REVIEW

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Regarding

a dissertation submitted for the award of the educational and scientific degree **"Doctor (PhD)"**
Professional field 7.1. Medicine
Doctoral program "Immunology"

Doctoral candidate: Dr. Martina Radoslavova Bozhkova
Form of doctoral study: full-time
Affiliated department: Department of Medical Microbiology and Immunology "Prof. Dr. Elisey Yanev", Faculty of Medicine, Medical University of Plovdiv

Dissertation title:
"Study of B-cell immune memory in COVID-19 and post-vaccination immunity"

Scientific supervisor: Assoc. Prof. Velizar Shivarov, MD, PhD

I. Introduction

This review has been prepared in my capacity as Chair of the Scientific Jury in connection with the public defense of the dissertation of Dr. Martina Radoslavova Bozhkova for the award of the educational and scientific degree "Doctor (PhD)".

The submitted set of materials – dissertation manuscript, abstract, publications, and accompanying documentation – fully complies with the requirements of the Act on the Development of the Academic Staff in the Republic of Bulgaria, its Implementing Regulations, and the internal regulatory documents of the Medical University of Plovdiv.

The dissertation was entirely developed by the doctoral candidate at the Department of Medical Microbiology and Immunology "Prof. Dr. Elisey Yanev" at the Faculty of Medicine, Medical University of Plovdiv, under the scientific supervision of Assoc. Prof. Velizar Shivarov, MD, PhD, and represents an independent, comprehensive, and scientifically sound research work. The statistical analysis was carried out at the Department of Social Medicine and Public Health, with the active participation of the doctoral candidate. The study was funded by one internal university project (COV-03/2021) and one national project (KP-06-N53/1), which emphasizes its significance at both institutional and national levels.

II. Brief profile of the doctoral candidate

Dr. Martina Bozhkova graduated in Medicine in 2019 from the Medical University of Plovdiv. In 2020, she began her specialization in Clinical Immunology at the Laboratory of Clinical Immunology at University Hospital "St. George" JSC, Plovdiv, as well as working as an Assistant at the Department of Medical Microbiology and Immunology "Prof. Dr. Elisey Yanev". After successfully passing an examination in 2022, she was enrolled as a full-time doctoral student at the Department with the topic: "Study of B-cell immune memory in COVID-19 and post-vaccination immunity".

Within the framework of her doctoral thesis, Dr. Bozhkova successfully developed an internal university research project (COV-03/2021).

With the aim of enhancing her professional competence related to the doctoral program's subject, she completed interactive courses and training in flow cytometry in June 2024 (Salamanca, Spain), April 2025 (Rostock, Germany), and June 2025 (Ghent, Belgium).

Dr. Bozhkova's scientific output includes publications in international journals indexed and abstracted in major global databases, including articles

with an impact factor. The results of the dissertation research have been presented at national and international scientific forums.

III. Relevance and Significance of the Scientific Problem

The topic of the dissertation is highly relevant and fully corresponds to contemporary scientific and clinical priorities in immunology and public health. The COVID-19 pandemic brought to the forefront questions related to the duration and quality of post-infectious and post-vaccination immunity, as well as the mechanisms underlying the formation and maintenance of immune memory.

In this context, the study of B-cell immune memory occupies a key position, as it represents the basis of long-term humoral immunity and effective protection against re-exposure to the virus. The comparative analysis of the immune response following natural infection and immunization with mRNA vaccines is of substantial importance both for fundamental immunology and for practical vaccine prophylaxis and the development of long-term immunization strategies.

The development of the dissertation during a period of intensive accumulation of international data, but with relatively limited national studies, confers additional value and originality to the research, particularly in the context of the Bulgarian population.

IV. Analysis of the Dissertation

The dissertation comprises a total of 160 pages and is structured into 12 logically interconnected chapters, prepared in accordance with established academic requirements for a doctoral dissertation. It includes an introduction, a comprehensive and analytical review of the current state of the scientific problem, clearly formulated aim and objectives, detailed materials and methods, results, discussion, conclusion, conclusions, and scientific contributions. The work is illustrated with 29 figures and 7 tables, which facilitate the comprehension and interpretation of the obtained results and are appropriately integrated into the text.

The bibliographic reference list includes 361 literature sources, predominantly publications from recent years reflecting the current state of

scientific research in the field. A significant proportion of the cited sources have been published within the last five years, demonstrating a targeted selection of contemporary scientific literature and good awareness of the dynamically evolving topic of immune responses in SARS-CoV-2 infection and following vaccination. The analysis and inclusion of publications by Bulgarian authors on the subject also make a positive impression.

The literature review is in-depth, well structured, and analytical. It addresses key concepts related to the development, differentiation, and functional heterogeneity of B lymphocytes, the mechanisms of the humoral immune response, the processes of formation and maintenance of B-cell immune memory, as well as contemporary data on the immunological characteristics of SARS-CoV-2 infection and post-vaccination immunity. Results from fundamental, clinical, and translational studies are presented, with the doctoral candidate demonstrating the ability for scientific synthesis and accurate interpretation of diverse and sometimes contradictory literature data. The review section logically substantiates the formulation of the aim and objectives of the dissertation.

The methodological section represents one of the pronounced strengths of the dissertation. A comprehensive and modern approach was applied to assess humoral and cellular immune responses, including serological methods (ELFA for determination of anti-RBD IgG), functional viral neutralization assays, B-ELISpot and T-ELISpot analyses, as well as multiparametric flow cytometry. Particularly high appreciation is warranted for the application of S1-specific tetramers for the direct detection and quantitative assessment of antigen-specific memory B cells, which requires a high level of methodological expertise and precision in experimental work. The methods are described clearly and in sufficient detail, ensuring reproducibility of the results.

The results are presented in a consistent and logical manner, supported by appropriate graphical and tabular materials. Data analysis is accurate and well substantiated, and the interpretation is compared with contemporary international scientific publications. The discussion demonstrates the ability for critical analysis and scientific synthesis, placing the obtained results within a broad immunological and clinical context. This confers completeness to the dissertation and clearly outlines its contribution to the study of B-cell immune memory in COVID-19 and post-vaccination immunity.

V. Scientific Contributions

The presented dissertation contains clearly defined scientific contributions of an original nature, which contribute to the expansion of existing knowledge in the field of clinical and experimental immunology. The conducted research is distinguished by a comprehensive and systematic approach to the assessment of immune memory following SARS-CoV-2 infection and mRNA vaccination. For the first time within the Bulgarian population, an in-depth multiparametric characterization of the B-cell immune response over time has been performed.

A major contribution of the dissertation is the integrated investigation of the humoral and B-cell components of the immune response through the combination of serological, functional, and cellular methods. This approach allows for a more precise interpretation of the relationship between serological markers and the actual capacity for immune protection.

A particularly significant scientific contribution is the implementation and application of multiparametric flow cytometry for detailed phenotypic characterization of B-cell subpopulations. In the dissertation, a flow cytometric panel was developed and optimized, enabling the simultaneous discrimination and analysis of naïve B cells, transitional forms, class-switched and non–class-switched memory B cells, as well as plasmablasts and plasma cells. This approach allows for the monitoring of structural changes in the B-cell repertoire at different time points following infection and vaccination.

An essential original contribution of the dissertation is the use of S1-specific tetramers for the direct detection and quantitative assessment of antigen-specific memory B cells. This represents a methodological challenge and a rarely applied approach in national scientific practice. Using this method, S1-specific memory B cells were identified and longitudinally monitored for the first time in a Bulgarian population, allowing for a direct assessment of antigen-specific B-cell immune memory independent of serological status.

The dissertation also contributes by tracking the dynamics of B-cell immune memory over an extended period following SARS-CoV-2 infection and immunization with different mRNA vaccine platforms. The temporal analysis of the data makes it possible to delineate distinct patterns of immune response and persistence of immune memory, as well as to draw well-founded comparisons between naturally acquired and post-vaccination immunity.

The obtained results have not only scientific and theoretical value but also practical significance for clinical immunology and vaccine prophylaxis, as they provide objective data on the duration and quality of B-cell immune memory. Taken as a whole, the dissertation represents a significant contribution to the study of the immune response in COVID-19 and provides a solid foundation for future research aimed at optimizing immunization strategies and immunological monitoring.

VI. Evaluation of the Publications and the Doctoral Candidate's Personal Contribution

The publication activity of Dr. Martina Radoslavova Bozhkova is directly related to the topic of the dissertation and concisely reflects the main scientific results of the conducted research. Publications in peer-reviewed international scientific journals indexed in established global databases are presented, including articles with an impact factor, which is indicative of the scientific value and relevance of the obtained results.

The doctoral candidate has made a substantial personal contribution at all stages of the dissertation research—from planning and experimental implementation, through data analysis and interpretation, to the preparation of scientific publications. The presentation of the results at national and international scientific forums further confirms their significance and the doctoral candidate's active participation in scientific exchange.

VII. Abstract

The abstract has been prepared in accordance with regulatory requirements and accurately reflects the content, structure, and main results of the dissertation. It follows the logic of the dissertation, clearly presenting the aim, objectives, applied methods, obtained results, and formulated scientific contributions. The abstract provides adequate and accessible information about the conducted research and allows for an objective preliminary assessment of the scientific value of the dissertation.

VIII. Critical Remarks

None.

IX. Conclusion

Based on the overall evaluation of the presented dissertation, its scientific significance, methodological rigor, and clearly formulated contributions, as well as the publication activity and personal contribution of the doctoral candidate, I consider that the dissertation fully meets all the requirements of the Act on the Development of the Academic Staff in the Republic of Bulgaria and the related secondary legislation for awarding the educational and scientific degree "Doctor (PhD)".

In my capacity as Chair of the Scientific Jury, I give my positive assessment and propose to the esteemed Scientific Jury to award the educational and scientific degree "Doctor (PhD)" to Dr. Martina Radoslavova Bozhkova in the doctoral program "Immunology".

January 3, 2026

Plovdiv

Reviewer:

Prof. Mariella Geneva-Popova, MD, PhD