

To The Chairman of Scientific Jury  
 Designated by Order  
 No. R-109-378/October 06, 2020  
 By the Rector of the Medical University-Varna

## REVIEW

**by Prof. Borislav Georgiev Georgiev**  
**Head of the Cardiology Clinic at the National Heart Hospital**  
**Member of the Jury for awarding the scientific and educational degree "PhD", designated**  
**by order No. R-378/October 06, 2020 by the Rector of the Medical university of Varna**

**Regarding:** Dissertation of Dr. Elena Stoyanova Marinova, PhD student on a self-study basis, Second Department of Internal Medicine, educational sector Endocrinology, Medical University "Prof. d-r Paraskev Stoyanov" Varna

Topic of the PhD thesis:

*Non-invasive assessment of arterial stiffness in patients with type 2 diabetes mellitus – correlation with some biomarkers*

Scientific tutors: Assoc. Prof. Mila Boyadzhieva and Prof. Branimir Kanazirev

The documents presented by Dr. Elena Stoyanova Marinova – dissertation, abstract, and additional documents are in accordance with the requirements of the regulation for acquisition educational and scientific degree "PhD" and rules of Medical university of Varna. I do not find any omission in the submitted documentation.

I declare that I have no conflict of interest with the candidate.

All presented materials are precisely arranged and described.

No evidence of plagiarism.

### **Brief CV data of the applicant**

Dr. Elena Stoyanova Marinova completed her higher medical education in 2008 at the Medical University "Prof. Paraskev Stoyanov" Varna. After winning a competition she started specialization in endocrinology and metabolic diseases at the University Hospital "St. Marina" Varna. Since 2012 she is a resident doctor at the Clinic of Internal Medicine and a part-time assistant at the Department of Propaedeutics of Internal Medicine of the Medical University of Varna. In 2015 she has acquired specialty in Endocrinology and metabolic diseases, and in 2016 she was elected a full-time assistant at the Department of Propaedeutics of Internal Medicine. In 2017, she conducted training for a highly specialized activity "ultrasound of the cervical region". In the same year she conducted a training course in Clinical Neurosonology.

### **Significance of the topic**

The topic of the dissertation is contemporary and relevant.

Arterial stiffness (AS) refers to non-traditional cardiovascular risk factors. It occurs in parallel with endothelial dysfunction in the course of the development of the atherosclerotic process, and there is ambiguity regarding the causal relationship between the two processes. It is known that the atherosclerotic process in diabetic patients begins earlier, progressively accelerates and leads to diffuse changes in the arterial vascular system, compared with patients without diabetes. Currently scientific interest is the measurement of AS as a tissue biomarker for

subclinical atherosclerosis. The accumulation of AS data in patients with T2DM would allow better management of cardiovascular complications and therapeutic intervention in the subclinical atherosclerosis phase.

Osteocalcin (OC) is a non-collagenous protein known in clinical practice as a marker for bone formation. It is synthesized by osteoblast cells and acts as a regulator of mineralization in the bone matrix. In the last decade, however, its role as a hormone involved in carbohydrate, energy and lipid metabolism has become known. Low concentrations of OC are associated with an increased risk of developing T2DM. Summarizing human data on the role of OC as a hormone that regulates glucose, energy and fat metabolism, the question arises about its relationship with the development of atherosclerotic vascular disease in patients with T2DM. The hypothesis of a link between OC and markers of atherosclerosis has been the subject of numerous studies in recent years.

**Structure of the dissertation:** The scientific work of Dr. Elena Marinova is formed on 136 pages according to the requirements and contains an introduction, literature review, aim and objectives, materials and methods, analysis of results and discussion, conclusions, contributions, and bibliography. A list of publications and scientific communications on the topic is attached. The dissertation is illustrated with 31 tables, 20 diagrams and 11 graphs.

**The introduction** is on 2 pages. **The literature review** is presented in 41 pages and shows very good awareness of the author regarding arterial stiffness and diabetes, metabolic disorders linking diabetes with medial vascular calcification, relationship of arterial stiffness with hemodynamics, ultrasound measurement of local arterial stiffness by echotracking, arterial stiffness and macrovascular disease in T2DM and role of osteocalcin in carbohydrate metabolism, vascular calcification and arterial stiffness.

The author has good awareness on the topic of the dissertation. Based on the literature review, she draws *conclusions*.

1. Arterial stiffness is a predictor of the development of cardiovascular disease determined by PWV. The predictive value of PWV is higher in high-risk populations such as patients with diabetes. PWV is considered a very important risk factor in the assessment of overall cardiovascular risk and provides information beyond traditional cardiovascular risk factors.

2. The presence of T2DM is associated with elevated cf-PWV values measured by applanation tonometry. There are no data to measure AS with the ET technique in patients with T2DM, which has advantages in this patient group, because of diabetes –related overweight and obesity.

3. The ultrasound methodology for measuring the parameters of local AS – “echo-tracking” is a new and non-invasive method for assessing cardiovascular risk and has a predictive value for future cardiovascular events. Data on PWV measured on the carotid artery are scarce in patients with T2DM.

4. Osteocalcin is a hormone, and one of its presumed target organs is the arterial vessel wall. The available data in its majority determine the connection of the OC with the processes of vascular calcification and AS.

5. It is important to take into account not only the total osteocalcin concentration (tOC), but the levels of different forms of osteocalcin – cOC and ucOC, because they have different biological effects. Low levels of ucOC and tOC are associated with an increased risk of developing T2DM. On the other hand, lower ucOC levels in patients with T2DM have been associated with an increased cardiovascular risk.

6. It is interesting whether the serum levels of ucOC and cOC in patients with T2DM have a correlation with the parameters of local carotid stiffness. The reported data on the inhibition of vascular calcification by OC are currently not convincing and further studies are needed.



7. Most publications do not take into account the effect of vitamin K2 on ucOC and cOC levels. Theoretically, vitamin K2 supplementation would change the ratio of ucOC and cOC. The effect of vitamin K2 supplementation on AS, and in particular AS in patient with T2DM, has been poorly studied.

**The bibliography** contains 207 cited titles, of which 1 is in Cyrillic and the rest in Latin.

Dr. Elena Marinova **aims** to obtain data on the local carotid AS in patients with T2DM without macrovascular complications by echotracking methodology, to reveal a link between the indicators of AS with glucometabolites, lipid, hemodynamic parameters and levels of serum osteocalcin, and to evaluate the effect of vitamin K2 supplementation in some individuals with diabetes. To achieve this aim she sets the following **tasks**:

1. To measure the local AS of the two carotid arteries, using echo-tracking technique, in patients with T2DM without established macrovascular complications and to compare with that in controls.
2. To look for a connection between the ET indicators and the age of the persons with T2DM and anthropometric, glucometabolite and lipid levels.
3. To analyze whether there is a relationship between the indicators of AS and hemodynamic parameters (CASP, SBP, DBP, PP, MAP) in patients with T2DM.
4. To determine the serum concentrations of ucOC and cOC in some patients with T2DM and to compare with those of healthy controls.
5. To look for a link between AS in individuals with T2DM and serum osteocalcin levels.
6. To look for a change in the values of AS and serum levels of OC in patients undergoing vitamin K2 supplementation.

**Methodical approach:** The study was conducted in the period October 2018 - September 2019 and included 100 patients (52 females and 48 males) with T2DM and 30 healthy controls (15 males and 15 females). The study included patients with diabetes without a history of proven macrovascular complications over the age of 18 years.

Statistical analysis includes various analyzes that are in line with the hypothesis and the aims of the study.

**Results:** The obtained results of Dr. Elena Marinova are diligently presented on 44 pages of the dissertation. The results are well illustrated. The obtained results correspond to the aims of the research.

**The discussion of the results** is presented in 15 pages and the obtained data are analyzed and compared, where possible, with other publications.

**Conclusions:** Dr. Elena Marinova offers 10 conclusions. They derive directly from the set tasks and from the conducted research.

1. 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
2. Arterial stiffness in patients with T2DM increases with age.
3. With the increase in waist circumference, in patients with T2DM, a significant increase in the values of PWV $\beta$ , Ep and  $\beta$ -stiffness index were observed. Their higher waist / height ratio is associated with increased carotid PWV $\beta$ .
4. HDL levels showed an inverse correlation with arterial compliance, and a positive relationship with serum ucOC and tOC levels in the T2DM group.

5. Higher glycated hemoglobin values were associated with significantly higher carotid PWV $\beta$  in the study groups.
6. CASP, PP, MAP and smoking independently correlated with PWV $\beta$  (L), and SBP and PP independently correlated with PWV $\beta$  (R) in patients with T2DM.
7. Pulse pressure in patients with T2DM significantly and independently correlates with PWV $\beta$  (R), PWV $\beta$  (L),  $\beta$ -stiffness index (R) and  $\beta$ -stiffness index (L).
8. Serum concentrations of cOC, ucOC and tOC in patients with T2DM are lower than controls, and for cOC this difference is significant.
9. 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.
10. In patients with T2DM who underwent vitamin K2 supplementation, there is a tendency to increase the hemodynamic parameters and ET parameters of AS. Significance of these changes was reached in CASP.

In the dissertation, conclusion 9 is somewhat vaguely stated as twice item 9, but it is clear that both sentences refer to carboxylated osteocalcin.

**Contributions:** The contributions are 8, divided into three groups - scientific-theoretical (3), scientific-practical (3) and confirmatory and are important for clinical practice.

➤ **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.

➤ **Scientific-practical contributions**

- The measurement of increased carotid stiffness by echo-tracking 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 measured on the left and right ACC, a higher number of correlation were found in the right ACC, which makes it more suitable for echo-tracking.

➤ **Confirmatory contributions**

- PWV increases with age
- Total and non-carboxylated osteocalcin are in lower concentrations in patients with T2DM.

**Publications:** In connection with the dissertation the author presents 3 publications in lists and 2 abstracts from scientific forums.

**The abstract** is presented in Bulgarian and English and the Bulgarian version contains 64 pages and reflects what is written in the dissertation. It is in accordance with the requirements.

**Conclusion:** I appreciate the work of Dr. Elena Stoyanova Marinova on the topic „*Non-invasive assessment of arterial stiffness in patients with type 2 diabetes mellitus – correlation with some biomarkers*“ as interesting in scientific terms and important for clinical practice.

I consider this dissertation meets the requirements for awarding the educational and scientific degree "PhD" according to Academic Staff Development Act in the Republic of Bulgaria and the Rules for the Academic Staff Development at the Medical University of Varna.

Based on the above merits of the dissertation of Dr. Elena Marinova, I strongly recommend to the honorable members of the Scientific Jury to vote positively and to award Dr. Elena Stoyanova Marinova the educational and scientific degree "PhD".

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