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## RATIO OF THE LEVEL OF NECROSIS AND APAPTOSIS OF PERIPHERAL BLOOD NEUTROPHILS

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**The purpose** of the work is to find out limits and ratio of the activity of apoptosis and necrosis of neutrophil granulocytes in venous peripheral blood in apparently healthy people. **Materials and methods.** 138 people living in the Arkhangelsk region were examined. The survey was conducted in accordance with the norms and rules of biomedical ethics approved by the Helsinki Declaration of the World Medical Association on the ethical principles of medical research (2013). The clinical analysis of peripheral blood was carried out on a Sysmex XS-500i hematological analyzer (Japan). The study of the content of apoptotic cells in the lymph suspension was carried out by the method of double staining with annexin V-FITC (An +/-) and propidium iodide (PI +/-). The results were evaluated on the Epics XL flow cytometer. In blood smears stained according to Romanovsky – Gimze, the neutrogramm structure was detected by microscopy. Serum concentrations of sFas-L, TRAIL, Nt-pro-BNP, cytokines, immunoglobulins were detected by a solid-phase immunoenzyme assay. The content of lymphocyte phenotypes was determined by double peroxidase labeling using monoclonal antibodies. The boundaries of the normal quantitative distribution were determined using the Shapiro-Wilk test. The statistical significance of differences between groups was assessed using Student's parametric t-test and Wilcoxon's test. **Results.** At increase in contents the necrotic neutrophils of AnV+/PI+ statistically authentically grows concentration the apoptotic cells of AnV+/PI- against the background of reduction of number of the circulating neutrophils, monocytes and T-helpers. The concentration of IgE and Nt-pro-BNP increases, but the level of IL-2 decreases. **Discussion.** The higher concentration of Nt-pro-BNP indicates a change in cell membrane activity and osmotic pressure. With increasing number of necrotic neutrophils, there is increase in the migration of all types of white blood cells in the tissue. In addition, the increase in neutrophil necrosis activity in apparently healthy residents of high latitudes creates a risk of autosensitization, which is manifested by increased levels of autoantibodies, including ds DNA. **Conclusion.** In apparently healthy people living in the North, at average, the total number of necrotic neutrophils is  $1,98 \pm 2,09 \times 10^9$  cl/l, apoptotic neutrophils –  $7,0 \pm 8,25 \times 10^9$  cl/l. Activation of neutrophil death by necrosis can become a pathogenetic mechanism for damage to cell membranes (nuclear, lysosomal or cellular), as well as the formation of T-helper immunodeficiencies.

**Key words:** necrosis and apoptosis of neutrophils, lymphocytes, cytokines, immunoglobulins, apparently healthy people.

Apoptosis and necrosis under physiological conditions, being programmed mechanisms of cell death during organism life, have significant differences [7, 27]. Apoptosis is morphologically manifested by signs of nucleus damage - chromatin agglomeration, karyorhexis and karyolysis with the formation of nuclear-free cells or apoptosis bodies, which are phagocytized by macrophages. Cell death by necrosis is due to cell lysis resulting from impaired membrane permeability, including nuclear, lysosomal, or cellular. Necrosis under physiological conditions is initiated by the inability of

the cell to provide active water transfer by osmotic exchange, which requires large energy consumption. Reduced efficiency of self-regulation of cell membrane function leads to alignment of osmotic pressure of cytoplasm and extracellular medium or cytoplasm and lysosomes, or nuclear space and cytoplasm. Membrane damage initiates complete (cytolysis) or partial destruction of cell structures by cell enzymes (autolysis, autophagy). In necrosis, the contents of the cytosol, cellular organelles, including lysosomes, appear in the intercellular medium and are able to initiate reactions of various systems, primarily kallikrein-kinin, complement, fibrinolysis and blood clotting. Cytokines play an important role in the regulation of cell death, but their role is not so unambiguous. Stimulation of neutrophils with IFN- $\alpha$  and IFN- $\gamma$  leads to a decrease in their apoptosis by tyrosine phosphorylation of STAT1 and STAT3, an increase in the level of mRNA cIAP2, activation of JAK2 [35]. In addition, IFN- $\gamma$ , acting on the receptors TLR2 and TLR4 inhibits the kinase p38 MAPK [14]. The action of IFN- $\gamma$  may depend on the number of receptors (IFN- $\gamma$ R2) on the surface of the neutrophil; at their high density, STAT-1 is rapidly activated, the level of IRF-1 is increased, and the programmed death of granulocytes is triggered. With a small amount of IFN- $\gamma$ R2 on the cell membrane, a weaker activation of STAT-1, a slower increase in the content of

IRF-1 is detected, and a neutrophil proliferation program is initiated [11]. Biffi W. L. et al. (1996) showed that IL-6 suppresses apoptosis of polymorphonuclear neutrophils through a mechanism involving a platelet activating factor, along with an increase in the level of cytosolic calcium [12]. According to L. Ottonello (2002), IL-6 has an anti-apoptotic effect, reducing the expression of the Bax protein and caspase-3 [33]. However, there is evidence that IL-6, with the participation of IFN- $\gamma$ , acting through its soluble receptors sIL-6R, causes apoptosis of granulocytes and initiates the secretion of CXCL5, CXCL6 chemokines to attract mononuclear leukocytes [22]. IL-10 prevents programmed death of myeloid progenitor cells, together with growth factors promotes their proliferation. IL-10 increases the tyrosine phosphorylation of the insulin receptor and stimulates the activity of 3-kinase / Akt and p70 S6 [15], thereby enhancing the antiapoptotic effect of ERK1 / 2 molecules [40]. According to other data, granulocytes, pre-treated with lipopolysaccharides, or TNF- $\alpha$  and IFN- $\gamma$ , interacting with IL-10 are prone to programmed death [24]. When IL-4 acts on neutrophils through the CD132 / IL-4R $\alpha$  receptors, phagocytosis and cell adhesion of granulocytes are enhanced, and their apoptosis is delayed [19]. It is well known about the proapoptotic activity of TNF- $\alpha$ , which is characterized by an increase in the lev-

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el of IA PI3K enzymes, an increase in the number of reactive oxygen species and caspase-3 within granulocytes [18]. The effect of stimulating apoptosis is directly dependent on the concentration of TNF- $\alpha$  [36]. However, there is information about the antiapoptotic effect of TNF- $\alpha$  on neutrophils [32]. Signaling is via TNFR1 receptors that bind to  $\delta$ -PKC; the resulting complex promotes the activation of ERK1 / 2 and NF $\kappa$ B, and the suppression of caspase 3 [25]. On the neutrophils themselves, an increase in the amount of membrane pro-TNF is observed [38], the circulation of anti-apoptotic proteins Mcl-1 and BFL-1 [16] is accelerated, which indicates autocrine regulation of the apoptosis process. The relationship between blood levels of brain natriuretic peptide (BNP) and the severity of heart failure is well understood. It is known that in patients with cardiovascular diseases, the concentration of TNF- $\alpha$  in plasma increases, the content of its cellular receptor and the readiness to trigger an apoptotic stimulus increase [21]. At the same time, TNF- $\alpha$ , through the increase in reactive oxygen species, induces nuclear factors of cell death, and it has also been shown that the synthesis of a soluble form of sFas-L is triggered with the participation of metalloproteinases, the activity of which is increased in patients with heart failure [28]. However, the question of the relationship between BNP and cytokines in the activation of cell death, which occurs both in health and in cardiovascular pathology, has not actually been studied. It is especially important to study these phenomena with respect to tissues and cells that provide regulatory and protective responses. Neutrophil granulocytes are the most numerous of the blood leukocytes. In humans, more than  $10^{11}$  neutrophil granulocytes are produced per day, and the turnover is tens of thousands of times less [17, 26]. Therefore, the regulation of neutrophil death is one of the most important processes for maintaining their optimal number and functional activity. In connection with the above, it was interesting to find out the limits and ratio of the activity of apoptosis and necrosis of neutrophil granulocytes in venous peripheral blood in apparently healthy people. The ratio of necrosis to apoptosis at the physiological level has not been apparently studied. At the same time, cell decay type may have different biological significance for differentiation, regeneration and renewal of cells and their level of activation [7]. It can be assumed that the change in the relationship between physiological levels of necrosis and apoptosis may be the cause of the

formation of immunodeficiency, auto-sensibilization, oncopathology, chronic course of the disease and even aging.

**Materials and methods.** The survey was conducted at 138 apparently healthy people aged 20 to 60 years living in the Arkhangelsk region. The examined persons did not suffer from acute infectious diseases, they showed no signs of autoimmune and lymphoproliferative processes. The survey was conducted in accordance with the norms and rules of biomedical ethics approved by the Helsinki Declaration of the World Medical Association on the ethical principles of medical research (2013).

Clinical analysis of peripheral blood was carried out on a Sysmex XS-500i hematological analyzer (Japan). The study of the content of apoptotic cells in the lymph suspension was carried out by the method of double staining with annexin V-FITC (An +/-) and propidium iodide (PI +/-). The results were assessed by staining or non-staining of cells: living cells - An- / PI-, early apoptosis - An + / PI-, late apoptosis - An + / PI +, necrosis - An- / PI +. The analysis of the results was performed by flow cytometry on an Epics XL flow cytometer (Beckman Coulter, USA); up to 5000 events were studied in each sample. In blood smears stained according to Romanovsky - Giemsa, the nuclear formula of neutrophils (neutrogram) was studied by microscopy (Meiji Techno, Japan). Up to 100 neutrophilic leukocytes were counted, among which cells with 1, 2, 3, 4, 5 and more segments of the nucleus were isolated [9]. To determine the phagocytic activity of neutrophils, 100  $\mu$ l of latex and 100  $\mu$ l of blood were mixed in a test tube with heparin, then the tube was incubated in a thermostat at + 37°C for 30 minutes. After the lapse of time, the supernatant fluid was taken, transferred

to a glass slide, and a smear was made. The resulting smear was stained according to Romanovsky-Giemsa. We took into account the data on 100 neutrophils, from which the phagocytic activity is calculated - the percentage of phagocytic neutrophils; phagocytic number - the average number of particles captured by one cell [2]. Serum levels of sFas-L, TRAIL, cytokines (TNF- $\alpha$ , IFN- $\gamma$ , IL-2, IL-4, IL-6, IL-10, IL-17F) were determined by the method of enzyme-linked immunosorbent assay (ELISA), immunoglobulins A, E, M, G (kits from Bender MedSystems, Austria), histamine (DRG, Germany), Nt-pro-BNP (Biomedica, Austria). The reaction was evaluated using a Multiskan MS photometer (Labsystems, Finland) and an Evolis automatic enzyme immunoassay analyzer (Bio-RAD, Germany). The content of lymphocyte phenotypes was examined by double peroxidase labeling using monoclonal antibodies («Sorbent», Russia). The results of the study were processed using the Statistica 6 application package (StatSoft, USA). Type of study retrospective, samples random, one-time. The general population is residents of the north of the European territory of Russia. The boundaries of the normal distribution of quantitative measures were determined using the Shapiro-Wilk test. The mean and standard deviation, median and lower, upper quartiles, were used in the analysis of the results. The validity of differences between groups was assessed using Student's parametric t-test for independent samples and Wilcoxon's nonparametric test. Statistical validity was assigned at  $p < 0.05$ .

**Results and discussion.** Among the general group of examined people of northerners, persons with a relatively increased and relatively reduced content of necrotic neutrophils AnV+/PI+ and apop-

#### Content of immunocompetent blood cells in practically healthy northerners depending on the content of dead neutrophils

Indicator, $\times 10^9$ cl/l	Necrosis		Apoptosis	
	> 3 % (1) 4.0(3.4-4.9)**	< 1 % (2) 0.8(0.75-0.9)	> 8% (3) 14.1(10.5-14.7)*	< 3% (4) 1.9(1.3-2.4)
	M $\pm$ m			
Leukocytes	6.9 $\pm$ 0.51**1-2	9.1 $\pm$ 0.58	7.64 $\pm$ 0.76*3-4	8.78 $\pm$ 0.65
Neutrophils	3.58 $\pm$ 0.33**1-2	5.51 $\pm$ 0.44	4.43 $\pm$ 0.49*3-4	5.4 $\pm$ 0.38
Lymphocytes	2.38 $\pm$ 0.15	2.87 $\pm$ 0.23	2.64 $\pm$ 0.27	2.63 $\pm$ 0.22
Monocytes	0.26 $\pm$ 0.04*1-2	0.42 $\pm$ 0.04	0.24 $\pm$ 0.04*3-4	0.36 $\pm$ 0.05
Eosinophils	0.21 $\pm$ 0.04	0.25 $\pm$ 0.04	0.26 $\pm$ 0.04	0.23 $\pm$ 0.04
	ME(Q1-Q3)			
AnV+/PI+	0.17(0.09-0.21)* 1-2	0.04(0.03-0.05)	0.07(0.05-0.10)	0.06(0.04-0.09)
AnV+/PI-	0.28(0.16-0.46)* 1-2	0.18(0.1-0.34)	0.5(0.41-0.68)**3-4	0.1(0.07-0.14)
% act. phagocytes	56(47-66)	50(45-56)	55(52-58)	55(48-67)

Note. Statistically significant differences: \* –  $p < 0.05$ ; \*\* –  $p < 0.01$ .

totot neutrophils AnV+/PI- blood were identified according to the results of laser flow cytometry: AnV+/PI+ >3% (n=35) и <1% (n=40),  $p=0,0011$ ; AnV+/PI- >8% (n=37) и <3% (n=36),  $p=0,0001$ . The median values, upper and lower quartile of the test cells are shown in Table.

It was found that in people with high levels of necrotic cells, the total number of white blood cells due to segmentonuclear neutrophils and monocytes is lower. The phagocytic activity of neutrophils does not decrease. Among the neutrophil granulocyte population, cell content with 2, 3, and 4 nucleus segments is reduced (Fig. 1). Concentrations of stick-nuclear neutrophils and cells with 5 or more nucleus segments did not actually differ. It has long been known that mainly mature neutrophils undergo autolysis, young cells autolysis more slowly [5, 10].

No significant differences in lymphocyte, eosinophil and basophil content have been established. Against the background of an increase in the content of necrotic neutrophil granulocytes, the concentration of apoptotic cells (AnV+/PI-;  $p<0,001$ ). In the ratio of the activity of apoptosis and neutrophil necrosis, the following patterns were revealed: with an increase in the level of neutrophil necrosis > 3%, the average value of the ratio is  $2,93\pm0,60$ , in the conditions of a decrease in the level of cellular necrosis < 1%, this ratio increases sharply  $7,95\pm1,76$ .

With increasing the neutrophil necrosis activity, the absolute content of circulating mature T-lymphocytes (from  $0,77\pm0,06$  to  $0,62\pm0,04\times10^9$  cl/l,  $p=0,038$ ) due to T-helpers (from  $0,69\pm0,06$  to  $0,54\pm0,04\times10^9$  cl/l,  $p=0,023$ ). The average total lymphocyte content and the remaining phenotypes CD8+, CD10+, CD16+, CD23+, CD25+, CD71+, HLADR, CD95+ remain virtually unchanged (Fig.

2). The reduction of circulating neutrophils, monocytes and T-helpers against the background of increased levels of necrotized neutrophils in the blood allows us to believe with some certainty that the migration of white blood cells in tissues under these conditions becomes more active. It seems that with the growth of necrotic leukocytes, there is an increase in the migration of all types of leukocytes into the tissue. However, due to the low blood concentrations of some of them (basophils, eosinophils, monocytes) and their predominant presence in tissues, the change in the level of these cells in the periphery is less pronounced.

With increasing the neutrophil necrosis activity, the content of the natriuretic peptide Nt-pro-BNP is higher [ $112,9(48,7-147,0)$  and  $27,4(17,1-50,4)$  fmol/ml, respectively;  $p=0,012$ ]. Statistically significant increase of natriuretic peptide concentration indicates change of cell membrane activity and osmotic pressure [3, 20]. Not revealed reactions to increase in activity of necrosis of neutrophils from IgM [ $3,1(2,7-3,6)$  and  $2,9(2,6-3,3)$  g/l,  $p=0,871$ ], IgG [ $8,8(3,9-10,9)$  and  $7,6(3,7-9,6)$  g/l,  $p=0,568$ ], IgA [ $1,2(1,0-1,5)$  and  $1,3(0,9-1,5)$  g/l,  $p=0,932$ ] and also in the maintenance of a histamine [ $0,96(0,47-1,27)$  and  $0,91(0,61-1,42)$  ng/ml,  $p=0,579$ ] and extracellular sFas-L [ $0,1(0,07-0,25)$  and  $0,07(0,04-0,12)$  ng/ml,  $p=0,531$ ].

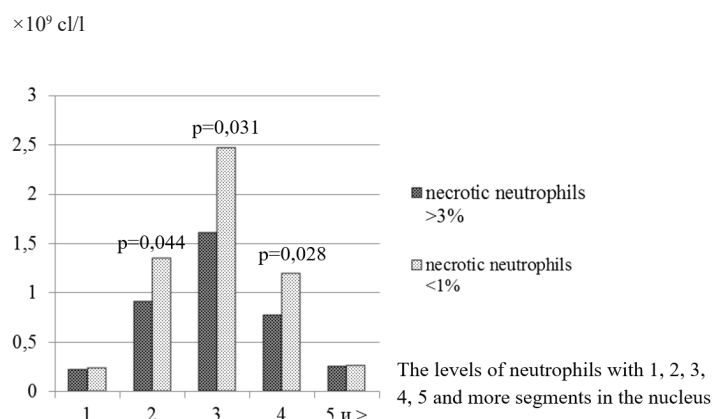
The cytokine profile of people with the level of granulocytes AnV+/PI+ more than 3% is characterized by falling of concentration of IL-2 from  $16,3(2,4-18,2)$  to  $7,8(4,2-12,7)$  pg/ml,  $p=0,016$ , against the background of rather stable maintenance of TNF- $\alpha$  [ $23,0(19,7-34,1)$  and  $25,8(17,9-32,0)$  pg/ml], IFN- $\gamma$  [ $19,9(19,6-20,1)$  and  $19,8(19,7-20,0)$  pg/ml], IL-4 [ $3,9(2,3-11,7)$  and  $5,6(2,3-7,9)$  pg/ml], IL-6 [ $7,8(5,3-13,5)$  and  $10,3(7,0-13,2)$

pg/ml], IL-10 [ $0,34(0,05-0,38)$  and  $0,98(0,09-2,0)$  pg/ml], IL-17F [ $47,8(39,1-53,7)$  and  $49,0(42,5-58,3)$  pg/ml],  $p>0,05$ .

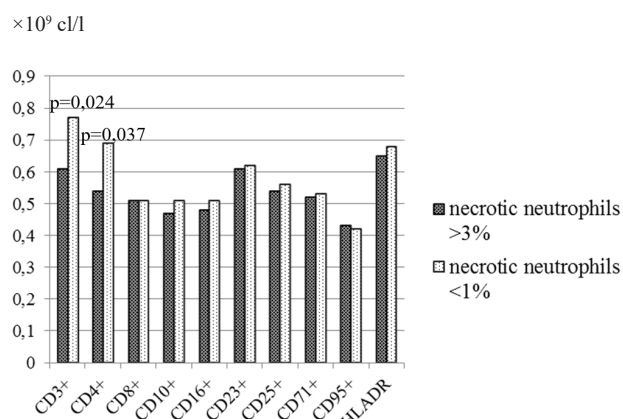
The higher IgE concentrations [from  $17,3(9,4-33,3)$  to  $31,1(12,5-59,9)$  IU/ml;  $p=0,023$ ] can be explained by the features of Ig of this class. IgE is a typical secretory Ig capable of rapid redistribution to the center of disadvantage effectively neutralizes, binds and reduces concentrations of antigen structures [23, 30]. Moreover, cytolysis with the participation of reagents occurs almost instantly.

The increase in neutrophil necrosis activity in apparently healthy residents of high latitudes creates a risk of auto-sensitization, which is manifested by increased levels of autoantibodies, including ds DNA [4, 8]. An increase in TRAIL concentrations [from  $11,4(4,4-56,6)$  to  $40,6(8,4-60,1)$  pg/ml,  $p=0,026$ ] associates with apoptosis activity.

Thus, with raising the death of polymorphonuclear neutrophil granulocytes of more than 3% leads to the decreased content of neutrophils circulating in peripheral venous blood without reducing their phagocytic activity. It is known that more than 60% of circulating neutrophils are potential phagocytes, they interact with the object of phagocytosis, form traps, produce active oxygen species and undergo apoptosis [6, 13]. Secreting neutrophils fully enter the circulating pool, have permeable membranes, produce less active oxygen species, and are practically not involved in apoptosis [1]. Autophagy regulates homeostasis in the endoplasmic reticulum [37]. In turn, the Toso gene inhibits autophagy and apoptosis, and IL-2 STAT5-dependent method suppresses formation of the Toso receptor [31]. Therefore, the reduction in IL-2 content under the conditions of increased necrosis and apoptosis, when the level of neutrophil death increases by more than



**Fig. 1.** Neutrogramm of peripheral blood of practically healthy people depending on the content of necrotic neutrophils



**Fig. 2.** Content of peripheral blood lymphocyte phenotypes in practically healthy people depending on necrotic neutrophil content



4 times, is not accidental. The ability to intraphagosomal degranulation gives neutrophils, the ability to regulate the functional activity of many immunocompetent cells, including lymphocytes, at autocrine and paracrine levels [34, 39]. Reduction of T-helper content in necrosis-increasing situations may result from increased lymphocyte migration and recycling activity.

**Conclusion.** In apparently healthy people living in the North, on average, the total number of necrotic neutrophils is  $1,98 \pm 2,09 \times 10^9$  c/l/l, apoptotic neutrophils –  $7,0 \pm 8,25 \times 10^9$  c/l/l. With increasing neutrophil necrosis  $> 3\%$  ( $0,16 \pm 0,08 \times 10^9$  c/l/l), people record an average level of apoptotic neutrophils  $0,29 \pm 0,21 \times 10^9$  c/l/l, in the situation of reducing necrosis activity  $< 1\%$  ( $0,04 \pm 0,03 \times 10^9$  c/l/l), the number of apoptotic neutrophils is equal to  $0,28 \pm 0,38 \times 10^9$  c/l/l. The activation of neutrophil death by necrosis can become a pathogenetic mechanism for damage to cell membranes (nuclear, lysosomal or cellular), as well as the formation of T-helper immune deficiencies.

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## EVALUATION OF PARAMETERS OF THE DYNAMIC COMPONENT OF POSTURAL BALANCE IN ELDERLY WOMEN

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The aim of the study was to develop centile tables to assess basic parameters of the dynamic components of the postural balance in women 60-74 years old. Three groups of women aged 60-64 years, 65-69 years, and 70-74 years, with a total number of 186 people, were examined. Evaluation of main indicators of the postural balance was carried out using the computer stabilometric complex "Balance Manager". The presented standards will help to increase the information content of research and objective analysis of the results obtained, as well as to evaluate the effectiveness of the implementation of measures to maintain normal, safe walk or prevention of mobility restriction.

**Key words:** dynamic component of postural balance, women, elderly age, centile grade.

**Introduction.** Domestic and international studies have shown that the main component of active longevity is the preservation of autonomy and mobility in the elderly. In order to minimize and prevent the loss of an independent, autonomous existence, specialists working with older people should timely determine the level

of risk that a future decrease in mobility can predict [2, 4, 6, 7]. The analysis of walking is a widely used indicator of the effectiveness of the functioning of lower extremities in particular, and the postural balance as a whole [3, 5, 6, 9, 10]. Changing the main parameters of a dynamic component of the postural balance is a clear indicator of the preservation of the dynamic component of the postural balance, since they are the most sensitive to future changes in the functional state of the body of the elderly women. However, in modern domestic literature data there is no information about standards for evaluating the main indicators of the dynamic component of the postural balance in elderly women [1, 8, 11-15]. The purpose of the study was to develop centile tables for evaluating the main parameters of the dynamic components of the postural balance in women 60-74 years old.

### Materials and research methods.

A cross-sectional study was conducted with the informed consent of participants. The study involved 186 women, aged 60-74 years, who were divided into age groups: 60-64 years old - 61 people (average age -  $62.8 \pm 1.3$  years), 65-69 years old - 63 people (average age -  $67.8 \pm 1.8$  years), 70-74 years - 62 people (average age -  $73.1 \pm 1.1$  years). All women in the course of the study were mobile and did not use additional means of support when walking. The following exclusion criteria were considered: a history of strokes, dementia, being registered in a neuropsychiatric dispensary, traumatic

brain injuries, acute and chronic diseases during the exacerbation period, as well as permanent residents in nursing homes.

The evaluation of the main parameters of the dynamic component of the postural balance was carried out using the Balance Manager computer stabilometric complex and included the following tests and parameters: Sit to Stand test (Weight Transfer Time, Rising Index, Sway Velocity), Walk Across (Step Width, Step Length, Speed), Tandem Walk (Step Width, Speed, End Sway), Step - Quick Turn (Time (with left and right legs), Sway (with left and right legs)), Step - Up and Over (Lift Up Index (with left and right legs), Movement Time (with left and right legs), Impact Index (with left and right legs)) [1-3].

Statistical processing of the obtained data was carried out using the application package SPSS 21.0 for Windows. For each of the studied indicators, the distribution of signs on normality was assessed using the Shapiro - Wilk criterion. For each of the studied indicators, arithmetic means (m), standard deviations (s), and values equal to 10, 25, 50, 75, and 90 centiles in each of the age groups were calculated.

**Results and discussion.** Tables 1-3 show the centile distribution of the main parameters of the dynamic component of the postural balance in women of the studied age groups, namely 60-64 years old, 65-69 years old, 70-74 years old. As a result of the data analysis with increasing age, in all the studied groups there is a uniform offset of median values of

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