

## STANDPOINT

From

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as a member of the scientific jury, according to Order № P-109-216 / 20.05.22 of the Rector of the Medical University of Varna "Prof. Dr. Paraskev Stoyanov" – in the procedure of defending for acquiring the scientific degree "**Doctor of Science**" by **Associate Professor Maria Negrinova Negreva, MD, PhD** in the field of higher education: 7. Health and sports, professional field: 7.1. Medicine, scientific specialty – Cardiology, on the topic:

**"Paroxysmal atrial fibrillation - early changes in the coagulation and fibrinolytic system"**

This opinion is in accordance with the requirements of the Law for development of the academic staff in the Republic of Bulgaria and the Regulations for its implementation, as well as with the Regulations for development of the academic staff at MU "Prof. Dr. Paraskev Stoyanov" - Varna. Associate Professor Dr. Maria Negreva presented all the necessary materials and documents on the defense procedure

### **General characteristics of the dissertation - relevance of the problem**

The presented dissertation on the above topic is dedicated to an advanced field in cardiology - alterations in hemostasis balance in the most common cardiac arrhythmia, atrial fibrillation, that contribute to the increased risk of thrombus formation, thromboembolization and its complications such as cerebrovascular and peripheral arterial incidents. A number of important pathophysiological mechanisms and anticoagulant approaches in this area continue to be widely open to complex scientific studies and discussions.

The dissertation is done mainly at the Medical University "Prof. Dr. Paraskev Stoyanov" - Varna, Faculty of Medicine, First Department of Internal Medicine, Department of Cardiology (since 2010). The main laboratory tests related to the dissertation were conducted jointly with the National Specialized Hospital for Active Treatment of Hematological Diseases - Sofia, Department of Hemostasis (activity of FII, FV, FVII, FVIII, FIX, FX, FXII, plasminogen,  $\alpha$ 2-antiplasmin; activity and levels of vWF; plasma levels of D-dimer) and Medical diagnostic laboratory "Lipogard" - Sofia (plasma levels of TF, FPA, F 1 + 2, vitronectin, FVIII and t-PA, as well as activity of FXI and PAI-1). An analytical view of the studied clinical characteristics and laboratory results can be seen, which leads to an in-depth assessment of the role of coagulation activation and impaired fibrinolysis in paroxysmal atrial fibrillation with an episode duration of  $\leq$  24 hours.

The dissertation contains 215 pages and is illustrated with 69 figures and 24 tables. The work includes eleven subdivided parts - introduction, literature review, purpose and objectives of the study, material and methods, results and discussion, conclusions, scientific contributions, bibliography, publications and participation in scientific forums related to the dissertation, citations related to the dissertation, reference for impact factor.

The literature review summarizes information from 577 references. The focus is on several key issues of atrial fibrillation - thromboembolic risk, anticoagulant prophylaxis and long-term clinical approach, periprocedural decision in cardioversion, prothrombotic changes

in atrial fibrillation, structure and normal function of the coagulation and fibrinolytic system, early markers of coagulation, fibrinolytic parameters.

Based on the presented data, a summary has been made. It is reported that a large number of studies often analyzed unilaterally coagulation activity or fibrinolysis, almost always in comorbid groups. The studies are mainly in patients with persistent and permanent forms of AF, and data in paroxysmal AF (PAF) are insufficient, especially in short episodes ( $\leq 24$  hours) of the disease. There is uncertainty about the exact "time window" when these alteration in the coagulation balance should be evaluated, the optimal cut-off values, as well as the time effect of the decision based on the dynamics of changes. The need for post-procedure anticoagulation in very low-risk patients (CHA2DS2-VASc score 0 for men or 1 for women) remains unclear. The predictive value of coagulation and fibrinolytic parameters for the manifestation of paroxysmal AF, as well as thromboembolic events is unclear.

The purpose of the work is logical and convincingly formulated - a comprehensive study on the coagulation and fibrinolytic system in the early hours (first 24 hours) of the clinical manifestation of paroxysmal AF. Assoc. Prof. Maria Negreva has formulated 9 tasks to achieve the goal of the dissertation, showing high competence and a complete view of the dissertation. These include the study of a number of parameters of the external coagulation pathway (plasma TF levels and coagulation activity of FVII), of the internal coagulation pathway (coagulation factors XII, XI, IX, VIII), common pathway for thrombin activation (prothrombinase complex factors - FX, FV, FII); plasma glycoprotein vWF, early markers for activation of coagulation and fibrin formation - D-dimer, levels of F1 + 2 and FPA, main indicators and inhibitors of fibrinolysis: plasminogen, t-PA, PAI-1,  $\alpha_2$ -antiplasmin and vitronectin. The results should be used to characterize the differences between the group with paroxysmal AF and control group, to search for a relationship between thromboembolic risk characteristics of patients, defined by CHA2DS2-VASc score, and the studied coagulation and fibrinolytic parameters, and to evaluate the possible correlation of the studied hemostasis parameters with the duration of the episode.

The design of the study covered a statistically significant groups – 51 patients with PAF and 52 controls, comparable by demographic and clinical characteristics, selected in the period from October 2010 to May 2012. The clinical evaluation of each participant was performed on the basis of available medical documentation, detailed medical history, physical examination, electrocardiogram, laboratory tests and transthoracic echocardiography, as well as required minimum routine laboratory tests - blood count, ASAT, ALAT and creatinine - tested in patients with admission to the hospital, and in the controls - up to two weeks before their inclusion in the study. Patients were monitored for arterial thromboembolic events (ischemic stroke, transient ischemic attack or systemic thromboembolism) through annual medical examinations until the end of December 2020.

### **Analysis of the scientific and scientific-applied achievements**

The dissertation of Associate Professor Dr. Maria Negreva includes a number of scientific and applied scientific contributions. I consider that the most significant and large achievement is the multilateral characterization of the coagulation and fibrinolytic status of patients in the early stages of paroxysmal atrial fibrillation (PAF). High prothrombotic activity was reported during the first 24 hours, respectively the first 6 hours, from paroxysmal atrial fibrillation (increased activity of FII, FVIII, FIX and FXII), prothrombin fragment F1 + 2, fibrinopeptide A and D-dimer as indirect markers for enhanced thrombin and fibrin formation. M.Negreva accepts that, even in its earliest manifestations, paroxysmal atrial fibrillation could be considered as a procoagulant condition despite the well-known traditional risk factors assessed by the CHA2DS2-VASc score. The presented results for early

prothrombotic phenotype give the author reason to recommend "timely periprocedural anticoagulation" to reduce thromboembolic risk. The significant activation of FXII and FXI found may lead to new personalized approaches to anticoagulation in specific groups of patients with AF. In PAF, there is also evidence of altered fibrinolytic activity, describing more compact and denser fibrin networks, relatively resistant to lysis with increased thromboembolic risk. In the study performed during the first 24 hours, elevated values of plasminogen and t-PA were found, which are associated with the highest predicted probability of the occurrence of PAF based on logistic regression and ROC analysis. On the other hand, plasma levels of TF, FVIII and vitronectin could be significant predictors of ischemic stroke.

#### **Submitted publications, included in the procedure**

Associate Professor Dr. Maria Negreva has submitted 10 publications related to the topic of the dissertation. Five of them are articles in prestigious international journals such as Minerva Cardiol Angiol, Cardiol Res, JAFIB, Arch Med Sci Atheroscler Dis, Medicine (Baltimore) and M.Negreva is first author in all of them. The total personal impact factor of publications related to the dissertation calculates 51,465, of which 3,151 is the impact factor of full-text publications and 48,314 is that of published abstracts in European Heart Journal - Participation in ESC Congresses in 2017 and 2018. A report on 8 citations of four papers from the publications is presented. Furthermore Associate Professor Maria Negreva has 5 articles in non-refereed journals with scientific review.

I am convinced that the scientific papers of Associate Professor Dr. Maria Negreva presents her significant contribution in an important field of cardiology, studying the mechanisms of coagulation changes in hemostasis balance in patients with paroxysmal atrial fibrillation.

#### **Recommendations**

I would like to recommend the present work be continued in the future with a study of the dynamics of the system involving groups of patients with stable recovery of sinus rhythm or persistence of AF.

#### **Conclusion**

The dissertation of Assoc. Prof. Dr. Maria Negreva is innovative for Bulgaria, in terms of assessing the role of activation of coagulation and changes of the fibrinolytic system in paroxysmal atrial fibrillation with an episode duration  $\leq 24$  hours, leads to more accurate evaluation of thromboembolic risk and outlines changes in clinical strategies.

I firmly confirm that the presented dissertation and the scientific papers attached to it, the quality and originality of the results and the scientific and applied contributions presented in them meet the requirements of ZRASRB, the Regulations for its implementation and the Regulations of the Medical University "Professor Dr. Paraskev Stoyanov" - Varna for the candidate's degree "Doctor of Science" in scientific field 7. Healthcare and sports, professional field 7.1. Medicine, Scientific specialty - Cardiology.

Based on the above, I recommend the scientific jury to award Associate Professor **Maria Negrinova Negreva, MD.PhD. scientific degree "Doctor of Science"** in the field of science 7. Health and sports, professional field 7.1. Medicine, Scientific specialty - Cardiology.

01 June 2022

  
Professor Ivanka Dimitrova Paskaleva, MD, PhD