

PEER REVIEW

of the dissertation for obtaining the scientific degree DOCTOR OF MEDICAL SCIENCES, on the topic: Early abnormalities in the coagulation and fibrinolytic system in paroxysmal atrial fibrillation.

Scientific specialty: Cardiology, code 03.01.47

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Reviewer: Prof. Toshko Lissitchkov, MD, PhD, DSc

(Decree №P-109-216/20.05.2022 of the Rector of Medical University of Varna and Minutes №1 of the meeting of the Scientific Jury)

Topicality of the problem: It is enough to review the latest version of the 2020 European Society of Cardiology Guidelines for the diagnosis and management of atrial fibrillation with 1492 literature sources used, to conclude that the problem is extremely relevant. It is an honor for Assoc. Prof. Negreva that, despite intensive research from various scientific circles, she has found a niche to contribute to global science and clinical practice.

I will not address this issue in the preamble of the review, as it is very well and logically developed in the review of the dissertation.

Structure of the dissertation: The entire work covers 213 standard typewritten pages, with classic structure:

Title page

Contents: 2 p.

Abbreviations used: 1 p.

Introduction: 2 p.

Literary review: 70 p.

Aim and tasks of the study: 2 p.

Materials and methods: 10 p.

Results and discussion: 80 p.

Conclusions: 2 p

Scientific contributions: 2 p

References: 27 p

Additional information about the scientific work

Publications and participation in scientific forums

Impact factor report

The dissertation is richly illustrated with 24 tables and 69 figures.

The structure of the dissertation is well balanced, and of course the main part is the author's own development: the results and their discussion.

The introduction outlines the **topicality of the problem and the niche**, in which, despite the huge number of studies and developments from various scientific circles in atrial fibrillation, is the periprocedural thromboembolic risk in short episodes of paroxysmal atrial fibrillation lasting less than 24 hours and the corresponding approach, periprocedural thromboprophylaxis.

The literary review can be divided into two: the first part deals with atrial fibrillation, epidemiological data and clinical characteristics, especially thromboembolic risk and principles of antithrombotic anticoagulant prophylaxis. Data on the different variants of atrial fibrillation, risks and attempts to stratify them, possible dissonance between current criteria and clinical incidence of thromboembolic complications are provided.

The second part deals with prothrombotic changes in hemostasis that occur during the various variants of atrial fibrillation, citing data from clinical and experimental studies on the coagulation and fibrinolytic systems. This section, for greater clarity on the problems that are found in atrial fibrillation, has been expanded by reviewing latest data and hypotheses about the physiology and disorders in these vital systems.

Data come from 577 literary sources: 5 in Cyrillic and 572 in Latin. The data used are from publications up to and including 2020, which shows their relevance.

As a note on the review, I would point out the lack of data from studies on coagulation inhibitors, as is the case with the fibrinolytic system.

Conclusion: the review outlines latest scientific achievements in this field, clarifying the relevance of the chosen problem and could be part of any training manual or monograph on these subjects.

The erudition and high competence of the author is evident from the critical discussion of the literature data and their incompleteness and inconsistency, especially in paroxysmal atrial fibrillation, a common clinical finding, both in terms of changes in

coagulation and fibrinolytic systems and peripheral risk assessment and refining the therapeutic approach as anticoagulant prophylaxis. **Thus, logically, we find the niche for the development of this dissertation.**

The aim of the present work is to study the coagulation status of patients with paroxysmal atrial fibrillation with an episode duration of less than 24 hours, examining the systems of coagulation and fibrinolysis.

To achieve this aim, 9 specific tasks have been set. After reviewing them, we see that their implementation would achieve the aim of the dissertation.

I would group these tasks in two directions:

First, establishment of significant changes in the coagulation and fibrinolytic systems in patients with short-term episodes of paroxysmal atrial fibrillation.

Second, determining their relationship with the established clinical criterion for thromboembolism CHA₂DS₂VASc score, episode duration, as well as predictive (prognostic) value for the manifestation of the disease and its main complication, thromboembolic events.

The set aim and tasks with the achieved original data are completely sufficient to meet the requirements for the degree of Doctor of Medical Sciences.

Materials and methods:

The study was conducted in the period October-May 2012 with 2 groups of participants: target 51 patients (26 men and 25 women) with a mean age of 59.84 years (31-77 years) with first manifestation of paroxysmal atrial fibrillation, selected from 338 screened patients, and controls: 52 (26 men and women) with mean age of 59.50 y (30-76 y), selected from 169 volunteers, meeting the study inclusion and exclusion criteria. Both types of criteria are carefully selected in order to form similar groups and, at the same time, to exclude diseases outside the target disease, intake of various medications, and other factors such as pregnancy, which would further affect the identified hemostasis changes and would compromise interpretation and their significance for the set aim and tasks. The exclusion criteria are in line with international recommendations and criteria for corresponding group of diseases.

The participants included in the patient group were monitored until the end of 2020 for a ten-year period for occurrence of ischemic stroke.

Laboratory studies on coagulation and fibrinolysis include a wide range of highly specialized studies: chronometric and photometric studies, as activity of relevant factors, and immuno-enzymatic, as antigens.

For the clinical states of the heart and assessment of heart cavities, different variants of echocardiographic methods were used in accordance with international recommendations.

To prove the significance of established data and changes, they are subjected to modern multifactorial statistical analysis.

Evaluation of this section: selected patients and controls are uniform and comparable according to accepted criteria, which makes the comparison of the found indicators significant for the set tasks. The spectrum of coagulation and fibrinolytic laboratory tests are quantitative, not qualitative, making their results highly significant.

Own results and discussion

The demographic characteristics of the two groups of participants in the study were analyzed in the materials and methods section. There are additional data on concomitant diseases and their therapy. There is also good comparability in routine laboratory tests: differences are insignificant. Atrial fibrillation attacks are with duration less than 24 hours with a mean of 8.14 hours, and blood samples for laboratory tests were most often taken 5 hours after the onset of the event.

Essential part of the work is the study of a wide range of coagulation and fibrinolytic components such as activity and antigen and their interpretation.

Significantly higher values, found in the group of patients compared to the controls:

- Tissue factor and factor seven, **these data are original** and show early initiation of the coagulation process in paroxysmal atrial fibrillation, occurring in its short episodes, less than 24 hours.
- Factors XII, XI. While according to current hypotheses, factors XII and XI are not directly related to physiological hemostasis, their activation is a risk factor for thrombosis. **The hypothesis of the dissertation** is the search for means to inhibit their activation, as a new option for antithrombotic prophylaxis.
- Early activation of factors IX as activity and VIII (as antigen and activity), Willebrand factor as activity and collagen-binding activity. These are **original data** for the target contingent of patients. These changes have also been hypothesized, that an early endothelial lesion may be the cause of high plasma levels of Willebrand factor, which is a protector of factor VIII and this leads to extended half-life and stabilization of its molecule.
- Factors X, V and II. These are also signs of increased blood clotting and increased risk of thrombosis.

- F1+2 and FPA/F1+2 is a reliable quantitative marker for the degree of thrombin formation, and FPA, for its activity. They are also assessed as risk indicators for thrombosis.
- Additional analysis of the above indicators does not show dependence on gender, age and body mass index as side effects.

Changes in the indicators of the fibrinolytic system.

- Higher values of plasminogen and tissue plasminogen activator were found in the group of patients compared to the control group.
- The activities of PAI-1, alpha2 antiplasmin and vitronectin were lower in the patient group compared to the control group.
- Higher levels of D- dimers were found in the patient group compared to the control group.

These data are original for the group of patients with brief episodes (less than 24 hours) of paroxysmal atrial fibrillation.

It has been hypothesized, that the activation of the fibrinolytic system expressed by increased values of plasminogen and t-PA and decrease of PAI-1 and alpha2-antiplasmin is the body's response to the state of hypercoagulation.

Additional analysis of the above indicators does not show dependence on gender, age and body mass index as side effects.

- The assessment of coagulation and fibrinolytic parameters, as a comparison between the low-risk and high-risk groups in terms of thromboembolic risk according to CHA₂DS₂ -VASc score did not show differences between them, which means that both groups have a similar risk of thromboembolic complications. This means reassessing the therapeutic approach to post-procedural anticoagulant prophylaxis after brief (≤ 24 hours) episodes of paroxysmal atrial fibrillation.
- The dependence of coagulation and fibrinolytic parameters on episode duration was studied, and patients were divided into 2 subgroups according to duration: less than 6 hours and more than 6 hours. The dynamic nature of the changes was established during the first 6 hours, while after the sixth hour such dynamics were absent. These data are of great clinical importance, a recommendation for early cardioversion instead of the current approach of wait-and-see behavior. This would reduce the degree of periprocedural risk of thromboembolic complications.
- The connection between hemostasis and manifestation of paroxysmal atrial fibrillation was sought. An ideal clinical model has been developed for this purpose, examining carefully selected patients.

- The possibility of predicting manifestation of the main complication of the disease, arterial thromboembolic events, has been studied. A large number of indicators were examined, which gives weight to the study. Undoubtedly, however, the small number of registered thromboembolic events (9 ischemic strokes) is impressive. This value is close to the expected, which confirms the adequacy of the established clinical model. At the same time, it raises the question of the applicability of the presented predictive models in real clinical practice. Therefore, this part of the results undoubtedly needs confirmation from large prospective clinical trials.

As a result, 9 conclusions were logically made, which are well argued and with which I agree!

Critical remarks on this section: As the changes in the fibrinolytic system are fully described, pro- and inhibitory factors, it would be good to consider the changes in the coagulation system in the same way. In this case, there are no data on coagulation inhibitors in these patients. The fact that this was the subject of the dissertation for the educational and scientific degree "Doctor" is not a procedural violation, without considering these results in detail, to be cited in their essential part.

Scientific contributions:

I would divide the contributions into two groups: laboratory contributions and clinically significant therapeutic and prognostic contributions

1. Laboratory contributions:

- Detailed characteristics of coagulation changes with highly sensitive quantitative methods in a constellation, consisting of 20 tests establishing quantitative changes in the coagulation and fibrinolytic systems in patients with short episodes (less than 24 hours) of paroxysmal atrial fibrillation. The results are unidirectional and significant in a sufficient number of patients and comparable controls in all required criteria.
- Convincing evidence of early significant hypercoagulation in brief episodes of paroxysmal atrial fibrillation has been established. This process shows progressive activity during the first 6 hours of the episode, after which no such progression is detected.
- The relationship between the periprocedural thromboembolic potential of brief episodes of paroxysmal atrial fibrillation and the thromboembolic risk profile of patients has been studied. It has been shown that there are no significant differences in the prothrombotic profile of the two groups of low and high risk patients according to the CHA₂DS₂-VASc score.

2. Clinically significant therapeutic and prognostic contributions.

- The established data on hypercoagulation and activated fibrinolytic system suggest remodeling of therapeutic behavior regarding periprocedural antithrombotic prophylaxis. It has been argued that the recommended conservative “watch and wait” approach increases the thromboembolic risk for the patient and is unsuccessful. This allows for upgrading the latest recommendations of the European Society of Cardiology, indicating the need for timely periprocedural anticoagulant prophylaxis to complete recovery of the coagulation profile in all patients with a short episode (less than 24 hours) of paroxysmal atrial fibrillation, including low-risk patients.
- Based on the proven dynamics of hemostatic changes in the first 6 hours of the paroxysmal atrial fibrillation episode, the earliest attempt to restore sinus rhythm is suggested.
- Dynamic changes in hemostatic potential in short-term episodes of paroxysmal atrial fibrillation justify a redefinition of the definition of "ideal candidate for acute cardioversion" proposed by recent European recommendations and narrowing down the duration of the episode to the first 6 hours of the episode.
- It is hypothesized that the identified changes in coagulation potential and, in particular, increased activity of factors XII and XI offer the possibility of a new type of antithrombotic prophylaxis by inhibiting their activity as an alternative to current therapeutic approaches.
- The clinical significance of hemostatic abnormalities beyond hemostasis was sought. The predictive potential of some indicators for manifestation of atrial fibrillation and its complication, stroke, has been shown. As the author himself critically notes, this aspect of clinical application can be accepted after further research.

The dissertation is written in coherent and clear Bulgarian, with an accurate design. It is sufficiently illustrated with a large number of figures and tables, which demonstrate the importance of the established data.

Thesis summary: The thesis summary is written on 96 pages and sufficiently informative and reflects in abbreviated form the essence of the dissertation, aim and tasks, results obtained with their discussion and evaluation, conclusions and scientific contributions, and all additional references.

I agree with the stated limitations of the study and the recommendations for future research.

From the Academic report we can see that parts of the dissertation were published in 5 articles indexed in Web of Science/Scopus, where the author is first author, 5 articles in other journals, 3 abstracts, presented at international congresses and indexed in Web of

Science/Scopus, 1 abstract, published in other journal. The author presents also 7 participations in congresses, symposiums and conferences.

I highly appreciate the autobiographical report. It shows the candidate's career development in time as a suitable combination of an excellent researcher and competent clinical cardiologist.

In conclusion: The reviewed dissertation on: **Early abnormalities in the coagulation and fibrinolytic system in paroxysmal atrial fibrillation** has all the merits for obtaining the degree of Doctor of Medical Sciences:

- The selected topic is of high clinical significance, such as morbidity and risk of fatal and potentially fatal complications.
- A sufficient number of patients with first short-term attack of paroxysmal atrial fibrillation and a control group comparable in all lateral indicators were studied.
- A constellation of highly specialized quantitative tests characterizing the coagulation and fibrinolytic potential and their changes has been selected, as the quantitative characteristics of the established indicators allow for their accurate characterization and processing.
- Original data has been found on a number of indicators, not found in previous studies
- The established quantitative changes in the characteristics of the coagulation and fibrinolytic systems are the basis for changes in therapeutic behavior: reassessment of thromboembolic risk in different categories of patients according to current criteria, recommendations for early active intervention instead of waiting behavior, possibility to build on current ESC recommendations from 2020
- An original scientific hypothesis for a new therapeutic approach to anticoagulant therapy has been presented as an alternative to current therapy with anti-factor Xa and anti-IIa drugs.
- It offers a basis for selection of certain coagulation markers as diagnostic and prognostic criteria for this type of clinical events.

Given the merits of the dissertation and high erudition and competence of the candidate, I strongly suggest to the Scientific Jury to award Assoc. Prof. Mariya Negrinova Negreva, MD, PhD the scientific degree DOCTOR OF MEDICAL SCIENCES in Cardiology, code 03.01.47.

May 27, 2022

Reviewer:


(Prof. T. Lissitchkov, MD, PhD, DSc)