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IMMUNE AND CYTOKINE RELATED DISORDERS, AORTIC STIFFNESS INDEX IN PATIENTS WITH ARTERIAL HYPERTENSION COMBINED TO GOUT

SUMMARY. Multiple data available indicate high prevalence of comorbid abnormalities in gouty arthritis patients, namely, high incidence of arterial hypertension, coronary artery disease, stroke, atherosclerosis of carotid arteries, vascular dementia. For instance, hypertension is found in 36-41% gout patients, and combined with metabolic syndrome it may reach 80%.

The purpose – studying features of clinical course, lipid profile and immune status, aortic stiffness in patients with combined hypertension and gout.

The study involved examination of 137 male patients with stage II hypertension, average age 56.9±3.4. All patients underwent echocardiography with estimation of the left ventricular mass index to verify hypertension stage, blood chemistry test with estimation of uric acid level, as well as lipid profile and immune status.

We have found significant disorders in the lipid profile of blood serum in patients with combined hypertension and gout. Positively higher percentage of activated T-cells was found in patients with combined hypertension and gout, both with early (CD3+CD25+) and late (CD3+HLA-DR+) activation marker, as well as those expressing FAS receptor, and ready to enter into apoptosis. We have identified abnormalities in adhesion and cooperation of immune competent cells, resulting in more intense activation of the same, effector functions and migration to the area of inflammation in the vessel wall.

Key words: arterial hypertension, gout, cytokines, cellular immunity, aortic stiffness.

The problem and its setting. Arterial hypertension (AH), ischemic heart disease, obesity, dyslipidemia, non-alcoholic fatty liver disease, violations of carbohydrate metabolism up to type 2 diabetes have become so prevalent all over the world that they have been combined into «diseases of civilization», or, as they are also called diseases, associated with atherosclerosis [1, p.13]. In addition, most of these diseases are components of the metabolic syndrome (MS). MS is a global disease of civilization, which is a combination of overweight or obesity with hypertension, lipid and carbohydrate metabolism disorders or diabetes mellitus [11, p.119]. Gout is one of the most common rheumatic diseases found in adults. Contemporary epidemiological studies indicate that its prevalence is growing not only in the countries with high living standards but also in the regions where it has formerly been deemed a fairly rare disease [2, p.15].

Analysis of recent research and publications. The latest trend is the more aggressive clinical course of gout, manifesting itself in a larger number of affected joints, nephrolithiasis and frequent development into chronic arthritis. At the same time occurrence of gout in females and in families, as well as nephrolithiasis has grown [2, p.21]. Multiple data available indicate high prevalence of comorbid abnormalities in gouty arthritis patients, namely, high incidence of arterial hypertension, coronary artery disease (CAD), stroke, atherosclerosis of carotid arteries, vascular dementia [6, p.15; 7, p.23]. For instance, hypertension is found in 36-41% gout patients, and combined with metabolic syndrome (MS) it may reach 80% [9, p.10]. In the USA 74% gout patients (6.1 million people) suffer from hypertension, and in the East-European countries hypertension is found in the ¾ of gout patients, total frequency of MS being 57% (from 15 to 77%) [9, p.10].

Gout is viewed as a systemic tophus-type disease characterized by deposition of monosodium urate crystals in various tissues and inflammation developing in patients affected by hyperuricemia, which is associated with ambient and/or genetic factors [2, p.22]. Gout progress is caused by uncontrolled hyperuricemia, which seems the only independent gout risk factor, with the risk growing simultaneously with hyperuricemia progress [9, p.11]. However, diagnostic relevance of hyperuricemia in identification of gout is not in any way equal to the same of monosodium urate crystals: frequency of identification of hyperuricemia in population exceeds frequency of gout by a large margin.

Identification of previously unsolved problems. The problem of hypertension combined with metabolic disorders is highly significant. Connection between uric acid level and cardiovascular diseases has been studied in at least 20 epidemiological and clinical trials involving over 100,000 patients with hypertension. In more than a half of the studies (11 out of 20) an independent connection was found between uricemia and risk of cardiovascular diseases, at least in main subgroups. Findings suggest that uric acid level contributes greatly to cardiovascular risk in patients with unattended hypertension. It was demonstrated that hyperuricemia increases the risk of hypertension development and progress. It was shown that rise in uric acid level by 1 mg/dl was accompanied by rise in hypertension risk by 13%, the risk greatly increasing in younger people and in females [6, p.15].

It was found that rise in uric acid level in patients with hypertension was an independent predictor for rise in risk of cardiovascular events (including fatal) and total mortality [1, p.13]. It was proved that the main cause of mortality in gout patients was specifically cardiovascular diseases, and it was shown that gout was connected with high risk of total and cardiovascular mortality [1, p.16] and with increased risk of development of myocardial infarction in gout patients [7, p. 24].

In view of growing incidence of gout in young and middle-aged patients, the problem of diagnostics and treatment of cardiovascular diseases in this category of patients is a subject of intense study. A number of studies indicate a tight link between insulin resistance and hyperuricemia and describe mechanisms by which hyperinsulinemia and insulin resistance may increase concentration of urates in blood serum [2, p.47].

The purpose – studying features of clinical course, lipid profile and immune status in patients with combined hypertension and gout.

Material and Method. The study involved examination of 137 male patients with stage II hypertension, average age 56.9±3.4. The main group consisted of 72 patients with combined hypertension and gout, and comparison group consisted of 65 patients with hypertension. The study also included examination of 35 healthy people of similar age and sex without any symptoms of hypertension or gout. Hypertension was diagnosed based on recommendations of the Ukrainian Association of Cardiologists [3, p.19], gout was diagnosed based on recommendations of the Decree No. 676 [4, p.9], and metabolic syndrome was identified based on IDF criteria, 2005 [5, p.31]. All patients underwent echocardiography with estimation of the left ventricular mass index (LV mass index) to verify hypertension stage, blood chemistry test with estimation of uric acid level, as well as lipid profile with estimation of total cholesterol and its fractions – high-density lipoproteins (HDL-C), low-density lipoproteins (LDL-C), very low-density lipoproteins (VLDL-C) and triglycerides (TG). The aortic stiffness index (ASI) was detected by the G.Radchenko, Yu.Sirenko, 2008 [10, p.20] in according to the formula ASI = PBP/ IV, where IV - impact volume, PBP - pulse blood pressure. Levels of proinflammatory and anti-inflammatory cytokines, Interleukin-17A (IL-17A), Interferon gamma (IFN-γ), content of soluble cell adhesion molecules sICAM-1 and sVCAM were determined using immunoassay kits certified in Ukraine and applying methods suggested by manufacturers Pro Con (Russia) and Diaclon (France), level of transforming growth factor beta (TGF-β) - using methods by Genzyme diagnostics. Content of lymphocyte subpopulations with CD3+CD25+, CD3+CD95+, CD3+HLA-DR+, CD54+, CD11b+ and CD62L+ phenotypes was determined by indirect immunofluorescence using monoclonal antibodies [8, p.29]. Student's t-test (variation statistics) was applied to statistical data analysis using Microsoft XP Excel applied software and specialized software STATGRAPHICS Plus v. 2.1.

Results and Discussion. Main results of the comprehensive examination of patients with combined abnormalities are given in Table 1. These data suggest that there were no reliable differences ($p>0.1$) in duration of the disease in patients of both groups, as well as in levels of office systolic and diastolic blood pressure, heart rate and LV mass index ($p>0.1$). Patients in the main group demonstrated positively higher levels of body mass index (BMI) with prevailing stage II obesity and waist circumference over 94 cm, which in combination with hypertension and lipid profile disorders became a reason for diagnosing them with MS. Thus, MS symptoms were found in 87.5% patients in the main group (63 pers.) and 63.08 % (41 pers.) patients in the comparison group. The main group also demonstrated positively higher rate of occurrence of concurrent chronic heart failure (CHF) (functional NYHA class I-II).

Table 1

Clinical, Instrumental and Laboratory Findings in Patients with Combined Hypertension and Gout (M±m)

Values	Main group (n=72)	Comparison group (n=65)	Control group (n=35)
Duration of hypertension, years	10.2 ± 1.2	10.5 ± 1.3	(-)
Systolic BP, mm Hg	167.7 ± 2.1*	163.5 ± 2.7*	125.6±4.7
Diastolic BP, mm Hg	102.7 ± 2.3*	98.1 ± 2.1*	75.2±3.7
Pulse, bpm	72.6±2.7	74.3±2.8	73.7±3.9
LV mass index, g/m^2	132.6±3.6*	130.4±3.7*	81.1±2.3
BMI, kg/m^2	32.7±1.9*	27.2±1.7* **	22.3±2.8
Aortic stiffness index	1.083±0,01	1.079±0,02	1.043±0,01
Concurrent CHF (NYHA FC I-II)	37 (51.4%)	22 (33.9%)	**(-)**
Uric acid	479.5±30.8*	297.6±25.9 **	265.5±12.3
Total cholesterol, mmol/l	7.03±0.31*	6.95±0.23*	4.90±0.41
TG, mmol/l	2.78±0.14*	2.14±0.11* **	1.18±0.12
LDL-C, mmol/l	3.72±0.19*	3.66±0.22*	2.81±0.35
HDL-C, mmol/l	1.01±0.03*	1.04±0.02*	1.49±0.21
VLDL-C, mmol/l	0.98±0.06*	0.82±0.07* **	0.51±0.07
Atherogenic index	5.78±0.11*	4.94±0.16* **	3.14±0.24

Notes: * - significance of differences in values compared with control group ($p<0.05$); **- significance of differences in values between groups; n – number of patients.

Based on analysis of the data given in Table 1, we can conclude that uric acid level in the main group of

patients was positively higher by 61.12% ($p<0.05$) than in the comparison group. Study of the lipid profile in the main group of patients showed significantly higher TG content – by 29.90% ($p<0.05$), VLDL-C – by 19.51% ($p<0.05$) than in the comparison group, as well as positively higher value of atherogenic index – by 17.01% ($p<0.05$). There were no significant differences in cholesterol and HDL-C. These data stemmed from common pathogenic mechanisms of atherosclerosis and hypertension development, role of atherosclerotic processes in blood pressure rise mechanisms. Certainly, such patients should be provided with follow-up care and not only diet, but also specific lipid-lowering therapy.

Changes in the arteries stiffness can be identified even before vascular disease. Stiffness of arteries can be both a marker of atherosclerotic process in the future, and directly affect the development of atherosclerosis and the formation of isolated systolic hypertension. There were no significant changes in ASI in groups of patients because the AH present in both of them.

Based on examination findings it was established that patients in the main group and in the comparison group had significant disorders of the immune status and cytokine profile of the blood serum (table 2).

Table 2

Immune and Cytokine Status in Patients with Combined Hypertension and Gout (M±m)

Values	Main group (n=72)	Comparison group (n=65)	Control group (n=35)
CD3+CD25+ lymphocytes, %	23.2±1.13	17.50±0.77**	14.71±0.39
CD3+HLA-DR+ lymphocytes, %	24.8±1.09	18.6±0.72**	15.4±0.72
CD3+CD95+ lymphocytes, %	15.7±0.63	11.8±0.44**	6.12±0.11
$CD54^+$ lymphocytes,%	19.2±0.73	15.1±0.48**	11.07±1.65
$CD11b^+$ lymphocytes,%	37.2±1.81	26.9±1.69**	21.5±1.4
$CD62L^+$ lymphocytes,%	40.5±1.73	35.6±1.69**	28.3±1.7
CD30+ lymphocytes, %	2.73±0.015*	2.69±0.018*	1.8±0.03
TNF-α, pg/ml	129.6±4.38	102.7±5.23**	42.3±4.9
IL-1β, pg/ml	111.8±5.24	96.6±5.35**	39.42±4.5
IL-6, pg/ml	70.4±2.41	69.9±2.93	10.31±2.3
IL-8, pg/ml	23.5±1.07	22.1±1.15	12.7±1.5
IFN-γ, pg/ml	48.6±2.96	47.9±2.13	96.4±8.6
IL-4, pg/ml	10.1±0.96	17.3±0.83**	25.42±3.3
IL-10, pg/ml	33.4±2.1	31.7±1.46	41.75±2.8
TGF-β, pg/ml	73.8±3.12	70.3±4.04	39.4±4.1
IL-17A, pg/ml	38.1±2.6	35.3±1.1	17.3±2.7
sVCAM, pg/ml	56.1±2.9	39.3±1.6**	18.6±1.9
sICAM-1, ng/ml	389.4±11.3	328.3±10.4**	275.5±17.29
CIC large (> 19 S), conventional units	21.17±0.49	27.02±0.41**	51.7±3.12
CIC medium (11-19S), conventional units	59.55±2.34	52.28 ± 2.6 **	34.54±2.02
CIC small (<11 S), conventional units	43.37±1.72	39.22±1.64**	10.94±1.13

Notes: **- significance of differences in values between groups; n – number of patients.

Based on analysis of the data given in Table 2, we can conclude that level of activated T-cells with CD3+CD25+ phenotype in the main group of patients was positively higher by 32.57% ($p<0.05$) than in the comparison group, which indicates that development of inflammation in the joints simultaneously with atherosclerotic inflammation and endothelium dysfunction generates a stronger immune response with obvious T-cell activation. Main group of patients also demonstrated positively higher content of activated lymphocyte subpopulations with CD3+HLA-DR+ phenotype exceeding reference range by 33.3% ($p<0.05$), which is a marker of late lymphocyte activation and confirms long duration of inflammation and intensity of immune response. High content of CD3+CD95+ lymphocytes (by 33.05%, ($p<0.05$)) as compared with the group of patients with isolated arterial hypertension is a consequence of pro-apoptotic effect of TNF-α.

The main group of patients also demonstrated positively higher content of activated lymphocyte subpopulations carrying various cell adhesion molecules and receptors thereof. For instance, content of CD54+ lymphocytes expressing cell adhesion molecule ICAM-1 exceeded the comparison group value by 27.15% ($p<0.05$), CD11b+ - by 38.29% ($p<0.05$), CD62L+ - by 13.76% ($p<0.05$). It is CD11b receptor that provides for adhesion of macrophages and neutrophils to the endothelial wall and acts as a ligand for ICAM-1, CD62L (L-selectin) provides for adhesion and adherence of lymphocytes to the endothelial wall [12, p.73].

We have also found high serum concentration of soluble vascular cell adhesion molecule sVCAM, the level of which in the main group exceeded the similar value in the comparison group by 42.75% ($p<0.05$), sICAM-1 - by 18.61% ($p<0.05$). Hence, more intense

inflammation with high levels of pro-inflammatory cytokines leads to stronger activation of immune competent cells and synthesis of cell adhesion molecules by immune competent cells. Moreover, high serum concentration of soluble vascular cell adhesion molecules sICAM-1 and sVCAM was combined with increased level of ICAM-1 (CD54+) receptor expression on peripheral blood lymphocytes, as well as CD11b (CD54 receptor ligand) and CD62L receptors, which provide for cell attachment to the endothelial wall.

Cell adhesion molecules play a key part in immune response processes and clearly regulate mechanisms of atherosclerotic inflammation development, since they are expressed on some cells of the immune system, bind to their counter receptors and attach to one another, which leads to local accumulation thereof, development of stasis and thrombosis in blood vessels. ICAM-1, VCAM and E-selectin are defined as early markers indicating inflammation activity, especially at the early stages of the disease. ICAM-1 belongs to immunoglobulin family, and its expression is increased by such cytokines as IL-2 and TNF-α. VCAM – vascular cell adhesion molecule – belongs to immunoglobulin family and is expressed on the surface of activated endothelium [12, p.128].

Patients with combined abnormalities, as well as patients with isolated hypertension demonstrated positively higher content of CD30+lymphocytes by 51.7% ($p<0.05$) and by 49.4% ($p<0.05$) respectively, which indicates type 2 T-helper immune response prevailing in atherosclerosis-associated diseases and development of autoimmune disorders associated with endothelium dysfunction and development of atherosclerotic inflammation.

It should be noted that the comparison group of patients with stage II isolated hypertension also demonstrated significant changes in the content of activated lymphocyte subpopulations as compared with values in healthy individuals. For instance, relative value of CD3+CD25+ lymphocytes was positively higher than the control group value by 18.97% ($p<0.05$), CD3+HLA-DR+ lymphocytes - by 20.78% ($p<0.05$), CD3+CD95+ lymphocytes - by 92.81% ($p<0.05$). The comparison group of patients also demonstrated significant increase in the value of lymphocytes expressing various cell adhesion molecules: level of CD54+ lymphocytes exceeded the control group value by 36.40% ($p<0.05$), CD11b+ - by 25.12% ($p<0.05$), CD62L+ - by 25.83% ($p<0.05$).

It is commonly known that hypertension causes thickening of the middle layer, narrowing of the lumen and increase in extracellular matrix [1, p.46]. Rise in the mass of smooth muscle cells increases the degree of vasoconstriction influenced by neurohormones and leads to rise in the total peripheral resistance, which in its turn contributes to stabilization and exacerbation of hypertension. Vessel wall thickening and its leukocytic infiltration by activated cells and cell adhesion molecules is a precondition for development and progress of atherosclerosis [1, p. 82].

TNF-α value in patients with combined hypertension and gout was 3.6 times higher ($p<0.05$) than the value in healthy individuals and exceeded the value in the comparison group by 26.19% ($p<0.05$); IL-1β – 2.84 times higher and by 15.73% ($p<0.05$) respectively. IL-6 value was 6.83 times higher than the reference range and had no significant differences from the comparison group value ($p>0.1$). Similar changes were found in IL-8 value, which was 1.85 times higher than the control group value ($p<0.05$), IL-17A - 2.2 times higher ($p<0.05$), and IFN-γ value was lower than the value in healthy individuals by 50.41% ($p<0.05$). At the same time we have also found significant rise in serum concentration of TGF-β in the main group – 1.87 times higher ($p<0.05$) than the control group value, and 1.78 times higher ($p<0.05$) than the comparison group value. These data may be due to the fact that TNF-α is viewed as a mediator of insulin resistance associated with obesity, which reduces activity of insulin receptor tyrosine kinase and inhibits activity of intracellular glucose transporters in muscle and adipose tissues. Symptoms of metabolic syndrome and possible development of non-alcoholic fatty liver disease in patients with hypertension also contribute to specific changes in the immune system, since a large scope of data concerning involvement of immune mechanisms in pathogenesis of atherosclerosis accumulated over the last years and immune theories of atherogenesis were suggested [12, p.91]. Lasting antigen persistence, namely low-density lipoproteins, both free and as a part of circulating immune complexes (CIC), is highly relevant for evolution of atherosclerosis, cytokine interactions between CD3+ lymphocytes and monocytes/macrophages. Phagocyte activation promotes release of hydrolytic enzymes, cytokines, chemokines and growth factors. Inflammation changes drastically further metabolism of low-density lipoproteins in the blood vessel wall – TNF-α and IL-1 enhance binding of low-density lipoproteins (LDL-C) with endothelium and smooth muscle cells [1, p.29]. TGF-β is produced by macrophages, lymphocytes and dendritic cells, and in liver – by liver-recruited macrophages and Kupffer cells, and plays a key part in immune homeostasis regulation, its main effects are connected with inhibition of T-cell and B-cell proliferation, namely cytotoxic CD8+ lymphocytes, type 1, 2 and 17 T-helpers, IL-2 and IL-12 secreted by T-cells. Defects in TGF-β signalling pathways contribute to increased proliferation and effector function of immune cells and may cause an uncontrolled immune response. In pathological conditions TGF-β is a fibrosis inducer and enhances collagen synthesis by hepatic stellate cells. The studies proved that TGF-β identified differentiation of stellate cells into microfibroblasts and directly influenced synthesis of collagens, tissue inhibitors of metalloproteinase 1, plasminogen activator inhibitor 1 and other factors [12, p.353], and hence its level in blood serum is highly relevant for identification of hepatic fibrosis at the early stages and development of the disease into hepatic cirrhosis and hepatocellular carcinoma.

Level of anti-inflammatory cytokines IL-4 and IL-10 in both groups was positively lower than the control group value. However, in patients with combined hypertension and gout IL-4 level was significantly lower in the main group by 71.29% ($p<0.05$) than in the comparison group, which is a compensatory response to

higher serum concentrations of pro-inflammatory cytokines.

In both groups of the patients we revealed imbalance of serum CIC with a significant prevalence of pathogenic CIC content. The main group of patients has the higher level of medium CIC content to 13.91% ($p<0,05$), and small - to 10.6% ($p<0,05$), than in comparison group. The level of physiological CIC large size was reduced in both groups.

Conclusions.

1. We have found significant disorders in the lipid profile of blood serum in patients with combined hypertension and gout and positively higher level of triglycerides and very low-density lipoproteins, as well as atherogenic index. Moreover, waist circumference in most of the patients in this group exceeded 94 cm, which in combination with hypertension and lipid profile disorders became a reason for diagnosing 87.5% patients in the main group (63 pers.) and 63.08 % (41 pers.) patients in the comparison group with MS.

2. The were no significant difference in ASI in both groups of patients. This index is the special marker of arterial hypertension, atherosclerosis.

3. Positively higher percentage of activated T-cells was found in patients with combined hypertension and gout, both with early (CD3+CD25+) and late (CD3+HLA-DR+) activation marker, as well as those expressing FAS receptor, and ready to enter into apoptosis. We have identified abnormalities in adhesion and cooperation of immune competent cells, resulting in more intense activation of the same, effector functions and migration to the area of inflammation in the vessel wall. Moreover, high level of pro-inflammatory cytokines (TNF-α, IL-1β, IL-6, IL-8) was found in blood serum of patients with combined hypertension and metabolic syndrome, combined with increased type 2 T-helper immune response (low serum concentration of IFN-γ), and type 17 T-helper immune response (high serum concentration of IL-17A). This type of abnormalities in the immune system is typical in patients with combined hypertension and gout.

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