

## OPINION

from **Assoc. Prof. Atanas Angelov Atanasov, MD, PhD**  
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of dissertation work for awarding  
the scientific and educational degree “**DOCTOR**”  
Field of higher education: 7. "Health and sport"  
Professional field: 7.1. "Medicine "  
Doctoral program: "Endocrinology”

Topic:

“Non-invasive assessment of arterial stiffness in patient with type 2 diabetes mellitus – correlation with some biomarkers”

**By Dr. Elena Stoyanova Marinova,**  
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Scientific advisers:

Prof. Branimir Kanazirev, MD, PhD and Assoc. Prof. Mila Boyadzhieva, MD, PhD

By order No. R-378/October 06, 2020 by the Rector of the Medical University of Varna I have been selected a member of the scientific jury and on the basis of protocol № 1/18.10.2020 I have been appointed to prepare an opinion on the procedure for awarding the educational and scientific degree "Doctor" by the candidate Dr. Elena Stoyanova Marinova.

**Brief data on the professional development and qualification of the doctoral student.** Dr. Elena Stoyanova Marinova graduated in medicine in 2008 at the Medical University Varna. Since 2009 she has been working as a doctor at the University Hospital “ St. Marina”. From 2013 to 2015 she was a part-time assistant

at the Department of Propaedeutics of Internal Medicine at the Medical University of Varna. Since 2016 she has been a full-time assistant in the same department. In 2015 she acquired a degree in Endocrinology.

### **Relevance of the topic.**

The identification of individuals at high cardiovascular (CV) risk is one of the great challenges in preventive cardiology. Atherosclerotic vascular diseases have a long asymptomatic period, which allows for early and successful preventive interventions. Using classical risk factors, traditional risk models such as Framingham Risk Score and SCORE give a 10-year of CV risk assessment. These risk models have their limitations. For this reason, in the last two decades, new biomarkers and imaging methods that can be used for individual risk stratification have been studied and included in the guidelines for cardiovascular prevention. The topic of the dissertation of Dr. Elena Marinova is especially relevant because it examines a lesser known non-traditional cardiovascular risk factor. Arterial stiffness (AS) occurs in parallel with endothelial dysfunction during the development of the atherosclerotic process, as there is ambiguity regarding the causal relationship between the two processes. There is evidence that arterial wall stiffness has an independent predictive value for cardiovascular events. Currently, data on the presence of AS and its clinical significance in patients with diabetes mellitus are scarce. Dr. Marinova is looking for a correlation between this new ultrasound marker for increased CV risk and various biomarkers - conventional and new, such as serum osteocalcin. In the last decade the hormonal nature of the bone protein osteocalcin and its participation in carbohydrate, energy and lipid metabolism has been established. Low concentrations of osteocalcin are associated with an increased risk of developing type 2 diabetes mellitus (T2DM). According to some authors, osteocalcin is involved in the processes of vascular calcification and arterial stiffness.

### **Structure of the dissertation:**

The dissertation of Dr. Elena Marinova is designed according to the requirements of 136 pages and is illustrated with 31 tables, 20 diagrams and 11 graphs. The dissertation is well balanced and includes an introduction and a literature review (43 pages), aim and tasks (1 page), materials and methods (6 pages), own results and discussion (55 pages), conclusions and contributions (3 pages). The references contains a total of 207 sources (1 in Cyrillic), of which 27% have been published in the last 5 years. The abstract includes 15 diagrams, 11 graphs and 31 tables.

## Literature review:

Literature review provides detailed information on the pathophysiological changes in the vascular wall in diabetes mellitus. Dr. Marinova discusses in detail the topic of arterial stiffness - from the biophysical basis of the process to AS and macrovascular complications in diabetes. Data on the different methods of measuring AS are also presented. In a separate chapter is presented and well illustrated high-frequency one-point echotracking method for measuring arterial stiffness. The role of osteocalcin in carbohydrate metabolism is considered, as well as the currently available data from studies, according to which osteocalcin can also be considered as a biomarker for an increased risk of developing diabetes. Evidence from numerous studies of the association between osteocalcin and the development of vascular calcification and arterial stiffness has been presented.

## Material and methods:

To solve the set tasks Dr. Marinova analyzed prospectively 100 consecutive patients with T2DM and 30 healthy controls. The inclusion and exclusion criteria for participation in the study and the methods used are presented very precisely. Dr. Marinova was not satisfied only to list the applied laboratory and instrumental tests. All steps from the venipuncture to the used equipment and units of measurement are considered in detail. The measurement of brachial arterial pressure, central aortic systolic pressure and ultrasound examination of the common carotid artery are described in detail and clearly. Both classical and modern statistical methods were used to interpret the data.

## Aim and tasks:

The doctoral student formulates the main goal of scientific work as a logical

**Results and discussion:** the most significant results that are found are the following:

- Patients with T2DM have significantly increased local AS of the carotid arteries, expressed by higher values of PWV $\beta$ , Ep and  $\beta$ -stiffness index and lower AC and AI, compared to healthy controls.
- Arterial stiffness in patients with T2DM increases with age.
- With the increase in waist circumference, in patients with T2DM, a significant increase in the values of PWV $\beta$ , Ep and  $\beta$ -stiffness index was observed.

- HDL levels showed an inverse correlation with arterial compliance, and a positive relationship with serum ucOC and tOC levels in the T2HD group.
- Higher glycated hemoglobin values were associated with significantly higher carotid PWV $\beta$  in the study groups.
- Serum concentrations of cOC, ucOC and tOC in patients with T2DM are lower than controls, and for cOC this difference is significant
- Carboxylated osteocalcin positively and independently correlates the  $\beta$ -stiffness index (R).
- Carboxylated osteocalcin significantly increases its serum concentrations after four weeks of vitamin K2 supplementation
- In patients with T2DM who have undergone vitamin K2 supplementation, there is a tendency to increase the hemodynamic parameters and ET parameters of AS.

**Conclusions:** the conclusions correspond to the set goal and tasks. The conclusions are well formulated and derived from the obtained results.

**Contributions:** of the proposed contributions I consider the most important are:

*1. Scientific-theoretical contributions*

- For the first time in Bulgaria arterial stiffness is measured in patients with T2DM using echo-tracking method
- Osteocalcin is studied for the first time in Bulgaria as a marker for arterial stiffness in patients with T2DM.
- For the first time in Bulgaria an intervention study is conducted looking for a change in carotid stiffness after vitamin K2 supplementation in patients with T2DM.

*2. Scientific- practical contributions*

- The measurement of increased carotid stiffness by echotracking method in patients with T2DM is non-invasive, fast and suitable for clinical practice.
- Early diagnosis at the stage of subclinical vascular damage would allow therapeutic decisions to be made in order to reduce cardiovascular complications in patients with T2DM.
- Although there are no statistically significant differences in PWV $\beta$  measured on the left and right ACC, a higher number of correlations was found in the right ACC, which makes it more suitable for the echo-tracking.

**Publications and scientific communication related to the dissertation work:** in connection with the dissertation, Dr. Marinova presents three publications and two participations in scientific conferences. I believe that the doctoral student has

potential and material on the topic that allows a larger number of publications, including in foreign journals.

**Critical notes:**

1. Some of the sources in the bibliography are not cited according to the generally accepted rules.
2. Only one source in Cyrillic is cited in the bibliography. There are other publications by Bulgarian authors on the topic of arterial stiffness.

**Conclusion:** The dissertation of Dr. Elena Marinova treats current issues in the field of endocrinology and preventive cardiology. The study conducted by Dr. Marinova has all the characteristics of a qualitative clinical study, the results of which should be sought to be published in international journals. Evidence of early changes in arterial stiffness has been presented in individuals with T2DM without atherosclerotic cardiovascular disease. Macrovascular complications of diabetes mellitus begin very early in the course of the disease and the development of preventive strategies is especially important. One such strategy may include measuring arterial stiffness by echo tracking. At this stage this modern method is not included among the recommended risk modifiers in the European and American guidelines for cardiovascular prevention. Therefore, it is necessary to accumulate more evidence and develop a unified protocol for the determination of AS. The results of Dr. Marinova's study again support the need for correction and targeted control not only of glycemia, but also of blood pressure, lipid profile and abdominal obesity in individuals with T2DM. With regard to osteocalcin, more studies are needed to clarify its importance as a potential biomarker for CV risk assessment. Only a randomized placebo-controlled study with a sufficient number of participants can answer the question of whether vitamin K2 supplementation can have a lasting and beneficial effect on arterial stiffness.

I believe that the dissertation of Dr. Marinova is a serious scientific study that fully meets the requirements for awarding educational and scientific degree "Doctor". This gives me reason confidently propose to the esteemed scientific jury to vote positively for the awarded educational-scientific degree of Dr. Elena Marinova.

06.11.2020, Varna

Assoc. Prof. Atanas Angelov, MD, PhD