

REVIEW

**by Prof. R. Rasho Kolev Rashkov, MD, PhD, DMSc
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Based on ordinance No. 1843/22.10.2020 of the Rector of Medical University – Plovdiv
I have been selected to be the member of a scientific panel for the dissertation thesis defense
procedure

for the acquisition of the scientific degree of “Doctor of science”
in higher education field 7 Healthcare and sport,
professional field 7.1. Medicine,
Scientific specialty of Rheumatology

Dissertation thesis author: Rositsa V. Karalilova, MD, PhD
Chief Assistant at the Department of Propaedeutics of Internal Diseases,
Medical University – Plovdiv
Rheumatology Clinic – University Hospital Kaspela, Plovdiv

Subject of the dissertation thesis:

**IMAGING AND SERUM BIOMARKERS FOR PULMONARY AND JOINT
INVOLVEMENT IN PATIENTS WITH SYSTEMIC SCLEROSIS**

1. Overall presentation of the process

The set of materials presented to me in print and electronic format are compliant with Art. 123 (1) of the Regulation on acquiring the scientific degree of “Doctor of science” at the Medical University – Plovdiv, and includes all required documents:

- Application to the Rector of the Medical University – Plovdiv to formally allow the dissertation thesis
- Report by an extended Department Board for preliminary discussion of the dissertation thesis detailing the resolution to begin the procedure and the appointment of a scientific panel
- European format CV
- Notarized copy of a diploma for an academic science doctorate degree
- Dissertation and Author’s abstract
- List of publications and research papers on the subject of the dissertation thesis
- Copies of scientific publications
- List of noted citations with copies of a selection of these
- Statement of authorship and authenticity of the applied documents
- Other documents, related to the procedure

Fifteen publications are presented on the topic of the dissertation thesis.

2. Brief biography and professional qualification

Rositsa V. Karalilova, MD, PhD is a Chief Assistant at the Department of Propedeutics of Internal Diseases, Medical University, Plovdiv. She was born on 10.10.1979. She graduated with a degree of Medicine in Plovdiv in 2005. She has worked as a resident doctor at the Rheumatology Clinic – University Hospital Kaspela, Plovdiv since 2005. In 2013 she became an Assistant at the

same Department and Chief Assistant in 2018, and continues to hold that position presently. She acquired her specialty in Rheumatology in 2014. In 2018, she defended a thesis on the subject of "Diagnostic capabilities of new ultrasonographic techniques for assessment of skin, joint, periarticular and lung involvement of systemic sclerosis" and acquired the academic research degree of PhD.

Karalilova, MD, PhD has published 15 full-length papers related to the dissertation as required by the rules of Medical University – Plovdiv, of which 6 are published in international journals, 3 of which with impact factor, being lead or first author of 5 of these.

Karalilova, MD teaches practical classes in Propedeutics of Internal Disease and Rheumatology to Bulgarian and international students. She takes part in specialized courses in rheumatology, musculoskeletal ultrasonography for post-graduate students and specialists. She participates in national and international qualification courses.

3. Relevance of the subject

The dissertation thesis, devoted to imaging and serum biomarkers for lung and musculoskeletal system involvement in systemic sclerosis, is the result of over 7 years of research by the author in the field of systemic sclerosis. Considering the fact that systemic sclerosis is the disease with the greatest mortality risk out of all rheumatic disease, as well as that with the least therapeutic advances made, there is undoubtedly a pressing need for early and timely assessment of internal organ involvement. The subject of biomarkers is relevant to modern science as part of the personalized approach to patients in every field of medical achievements with the correlation between ultrasound studies and serum biomarkers in systemic sclerosis being developed for the first time not only in Bulgaria but also globally. The inclusion of micro-RNA testing into the project is a particularly original idea and has significant contribution to Bulgarian and European science.

4. Problem knowledge

The presented information is complete and shows insight into the problem. An analytical review is performed with regard to modern knowledge of molecular and cellular mechanisms underlying the pathogenic pathways in this disease and a summary is provided on international progress and unsolved issues. These data show excellent awareness by Karalilova, PhD of the subject of study.

5. Study objective and methods

The objective of the dissertation thesis is to uncover some of the cellular and molecular mechanisms for joint and pulmonary involvement in SSc and to establish a panel of biomarkers with possible organ association. The capabilities of a set of 8 serum biomarkers are examined – the expression levels of 5 micro-RNAs and 3 high-tech imaging biomarkers, for early identification of changes in systemic sclerosis and their predictive ability is assessed. The potential regulation of a portion of the investigated serum biomarkers is also considered.

The 5 tasks that are set enable the achievement of the objective. The most modern diagnostic methods in the field are employed – quantitative polymerase chain reaction, expression levels of 5 micro-RNAs, ultrasonography (imaging biomarker) and relationships between these biomarkers.

The methods utilized, the testing protocol, follow-up handling and statistical analyses of the resulting data are suitable and contribute to the achievement of the set objective and tasks.

6. Characteristics and assessment of the dissertation thesis

The dissertation thesis is written out on 200 standard typing pages and structured in the accepted manner in chapters. It includes 34 tables, 34 figures and 15 high-quality attachments.

The introduction (2 pages) is clearly formulated, specifically reflecting the unresolved problems in the field of systemic sclerosis and the need to research the examined biomarkers.

The literature overview (68 pages) is detailed, complete and in-depth knowledge and insight of Karalilova, MD on the essence of the examined topic. It is modern, up-to-date and fulfills the set objective and tasks of the dissertation thesis. The conclusions of the literature review summarize quite well the unresolved problems related to this disease and logically point towards the need for scientific effort in this regard. The actual value of the dissertation thesis lies also in the combined approach towards early diagnosis through a combination of objective serum and imaging biomarkers for lung and joint involvement in patients with systemic sclerosis; as well as the predictive ability of these biomarkers for potential internal organ involvement, which could lead to timely therapeutic interventions.

The objective of the dissertation thesis is clearly defined and specific, and corresponds to the set tasks.

The chapter "Materials and Methods" (11 pages) details the demographics and clinical characteristics of the examined persons. A total of 95 participants are included in the study, of whom 31 patients with dcSSc, 18 with lcSSc, 10 with VEDOSS, 13 with OVERLAP syndrome and 23 healthy volunteers. The methods used are complex, modern, reliable, validated, enable a comprehensive assessment and provide the opportunity to acquire objective results. Clinical and paraclinical methods are applied, such as determining serum concentrations of the investigated biomarkers (IL-6, YKL-40, TGF- β 1, ICAM-1, CXCL-4, IL-12p40, TNF α , IL-17A), correlation analyses are performed between the levels of these biomarkers and imaging tests – ultrasonographic assessment of skin, joints and tendons, lungs, X-ray imaging of the lungs, echocardiography, capillaroscopy. In a separate survey of 41 patients with systemic sclerosis and 13 healthy volunteers, expression levels of inflammation-related genes and expression levels of several micro-RNAs are determined. The statistical methods used to process the data are modern and enable insightful interpretation of the obtained results.

The results (37 pages) are presented in two aspects. A comparative assessment of serum cytokine concentrations in patients and controls is performed. Correlation analyses are performed on the serum levels of the specified biomarkers and the ultrasonographic data about joint, periarticular structures, pulmonary involvement and digital ulceration. The prognostic role of IL-6, YKL-40, TGF- β 1, ICAM-1, CXCL-4, IL-12p40, TNF α and IL-17A is assessed as biomarkers when determining joint and pulmonary involvement in systemic sclerosis patients. In a separate substudy of 41 patients and 13 healthy controls, the gene expression of inflammation response genes is determined for YKL-40, the expression levels of micro-RNAs (miR-24, miR-30c, miR-125a, miR-153, miR-214) are examined with regard to their assumed participation in the regulation of the expression of individual genes in SSc. The presented results are illustrated in detail, having direct connection to the tasks set at the beginning of the dissertation thesis.

The obtained results are original and valuable from a scientific and practical point-of-view.

The analysis of the results is thorough and detailed. The obtained significant correlations between some of the examined serum and imaging biomarkers, and the lack thereof between others, the established predictive role of some of these biomarkers for internal organ involvement all demonstrate the significance of the dissertation as it is the first time this is done in Bulgaria. The analysis of the expression levels of miRNAs (miR-24, miR-30c, miR-125a, miR-153, miR-214) contributes greatly to the quality of the scientific study.

In the chapter "Discussion" (21 pages) an analytical, critical and objective comparison and contrast is performed between the obtained results from the comparative analysis among patients and healthy controls against those in global literature; the possible reasons for the differences and concurrences are discussed in detail. The limitations of the dissertation are critically described and the potential future directions for development of the examined problem are outlined.

The conclusions are properly supported, clearly formulated and correspond to the set objective and the performed tasks, while reflecting the analysis of the results. The relevance of the obtained practical data is summarized.

The literature references include 917 titles – 7 in Cyrillic and 910 in Latin script. This once again emphasizes the importance and relevance of the considered problem at a global level and the need for its development in Bulgaria.

7. Contributions and significance of the study for science and practice

The DMSc candidate has specified 5 original contributions, 2 practical scientific ones and 2 confirmatory contributions. The contributions are original for Bulgarian rheumatology and some of them also internationally.

Currently, 4 of the publications related to the dissertation have been cited 17 times in global literature.

On the other hand, for some of the methods used in the dissertation, Karalilova, MD has facilitated their routine implementation in practice with diagnostic, prognostic and follow-up applications related to patients with systemic sclerosis.

8. Evaluation of the publications on the dissertation thesis

Karalilova, MD, PhD has presented 15 publications related to the dissertation subject, of which 6 in international journals, 3 of which with Impact factor, being lead or first author in 5 of the papers. The above is evidence that the included publications fully meet the requirements for recognition of the scientific degree “Doctor of science”, as set out in the Rules of Medical University – Plovdiv.

9. Author’s abstract

The Author’s abstract is written out on 76 pages and presents in short the key points of the dissertation thesis. It is structured according to the requirements and enables the clear and sufficiently complete examination of the subject matter.

10. Critical notes and recommendations

The literature reference in Cyrillic is relatively short compared to the extensive one in Latin script. Some of the tables and figures essentially provide the same information.

11. Conclusion

The dissertation thesis “Imaging and Serum Biomarkers for Pulmonary and Joint Involvement in Patients with Systemic Sclerosis” by Rositsa Valerieva Karalilova, MD, PhD, Chief Assistant at the Department of Propaedeutics of Internal Diseases, Medical University – Plovdiv, Rheumatology Clinic – University Hospital Kaspela, Plovdiv is the first in Bulgaria **complex** study of patients with systemic sclerosis using modern serum and imaging biomarkers with regard to the assessment of their diagnostic and prognostic role, analysis of expression levels of micro-RNAs with potential involvement in the regulation of certain proteins. The thesis is relevant, original, in-depth and has clearly formulated objective, tasks and results based on self-sourced clinical materials. It demonstrates the serious contribution of the thesis and complies with scientific interest and the need for research in this field of medicine. The national requirements for the defense of a dissertation thesis for “Doctor of science” have been met in accordance with the Regulations on the conditions and rules for acquiring scientific degrees and holding academic positions at Medical University – Plovdiv with 50 points from indicator group A; 100 points from indicator group B out of 100 required; 157,17 points from indicator group D out of 100 required, 430 points from indicator group E out of 100 required.

The dissertation thesis shows that Rositsa Karalilova, MD, PhD has in-depth theoretical knowledge and professional skills in rheumatology.

In view of the above, I am confident in giving my **positive assessment** and suggest to the respectable scientific panel to vote positively when awarding the scientific degree of “Doctor of

science” to Rositsa V. Karalilova, MD, PhD in the higher education field 7. Healthcare and sport, professional field 7.1. Medicine, Scientific specialty of Rheumatology.

02.11.2020
Sofia

Prof. R. Rasho Kolev Rashkov, MD, PhD, DMSc

