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THE RELATIONSHIP BETWEEN SERUM URIC ACID LEVEL AND CONCENTRATION OF PROANGIOGENIC MONONUCLEAR PROGENITOR CELLS IN CHRONIC HEART FAILURE PATIENTS

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Abstract. *Background: Serum uric acid is considered as a marker of nature progression of chronic heart failure mediated cardiovascular remodelling. Progression of chronic heart failure associates with declining of circulating mononuclear progenitor cells in the peripheral circulation. The objective of this study was to evaluate the interrelationship between SUA concentrations and proangiogenic MPCs in ischemic chronic heart failure patients.*

Design and Methods: The study population was structured retrospectively after determining the coronary artery disease (CAD) by contrast-enhanced spiral computed tomography angiography in 126 subjects (54 male, 62 women), aged 48 to 62 years, with mild-to-severe ischemic chronic heart failure. Serum uric acid level was measured by enzymatic methods, NT-pro-BNP level was examined by immunoelectrochemiluminesence method. Circulating mononuclear progenitor cells were determined as CD 34$^+$ cells by the flowcytometric technique using High-Definition Fluorescence Activated Cell Sorter methodology. All biomarkers were measured at baseline.

Results: Concentrations of serum uric acid were distributed by quartiles (Me; IQR): QI=20.11 (19.06; 22.33) mmol/l; QII=27.53 (23.2; 31.10) mmol/l; QIII=35.80 (32.0; 39.0) mmol/l; and QIV=44.9 (40.00; 49.60) mmol/l. We found an independent impact of serum uric acid on counts of CD14$^+$CD309$^+$ circulating mononuclear progenitor cells (r=-0.388; P=0.001) and CD14$^+$CD309$^+$Tie2$^+$ circulating mononuclear progenitor cells (r=-0.414; P=0.001), but on CD45$^+$CD34$^+$ circulating mononuclear progenitor cells (r=-0.214; P=0.22) and CD45$^-$CD34$^+$ circulating mononuclear progenitor cells (r=-0.16; P=0.16) did not. Cox proportional adjusted Odds Ratios analyses for CD14$^+$ CD309$^+$ and CD14$^+$CD309$^+$Tie2$^+$ circulating mononuclear progenitor cells by serum uric acid Quartiles (Q) has showed that high Q (Q3 and Q4) of serum uric acid versus low Q (Q1 and Q2) associated with increased risk of depletion of both CD14$^+$CD309$^+$ and CD14$^+$CD309$^+$Tie2$^+$ circulating mononuclear progenitor cells. The ROC analysis has been showed that there was the cut-off point for the serum uric acid level with the best prognostic potential on the risk of decreasing circulating mononuclear progenitor cells in both models equal 31.5 mmol/l.

Conclusion: Circulating level of proangiogenic circulating mononuclear progenitor cells is declined progressively depended on quartiles of serum uric acid level in chronic heart failure subjects. We suggest that mild elevation of serum uric acid might be considered as a predictor of lowed proangiogenic circulating mononuclear progenitor cells in chronic heart failure patient population.

Key words: *chronic heart failure; serum uric acid; circulating mononuclear progenitor cells; predictive value.*

Chronic heart failure (CHF) has been remained a potential fatal complication of any cardiovascular diseases and it is characterized by a systemic inflammatory response that leads to end organ damage [24]. Serum uric acid (SUA) has been shown to be an independent predictor of outcome in the general population and in patients with CHF, the metabolic syndrome, type 2 diabetes mellitus (T2DM), and atherosclerosis, chronic kidney disease [9, 13]. Although sustained hyperuricaemia is considered as independent adverse factor in CHF-related mortality [14], a causal role of SUA is not yet to be established [17]. It has been suggested that there is a relationship between SUA as a "phenotypical" marker of metabolic disorders and a tenderness of reparative processes affected vascular wall and contributed endothelial function. However, less is known about the association between SUA level and circulating mononuclear progenitor cells (MPCs), which have an effect on angiogenesis and tissue reparation [24]. Currently it is well established that MPCs might be recruited resulting in pro-inflammatory cytokines production that are suitable for CHF [11, 26]. Substantially, many studies have demonstrated that level of MPCs is declined progressively in the peripheral circulation with increasing severity of symptomatic CHF [5, 16, 30,]. However, we have been postulated that depletion numerous and functional disability of MPCs in circulation may link SUA with inflammatory response and outcomes in CHF. Therefore, CD34$^+$ MPC populations are not related to cardiovascular remodelling or clinical outcome in CHF patients [5, 8]. Recent evidence suggests circulating proangiogenic CD14$^+$CD309$^+$ and CD14$^+$CD309$^+$Tie^{2+} MPCs levels are decreased in patients with stable CHF [3], but an association of MPCs level with SUA irrespective to clinically significant hyperuricemia is still not investigated.

The objective of this study was to evaluate a relationship between serum uric acid level and circulating proangiogenic MPCs in patients with ischemic mild-to-severe CHF.

Design and Methods

Study population. The study population was structured retrospectively after determining the coronary artery disease (CAD) by contrast-enhanced spiral computed tomography angiography in 126 subjects with symptomatic ischemic mild-to-severe CHF. Chronic heart failure (CHF) was diagnosed according to current clinical guidelines [21]. All patients were Caucasians, have given their written informed consent for participation in the study and met the following inclusion criteria: Q-wave and non-Q-wave MI within 3 months prior to study enrolment; severe kidney and liver diseases that may affect

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clinical outcomes; malignancy; creatinin plasma level above 440 μmol/L; estimated glomerular filtration rate (GFR) < 35 ml/min/м^2; brain injury within 3 months prior to study enrolment; pulmonary edema; tachyarrhythmia; valvular heart disease; thyrotoxicosis; ischemic stroke; intracranial hemorrhage; acute infections; surgery; trauma; all the ischemic events within 3 previous months; inflammations within a previous month; neoplasm; pregnancy; implanted pacemaker, any disorder that, according to investigators, might discontinue patient's participation in the study; and patient's refusal to participate in the study or to give his consent for it.

The study was approved by an institutional review committee. The investigators followed strictly all the requirements to clinical trials in conformity with the World Medical Association Declaration of Helsinki, 1964, *Good Clinical Practice* provided by International Conference on Harmonization (GCP-ICH), Council of Europe Convention for the Protection of. Human Rights and Dignity of the Human Being in view of using achievements in biology and medicine, Convention on Human Rights and Biomedicine, including Additional Protocol to the Convention on Human Rights and Biomedicine, concerning Biomedical Research, and legislation of Ukraine.

Methods for visualization of coronary arteries. Multispiral computed tomography angiography and/or angiographic study have been carried out to verify the ischemic origin of CHF and have been performed for all patients prior to their inclusion in the study. When atherosclerotic lesions of the coronary arteries were verified, patients were subjected to conventional angiographic examination provided indications for revascularization were available. CAD was considered to be diagnosed upon availability of previous angiographic examinations carried out not later than 6 months ago provided no new cardiovascular events occurred for this period, and the procedure are available for assay. The coronary artery wall structure was measured by means of contrast spiral computed tomography angiography [4] on Somatom Volum Zoom scanner (Siemens, Erlangen, Germany) with two detector rows when holding patient's breathe at the end of breathing in. After preliminary native scanning, non-ionic contrast Omnipak (Amersham Health, Ireland) was administered for the optimal image of the coronary arteries. To reconstruct the image, 0.6-mm-width axial tomographic slices were used.

Echocardiography examination. Transthoracic ultrasonic echocardiography was performed according to a conventional procedure on ACUSON apparatus, SIEMENS, Germany, in B-mode regimen and tissue Doppler echocardiography regimen from parasternal, subcostal, and apical positions over the short and long axis with sensor P of 5 MHz. Left ventricular end-diastolic and end-systolic volumes were measured by modified Simpson's planimetric method. Left ventricular ejection fraction (LVEF) was assessed in compliance with the requirements of American Society of Echocardiography [25]. Tissue Doppler echocardiography was carried out in 4-, 3- and 2-chamber projections in each of 16 segments of the left ventricle and in 4 spots of the mitral annulus: at the base of posterior septal, lateral, inferior, and anterior left ventricular walls [23]. Peak systolic (Sm), early diastolic (Em), and late diastolic (Am) myocardial velocities were measured in the mitral annulus area, followed by calculating velocity of early diastolic left ventricular filling (E) to Am (E/Am) ratio and to Em (E/Em) ratio.

Calculation of glomerular filtration rate. Calculation of glomerular filtration rate (GFR) was carried out using MDRD-6 formula [18].

Blood Sampling and Biomarker Measurements. Venous blood samples were drawn in the fasting state in the morning (at 7-8 a.m.) at baseline into cooled silicone test tubes to detect serum uric acid, N-terminal pro -brain natriuretic peptide (NT-pro-BNP), total cholesterol and cholesterol fractions, any biochemical parameters. Samples were processed according to the recommendations of the manufacturer of the analytical technique used. They were centrifuged upon permanent cooling at 6,000 rpm for 3 minutes. Then, plasma was refrigerated immediately to be stored at a temperature not higher than -35^{o}C.

Serum uric acid level measurement. Serum uric acid level was measured by enzymatic methods using chemical analyzer Beckman Synchron LX20. Analytical Range average for serum uric acid was 0.5-82 mmol / L.

NT-pro-Brain Natriuretic Peptide level measurement. NT-pro-BNP level was measured by immunoelectrochemiluminesence method using sets by R&D Systems (USA) on Elecsys 1010 analyzer (Roche, Mannheim, Germany). Calibration of the assay was performed according to the manufacturer's recommendations and values were normalized to a standard curve.

Cholesterol level measurement. Concentrations of total cholesterol (TC) and high density lipoprotein (HDL) cholesterol were determined with Dimension Clinical Chemistry System (Dade Behring Inc, Newark, NJ). Low density lipoprotein (LDL) cholesterol was calculated using Friedewald formula [7].

Circulating EPCs. The flow cytometric technique (FCT) was used for predictable distinguish circulating cells subsets, which depend on expression of CD45, CD34, CD14, Tie-2, and VEGFR2, using High-Definition Fluorescence Activated Cell Sorter (HD-FACS) methodology [29] Accordingly, the cells in question were phenotyped on the basis of their forward scatter characteristic (FSC) and side scatter characteristic (SSC) profiles. The cells were directly stained and analyzed for the phenotypic expression of surface proteins using anti-human monoclonal antibodies, including anti-CD45 FITS (BD Biosciences, USA), anti-CD34 FITS (BD Biosciences, USA), anti-VEGFR-2 known as anti-CD309 (BD Biosciences, USA), anti-Tie2 (BD Biosciences, USA) and anti-CD14 (BD Biosciences, USA). The fluorescence minus one technique was used to provide negative controls and establish positive stain boundaries. After lysis of erythrocytes with UTILIZE wash solution, the samples were centrifuged at 200 g for 15 min; then they were washed twice with PBS and fixed immediately.

Double- or triple-positive events were determined using Boolean principles ('and', 'not', 'or', etc.). Circulating EPCs are defined as CD34 / VEGFR2 positive cells in lack of CD45 expression. 500,000 events were analyzed from each tube. For CD14+ populations, coexpression with Tie-2- and/or VEGFR-2- was determined using quadrant analysis. Standardized cell counts were presented as a percentage of total white blood cells count, which were identified as the total number of all $CD45^+$ cells.

Statistical Analysis. Statistical analysis of the results obtained was carried out in SPSS system for Windows, Version 20 (SPSS Inc, Chicago, IL, USA). The data were presented as mean (M) and error of mean (±m) or 95% confidence interval (CI); median (Me) and interquartile range (IQR). To compare categorical variables between groups, Chi^2 test ($\chi 2$) and Fisher F exact test were used. The circulating NT-pro-BNP and SUA level in the blood failed to have a normal distribution, while distribution of the total cholesterol and cholesterol fractions had a normal character (estimated by means of Kolmogorov-Smirnov test) and was not subjected to any mathematical transformation. Concentrations of SUA were distributed by quartiles (Me; IQR): QI=20.11 (19.06; 22.33) mmol/l; QII=27.53 (23.2; 31.10) mmol/l; QIII=35.80 (32.0; 39.0) mmol/l; and QIV=44.9 (40.00; 49.60) mmol/l. Kruskal–Wallis test was used for difference in medians across quartiles of SUA. The factors, which could be associated potentially with MPCs declining, were determined by univariate and then multivariate regression analysis. Cox proportional multivariate Odds Ratio (OR) and 95% CI were calculated for all independent predictors of MPCs declining. Receiver operating characteristic (ROC) curves were configured to establish cut-off points of SUA level that optimally predicted decreased MPCs. A calculated difference of $P<0.05$ was considered significant.

Results and discussion

General characteristics of study patient population. Table 1 shows a general characteristic of the patients included in the study. As one can see from Table 1, no substantial age and gender differences were found among persons involved in the study. Patients with CHF were distributed in NYHA class I, II, and III (30.2 %; 38.1 %; and 31.7 % respectively), and they, however, had hyperlipidaemia (44.4 %), arterial hypertension (66.7 %), T2DM (36.5 %). Excepted eGFR value and creatinin level patients with different quartiles of SUA were similar in Framingham General Cardiovascular Risk, NYHA classes; proportion of comorbidities incidences; body mass index; hemodynamic performances; fasting glucose; HbA1c; NT-pro-BNP, lipids level. Compared with SUA quartiles I-III, patients with QIV SUA level had higher rate of premature CAD in family anamnesis ($P<0.05$).

Baseline angiographic and treatment characteristics of patients with CHF are presented in Table 2. Coronary arteries with plaques were determined in 36.5 %; 33.3 %; and 20.2 % for 1 vessel, 2 vessels, 3 and more vessels respectively. All the CHF patients were informed about coronary angiography, and they were treated according to current clinical guidelines with diet, lifestyle modification, and drug therapy that included ACE inhibitors / ARBs, beta-blockers, mineralocorticoid antagonists, aspirin or other antiagregants, ivabradin, diuretics, as well as statins and metformin if needed. No significant difference between patients related to coronary arteries with plaques determined depending SUA quartiles were found. ACEI/ARBs and aspirin were given for all patients across SUA quartiles in similar proportions. Compared with QI SUA cohort, patients with QII-IV SUA cohorts had a higher prescribing rate of beta-blockers, mineralocorticoid antagonists diuretics ($P<0.05$), but lower prescribing rate of i/f channel blocker ivabradin, statins ($P<0.05$).

Determination of serum uric acid level in the study patient population. For all CHF subjects, the median level of SUA was 31.00 mmol/l (95 % CI = 22.76 - 41.89 mmol/l). SUA level was categorized into quartiles (Me; 95 % CI) based upon their distribution among all patients. No significant difference in SUA between women and men with CHF (Me =26.40 mmol/l; 95 % CI = 23.51 - 38.70 mmol/l and Me =28.70 mmol/l; 95 % CI = 24.31 - 39.20 mmol/l; P=0.46 respectively) was found.

Circulating MPCs level in the study patient population. Table 3 shows the incidence of various phenotypes of circulating $CD34^+$ MPCs. There was a significant change in level of circulating MPCs depended on quartiles of SUA. Subjects with higher SUA quartile had significantly lower MPCs counts when compared with patient with low quartiles.

The authors have found a closely positive association between $CD45^+CD34^+$ MPCs count and the LVEF (r=0.686; P=0.001), and a negative association with the E/Am ratio (r=-0.566; P=0.001), the E/Em ratio (r=-0.568; P=0.001), eGFR (r=-0.561; P=0.025), SUA (r=-0.482; P=0.001), and the NT-pro-BNP level (r=-0.353; P=0.001). Circulating $CD45^-CD34^+$ MPCs count was associated as a negative linear regression with T2DM (r=-0.614; P=0.001), SUA (r=-0.466; P=0.001), hypertension (r=-0,240; P=0,026), the NT-pro-BNP level (r=-0.605; P=0.002), eGFR (r=-0.423; P=0.012), adherence to smoking (r=-0.222; P=0.040). A positive association was found between the $CD45^-CD34^+$ MPCs count and LVEF (r=0.723; P=0.001), the E/Am ratio (r=0.52; P=0.0024) and the E/Em ratio (r=0.60; P=0.001). The $CD14^+$ $CD309^+$ subpopulation count was associated positively with LVEF (r=0.785; P=0.001), the E/Em ratio (r=0.52; P=0.001), the E/Am ratio (r=0.48; P=0.001); and it was associated negatively with the NYHA class (r=-0.622; P=0.001), T2DM (r= -0.521; P=0.001), SUA (r=-0.508; P=0.001), NT-pro-BNP level (r=-0.362; P=0.001), hypertension (r= -0.320; P=0.005), the total cholesterol level (r=-0.260; P=0.04), adherence to smoking (r=-0.259; P=0.042) and patient's age (r=-0.254; P=0.002). The $CD14^+CD309^+Tie2^+$ subpopulation count showed a positive association with LVEF (r=0.639; P=0.001), the E/Em ratio (r=0.52; P=0.001), eGFR (r= 0.486; P=0.002); and a negative association with the NYHA class (r=-0.657; P=0.001), SUA (r=-0.628; P= 0.001), T2DM (r=-0.610; P=0.001), the NT-pro-BNP level (r=-0.373;

Table 1. General Characteristics of Study Patients

	Patients with CHF across all quartiles of SUA (n=126)	Quartile I (19.06 - 22.33 mmol/l)	Quartile II (23.2 - 31.10 mmol/l)	Quartile III (32.0 - 39.0 mmol/l)	Quartile IV (40.00 - 49.60 mmol/l)	P value
Age, years	58.34±9.60	57.70±6.10	57.40±6.76	60.30±4.20	62.60±6.22	0.42
Male, n (%)	74 (58.7%)	17 (44.7%)	21 (65.6%)	18 (66.7%)	18 (62.0%)	0.28
Framingham General Cardiovascular Risk, %	23 (16 - 27)	22 (15 - 26)	24 (16 - 30)	23 (17 - 31)	23 (16 - 30)	0.75
NYHA class I, n	38 (30.2%)	11 (29.0%)	9 (28.1%)	8 (29.6%)	10 (34.4%)	0.42
NYHA class II, n	48 (38.1%)	14 (36.8%)	12 (37.5%)	10 (37.0%)	12 (41.4%)	0.44
NYHA class III, n	40 (31.7%)	13 (34.2%)	11 (34.4%)	9 (33.3%)	7 (24.1%)	0.48
Arterial hypertension, n (%)	84 (66.7%)	25 (65.8%)	22 (68.8%)	18 (66.7%)	19 (65.5%)	0.86
Hyperlipidaemia,	56 (44.4%)	17 (44.7%)	15 (46.9%)	12 (44.4%)	12 (41.3%)	0.79
T2DM, n (%)	46 (36.5%)	14 (36.8%)	12 (37.5%)	10 (37.0%)	10 (34.5%)	0.80
Premature CAD, n (%)	12 (9.5%)	3 (7.9%)	3 (9.3%)	2 (7.40%)	4 (13.9%)	<0.01
Smoking, n (%)	26 (20.6%)	8 (21.0%)	6 (18.8%)	5 (18.5%)	7 (24.1%)	0.42
Body mass index, kg/m^2	24.1 (95% CI 21.6 – 28.7)	23.3 (95% CI 20.1 – 25.1)	25.0 (95% CI 20.8 – 27.2)	24.6 (95% CI 19.4 – 25.9)	25.2 (95% CI 19.5 – 25.5)	0.58
Mean systolic BP,	130.90±8.41	127.30±5.98	133.80±6.12	129.20±6.34	128.10±4.93	0.44
Heart rate, beat	70.52±3.34	68.56±5.11	70.44±5.68	71.36±4.66	70.16±5.12	0.52
LV EF, %	43.80±0.77	44.10±0.94	43.50±0.97	43.60±0.79	43.10±0.85	0.28
E/Am, U	16.6±0.94	16.3±0.82	16.5±0.76	16.5±0.82	17.1±0.72	0.48
E/Em, U	16.6±1.00	16.2±0.89	16.6±0.72	17.2±0.55	17.0±0.56	0.46
eGFR, ml / min / m^2	82.3 (95% CI = 68.7 – 102.6)	93.5 (95% CI = 88.3 – 100.3)	86.1 (95% CI = 68.3 – 104.1)	83.5 (95% CI = 68.3 – 112.6)	76.2 (95% CI = 61.1 – 98.3)	0.045
HbA1c, %	6.8 (95% CI=4.1-9.5)	6.8 (95% CI=3.9-8.9)	6.9 (95% CI=3.5-9.6)	6.8 (95% CI=3.7-8.9)	6.9 (95% CI=3.8-9.2)	0.86
Fasting glucose, mmol/L	5.20 (95% CI=3.3-9.7)	5.11 (95% CI=3.2-8.5)	5.28 (95% CI=3.1-8.9)	5.21 (95% CI=3.0-9.5)	5.17 (95% CI=3.2-9.0)	0.87
Creatinin, µmol/L	72.3 (95% CI = 58.7 – 92.6)	70.7 (95% CI = 53.1 – 98.5)	71.1 (95% CI = 55.7 – 108.2)	73.7 (95% CI = 53.8 – 109.5)	88.1 (95% CI = 63.0 – 134.2)	0.048
TC, mmol/L	5.1 (95% CI = 3.9 - 6.1)	5.0 (95% CI = 3.7 - 6.4)	5.1 (95% CI = 3.8 - 6.3)	5.0 (95% CI = 3.9 - 6.0)	5.0 (95% CI = 3.7 - 6.2)	0.12
HDL cholesterol, mmol/L	0.91 (95% CI = 0.89 - 1.12)	0.95 (95% CI = 0.92 - 1.14)	0.94 (95% CI = 0.88 - 1.12)	0.91 (95% CI = 0.86 - 1.13)	0.90 (95% CI = 0.83 - 1.10)	0.12
LDL cholesterol, mmol/L	3.23 (95% CI = 3.11 - 4.4)	2.95 (95% CI = 2.84 - 4.6)	3.15 (95% CI = 2.90 - 4.6)	3.24 (95% CI = 3.01 - 4.7)	3.265 (95% CI = 2.98 - 4.64)	0.64
NT-pro-BNP, pg/mL	1533.6 (95% CI = 644.5 – 2560.6)	1263.9 (95% CI = 688.2 – 2120.4)	1446.2 (95% CI = 612.6 – 2873.5)	1590.6 (95% CI = 622.4 – 2710.2)	1873.5 (95% CI = 711.2 – 2790.4)	0.22

N o t e : CI – confidence interval, T2DM - type 2 diabetes mellitus, eGFR – estimated glomerular filtration ratio, TC – total cholesterol, HbA1c – glycated haemoglobin, LDL – low-density cholesterol, HDL – high-density cholesterol, BP – blood pressure, LV EF – left ventricular ejection fraction, U – unit, Em - early diastolic myocardial velocity, Am - late diastolic myocardial velocity, E – peak velocity of early diastolic left ventricular filling.

Table 2. Baseline Angiographic and Treatment Characteristics of patients with CHF depending quartiles of serum uric acid

Variables	Patients with CHF across all quartiles of SUA (n=126)	Quartile I (19.06 - 22.33 mmol/l)	Quartile II (23.2 - 31.10 mmol/l)	Quartile III (32.0 - 39.0 mmol/l)	Quartile IV (40.00 - 49.60 mmol/l)	P value
Coronary arteries with plaques determined						
1 vessel, n (%)	46 (36.5%)	12 (31.6%)	13 (40.6%)	11 (40.7%)	10 (34.5%)	0.66
2 vessels, n (%)	42 (33.3%)	13 (34.2%)	10 (31.3%)	9 (33.3%)	10 (34.5%)	0.72
3 vessels and more, n (%)	38 (30.2%)	13 (34.2%)	9 (28.1%)	7 (25.9%)	9 (31.0%)	0.73
Medications						
ACEI/ARBs, n (%)	126 (100%)	38 (100%)	32 (100%)	27 (100%)	29 (100%)	0.52
Aspirin, n (%)	98 (77.8%)	31 (81.6%)	25 (65.8%)	22 (81.5%)	20 (69.0%)	0.54
Other antiagregants, n (%)	6 (4.8%)	2 (5.2%)	1 (3.1%)	1 (3.7%)	2 (6.9%)	0.86
Beta-blockers, n (%)	104 (82.5%)	16 (42.1%)	32 (100%)	27 (100%)	29 (100%)	<0.05
Ivabradin, n (%)	37 (29.4%)	22 (57.9%)	12 (37.5%)	2 (7.4%)	1 (3.4%)	<0.05
Mineralocorticoid antagonists, n (%)	52 (41.3%)	4 (10.5%)	19 (59.4%)	14 (51.9%)	15 (51.7%)	<0.05
Diuretics, n (%)	106 (84.1%)	19 (50.0%)	25 (78.1%)	27 (100%)	29 (100%)	<0.05
Statins, n (%)	94 (74.6%)	33 (86.8%)	28 (87.5%)	22 (81.5%)	11 (37.9%)	<0.05
Metformin, n (%)	41 (32.5%)	9 (23.7%)	11 (34.3%)	12 (44.4%)	9 (31.0%)	0.054

N o t e : CI – confidence interval, ACEI – angiotensin-converting enzyme inhibitor, ARBs – angiotensin-2 receptor blockers.

P=0.001), a l*ow-density lipoprotein* cholesterol (r=-0.354; P=0.001), the total cholesterol level (r=-0.258; P=0.043), adherence to smoking (r=-0.285; P=0.042), body mass index (r= -0.272; P=0.046).

Association between SUA level and biomarkers. The univariable linear correlation between SUA and $CD45^+CD34^+$ MPCs, $CD45^-CD34^+$ MPCs, $CD14^+CD309^+$ MPCs, $CD14^+CD309^+Tie2^+$ MPCs, NT-pro-BNP concentration, NYHA class, LVEF, T2DM, eGFR was evaluated. A significant positive relationship was found between SUA level and NYHA class (r=0.612; P=0.001); T2DM (r=0.462; P=0.001), NT-pro-BNP (r=0.612; P=0.001), diuretics (r = 0.37, P<0.01), body mass index (r=0.34, P<0.05), hyperlipidaemia (r= 0.32, P<0.05), age (r=0.30, P<0.01), male sex (r= 0.29, P<0.05), and inverse association was obtained between SUA level with eGFR (r=-0.476; P=0.002), LVEF (r=-0.42; P=0.001), $CD45^+CD34^+$ MPCs (r=-0.388; P= 0.001); $CD45^-CD34^+$ MPCs (r=-0.41; P=0.001); $CD14^+CD309^+$ MPCs (r=-0.397; P=0.001); $CD14^+CD309^+Tie2^+$ MPCs (r=-0.442; P=0.001). We did not find a significant association with the other biomarkers examined. Multivariable linear regression analyses were performed for $CD34^+$ phenotypes of MPCs, adjusted for eGFR, BMI, LVEF, NYHA, diuretics, and T2DM. We found an independent impact of SUA on counts of $CD14^+CD309^+$ MPCs (r=-0.388; P=0.001) and $CD14^+CD309^+Tie2^+$ MPCs (r=-0.414; P=0.001), but on $CD45^+CD34^+$ MPCs (r=-0.214; P=0.22) and $CD45^-CD34^+$ MPCs (r=-0.16; P=0.16) did not.

Cox proportional adjusted Odds Ratios analyses for $CD14^+CD309^+$ and $CD14^+CD309^+Tie2^+$ MPCs by SUA Quartiles (Q) has showed that high Q (Q3 and Q4) of SUA versus low Q (Q1 and Q2) associated with increased risk of depletion of both $CD14^+CD309^+$ and $CD14^+CD309^+Tie2^+$ MPCs (Table 4).

The predictive value of SUA level with respect to the MPCs with phenotypes $CD14^+CD309^+$ and $CD14^+CD309^+Tie2^+$ in the patients with CHF was performed using ROC-analysis, the results of which are presented in Fig. 1. The findings suggest a high predictive power of SUA in the both models for declining of $CD14^+CD309^+$ and $CD14^+CD309^+Tie2^+$ MPCs in CHF patients. The estimated AUCs (area under curves) were 0.631 (sensitivity=63.9%; specificity=56.2%) and 0.687 (sensitivity=72.2%; specificity=52.9%) respectively. In

Table 3. Concentrations of MPCs in relation to SUA quartiles

Cell phenotypes, % (median; IQR)	CHF patients (n=126)	SUA quartiles				P value
		Quartile I (19.06 - 22.33 mmol/l)	Quartile II (23.2 - 31.10 mmol/l)	Quartile III (32.0 - 39.0 mmol/l)	Quartile IV (40.00 - 49.60 mmol/l)	
$CD45^+CD34^+\times10^{-4}$, %	1.282 (1.21 – 1.528)	1.77 (1.58 – 1.93)	1.72 (1.53 – 1.91)	1.45 (1.21 – 1.68)	1.05 (0.80 – 1.17)	<0.01
$CD45^-CD34^+\times10^{-4}$, %	0.727 (0.54 – 0.913)	1.01 (0.91 – 1.15)	0.91 (0.81 – 1.01)	0.83 (0.72 – 0.93)	0.63 (0.33 – 0.86)	<0.01
$CD14^+CD309^+\times10^{-4}$, %	29.18 (15.00 – 34.50)	43.9 (33.7 – 54.12)	37.2 (28.8 – 45.59)	28.0 (17.48 – 37.2)	14.0 (11.1 – 19.86)	0.02
$CD14^+CD309^+Tie^{2+}\times10^{-4}$, %	0.67 (0.21 – 1.10)	0.86 (0.74 – 0.98)	0.82 (0.73 – 0.92)	0.67 (0.58 – 0.76)	0.37 (0.29 – 0.56)	0.01

Table 4. Cox proportional adjusted Odds Ratios analyses for $CD14^+CD309^+$ and $CD14^+CD309^+Tie2^+$ MPCs by SUA Quartiles

SUA Quartiles	SUA, mmol/L		Odds Ratios	95% CI	P value
	Mean value	95% CI			
		For $CD14^+CD309^+$ MPCs			
Q1	20.11	19.06 - 22.33	1.0	-	-
Q2	27.53	23.2 - 31.10	1.02	0.88-1.11	0.24
Q3	35.80	32.0 - 39.0	1.18	1.06-1.29	0.001
Q4	44.9	40.00 - 49.60	1.24	1.12-1.46	0.002
		For $CD14^+CD309^+Tie2^+$ MPCs			
Q1	20.11	19.06 - 22.33	1.0	-	-
Q2	27.53	23.2 - 31.10	1.08	1.00-1.20	0.054
Q3	35.80	32.0 - 39.0	1.22	1.11-1.34	0.001
Q4	44.9	40.00 - 49.60	1.38	1.20-1.55	0.001

N o t e : All the respective biomarker-models are adjusted for eGFR, BMI, LVEF, NYHA, diuretics, and T2DM.

this case, the cut-off point for the SUA level that had the best prognostic potential on the risk of decreasing MPCs in both models was 31.5 mmol/L. Thus, these data suggest that for the CHF patient elevation of SUA might be considered as a predictor of lowed proangiogenic MPCs.

Previously reports have been predominantly elucidated a relationship between cardiovascular outcomes and documented hyperuricemia in patients with acute and chronic heart failure [13, 19, 20]. The effects of SUA on all-cause mortality at different SUA cut-offs in CHF patient population was evaluated using meta-regression.

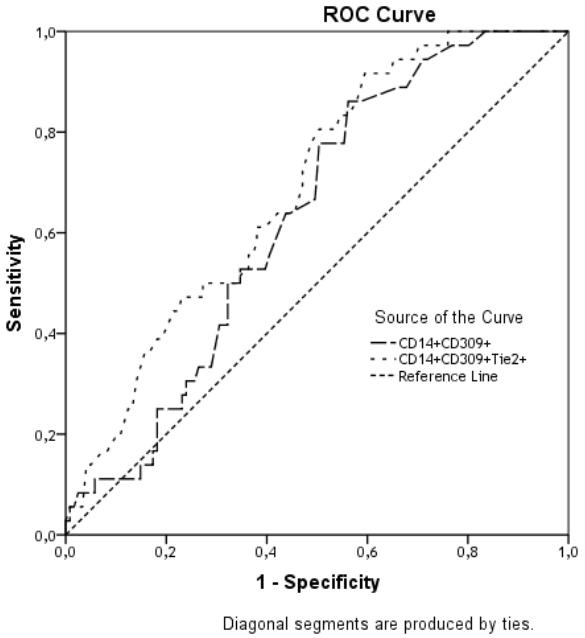

Figure 1: The predictive power of SUA in the both models for declining of $CD14^+CD309^+$ and $CD14^+CD309^+Tie2^+$ MPCs in CHF patients. Results of the Receive Operation Characteristic analysis

There was a linear association between SUA after 7 mg/dL and mortality [27]. Arguing against a pure protective role of SUA in cardiovascular disease [1], we found that levels of SUA remained independently associated with lowed proangiogenic MPCs after adjusting for parameters with known impact on concentrations of MPCs. Moreover, even tendency to increase of SUA in CHF patient population associated with progressively declining proangiogenic MPCs, which have a tremendous tissue repair capacity. Probably, these findings might be taken into consideration to be explaining controversial role of SUA in CHF evolution and outcomes. Really, significant association between high SUA level and BMI, diuretic use, some biomarkers, such as NT-pro-BNP, as wells as with hemodynamic performances (E/Ea and LVEF) even beyond declining eGFR was frequently noted in recent investigations [12, 27]. Amin A, Vakilian F, Maleki M. (2011) [2] reported that mild elevated SUA levels in patients with systolic CHF is associated with impaired clinical and hemodynamic profile and might be used as a noninvasive indicator of elevated left ventricular filling pressures. Misra D, Zhu Y, Zhang Y, Choi HK (2011) have been evaluated the independent impact of CHF status (compensation or decompensation) on SUA levels among men with high cardiovascular risk profile [22]. Investigators found that mild elevated SUA associated with increased risk of CHF decompensation (OR=1.67; 95 % CI 1.21 to 2.32). Although hyperuricemia predominantly affects men, in our study we have not received a confirmation of differences in SUA between men and women with CHF. Noted, that there was not a documented hyperuricaemia (SUA≥6 mg/dL for women and ≥8 mg/dL for men) especially required treatment in patients enrolled in the study. However, the interpretation of SUA levels for individual CHF patients may be confusing, but even small increased SUA levels at symptomatic

Area Under the Curve

Test Result Variables	Area	Std. Error[a]	Asymptotic Sig.[b]	Asymptotic 95% Confidence Interval	
				LowerBound	Upper Bound
$CD14^+CD309^+$	0.631	0.047	0.017	0.540	0.722
$CD14^+CD309^+Tie2^+$	0.687	0.046	0.001	0.598	0.776

The test result variables: $CD14^+CD309^+$, $CD14^+CD309^+Tie2^+$ has at least one tie between the positive actual state group and the negative actual state group.

a. Under the nonparametric assumption

b. Null hypothesis: true area = 0.5

CHF might be discussed as a marker of endothelial dysfunction and, probably, as an indicator of tissue repair disorders. Current evidence suggests that SUA could be a marker of oxidative damage in several settings distinguished CHF, such as overweight, obesity, diuretic use. We confirmed a slight linear association SUA with BMI and diuretic use, but direct effect of BMI and diuretic use on number of circulating MPCs was not found. It has predisposed that SUA may realize their capacity for modulating tissue damage through other mechanisms irrespective SUA clearance. Therefore, it is not clear whether this increase in SUA levels may be a counter-regulatory process or a pathophysiological detrimental factor. Because SUA is a product of xanthine oxidase (XO), apoptosis and tissue hypoxia that are suitable for CHF lead to increased purine catabolism, which, in turn, increases XO activity and subsequently SUA levels [28]. Indeed, has a significant association with poor outcomes in CHF patients without CKD but not in those with CKD [6, 15, 32], suggesting that hyperuricaemia may predict poor outcomes when it is primarily a marker of increased XO activity, but not when it is primarily due to impaired renal excretion of uric acid [32]. In controversy of data presented by Filippatos GS et al (2011) [6], no association between SUA and BMI was found in our study. Diuretics, widely used to treat CHF, increase SUA by stimulating the reabsorption of sodium and urate in the proximal tubule. Although we obtained an association between SUA and diuretics administration, the direct effect of diuretics in depletion of MPCs in patient study population was not determined. Additionally, increased SUA might also associated with coronary artery disease and with its risk factors, such as obesity, hypertension, hypertriglyceridemia, dyslipidaemia and T2DM, and worsen renal function. Multivariable linear regression analyses that was performed for $CD34^+$ phenotypes of MPCs with adjustment for eGFR, LVEF, NYHA, diuretics, and T2DM, has been showed an independent impact of SUA on counts of $CD14^+CD309^+$ MPCs and $CD14^+CD309^+Tie2^+$ MPCs. We suggest that tissue ischemia determines an increase in XO, which leads to an increase in SUA levels, and mediates suppression of recruitment, mobbing, differentiation and functional status of MPCs through Akt/STAT/MAP-kinase mechanisms, that is reflection of chronic inflammatory, oxidative stress and, probably, catabolic state suitable for CHF [28, 31]. Finally, significant confounder impacting SUA levels on population of proangiogenic MPCs with involving several pathogenetic mechanisms are predisposed. It is possible to address to new investigations whether relationships between SUA and proangiogenic MPCs are multidimensional, or if they can be associated with clinical outcomes.

Conclusion. Circulating level of proangiogenic MPCs is declined progressively depended on quartiles of SUA level in CHF subjects. We suggest that mild elevation of SUA (>31.5 mmol/L) might be considered as a predictor of lowed proangiogenic MPCs in CHF patient population.

Abbreviations:
BMI: Body Mass Index; CAD: Coronary Artery Disease; T2DM: Type two Diabetes Mellitus; eGFR: Estimated glomerular filtration rate; HbA1c: Glycated hemoglobin; CHF: Chronic Heart Failure; LVEF: Left Ventricular Ejection Fraction; SUA: serum uric acid; MPCs – mononuclear progenitor cells; LDL: low-density cholesterol, HDL: high-density cholesterol.

Conflict of interests: The Authors declare that they have no competing interests.

Study restrictions: This study has some restrictions. The authors believe that a greater cohort is to be desirable to improve the power of the study. There is a variation in the definition of EPCs, the number of existing cardiovascular risk factors in various patients, and in the interaction between EPCs and other hematopoietic progenitor, inflammatory cells or platelets. The authors suppose that these restrictions might have no significant impact on the study data interpretation.

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Ethical principles. The investigators followed strictly all the requirements to clinical trials in conformity with the World Medical Association (WMA) Declaration of Helsinki, 1964, *Good Clinical Practice* proveided by International Conference on Harmonization (GCP-ICH), Council of Europe Convention for the Protection of Human Rights and Dignity of the Human Being in view of using achievements in biology and medicine, Convention on Human Rights and Biomedicine, including Additional Protocol to the Convention on Human Rights and Biomedicine, concerning Biomedical Research, and legislation of Ukraine.

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The author contributions. AB conceptualized and designed the study and carried out the analysis and interpretation of data. AK performed visualization procedures, carried out the biochemical analysis including the determination of proangiogenic MPCs, and participated to the acquisition and analysis of data. All authors read and approved the final manuscript.

А.Е. Березин, А.А. Кремзер

Взаимоотношение между сывороточным уровнем мочевой кислоты и концентрацией проангиогенных мононуклеарных прогениторных клеток у пациентов с хронической сердечной недостаточностью

Мочевая кислота сыворотки рассматривается как маркер прогрессирования хронической сердечной недостаточности, связанный с сердечно-сосудистым ремоделингом. Прогрессирование хронической сердечной недостаточности ассоциируется со снижением циркулирующих мононуклеарных прогениторных клеток в периферической крови. Целью данно-

го исследования было оценить взаимосвязь между концентрацией мочевой кислотой сыворотки и циркулирующими мононуклеарными прогениторными клетками у больных с хронической сердечной недостаточностью ишемического генеза.

Материалы и методы: В исследование было ретроспективно включено 126 пациентов (54 мужчин, 62 женщины) в возрасте от 48 до 62 лет, с хронической сердечной недостаточностью ишемического генеза. Диагноз ишемической болезни сердца был верифицирован с помощью контрастно усиленной спиральной компьютерной томографии/ ангиографии. Уровень мочевой кислоты сыворотки измерялся ферментативным методом, уровень NT-pro-BNP был определен с помощью иммуноферментного анализа. Циркулирующие мононуклеарные прогениторные клетки были определены как CD 34^+ клетки методом проточной цитометрии. Все биомаркеры были измерены в начале исследования.

Результаты: Концентрации мочевой кислоты сыворотки были распределены по квартилям (Me; IQR): QI=20,11 (19,06; 22,33) ммоль/л; QII=27,53 (23,2; 31,10) ммоль/л; III квартиль = 35,80 (32,0; 39,0) ммоль/л; и IV квартиль=44,9 (40,00; 49,60) ммоль/л. Мы выявили независимое влияние мочевой кислоты сыворотки на количество $CD14^+$ $CD309^+$ циркулирующих мононуклеарных прогениторных клеток (r=-0,388, p = 0,001) и $CD14^+$ $CD309^+$ $Tie2^+$ циркулирующих мононуклеарных прогениторных клеток (r=-0,414, p=0,001), и не выявили влияния на $CD45^+$ $CD34^+$ циркулирующих мононуклеарных прогениторных клеток (r=-0,214, p=0,22) и $CD45^-$ $CD34^+$ циркулирующих мононуклеарных прогениторных клеток (r=-0,16, p=0,16).

Анализ Cox пропорциональных соотношений для $CD14^+$ $CD309^+$ и $CD14^+$ $CD309^+$ $Tie2^+$ циркулирующих мононуклеарных прогениторных клеток по квартилям МКС (Q) показал, что высокая Q (Q3 и Q4) из мочевой кислоты сыворотки против низкой Q (Q1 и Q2), связана с повышенным риском истощения $CD14^+$ $CD309^+$ и $CD14^+$ $CD309^+$ $Tie\text{-}2^+$ циркулирующих мононуклеарных прогениторных клеток.

При использовании ROC анализа было установлено, что точка cut-off для концентрации мочевой кислоты с наилучшим прогностическим потенциалом, свидетельствующая о снижении уровня циркулирующих мононуклеарных прогениторных клеток, равна 31,5 ммоль/л.

Вывод: Уровень снижения циркулирующих мононуклеарных прогениторных клеток зависит от квартилей уровня мочевой кислоты сыворотки у пациентов с хронической сердечной недостаточностью. Мы предполагаем, что умеренное увеличение уровней мочевой кислоты сыворотки может рассматриваться как предиктор снижения проангиогенных циркулирующих мононуклеарных прогениторных клеток в популяции пациентов с хронической сердечной недостаточностью. (Арх. клин. эксп. мед. – 2014. – Т. 23, № 1. – С. 8-16)

Ключевые слова: хроническая сердечная недостаточность; мочевая кислота сыворотки; циркулирующие мононуклеарные прогениторные клетки; прогностическая ценность.

О.Є. Березін, О.О. Кремзер

Взаємовідносини між сироватковим рівнем сечової кислоти ТА концентрацією проангіогенних мононуклеарних прогеніторних клітин у пацієнтів з хронічною серцевою недостатністю

Сечова кислота сироватки розглядається як маркер прогресування хронічної серцевої недостатності, пов'язаний із серцево-судинним ремоделінгом. Прогресування хронічної серцевої недостатності асоціюється зі зниженням циркулюючих мононуклеарних прогеніторних клітин в периферичній крові. Метою даного дослідження було оцінити взаємозв'язок між концентрацією сечової кислоти сироватки і циркулюючих мононуклеарних прогеніторних клітин у хворих з хронічноюї серцевою недостатністю ішемічного ґенезу.

Матеріали і методи: У дослідження було ретроспективно включено 126 пацієнтів (54 чоловіків, 62 жінки) віком від 48 до 62 рокі, з хронічної серцевої недостатності ішемічного ґенезу. Діагноз ішемічної хвороби серця був верифікований за допомогою контрастно посиленої спіральної комп'ютерної томографії / ангіографії. Рівень сечової кислоти сироватки вимірювався ферментативним методом, рівень NT-pro-BNP був визначений за допомогою імуноферментного аналізу. Циркулюючі мононуклеарні прогеніторні клітини були визначені як CD 34^+ клітини методом проточної цитометрії. Всі біомаркери були виміряні на початку дослідження.

Результати: Концентрації сечової кислоти сироватки були розподілені за квартилями (Me; IQR): QI=20,11 (19,06; 22,33) ммоль/л; QII=27,53 (23,2; 31,10) ммоль/л; III квартиль=35,80 (32,0; 39,0) ммоль/л; і IV квартиль=44,9 (40,00; 49,60) ммоль/л. Ми виявили незалежне вплив МКС на кількість $CD14^+$ $CD309^+$ циркулюючих мононуклеарних прогеніторних клітин (r=-0,388 , p=0,001) і $CD14^+$ $CD309^+$ $Tie2^+$ циркулюючих мононуклеарних прогеніторних клітин (r=-0,414, p= 0,001), і не виявили впливу на $CD45^+$ $CD34^+$ циркулюючих мононуклеарних прогеніторних клітин r=-0,214, p=0,22) і $CD45^-$ $CD34^+$ циркулюючих мононуклеарних прогеніторних клітин (r=-0,16, p=0,16) .

Аналіз Cox пропорційних співвідношень для $CD14^+$ $CD309^+$ та $CD14^+$ $CD309^+$ $Tie2^+$ циркулюючих мононуклеарних прогеніторних клітин по квартилях сечової кислоти сироватки (Q) показав, що висока Q (Q3 і Q4) з сечовою кислотою сироватки проти низької Q (Q1 і Q2), пов'язана з підвищеним ризиком виснаження як $CD14^+$ $CD309^+$ та $CD14^+$ $CD309^+$ $Tie2^+$ циркулюючих мононуклеарних прогеніторних клітин.

При використанні ROC аналізу було встановлено, що точка cut-off для концентрації сечової кислоти сироватки з найкращим прогностичним потенціалом, що свідчить про зниження рівня циркулюючих мононуклеарних прогеніторних клітин, дорівнює 31,5 ммоль/л.

Висновок: Рівень зниження циркулюючих мононуклеарних прогеніторних клітин залежить від квартилей рівня сечової кислоти сироватки у пацієнтів з хронічною серцевою недостатністю. Ми припускає-

мо, що помірне збільшення рівнів сечової кислоти сироватки може розглядатися як предиктор зниження проангіогенних циркулюючих мононуклеарних прогеніторних клітин у популяції пацієнтів з хронічною серцевою недостатністю. (Арх. клін. експ. мед. – 2014. – Т. 23, № 1. 8–16 С.)

Ключові слова: хронічна серцева недостатність; сечова кислота сироватки; циркулюючі мононуклеарні прогеніторні клітини; прогностична цінність.

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