SUMMARY

Cardiovascular diseases belong to the group of civilization diseases and are the most common causes of death in the medium and highly developed countries. The most prevalent cardiovascular diseases include atherosclerosis and chronic venous disease. Many environmental, genetic and epigenetic factors influence the development of these diseases. Common risk factors for atherosclerosis and chronic venous disease include: age, diabetes, obesity and smoking.

Atherosclerosis clinically manifests in arterial atherosclerotic plaques that disturb blood flow and impair blood supply to the tissues. The progressive increase in the size of atherosclerotic plaques can lead to chronic ischemic syndromes, including low extremities arterial disease.

Chronic venous disease is caused by impaired blood flow and venous hypertension determined by malfunctions of venous valves and muscle pumps, as well as by reflux and vein occlusion in the lower limbs.

Low extremities arterial disease and chronic venous disease are characterized by a high risk of life-threatening complications and the insufficiency of diagnostic methods detecting early metabolic symptoms of these diseases.

MiRNAs are short, single-stranded RNA molecules that regulate gene expression. MiRNAs are important factors affecting the course of signal transduction pathways in cells, deciding about the normal or pathological course of physiological processes.

The aim of our study was to: indicate changes in miRNA and gene expression in patients with low extremities arterial disease and chronic venous disease compared to healthy controls, to select potentially biomarker miRNAs and genes for these diseases, and to identify regulatory relationships between miRNAs and genes shown as potential biomarkers of low extremities artery disease and chronic venous disease.

The biological material used in our studies were peripheral blood mononuclear cells (PBMCs) isolated from venous blood from study participants. MiRNA and gene expression profiles in PBMC were obtained using Next Generation Sequencing (NGS) method with Ion Torrent technology. Statistical significance of changes in miRNA and genes expression in the group of patients with low extremities arterial disease and chronic venous disease compared to the control group was assessed using the DESeq2 and UVE-PLS methods implemented in the R programming environment.

Using the DESeq2 method, we indicated 1 181 miRNA transcripts and 17 868 genes with differential expression in patients with low extremities arterial disease as well as 1 034 miRNA transcripts and 23 204 genes with differential expression in patients with chronic venous disease was demonstrated, compared to the control group. Statistical significance ($p < 0.05$) were demonstrated for 231 miRNA transcripts (for 209 miRNA) and 221 genes in the group of patients with low extremities arterial disease as well as for 96 miRNA transcripts (for 85 miRNA) and 2719 genes in the group of patients with chronic venous disease.

Using the UVE-PLS method, we indicated 86 miRNA transcripts (for 81 miRNA) and 14 genes with differential expression in patients with low extremities arterial disease, as well as 48 miRNA transcripts (for 41 miRNA) and 74 genes with differential expression in patients with chronic venous disease.

We demonstrated 28 miRNAs and 14 genes whose statistically significant changed expression in the group of patients with low extremities arterial disease compared to control group was confirmed by both DESeq2 and UVE-PLS.

We demonstrated 31 miRNAs and 62 genes whose statistically significant changed expression in the group of patients with chronic venous disease compared to control group was confirmed by both DESeq2 and UVE-PLS.

Among demonstrated 59 miRNAs and 76 genes, there were 7 miRNAs (hsa-miR-34a-5p, hsa-miR-122-5p, hsa-miR-3591-3p, hsa-miR-548d-5p, hsa-miR-548aa, hsa-miR-548t-3p, hsa-miR-30e-3p) and one gene (CDS2), which has been assigned to both low extremities arterial disease and chronic venous disease.

In order to assess the diagnostic value of demonstrated 28 miRNAs and 14 genes selected for low extremities arterial disease and 31 miRNAs and 62 genes selected for chronic venous disease, the ROC (Receiver Operating Characteristics) analysis was performed. The area under the ROC curves for 28 miRNAs selected for low extremities arterial disease was above 0.8, while the area under the ROC curves for the 14 genes selected for low extremities arterial disease was 1 for 11 genes, 0.982 for 2 genes and 0.964 for one gene.

The area under the ROC curves for 31 miRNAs selected for chronic venous disease was within the range of 0.757-0.930, while the area under the ROC curves for 62 genes selected for chronic venous disease was 1 for 60 genes and 0.98 for 2 genes. The results of the ROC analysis confirmed the high predictive value of changed expression of selected miRNAs and genes in discrimination of low extremities arterial disease and chronic venous disease. Obtained results allow to propose 28 miRNAs and 14 genes as biomarkers of low extremities arterial

disease and also allow to propose 31 miRNAs and 62 genes as biomarkers of chronic venous disease.

Using the multiMiR package we demonstrated 8 validated and 71 the most predictive interactions, occurring between proposed miRNA and gene biomarkers associated with low extremities arterial disease. There were also 12 validated and 51 the most predictive interactions, occurring between proposed miRNA and gene biomarkers associated with chronic venous disease.

Functional analysis of genes regulated by proposed miRNA biomarkers of low extremities arterial disease demonstrated the association of these genes with atherosclerosis, neurological diseases and addiction to chemical substances.

Functional analysis of genes regulated by proposed miRNA biomarkers of chronic venous disease demonstrated the association of these genes with cardiovascular diseases, neurological diseases, kidney diseases and addiction to chemical substances.

The results obtained in our studies confirm the role of miRNA-dependent epigenetic regulation in the etiopathogenesis of low extremities arterial disease and chronic venous disease. Potential biomarkers of arterial disease of the lower limbs and chronic venous disease proposed in our studies can be used in differentiation, diagnosis and therapy of these diseases.

Obtained results do not fully explain the effect of changes in miRNA and genes expression on the course of low extremities arterial disease and chronic venous disease. Still exists the need for further comprehensive studies on the role of miRNA regulation in etiopathogenesis of low extremities arterial disease, chronic venous disease, as well as in other cardiovascular diseases.