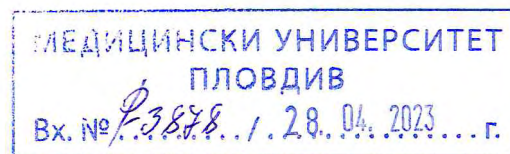


To the Chairman
of the Scientific Jury



REVIEW

by Prof. Adelaida Lazarova Ruseva, MD

Head of the Department "Clinical Laboratory, Clinical Immunology and Allergology",
MU-Pleven,

Head of Clinical Laboratory, UMBAL "Dr. G. Stranski", Pleven

External member of a scientific jury for the defense of a dissertation work for the acquisition of the degree "Doctor" in the scientific specialty "Clinical Laboratory" on the basis of order No. R-819 of 04/03/2023 of the Rector of the Medical University, Plovdiv.

Topic: "Clinical-laboratory assessment of coagulation and fibrinolysis in patients with malignant diseases".

Author: Dr. Snezhana Stoyanova Stoencheva, doctoral student of independent training in the field of higher education 7. Health care and sports, by professional direction 7.1. Medicine, in the "Clinical Laboratory" department, Faculty of Pharmacy, MU Plovdiv, Scientific supervisors: Assoc. Prof. Tanya Deneva, MD. and Prof. Jeanette Grudeva-Popova, M.D.

Brief introduction of the candidate:

Dr. Snezhana Stoencheva graduated in 1999 from Plovdiv Language High School with a major in English. In the period 2000-2006, she received her higher education at Plovdiv Medical University. She began her work experience in 2007 at MDL "Astralab" OOD - Gorna Oryahovitsa. Since March 2012 and currently works as a doctor in the Central Clinical Laboratory of UMBAL "St. Georgi", the city of Plovdiv.

Dr. Stoencheva has been a clinical laboratory specialist since 2013. Since the beginning of 2015, she has been appointed as an assistant in the Department of Clinical Laboratory, Faculty of Pharmacy of MU Plovdiv. She conducts clinical laboratory exercises for students majoring in "Medicine", 3rd year - in Bulgarian and English; practical exercises in clinical chemistry for students of the "Pharmacy" specialty, III year, as well as lectures and exercises in clinical laboratory for specialized doctors. Dr. Stoencheva is responsible for the educational activities of the Department of Clinical Laboratory. She has participated in 4 intra-university projects, in 10 articles, in 1 teaching aid and has 13 citations.

Relevance of the topic of the dissertation:

According to the WHO, malignant diseases are the second leading cause of death in developed countries, second only to cardiovascular diseases. Abnormalities in coagulation status are found in up to 50% of all patients with neoplastic diseases and in up to 90% of those with metastases. Thrombosis is believed to be the second most common cause of death in these patients.

As early as 1865, the French physician Armand Trousseau reported the occurrence of "mysterious" thrombotic disorders in cancer patients. Later, his name was used to denote the manifestation of thrombophlebitis in patients with malignant neoplasia - Trousseau syndrome. This marks the beginning of the establishment of an important link between malignant diseases and hypercoagulable conditions.

Today it is already known that the hemostatic system can play an important role in the pathogenesis of cancer. A large body of evidence indicates that cellular and circulating hemostatic factors play an active role in major aspects of tumor biology, primarily angiogenesis. In this regard, a number of experimental strategies have been developed, including pharmacological modulation of platelet function and of various components of the coagulation cascade, as well as the use of genetically modified animals showing altered expression or activity of these components.

The enormous progress achieved in the study of the relationship between hemostasis and malignant diseases in itself proves the relevance of this problem, which is the basis of Dr. Stoencheva's dissertation work.

Structure and content of the dissertation:

The dissertation is presented on 136 pages with an optimal ratio between the individual parts: introduction, literature review, aim and tasks, material and methods, results, discussion, conclusions and contributions. Contains 24 tables and 71 figures. The bibliography includes 252 titles, of which 3 are in Cyrillic.

The **literature review** is detailed and comprehensive, structured well and presented in a volume of 25 pages. Logically, it begins with epidemiological data regarding malignancies, emphasizing the high incidence of malignancy particularly in patients with venous thromboembolism (VTE). The possibility that chemotherapy is a risk factor for the development of VTE is reflected. Ambiguous scientific results regarding the benefit of antithrombotic prophylaxis are also presented. The different models of coagulation are presented in chronological order: from the protein-centered cascade model to the cell-based model. Due to the fact that the hemostatic system is anatomically and functionally an integral part of the vascular system, coagulation and angiogenesis appear to be inextricably linked to thrombotic events in the presence of carcinoma. The importance of various protein molecules as angiogenic activators is also presented. Of the substances that tumor cells express, a separate place is assigned to the Tissue factor, as a factor with a marked procoagulant action. The role of Thrombin and Fibrinogen/Fibrin influencing angiogenesis is also commented. In patients with carcinoma, reduced activity of natural inhibitors of blood coagulation may increase procoagulant activity. The current understanding of the relationship between the fibrinolytic system and tumor diseases is also presented. There is space for the main markers of activation of coagulation and/or fibrinolysis: D-Dimer, Prothrombin fragment 1+2 and the Thrombin/Antithrombin complex.

The purpose of the dissertation work is clearly defined - to track the changes in basic laboratory indicators of coagulation and fibrinolysis in patients with malignant diseases of systemic and/or radiotherapy, to evaluate their diagnostic reliability in the specific diseases. The set goal is achievable through excellently formulated 5 tasks related to: determining the levels and dynamics in the values of Fibrinogen, D-Dimer,

F1+2, TAT, AT III, TF and t-PA in individual groups of patients and conducting a comparative analysis; evaluation of the correlation dependence between the studied indicators and changes in hemostasis; determining the diagnostic reliability of the evaluated indicators.

In the "**Materials and methods**" section, data on: the design of the scientific study, criteria for inclusion and exclusion of the researched persons; requirements for the biological material, as well as a description of the research methods and statistical processing of the results are presented with extreme precision.

In connection with the realization of the set goal, the doctoral student presents the **results** of her work in 3 identical sections, referring to solving the set tasks in the three groups of patients with: mammary gland carcinoma, lung carcinoma and non-Hodgkin's lymphoma. Data from the descriptive characteristics of coagulation and fibrinolysis indicators in controls and patients from the three monitored groups during Visits 1-3, comparative characteristics, dynamics and changes by stages and visits of laboratory indicators in patients and healthy controls are presented. Correlation dependence between individual parameters in patients and healthy controls was investigated. Based on ROC curves, sensitivity and specificity of the studied indicators were calculated with a view to determining their diagnostic reliability.

The results of the dissertation work are **discussed** in detail on the basis of existing data indicated in the literature review. As a result of the research and analysis, 7 important **conclusions** of great practical value were made. The use of standard screening tests (aPTT, PT, TT) are not informative enough to detect changes in the hemostasis system. Because of the proven link between carcinogenesis and blood coagulation disorders, higher levels of TF, TAT, F1+2, D-Dimer, fibrinogen and lower AT III activity are found in patients with malignant diseases compared to healthy controls. The results of measuring t-PA are interesting. In all three patient groups, the values of this indicator were higher compared to the healthy controls. Indeed, the concentration and expression of fibrinolytic markers may differ between different tissues and different cancer types. The presence of divergent results may also be due to different sizes of the studied populations, different cut off values, as well as

differences in the used reagents and analysis systems. Therefore, the publication of the results and the corresponding conclusions of this work are of great importance in helping clinicians to correctly assess the utility of measuring these indicators as markers of disorders in hemostasis processes before and during systemic therapy. The application of the systemic therapy significantly changes the dynamics of the investigated indicators. The longitudinal follow-up proved that the cytotoxic effect of the used drugs had a suppressive effect on the values of TF, TAT, PF1+2, D-Dimer and t-PA. Despite the decrease in their levels during the course of therapy, these indicators remained higher compared to the control group, due to which the prothrombotic state persisted. The results of Dr. Stoencheva's dissertation show a missing correlation between the coagulation and fibrinolysis indicators in healthy individuals, but the presence of statistically significant dependencies in the patient groups. The PhD student assumes that this complex alteration of interactions between hemostatic and fibrinolytic factors is induced by the tumor process itself. Based on the ROC analysis, Dr. Stoencheva found that the indicators TAT, D-Dimer and F 1+2 have the necessary diagnostic reliability to be used as indicators for distinguishing patients with an increased risk of thrombotic complications.

Based on the conclusions of the dissertation, the doctoral student formulated 5 **contributions** mainly of a practical and applied nature. They are meaningfully and factually conditioned by the obtained results and their analysis. For the first time in Bulgaria, the following coagulation activation markers TAT and PF 1+2 were investigated and evaluated in patients with malignant diseases in order to assess their diagnostic reliability; t-PA as a marker of fibrinolysis activation in patients with malignant diseases; the change in markers of activation of coagulation and fibrinolysis in the course of therapy in patients with malignant diseases. The relationship between malignant diseases and blood coagulation disorders has been confirmed, as well as the significance of the study of hemostasis indicators as part of the multimarker approach to assess the risk of thrombotic complications.

The presented **abstract** is written on 54 pages. It contains 48 figures and 16 tables, was prepared according to generally accepted rules and reflects the results presented in the dissertation work.

In connection with the dissertation work, the doctoral student presents 3 publications in scientific journals: "BJSTR", 2019; "Bratislava medical journal", 2023 and "Knowledge - International Journal", 2023. She is the first author in all three publications. 7 participations in scientific forums are also indicated.

In conclusion:

I firmly believe that with this work, Dr. Snezhana Stoencheva proves that she possesses the necessary qualities to acquire the educational and scientific degree "Doctor". This gives me the reason to give a **POSITIVE REVIEW** for the dissertation work on the topic: "Clinical-laboratory assessment of coagulation and fibrinolysis in patients with malignant diseases" for awarding the educational and scientific degree "Doctor" in the scientific specialty "Clinical Laboratory" to Dr. Snezhana Stoencheva.

April, 28, 2023

Prof. A. Ruseva, PhD

Заличено на основание
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