

## PEER REVIEW

Of the dissertation for the award of the scientific degree DOCTOR OF SCIENCE with the title:  
**EARLY DEVIATIONS IN THE COAGULATION AND FIBRINOLYTIC SYSTEM IN PAROXYSMAL ATRIAL FIBRILLATION**

**Author of the dissertation:**

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**Member of the Scientific Jury, reviewer:**

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The review was prepared in accordance with the Law for Development of Academic Staff, the Regulations for its Application and Regulations on the Terms and Conditions for Acquiring Scientific Degrees and Holding Academic Positions of the Medical University of Varna. The Scientific Jury for the public defense of the dissertation was determined by the decree of the Rector of the Medical University of Varna №P-109-216/20.05.2022. The dissertation was discussed and proposed for defense at the department council of the First Department of Internal Medicine at the Medical University of Varna.

The presented materials on paper/electronic media were in accordance with the procedure for acquiring the scientific degree Doctor of Science at the Medical University of Varna; Regulations of the Medical University of Varna

**Structure of the dissertation:**

The dissertation consists of 213 standard pages, of which:

1. Title page: 1
2. Acronyms: 1
3. Contents: 2
4. Introduction: 2
5. Literary review: 70
6. Aims, tasks: 2

7. Methodology: 15
8. Own results and discussion: 80
9. Conclusions: 2
10. Contributions: 3
11. References: 28
12. Publications, citations, impact factor report: 6

The ratio review: methodology: results-discussion is optimal, respectively 35: 20: 45% . The dissertation contains a total 69 figures and 24 tables. The dissertation includes 577 literary sources, of which 5 in Cyrillic and 572 in Latin. All cited titles are directly related to the studied problem.

**Characteristic features of the work:**

Atrial fibrillation is the most common arrhythmia in modern society and it is no coincidence that in recent years there has been significant progress in the study of pathophysiological mechanisms underlying the arrhythmia, search for pharmacological agents to modify the observed imbalance at the cellular level and all this through the prism of designed and conducted studies on stable follow-up endpoints. In this aspect, the study of the mechanisms of thrombosis and introduction of pharmacological agents aimed at its modification is a typical example of successful practical implementation of strategies that change the outcome of the disease and improve long-term prognosis. No less important is the issue of determining patient risk characteristics, predetermining an increased risk of thrombosis, with currently dominating strategy of defining low and high risk of thromboembolic events as a pragmatic tool for choosing therapeutic regimens. The coagulation and fibrinolytic systems have a leading and responsible role in the process of thrombosis in atrial fibrillation and this determines the significant experimental and clinical interest in them. Knowing the precise mechanisms of their interaction is the only way to objectively assess the thromboembolic potential of the disease and to optimize anticoagulant strategy. Of particular interest are the early stages of the development of atrial fibrillation, its shorter duration before the accumulation of burden of risk factors and occurrence of irreversible electrical and structural remodeling of the atria.

These data motivate the choice of topic by the candidate: EARLY DEVIATIONS IN THE COAGULATION AND FIBRINOLYTIC SYSTEM IN PAROXYSMAL ATRIAL FIBRILLATION.

**The literary review** is 68 pages long and presents the author as a leading expert on this issue. Epidemiological data and assessment of thromboembolic risk in atrial fibrillation, structure and normal functioning of the coagulation and fibrinolytic system, data from experimental and clinical studies on the coagulation and fibrinolytic system in atrial fibrillation and assessment of short-term and long-term thromboembolic risk and management strategies.

**Factual preconditions for the study** are derived from the review in the form of a conclusion. These are the main prerequisites:

- Thrombosis in atrial fibrillation is a structural manifestation of hypercoagulability due to an imbalance between the multicomponent and precisely interregulated coagulation and fibrinolysis systems.
- Conducted studies on the coagulation and fibrinolytic system in atrial fibrillation are mainly on its persistent and permanent form with very little data in short episodes ( $\leq 24$  hours) of the disease.
- The periprocedural thromboembolic potential of brief episodes ( $\leq 24$  hours) of paroxysmal atrial fibrillation and the need for postprocedural anticoagulation in very low-risk patients (CHA<sub>2</sub>DS<sub>2</sub>-VASc score 0 for men/1 for women) remain unclear.
- The predictive value of coagulation and fibrinolytic parameters for the manifestation of paroxysmal atrial fibrillation, as well as thromboembolic events related to it is unclear.

**The aim of the study** is formulated precisely and clearly: To study the coagulation status of patients with paroxysmal atrial fibrillation and episode duration  $\leq 24$  hours, examining the systems of coagulation and fibrinolysis.

To achieve it, 9 **main tasks** have been identified:

1. To study the extrinsic pathway of hemocoagulation by examining plasma TF levels and FVII coagulation activity.
2. To study the intrinsic pathway of hemocoagulation by examining XII, XI, IX, VIII coagulation factors and vWF plasma glycoprotein.
3. To study the common pathway of the coagulation cascade by determining:
  - 3.1. activity of the main elements of the prothrombinase complex: FX and FV, as well as thrombin itself (FIIa).
  - 3.2. F1+2 and FPA levels as early markers of haemocoagulation.

4. To study main regulators and indicators of fibrinolysis: plasminogen, t-PA, PAI-1,  $\alpha$ 2-antiplasmin and vitronectin, as well as plasma levels of the specific end product of fibrinolysis: D-dimer.
5. To analyze the power of t-test for testing the hypothesis for equality of mean values of coagulation and fibrinolytic indicators for the studied sample size of patient and control groups.
6. To study the influence of thromboembolic risk characteristics of patients, defined by CHA2DS2-VASc score, on coagulation and fibrinolytic indicators.
7. To search and estimate possible functional dependences of the values of studied hemostatic indicators on duration of the episode of paroxysmal atrial fibrillation.
8. To assess the prognostic value of coagulation and fibrinolytic indicators using the probability of manifestation of paroxysmal atrial fibrillation.
9. To analyze the predictive value of studied coagulation and fibrinolytic indicators for manifestation of arterial thromboembolic events.

**The methodology of the study** , which includes complex clinical-laboratory and instrumental instruments, is very impressive. The study was conducted at the First Clinic of Cardiology with Intensive Care Unit at the University Hospital St. Marina, Varna on the basis of approval by the Research Ethics Committee at the same hospital (№35/29.10.2010) and Medical University of Varna (№9/14.10.2010), and in accordance with the requirements of the Declaration of Helsinki (The World Medical Association Declaration of Helsinki, 2008). The study included 51 patients with atrial fibrillation (26 men and 25 women) with a first episode of paroxysmal atrial fibrillation aged no more than twenty-four hours and a mean age of  $59.84 \pm 1.60$  years (31-77 years) and 52 controls, men and women in equal numbers: 26 (50%), mean age  $59.50 \pm 1.46$  years (30-76 years) without anamnestic or ECG data for atrial fibrillation to date. A total of twenty coagulation and fibrinolytic parameters were studied in each participant, namely: activity of FII, FV, FVII, FVIII, FIX, FX, FXI, FXII, plasminogen,  $\alpha$ 2-antiplasmin, PAI-1; vWF activity and levels; plasma levels of TF, FVIII, FPA, F 1 + 2, t-PA, vitronectin, D-dimer. Patients were monitored for ischemic stroke through annual medical examinations until the end of December 2020 or earlier in case of discontinuation of monitoring for reasons other than stroke, but before it occurred (refusal to participate, death or cancer detection).

**The results obtained** are convincing, well illustrated with tables and figures. They are presented clearly and accurately, and thoroughly compared with results from other sources. The statistical methods, used to obtain them, are completely appropriate. In the separate subchapters there is a detailed analysis of the observed dependencies. The conclusion corresponds to the obtained results. The same applies to the conclusion made. With the help of the obtained data, clinical and laboratory data for the development of hypercoagulability in the first 24 hours of paroxysmal atrial fibrillation due to significant deviations in the coagulation and fibrinolytic system are established. Short ( $\leq 24$  hours) episodes of the disease are clearly defined as a prothrombotic state, even in low-risk thromboembolic characteristics of patients (CHA<sub>2</sub>DS<sub>2</sub>-VASc score = 0 in men/1 in women) with episode duration having an effect on established hemostatic deviations. The first 6 hours of the disease appear as the time interval defining a lower periprocedural thromboembolic risk. Monitoring of patients and the search for prognostic indicators provides opportunities for clinical application of some hemostatic indicators beyond hemostasis assessment, namely, ability to predict the manifestation of paroxysmal atrial fibrillation by plasma activity of plasminogen and plasma levels of t-PA and its complication, ischemic brain stroke, by plasma levels of TF, FVIII and vitronectin. This also defines the contributions of the work, which are of an original nature.

**I highly appreciate the work of Dr. Negreva in several areas:**

- 1) There is a real need for a clinical trial to identify changes in the coagulation and fibrinolytic system among patients with paroxysmal AF of short duration, assessment of clinical significance and dynamic follow-up.
- 2) Significant activity was performed in the selection of patients with paroxysmal atrial fibrillation in the absence of classical risk factors and conditions leading to changes in coagulation status, still again noteworthy is the fact that the majority of patients included have hypertension often requiring medical treatment.
- 3) The study uses a wide range of tests that allow detailed analysis of early changes of coagulation activity in the initial stage of atrial fibrillation. As with any clinical-laboratory design study, the real clinical potential would be clearer in prospective dynamic studies.
- 4) In order to clarify the need and optimize the solution for postprocedural anticoagulant prophylaxis, the dependence of hemostatic deviations on thromboembolic risk profile of patients, defined by CHA<sub>2</sub>DS<sub>2</sub>-VASc score, as well as the time characteristics of the atrial

fibrillation episode was sought; In this respect, data on sex differences in coagulation indicators outside the inclusion of women in the risk scale would be interesting.

5) A connection with disease dynamics and risk of ischemic accidents in the long run was sought.

6) Innovative aspect and projection into the future: an unresolved issue in the world literature is analyzed.

The results of the dissertation confirm and enrich the knowledge about the importance of the coagulation and fibrinolytic system in patients with early stages of atrial fibrillation, while paving the way for future research and challenges in their clinical applicability.

On the topic of the dissertation, the candidate has **published** 5 full-text publications indexed in Web of Science/Scopus, as well as 5 full-text publications in other peer reviewed journals. Fragments of the dissertation were presented at scientific forums in Bulgaria and abroad, as evidenced by the abstracts of the forums, most of which are in impact factor journals. Based on these facts, the scientific activity in the period of preparation of the work is assessed as optimal and corresponds to the scientometric indicators of the Medical University of Varna for clinical and diagnostic units.

#### **Conclusion:**

In conclusion, the dissertation presented by **Assoc. Prof. Mariya Negrinova Negreva, MD, PhD** contains scientific, scientific applied and applied results, which represent an original contribution to science and meet the requirements for awarding the degree DOCTOR OF SCIENCE.

On these grounds, I propose to the esteemed members of the Scientific Jury to vote positively and propose to the Rector of The Medical University of Varna **to award the scientific degree DOCTOR OF SCIENCES** in the scientific specialty of cardiology to **Assoc. Prof. Mariya Negrinova Negreva, MD, PhD**, First Department of Internal Medicine at the Medical University of Varna.



01.06.2022

Sofia

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