Background. Apoptosis is a natural process of genetically programmed cell death, which, however, is exposed to a number of external and internal factors. According to modern concepts, the number of cells, which are exposed to programmed death, depends on the ratio of activators and inhibitors of apoptosis, thus it can be considered as the result of imbalance of pro- and antiapoptotic factors in the body that affect certain cells. Dysregulation of apoptosis leads to various diseases coming from intensification or, conversely, inhibition of apoptosis. Vice versa, diseases, in particular critical ones, lead to evident pathology of apoptosis.

Chronic metabolic stress because of hyperglycemia induced by sensory decrement of insulin with underlying type 2 diabetes mellitus (DM) affects cellular homeostasis of almost all cell types. Intracellular calcium stores such as the endoplasmic reticulum and mitochondria together with plasma membrane systems that regulate the concentration of calcium, are of first importance in calcium signaling. Excess accumulation of Ca\(^{2+}\) by mitochondria in case of diabetes may interrupt the synthesis of ATP and significantly change the ratio of ATP / ADP. There is also an evidence that in case of diabetes mitochondrial pores might open, what causes rapid and massive release of Ca\(^{2+}\) into cytosol. This leads to disorder in various cell functions and development of apoptosis. ATP production decrement that occurs interrupts mechanisms of Ca\(^{2+}\) homeostasis even more, particularly Ca\(^{2+}\) pumps ER and the plasma membrane.

The increase of calcium ions concentration in the cytosol may occur due to either strengthening of its input from intercellular space through the plasma membrane, or intracellular homeostasis disorder, depending on the function of mitochondria and endoplasmic reticulum. The increase of intracellular Ca\(^{2+}\) leads to the formation of cyclophylin D-ANT complex, which induces pores opening and apoptosis processes initiation. The close connection of calcium ions with the processes of cell apoptosis suggests its being an important marker and initiator of this process.

The death of red blood cells is characterized by some common for apoptosis features. To distinguish the death of red blood cells from apoptosis of nuclear cells, some authors suggest the term «Eryptosis» (Lang, K.S. et al., 2005). It is well known that the shape and flexibility of red blood cells strongly depends on calcium ions – the growth of internal Ca\(^{2+}\) leads to changes in their shape and volume, cell rigidity and hemolysis strengthening. Eryptosis might be caused by energy shortage, osmotic shock or oxidative stress. Energy shortage (decreased activity of calcium-ATPase) leads to decrease of calcium, which in turn accelerates the transmembrane movement of potassium and chloride that causes cellular dehydration. Energy shortage also reduces glutathione reserves and thereby reduces the antioxidant protection of red blood cells, which similarly activates cationic channels, affecting the flow of calcium. Atherogenic and
biochemical changes in the blood, in case of coronary artery disease, namely lipid storage disease with subsequent activation of lipid peroxidation, hyperperoxidation and oxidative stress with formation of free radicals, lead to the transition of oxyhemoglobin to methemoglobin, increase of protease, caspases, externalization of phosphatidylserine, increase of intracellular calcium followed by initiation of apoptosis and disruption of the structure and function of red blood cells.

The last decade was marked by radical transformation of the idea about neutrophilic granulocytes. While initially neutrophils were considered only as cell macrophages as well as «vanguard of white blood cells», that are first to take up the struggle with a causative agent in the focus of inflammation (short-lived, non-specific, only capable of phagocytosis or exocytosis of cells that can be killers of their damaged tissues), the present-day knowledge describes them as a specific weapon that is quickly mobilized and directly involved into activation of endothelium, monocytes, macrophages, thrombocytes, dendritic cells and T-lymphocytes. In other words, neutrophils are the main managers of immune protection that respond to any metabolic and alternative processes that occur in the body.

**Objective.** Find out ultrastructural changes and the state of cellular immunity in patients with acute coronary syndrome (ACS) and type 2 DM, and identify the association between increase of intracellular calcium in erythrocytes and calcification of coronary arteries for diagnosis improvement and correction of biochemical changes in the blood that conduce to coronary artery disease.

**Materials and methods.** The study involved 20 patients with coronary artery disease who were hospitalized in the cardiology department of Lviv Emergency Hospital (Department of Family Medicine FPGE of DanyloHalytskyLviv National Medical University), including 8 persons suffering from type 2 DM with different severity, 12 persons suffering from obesity of I-III level and 2 more with occupational hazards. The control group included 10 almost healthy persons. The average age of patients was 65 (±5.6) years. Both groups were compared in accordance with age and gender characteristics. The patients with coronary artery disease underwent multispiral computed tomography on tomographic scanner SomatomVolum Zoom (Siemens, Germany) according to A. Agatston method using the program Calcium scoring. Quantitative analysis calcification of coronary arteries was performed by algorithm offered by A. Agatston et al. Calcium amount was calculated for individual segments of coronary arteries with calculation of total calcium index. Cytological determination of calcium salt in erythrocytes was carried out in direct smears of peripheral blood using alcohol solution of Alizarin Red S using McGee-Russell method. Also the level of overall and ionazed calcium in plasma and blood pH by hardware
(Electrolytes Analyzer E-lyte 5, ELT-1000) were measured. Immunological studies of leukocytes apoptosis (neutrophils, monocytes and lymphocytes) were performed using a set ApoLect to identify apoptotic and necrotic-modified cells in whole blood (Immunology Department Laboratory of DanyloHalytskyLviv National Medical University). Ultrastructural signs of leukocytes apoptosis were also studied using electron microscopy of blood samples of those patients.

**Results.** While carrying out immunological studies a significant increase in the number of apoptotically altered monocytes mainly as well as lymphocytes and appearance of necrotic altered blood cells in patients with ACS and accompanied with type 2 DM were revealed. Classic ultrastructural signs of leukocytes apoptosis were displayed through their excessive osmiophilia, condensation of nuclear heterochromatin, and pycnosis and karyorhexis, appearance of microbubble, vacuolization of endoplasmic reticulum and the Golgi complex, and also the presence of small, rounded mitochondria. Apoptosis of leukocytes positively correlated with the severity of coronary artery disease risk factors as well as the age of the patients.

In the body of patients with high and very high index of calcification of the coronary arteries (from 400 to 1599) the presence of calcium granules in red blood cells of various sizes and shapes, of violet-blue color and also the alteration of the shape and size of red blood cells can be cytologically observed. From biochemical view the decrease of ionized calcium level in blood serum in case of pH shift towards acid side was noticed.

The presence of type 2 DM of medium or severe degree significantly increased level of calcification of both coronary vessels and red blood cells. In the body of healthy people without risk factors of coronary artery disease deposits of calcium in erythrocytes were not observed.

**Conclusions.**

1. Presence in senior and elderly patients with CHD of risk factors such as type 2 DM, hypertension, atherogenic dyslipidemia, and obesity, smoking and occupational hazards – leads to activation of apoptosis of leukocytes, particularly neutrophils and monocytes/macrophages. They can be observed by modern specific immunological tests, and electron microscopic studies.
2. High (400-999) and very high (over 1000) coronary arteries calcification index is associated with calcification of the vast majority of red blood cells and apoptosis and calcification of a significant percentage of peripheral blood leukocytes, which correlates with the presence and severity of coronary artery disease.
3. Detected correlation between calcification of erythrocytes and leukocytes and calcification of coronary arteries is a marker of progression of coronary artery disease and it may facilitate the search of preventive and therapeutic remedies to optimize the treatment of this group of patients.