

EFFECTS OF HIGH DOSES OF ALLOPURINOL ON SERUM URIC ACID AND CARDIAC BIOMARKERS IN CHRONIC HEART FAILURE

ADRIANA ILIEȘIU^{1*}, ALEXANDRU CÂMPEANU¹, DACIANA MARTA², GABRIELA USCOIU¹, IRINA PÂRVU¹, ANA CIOBANU¹, MARIANA JINGA³, GABRIELA GHEORGHE¹

¹"Carol Davila" University of Medicine and Pharmacy, "Prof. Dr. Th. Burghel" Clinical Hospital, Department of Internal Medicine and Cardiology, 20th Panduri Rd, Bucharest, Romania

²"Victor Babes" National Institute of Pathology and Biomedical Research, 99-101th Splaiul Independentei St, Bucharest, Romania

³"Dr. Carol Davila" Central Military Emergency Hospital, Department of Internal Medicine, 88th Mircea Vulcanescu St., Bucharest, Romania

*corresponding author: adilies@yahoo.com

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Abstract

Allopurinol, by xanthine oxidase inhibition, could be a therapeutic option in patients with chronic heart failure (CHF). The purpose of the study was to assess the effects of high dose allopurinol on serum uric acid (sUA) and cardiac biomarkers, N-terminal pro-brain natriuretic peptide (NT-proBNP), growth differentiation factor-15 (GDF-15) and osteopontin (OPN), in patients with CHF. In 57 patients with stable CHF, New York Heart Association (NYHA) class III and reduced systolic function, under optimal medical therapy, ejection fraction (EF) and E/e' ratio as markers of left ventricular function were assessed by echocardiography. sUA, NT-proBNP, GDF-15 and OPN were measured before and after 6 months of treatment with allopurinol 600 mg/day. Patients with elevated filling pressures (E/e' $\geq$ 13) at baseline had lower EF and higher levels of NT-proBNP, GDF 15 and sUA. After 6 months, for 23 patients (40%) who improved their NYHA class, also the levels of NT-proBNP and GDF 15 decreased, along with lower sUA values. OPN was elevated at baseline and increased even further after 6 months, being unaffected by the CHF clinical course. Treatment with high doses of allopurinol was favourable, but heterogeneous effects were registered on cardiac biomarkers in CHF patients. Since the therapeutic benefits were associated with important sUA level decrease, sUA is a useful marker of CHF outcome and efficiency of allopurinol treatment.

Rezumat

Allopurinolul, prin inhibarea xantinoxidazei, ar putea fi o opțiune terapeutică la pacienții cu insuficiență cardiacă cronică (ICC). Scopul studiului a fost de a evalua efectele allopurinolului în doze mari asupra nivelului acidului uric seric (AUS) și a biomarkerilor cardiaci, N-terminal pro-brain natriuretic peptide (NT-proBNP), factorul 15 de diferențiere a creșterii (GDF-15) și osteopontina (OPN), la pacienții cu ICC. Au fost evaluați ecocardiografic, înregistrându-se fracția de ejeție (FE) a ventriculului stâng și raportul E/e', marker al funcției diastolice ventriculare, 57 de pacienți cu ICC stabilă, clasa III conform *New York Heart Association* (NYHA) și funcție sistolică scăzută, sub tratament medical optim. Au fost determinate AUS, NT-proBNP, GDF-15 și OPN înainte și după 6 luni de tratament cu allopurinol 600 mg/zi. Pacienții cu presiuni de umplere crescute (E / e' $\geq$ 13) au avut la includere FE mai mică și valori mai mari de NTproBNP, GDF 15 și AUS. După 6 luni, la 23 de pacienți (40%), odată cu ameliorarea clasei NYHA, au scăzut atât NT-proBNP cât și GDF 15, în paralel cu scăderea mai importantă a AUS. Nivelul OPN, crescut la includerea în studiu, a continuat să crească după 6 luni, nefiind influențat de evoluția ICC. Tratamentul cu allopurinol în doze mari are efecte favorabile, dar heterogene, asupra biomarkerilor cardiaci în ICC. Deoarece beneficiile terapeutice s-au asociat cu scăderea nivelului AUS, acesta este un marker util de evoluție și de eficiență a tratamentului cu allopurinol în ICC.

Keywords: chronic heart failure (CHF), serum uric acid (sUA), allopurinol, NT-proBNP, GDF15, osteopontin

Introduction

Chronic heart failure (CHF) has become a major epidemic disease, with high morbidity and mortality. Several cardiac biomarkers might prove beneficial to identify patients at higher risk and to improve the heart failure therapy. Beside natriuretic peptides, validated CHF biomarkers, other prognostic biomarkers have emerged.

Growth differentiation factor 15 (GDF-15) is a cardioprotective cytokine, member of the Transforming Growth Factor Beta cytokine superfamily. Although not expressed in the normal heart, its level increases in cardio myocytes and other cardiovascular cells during incident cardiovascular stress [1]. GDF15 is not useful for the CHF diagnosis, but it provides independent prognostic information [2].

Osteopontin (OPN) is an extracellular matrix protein overexpressed in a variety of diseases. The normal heart expresses low levels of OPN, however OPN levels increase markedly in patients with CHF and predict detrimental cardiac remodeling and dysfunction [3].

Serum uric acid (sUA) is the end-product of xanthine oxidoreductase. Since xanthine oxidase upregulation appears to be a major source of oxidative stress in response to hypoxia and inflammation, sUA level is an important index of impaired oxidative metabolism and a metabolic biomarker of a poor prognosis in CHF [4, 5].

Allopurinol, by inhibiting xanthine oxidase, decreasing oxidative stress and lowering sUA, could be a therapeutic option in CHF on top of optimal medical treatment. However the usual dose of xanthine-oxidase inhibitors has generated conflicting results in clinical studies [4, 6-8]. At the moment, there is no data regarding the effects of high dose allopurinol on clinical outcomes and cardiac biomarkers in CHF patients.

The purpose of the study was to assess the effects of a high dose, 600 mg/day of allopurinol, on sUA level and on biomarkers of cardiovascular stress, NT-proBNP and GDF15, and extracellular matrix remodeling, osteopontin (OPN), in patients with CHF with reduced systolic function.

Materials and Methods

Study population

Fifty-seven patients, 43 men and 14 women, with a mean age of 60 ± 12 years, with stable CHF NYHA class III and EF < 40% were included in the study. Exclusion criteria were: decompensated heart failure, recent myocardial infarction, significant valvular disease, impaired kidney function and severe pulmonary or liver diseases. The patients had been treated with optimal CHF medical therapy (beta-blockers, angiotensin converting enzyme inhibitors, furosemide, spironolactone and digoxin) for at least 6 months prior to study initiation, and no patients were taking urate lowering medication. The study took place between May 2011 and April 2012. The patients were informed about the methodology and purpose of the current research study and subsequently signed a written informed consent. The study was approved by the local Ethics Committee.

A comprehensive clinical and echocardiographic examination and laboratory tests were performed in all patients.

Echocardiographic Data

The echocardiography was performed using an Aloka 4000 ultrasound system with a 2.5 MHz probe. The left ventricular (LV) end-diastolic volume (LVEDV), the LV end-systolic volume

(LVESV) and EF were assessed using the Simpson method. The mitral peak velocity of early filling (E) and the peak velocity of late filling (A) were evaluated. The septal and lateral annular velocities (e') were measured using tissue Doppler echocardiography. The LV diastolic function and LV filling pressures were estimated using the ratio between E and the averaged septal and lateral e' , expressed as E/ e' ratio. An E/ e' ≥ 13 was considered a marker of elevated LV filling pressures.

Biochemical assessments

sUA levels were assessed using an enzymatic-colorimetric method using a Roche Diagnostics kit (normal range: ≤ 7.2 mg/dL).

Creatinine was determined using a kinetic enzymatic colorimetric method (Jaffe) (normal range 0.8-1.2 mg/dL). The estimated glomerular filtration rate (eGFR) was calculated using the Modification of Diet in Renal Disease (MDRD) equation.

NT-proBNP was determined using Roche Cardiac Reader kit (normal range <125pg/mL).

Growth-differentiation factor 15 (GDF 15) concentrations was determined using R&D Systems human GDF-15 Quantikine Elisa (enzyme linked immunosorbent assay) kit (normal range: 319 ± 90 pg/mL).

Osteopontin (OPN) was determined using "Quantikine Human Osteopontin (OPN) Immunoassay" R&D Systems DODT00 ELISA kit (normal range: 0.312 - 20 ng/mL).

The clinical examination, echocardiography and the laboratory tests for sUA and cardiac biomarkers were assessed before and after six months of treatment with 600 mg/day allopurinol along with the CHF optimal medical treatment.

Data analysis

Results are presented as mean $\pm$ standard deviation (SD) for continuous numerical variables and percentages for categorical variables. The χ^2 test, Fisher test were used to compare categorical variables, while Student t test was the test of choice for numerical variables. Significant correlations between continuous variables were evaluated using Pearson's method. A p value less than 0.05 was considered statistically significant. Statistical analysis was performed using SPSS statistics, version 21.0.

Results and Discussion

sUA and cardiac biomarkers at baseline

Among the fifty-seven patients with NYHA III stable CHF with reduced EF (< 40%), 43 patients (75%) had ischemic cardiomyopathy (Table I). 18 (32%) CHF patients had permanent atrial fibrillation. The mean level of creatinine was 1.07 ± 0.19 mg/dL and the estimated glomerular filtration rate (eGFR) was 75.7 ± 16.05 mL/min/1.73 m².

Table I

Demographics, clinical characteristics, pharmacological treatment and echocardiographic parameters of the study population

Demographic and Clinical Data	
Mean age (years)	60 (± 12)
Men (No.)	43 (75%)
Functional NYHA class III (No.)	57 (100%)
Ischemic CHF (No.)	43 (75%)
Atrial fibrillation (No.)	18 (32%)
Systolic BP (mmHg)	126 $\pm$ 18
Diastolic BP (mmHg)	78 $\pm$ 11
Heart rate (beats/min)	70 $\pm$ 13
Serum creatinine	1.07 $\pm$ 0.19
eGFR (mL/ min/1.73 m ²)	75.7 $\pm$ 16.0
Serum Uric Acid (mg/dL)	7.9 $\pm$ 2.2
Pharmacological treatment	
Beta-blocker (Bisoprolol 5-10 mg/day)	57 (100%)
ACEI (Trandolapril 2-4 mg/day)	57 (100%)
Loop diuretics (Furosemide 40 mg/day)	48 (84%)
Spironolactone (25-50 mg/day)	27 (47%)
Digoxin (0.25 mg/day)	20 (35%)
Echocardiographic parameters (mean value)	
LVEF %	33 $\pm$ 6
E (m/s)	0.7 $\pm$ 0.2
Mean e' (cm/s)	6.5 $\pm$ 1.7
E/e'	11.5 $\pm$ 6.7

ACEI – Angiotensin converting enzyme inhibitors, BP – Blood Pressure, eGFR - estimated glomerular filtration rate, LVEF – left ventricular ejection fraction, E - peak velocity of early mitral filling, e' –tissue Doppler averaged annular velocities

All patients were treated with beta-blockers and angiotensin converting enzyme inhibitors, 48 patients (84%) with loop diuretics (furosemide), 27 patients (47%) with spironolactone and 20 patients (35%) with digoxin.

The mean LVEF was 33.2 ± 6.1 % and the mean E/e' ratio was 11.5 ± 6.7 . 38 patients (66%) had the $E/e' \geq 13$, while 19 patients (34%) had $E/e' < 13$ (Table I).

Patients with $E/e' \geq 13$ had significantly lower EF (29.8 ± 3.9 vs. 34.6 ± 4.4 %, $p = 0.001$) and higher NT-proBNP (2531 ± 709 vs. 643.8 ± 430 pg/mL, $p < 0.003$) and GDF15 (1173 ± 478 vs. 921 ± 383 pg/mL, $p = 0.03$) values. OPN levels were increased (42.9 ± 16 vs. 22.68 ± 4.32 ng/mL normal range), without significant difference with respect to E/e' ratio (Table II).

Thirty-five patients (61%) were hyperuricaemic. The mean sUA for the entire group was 7.9 ± 2.2 mg/dL, higher in patients with $E/e' \geq 13$ (8.6 ± 2.2 vs. 7.3 ± 1.4 mg/dL, $p = 0.08$).

Table II

Mean values of EF, cardiac biomarkers and serum uric acid (sUA) before allopurinol treatment in the global study population and according to E/e' ratio

Parameter	Global Study Population	Filling pressures		p
		E/e' < 13*	E/e' > 13*	
EF (%)	33 $\pm$ 6	34 $\pm$ 4	30 $\pm$ 4	0.001
sUA (mg/dL)	7.9 $\pm$ 2.2	7.3 $\pm$ 1.4	8.6 $\pm$ 2.2	0.08
NT-proBNP (pg/mL)	1625 $\pm$ 1268	643 $\pm$ 430	2531 $\pm$ 709	0.003
GDF (pg/mL)	1047 $\pm$ 505	921 $\pm$ 383	1173 $\pm$ 478	0.03
OPN (ng/mL)	49 $\pm$ 14	43 $\pm$ 16	55 $\pm$ 10	0.28

p* for comparison between $E/e' < 13$ and $E/e' \geq 13$; $p < 0.05$

sUA and cardiac biomarkers after six months of 600 mg/day allopurinol treatment

After 6 months of a high dose allopurinol treatment, forty-nine patients were included in the final analysis, while eight patients (14%) did not complete the study. One patient had a stroke, one patient received cardiac resynchronisation therapy and two patients (3.5%) died. Four patients (7%) had side effects during allopurinol treatment, allergodermia in three patients and alopecia in one patient, all reversible after allopurinol discontinuation. Kidney function did not worsen in any of the patients on high doses of allopurinol.

After 6 months, 23 patients (41%) improved the functional NYHA class and 26 patients (46%) were clinically stationary (NYHA class III). The sUA, NT-proBNP and GDF15 mean values decreased,

while OPN significantly increased after 6 months of a high dose of allopurinol treatment (Figure 1).

Compared to NYHA class III patients, the patients that registered a clinical improvement had significantly higher EF (48 ± 12.1 vs. 25.1 ± 3.1 %, $p < 0.001$) and lower NT-proBNP (670 ± 487 vs. 1143 ± 879 pg/mL, $p < 0.001$) (Table III). GDF15 decreased in NYHA class II patients and increased even further in NYHA class III patients (856 ± 170 vs. 1240 ± 492 pg/mL, $p = 0.04$). OPN continued to increase in all patients, without significant difference with respect to NYHA functional class (56.6 ± 25.7 mean value, 58.3 ± 35.8 vs. 55.6 ± 18.7 ng/mL, $p = 0.74$).

After six months, the mean sUA level decreased from 7.9 ± 2.2 mg/dL to 4.4 ± 1.6 mg/dL. Patients that registered a symptomatic improvement had significantly lower sUA levels than patients with

NYHA functional class III (4.2 ± 1.6 vs. 5.5 ± 1.3 mg/dL, $p = 0.04$) (Table III).

In patients with stable CHF and NYHA functional class III, sUA and cardiac biomarkers were markedly increased above the normal range one year before the inclusion in the study, despite optimal CHF medical therapy.

In this study, hyperuricaemia was highly prevalent (61%). The mean sUA was increased (7.9 ± 2.2 mg/dL), patients with more severe CHF, advanced diastolic dysfunction and lower EF having higher sUA values (8.6 ± 2.2 mg/dL) (Table II). Since the kidney function was normal in CHF patients, it can be assumed that hyperuricaemia is the result of

increased production rather than decreased renal clearance. Accordingly, hyperuricaemia reflects xanthine oxidase enzymatic upregulation, produced along with multiple reactive oxygen species [9-11]. In a large cohort of patients with heart failure it has been demonstrated that sUA is an important marker of impaired oxidative metabolism and prognosis only in patients with normal kidney function, when it is the result of increased xanthine oxidase activity [5]. Oxidative stress and proinflammatory cytokines has been implicated in other cardiovascular diseases, in vascular aging [12] and in diabetes mellitus [13].

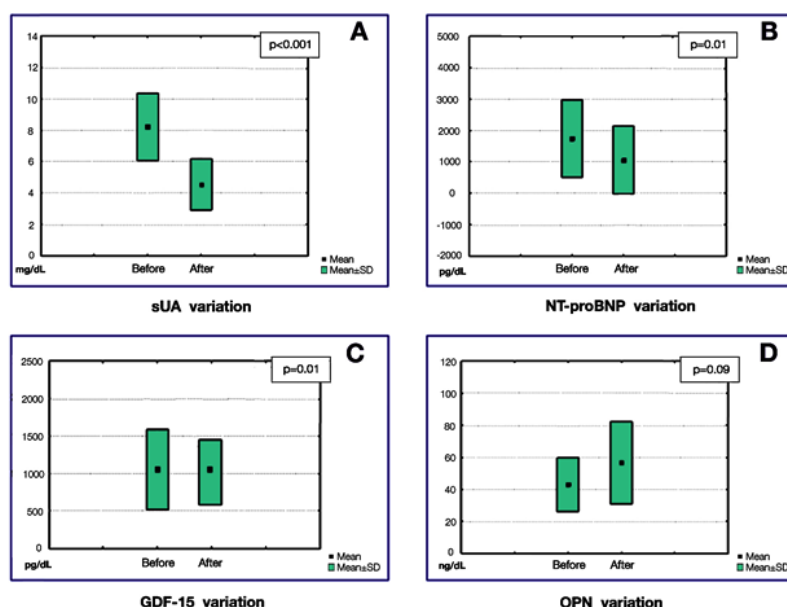


Figure 1.

Variation of sUA (A) and NT-proBNP (B), GDF15 (C) and OPN (D) levels in the global study population at the study beginning and after 6 months of treatment with allopurinol 600 mg/day

p* for comparison between mean values of sUA and cardiac biomarkers; $p < 0.05$

Table III

Mean values of EF, cardiac biomarkers and serum uric acid (sUA) before and after 6 months of 600 mg/day allopurinol treatment in the global study population and according to NYHA class

	Before Allopurinol Treatment	After 6 months of 600mg/day Allopurinol Treatment			p
		Global Study Population	NYHA II (23 patients) *	NYHA III (26 patients) *	
EF (%)	33 ± 6	42 ± 14	48 ± 12	25 ± 3	< 0.001
SerumUA(mg/mL)	7.9 ± 2.2	4.4 ± 1.6	4.2 ± 1.6	5.5 ± 1.3	0.04
NTproBNP (pg/mL)	1625 ± 1268	997 ± 853	670 ± 487	1143 ± 879	< 0.001
GDF15 (pg/mL)	1047 ± 505	1033 ± 423	856 ± 170	1240 ± 492	0.04
OPN (ng/mL)	49 ± 14	57 ± 25	58 ± 35	55 ± 19	0.74

p* for comparison between NYHA class II and NYHA class III; $p < 0.05$

The mean GDF15 level was considerably increased at the initiation of the study (941 ± 84 versus 319 ± 90 pg/mL normal range), significantly higher in severe heart failure with increased LV filling pressures and higher NT-proBNP (1173 ± 478 pg/mL, $p = 0.03$) (Table II). In a study conducted by

Kempf, about 75% of 455 patients with CHF had GDF-15 levels above the upper limit of the normal range, irrespective to heart failure aetiology [14]. In Val-HeFT study, high GDF 15 levels were found in 85% of 1734 CHF patients. GDF 15 was associated with severe symptoms and was strongly related

with BNP, eGFR, high-sensitivity troponin T and sUA [2]. The relation between GDF-15 and NT-proBNP in heart failure supports the hypothesis that both biomarkers are induced by biomechanical stress [14, 15]. In heart failure patients with preserved EF and elevated filling pressures, GDF15 was significantly increased. Combined GDF15 and NT-proBNP improved the heart failure diagnostic accuracy [16]. GDF15 is expressed in myocytes and other cardiovascular cells not only by mechanical stress, but also by metabolic stress (cardiac ischemia, oxidative stress, inflammation) [17-19]. In the ARISTOTLE study, in patients with atrial fibrillation, GDF-15 was an independent risk factor for major bleeding, mortality, and stroke. The authors concluded that GDF 15 is a marker of oxidative stress and inflammation [20]. Similar variations between GDF15 and sUA have recently been found in patients with left-sided heart failure [12]. Tissue hypoxia, impaired oxidative metabolism, and inflammatory cytokine activation are the same strong inducers of increased GDF 15 expression and sUA elevation [21, 22].

In this study, OPN levels were higher than the normal range (42.9 ± 16.7 ng/mL), irrespective to LV filling pressures (55.3 ± 10 ng/mL vs. 22.6 ± 4.3 normal range) in CHF patients (Table III). OPN is a member of matricellular proteins exerting regulatory functions and is critically involved in cardiac adaptation to biomechanical strain, cardiac remodeling, fibrosis and myocardial dysfunction [23]. OPN was significantly elevated in 420 patients with heart failure and systolic dysfunction, with higher levels in patients with moderate - severe heart failure, NYHA class III-IV [3]. In an acute heart failure setting, OPN added prognostic value to NT-proBNP and identified patients with high risk of mortality and rehospitalisation [24].

The persistence of elevated cardiac biomarkers in CHF despite optimal medical therapy suggests that conventional medication only has a partial influence on complex mechanisms involved in the disease progression. Treatment with xanthine-oxidase inhibitors, by improving the oxidative metabolism and inflammation, is a rational therapeutic option in CHF.

After six months of a high dose allopurinol treatment, 46% of CHF patients had a favourable clinical evolution (class NYHA II). Patients with symptoms improvement had higher EF (48 ± 12 % vs. 25 ± 3 %, $p < 0.001$) and lower NT-proBNP values (670 ± 487 vs. 1143 ± 879 pg/mL, $p < 0.001$) (Table III). The benefit of xanthine-oxidase inhibitors on the left ventricular function has also been demonstrated in high profile studies on CHF, in which EF increased and there were registered favourable effects on natriuretic peptide levels [6, 25, 26].

The decrease in sUA levels under high doses of allopurinol was sustained, prompt and significant, from 7.9 ± 2.2 mg/dL at baseline to 4.4 ± 1.6 mg/dL after six months. Patients with improvement in heart failure symptoms had significantly lower sUA levels (4.2 ± 1.6 vs. 5.5 ± 1.3 mg/dL, $p = 0.04$) with a decrease over 35% compared to patients who remained in the same functional CHF class. In a large cohort of patients with CHF, the treatment with allopurinol was independently associated with an improved rate of survival at 1.4 years [4].

GDF15 decreased in patients with improvement in CHF functional status (865 ± 170 versus 1240 ± 492 pg/mL, $p = 0.04$) (Table III). Moreover, the decreased level of GDF15 on high dose allopurinol treatment was associated with a better hemodynamic profile and considerably greater reduction in sUA. The similar variation of sUA and GDF15 after 6 months of high doses of allopurinol treatment suggests that allopurinol acts on the same mechanism responsible for increased sUA production and GDF 15 expression. To our knowledge, this is the first study demonstrating the efficacy of high-dose allopurinol in decreasing GDF 15 levels while improving the cardiac function and CHF symptoms.

GDF 15 levels continued to increase in patients who remained in the same functional CHF class after six months. In VAL-HeFT study, consistent with the observations at baseline, the increases in GDF-15 over the course of 12 months were independently associated with increasing levels of biomarkers reflecting cardiac strain (BNP), injury (troponin T), inflammation (uric acid) and worsening renal function [2, 12]. GDF 15 was the most predictive marker for mortality, even stronger than NT-proBNP in patients with advanced chronic heart failure [27].

OPN continued to increase after six months in CHF patients included in the study, regardless of the clinical course of the disease. Allopurinol treatment did not influence the OPN expression. The progressive increase of OPN levels expresses an unsuccessful attempt to ameliorate the myocardial matrix remodeling by allopurinol. Since the functional significance of increased OPN in CHF is not fully understood, there is a need for better understanding the complex mechanisms involved in OPN expression in order to provide new therapeutic targets.

The results of the present study emphasize the beneficial, but unequal effects, of allopurinol treatment on cardiac biomarkers which reflect the myocardial mechanical (NT-proBNP) and metabolic stress (GDF15 and sUA) in CHF.

Conclusions

Hyperuricaemia, highly prevalent in heart failure, is a marker of xanthine oxidase activation and increased oxidative stress, important pathogenic mechanisms involved in heart failure severity and progression.

The high dose allopurinol treatment has beneficial, but heterogeneous effects on heart failure pathogenic mechanisms and cardiac function. In patients with a favourable clinical outcome, allopurinol significantly decreased serum uric acid and GDF 15 levels, a new biomarker of oxidative stress and inflammation.

In summary, high doses of allopurinol should be associated to the conventional treatment in chronic heart failure, serum uric acid being an available and accessible metabolic biomarker, for the assessment of treatment efficacy.

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References

- Kempf T., Wollert K.C., Growth-differentiation factor-15 in heart failure. *Heart Fail. Clin.*, 2009; 5(4): 537-547.
- Anand I.S., Kempf T., Rector T.S., Tapken H., Allhoff T., Jantzen F., Serial measurement of growth-differentiation factor-15 in heart failure: relation to disease severity and prognosis in the Valsartan Heart Failure Trial. *Circulation*, 2010; 122(14): 1387-1395.
- Rosenberg M., Zugck C., Nelles M., Juenger C., Frank D., Remppis A., Osteopontin, a new prognostic biomarker in patients with chronic heart failure. *Circ. Heart Fail.*, 2008; 1(1): 43-49.
- Gotsman I., Keren A., Lotan C., Zwas D.R., Changes in uric acid levels and allopurinol use in chronic heart failure: association with improved survival. *J. Card. Fail.*, 2012; 18(9): 694-701.
- Vaduganathan M., Greene S.J., Ambrosy A.P., Mentz R.J., Subacius H.P., Chioncel O., Relation of serum uric acid levels and outcomes among patients hospitalized for worsening heart failure with reduced ejection fraction (from the efficacy of vasopressin antagonism in heart failure outcome study with tolvaptan trial). *Am. J. Cardiol.*, 2014; 114(11): 1713-1721.
- Cingolani H.E., Plastino J.A., Escudero E.M., Mangal B., Brown J., Pérez N.G., The effect of xanthine oxidase inhibition upon ejection fraction in heart failure patients: La Plata Study. *J. Card. Fail.*, 2006; 12(7): 491-498.
- Hare J.M., Mangal B., Brown J., Fisher C.Jr., Freudenberger R., Colucci W.S., Impact of oxypurinol in patients with symptomatic heart failure. Results of the OPT-CHF study. *J. Am. Coll. Cardiol.*, 2008; 51(24): 2301-2309.
- Struthers A.D., Donnan P.T., Lindsay P., McNaughton D., Broomhall J., MacDonald T.M., Effect of allopurinol on mortality and hospitalisations in chronic heart failure: a retrospective cohort study. *Heart*, 2002; 87(3): 229-234.
- Gotsman I., Glantzounis G.K., Tsimoyiannis E.C., Kappas A.M., Galaris D.A., Uric acid and oxidative stress. *Curr. Pharm. Des.*, 2005; 11(32): 4145e51.
- Leyva F., Anker S.D., Godsland I.F., Teixeira M., Hellewell P.G., Kox W.J., Uric acid in chronic heart failure: a marker of chronic inflammation. *Eur. Heart J.*, 1998; 19(12): 1814-1822.
- Anker S.D., Doehner W., Rauchhaus M., Sharma R., Francis D., Knosalla C., Uric acid and survival in chronic heart failure: validation and application in metabolic, functional, and hemodynamic staging. *Circulation*, 2003; 107(15): 1991-1997.
- Băcanu C., Margină D., Ilie M., Mitrea N., Assessment of age-related cardiovascular disease using redox stress markers. *Farmacia*, 2013; 61(5): 931-938.
- Mohamad M.A., Mitrea N., Nicolae A.C., Constantinescu M.Z., Drăgoi C.M., Arsene A.L., Barbu C.G., The dynamics of adiponectin and leptin on metabolic syndrome patients and age matched healthy subjects. *Farmacia*, 2014; 62(3): 532-545.
- Kempf T., von Haehling S., Peter T., Allhoff T., Ciccoira M., Doehner W., Prognostic utility of growth differentiation factor-15 in patients with chronic heart failure. *J. Am. Coll. Cardiol.*, 2007; 50(11): 1054-1060.
- Lankeit M., Kempf T., Dellas C., Cuny M., Tapken H., Peter T., Growth differentiation factor-15 for prognostic assessment of patients with acute pulmonary embolism. *Am. J. Respir. Crit. Care Med.*, 2008; 177(9): 1018-1025.
- Stahrenberg R., Edelmann F., Mende M., Kockskämper A., Dungen H.D., Lüers C., The novel biomarker growth differentiation factor 15 in heart failure with normal ejection fraction. *Eur. J. Heart Fail.*, 2010; 12(12): 1309-1316.
- Kempf T., Eden M., Strelau J., Naguib M., Willenbockel C., Tongers J., The transforming growth factor-beta superfamily member growth-differentiation factor-15 protects the heart from ischemia/reperfusion injury. *Circ. Res.*, 2006; 98(3): 351-360.
- Xu J., Kimball T.R., Lorenz J.N., Brown D.A., Bauskin A.R., Klevitsky R., GDF15/MIC-1 functions as a protective and antihypertrophic factor released from the myocardium in association with SMAD protein activation. *Circ. Res.*, 2006; 98(3): 342-350.
- Kempf T., Wollert K.C., Growth-differentiation factor-15 in heart failure. *Heart Fail. Clin.*, 2009; 5(4): 537-547.
- Wallentin L., Hijazi Z., Andersson U., Alexander J.H., De Caterina R., Hanna M., Growth differentiation factor 15, a marker of oxidative stress and inflammation, for risk assessment in

- patients with atrial fibrillation: insights from the Apixaban for Reduction in Stroke and Other Thromboembolic Events in Atrial Fibrillation (ARISTOTLE) trial. *Circulation*, 2014; 130(21): 1847-1858.
21. Schlittenhardt D., Schober A., Strelau J., Bonaterra G.A., Schmiedt W., Unsicker K., Involvement of growth differentiation factor-15/macrophage inhibitory cytokine-1 (GDF-15/MIC-1) in oxLDL-induced apoptosis of human macrophages *in vitro* and in arteriosclerotic lesions. *Cell Tissue Res.*, 2004; 318(2): 325-333.
 22. Zimmers T.A., Jin X., Hsiao E.C., McGrath S.A., Esquela A.F., Koniaris L.G., Growth differentiation factor-15/macrophage inhibitory cytokine-1 induction after kidney and lung injury. *Shock*, 2005; 23(6): 543-548.
 23. Singh M., Dalal S., Singh K., Osteopontin: At the cross-roads of myocyte survival and myocardial function. *Life Sci.*, 2014; 118(1): 1-6.
 24. Behnes M., Brueckmann M., Lang S., Espeter F., Weiss C., Neumaier M., Diagnostic and prognostic value of osteopontin in patients with acute congestive heart failure. *Eur. J. Heart Fail.*, 2013; 15(12): 1390-1400.
 25. Gavin A.D., Struthers A.D., Allopurinol reduces B-type natriuretic peptide concentrations and haemoglobin but does not alter exercise capacity in chronic heart failure. *Heart*, 2005; 91(6): 749-753.
 26. Santhanakrishnan R., Chong J.P., Ng T.P., Ling L.H., Sim D., Leong K.T., Growth differentiation factor 15, ST2, high-sensitivity troponin T, and N-terminal pro brain natriuretic peptide in heart failure with preserved vs. reduced ejection fraction. *Eur. J. Heart Fail.*, 2012; 14(12): 1338-1347.
 27. Lok D.J., Klip I.T., Lok S.I., Bruggink-André de la Porte P.W., Badings E., van Wijngaarden J., Incremental prognostic power of novel biomarkers (growth-differentiation factor-15, high-sensitivity C-reactive protein, galectin-3, and high-sensitivity troponin-T) in patients with advanced chronic heart failure. *Am. J. Cardiol.*, 2013; 112(6): 831-837.