

OPINION

In connection with dissertation labor for award on educational and scientific DOCTOR degree on topic: **“Blood levels of circulating long-chain non-coding ribonucleic acids-LncRNAs in cardiovascular diseases ”**

Author on the dissertation:

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The opinion was prepared in accordance with the Academic Staff Development Act (ASDA), The Rules for application of ZRAS (PRZRAS) and The Rules for the conditions and the order for acquisition on scientific degrees and borrowing on academic positions (PURPNSZAD) at MU-Sofia. The scientific jury for the public defense of the dissertation was determined by order of the Rector of Medical University - Varna No. P-109-161/14.03.2025 Mr.

The presented set materials and documents from PhD Yordanka Doneva-Kashlova on paper and electronic carrier is compliant with requirements on The law for development of the academic staff in the Republic of Bulgaria and the Regulations for its implementation, as well as and with the rules on MU- Varna from Procedure for acquisition on OHC "doctor".

Dr. Yordanka Georgieva Doneva-Kashlova is born on 01/19/1985 year He graduated from the Medical University of Varna in 2013. Since 2020 with order on The Rector on Medical University - Varna Dr. Yordanka Doneva-Kashlova has been enrolled as a full-time doctoral student in internal medicine at the Department of Propaedeutics of Internal Medicine at Medical University - Varna. Order for enrollment on The Rector on MU- Varna (No. P- 109-74/31.01.2020 year). In 2018, after successfully passing the state exam, Dr. Kashlova obtained a specialty in Internal Medicine. From 2015 to 2017 and since 2024, she has been a part-time assistant at the Department of Propaedeutics of Internal Medicine, MU-Varna. Since 2021, she has been enrolled in a specialization in "Endocrinology and Metabolic Diseases"

Dr. Kashlova is fulfilled in deadline all staked in the individual curriculum tasks and activities. She has successfully passed the doctoral minimum exam in the specialty and by methodology on scientific research.

With advances in sequencing technology, genomic engineering, and RNA technologies for quantitative polymerase chain reaction, new lncRNAs (long non-coding ribonucleic acids) are constantly being discovered in various fields of medicine, as well as in-depth analysis of already established ones. The number of lncRNAs and their importance for the cardiovascular system is not small. Many lncRNAs have been described, specifically expressed in cardiac tissue and expressed to varying degrees in CVD. Their specific expression profile varies depending on the dynamics of the disease development.

There is also a significant number of studies demonstrating the involvement of long-chain RNAs in the pathogenesis and pathophysiology of socially significant diseases such as myocardial infarction and heart failure.

For example, some RNA molecules have been described with increased expression in the early phase of myocardial infarction and heart failure, while others are associated with decreased expression. A correlation of long-chain RNAs with various laboratory and clinical parameters has also been established. This fact gives rise to a wide field for discussion and creates the possibility of using them as non-invasive biomarkers for the precise diagnosis, prognosis and stratification of diseases in the field of cardiovascular pathology. The development of new approaches and the in-depth analysis of the dynamic regulation of lncRNAs also allows for the introduction of new therapeutic strategies.

The indicated data motivate the choice on topic from PhD Kashlova - "Blood levels of circulating long-chain non-coding ribonucleic acids- lncRNAs in cardiovascular diseases". The literature review as systematics and analytical ability in viewing of The available information presents the doctoral student as a leading expert on the issue. The essence of the class of long non-coding ribonucleic acids (lncRNAs), their participation in the pathogenesis and development of cardiovascular diseases, their potential for diagnosis and creation of new therapeutic strategies for these diseases are consistently covered. The factual premises for the study are derived from the overview in the form of a conclusion.

The goal of the dissertation is formulated clearly and precisely – to study and analyze the plasma expression of long-chain non-coding ribonucleic acids – lncRNA Wisper and lncRNA NRF, in patients with heart failure and patients with acute myocardial infarction with ST elevation.

For its implementation has been carried out tracking on sufficient number patients — over

80, who are divided into three groups as follows: with acute myocardial infarction (STEMI); patients with heart failure NYHA class III – IV and persons without cardiovascular disease. Used are rich set from methods — laboratory methods: RNA isolation from plasma, reverse transcription and RT-qPCR; echocardiographic methods for assessing left ventricular ejection fraction using the Simpson method, as well as statistical methods: descriptive analysis, Kolmogorov–Smirnov test / Shapiro Wilk test, graphical method, correlation analysis, pairwise comparison, etc.

The main results of Dr. Kashlova's work can be summarized in the following guidelines:

- Significantly higher levels of **lncRNA Wisper expression were found** in patients with heart failure compared to the control group. Plasma levels of lncRNA Wisper expression differentiated patients with HF from the control group with 80% sensitivity and 67% specificity. The results obtained indicate the potential role of the studied RNA as a sensitive marker for distinguishing patients with heart failure from healthy individuals.
- The results obtained show that **lncRNA Wisper** increases during the first 12 hours of the onset of AMI. Although it may be a biomarker for myocardial necrosis, it does not seem to follow the dynamics of plasma troponin levels, whose characteristic change, its correlation with the area of myocardial necrosis, as well as the reliability and accessibility of its study make it an indispensable biomarker in AMI. lncRNA Wisper at first glance has no advantages for the diagnosis of acute myocardial infarction, but it may have value in another aspect. There is evidence that lncRNA Wisper is a regulator of fibrosis and has a role in the activation of fibroblasts. If we assume that lncRNA Wisper starts these processes at such an early stage of the onset of AMI, it is possible that it has the value of a predictor of the development of fibrosis and its degree in the chronic phase of myocardial infarction with the corresponding clinical manifestation.
- Expression of **lncRNA NRF** in myocardial infarction - plasma expression levels of lncRNA NRF are significantly higher in patients with AMI. This result correlates with the known data on the involvement of lncRNA NRF in the regulation of necroptosis as a major pathophysiological process in AMI
- Expression of **lncRNA NRF** in heart failure - analysis of plasma levels of lncRNA NRF in patients with HF shows a marked tendency for increase, but does not reach a significant difference compared to the control group. A possible reason for this is

that in different CVDs a different type of cell death prevails. For example, in myocardial infarction necrosis prevails, in the implementation of which Lnc RNA NRF is directly involved. In chronic heart failure the leading form of cell death is apoptosis.

The dissertation is presented in a volume of 120 pages and contains 28 figures and 21 tables. The bibliography includes 299 titles.

On the topic of the dissertation, the candidate has published two works, accepted and printed in journals " Cardiovascular Diseases, 54, 2023, No. 1" and " Journal of IMAB - Annual Proceeding (Scientific Papers). 2021 Apr-Jun;27 ".

On the basis on these facts scientific activity in the period on preparation on labor is evaluates as optimal.

Conclusion:

In conclusion, the dissertation presented by Dr. Yordanka Georgieva Doneva-Kashlova contains scientific, scientifically applied and applied results that represent an original contribution to science and exceed the requirements for awarding the educational and scientific degree "DOCTOR". The dissertation work shows that the doctoral student owns in-depth theoretical knowledge and professional skills in the scientific specialty, demonstrating qualities and skills for independent conduct and discussion on scientific research.

On these grounds, I propose to the esteemed members of the Scientific Jury to vote positively and propose to the Rector of MU Varna to award the scientific and educational degree "doctor" in the doctoral program in cardiology, to Dr. Yordanka Georgieva Doneva-Kashlova , Department of Propaedeutics of Internal Medicine, Medical University — Varna.

25.04.2025 Mr.

проф. д-р Арман Постаджиян, д.м.

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Заличено на основание чл. 5,
§1, б. „В“ от Регламент (ЕС)
2016/679