and CRP levels and the prevalence of smoking and alcohol consumption between

		Patients (n=53)	Controls (n=13)	*P*
Age (years)		67.94 ±11.81	55.38 ± 10.69	.001
BMI (kg/m²)		26.2 (24.2-30)	29.3(26.6–30.3)	.273
Gender (female)		15 (28.3)	9 (69.23)	.008
Smoking		15 (28.3)	4 (30.77)	.09
Alcohol consumption		10 (18.87)	2 (15.38)	.08
HF type	Acute	14 (26.42%)	-	-
	Chronic compensated	19 (35.85%)		
	Chronic decompensated	20 (37.74%)		
NYHA class	II	25 (47.17%)	-	-
	III	19 (35.85%)		
	IV	9 (16.98%)		
Medication	BB	2 (3.77%)	1 (7.69%)	.185
	ACE-inhibitor	11 (20.75%)	0 (0)	
	BB+ACE-I	28 (52.83%)	7 (53.85%)	
	None	12 (22.64%)	5 (38.46%)	
LV end-diastolic volume (ml)		146 (120-190)	-	-
LV mass (g/m²)		156 (130-181)	87 (80 - 100)	.001
EF (%)		25 (20 - 35)	55 (55 - 60)	.001

Table 1
DEMOGRAPHIC, CLINICAL AND BIOLOGICAL PARAMETERS FOR STUDY GROUP AND CONTROL GROUP

MR	Absent/mild	15 (28.3%)	13 (100)	.001
	Moderate	18 (33.96%)	0 (0)	
	Severe	20 (37.74%)	0 (0)	
PAH	Absent	25 (47.17%)	13 (100)	.007
	Mild	12 (22.64%)	0 (0)	
	Moderate	12 (22.64%)	0 (0)	
	Severe	4 (7.55%)	0 (0)	
QRS duration (ms)		120 (80-140)	80 (80-100)	.012
NT-proBNP (pg/mL)		1241(875-1531)	254 (100- 334)	.001
Uric acid (mg/dL)		7.5 (6.5– 8.9)	4.6 (3.8 – 5.2)	.001
Total cholesterol (mg/dL)		155 (140-174)	212 (181-219)	.001
LDL-cholesterol (mg/dL)		93 (72 - 106)	135 (109-157)	.001
HDL-cholesterol (mg/dL)		41 (34 - 48)	50 (41 - 62)	.047
Triglycerides (mg/dL)		108 (87-144)	89 (69 - 112)	.116
CRP (mg/dL)		0.5 (0.4–0.8)	0.4 (0.3 – 0.7)	.142
CLcr (mL/min)		72.73±32.87	103.89±23.54	.002
Adjusted CLcr (mL/min/1.73m²)		65.65 ± 26.08	97.54 ± 19.92	.001

the two groups. We found HF patients had LV structural alterations – increased LV mass, lower EF, longer QRS duration – and higher prevalence of MR and PAH compared to patients in the control group.

Urinary 8-*iso*-$PGF_{2\alpha}$ levels were significantly higher in HF patients compared to the control group (267.32 pg/μmol creatinine vs. 19.82 pg/μmol creatinine, $p<0.001$). The highest urinary 8-*iso*-$PGF_{2\alpha}$ levels were found in acute HF, followed by decompensated HF while compensated HF patients had the lowest, although without statistical significance – 337.1 pg/μmol creatinine (167.8-730.8) vs 271.1 pg/μmol creatinine (183.2 - 483.6) vs 216.7 pg/μmol creatinine (60.4 - 304.7). Each type of HF patients had significantly higher levels of 8-*iso*-$PGF_{2\alpha}$ compared to the control group (table 2).

Regardless of the group, women had significantly lower levels of 8-*iso*-$PGF_{2\alpha}$ compared to men (108.97 pg/μmol creatinine vs. 253.57 pg/μmol creatinine, $p<0.001$). Age, type of medication, presence/absence of smoking and alcohol consumption had no significant effect on the urinary 8-*iso*-$PGF_{2\alpha}$ levels (data not shown).

Although patients in NYHA class IV had the highest levels of 8-*iso*-$PGF_{2\alpha}$, the differences between different NYHA classes were not significant. Urinary 8-*iso*-$PGF_{2\alpha}$ levels increased with the lowering of EF; patients with EF > 36% had the lowest level (table 3).

8-iso-$PGF_{2\alpha}$ levels in patients with concentric LV hypertrophy (RWT ≥ 0.42) were 213.59 pg/μmol creatinine lower compared to those with eccentric LV hypertrophy (RWT < 0.42) ($p=0.014$).

Patients with severe MR and moderate/severe PAH had the highest levels of urinary IsoP compared to other grades of MR and PAH, respectively. We found no significant differences between patients with no or mild MR compared to moderate MR and between absent PAH compared to mild PAH.

Urinary 8-*iso*-$PGF_{2\alpha}$ levels had a significant negative correlation with the body mass index (BMI) ($r = -0.43$, $p < 0.001$) and creatinine clearance but not with uric acid, CRP, total cholesterol, LDL-cholesterol, HDL-cholesterol and triglycerides.

We found a positive correlation between urinary 8-*iso*-$PGF_{2\alpha}$ and plasma NT-proBNP levels ($r=0.29$, $p= 0.019$). Several echocardiographic parameters specific to LV remodeling and LV systolic performance correlated to the 8-*iso*-$PGF_{2\alpha}$ levels (table 4). Significant positive correlations were found between urinary 8-*iso*-$PGF_{2\alpha}$ levels and LV mass (absolute) ($r=0.38$, $p=0.002$), LV mass (indexed) ($r=0.47$, $p=0.001$), end-diastolic LV diameter ($r=0.39$, $p=0.001$), respectively. Significant negative correlations were found between 8-*iso*-$PGF_{2\alpha}$ and RWT ($r=-0.31$, $p=0.01$), LV EF ($r=-0.55$, $p<0.001$), respectively.

Table 2

PAIRED COMPARISONS FOR 8-ISO-PGF2α IN HF AND CONTROL GROUPS

Comparison pair	Difference	95% CI	*p*
pair (1, 2)	0.462	0.219 - 0.72	.99
pair (1, 3)	0.346	0.146 – 0.62	.53
pair (1, 4)	0.171	0.006 – 0.51	.043
pair (2, 3)	0.389	0.186 – 0.64	.75
pair (2, 4)	0.065	0.012 – 0.29	.0005
pair (3, 4)	0.138	0.036 – 0.41	.006

1: acute HF, 2: compensated HF, 3: decompensated HF, 4: control

Parameter	Group	Patients (number)	Urinary 8-*iso*-PGF$_{2\alpha}$	p
EF				
<25	1	15	450.53 (246.96 – 752.43)	.000*
25-29	2	14	312.80 (49.28 – 560.21)	.001[&]
30-35	3	13	199.99 (123.64 – 471.87)	.004[$]
>36	4	24	23.49 (17.26 - 157.81)	0.02[#]
MR				
Absent/mild	1	15	199.99 (40.36 -316.88)	.051[▼]
Moderate	2	18	253.70 (184.35 – 623.81)	.0023[&]
Severe	3	20	428.41 (143.08 – 644.92)	.0016[a]
PAH				
Absent	1	25	134,93 (85.38 – 266.15)	.048[▼]
Mild	2	12	253,70 (60.43 – 667.08)	.003[&]
Moderate/severe	3	16	466,94 (258.06 – 707.24)	.0001[a]

[*]comparing groups 1 and 2; [&] comparing groups 2 and 3; [&] comparing groups 3 and 4; [#] comparing groups 4 and 1; [a] comparing groups 1 and 3

Table 3
CORRELATIONS BETWEEN URINARY 8-ISO-PGF$_{2\alpha}$ AND ECHOCARDIOGRAPHIC PARAMETERS IN SEVERITY SUBGROUPS

Parameter	8-iso-PGF2α	p
Clinical		
Age	0.16	.206
BMI	-0.43	.001*
Biological		
CRP	0.20	.11
Total cholesterol (mg/dL)	0.16	.66
Triglycerides (mg/dL)	0.12	.51
LDL-cholesterol (mg/dL)	0.31	.36
HDL-cholesterol (mg/dL)	0.24	.51
Creatinine clearance(ml/min/m^2)	- 0.29	.018*
Uric acid (mg/dl)	0.22	.49
NT-proBNP (ng/ml)	0.29	.019*
ECG		
QRS duration (ms)	0.11	.69
Echocardiographic		
LV mass (g)	0.38	.002*
Indexed LV mass (g/m$^{2)}$	0.47	.0001*
Relative wall thickness (RWT)	- 0.31	.018
End-diastolic LV diameter (mm)	0.39	.001*
EF (%)	-0.55	.001*

Table 4
CORRELATIONS BETWEEN SERUM 8-ISO-PGF$_{2\alpha}$ LEVELS AND CLINICAL, BIOLOGICAL AND ECHOCARDIOGRAPHIC PARAMETERS IN HF PATIENTS

*P values <0.05 show a statistically significant association

Univariate regression identified the predictive markers of increased OS in HF patients: male sex, moderate MR, moderate/severe PAH, LV mass and RWT (table 5).

When these markers were introduced in multiple variables regression models, only moderate/severe MR and moderate/severe PAH remained predictive for the level of OS in HF patients ($F = 7,6084$, $p < 0.001$) (table 6).

In our study the level of OS– assessed by 8-*iso*-PGF$_{2\alpha}$ – was increased in all HF patients compared to those from the control group, similar to published results that show the OS is higher in chronic diseases, particularly in HF [17]. Acute HF patients had the highest urinary 8-*iso*-PGF$_{2\alpha}$ levels, possibly due to the

Table 5
UNIVARIATE REGRESSION ANALYSIS FOR 8-ISO-$PGF_{2\alpha}$

	B	R2	(95% CI)	*p*
Sex (male vs. female)	0.8276	0.061	(0.0147 – 1.6406)	.046
Group (HF vs. control)	2.36	0.339	(1.54 – 3.19)	.001
RWT (concentric vs eccentric)	-6.39	0.105	(-11.05- 1.72)	.008
Indexed LV mass (g/m2)	0.014	0.187	(0.007 – 0.022)	.001
Moderate/severe MR (present vs. absent)	0.704	0.046	(-0.092 – 1.502)	.028
Moderate/severe PAH (present vs. absent)	1.37	0.133	(0.49 – 2.25)	.003

Table 6
MULTIVARIATE REGRESSION MODEL FOR 8-ISO-$PGF_{2\alpha}$

	B	(95% CI)	
Sex (male vs. female)	0.27	(-0.4163 – 0.9729)	.435
RWT (concentric vs eccentric)	- 1.23	(-5.8179 – 3.3477)	.599
Indexed LV mass (g/m2)	0.002	(-0.0067 – 0.0119)	.584
Moderate/severe MR (present vs. absent)	- 0.88	(-1.604 - -0.1637)	.019
Moderate/severe PAH (present vs. absent)	0.92	(0.0701 – 1.7793)	.038

major acute imbalance in neurohormonal status and homeostasis. The lowest urinary 8-*iso*-$PGF_{25\alpha}$ levels were found in chronic compensated HF patients, suggesting a decrease in the level of OSafter the acute phase of the disease, probably achieved through HF-specific medication and reaching a better hemodynamic status. However, parallel to the worsening of the clinical status and progression of NYHA class, the level of OS increases [18], also shown by our results.

However, the levels of OS in compensated HF patients are significantly higher than in the control patients. Because the control group in our study consisted of hypertensive patients under neurohormonal medication (beta-blockers and/or ACE-I), this allows us to hypothesize that the effect of neurohormonal medication (including aldosterone antagonists) is limited and additional therapeutic options are required to reduce the level of OS in these patients.

Several CV risk factors influence the OS level, such as smoking that significantly increases xanthine oxidase and malondialdehyde levels [19] and decreases vitamins C and E (antioxidants) levels. In our study, age, alcohol and smoking had no major influence on the 8-*iso*-$PGF_{2\alpha}$ levels, suggesting that OS in HF patients is caused by the disease itself and its severity and less by the additional factors.

In our study the cause of HF was ischaemic or dilated cardiomyopathy – both having myocite injury and cardiac remodeling as cornerstones, which causes the progression towards HF [20, 21]. ROS levels – increased both in ischemic heart disease [18] and dilated cardiomyopathy [17, 22] – induce myofibrillar contractile dysfunction through a number of mechanisms, including decreased nitric oxide bioavailability, decreased â-adrenoreceptor expression [23] and ion channel activity [24], activation of protein kinases [25] and oxidation of myofibrillar proteins [26]. Besides, our results show the increase in the level of OS parallels the worsening of LV contractile performance (assessed by EF and LV end-diastolic diameter). We believe the most significant result of our study to be the association between LV remodeling (characterized by dilatation, eccentric remodeling and increased myocardial mass) and increased level of oxidative stress. To the best of our knowledge, this is the first study to present this correlation between the level of OS and LV hypertrophy and also that OS is higher in eccentric than in concentric LV hypertrophy.

Besides, LV remodeling, regardless of neurohormonal activation, is a contributor to HF progression through myocyte degeneration and fibrosis [26] associated to cardiomyocyte reprogramming of the molecular expression and activation of cytokines and free radicals secretion. Hemodynamic stress of volume and pressure overload caused by severe MR and PAH further deteriorate the molecular myocardial environment by releasing ROS and increasing the level of OS, as shown by the results in our study.

Changes in NT-proBNP and 8-iso-$PGF_{2\alpha}$ were similar in HF patients, suggesting their dynamics are parallel in these patients and the main cause of their changes is the cardiac dysfunction itself. The positive correlation between 8-*iso*-$PGF_{2\alpha}$ and NT-proBNP (the *de facto* diagnostic and prognostic marker for HF) is another argument supporting the idea that the level of free radicals is higher in patients with advanced HF and measuring the end-products of aerobic metabolism could further improve the evaluation of disease severity and predict the outcome (with the caveat of further studies needed).

In chronic HF the urinary 8-iso-$PGF_{2\alpha}$ levels correlate with NYHA class [28], plasma BNP and IL-6 levels [17], thus prompting its possible addition to the list of negative prognostic markers [17, 29, 30] alongside other inflammation [31] and OS [32-34] markers.

The limitations of our study are caused by the rather small number of patients included. We tried to overcome the lack of specificity of the markers of OS by assembling a homogeneous patient group with no other chronic illnesses that could influence the ROS level, except for HF secondary to ischaemic or dilated cardiomyopathy.

Conclusions

In our HF group the level of OS is increased regardless of the type of HF (acute or chronic), the level of HF compensation and the neurohormonal medication. Eccentric cardiac remodeling, MR severity and PAH severity are independent predictors of OS in these patients. The prognostic value of the OS level needs further evaluation.

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