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Total Antioxidant Capacity, Uric Acid, and Bilirubin in Patients with Heart Failure due to Non-Ischemic Cardiomyopathy

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ABSTRACT | The impairment of cardiac contractility in chronic heart failure might be caused by an increased level of reactive oxygen species (ROS) and/or a decrease in the antioxidant defense. Therefore, we studied total antioxidant capacity (TAC), uric acid, bilirubin, and malondialdehyde (MDA) concentrations in relation to the hemodynamic status in patients with stable heart failure. As many as 216 right heart catheterization procedures were performed in 107 patients with non-ischemic cardiomyopathy. TAC, uric acid, bilirubin, and MDA concentrations were analyzed. A comparison of groups established on the basis of the median of cardiac index (CI of 2.1 l/min/m^2) and mixed venous oxygen saturation (SvO_2 of 62%) was done. The results showed that significantly higher uric acid and bilirubin levels were determined in the group with CI < 2.1 l/min/m^2 and in the group with SvO_2 < 62%. TAC was higher in patients with lower CI. MDA concentration was similar in all subgroups. TAC correlated with stroke volume ($p < 0.05$), and pulmonary and systemic vascular resistance (SVRI) ($p < 0.05$). Both uric acid and bilirubin correlated significantly and positively with all hemodynamic parameters of pulmonary circulation and SVRI. Additionally, positive correlations between uric acid and TAC ($p < 0.001$) and bilirubin and uric acid ($p < 0.001$) were detected. On the other hand, a negative correlation between TAC and MDA ($p < 0.01$) was observed. In conclusion, this study revealed a novel relationship between redox state and severity of heart failure. An increase of antioxidant parameters in patients with low CI and low SvO_2 may suggest an increased oxidative stress intensity and elevated compensatory mechanism in stable heart failure due to non-ischemic dilated cardiomyopathy.

KEYWORDS | Bilirubin; Heart failure; Total antioxidant capacity; Uric acid; Redox state; Malondialdehyde

ABBREVIATIONS | ACE, angiotensin-converting enzyme; CI, cardiac index; DCM, dilated cardiomyopathy; HA, arterial hypertension; IQR, interquartile range; ICD, implantable cardioverter-defibrillator; LVEDD, left ventricle end-diastolic diameter; LVEDV, left ventricle end-diastolic volume; LVEF, left ventricle ejection fraction; mABP, mean arterial blood pressure; MDA, malondialdehyde; mPAP, mean pulmonary artery pressure; NT-proBNP, N-terminal pro-B-type natriuretic peptide; NYHA, New York Heart Association; PVRI, pulmonary vascular resistance index; PWP, pulmonary wedge pressure; RHC, right heart catheterization; SVI, stroke volume index; SvO_2, mixed venous blood saturation; SVRI, systemic vascular resistance index; TAC, total antioxidant capacity; TPG, transpulmonary gradient; XO, xanthine oxidoreductase

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CONTENTS

1. INTRODUCTION

Excessive production of reactive oxygen species (ROS) or insufficient antioxidant protection generates a condition known as oxidative stress. Oxidative stress plays an important role in the pathological processes of many diseases [1, 2]. ROS generation is closely related to oxygen consumption. Human heart uses a relatively large amount of oxygen and cannot produce enough energy in anaerobic conditions. In the case of ischemia or hypoxia, mitochondrial electron transport is imbalanced. It leads to adenosine triphosphate (ATP) depletion, acidosis, mitochondrial depolarization, intracellular Ca^{2+} overload, and cell death [3]. Decreased oxygen level during either isolated hypoxia or ischemia-associated hypoxia is also a major determinant of myocardial gene expression [4].

Besides mitochondrial respiratory chain, ROS can be produced by xanthine oxidase (XO), reduced nicotinamide adenine dinucleotide phosphate (NADPH) oxidases, lipoxygenase, cytochrome P450 enzymes, nitric oxide synthases, peroxidases, and other hemoproteins [5–7]. All these enzyme systems are present in three major cardiac cell types, namely, cardiac myocytes, fibroblasts, and endothelial cells. Moreover, an increase of circulating or endothelium-bound XO after ischemia-reperfusion can lead to heart damage even though its activity in the cardiac tissue is low [8].

In the end-stage of heart failure and in animals with experimental heart failure an excess formation of superoxide anion or hydroxyl radical using electron paramagnetic resonance spectroscopy (EPR) was demonstrated [9, 10]. One of the methods of oxidative stress detection may be a measurement of organic molecules which are produced as a result of harmful ROS influence on the integrity of biological tissue, such as malondialdehyde (MDA). MDA is one of the small-molecular-weight species created by the fragmentation of polyunsaturated fatty acids which are being attacked by ROS. It is a generally accepted index of lipid peroxidation [11].

Uric acid is the final product of enzymatic degradation of purine nucleosides. The level of this acid may show an activity of circulating XO, an important source of oxygen free radicals. In experimental studies the antioxidant ability of uric acid to protect the erythrocyte membrane against lipid oxidation was demonstrated [12]. Another metabolic pathway which can influence the redox status is heme degradation with reduction of biliverdine by biliverdine reductase to bilirubin and subsequent oxidation of bilirubin to biliverdine by ROS [13].

There are different enzymatic ways to protect against oxidative damage. These include superoxide dismutase (SOD), catalase, glutathione peroxidase (GPx), heme oxygenase 1 (which breaks down the pro-oxidant heme and forms the cytoprotectants carbon monoxide and bilirubin), and haptoglobin (which binds free heme groups in the extracellular interstitium) [14, 15]. The non-enzymatic substances (such as vitamin E and vitamin C and as mentioned above, bilirubin and uric acid) are engaged in ROS

inactivation [9, 16]. Total antioxidant capacity (TAC) is an integrated parameter rather than a simple sum of measurable antioxidants. It reflects the cumulative action of all antioxidants present in the blood plasma [17]. The impairment of cardiac contractility and function in chronic heart failure might be caused by an increased ROS level and/or a decrease in the antioxidant status. Such disturbances were described as the reasons of alcohol-mediated and anthracycline-induced cardiomyopathies [18]. Therefore, we studied the total antioxidant status, uric acid, bilirubin, and MDA in relation to hemodynamic status in patients with stable heart failure due to non-ischemic cardiomyopathy.

2. STUDY GROUPS AND METHODS

2.1. Patients

We analyzed the results of 107 patients (mean age: 46.5 ± 11.0 years old) with heart failure of non-ischemic origin (non-atherosclerotic lesions in coronary angiography) diagnosed as dilated cardiomyopathy (DCM). They underwent right heart catheterization (RHC) (as a routine assessment) according to our heart transplantation protocol based on the Guidelines for Heart Transplantation [19].

Accordingly, due to the dynamic nature of a heart failure, patients on the heart transplantation waiting list, as well as patients in too good conditions for transplantation at first, were regularly re-evaluated. Thus, in this group, within five years eight patients underwent 5 RHC, fourteen patients underwent 4 RHC, fifteen patients underwent 3 RHC, sixteen patients underwent 2, and fifty-four patients underwent one RHC, and the total number of the RCH procedures was 227.

Finally, we excluded 11 catheterizations because of incomplete laboratory data. At the time of assessment all patients were clinically stable; most of them received optimal conventional heart failure therapy (including angiotensin-converting enzyme inhibitors, β-blockers, mineralocorticoid receptor antagonists, digitalis, and diuretics) for at least one month. All patients in this study were nonsmokers and had not taken any vitamin and/or antioxidant supplements for at least 3 days before the study.

The study protocol was approved by the Bioethics Committee of Medical University of Silesia. The informed consent in written form was obtained from all enrolled patients.

2.2. Clinical Assessments

Noninvasive clinical assessment included physical examination, electrocardiography (ECG), and echocardiography. The New York Heart Association (NYHA) classification was used to assess the functional capacity. Echocardiographic images were taken in a standard way as was recommended by the American Society of Echocardiography Committee. Left ventricular end-diastolic volume (EDV) and end-systolic volume (ESV) were obtained from the apical 4- and 2-chamber views by the modified Simpson's method. Left ventricular ejection fraction (LVEF) was calculated in a standard way ([EDV − ESV] × 100 ÷ EDV) to assess ventricular systolic function.

2.3. Cardiac Catheterization Procedure and Hemodynamic Measurements

RHC was performed with the use of Swan–Ganz catheter (Edwards Lifesciences, Warsaw, Poland) administered under local anesthesia (1% lignocaine) via the right jugular vein into pulmonary artery. Patients were fasting, awake, and not sedated. All medications were withheld in the morning of the investigation. Then, two samples of mixed venous blood (SvO_2) were collected in order to determine its saturation. After 20 min of stabilization of circulation parameters, pulmonary wedge pressure (PWP), systolic pulmonary artery pressure (sPAP), diastolic pulmonary artery pressure (dPAP), and right atrium pressure (RAP) were measured. The cardiac output was measured by thermodilution with the use of rapid bolus injection of 10 ml of cold saline. Systolic (sABP) and diastolic (dABP) systemic arterial pressure were measured in a noninvasive way. Hemodynamic parameters were checked five times. Mean values were used for the final evaluation. Acquired data enabled the calculation of mean pulmonary artery pressure (mPAP), mean systemic arterial pressure (mABP), pulmonary vascular resistance index (PVRI), and systemic vascular resistance index (SVRI). mPAP (mm Hg) is the sum of dPAP and one-third of a subtraction of sPAP and dPAP in pulmonary artery (mPAP = dPAP + [sPAP − dPAP] ÷ 3). mABP (mm Hg) is the sum of diastolic arterial

TABLE 1. The demographic and clinical data of all patients at baseline	
Sex (female): n (%)	16 (14.95)
Age (years): median ± IQR	49.15 ± 18.10
BMI (kg/m^2): median ± IQR	27.57 ± 6.56
NYHA class: I–II / III–IV (%)	68 / 39 (63.55 / 36.45)
Duration of illness (years): median ± IQR	2.37 ± 5.02
Arterial hypertension: n (%)	26 (24.3)
Type 2 diabetes: n (%)	13 (12.2)
Atrial fibrillation: n (%)	25 (23.4)
LVEF (%): median ± IQR	22.0 ± 11.0
LVEDV (ml): median ± IQR	200.0 ± 90.0
LVEDD (mm): median ± IQR	68.0 ± 12.0

Note: IQR, interquartile range; BMI, body mass index; NYHA class, New York Heart Association functional class; LVEF, left ventricle ejection fraction; LVEDV, left ventricle end-diastolic volume; LVEDD, left ventricle end-diastolic diameter.

TABLE 2. Laboratory data and hemodynamic parameters (median ± IQR)	
Laboratory Data	
NTproBNP (pg/ml)	1051 ± 1642
Hemoglobin (g/l)	145.0 ± 21,0
Creatinine clearance (ml/min)	113.0 ± 45.81
Sodium (mmol/l)	137.0 ± 4.00
Cholesterol (mgdl)	184.5 ± 64.0
HDL (mg/dl)	42.95 ± 15.5
LDL (mg/dl)	112.7 ± 33.0
Triglycerides (mg/dl)	127.0 ± 77.0
ALAT (IU/l)	26.0 ± 13.0
ASPAT (IU/l)	23.0 ± 12.0
Bilirubin (µmol/l)	14.40 ± 11.0
Uric acid (µmol/l)	432.0 ± 157.0
TAC (mmol Trolox Eq/l)	1.23 ± 0.27
MDA(µmol/l)	4.18 ± 1.98
SvO_2 (%)	62.00 ± 15.0
Hemodynamic Parameters	
Mean pulmonary artery pressure (mm Hg)	24.10 ± 17.53
Mean arterial blood pressure (mm Hg)	93.50 ± 22.30
Pulmonary wedge pressure (mm Hg)	18.50 ± 15.50
Transpulmonary gradient (mm Hg)	7.63 ± 5.90
Stroke volume index, (ml/m^2)	27.78 ± 16.03
Cardiac index (l/min/m^2)	2.11 ± 0.70
Pulmonary vascular resistance index (dyn·s·cm^{-5}/m^2)	291.0 ± 272.9
Systemic vascular resistance index (dyn·s·cm^{-5}/m^2)	3184 ± 1349

Note: NT-proBNP, N-terminal pro-B-type natriuretic peptide; HLD, high-density lipoprotein; LDL, low-density lipoprotein; ALAT, alanine transaminase; ASPAT, aspartate transaminase; TAC, total antioxidant capacity; MDA, malondialdehyde; SvO_2, mixed venous blood saturation; IQR, interquartile range.

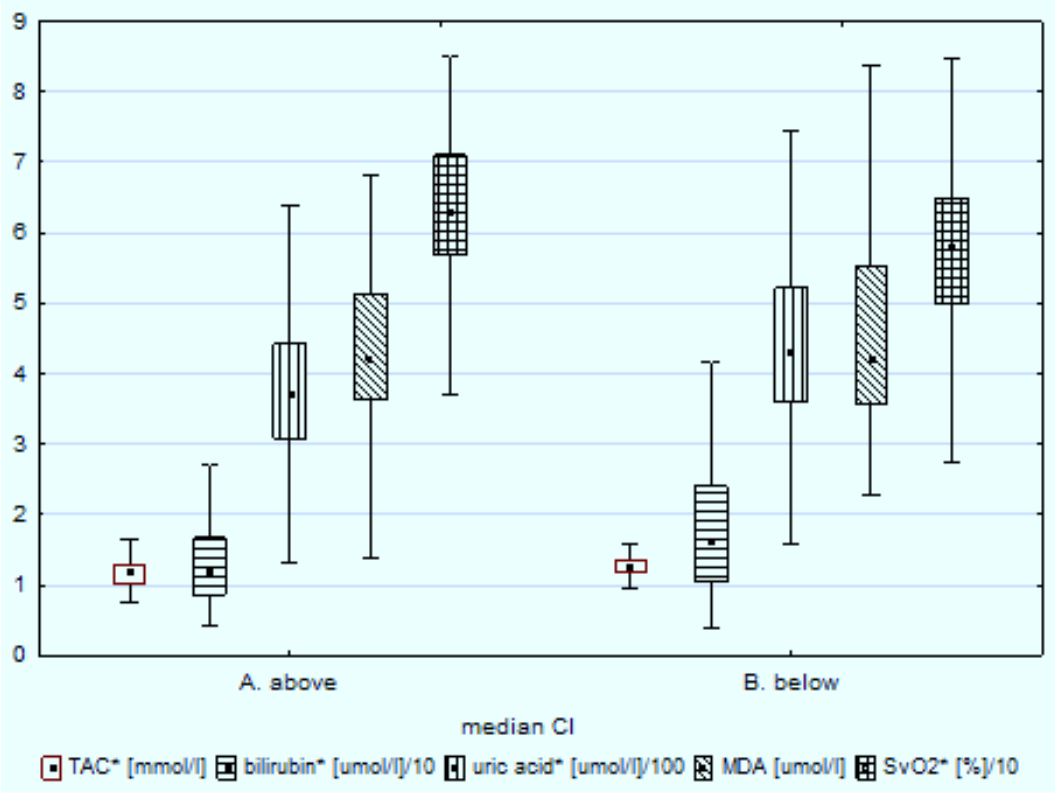

FIGURE 1. Comparison of redox parameters in groups established based on median of cardiac index (CI). The median of CI was calculated to be 2.1 l/m^2. The parallel box plots data represent median (25%–75%) and 1.5 IQR of total antioxidant capacity (TAC), bilirubin/10, uric acid/100, malondialdehyde (MDA), mixed venous blood oxygenation/10 in patients with higher (panel A on the left side) and lower (panel B on the right side) cardiac index. *, $p < 0.05$ versus corresponding group.

blood pressure (dABP) and one-third of a subtraction of systolic arterial blood pressure (sABP) and dABP (mABP = dABP + [sABP − dABP] ÷ 3). PVRI (dyna·s·cm^{-5}/m^2) equals quotient of subtraction mPAP, PWP, and CI (PVRI = 79 × [mPAP − PWP] ÷ CI). (iv) SVRI [dyn·s·cm^{-5}/m^2] equals quotient of subtraction mABP, RAP, and CI (SVRI = 79 × [mABP − RAP] ÷ CI). Blood pressure parameters

TABLE 3. Comparison of the levels of redox parameters in groups established based on cardiac index (median of 2.1 l/min/m^2)

Redox Parameter	Group (CI < median) median ± IQR	Group (CI > median) median ± IQR	p Value
TAC (mmol Trolox Eq/l)	1.27±0.16	1.20 ± 0.27	< 0.005
Bilirubin (µmol/l)	16.05 ± 13.45	11.90 ± 8.00	< 0.001
Uric acid (µmol/l)	4.30 ± 1.61	3.70 ± 1.34	< 0 .01
MDA (µmol/l)	4.20 ± 1.95	4.19 ± 1.50	> 0.10

Note: TAC, total antioxidant capacity; MDA, malondialdehyde; CI, cardiac index; IQR, interquartile range.

were expressed in millimeters of mercury (mm Hg), CI as liters per minute per square meter (l/min/m^2). Resistance was expressed in the unit of dyn·s·cm^{-5}.

2.4. Biochemical Methods

Blood samples for laboratory assessments were obtained from the patients at the time of RHC. Serum was separated by centrifugation at 1500 *g* for 10 min and frozen at −70°C. Uric acid concentration was measured with the colorimetric method (Cobas 6000e501, Roche, Basel, Switzerland). NT-proBNP was measured with the use of chemiluminescence method (Cobas 6000e501, Roche)). Additionally, blood hemoglobin, serum creatinine, bilirubin, and uric acid concentrations were determined with the use of routine techniques. MDA was measured according to the method described by Ohkawa et al. based on the reaction of thiobarbituric acid and spectrofluorometric detection at an excitation wavelength of 515 nm and an emission wavelength of 552 nm. MDA concentration was calculated from the standard curve, prepared from 1,1,3,3-tetraethoxypropane [20]. Serum TAC levels were determined with the method described by Erel [21]. In this method, the antioxidative effect of the sample against the formation of the potent free radical form of 2,2′-azinobis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) is measured. The reaction is monitored spectrophotometrically. The results are expressed as mmol trolox equivalents/liter (Eq/l). Trolox is a water-soluble analog of vitamin E. The redox parameters were compared between established subgroups depending on value of (1) cardiac index and (2) mixed venous oxygen saturation, reflecting the balance between oxygen delivery and oxygen demand (value below and above the median).

2.5. Statistical Analysis

Normality of the distribution of the continuous data was analyzed by Shapiro–Wilk test. The data were presented as median and interquartile range (because of abnormal distribution of most of the data) and were compared with the use of Kolmogorov–Smirnov test. Categorical data were presented as absolute numbers and percentage. Spearman correlation coefficient was calculated for particular parameters. Results were considered statistically significant if $p < 0.05$. Lack of statistical significance was presented as NS (nonsignificant). Statistical analysis was performed with Statistica 10.0 software (Statsoft Inc., Tulsa, OK, USA).

3. RESULTS

3.1. Clinical, Laboratory, and Hemodynamic Characteristics

One hundred and seven patients, aged 49.15±18.10 years old, with heart failure caused by DCM were enrolled into the study (16 patients were women). Finally, 216 measurements were analyzed. At the beginning (the first RHC) 26 patients had treated hypertension and 13 patients had non-insulin-dependent diabetes. The median time from the onset of heart failure symptoms to first analyzed RHC was 2.37 years. Everybody in the group of DCM patients was

TABLE 4. Comparison of the levels of redox parameters in groups established based on mixed venous blood oxygenation (median of 62.0%)

Redox Parameter	Group (SvO_2 < median) median ± IQR	Group (SvO_2 > median) median ± IQR	p Value
TAC (mmol Trolox Eq/l)	1.21 ± 0.26	1.24 ± 0.19	> 0.10
Bilirubin (µmol/l)	15.0 ± 14.10	12.90 ± 8.40	< 0.05
Uric acid (µmol/l)	432.0 ± 170.0	385 ± 127.0	< 0.05
MDA (µmol/l)	4.36 ± 1.73	3.99 ± 1.55	> 0.10

Note: TAC, total antioxidant capacity; MDA, malondialdehyde; SvO_2, mixed venous blood oxygenation; IQR, interquartile range.

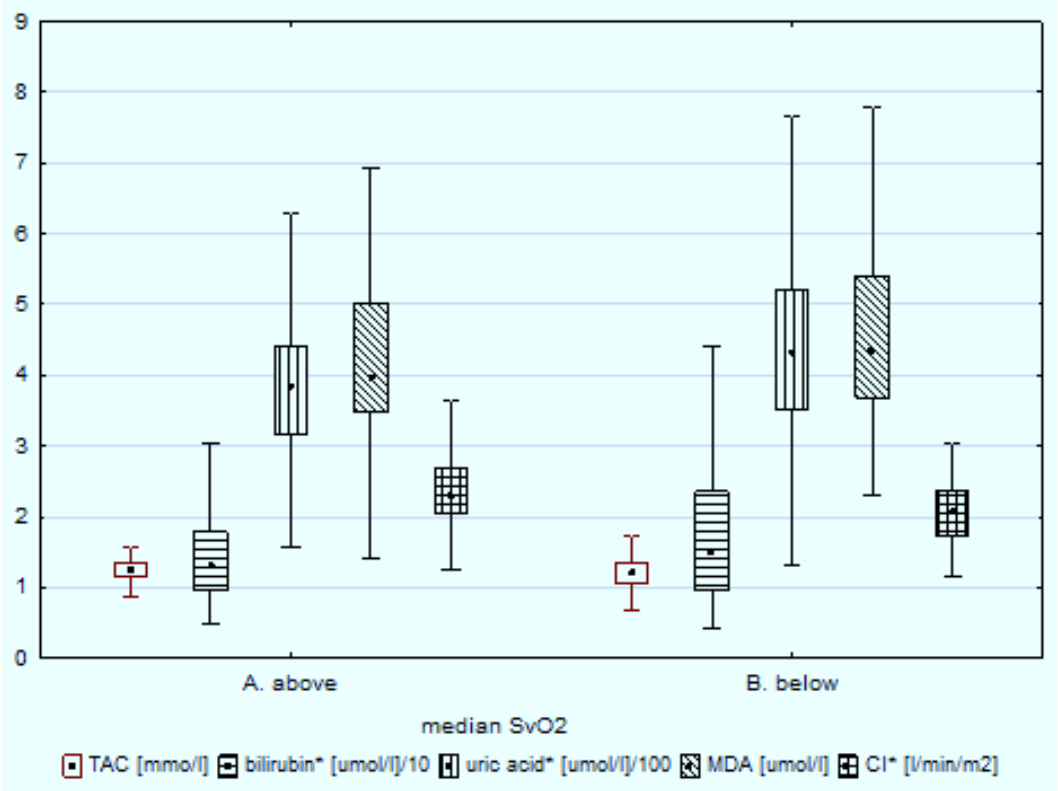

FIGURE 2. Comparison of redox parameters in groups established based on median of mixed venous blood oxygenation (SvO_2). The median of SvO_2 was calculated to be 62%. The parallel box plots data represent median (25%–75%) and 1.5 IQR of total antioxidant capacity (TAC), bilirubin/10, uric acid/100, malondialdehyde (MDA), cardiac index (CI) in patients with higher (panel A on the left side) and lower (panel B on the right side) mixed venous blood oxygenation. *, $p < 0.05$ versus corresponding group.

characterized by typical echocardiographic features of impairment of left ventricle systolic function. LVEF was severely depressed. The majority of patients were treated with β-blockers (91.6%) and either an angiotensin-converting enzyme (ACE) inhibi-inhibitor (94.4%) or an angiotensin receptor blocker (30.8%) and a mineralocorticoid receptor antagonist (87.9%). Some of the patients received digoxin (58.9%), loop and/or thiazide diuretics respectively (60.8% and 19.6%). Demographic and clinical data of all patients at baseline are presented in **Table 1**. All laboratory parameters with redox biomarkers and results of RHC are shown in **Table 2**.

The N-terminal pro-B-type natriuretic peptide (NT-proBNP) level was markedly elevated. There were no abnormalities in the median value of creatinine clearance and the concentrations of sodium, transaminases, and hemoglobin. Hemodynamic measurements showed elevated pulmonary wedge pressure causing a slight increase of pulmonary artery pressure hypertension with elevated PVRI. At the same time, reduced stroke volume and cardiac index were detected, which led to the increase of SVRI and the reduction of SvO_2.

3.2. Comparison of Redox State in Patients with Heart Failure Stratified by Cardiac Index

The group of patients with lower cardiac index showed higher levels of bilirubin, uric acid, and TAC compared to the group with cardiac index above median. There was no significant difference in the

TABLE 5. Spearman correlations between redox biomarkers and hemodynamic parameters

Hemodynamics	Uric Acid	Bilirubin	TAC	MDA
mPAP	0.284 ($p < 0.001$)	0.298 ($p < 0.001$)	0.054 (NS)	0.092 (NS)
mABP	0.275 ($p < 0.001$)	–0.007 (NS)	0.075 (NS)	0.100 (NS)
PWP	0.243 ($p < 0.001$)	0.269 ($p < 0.001$)	0.012 (NS)	0.097 (NS)
TPG	0.225 ($p < 0.001$)	0.197 ($p < 0.01$)	0.090 (NS)	0.007 (NS)
SVI	–0.387 $p < 0.001$	–0.422 ($p < 0.001$)	–0.139 ($p < 0.05$)	–0.050 (NS)
CI	–0.362 ($p < 0.001$)	–0.347 ($p < 0.001$)	–0.207 ($p < 0.01$)	0.000 (NS)
PVRI	0.335 ($p < 0.001$)	0.311 ($p < 0.001$)	0.148 ($p < 0.05$)	–0.002 (NS)
SVRI	0.388 ($p < 0.001$)	0.184 ($p < 0.01$)	0.156 ($p < 0.05$)	0.001 (NS)

Note: mPAP, mean pulmonary artery pressure; mABP, mean arterial blood pressure; PWP, pulmonary wedge pressure; TPG, transpulmonary gradient; SVI, stroke volume index; CI, cardiac index; PVRI, pulmonary vascular resistance index; SVRI, systemic vascular resistance index; NS, not significant.

MDA concentration between the groups (**Table 3** and **Figure 1**).

3.3. Comparison of Redox State in Patients Stratified by Mixed Venous Blood Saturation

The group with SvO_2 below median had significantly higher concentrations of uric acid and bilirubin. The TAC and MDA levels did not differ significantly (**Table 4** and **Figure 2**).

3.4. Correlations between Redox Biomarkers and Hemodynamic Parameters

Both uric acid and bilirubin concentrations had a significant, positive correlation with all hemodynamic parameters of pulmonary circulation and SVRI. On the other hand, the correlation with stroke volume index and cardiac index was negative. There was a positive correlation between uric acid and mABP (but not bilirubin). A negative correlation of TAC and CI was also indicated (**Figure 3**) in contrast to their positive correlations with pulmonary and systemic resistance. There was no significant correlation between MDA and measured hemodynamic parameters (**Table 5**).

3.5. Correlations between Redox Biomarkers

SvO_2 negatively correlated with uric acid, bilirubin, and also MDA. TCA correlated positively with uric acid (**Figure 4**) concentration and negatively with MDA (**Figure 5**). Additionally, a positive correlation between bilirubin and uric acid (**Table 6**), but not between bilirubin and TCA (**Figure 6**) was found.

4. DISCUSSION

It is obvious that heart failure conditions, both in the heart itself and in other tissues, can lead to the formation of harmful ROS. A high level and/or an inadequate removal of ROS, especially superoxide anion, lead to oxidative stress [22]. The investigations in humans and animal models of heart failure have provided evidence that oxidative stress is increased in heart failure and it contributes to disease progression. High metabolic activity of the mitochondria-rich myocardium makes these findings obvious [23]. Despite the advances in the treatment of heart failure, mortality of these patients remains at a high level. Evaluation of biochemical pathways for targeting new therapy seems to be of a great importance. RHC is a gold standard for diagnosis of abnormalities in pulmonary circulation [24]. Additionally, during cardiac catheterization the data necessary to calculate cardiac output, systemic vascular resistance, and sample for SvO_2 measurement can be obtained. In the present study, we analyzed the results of 107 patients (admitted to hospital for routine procedures) with severe left ventricle systolic dysfunction. Taking into account that redox status undergoes constant changes, we decided not to average all the measurements of one patient (mean time between RHC was 13.62 ± 2.75 months). On the contrary, we analyzed each assessed redox marker on the day of the proce-

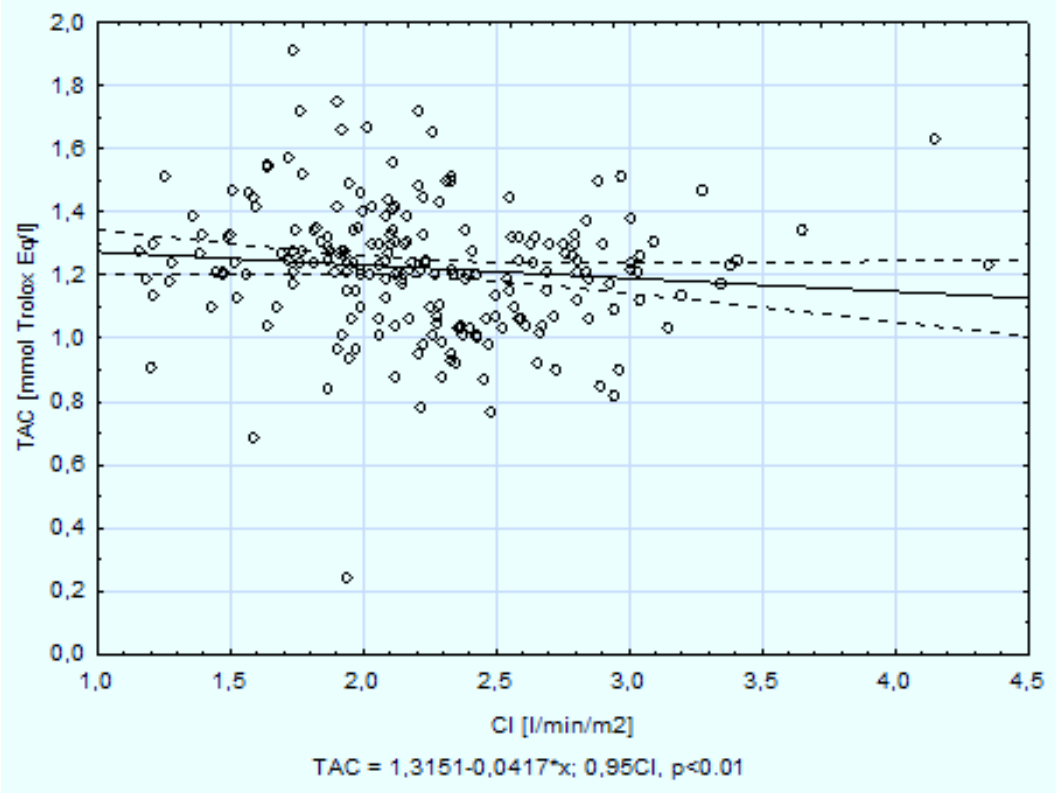

FIGURE 3. The correlation between total antioxidant capacity (TAC) and cardiac index (CI). The scatterplot shows a negative linear relationship between TAC and CI in 216 of simultaneous evaluations. The correlation coefficient r = 0.207 ($p < 0.01$).

dure as a single measurement in correlation to corresponding RHC results (216 procedures). We decided to evaluate the antioxidant defense by measuring the TAC in the serum. Some researchers suggest that TAC may be more useful, less time-consuming, and less expensive than the assessment of particular antioxidants. Since antioxidative effects of the plasma antioxidant components are additive, their synergistic interaction could be determined [21]. We also analyzed simple, inexpensive, and routine assays to measure plasma concentrations of uric acid and bilirubin. At the same time, MDA, as one of ROS products, was assessed.

To our knowledge, this is the first study assessing some parameters of redox state in relation to severity of heart failure expressed by invasively measured CI in stable patients with non-ischemic cardiomyopathy. Patients with CI < 2.1 l/min/m^2 had higher concentrations of TAC in comparison to the group with less decreased CI. The group with lower CI had also higher concentrations of uric acid and bilirubin. However, the concentration of MDA was similar in both groups. It could suggest that the existing antioxidant defense in stable patients was sufficient to inactivate the production of ROS. The inverse correlation between TAC and MDA in the present group (–0.242, $p < 0.01$) can be a proof. There is evidence of oxidative stress during surgical reperfusion of the whole heart and it is related to transient left ventricular dysfunction [25]. On the contrary, a study by Kunt et al. indicated that in patients who developed low cardiac output syndrome during the coronary bypass surgery, the TAC presented a sharp decrease [26]. This difference may be caused by the fast antioxidant consumption in the conditions of ischemia/reperfusion during cardiac surgery which are different from the

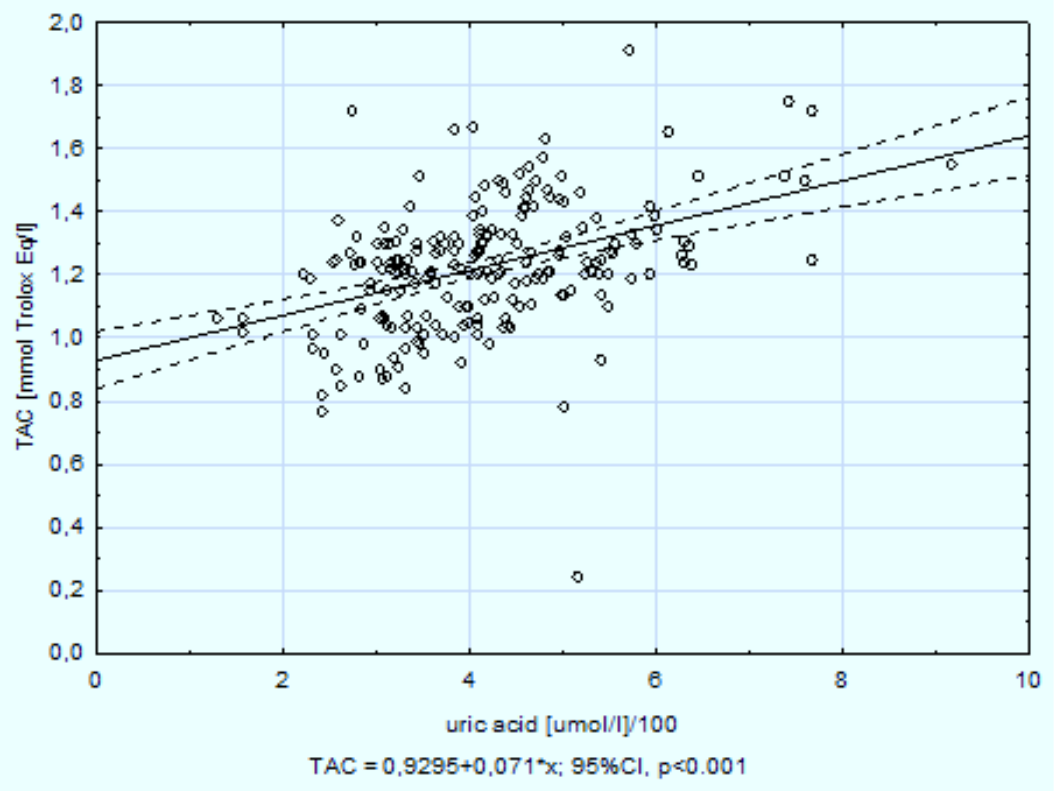

FIGURE 4. The correlation between total antioxidant capacity (TAC) and uric acid. The scatterplot shows a positive linear relationship between TAC and uric acid in 216 of simultaneous evaluations. The correlation coefficient r = 0.414 ($p < 0.001$).

conditions in chronic hypoxia in heart failure. The positive correlations between TAC and uric acid, and between TAC and bilirubin are not surprising since these small molecules are TAC components, like albumin, vitamin E, and vitamin C [21, 27, 28]. The fact that increased bilirubin or uric acid concentrations are related to adverse outcomes makes it difficult to view these parameters as beneficial antioxidants [29, 30]. They are rather markers of the intensity of ROS production under hypoxic conditions. In this context, XO was suggested to be the second major source of ROS formation in human myocardium, and uric acid, the product of XO, is increased in the failing human heart [31]. Previous studies also showed an increased uric acid level in patients with tissue hypoxemia caused by an obstructive sleep apnea or chronic obstructive pulmonary disease (COPD) [32, 33].

The correlation between TAC and NTproBNP may reflect an increase in antioxidative mechanisms in patients with more advanced heart failure. Both uric acid and bilirubin levels were higher in patients with low CI and SvO_2. They also correlated with heart failure severity which was expressed by an increased NTproBNP concentration. In a previous paper we showed correlations between the enzymatic antioxidant manganese superoxide dismutase (MnSOD) and NTproBNP and left ventricular ejection fraction. However, we did not find any correlation with CI and SvO_2 [34]. The association with abnormal hemodynamic parameters, reflecting negative remodeling of hemodynamics of both systemic and pulmonary circulation, was indicated. Similar correlations between CI, NTproBNP, and bilirubin were found by McGovan et al. All these parameters were independent predictors of outcome in heart failure

TABLE 6. Spearman r correlation between biomarkers

	Uric Acid	Bilirubin	TAC	MDA	SvO_2	NT–proBNP
Uric Acid		0.335 (p < 0.001)	0.414 (p < 0.001)	–0.006 (NS)	–0.174 (p < 0.05)	0.231 (p < 0.001)
Bilirubin	0.335 (p < 0.001)		0.132 (NS)	0.034 (NS)	–0.178 (p < 0.05)	0.370 (p < 0.001)
TAC	0.414 (p < 0.001)	0.132 (NS)		–0.242 (p < 0.01)	0.106 (NS)	0.220 (p < 0.001)
MDA	–0.006 (NS)	0.034 (NS)	–0.242 (p < 0.01)		–0.162 (p < 0.05)	–0.039 (NS)
SvO_2	–0.174 (p < 0.05)	–0.178 (P < 0.001)	0.106 (NS)	–0.162 (p < 0.05)		–0.247 (P < 0.001)
NT–proBNP	0.231 (P < 0.001)	0.370 (P < 0.001)	0.220 (P < 0.001)	–0.039 NS	–0.247 (P < 0.001)	

Note: TAC, total antioxidant capacity; MDA, malondialdehyde; SvO_2, mixed venous blood oxygenation; NT-proBNP, N-terminal pro-B-type natriuretic peptide.

patients (median CI of 2.0 l/min/m^2) [35]. The increase of bilirubin concentration in patients with heart failure can be partially explained by an increase in central venous pressure, which leads to passive hyperemia and liver dysfunction (congestive hepatopathy). In this case, the increase in the serum activity of aspartate transaminase (ASPAT) and alanine transaminase (ALAT) (sensitive indicators of liver cell damage) can be observed. The positive correlation of bilirubin concentration and RAP was shown; however, there was no correlation between ALAT activity and RAP in the study group. With regard to the above inconsistency, liver dysfunction is not the only reason for the increase of bilirubin concentration in the studied patients. The oxidative stress can exert an impact on it too. No correlations between uric acid and MDA and between bilirubin and MDA were shown. Uric acid is a powerful scavenger of carbon-centered and peroxyl radicals in the hydrophilic environment, but it is not able to scavenge lipophilic radicals and it cannot break the radical chain propagation within lipid membranes [36]. Similarly, in Gilbert syndrome the elevated bilirubin concentration was associated with an improved resistance to serum oxidation, but it did not influence the MDA concentration [37]. These findings could explain the observation of no correlation between uric acid and bilirubin with MDA despite the negative correlation of TAC and MDA. Perhaps, this is caused by antioxidative properties of other TAC lipophilic components like vitamin E. On the other hand, lack of any correlation between MDA and all hemodynamic parameters of pulmonary circulations suggests that lipid peroxidation plays no role in pulmonary hemodynamic remodeling in heart failure. It should be emphasized that patients did not take antioxidants, but they received the optimal treatment for heart failure. Both ACE inhibitors and some β-blockers have proven antioxidant activity [38].

In conclusion, this study revealed the relationship between redox state and severity of heart failure. Especially, the antioxidant parameters were associated with abnormalities in systemic and pulmonary circulation. We should remember that uric acid and bilirubin also have pro-oxidative properties and sometimes may not have any beneficial role in maintaining an antioxidant-pro-oxidant balance [39, 40]. The increase of antioxidant parameters in patients with low CI and low SvO_2 in resting conditions, paradoxically, may reflect rather oxidative stress intensity than compensatory mechanism. Despite the undeniable importance of oxidative stress in heart failure and promising results of antioxidant-based therapies in animal studies [41], the use of antioxidants did not benefit the prognosis of heart failure patients [42]. The inadequate appreciation of the ROS generation, the choice of the inappropriate antioxidant therapy, or incomplete understanding of antioxidant defenses in humans may be responsible for this defeat.

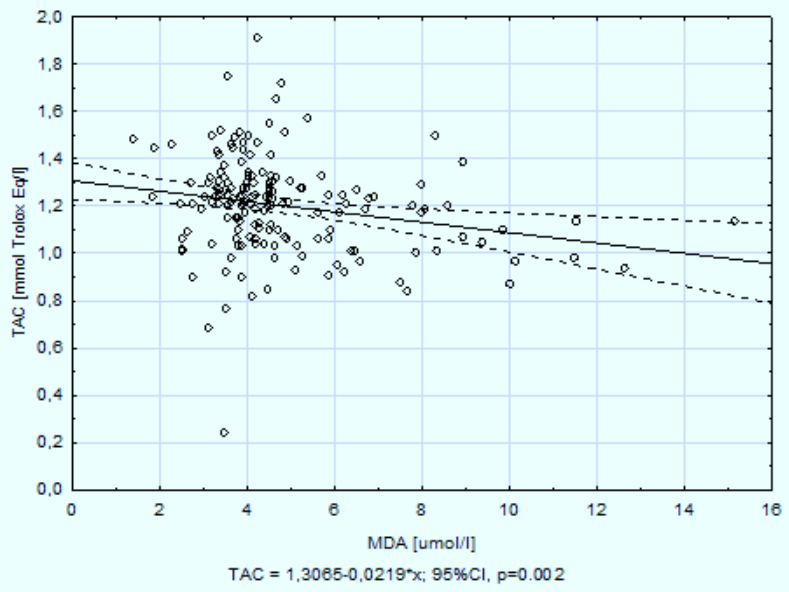

FIGURE 5. The correlation between total antioxidant capacity (TAC) and malondialdehyde (MDA). The scatterplot shows a negative linear relationship between TAC and MDA in 216 of simultaneous evaluations. The correlation coefficient r = –0.242 ($p < 0.01$).

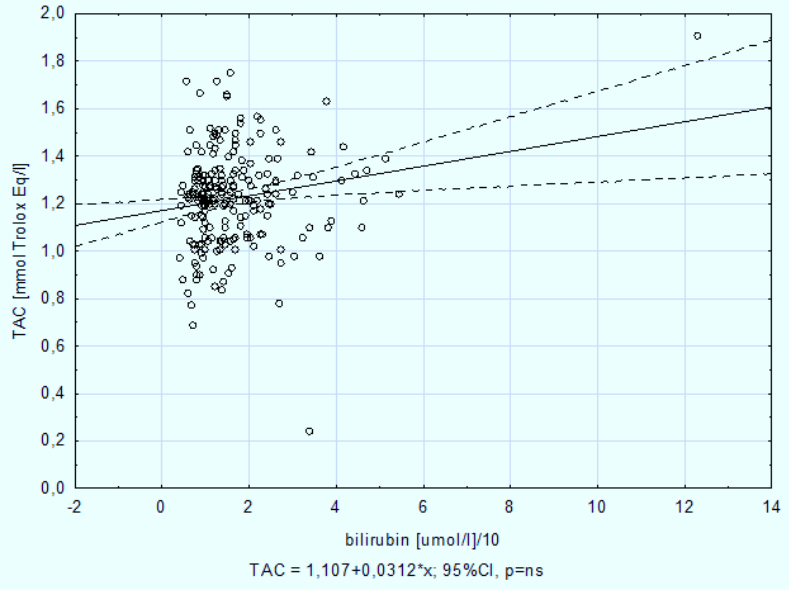

FIGURE 6. The correlation between total antioxidant capacity (TAC) and bilirubin. The scatterplot shows a nonsignificant linear relationship between TAC and bilirubin in 216 of simultaneous evaluations. The correlation coefficient r = 0.132 ($p > 0.05$).

CONFLICTS OF INTEREST

The authors declare no conflict of interest.

REFERENCES

1. Dursun F, Vural Ozec A, Aydin H, Topalkara A, Dursun A, Toker MI, et al. Total oxidative stress, paraoxonase and arylesterase levels at patients with pseudoexfoliation syndrome and pseudoexfoliative glaucoma. *Int J Ophthalmol* 2015; 8(5):985–990. doi: 10.3980/j.issn.2222-3959.2015.05.24.
2. Wang D, Feng JF, Zeng P, Yang YH, Luo J, Yang YW. Total oxidant/antioxidant status in sera of patients with thyroid cancers. *Endocr Relat Cancer* 2011; 18(6):773–782. doi: 10.1530/ERC-11-0230.
3. Chen Q, Moghaddas S, Hoppel CL, Lesnefsky EJ. Ischemic defects in the electron transport chain increase the production of reactive oxygen species from isolated rat heart mitochondria. *Am J Physiol Cell Physiol* 2008; 294(2):C460–466. doi: 10.1152/ajpcell.00211.2007.
4. Huang Y, Hickey RP, Yeh JL, Liu D, Dadak A, Young LH, et al. Cardiac myocyte-specific HIF-1alpha deletion alters vascularization, energy availability, calcium flux, and contractility in the normoxic heart. *FASEB J* 2004; 18(10):1138–1140. doi: 10.1096/fj.04-1510fje.
5. Seshiah PN, Weber DS, Rocic P, Valppu L, Taniyama Y, Griendling KK. Angiotensin II stimulation of NAD(P)H oxidase activity: upstream mediators. *Circ Res* 2002; 91(5):406–413.
6. Xia Y, Tsai AL, Berka V, Zweier JL. Superoxide generation from endothelial nitric-oxide synthase: a Ca^{2+}/calmodulin-dependent and tetrahydrobiopterin regulatory process. *J Biol Chem* 1998; 273(40):25804–25808.
7. Xia Y, Roman LJ, Masters BS, Zweier JL. Inducible nitric-oxide synthase generates superoxide from the reductase domain. *J Biol Chem* 1998; 273(35):22635–22639.
8. Nielsen VG, Weinbroum A, Tan S, Samuelson PN, Gelman S, Parks DA. Xanthine oxidoreductase release after descending thoracic aorta occlusion and reperfusion in rabbits. *J Thorac Cardiovasc Surg* 1994; 107(5):1222–1227.
9. Sam F, Kerstetter DL, Pimental DR, Mulukutla S, Tabaee A, Bristow MR, et al. Increased reactive oxygen species production and functional alterations in antioxidant enzymes in human failing myocardium. *J Card Fail* 2005; 11(6):473–480. doi: 10.1016/j.cardfail.2005.01.007.
10. Tsutsui H, Ide T, Kinugawa S. Mitochondrial oxidative stress, DNA damage, and heart failure. *Antioxid Redox Signal* 2006; 8(9–10):1737–1744. doi: 10.1089/ars.2006.8.1737.
11. Diaz-Velez CR, Garcia-Castineiras S, Mendoza-Ramos E, Hernandez-Lopez E. Increased malondialdehyde in peripheral blood of patients with congestive heart failure. *Am Heart J* 1996; 131(1):146–152.
12. Ames BN, Cathcart R, Schwiers E, Hochstein P. Uric acid provides an antioxidant defense in humans against oxidant- and radical-caused aging and cancer: a hypothesis. *Proc Natl Acad Sci USA* 1981; 78(11):6858–6862.
13. Jansen T, Hortmann M, Oelze M, Opitz B, Steven S, Schell R, et al. Conversion of biliverdin to bilirubin by biliverdin reductase contributes to endothelial cell protection by heme oxygenase-1: evidence for direct and indirect antioxidant actions of bilirubin. *J Mol Cell Cardiol* 2010; 49(2):186–195. doi: 10.1016/j.yjmcc.2010.04.011.
14. Clark JE, Foresti R, Green CJ, Motterlini R. Dynamics of haem oxygenase-1 expression and bilirubin production in cellular protection against oxidative stress. *Biochem J* 2000; 348 Pt 3:615–619.
15. Levy AP, Asleh R, Blum S, Levy NS, Miller-Lotan R, Kalet-Litman S, et al. Haptoglobin: basic and clinical aspects. *Antioxid Redox Signal* 2010; 12(2):293–304. doi: 10.1089/ars.2009.2793.
16. Bruno RS, Leonard SW, Atkinson J, Montine TJ, Ramakrishnan R, Bray TM, et al. Faster plasma vitamin E disappearance in smokers is normalized by vitamin C supplementation. *Free Radic Biol Med* 2006; 40(4):689–697. doi: 10.1016/j.freeradbiomed.2005.10.051.
17. Ghiselli A, Serafini M, Natella F, Scaccini C. Total antioxidant capacity as a tool to assess redox status: critical view and experimental data. *Free Radic Biol Med* 2000; 29(11):1106–1114.
18. Jaatinen P, Saukko P, Hervonen A. Chronic ethanol exposure increases lipopigment accumulation in human heart. *Alcohol Alcohol* 1993; 28(5):559–569.
19. de Jonge N, Kirkels JH, Klopping C, Lahpor JR, Caliskan K, Maat AP, et al. Guidelines for heart transplantation. *Neth Heart J* 2008; 16(3):79–87.
20. Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Anal Biochem* 1979; 95(2):351–358.
21. Erel O. A novel automated direct measurement

method for total antioxidant capacity using a new generation, more stable ABTS radical cation. *Clin Biochem* 2004; 37(4):277–285. doi: 10.1016/j.clinbiochem.2003.11.015.

22. Fukai T, Folz RJ, Landmesser U, Harrison DG. Extracellular superoxide dismutase and cardiovascular disease. *Cardiovasc Res* 2002; 55(2):239–249.
23. Sawyer DB. Oxidative stress in heart failure: what are we missing? *Am J Med Sci* 2011; 342(2):120–124. doi: 10.1097/MAJ.0b013e3182249fcd.
24. Galie N, Humbert M, Vachiery JL, Gibbs S, Lang I, Torbicki A, et al. 2015 ESC/ERS Guidelines for the diagnosis and treatment of pulmonary hypertension: The Joint Task Force for the Diagnosis and Treatment of Pulmonary Hypertension of the European Society of Cardiology (ESC) and the European Respiratory Society (ERS): Endorsed by: Association for European Paediatric and Congenital Cardiology (AEPC), International Society for Heart and Lung Transplantation (ISHLT). *Eur Respir J* 2015; 46(4):903–975. doi: 10.1183/13993003.01032-2015.
25. Ferrari R, Agnoletti L, Comini L, Gaia G, Bachetti T, Cargnoni A, et al. Oxidative stress during myocardial ischaemia and heart failure. *Eur Heart J* 1998; 19 Suppl B:B2–11.
26. Kunt AS, Andac MH. Decrease of total antioxidative capacity in developed low cardiac output syndrome. *Oxid Med Cell Longev* 2012; 2012:356301. doi: 10.1155/2012/356301.
27. Bazvand F, Shams S, Borji Esfahani M, Koochakzadeh L, Monajemzadeh M, Ashtiani MT, et al. Total antioxidant status in patients with major beta-thalassemia. *Iran J Pediatr* 2011; 21(2):159–165.
28. Zablocka-Slowinska K, Porebska I, Golecki M, Kosacka M, Pawelczyk K, Pawlik-Sobecka L, et al. Total antioxidant status in lung cancer is associated with levels of endogenous antioxidants and disease stage rather than lifestyle factors: preliminary study. *Contemp Oncol (Pozn)* 2016; 20(4):302–307. doi: 10.5114/wo.2016.61850.
29. Anker SD, Doehner W, Rauchhaus M, Sharma R, Francis D, Knosalla C, et al. Uric acid and survival in chronic heart failure: validation and application in metabolic, functional, and hemodynamic staging. *Circulation* 2003; 107(15):1991–1997. doi: 10.1161/01.CIR.0000065637.10517.A0.
30. Allen LA, Felker GM, Pocock S, McMurray JJ, Pfeffer MA, Swedberg K, et al. Liver function abnormalities and outcome in patients with chronic heart failure: data from the Candesartan in Heart Failure: Assessment of Reduction in Mortality and Morbidity (CHARM) program. *Eur J Heart Fail* 2009; 11(2):170–177. doi: 10.1093/eurjhf/hfn031.
31. Sakai H, Tsutamoto T, Tsutsui T, Tanaka T, Ishikawa C, Horie M. Serum level of uric acid, partly secreted from the failing heart, is a prognostic marker in patients with congestive heart failure. *Circ J* 2006; 70(8):1006–1011.
32. Drager LF, Lopes HF, Maki-Nunes C, Trombetta IC, Toschi-Dias E, Alves MJ, et al. The impact of obstructive sleep apnea on metabolic and inflammatory markers in consecutive patients with metabolic syndrome. *PLoS One* 2010; 5(8):e12065. doi: 10.1371/journal.pone.0012065.
33. Bartziokas K, Papaioannou AI, Loukides S, Papadopoulos A, Haniotou A, Papiris S, et al. Serum uric acid as a predictor of mortality and future exacerbations of COPD. *Eur Respir J* 2014; 43(1):43–53. doi: 10.1183/09031936.00209212.
34. Wojciechowska C, Romuk E, Tomasik A, Skrzep-Poloczek B, Nowalany-Kozielska E, Birkner E, et al. Oxidative stress markers and C-reactive protein are related to severity of heart failure in patients with dilated cardiomyopathy. *Mediators Inflamm* 2014; 2014:147040. doi: 10.1155/2014/147040.
35. MacGowan GA, Neely D, Peaston R, Wrightson N, Parry G. Evaluation of NT-proBNP to predict outcomes in advanced heart failure. *Int J Clin Pract* 2010; 64(7):892–899. doi: 10.1111/j.1742-1241.2010.02388.x.
36. Muraoka S, Miura T. Inhibition by uric acid of free radicals that damage biological molecules. *Pharmacol Toxicol* 2003; 93(6):284–289.
37. Bulmer AC, Blanchfield JT, Toth I, Fassett RG, Coombes JS. Improved resistance to serum oxidation in Gilbert's syndrome: a mechanism for cardiovascular protection. *Atherosclerosis* 2008; 199(2):390–396. doi: 10.1016/j.atherosclerosis.2007.11.022.
38. Kukin ML, Kalman J, Charney RH, Levy DK, Buchholz-Varley C, Ocampo ON, et al. Prospective, randomized comparison of effect of long-term treatment with metoprolol or carvedilol on symptoms, exercise, ejection fraction, and oxidative stress in heart failure. *Circulation* 1999; 99(20):2645–2651.
39. Kapitulnik J. Bilirubin: an endogenous product of heme degradation with both cytotoxic and cytoprotective properties. *Mol Pharmacol* 2004; 66(4):773–779. doi: 10.1124/mol.104.002832.

40. Sinha S, Singh SN, Ray US. Total antioxidant status at high altitude in lowlanders and native highlanders: role of uric acid. *High Alt Med Biol* 2009; 10(3):269–274. doi: 10.1089/ham.2008.1082.
41. Hamblin M, Smith HM, Hill MF. Dietary supplementation with vitamin E ameliorates cardiac failure in type I diabetic cardiomyopathy by suppressing myocardial generation of 8-iso-prostaglandin F2alpha and oxidized glutathione. *J Card Fail* 2007; 13(10):884–892. doi: 10.1016/j.cardfail.2007.07.002.
42. Keith ME, Jeejeebhoy KN, Langer A, Kurian R, Barr A, O'Kelly B, et al. A controlled clinical trial of vitamin E supplementation in patients with congestive heart failure. *Am J Clin Nutr* 2001; 73(2):219–224.