STANDPOINT

On behalf of Assoc. Prof. Dr. Elena Svetlozarova Dimitrova, MD, PhD

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Regarding the dissertation

BIOCHEMICAL AND ULTRASOUND MARKERS OF EARLY CARDIOVASCULAR DAMAGE IN PATIENTS WITH SPONDYLOARTHRITIS

By Dr. Miroslav Penev Markov for the attainment of the educational and scientific degree "Doctor" in the specialty of "Internal medicine"

The standpoint is prepared in accordance with the Law on Academic Staff Development, the Regulation on the Implementation of the Development of Academic Staff Act, and the Regulation for the Acquisition of Scientific Degrees and Academic Positions at Medical University - Varna. The scientific jury for the public defense of the dissertation is appointed by an order from the Rector of Medical University - Varna №P-109-426/06.10.2025.

The set of materials and documents submitted by Dr. Miroslav Penev Markov, in both paper and electronic form, complies with the requirements of the Law on Academic Staff Development in the Republic of Bulgaria and its implementing regulations, as well as with the rules of the Medical University of Varna for the procedure for obtaining the educational and scientific degree "Doctor." The dissertation consists of 144 standard pages, divided into 13 chapters as follows: Introduction; Literature Review; Aim and Objectives; Materials and Methods; Results; Discussion; Conclusions; Contributions; Limitations; Summary; List of Publications related to the dissertation; Acknowledgements; Bibliography. The dissertation includes 33 figures and 30 tables. The bibliography contains 363 references, a substantial part of which are from the past ten years, and it also includes publications by Bulgarian authors.

Although the **topic** of the dissertation is not entirely new, it remains relevant due to the need to gather further data regarding atherogenesis in patients with chronic inflammatory diseases, particularly the validation of noninvasive methods for assessing arterial stiffness and serum biomarkers predicting subclinical atherosclerosis.

The **literature review** is comprehensive and well-structured, presenting the epidemiology and pathogenesis of various spondyloarthritides, as well as the relationship

between chronic inflammation and the atherosclerotic process. The role of adhesion molecules, as well as the significance and methods for assessing arterial stiffness, is thoroughly discussed.

The **aim** of the dissertation is clearly defined—to evaluate the role of vascular ultrasound parameters and adhesion molecules as markers of early atherosclerosis in patients with ankylosing spondylitis and psoriatic arthritis. The study seeks to determine whether the inflammatory process underlies the development of vascular changes and whether there are specific differences between the two nosological entities and healthy controls. It also analyzes the interaction between inflammatory activity, lipid metabolism, and arterial stiffness in the context of cardiovascular risk.

To achieve this aim, the study defines the following specific **objectives**:

1. To compare vascular ultrasound parameters (CIMT, β-stiffness, PWV, AI, ACo, EP) among patients with ankylosing spondylitis, psoriatic arthritis, and healthy controls to assess arterial stiffness.
2. To analyze and compare the levels of adhesion molecules VCAM-1 and ICAM-1 among the three study groups regarding their role as markers of endothelial dysfunction.
3. To evaluate the relationship between disease activity (ASDAS-CRP, BASDAI, DAS28), inflammatory markers (CRP, ESR), and vascular parameters in each group and to identify potential differences among them.
4. To compare the lipid profile, atherogenic indices, and cardiovascular risk scores among the groups and to explore their relationships with vascular changes and adhesion molecules.
5. To assess the influence of demographic and clinical factors (sex, age, arterial hypertension, smoking, therapeutic status) on vascular, laboratory, and clinical parameters in patients with spondyloarthritis.

The **study** was conducted at University Hospital "St. Marina" – Varna and the Medical University of Varna, in the Departments of Internal Medicine, Rheumatology, Clinical Immunology, and Clinical Laboratory, involving a total of 154 participants. They were divided into three groups—patients with ankylosing spondylitis (AS), psoriatic arthritis (PsA), and a control group of healthy volunteers without evidence of inflammatory joint disease or established cardiovascular pathology. All participants were selected based on preliminary

screening performed at the Departments of Internal Medicine and Rheumatology of "St. Marina" University Hospital during 2023–2024. Patients were followed up either on an outpatient basis or during hospitalization under clinical procedure No. 42, with diagnosis and treatment consistent with established EULAR standards and recommendations.

All participants signed informed consent prior to inclusion. Ethical approval was obtained from the Ethics Committee of the Medical University of Varna, in accordance with regulatory and ethical standards for conducting research involving human participants. Data were collected through direct clinical examination, structured interviews, and review of available medical documentation. For patients with AS and PsA, information was gathered from outpatient records, hospital discharge summaries, laboratory results, and imaging studies. For the control group, data were obtained through anamnesis, physical examination, and standardized testing. For each participant, an individual registration form was completed, including demographic data, clinical diagnosis (for patients), disease duration, medication, smoking status, blood pressure values, laboratory and ultrasound parameters, and disease activity indices. Instrumental studies included ultrasound assessment of carotid intima-media thickness (CIMT)—a recognized morphological marker of early subclinical atherosclerosis—and measures of arterial stiffness: β-stiffness index, pulse wave velocity β (PWVβ), arterial compliance (ACo), augmentation index (AIx), and pressure-strain elastic modulus (Ep). Laboratory tests included lipoproteins (total cholesterol, HDL-C, LDL-C, TG), C-reactive protein, ESR, and adhesion molecules (sVCAM-1, sICAM-1). Atherogenic and hemodynamic indices, as well as cardiovascular risk scores, were also calculated. Inflammatory activity was assessed using validated composite indices specific to each disease. Patients with known atherosclerotic cardiovascular disease, heart failure, arrhythmias, diabetes mellitus, chronic kidney disease, or other conditions affecting the studied parameters were excluded. Appropriate statistical methods were applied for data processing and analysis.

The **results**, presented over 55 well-structured pages, are clearly illustrated. The groups are well balanced in most demographic characteristics, with differences in sex and age reflecting disease-specific patterns. Special attention is given to treatment regimens, disease activity, and atherogenic indices, which indicate higher atherogenicity among patients with inflammatory diseases. Cardiovascular risk was assessed using the Framingham score system and the newer SCORE2/SCORE2-OP systems, with the latter appearing to better reflect actual

risk despite a lack of statistically significant differences among groups. Differences in ultrasound parameters indicate more pronounced vascular alterations in inflammatory conditions. Data on adhesion molecules are also of interest, showing no substantial differences among groups. In AS patients, the study once again demonstrates the link between high disease activity, systemic inflammation, and early changes in vascular stiffness and endothelial dysfunction. Particularly valuable for future research are the results concerning the effects of various biologic therapies on vascular and inflammatory parameters and the potentially distinct inflammatory and vascular profiles across treatment subgroups. Correlation analyses between ultrasound measures and traditional cardiovascular risk factors are presented as well. The study confirms the role of hypertriglyceridemia in vascular damage and highlights carotid IMT as a sensitive marker of lipid-mediated subclinical atherosclerosis. At the same time, some traditional atherogenic indices appear to have limited predictive value for arterial stiffness in inflammatory arthropathy populations.

The **discussion** provides a logical and in-depth analysis of the results, including comparisons with existing literature. Findings are summarized in six main conclusions. The **conclusions** fully correspond to the obtained results.

The **contributions** of the dissertation are grouped into three categories: theoretical, practical, and confirmatory. The findings confirm, expand, and enrich current knowledge regarding spondyloarthritis, the pathogenesis of chronic inflammation, and its role in the development of atherosclerosis. For the first time in Bulgaria, both structural (CIMT) and functional (β-stiffness, PWV, AI, ACo, EP) parameters of arterial stiffness have been simultaneously assessed in patients with ankylosing spondylitis and psoriatic arthritis using echotracking methodology, combined with the evaluation of endothelial activation markers ICAM-1 and VCAM-1. Furthermore, this is the first comparative assessment in Bulgaria of the effects of different targeted therapies (anti-TNF, anti-IL-17, JAK inhibitors) on endothelial function and arterial elasticity in patients with spondyloarthropathies. The limitations of the current study are explicitly stated.

The main results of the dissertation have been published in two scientific papers and presented at two scientific forums.

In conclusion, the dissertation presented by Dr. Miroslav Penev Markov contains results that fully meet the requirements for awarding the educational and scientific degree

"Doctor." The dissertation demonstrates that the PhD candidate possesses deep theoretical knowledge in the specialty and exhibits the skills and competence required to conduct independent scientific research. **On these grounds, I propose that the esteemed members of the Scientific Jury vote in favor of awarding Dr. Miroslav Penev Markov the educational and scientific degree "Doctor" in the specialty of "Internal medicine".**

27/10/2025

Assoc. Prof. Elena Dimitrova, MD, PhD

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