OPINION

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Subject: Dissertation for the award of the scientific degree "Doctor of Science" in the field of higher education 7. Health and sports, professional field 7.1. Medicine, scientific specialty Cardiology

of Assoc. Prof. Dr. Maria Negrinova Negreva, MD, PhD
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Dissertation topic: "Paroxysmal atrial fibrillation - early abnormalities in the coagulation and fibrinolytic system"

By order of the Rector of MU-Varna № 109-216 / 20.05.2022 and on the basis of a decision of the Faculty Council of the Faculty of Medicine under protocol № 65/17.05.2022 I was elected a member of the **scientific jury** and after at its first meeting I was appointed to prepare an **opinion** on the above dissertation. The presented documents correspond to the Regulations for development of the Academic Staff of MU-Varna.

1. Biographical data and professional development

Assoc. Prof. Dr. Maria Negreva completed her secondary education in 2000 at the First Language High School in a class with primary language English and her higher education in 2006 at the Medical University "Prof. Dr. P. Stoyanov"-Varna. After obtaining a Master's degree in Medicine, from 2006 to 2008 she worked as an intern at the First Clinic of Cardiology with ICU at the University Hospital "St. Marina"-Varna. In 2008 she was elected Assistant Professor of Internal Medicine at the First Department of Internal Medicine, Faculty of Medicine, which she held until 2016. In 2013 she acquired a clinical specialty in Cardiology.

After successfully defending her dissertation on "Dynamics in oxidative status in patients with paroxysmal atrial fibrillation", in 2015 she received educational and scientific degree "Doctor" in the scientific specialty "Cardiology". Since 2016 she has held the academic position of "Associate Professor" in Cardiology at the First Department of Internal Medicine, and since 2018 she has been the head of the Board of Cardiology in the same Department. Assoc. Prof. Dr. Negreva has an additional professional qualification in echocardiography - fundamental and expert level. She specializes in echocardiography and electrophysiology in Italy and Germany.

The main research interests of Assoc. Prof. Maria Negreva are related to rhythm and conduction disorders and especially atrial fibrillation. In-depth research has been associated with oxidative and coagulation changes in patients with AF and restored sinus rhythm, as well as with the onset of echocardiographic changes in these patients. In this direction are the main publications and the topic of her dissertation. She has over 60 full-text publications and 35

participations in scientific forums, mainly related to her research interests in arrhythmology. She participated in a project financed by the Research Fund of the Ministry of Education and Science. Assoc. Prof. Negreva is a research supervisor of two PhD students and a scientific consultant of one doctoral student. She teaches internal medicine and cardiology to medical students and specializing physicians in Cardiology at MU-Varna. She has published 1 monography and participated in the preparation of 5 textbooks.

She masters English, French and German languages.

2. Relevance of the problem

Atrial fibrillation (AF) is the most commonly diagnosed arrhythmia in clinical practice. Its incidence has increased worldwide over the last twenty years and reaches 3% of the total population over the age of 20. It increases with age, and over the age of 80 the prevalence is over 10%. AF increases the risk of adverse outcomes in the patients and they have twice higher mortality and morbidity. The main clinical problem of the disease are thromboembolic complications - cerebrovascular and extracardiac vascular accidents. Stroke is five times more common in patients with AF than in people without arrhythmia at the same age and sex. The long-term thromboembolic risk and subsequent long-term anticoagulant therapy place demands on the search for prognostic factors in individual patients to predict the need and duration of treatment. The modern predicting systems have been validated in large epidemiological and clinical studies and are used in everyday practice. However, all of them are not enough to refine the individual characteristics of individual patients and to personalize treatment, as the residual risk is still high, especially in the high-risk group. Experimental and clinical studies on the coagulation and fibrinolytic system of patients with AF have shown significant hyperactivity of the coagulation system in patients with prolonged AF, but few studies are done on changes in the fibrinolytic system. In addition, these changes in both systems remain unstudied in episodes of AF <24 hours from onset. The predictive value of coagulation and fibrinolytic parameters for paroxysmal atrial fibrillation, as well as thromboembolic events related to it, is unclear.

These unresolved issues naturally direct the research of Assoc. Prof. Negreva in this direction. In view of the clinical significance of atrial thromboembolic events in AF and the indisputable need for precise anticoagulation in these patients are an argument in favour of the need for a comprehensive study of the coagulation and fibrinolytic system in the early hours (first 24 hours) of the clinical manifestation of paroxysmal atrial fibrillation.

3. Structure and content of the dissertation

The dissertation contains 217 standard pages. It is properly structured and contains the major compartments: introduction; literature review; aim, tasks; material and methods; results and discussion; conclusions; scientific contributions; references; publications and participations related to the dissertation and noticed citations of the papers in the scientific literature, with the respective impact factor.

The **literature review** is comprehensive and is written in correct Bulgarian on 69 pages. It provides an in-depth analysis of the research problem in the light of the latest research. Its content is entirely focused on the main goal and objectives of the dissertation. The main debatable and unsolved problems in connection with coagulation and fibrinolysis in AF are clear, which are the main motive for the development of the dissertation.

The **aim** is clear and concise: to study the coagulation status of patients with paroxysmal atrial fibrillation and the duration of the episode ≤24 hours, by studying the systems of coagulation and fibrinolysis. It leads logically and reasonably to **9** specific and clear **tasks** directly related to achieving the main goal of the scientific work.

The section **"Material and Methods"** is presented very accurately on 16 pages. The design of the study is described precisely - a prospective study of 51 patients with the first attack of paroxysmal atrial fibrillation, equally men and women, and 52 controls without arrhythmia at the same age, gender and BMI. The inclusion and exclusion criteria are clearly stated. The main clinical, laboratory and ultrasound indicators of both groups are characterized in detail. The method of blood sampling, their storage and the methods for examination of all twenty coagulation and fibrinolytic parameters are specified in detail. Reliable and adequate statistical methods have been used for data analysis, which guarantee the reliability of the obtained results and the conclusions made.

The **results** of the individual research are presented in detail and appropriately illustrated with 69 figures and 24 tables. In the **discussion**, in parallel with the presentation of the obtained results, the significance of the obtained results, their advantages and disadvantages are discussed, and they are compared with what is known so far in the international literature.

The analyses begin with a comparison of the two groups, which shows that there are no significant differences in the demographic characteristics and ultrasound indicators of the participants. Deviations in coagulation have been reported in patients with AF. TF and FVII levels were significantly higher in the patient group, indicating significant activation of the external pathway of the coagulation cascade in the first 24 hours after the onset of the arrhythmia. Increased coagulation activity of FXII, FXI and FIX, as well as FVIII, vWF and collagen-binding activity of glycoprotein has been observed in patients with paroxysmal atrial fibrillation. Their established activation provides opportunities to seek new pharmacological approaches for adequate and safe anticoagulation in the disease. Higher levels of other coagulation factors were also measured, such as major elements of the prothrombinase complex FX and FV and of thrombin itself (FIIa), as well as F1+2 and FPA in the first hours of AF. The generalized analysis of the results gives an objective reason to assume that the short (≤24 hours) episodes of paroxysmal atrial fibrillation are associated with significantly increased activity of the coagulation system, which has its own specifics and systemic nature. Hypercoagulability has been observed, which is an indisputable prerequisite for thrombosis in short (≤24 hours) episodes of paroxysmal atrial fibrillation.

Examination of the fibrinolytic system in patients with paroxysmal AF also shows important abnormalities. Plasminogen activity, t-PA and D-dimer levels were higher in patients with AF compared to healthy controls, while PAI-1 activity and α2-antiplasmin and vitronectin plasma levels were lower. These results indicate that early changes in fibrinolytic activity due to increased plasmin synthesis and decreased activity of the fibrinolytic inhibitors PAI-1 and α2-antiplasmin are most likely to be a pathophysiological response to hypercoagulation.

A detailed assessment of the adequacy of the sample used was made by analysing the power of the t-test. It shows that the number of selected participants for each group is sufficient and adequate. The following chapter assesses the relationship between the risk profile for embolic events in patients with AF using the CHA2DS2-VASc model and coagulation and fibrinolytic status disorders. Only α2-antiplasmin activity was found to be higher in patients

with moderate and high thromboembolic risk, while other indicators were indistinguishable between the two groups of patients with AF. It is logical to conclude that the clinical manifestation of short (≤24 hours) episodes of paroxysmal AF is associated with significant prothrombotic abnormalities in coagulation status that are not predetermined by the worsening thromboembolic risk profile of patients assessed by the CHA2DS2-VAS system. The latter is a major determinant of thromboembolic risk in the long run.

Data from correlation and regression analyses confirm that longer episodes of arrhythmia are associated with significantly more pronounced deviations in key coagulation parameters. Procoagulant abnormalities in short episodes (≤24 hours) of paroxysmal atrial fibrillation are not static but dynamic in nature, with the most pronounced changes in the first six hours of illness. The duration of the episode proved to be a critical predictor of the dynamics of procoagulant abnormalities during the first six hours of clinical arrhythmia. Based on the constructed logistic models and their calculated correctness, seven of the studied twenty coagulation and fibrinolytic indicators proved to correctly classify the observed cases from controls. Age, CHA2DS2-VASc score, elevated TF and FVIII levels, and low vibronectin levels, which were assessed by multivariate survival analysis, have predictive value for increased risk of stroke.

The dissertation also presents **nine conclusions** that summarize the observed results. **Scientific contributions** of original fundamental and clinical character also stand out:

1. The first clinical study on coagulation balance in short episodes of paroxysmal atrial fibrillation lasting ≤24 hours was performed. An objective analysis of the studied twenty indicators of the coagulation and fibrinolytic system was performed.

2. Direct and convincing evidence for the development of early significant systemic hypercoagulability in the course of brief (≤24 hours) episodes of paroxysmal AF is presented. It objectively shows an increased periprocedural thromboembolic risk in these episodes.

3. For the first time the connection between the periprocedural thromboembolic potential of the short (≤24 hours) episodes of paroxysmal AF and the thromboembolic risk characteristic of the patient was directly studied.

4. The first six hours of paroxysmal atrial fibrillation have been shown to have significantly lower procoagulant activity.

5. The presented results not only agree with the latest recommendations of the European Society of Cardiology for the treatment of short (≤24 hours) episodes of paroxysmal atrial fibrillation, published in 2020, but significantly build on them.

6. The studied clinical and laboratory indicators provide opportunities for their use beyond the hemostasis assessment, the introduction of which in clinical practice requires additional studies:

• creation of new effective anticoagulants, based on the presented facts about FXI and FXII, with expected greatly reduced haemorrhagic risk compared to the established anti-FIIa and anti-FXa;

• use of some of the studied indicators as markers for prediction of paroxysmal atrial fibrillation;

• predicting the main complication of paroxysmal atrial fibrillation - ischemic stroke.

The **bibliography** includes 577 literature sources, of which 5 in Cyrillic and 572 in Latin.

In connection with the dissertation, 10 **published full-text articles** were presented - 5 in referred and indexed journals and 5 in others, 4 published abstracts of participation in international congresses and 7 participations in congresses, conferences and symposia in Bulgaria and abroad. In 7 of the full-text articles and in all the abstracts Assoc. Prof. Negreva is the first author, and in 3 she is the second. Her publications have been **cited** in the international referred literature 8 times.

4. Conclusion

The dissertation of Assoc. Prof. Dr. Maria Negrinova Negreva examines an up-to-date problem in cardiology of fundamental and clinical importance - the role of changes in coagulation in the early hours of development of paroxysmal AF. There are a sufficient number of patients studied, which is statistically proven. Modern research methods adequate to the purpose and tasks are used. The obtained results are well summarized and analyzed and lead to important conclusions and significant contributions.

The dissertation meets the requirements for obtaining the scientific degree "Doctor of Science", laid down in the Law of Development of the Academic Staff in Republic of Bulgaria and the Regulations for the development of the academic staff of MU-Varna.

I am convinced of my positive assessment and recommend to the esteemed members of the scientific jury to award Assoc. Prof. Dr. Maria Negrinova Negreva, MD, the degree of Doctor of Science in Cardiology.

30.05.2022
Varna

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/ prof. Dr. Yoto Trifonov Yotov, MD /