

PEER REVIEW

by Prof. Svetoslav Georgiev, MD, PhD
Vice-Rector of Career Development
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Regarding: Dissertation for the award of the scientific degree Doctor of Science in the scientific specialty: Cardiology; professional field: 7.1. Medicine; field of higher education: 7. Healthcare and Sports; with a title: **EARLY DEVIATIONS IN THE COAGULATION AND FIBRINOLYTIC SYSTEM IN PAROXYSMAL ATRIAL FIBRILLATION**, written by: Assoc. Prof. Maria Negrinova Negreva, MD, PhD at the First Department of Internal Medicine, Education sector of Cardiology, Faculty of Medicine, Medical University of Varna.

I. Procedure for declaring a public defense

The dissertation was discussed and directed for public defense by the Department Council of the First Department of Internal Medicine at the Medical University of Varna. By decree No. P-109-216/20.05.2022 from the Rector of MU-Varna, a Scientific Jury was approved for conducting the defense of the dissertation. I was elected Chairman of the Jury and reviewer. I have received all necessary documents for writing the review from the Career Development Center of the Medical University of Varna. The review was prepared in accordance with the requirements of the Law for the Development of Academic Staff in Bulgaria, Regulations for its implementation, and the Regulations for the Development of Academic Staff of the Medical University of Varna.

II. Brief biographical data about the author of the work

Assoc. Prof. Maria Negreva, MD, PhD was born in 1981. She graduated with a Master's degree in Medicine from the Medical University of Varna in 2006. In 2015 she defended her PhD in Cardiology. Since 2006 she has been working at the First Clinic of Cardiology as a resident (2006-2008), assistant physician (2008-2016) and associate professor of cardiology (2016 - present). Since 2018 she has been the Head of the Educational Sector of Cardiology at the First Department of Internal Medicine.

Assoc. Prof. Negreva is a co-author of 5 textbooks for students and specializing doctors. She is a research supervisor to two doctoral students in cardiology. She speaks English, French and German.

III. Scientometric analysis of the dissertation

The candidate presents 10 full-text scientific publications associated with the dissertation, all of them published in international journals. 5 articles are indexed in the Web of Science/Scopus databases, two of them in journals with impact factor. She is the first author in 7 publications and second author in 3. She presents also 3 abstracts from international congresses, two of which were published in journals with an impact factor. Assoc. Prof. Negreva is the first author in these abstracts. There are 8 positive citations of the presented publications in scientific databases by international authors. The total impact factor of Assoc. Prof. Negreva of the journals, where the publications associated with the dissertation are published, is 51.465.

The scientometric analysis shows that the candidate fulfills in excess all minimum requirements of the Medical University of Varna for acquiring the “Doctor of Science” degree.

IV. Structure of the dissertation

The dissertation consists of 179 standard pages, without the literature review. It is structured according to the traditional model adopted in our country, so I will not describe its structure. I will only point out that the Results and Discussion section is 80 pages, ie. the dominant part of the work, illustrated with 24 tables and 69 figures. The ratio of text to illustrative materials is balanced and easily understood.

V. Relevance and significance of the dissertation

The dissertation of Assoc. Prof. Maria Negreva is the first systematic scientific study of the coagulation status in paroxysmal atrial fibrillation with episode duration of ≤ 24 hours, which is clear from the literature in available global medical databases. The topic of the dissertation is completely relevant, given the following main facts:

1. There is still a lack of clarity about coagulation balance in brief (≤ 24 hours) episodes of paroxysmal atrial fibrillation;
2. The periprocedural thromboembolic potential of these episodes continues to be subject of scientific discussions in the most recent European and American guidelines for the treatment of atrial fibrillation. There is still no definite opinion on their post-procedural anticoagulant approach.
3. The obtained results are a good basis and can be used for studies on the application of hemostatic indicators beyond hemostatic assessment: to predict the manifestation of paroxysmal atrial fibrillation and arterial thromboembolic complications associated with it.

These facts are in themselves an objective and sufficient reason to accept the aim of the dissertation formulated by Assoc. Prof. Maria Negreva as modern and significant in theoretical

and practical terms. 9 logically derived tasks were formulated, aimed at detailed knowledge of coagulation status in the course of short (≤ 24 hours) episodes of paroxysmal atrial fibrillation, its periprocedural potential, factors influencing it, as well as its predictive value for manifestation of the disease and arterial thromboembolic complications associated with it.

I accept that the formulated aim and tasks can logically present new and essential data for optimizing the approach to short (≤ 24 hours) episodes of paroxysmal atrial fibrillation.

The conducted research is characterized by completeness and comprehensiveness due to implementation of the well-formulated tasks. The simultaneous study of twenty indicators of coagulation and fibrinolysis, carefully selected and logically associated, clearly outlines the development of coagulation imbalance in short episodes (≤ 24 hours) of paroxysmal atrial fibrillation and intimate mechanisms of the deviations. Particularly impressive is the simultaneous determination of plasma levels and activity of some indicators, which is a confirmation of the in-depth and modern approach to solving the tasks.

The study is an original scientific work, implemented in modern laboratories with modern laboratory tests.

VI. Literary scope and methodology of the dissertation

The literature review is written systematically and clearly, on a good scientific level, showing excellent knowledge of literature and competence of the candidate to summarize and analyze published results. The presented conclusions from the literature review clearly outline the need for the conducted research.

The list of cited literature includes 577 titles, of which 5 are in Cyrillic and 572 in Latin. I am strongly impressed by the large relative share of cited sources from the last 10 years: 242 sources (100 of them in the last 5 years).

Assoc. Prof. Maria Negreva structures the literary review of her dissertation in 5 sections. The first section, Historical Notes, is a natural introduction to the problem of the "atrial fibrillation-thromboembolism" relationship over time. The next two sections clearly outline the significant thromboembolic potential of paroxysmal atrial fibrillation in the light of modern cardiology, the effective long-term anticoagulant approach to it using the CHA₂DS₂-VASc score and the lack of a clear definition of periprocedural thromboprophylaxis. In the course of brief (≤ 24 hours) episodes of paroxysmal atrial fibrillation. The fourth section of the Literary Review is of particular interest. It summarizes the results of studies on the coagulation and fibrinolytic system in atrial fibrillation, clearly outlining the problem of paroxysmal atrial fibrillation: insufficient and contradictory data on the activity of both systems in short (≤ 24 hours) episodes of the disease and lack of knowledge of the pathophysiological substrate in these episodes. A separate chapter presents modern understandings of the structure and normal functioning of the coagulation and fibrinolytic system. The close connection and precise mutual regulation

between the two systems is clearly shown, as well as the need for their simultaneous detailed study in states of thrombosis. This is how the selection of the hemostatic indicators for achieving the aim of the dissertation is justified.

It is worth noting the precise, well-planned selection of the material in order to achieve the aim of the study. A patient group of 51 patients (26 men, 25 women) and a control group of 52 volunteers (26 men, 26 women) were studied. The analyzed groups are very well balanced in terms of demographic and clinical indicators. The precise selection of the participants on the basis of many exclusion criteria allows for an objective comparison of the two groups, which would contribute to the reliability of the conclusions and established causal relationships. The techniques for blood sampling, obtaining and storing plasma and serum are clearly described. Three main methods were used in laboratory practice: kinetic method, photometric method and ELISA, and their features are clearly presented. I can also point out the variety of methods used in statistical analysis, incl. power analysis, presenting the adequacy of the sample size and the correctness of the conclusions made related to the hypothesis of equality of mean values of the studied hemostatic parameters. Its use in itself is proof of the in-depth statistical approach to achieving the formulated aim.

The thesis summary of the dissertation presents the main sections of the work.

VII. Main results and contributions of the dissertation

As is well known, the value of the results and resulting contributions are determined by a number of factors. One of them is undoubtedly the importance of the studied indicators and clinical model in which they are obtained. In this sense, the presented dissertation undoubtedly has two distinctive features: it examines a large number of highly informative hemostatic indicators, fourteen coagulation and six fibrinolytic parameters in a population, whose selection provides optimal opportunity for correctness of conclusions and causal relationships. This gives me reason to assume that the results obtained are not only significant, but also a correct basis for the conclusions made. High procoagulant activity has been unequivocally shown in paroxysmal atrial fibrillation, which occurs in short (≤ 24 hours) episodes of the disease. Conclusions are made about the specific features of the coagulation process in the disease. Of particular interest are the results related to the role of thromboembolic patient characteristics and duration of the episode of atrial fibrillation for the coagulation balance. They allow for contributions of direct clinical significance. The results of the dissertation work enrich the knowledge about hemostasis in short episodes of atrial fibrillation and corresponding clinical approach.

The main scientific contributions on the coagulation and fibrinolytic system in short (≤ 24 hours) episodes of paroxysmal atrial fibrillation are six, from which I will highlight the following points:

- Early significant systemic hypercoagulability develops during short (≤ 24 hours) episodes of paroxysmal atrial fibrillation, both in low-risk patients (CHA₂DS₂ -

VASc score = 0 for men or 1 for women) and in those with increased risk (CHA₂DS₂-VASc score ≥ 2 for both sexes).

- The need for timely *periprocedural anticoagulant therapy to complete restoration of the hemostatic profile in all patients with a short (≤ 24 hours) episode of paroxysmal atrial fibrillation*, incl. for very low risk (CHA₂DS₂-VASc score = 0 for men or 1 for women) is clearly outlined.
- Arguments are presented on the need for *the earliest possible attempt to restore sinus rhythm in paroxysmal atrial fibrillation, which would lead to limitation of procoagulant changes*.
- Objective evidence has been provided to achieve effective anticoagulation with anti-FXII and anti-FXI drugs with expectations of greatly reduced hemorrhagic risk compared to established anti-FIIa and anti-FXa drugs.

Contributions related to the predictive value of hemostatic indicators for paroxysmal atrial fibrillation and associated ischemic stroke are interesting, but require confirmation from clinical trials.

The formulated contributions are mainly of an applied nature. They belong to the category of obtaining and proving new facts, which gives the work a high scientific value.

VIII. Conclusion

The dissertation of Assoc. Prof. Maria Negreva on the topic of **Early deviations in the coagulation and fibrinolytic system in paroxysmal atrial fibrillation** is very well structured, with clearly defined objectives, proper selection of material, critical evaluation of results and precisely formulated conclusions. It fully meets the requirements of the Law on the Development of the Academic Staff of Bulgaria, the Regulations for its implementation and Regulations for Development of the Academic Staff of the Medical University of Varna. The in-depth nature of the study, results and contributions of mostly applied nature allow me to give a positive assessment of the dissertation and strongly recommend to the esteemed members of the Scientific Jury to award the scientific degree "Doctor of Science" in the scientific specialty cardiology to Assoc. Prof. Maria Negreva, MD, PhD.

Date: May 26, 2022.

Signature:.....

/Prof. Svetoslav Georgiev, MD, PhD/