ORIGINAL ARTICLE

PARAMETERS OF ENDOTHELIAL DYSFUNCTION AND IMMUNE RESPONSE IN PATIENTS WITH RHEUMATOID ARTHRITIS WITH AND WITHOUT ISCHEMIC HEART DISEASE

DOI: 10.36740/WLek202208208

Anna G. Husarchuk[1], Tetiana O. Ilashchuk[1], Iryna V. Okipniak[1], Valentyna L. Vasiuk[1], Oksana M. Mykytiuk[1], Liudmyla V. Mikulets[1], Oksana V. Stasyshena[2]

[1]BUKOVINIAN STATE MEDICAL UNIVERSITY, CHERNIVTSI, UKRAINE

[2]SHUPYK NATIONAL HEALTHCARE UNIVERSITY OF UKRAINE, KYIV, UKRAINE

ABSTRACT

The aim: To determine changes in endothelial dysfunction and immunological response in patients with rheumatoid arthritis with and without coronary heart disease.

Materials and methods: The study involved 151 patients with RA and coronary heart disease. The ED was assessed by examining the NO system in RA for determination of the content in the blood of the product of NO synthase – the final metabolites of NO_2^- and NO_3^- and the level of VEGF and CD28 in the serum.

Results: The results of the study of the levels of metabolites NO_2^- and NO_3^- in the first and second groups showed their increased content. Analysis of the content of VEGF in the blood for patients with different durations of the disease showed that the concentration of the studied protein grows larger with increasing duration of the disease. The maximum of sCD28 concentration was found in middle-aged patients, and the minimum (the difference was significant) – in the elderly.

Conclusions: As the duration of the disease increases, the content of VEGF in the blood of patients increases, which, at the same time, did not show age dependence on RA and did not change further with concomitant coronary heart disease. Detected concentrations of sCD28 are higher in patients with less prolonged RA, and begin to decrease with increasing duration of the disease.

KEY WORDS: rheumatoid arthritis, coronary heart disease, markers of inflammation, nitric oxide, endothelial dysfunction

Wiad Lek. 2022;75(8 p2):1985-1990

INTRODUCTION

In spite of achievements of medical science etiology of rheumatoid arthritis (RA) as an autoimmune disease remains unknown, where chronic erosive arthritis results in systemic lesions of the internal organs [1]. Scientists determine that the main cause of reduced life span of patients with RA is cardiovascular diseases (CVD) including acute disturbed cerebral circulation, and ischemic heart disease (IHD) [2]. The most important function of the coronary artery (CA) endothelium is adaptive regulation of the coronary blood circulation according to the myocardial requirements in oxygen [3]. Endothelial dysfunction (ED) is considered as a predictor of CVD, and it is one of the diagnostic criteria of early detection of atherosclerotic vascular lesions. IHD as one of CVDs plays a leading role in ED pathogenesis in patients with RA. V. I. Mazurova and V. A. Yakusheva, having examined patients with RA and IHD found that the most severe cases of IHD were observed in patients with systemic signs and duration of the disease > 5 years [4].

Early detection of biomarkers in the examined samples is an important diagnostic and preventive measure for patients with IHD to eliminated atherosclerotic process with underlying hyperlipidemia and real assessment of endothelial dysfunction degree [5]. Biomarkers are compounds of an organic nature, which presence in the focus of inflammation is indicative of certain physiological or pathological processes in the human body. Biomarkers can be found either on biochemical or molecular-genetic levels. Though biomarkers are being actively examined now, the level of their investigation is not sufficient. Therefore, the research deals with the study of the parameters of ED and immune response in patients with RA with and without IHD. Hyperactivity of renin-angiotensin-aldosterone system (RAAS) with an increased secretion of angiotensin-converting enzyme (ACE) is a cause of ED, therefore continuous RAAS activation is the most important factor leading to ED in patients with RA and IHD [6].

In this respect in modern cardiology a deliberate assessment of the role of the tissue RAAS in maintenance of normal endothelial function and physiological state of vessels determine the choice of pharmacotherapy of CVD including IHD. One of such drugs is ACE inhibitor *Perindopril*, which produces a correcting effect on the endothelial function in patients with RA and IHD, eliminates complications of coronary and cerebral atherosclerosis [7].

Endothelial functions are known to be disturbed earlier than clinical and morphological signs of atherosclerosis occur, therefore ED signs are the first causes promoting development of CVD. For example, tumor necrosis fac-

tor-α (TNF-α), a mediator of inflammation in joints, is associated with ED and severity of atherosclerotic lesion of the carotid arteries.

Nitrogen monoxide (NO) as a vasodilator, participates in regulation of the body physiological processes, where by means of nicotinamide adenine dinucleotide (NAD)- and nicotinamide adenine dinucleotide phosphate (NADP)-dependent oxidase is transformed into nitrogen superoxide, which is one of the main oxidant of low-density lipoproteins (LDLP). The vasodilator NO is formed in oxidation-reduction reaction (redox reaction) of L-arginine with endothelial and inducible NO-synthase. Further mechanism of NO transformation is accompanied by oxidation to nitrite- (NO_2^-) and nitrate ions (NO_3^-), for example, in decomposition reaction of nitrosothiols (RS–N=O), formation of peroxynitrite (RONOO) followed by protein nitrosylation [8].

Nitrogen monoxide inhibits proliferation and migration of the smooth muscle cells, aggregation and adhesion of the blood corpuscles on the endothelial cells. At the same time, NO plays an important role in RA pathogenesis at the expense of exacerbation of processes of free radical damage of molecules in the tissues of patients due to its high content [9].

A biological role of nitrogen monoxide and its metabolites confirms their important value in redox molecular processes in the body, and a high level of inflammation markers and pro-inflammatory cytokines (ESR, C-reactive protein (CRP), interleukin-6 (IL-6)) are indicators of inflammatory processes for patients with RA. Moreover, more intensive inflammatory process with RA is associated with growth of hard arterial wall, reduced elasticity of major and minor arteries in combination with IHD and without it.

Analysis of vascular endothelial growth factor (VEGF) as a protein marker for stimulation of vasculogenesis and angiogenesis found its increased content in the blood of patients with IHD. Since it stimulates growth and migration of vascular endothelium cells for the formation of the basal lamina, it can influence on the processes of chronic inflammation promoting collagen deposits in the place of inflammation, remodeling the cartilaginous and osseous tissues, stimulating osteoclastogenesis, forming subchondral cysts and provoking RA and osteoporosis. Now VEGF present in the focus of inflammation is a possible marker of the blood plasma inducing synthesis of TNF-α and IL-1β, IL-6 by mononuclear leukocytes of the synovial fluid [10].

Combination of VEGF nitrogen monoxide results in vasodilation and increased permeability of the vascular wall, and loss of the extracellular matrix of the walls followed by further induction of atherogenesis. Thus, this index is potentially sensitive marker of the condition of patients with RA and IHD, and it requires further investigation.

According to the theory of «immune aging» RA development occurs in association with CVD, lung diseases, osteoporosis and disturbed cognitive functions. Aging of immune cells is associated with reduced diversity of T-cell receptors, lower expression of CD28 molecule, shortening and erosion of telomeres. CD28-molecular surface of T-lymphocytes with RA and deep aging increases at the expense of $CD4^+/CD28^-$ $iCD8^+/CD28^-$leukocytes. $CD28^-$ T-cells are included into terminally differentiated effector memory cells, which synthesize abnormally large amounts of interferon (IFN)-γ, TNF-α, IL-1β, and IL-6 [11].

CD28 molecules are very important for the regulation of interaction of stimulating and inhibiting signals in T-cells. Thus, in case of RA the concentration of CD8+/CD28+ T-cells essential for physiological immune response decreases [12]. Nowadays the study of the content of these cells is limited due to expensiveness and lack of special equipment (flow cytometry), therefore, in our research we assessed the content of «cast-off» molecules – soluble fraction of CD28 – sCD28. Analyzing literature data it should be noted that the content of soluble fraction sCD28 in the blood can be a marker for patients with RA, and its parameter is correlated by the stage of the disease and presence/absence of exacerbation.

THE AIM

The aim of the research is to analyze changes in the parameters of ED and immune response in patients with RA with and without IHD.

MATERIALS AND METHODS

151 patients with RA and IHD were examined and divided into 3 groups.1 group – 60 patients with RA, 2 group – 30 patients with RA and IHD, 3 group – 61 patients with IHD. The control group included 22 patients.

RA in the groups included in the research developed in different ways: the first symptoms were registered at the age of 47,8±9,12 years (patients with RA) and 39,1±8,01 years (patients with RA and IHD). At the same time, a tendency of various average duration of RA was noticed: 2 group (RA+IHD) – 13,9±6,8 years, 1 group (RA without IHD) – 10,8±5,6 years (22,5% lower). The 3th group (IHD) included patients with an average age of 66,3±5,41 years. That is, the longer was the period of RA and the earlier was the development of pathology, the earlier IHD occurred.

ED level was assessed by means of detection of NO-synthetase content in the blood with RA, as a final metabolite of NO_2^- and NO_3^-. The level of VEGF and CD28 in the blood serum was determined by means of immune-enzyme method (sets «Human VEGF1» and «Human CD28» (Wuhan Fine Biotech Co., Ltd.)).

The results obtained were statistically processed by means of the packages of the applied programs Microsoft Excel, Statistica for Windows 6.0. Quantitative data were compared by Wilcoxon-Mann-Whitney U-test or Crackel-Wallis criterion for independent sampling and Wilcoxon T-criterion for dependent sampling. Qualitative data were compared by Xi-square criterion or Fisher test for independent sampling and by McNemar's test for dependent sampling.

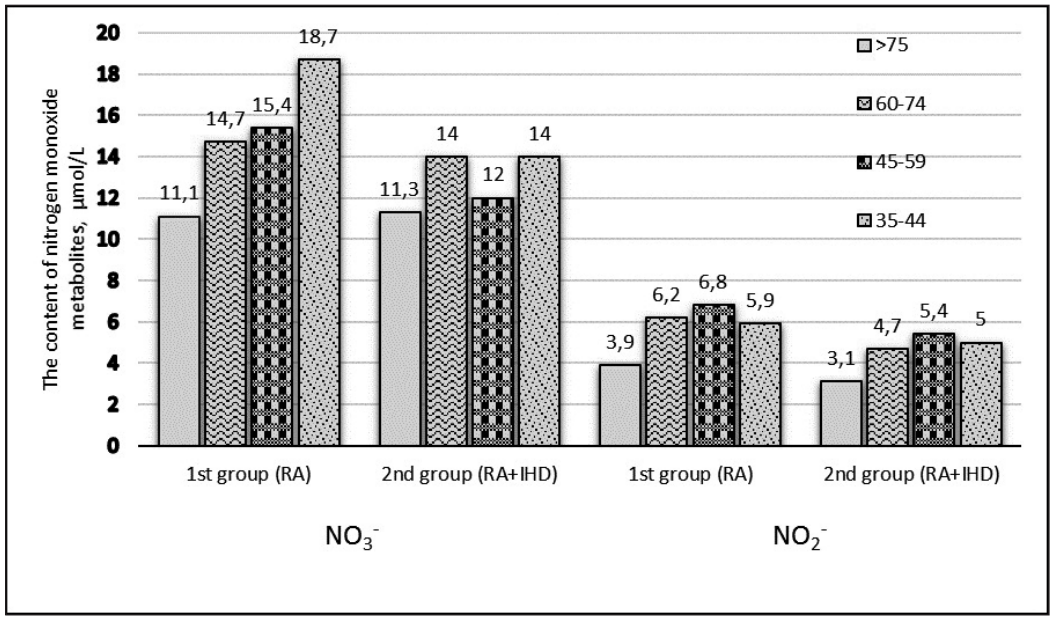

Fig. 1. The content of nitrogen monoxide metabolites in the blood of patients (1st and 2nd groups) with RA and IHD depending on age

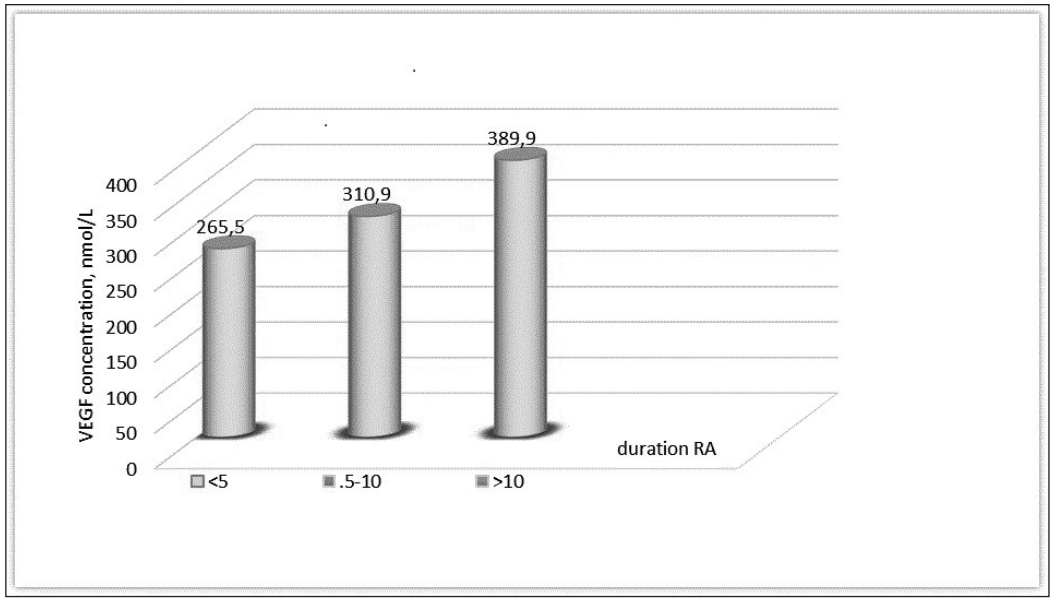

Fig. 2. VEGF concentration in patients with RA depending on duration of the disease

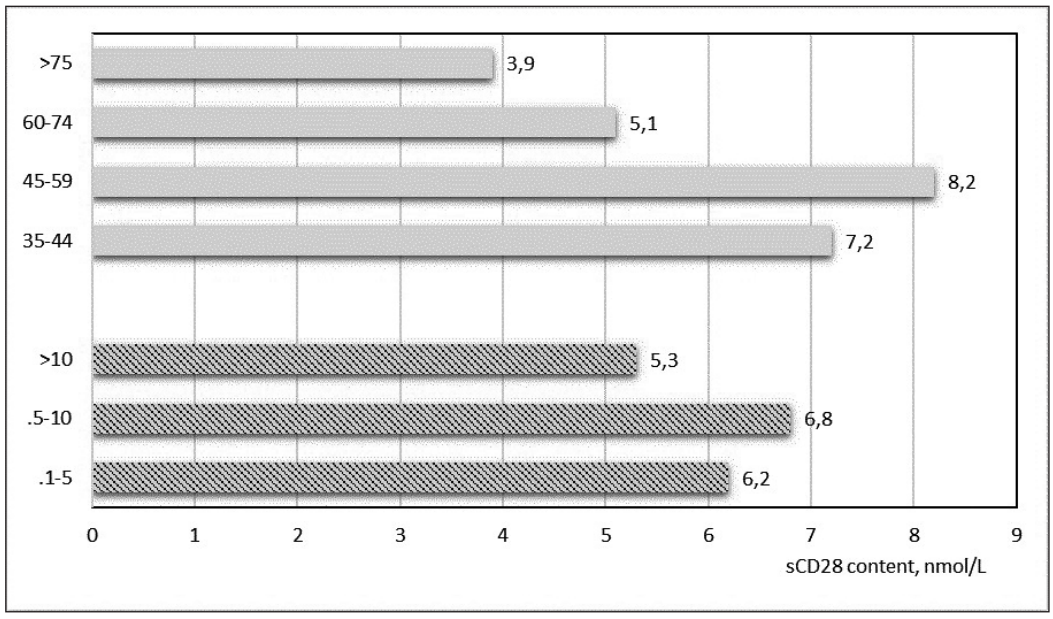

Fig. 3. The content of CD28 marker in the blood plasma of patients with RA (concerning the age of patients and duration of the disease)

Table I. Characteristics of patients from the groups of the research

	males	females	age	RA duration	seropisitive	seronegative
1 group (RA without IHD) n = 60	29 (47,5%)	31 (50,8%)	61,4	13,1±0,8	45 (75,0%)	15 (25,0%)
2 group (RA+IHD) n = 30	11 (31,4%)	24 (68,6%)	56,1	11,1±1,4	25 (71,4%)	6 (17,1%)
3 group (IHD) n = 61	29 (47,5%)	31 (50,8%)	66,3			
p RA and IHD, RA	0,107		p<0,05	p<0,05	0,703	0,373
p RA and IHD, IHD	1		p<0,05			
p RA,IHD	0,107		p<0,01			

Table II. Results of measuring the content of metabolites NO2– and NO3–, VEGF and CD28 molecule in the groups of the research

Objects of the research	NO_2^-, mcmol/L	NO_3^-, mcmol/L	VEGF, nmol/L	CD28, nmol/L
1 group (RA without IHD) n = 60	6,1±0,31	16,8±1,33	330,85±19,08	6,1±0,87
2 group (RA+IHD) n = 30	5,2±0,12	14,2±1,64	353,51±17,78	6,1±0,91
3 group (IHD) n = 61	7,1±1,04	12,3±2,01	294,65±31,11	-
Control group, n = 22	4,3±0,61	13,0±1,48	200,9±40,11	4,2
p_1	p>0,05	p>0,05	p>0,05	p>0,05
p_2	p>0,05	p<0,05	p>0,05	-
p_3	p<0,05	p<0,05	p<0,05	p<0,05
P_4	p<0,05	p<0,05	p<0,05	p<0,05

Note.
n – number of patients in the group,
p_1 – probability of changes in comparison of patients with RA and RA+IHD;
p_2 – probability of changes in comparison of patients with IHD and RA+IHD;
p_3 – probability of changes in comparison of the control group and patients with RA;
p_4 – probability of changes in comparison of the control group and patients with RA+IHD.

RESULTS

At the beginning of the study the proportion of patients in the groups according to their sex, age, duration of the disease are presented (Table I).

In every group examined the patients were selected practically in proportional ratio by gender. 1st group included 29 men (47,5%), and 31 women (50,8%). 2nd group included 11 men (31,4%), and 24 women (68,6%). Duration of RA in both groups of patients was on an average 12,6 years.

Results of the study of metabolites NO_2^- and NO_3^- in the first and second groups were indicative of their elevated level in comparison with the control group of patients (Table II).

The content of nitrite ions (NO_2^-) in the blood was 20,9% and 41,8% higher for the patients from the first and second groups respectively; the content of nitrate ions (NO_3^-) was 16,9% and 29,2% higher for the patients from the control group and the third group in comparison with healthy individuals respectively.

The figures in Table II indicate that there are no reliable differences between the levels of metabolites in patients from the 1st (RA without IHD) and 2nd (RA + IHD) groups, but there is a tendency to lower content of nitrites (14,7%) and nitrates (15,4%) in the blood of patients from the 2nd group; at the same time, the parameters obtained are higher than those for the 3rd group (IHD) (Fig. 1).

Figure 1 demonstrates that the highest content of nitrite ions (NO_2^-) is found among patients of the middle age, both with RA and IHD. The content of nitrite ions (NO_2^-) reduces with age, it becomes even lower than the parameters of healthy individuals (9,5% lower for the 1st group, and 26,7% lower for the 2nd group). These results do not contradict the previous literary data dealing with investigation of patients with RA, gout or psoriasis arthritis, systemic lupus erythematosus. At a young and middle age in the background of less pronounced ED the content of NO in the blood of patients with RA might increase due to activity of inducible isoform of NO-synthase, which is synthesized in excess by the connective tissue in the focus of inflammation with RA and stimulated by higher level of inflammatory cytokines.

When inflammation is acute or chronic, the content of nitrate ions (NO_3^-) increases rapidly due to non-radical decay of peroxynitrite. NO excess inhibits activity of the

endothelial isoform of NO-synthase and complicates ED. With age of patients with RA and IHD ED becomes more intensive, and constitutional active isoforms of enzymes and stimulation of iNO-synthase are minimal. Therefore, the total content of NO metabolites decreases to the parameter lower than that of the patients of younger age.

Analysis of VEGF content in the blood of patients from the 1st and 2nd groups (Table 2) before treatment did not find any reliable differences for them, but this parameter was found to be higher than for healthy individuals in both groups: 76,3% higher for patients from the 1st group and 65,1% higher for the 2nd group. The correlation of VEGF parameter depending on the patients' age was not found either, since the difference was minimal, statistically unreliable and it was lower than 5% (Fig. 2).

Meanwhile, analysis of VEGF content in the blood of patients with various duration of the disease showed that concentration of the protein examined becomes higher when the disease lasts longer. For patients from the 1st group (RA), where the disease lasted up to 5 years, VEGF content 32,5 % increased or to 265,5 nmol/L in comparison with the control group; when RA lasted for 5-10 years VEGF content 54,9% increased or to 310,9 nmol/L, and when the disease lasted longer than 10 years the parameter 94,5% increased to 389,9 nmol/L.

The results obtained concerning correlation of VEGF concentration with the parameters that determine RA activity (ESR, CRP) correlate with the data found in literature confirming the opinion that increased activity of VEGF-mediated processes occurs when RA with IHD lasts longer and patients are older.

We examined sCD28 content in the blood of patients with RA and analyzed the content of this parameter in patients of different age and duration of the disease.

Analysis of the results obtained of average values of sCD28 concentration in the blood of patients from the 1st and 2nd groups did not find differences in the parameters (Table II), but the content of this molecule in samples was twice higher than that of the control group. Therefore, further examination of sCD28 content was made for the two groups (RA) and (RA+IHD) (Fig. 3).

This parameter decreased to 5,3 nmol/L for patients with duration of RA more than 10 years (26,7% lower for patients with duration of RA under 10 years). Analysis of sCD28 content in samples found that maximal concentration was determined among middle age patients, and minimal one (the difference was reliable) – at the elderly age (Fig. 3).

DISCUSSION

Our research does not contradict the hypothesis that with early RA sCD28 marker increases, and when the disease lasts for a long time it decreases. High sCD28 content in the blood of patients with RA is indicative of participation of the regulator mechanism in the compensation of T-cell activation with RA, and at the same time sCD28 inhibits undesirable antigen-antibody interrelations of T-lymphocytes. Reduction of sCD28 content with age is associated with the effect of aging processes on the immune system [12].

The content of NO metabolites in the blood of patients was found to increase at the young and middle age in the background of less pronounced ED and shorter duration of RA. The content of nitrate ions (NO_3^-) in the blood formed in acute or chronic inflammation changes especially considerably. The highest content of nitrite ions (NO_2^-) is observed in middle-aged patients with both RA and coronary heart disease. With increasing age, the content of nitrite ions (NO_2^-) decreases and becomes even lower than that of healthy people. ED intensifies with age, comorbid IHD and in the background of long RA, when the mechanisms of nitrogen monoxide synthesis become so weak that the total content of NO metabolites becomes lower than the parameters of sick people of younger age [8].

When duration of the disease is longer, VEGF content in the blood of patients increases. At the same time, it does not find age dependence with RA and did not change additionally with comorbid IHD [10]. Therefore, it is chronic inflammatory process with RA that becomes the major factor in detection of levels of this regulator protein. sCD28 concentrations are higher in patients with shorter period of RA, they begin to decrease when the disease lasts longer. Elevated parameters in patients of young and middle age enable to consider them as potential biomarkers of the disease activity [11].

CONCLUSIONS

As the duration of the disease increases, the content of VEGF in the blood of patients increases, which, at the same time, did not show age dependence on RA and did not change further with concomitant coronary heart disease. Detected concentrations of sCD28 are higher in patients with less prolonged RA, and begin to decrease with increasing duration of the disease.

Prospects of further studies include the study of immune status parameters in patients suffering from RA with and without IHD, their use as biomarkers of the disease and examination of their interrelations with the parameters of the functional state of the vascular endothelium.

REFERENCES

1. Kravchun P. H., Shelest O. M., Kovalova Yu. O. Markery funktsii endoteliiu i zapalennia u khvorykh na ishemichnu khvorobu sertsia [Markers of endothelial function and inflammation in patients with coronary heart disease]. Medytsyna sohodni i zavtra. 2012; 1: 11-17. (In Ukrainian).
2. Piepoli M. F., Hoes A. W. European Guidelines on cardiovascular disease prevention in clinical practice: The Sixth Joint Task Force of the European Society of Cardiology and Other Societies on Cardiovascular Disease Prevention in Clinical Practice (constituted by representatives of 10 societies and by invited experts). Developed with the special contribution of the European Association for Cardiovascular Prevention & Rehabilitation (EACPR) Eur. Heart J. 2016; 37: 2315–2381.
3. Kruglyi L.B., Zairova A.R., Fomicheva O. A. Signs of arterial stiffness in patients with rheumatoid arthritis and coronary heart disease. Nauchno-Prakticheskaya Revmatologiya, Rheumatology Science and Practice. 2017; 55(4): 382-387 doi: 10.14412/1995-4484-2017-382-387.

4. Iakusheva V.A. Vplyv systemnykh proiaviv revmatoidnoho artrytu i yoho tryvalosti na perebih ishemichnoi khvoroby sertsia [Influence of systemic manifestations of rheumatoid arthritis and its duration on the course of coronary heart disease]. Naukovo-praktychna revmatolohiia. 2003; 2: 117. (In Ukrainian).
5. Puentes-Osorio Y., Amariles P. Potential clinical biomarkers in rheumatoid arthritis with an omic approach // Autoimmun. Highlights. 2021; 12(1): 9.
6. Hansildaar R., Vedder D., Baniaamam M., Tauscheet A-K. Cardiovascular risk in inflammatory arthritis: rheumatoid arthritis and gout. Lancet Rheumatol. 2021; 3(1): 58–70.
7. Fushtei I.M., Sid Ye.V., Ryndina N. H. Korektsiia endotelialnoi dysfunktsii u khvorykh na arterialnu hipertenziiu v poiednanni z revmatoidnym artrytom [Correction of endothelial dysfunction in patients with hypertension in combination with rheumatoid arthritis]. Visnyk problem biolohii i medytsyny. 2019; 4 (2): 216-221. (In Ukrainian).
8. Se Hee Kim, Sang-Heon Lee. Cardiovascular disease risk calculators to reflect the subclinical atherosclerosis of coronary artery in rheumatoid arthritis: a pilot study. BMC Rheumatol. 2021; 5(1): 39.
9. Taylor P.C., Atzeni F. The Key Comorbidities in Patients with Rheumatoid Arthritis: A Narrative Review. J. Clin. Med. 2021; 10(3): 509.
10. Knyazeva A. K., Komarova E. B., Bludova N. G. Endothelial dysfunction in patients with rheumatoid arthritis and comorbidity. Sovremennaya Revmatologiya, Modern Rheumatology Journal. 2017; 11 (4): 25–29. doi: 10/14412/1996-7012-2017-4-25-29.
11. Romano S., Salustri E. Cardiovascular and Metabolic Comorbidities in Rheumatoid Arthritis. CurrRheumatol Rep. 2018; 20(12): 81.
12. Behl T., Kaur I., Sehgal A., Zengin G. The Lipid Paradox as a Metabolic Checkpoint and Its Therapeutic Significance in Ameliorating the Associated Cardiovascular Risks in Rheumatoid Arthritis Patients. Int. J. Mol. Sci. 2020; 21(24): 95-105.

«Drug correction of metabolic and immunological disorders, intensity of systemic inflammation and endothelial dysfunction in patients with a combined course of cardiovascular and bronchoobstructive diseases, musculoskeletal disorders and chronic dermatoses». State registration number: 0120U101550 and «Substantiation and development of medical and social technologies for the prevention of major non-infection diseases» (2020-2024, State registration number: 0120U102625).

ORCID and contributionship:
Anna G. Husarchuk: 0000-0001-9088-5495[A-D]
Tetiana O. Ilashchuk: 0000-0002-0094-8315[E,F]
Iryna V. Okipniak: 0000-0002-3428-6487[B,E]
Valentyna L. Vasiuk: 0000-0003-2037-2162[B,E]
Oksana M. Mykytiuk: 0000-0001-8264-9433[D,E]
Liudmyla V. Mikulets: 0000-0002-1729-4151[C]
Oksana V. Stasyshena: 0000-0002-2992-4640[B]

Conflict of interest:
The Authors declare no conflict of interest.

CORRESPONDING AUTHOR
Anna G. Husarchuk
Bukovinian State Medical University
2 Teatralnaya sq., 58000 Chernivtsi, Ukraine
tel: +380952016700
e-mail: husarchuk.anna@bsmu.edu.ua

Received: 17.11.2021
Accepted: 26.05.2022

A – Work concept and design, **B** – Data collection and analysis, **C** – Responsibility for statistical analysis, **D** – Writing the article, **E** – Critical review, **F** – Final approval of the article