

Proteomic, metabolomic and lipidomic blood analysis in the investigation of atherosclerosis progression in chronic kidney disease

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ABSTRACT

Chronic kidney disease (CKD) is characterized by prolonged, progressive and irreversible loss of kidney function, which requires a renal replacement therapy in the last stage. The disorder causes the accumulation of intermediate products of metabolism in the body, which exposes patients to the development of many accompanying diseases. One of them is the rapidly progressive CKD-related atherosclerosis (CKD-A) and its consequences in the form of cardiovascular disease (CVD) as well as its complications, which are the major cause of mortality among CKD patients. Several studies have shown that nature of atherosclerotic lesions appearing in dysfunctional blood vessels in CKD differs from those in classical CVD, suggesting the involvement of other molecular mechanisms in the development of CKD-A. However, the pathomechanism of CVD acceleration in CKD has not been fully elucidated so far.

The main goal of the doctoral dissertation was an attempt to extend the knowledge about the processes and pathways involved in the development of CKD-A. The results presented in this doctoral dissertation were published as the review article and two experimental papers. In review, the versatile utilization of various "omics" approaches, with particular emphasis on proteomics for the study of CKD, was discussed. Moreover, the potential of using systems biology and integrative studies to analyze the pathomechanism underlying CKD-A progression, was emphasized. Then, a comprehensive proteome analysis of the leukocyte fraction as well as the metabolomic and lipidomic analysis of plasma using methods based on mass spectrometry (MS), were performed. The biological material was obtained from two groups of patients with different severity of CKD and thus different progression of atherosclerosis, and two groups with classical CVD, also different in the context of atherosclerosis progression, but without any symptoms of renal dysfunction. As a reference, material collected from healthy volunteers, was used. The obtained results were partially verified at the level of proteins and mRNA utilizing the techniques such as ELISA, western blot, ddPCR and MS targeted analysis in the MRM mode. The performed statistical and bioinformatics analyzes allowed to indicate the processes and molecular pathways involved in the development and progression of CKD-A.

The identified changes in protein accumulation revealed disturbances in the inflammatory mechanisms in CKD-A related to the process of cells transmigration through the vascular endothelium. It has been shown that in CKD mobilization and adhesion of leukocytes on the endothelial surface may be increased, but the processes responsible for the reorganization of the cytoskeleton and cell polarization, necessary to complete the migration process are most likely inhibited. In this situation, cell aggregation on the endothelial surface, local inflammation and endothelial dysfunction, promoting atherogenic processes, might be observed. As a consequence the intensification of programmed cell death strongly correlated with changes at the level of adhesive molecules, was observed.

The unique lipidomic profile of plasma in CKD-A and CVD was also demonstrated for the first time. The reduced level of accumulation of sphingomyelins, cholesterol and its esters, phosphatidylcholines, ceramides and phosphatidylethanolamines in CKD compared to CVD, were indicated. An increased level of triacylglycerol accumulation in CKD was also demonstrated, but a detailed analysis of compounds that are substrates for these lipids, and thus LDL and HDL, showed that in this case, disturbances in the synthesis or degradation of these molecules may play a much

greater role than their level, which may affect their atherogenic and anti-atherogenic properties. The performed analyzes of correlation suggested that the unique lipid profile may also be associated with the severity of inflammation accompanying renal dysfunction.

In conclusion, a lot of changes at the level of proteins, lipids and low-molecular-weight metabolites, were demonstrated, indicating, among others, disturbance of inflammatory mechanisms, in particular systemic inflammation, migration, apoptosis and the coagulation process, and thus endothelial and leukocyte homeostasis in CKD-A. A direct comparison of the accumulation of molecules in patients with classical CVD and CKD provides better understanding the molecular mechanism of accelerated CKD-A development. The presented results support the postulated "reverse epidemiology" theory in CKD, and future research should focus, among other, on mechanistic studies of the diapedesis process of individual leukocyte subpopulations in CKD.