**REVIEW**

**From Prof. Dr. Lachezer Nachev Grozdinski, DSc**

**Head of the Clinic of Angiology and Phlebology,**

**Acibadem City Clinic University Hospital**

of dissertation for obtaining the educational and scientific degree "PHD"

in the field of higher education 7. Healthcare and sports, professional field 7.1. Medicine, doctoral program "Angiology"

Author: Dr. Elitsa Rashkova Gerova - Micic

Form of doctoral studies: Regular preparation

Scientific Unit: Clinic of Angiology, Acibadem City Clinic UMHAT Tokuda EAD

Topic: "Multifocal atherosclerosis - diagnostic and prognostic markers in different vascular basins"

Scientific adviser: Prof. Dr. Milena Staneva Staneva, PhD.

1. General presentation of the procedure and the doctoral student

At a meeting of the Scientific Council of Acibadem City Clinic UMHAT Tokuda EAD (Minutes №40 / 10.02.2022) and by order № 15-05-71 / 04.04.2022 of the Executive Director and Procurator of the hospital, I was appointed for external member of the Scientific Jury in connection with the dissertation of Dr. Elitsa Gerova - Micic. I am determined to submit a Review.

There are no gaps in the documentation attached by Dr. Gerova, the requirements of ZRASRB, PPZRASRB and the Regulations on the terms and conditions for obtaining scientific degrees and holding academic positions in Acibadem City Clinic University Hospital Tokuda EAD are met.

I declare that I have no conflict of interest with the author of the dissertation.

Dr. Elitsa Gerova graduated from the Secondary School "Ivan Vazov" in Plovdiv, and medicine at the Medical University of Sofia in 2014. Since 2014 - she is currently a doctor in the Department of Vascular Surgery, University Hospital "Sofiamed", Sofia. Dr. Gerova acquired a degree in Angiology in 2020. She holds a certificate of professional qualification in "Ultrasound Vascular Diagnostics".

She is a member of the Bulgarian Medical Association, the European Society of Vascular Surgery and the Bulgarian Society of Vascular and Endovascular Surgery and Angiology.

2. Relevance of the topic

The topic of the dissertation is well chosen and relevant. Based on the fact that atherosclerosis, which is a multifocal process that is asymptomatic for a long time and often its first clinical manifestation can lead to severe organ damage or death, one of the directions in the modern fight against it is to detect sensitive markers that allow early to diagnosis and treatment. In this regard, ultrasound methods examining DIM, plaques and stenoses of the carotid and femoral arteries, calculation of the foot-brachial index (ABI), measurement of the diameter of the abdominal aorta allow the development of highly sensitive markers for multifocal atherosclerosis.

Although several dissertations have been defended on similar topics in Bulgaria, there is still insufficient data in the literature on the prognostic value of the applied ultrasound methods in patients with peripheral arterial disease (PAD). The practical orientation of the work is valuable, which is evident in the establishment of structural changes in all arteries (carotid, iliac, femoral, foot and abdominal aorta) in patients with PAH, followed for 2 years, these changes are markers of MFA.

3. Knowledge of the problem

The doctoral student knows the state of the problem and creatively evaluates the literary material.

4. Research methodology

The chosen research methodology allows achieving the set goal and obtaining an adequate answer to the tasks solved in the dissertation.

5. Characteristics and evaluation of the dissertation and contributions

Dr. Gerova's dissertation is written in a volume of 170 pages, illustrated with 92 tables and 19 figures. Includes the following sections: Contents - 3 pages, Abbreviations and symbols used - 2 pages; Introduction and literature review - 21 pages; Purpose and tasks - 1 page; Material and methods - 16 pages; Own results and discussion - 98 pages; Conclusions and recommendations - 3 pages; Bibliography - 19 pages; Appendix - 4 pages. The ratio between the review, methodological part and results and the discussion corresponds to the norm. The bibliography contains 208 literary sources, of which 15 in Cyrillic and 193 in Latin. All the cited titles are directly related to the studied problem.

In the introduction the problem is well formulated and the purpose of research in the dissertation is substantiated.

The literature review is written competently and shows a good knowledge of the problem in its various aspects, but is not focused on the specific topic, but discusses theories of the occurrence and development of atherosclerosis. Risk factors are considered only for coronary heart disease apply to what extent to other forms of atherosclerosis. An overview of peripheral artery atherosclerosis includes an overview of abdominal aortic aneurysms. It is known that the latter are a disease other than atherosclerosis and from this point of view have no place in the review.

The literature review ends with an analysis of the unresolved and controversial issues that give the author a reason to conduct the study.

The aim of the dissertation is well formulated in accordance with the topic of the dissertation and the presented literature review. Dr. Gerova aims to study the multifocal nature of atherosclerosis as a pathological process in order to identify and offer prognostic and diagnostic ultrasound markers.

 To achieve this goal the doctoral student sets 6 tasks:

1. To study the incidence of multifocal atherosclerosis in patients with peripheral atherosclerosis.

2. To determine the clinical characteristics and risk profile of patients with peripheral and multifocal atherosclerosis

3. To study with ultrasound methods the morphological (DIMC, plaques, stenoses) changes of the carotid and peripheral arteries (femoral, foot arteries and abdominal aorta) in patients with peripheral atherosclerosis.

4. To determine the relationship between atherosclerotic risk factors and data from ultrasound morphological changes of the carotid and peripheral arteries in patients with multifocal atherosclerosis.

5. To analyze the morphological changes of the carotid, femoral, costal arteries and abdominal aorta and to evaluate as markers and predictors of atherosclerosis.

6. To develop and propose a set of diagnostic and prognostic markers to optimize the screening and diagnosis of multifocal atherosclerosis

The section "Materials and methods" describes in detail the included 240 patients aged 32-91 years in the period July 2017 to July 2020. All patients are divided into four groups, depending on the affected areas : Group 1 - "Control", including 40 patients without clinical and Doppler sonographic data for atherosclerosis. Group 2 - patients only with chronic arterial insufficiency of the limbs (HANK) - pronounced atherosclerosis in one pool. 68 patients with HANK with no evidence of coronary or carotid atherosclerosis were included. Group 3 consisting of 97 patients with HANK, IBS and SMEs, which is the most numerous group and covers 97 patients or 40% of all subjects, of whom 72 men aged 67.8 and 25 women aged 68.3 This group is divided into two subgroups depending on the extent of the atherosclerotic process, involving two basins simultaneously. 3rd grade - patients with HANK and accompanying carotid atherosclerosis, 3rd grade - patients with HANK and coronary atherosclerosis (CHD). Group 4 - 35 patients with HANK, MSB and coronary heart disease - in this group the atherosclerotic process involves three basins of carotid arteries, peripheral arteries and coronary arteries. Patients were tested for risk factors for atherosclerosis (hypertension, diabetes, smoking, alcohol consumption, body mass index (BMI), dyslipidemia, familial burden) and experienced revascularization interventions. Computed tomography angiography of carotid arteries, as well as from renal to foot arteries, color-coding duplex scanning (CCDS) for morphological changes (thickness of intima media - DIMC, plaques, stenosis, thrombosis) of carotid, femoral, foot, of ABI and diameter of the abdominal aorta. Coronary angiography has been performed in patients with clinical evidence of coronary heart disease or suspected coronary heart disease.

 Over a 24-month period, 120 HANK patients who survived revascularization were followed by ABI, DIM, plaque, stenosis, and thrombosis of the carotid arteries and lower limb arteries, ABI was measured, and the effect of statin and fibrate therapy on progression of the atherosclerotic process.

Statistical analysis includes various analyzes that are in line with the set goals. Data were processed with IBM SPSS Statistics v.23.

Results and discussion: The presented results are well illustrated and meet the objectives of the study. Dr. Gerova analyzes the data obtained and compares them, where possible, with other publications on the subject. Based on the obtained results and their analyzes, the most significant result of this research work is an Algorithm for optimizing the diagnosis and therapeutic behavior in patients with multifocal atherosclerosis.

Conclusions: Dr. Elitsa Gerova offers 17 conclusions. They derive directly from the set tasks and from the conducted research:

1. The risk profile of patients for prediction and development of atherosclerotic process is determined by the following factors: male gender, age over 65 years, the presence of AH, diabetes, family history, exercise, dyslipidemia. The at-risk patient is a middle-aged man of 68 years with HANK, AH, family burden. In the presence of these factors, a statistically significant dependence is established for the development of a multifocal atherosclerotic process.

2. An important factor in determining the possibility of developing atherosclerotic process in the cerebrovascular basin in patients with HANK is the statistically significant inversely proportional relationship between CA DIM and ABI - the lower the ABI, the higher the DIM of CA, most often on CCAbif. and ICA.

3. The established directly proportional relationship between the DIM of CA and FCA with the components of the lipid profile and mainly with the triglycerides - the higher the values ​​of the triglycerides, the higher the DIM increases the CCA, CCA bif. , ICA, FCA can be successfully used in patient follow-up and for proper assessment of therapy.

4. The analysis confirms that for CA DIM, with the exception of DIM-FCA, a statistically significant difference in DIM levels was found between patients with and without SMEs.

5. The established statistically significant relationship between the increase in CA DIM and the presence / asymptomatic coronary atherosclerosis is defined as an important factor in the prediction of coronary atherosclerosis.

6. Higher levels of fibrinogen and lipid profile have been found in more severe atherosclerosis (lower ABI or multifocal involvement). These indicators can be used as markers to control the atherosclerotic process.

7. A high incidence (55%) of multifocal atherosclerosis has been reported in patients with HANK. Affection in two areas (HANK and IBS / HANK and SME) is diagnosed in 40.4%, and in three (HANK and IBS and SME) - in 14.6%.

8. Diabetes mellitus is a risk factor for HANK and its severity and duration are associated with a more advanced stage of vascular involvement.

9. Patients with HANK have pathological structural changes in the following ultrasound parameters: DIM ICAb, DIM ICA, DIM FCA, presence of plaques, stenoses, thrombosis of carotid and femoral arteries, ABI <0.9, as a scar and markers of a multifocal atherosclerotic process.

10. There is an inverse relationship between ABI levels and the likelihood of experiencing IMI - the lower the ABI levels, the higher the likelihood of experiencing IMI. When ABI reaches levels of about 0.2 then the probability of surviving IMI becomes over 50%.

11. Patients with high levels of AMD are most often men between the ages of 69 and 70 who use alcohol and cigarettes, with an obesity index of 26-27, with a family history of hypertension, and with stage III obesity. HANK. With cholesterol levels of 5.60-5.70, triglycerides 1.9-2.0, HDL 1.3-1.4 and LDL 2.9.

12. The first five risk factors in all patients are family history, hypertension, male gender, smoking and alcohol. The results of the studied quantitative factors are different. For survivors of IMI and AMI, the most important quantitative factors are LDL and HDL, and for patients with high CA and FCA AMD, age and cholesterol levels.

13. With statin or fibrate treatment, the percentage of adverse vascular events is definitely manageable, reducing the residual risk in patients with atherogenic dyslipidemia and hypertriglyceridemia.

14. The presence of plaque or stenosis of CA contributes to an increased risk of HANK, IMI or CHD in the range of 4-8%.

15. In the case of more aggressive secondary prophylaxis with a statin, antiplatelet agent and ACE inhibitor, such as group IV therapy, the weakest progression of the atherosclerotic process is found.

16. In patients with HANK, stenosis of the aoiliac segment is found in almost 1/3 of the patients. Aortic segment thrombosis is diagnosed in just over 1/10. In patients with SME, thrombosis of the aorto-iliac segment is diagnosed statistically significantly more often (20%) than those without SME (9.2-9.7%). Aorto-iliac segment thrombosis can be used as a predictor of SMEs.

17. Thrombosis of the femoropopliteal segment was found in almost half of the examined patients. Stenosis of the femoropopliteal segment was found to be bilateral much less frequently. There is a tendency for a higher incidence of femoropopliteal segment thrombosis in patients with coronary heart disease (58.5%) compared to those without (45.2%), as well as in patients with SME (57.0%) compared to those with without (44.0%). The significant association between femoropopliteal segment thrombosis on the left and coronary heart disease and / or MSD may be a predictor of multifocal atherosclerosis (MSD and / or coronary heart disease).

Criticisms of the dissertation

The literature review is not focused on the specific topic - multifocal atherosclerosis and diagnostic and prognostic markers. Too much attention is paid to the risk factors of atherosclerosis, which is not actually the main object of the dissertation. There is not enough clarity about the nature of coronary pathology in gr. 3a - HANK and IBS and gr. 4 - HANK, IBS and SME. There is talk of a number of affected coronary branches, but not of its severity - the degree of stenosis and the presence of thrombosis. On the other hand, in the groups without coronary atherosclerosis, it is not specified to what extent coronary angiography diagnoses mild stenosis. Echo-Doppler diagnoses plaques in the carotid and femoral arteries over 1.5 mm. thickness. If coronary angiography does not have the same sensitivity, it omits the diagnosis of initial coronary atherosclerosis.

The results of statin treatment in patients in group V, in the absence of a control group are uncertain and biased. Group V itself was selected from patients who underwent various vascular revascularizations, indicating an advanced stage of multifocal atherosclerotic process. However, no case of stroke, heart attack, or amputation gangrene or death has been reported in the two-year follow-up. This does not sound realistic and calls into question the objectivity of the study.

In general, the topic studied in the dissertation has been the subject of several similar dissertations in Bulgaria and examines facts known to world science. But the comprehensive study of structural vascular changes in patients with HANK, proving the multifocal nature of the atherosclerotic process and identifying markers for its detection is undoubtedly valuable in this scientific work. The development of an algorithm for optimizing the diagnosis and therapeutic behavior in patients with multifocal atherosclerosis is an indisputable scientific contribution.

Contributions: The contributions are reflected in the Abstract and are 10, divided into two groups - original (7) and confirmatory (5) and are important for vascular medicine in our country.

Based on the research material, the following contributions can be recognized:

With original character:

1. Pathological structural changes in the following ultrasound parameters were found in patients with HANK: DIM ICAb, DIM ICA, DIM FCA, presence of plaques, stenoses, thrombosis of carotid and femoral arteries, ABI <0.9, as a sign and markers of multifocal atherosclerotic process.

2. Based on the established results and statistical dependencies, an algorithm has been developed to optimize the diagnosis and therapeutic behavior in patients with multifocal atherosclerosis.

Confirmatory:

1. It is confirmed that the classic risk factors for HANK are essential for the development of multifocal atherosclerosis - male, over 68 years of age, hypertension, diabetes, smoking, family history, dyslipidemia.

2. The first morphological changes in the arterial wall can be visualized by B-mode ultrasonography. It is confirmed that this non-invasive method is one of the best for detecting the early stages of atherosclerosis, as it is easy to apply, the equipment is available in many places, and its resolution is better than that of magnetic resonance imaging and CT .

3. It has been proven that the presence of plaque or stenosis of CA contributes to an increased risk of HANK, IMI or CHD in the range of 4-8%.

4. The proposed prognostic markers used in the subclinical stage of the atherosclerotic process contribute to the identification of high-risk patients and to the optimization of their prevention and early treatment.

5. A set of diagnostic and prognostic markers for optimizing the screening and diagnosis of multifocal atherosclerosis - thickening of DIM over 1 mm, plaques of carotid and / or femoral arteries, stenosis and / or thrombosis of carotid and / or femoral arteries, ABI below 0.9, dilatation and aneurysm of the abdominal aorta.

The abstract is presented on 69 pages and reflects what was written in the dissertation.

7. Assessment of the publications and personal contribution of the doctoral student

 The doctoral student has attached 2 publications and three abstracts of reports presented at scientific forums (1 of which was published in a journal referenced in the Web of Science). This scientific activity is sufficient and covers the minimum required points for the scientific degree "PhD" of NACID.

CONCLUSION

The dissertation contains scientific, scientific-applied and applied results that expand our knowledge in the field of multifocal atherosclerosis. It is written in sound medical language, without significant errors.

Dr. Elitsa Gerova meets all the requirements of the Law for the Development of the Academic Staff in the Republic of Bulgaria (ZRASRB), the Regulations for the implementation of the ZRASRB and the Regulations of Acibadem City Clinic Tokuda Hospital EAD. The presented materials and dissertation results fully comply with the specific requirements adopted in connection with the Regulations of Acibadem City Clinic UMHAT Tokuda EAD for application of ZRASRB.

Due to the above, I give my positive assessment and recommend to the members of the Scientific Jury to evaluate positively the dissertation "Multifocal atherosclerosis - diagnostic and prognostic markers in different vascular basins" and to award Dr. Elitsa Rashkova Gerova - Micic the scientific and educational degree “PhD”In the scientific specialty “ Angiology ”.

June 18, 2022 Prepared the review:

city ​​of Sofia Prof. Dr. Lachezar Grozdinski, DSc