OPINION

by Assoc. Prof. Irena Ivanova Gencheva – Angelova, MD, PhD
Department of "Clinical Laboratory, Clinical Immunology and Allergology",
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of a dissertation for awarding the educational and scientific degree 'Doctor'
professional direction 7.1 Medicine
doctoral program "Clinical Laboratory"

Author: Snezhana Stoyanova Stoencheva, MD
Form of doctoral studies: independent program
Department: "Clinical Laboratory", Faculty of Phramacy, MU - Plovdiv
Topic: "Clinical laboratory assessment of coagulation and fibrinolysis in patients with malignant diseases"
Research supervisor: Assoc. Prof. Tanya Deneva, MD, PhD, MU - Plovdiv
Prof. Zhanet Grudeva – Popova, MD, PhD, MU - Plovdiv

1. General presentation of the procedure and the doctoral student

The set of materials presented to me on paper and electronic media is in accordance with Art. 70 (1) of Section I. Acquisition of educational and scientific degree "DOCTOR" and scientific degree 'DOCTOR OF SCIENCES" at MU - Plovdiv; Regulations of MU - Plovdiv dated 28.01.2021 and includes the following documents:

- Application to the Rector of MU - Plovdiv for disclosure of the procedure for the defence of a dissertation work
- a European format curriculum vitae signed by the doctoral student
- a notarized copy of a higher education diploma
- orders for enrolment in doctoral studies, interruption of studies (due to maternity) and for continuation of studies; for deduction with right of defence
- an order for conducting an exam from the individual plan and a corresponding protocol for a passed exam or doctoral minimum in the specialty
- protocol from the departmental council for the preliminary discussion of the pre-dissertation work and the decisions taken for the disclosure of the procedure and for the composition of the scientific jury
- dissertation work
- abstract
- a list of scientific publications on the topic of the dissertation
- copies of scientific publications
- list of participations in scientific forums
- list of noticed citations
- declaration of originality and authenticity of the attached documents
- other documents related to the course of the procedure

The doctoral student has attached 3 publications in connection with the dissertation.

The documents meet the requirements for the implementation of the dissertation defence procedure.

Biographical data of the doctoral student:

Snezhana Stoencheva, MD was born on 03.10.1980. In 1999 she graduated from Plovdiv Language High School, majoring in English, and in 2006 Medicine from MU - Plovdiv. From 2009 to 2013 specialized in Clinical Laboratory, and in 2013 acquired a specialty in Clinical Laboratory. From 2015 until present, she has been an assistant at MU - Plovdiv, Faculty of Pharmacy in the Department of Clinical Laboratory. Since June 2021, she is a PhD student on independent program at the Department of Clinical Laboratory, MU - Plovdiv in professional direction 7.1 Medicine.

2. Relevance of the topic

Malignant diseases occupy a large share in human pathology. According to the World Health Organization, they are the second cause of death in developed countries, giving way to cardiovascular diseases.

A common complication in patients with malignant diseases is thrombosis. Abnormalities in coagulation status are found in up to 50% of patients with malignancy and in up to 90% of those with metastases, with thrombosis being the second most common cause of death. Disturbances in the coagulation status are often the first sign of malignancy.

Coagulopathy and angiogenesis in the presence of carcinoma appear to be anatomically and functionally related. They predispose malignancies to an increased risk of thrombotic events or bleeding complications.

In Bulgaria, there are still no systematic studies focused on the clinical laboratory assessment of haemostasis abnormalities in oncological diseases.

The present study describes and monitors changes in laboratory indicators of activation of coagulation and fibrinolysis - fibrinogen (Fib), thrombin -antithrombin complex (TAT), tissue factor (TF), prothrombin fragment 1+2 (F1+2), Antithrombin III (AT III), D-dimer (D-dimer) and tissue plasminogen activator (t-PA) in patients with malignant diseases, and evaluates the diagnostic reliability of the investigated markers. This would assist clinicians in thrombogenic risk assessment, diagnosis, treatment and prevention of thrombotic complications.

The study of coagulation activation and fibrinolysis indicators together with routine haemostasis tests in clinically healthy individuals and in patients with malignant pathology would allow the discovery of a marker or a complex of markers that best reflects changes in the haemostasis system and the risk of thrombotic events.

All this defines the topic of the dissertation work as current, original and useful for the clinical practice. The results are important for the development of science and practical behaviour.

3. Knowing the problem

It is clear from the extensive literature review that the doctoral student knows the state of the problem very well and presents a thorough analysis.

4. Research methodology

The nature of the study is single-centre, prospective, controlled and longitudinal. The study included 185 people aged 18-70, divided into 3 groups: with breast carcinoma, with lung

carcinoma and with non-Hodgkin's lymphoma (NHL). 65 clinically healthy volunteers, 30 women and 35 men, were also studied. The inclusion and exclusion criteria in the study are well defined. The target group of patients was followed prospectively within three scheduled visits. In all subjects, the biological material was taken according to the standard requirements for patient preparation and venous blood collection for clinical laboratory studies. A survey method, clinical-diagnostic methods, instrumental methods and a large panel of clinical laboratory studies were used to collect the scientific information. The systematization, processing and analysis of primary data in the form of quantitative and qualitative variables was implemented with the statistical package of social science software IBM SPSS Statistics v. 26.

The chosen research methodology allows achieving the set goal and obtaining an adequate answer to the tasks solved in the dissertation work.

5. Characterization and evaluation of the dissertation work and contributions

The presented dissertation covers a total of 136 pages, illustrated with 24 tables and 71 figures, and is organized in the following sections: Title page, Contents (3 pages), Abbreviations used (1 page), Introduction (2 pages), Literature review (23 pages), Aim and tasks (1 page), Material and Methods (12 pages), Results (58 pages), Discussion and conclusions (7 pages), Contributions (1 page). The bibliography contains 252 literary sources, of which 3 are in Cyrillic and 249 are in Latin.

The structure and content are presented correctly and in detail. The most frequently used abbreviations are listed. The individual chapters and sub-chapters are properly designed, which gives clarity and clarity to the dissertation work.

The literature review is very well structured and includes up-to-date information on the topic. Epidemiological data on thrombosis are presented in detail. They summarize some particular aspects of the thrombotic process in patients with malignant diseases. The impact of anticoagulants on the tumour process and pulmonary thromboembolism as a major problem in patients with malignancies is reviewed. A detailed review of haemostasis indicators, which are the subject of the doctoral student's research, and the sought-after relationship with angiogenesis and carcinogenesis, has been made. The PhD student knows the state of the problem very well and presents a thorough analysis.

The purpose of the dissertation work, namely to monitor the changes in the laboratory indicators of coagulation and fibrinolysis - fibrinogen (Fib), thrombin-antithrombin complex (TAT), tissue factor (TF), prothrombin fragment 1+2 (F1+2), Antithrombin III (AT III), D-dimer (D-dimer) and tissue plasminogen activator (t-PA) in patients with malignant diseases on systemic and/or radiotherapy, to evaluate their diagnostic reliability in the specific diseases, and related tasks are clearly and precisely worded.

Results: The study included 185 people aged between 18-70 years, divided into 3 groups: with breast carcinoma (n=38), with lung carcinoma (n=42) and with non-Hodgkin's lymphoma (NHL) (n= 40). 65 clinically healthy volunteers, 30 women and 35 men, were also studied. The PhD student derived demographic and clinical data for each study group of patients and healthy controls. Results of clinical laboratory indicators of coagulation and fibrinolysis, namely fibrinogen, thrombin-antithrombin complex, tissue factor, prothrombin fragment 1+2, AT III,

D-dimer and t-PA, in the patients of the respective group compared with healthy controls. The indicators of coagulation and fibrinolysis in the applied treatment strategy were studied in dynamics. The changes of the studied indicators in the patients of the three groups by stages and visits were evaluated. The correlation dependence between the investigated parameters in the patients of the three groups and in healthy controls was sought. Last but not least, the diagnostic reliability of the indicators was evaluated. A comparative analysis of the results of the investigated coagulation and fibrinolysis indicators was made between patients with mammary gland carcinoma, lung carcinoma and non-Hodgkin's lymphoma.
The t-test, Mann-Whitney test, one-way ANO-VA, repeated-measures ANOVA, Shapiro-Wilk test, Spearman rank correlation coefficient, Kruskal-Wallis test, Friedman test, Wilcoxon test, ROC curves were used to process the obtained data. A significance level of <0.5 was adopted for all tests.
The doctoral student's own research is presented consistently, described comprehensively and illustrated with a sufficient number of tables and figures.
In the "Discussion" section, the dissertation student makes a thorough analysis of the obtained results. Evaluates in detail the comparative analysis of results for fibrinogen, thrombin-antithrombin complex, tissue factor, prothrombin fragment 1+2, AT III, D-dimer and t-PA in the three target groups of patients. It evaluates the correlation and statistical significance of the obtained results. In the course of the longitudinal follow-up of the indicators in the course of the therapy, it was also established that the time factor (the visit) also has an influence. Last but not least, the doctoral student also discusses the influence of the therapy on the investigated parameters of coagulation and fibrinolysis.

In the dissertation, **7 conclusions** are summarized, which are in accordance with the obtained results and are logically deduced from the set tasks.
The dissertation student has defined a total of **5 contributions**, two of them of a confirmatory nature and three – original for Bulgaria. All contributions are welcome.

6. Evaluation of the publications and personal contribution of the doctoral student
The list of scientific works related to the dissertation includes **3 articles** in scientific journals, one of which in a journal with an impact factor (Bratislava medical journal, IF2021=1.564). In all three publications, Stoencheva, MD is the first author. The dissertation student has **7 scientific announcements** in scientific forums, in 4 of which she is again the first author.
In the conducted dissertation research, the doctoral student personally participated in carrying out the studies, their summary and analysis. Stoencheva, MD was also a leading researcher in a scientific project of the MU - Plovdiv, "Clinical-laboratory evaluation of coagulation and fibrinolysis in patients with malignant diseases".
7. Abstract
The abstract has a total volume of 54 pages and, in terms of content and quality, fully presents the results of the scientific research in the dissertation.

CONCLUSION
Dr. Stoencheva's dissertation contains scientific-applied and applied results that represent an original contribution to science and meet all the requirements of the Law on the Development

of Academic Staff in the Republic of Bulgaria (LDASRB), the Regulations for the Implementation of LDASRB and the Rules of the Ministry of Education - Plovdiv. The presented materials and dissertation results fully correspond to the specific requirements adopted in connection with the Regulations of the Ministry of Education - Plovdiv for the application of the LDASRB.

The dissertation shows that the doctoral student, Dr. Snezhana Stoyanova Stoencheva, possesses in-depth theoretical knowledge and professional skills in the scientific specialty Clinical Laboratory, demonstrating qualities and skills for independent conduct of scientific research.

Due to the mentioned above, I confidently give my **positive assessment** of the conducted research, presented by the above-reviewed dissertation work, abstract, achieved results and contributions, and I propose to the honourable scientific jury to award the educational and scientific degree "Doctor" to Dr. Snezhana Stoyanova Stoencheva in the doctoral program in Clinical Laboratory.

28.04.2023

Prepared the opinion: .....

(Assoc. Prof. Irena Gencheva, MD, PhD)