Review

on the dissertation work of doctor Yordanka Georgieva Doneva-Kashlova - PhD student in full-time form of training in the doctoral program "Internal Diseases", enrolled by order No.109-74/31-01-2020.

for the award of the scientific and educational degree "Doctor"

Reviewer Prof. Dr. Hristo Blagoev Tsekov, PhD, Consultant at the St. Anna“, гр. Varna

Thesis topic:

"Blood levels of circulating long-chain non-coding ribonucleic acids - lnc RNAs in cardiovascular disease"

The topic of this thesis is topical due to the fact that it contributes to the knowledge of the importance of PHK molecules in the control of biological processes.

A number of biomarkers have entered modern cardiology as indicators of deviations in biological processes - such as oxidative status and antioxidative potential. Neurohormonal changes leading to deterioration of H.S.H. are identified. On the other hand, intracellular and extracellular enzymes relevant to the pathogenesis of LV dysfunction are investigated. The role of natriuretic peptide, heme oxygenase-1, metalloproteinase-9, and galectin-3 in ventricular remodeling processes was demonstrated. A number of biomarkers of myocardial necrosis and apoptosis have entered practice. Unfortunately, despite their high specificity and sensitivity, they sometimes give heterogeneous results.

New studies demonstrate the involvement of long-chain non-coding ribonucleic acids in the development of apoptosis, fibrosis and cardiac remodeling. This highlights the potential of these molecules as both therapeutic targets and biomarkers in cardiovascular disease.

The overall mechanisms of synthesis and regulation of long-chain PHKs are still unclear. Through diverse molecular mechanisms, they are involved in the activation and inhibition of gene expression. Once expressed, they may be involved in the development of cardiovascular pathology.

Long-chain PHKs lead to dynamic transcription, differentiation, and maturation of cardiac cells due to cardiac gene expression. Overexpression stimulates cardiomyocyte proliferation and enhances cardiac regenerative response. The PHK molecule inhibits apoptosis and regulates cardiac remodeling by affecting specific gene loci. This opens the possibility of long-chain PHK being a biomarker for early diagnosis of CH and OMU.

Undoubtedly, this results in the fact that the topic of the dissertation is contemporary and topical. The literature review in its comprehensiveness has the character of a monograph.

The dissertation is presented in 120 pages and contains 28 figures and 21 tables. The references include 299 titles.

Dr. Kashlova aimed to investigate and analyze plasma expression of long-chain non-coding PHK in patients with CH and acute MI with ST elevation.

Three groups were included in the study:

- The first one was with ST elevation AMI - 37 patients

- The second - with exacerbated XCH - 28 patients
- The third is a control group - 15 healthy

The studies were performed in the reference laboratories of MU-Sofia. The diagnosis of the patients was made by echocardiographic methods and standard clinical approach.

Seven statistical methods were used for statistical analysis. Differences with $p \leq 0.05$ were assumed to be statistically significant in data processing.

In the general characteristics of the patients, their comorbidity was assessed. In patients with HF, 17.9% had CKD and 39.3% had DM. In patients with MI in 100% CKD, in 21,6% HTN and in 75,7% dyslipidemia.

In patients with HF, the levels of Inc RNAs were significantly higher compared to the control group. This correlates with a low ejection fraction (fi<40%) with most likely more pronounced myocardial fibrosis. We looked for changes in Inc RNA expression in the presence of H.D. No significant correlation was found. Similar results were found for other comorbidities, such as CKD and the presence of dysmipidemia.

Inc RNA expression levels were significantly higher among the AMI group compared to the control group. With a sensitivity of 80% and specificity of 71%. In both HF and AMI, no significant correlation with comorbidities was found.

Plasma excretion of Inc RNA NRF was increased in both HF and AMI. The mean was highest in MI, followed by the mean with HF and lowest in the control group. The results were similar in comorbid diseases. They did not change the overall trend.

A large set of long-chain RNAi molecules has been described. The biomarker potential of five circulating RNAi has been evaluated. Dr. Kashlova is studying non-coding RNAi for the first time in this country. Their dynamics are associated with both necrotic processes and the excessive development of fibrosis. The course is always established by facts that are difficult to explain. Why is it that elderly patients, in whom myocardial fibrosis is expected to be more pronounced, express Inc RNA to a lesser extent than younger patients.

Perhaps here is the place to mention the great Goethe who said - to explore the explorable, and to be humble before the unexplored.

Although Inc RNA has no advantages over MI in troponin dynamics, it has a role in fibroblast activation and has value as a predictor of fibrosis development.

Dr. Kashlova's study opens another opportunity to refine the diagnosis, therapeutic approach and prognosis of the most severe cardiac pathology.

My critical note refers to the presented HF patients as a monolithic group. There are different types of HF. HF with preserved FI, with systolic and segmental left ventricular dysfunction, with diastolic dysfunction. This is challenge enough for Dr. Kashlova to continue her research in this direction.

With her development Dr. Kashlova updates our knowledge, which is the main argument for my positive vote, which I believe will be shared by the other members of the Scientific Jury.

Prof. Dr. Hristo Blagoev Tsekov PhD.