STATEMENT

by Assoc. Prof. Eleonora Georgieva Dimitrova- Gospodinova, MD, PhD, Head of the Department of Oncology, Medical University "Prof. Dr. Paraskev Stoyanov"- Varna

According to the Order of the Rector of MU-Varna № R-109-161/ 14.03.2025, I have been elected as a member of the Scientific Jury, and on the basis of Protocol № 1/ 26.03.2025 I have been appointed to prepare an opinion on the procedure for the acquisition of educational and scientific degree "Doctor" in the field of higher education 7. Health and Sport, professional field 7.1 Medicine, doctoral programme in the specialty Internal Medicine.

On the PhD work entitled: "**Blood levels of circulating long noncoding ribonucleic acids - LncRNAs in cardiovascular diseases**".

Author: Yordanka Georgieva Doneva-Kashlova, MD

Scientific supervisor: Assoc. Prof. Dr. Veselin Dobrev Valkov, MD, PhD

1. Significance of the problem, formulation of the aim and objectives

The increasing incidence of cardiovascular diseases, their significant health and social and economic consequences determine the need for the implementation, use and improvement of more and more diagnostic and therapeutic modalities. Of particular importance is the prevention, early diagnosis and timely treatment. In this aspect, the validation of new diagnostic procedures and biomarkers is imperative. Biomarker testing has been shown to occupy an important place in the diagnostic algorithm, therefore the development of various highly specific and affordable markers is essential.

Advances and constant developments in science provide opportunities to assess cell physiology on a molecular level. So far troponin as a specific marker of tissue injury appears to be the most valuable biomarker for cardiovascular disease(CVDs) in general. The recognition and accumulated knowledge of the genome of cells provides new opportunities, both for diagnosis and targeted treatment.

In this sense, a subject of the present study are long noncoding RNAs- Lnc RNAs-represent an interesting and understudied class of potential biomarkers for both cardiovascular and many other diseases, including cancer.

The literature contains a considerable number of publications on the regulatory role of LncRNAs in both the physiology and pathogenesis of CVDs. However, their potential as biomarkers for different cardiovascular diseases has not yet been fully explored. The known tissue specificity of LncRNAs provides hope in this direction.

All these facts led to the definition of a specific aim of the present study: to investigate and analyze the plasma expression level of two long noncoding ribonucleic acids- lncRNA Wisper and lncRNA NRF, in patients with heart failure (HF) and patients with ST-elevation acute myocardial infarction (STEMI). ".

Five objectives were clearly formulated to serve the purpose of the study.

2. Structure of the PhD work

The present PhD work contains 120 pages :„Introduction"-1 page., „Literature review"- 41 pages, „Methodology and organization of the study"- 10 pages, „Results"- 32 pages, „Discussion"- 8 pages, „Conclusions, and contributions"-2 pages, „Conclusion"- 1 page, „Scientific publications and contributions related to the PHD work"-1 page.

The PhD work is illustrated with 21 tables and 28 figures, there is 1 appendix.

The bibliography includes 299 titles, of which 3 in Cyrillic and 296 in Latin.

The structure of the PhD work is well balanced and organized. The individual sections have a logical sequence and complex content. The scientific work meets the requirements of the Law of the Development of the Academic Staff in the Republic of Bulgaria.

3. Literature

The PhD student presents a thorough analysis of the scientific information. Data on the epidemiology, main diagnostic approaches and potential markers in the diagnosis and treatment of heart failure, myocardial infarction and other cardiovascular diseases are presented.

A classification of noncoding RNAs, their physiological and pathophysiological role in cardiovascular pathology is presented.

4. Study design and methodology

The study is retrospective in nature. The biological material (blood samples) were collected from patients hospitalized at"St. Marina Hospital", treated in the Clinic of Internal Medicine and in the First Clinic of Cardiology with Intensive Cardiology Unit in the period 2014-2016.

Patients in the study were divided into three groups as follows: first group - patients with acute myocardial infarction with ST elevation myocardial infarction (STEMI) - n=37; second group - patients with a diagnosis of heart failure NYHA class III-IV - n=28; third group – control individuals without cardiovascular disease, meeting the inclusion and exclusion criteria, used as a control group - n=15.

Plasma expression level of long noncoding circulating RNA molecules - lncRNA Wisper and lncRNA NRF was analysed by the method of real-time reverse transcription polymerase-chain reaction(RT-qPCR). The methods used for statistical processing of the obtained results are described in detail.

5. Correlation between objective, results and conclusions

There is a correspondence between the stated aim, the formulated five objectives and the obtained results.Two hypotheses were formulated based on the stated aim and the data from the studied literature.The data from the conducted study are illustrated in tables and figures. The results demonstrate that plasma expression levels of the first RNA-Lnc Wisper are significantly increased in patients with acute myocardial infarction as well as in those with heart failure. Their expression is not affected by the presence of diabetes mellitus, dyslipidemia, renal failure. Their value correlates inversely with age in patients with heart failure On the other hand, the second RNA-Lnc NRF demonstrates the potential to differentiate patients with myocardial injury from individuals without cardiovascular disease.

6. Analysis of conclusions and contributions

Six conclusions are formulated, synthesizing the most important of the results obtained. The conclusions are specific and clear.

Contributions of scientific and theoretical nature are defined. The main contributions of the PhD work include the following:

- for the first time in Bulgaria the expression of lnc RNA Wisper and lncRNA NRF in human plasma of patients with acute myocardial infarction and patients with heart failure was studied and the results were compared with a control group.

- plasma expression level of lncRNA Wisper was found to be increased in heart failure as well as in acute myocardial infarction.

- age was revealed to influence the plasma expression of lncRNA Wisper.

- the potential predictive value of lncRNA Wisper for the development of fibrosis in heart failure patients is reported for the first time in Bulgaria

- the increased expression of lncRNA NRF in patients with acute myocardial infarction compared to a control group was analyzed.

- it is reported the value of lncRNA Wisper as a biomarker for myocardial fibrosis, which is not influenced by additional factors such as gender and the most common comorbidity accompanying cardiovascular disease.

- reported potential of lncRNA NRF as a biomarker for acute myocardial necrosis.

The PhD student presents two scientific publications related to the thesis.

The abstract consists of 58 pages, containing all the main chapters of the thesis.

7. Conclusion

The PhD work of Dr. Yordanka Georgieva Doneva-Kashlova represents a thorough scientific development dedicated to the pursuit of refining endemically prevalent diseases with still poorly studied and innovative methods. The topic is topical, extensive and complex. The methods applied in the study are consistent with the formulated aim and objectives, the results are unambiguous, the conclusions are clearly formulated. There are contributions of theoretical and clinical-applied value.

The dissertation on the topic "Blood levels of circulating long noncoding ribonucleic acids-LncRNAs in cardiovascular diseases" meets the requirements for the degree of PhD.

On the basis of all the above, I give a positive assessment of the PhD work of Yordanka Georgieva Doneva-Kashlova and propose to the members of the esteemed Scientific Jury to award her the educational and scientific PhD degree.

11.04.2025

Assoc. Prof. Eleonora Georgieva Dimitrova- Gospodinova, MD, PhD