

REVIEW

by Assoc. Prof. Petya Asenova Dimitrova, PhD

Laboratory of Experimental Immunotherapy, Department of Immunology, Stephan Angeloff
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of Doctoral Thesis for obtaining an educational and scientific degree “Doctor of Philosophy”
(PhD)

professional field: 7.1 Medicine

doctoral program: Immunology

Author: Martina Radoslavova Bozhkova, MD

Form of doctoral studies: a full-time PhD student

Faculty: Department of Medical microbiology and immunology “Prof. Dr. Elissay Yanev”,
Faculty of Medicine

Topic: **Study of B-cell immune memory in COVID-19 and post-vaccination immunity**

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

1. General description of the presented materials according the PhD procedure

Dr. Martina Radoslavova Bozhkova, MD has presented and submitted her Doctoral thesis in paper and online, including all relevant documents, according to the requirements of the Art. 70 (1) of Section I. Acquisition of the educational and scientific degree "DOCTOR OF PHILOSOPHY " and the scientific degree "DOCTOR OF SCIENCES" at MU-Plovdiv; Regulations of MU-Plovdiv from 28.01.2021 and including the following documents: application for admission to PhD thesis defense, a CV, copy of completed master's degree, certificate for PhD enrolment, records of exams and courses in the specialty, summary for credits, order for conducting an exam from the individual plan and a corresponding protocol for passing the exam or doctoral minimum in the specialty, protocol and preliminary review of pre-defense, the decisions for opening the procedure and for the composition of the scientific jury, dissertation thesis, abstract, list and copy of scientific publications, related to the topic of the PhD thesis, declaration of originality.

The doctoral student has presented **three publications** related to the Doctoral thesis.

2. Brief outline of the CV of the doctoral student

Dr. Martina Radoslavova Bozhkova was born on 29 July 1994 in Plovdiv. She graduated in medicine with honors in 2019 at the Medical University – Plovdiv. Since October 2020, she has been working in the Department of Medical Microbiology and Immunology “Prof. Dr. Elisei

Yanev”, Faculty of Medicine, Medical University – Plovdiv as an assistant professor. Since 2022, she was enrolled in a full-time PhD study at the Department. In December 2024, she acquired a specialty in Clinical Immunology.

3. Relevance of the topic and research goals and objectives

The COVID-19 pandemic presented a huge challenge, both in terms of studying the virus, its mutations and mechanism of replication and reproduction, and in terms of evaluating the immune response in severe and mild infections, and after vaccination and effective therapy. The society in Bulgaria and other countries worldwide was divided "for and against" the vaccination against SARS CoV-2, there were also speculative lack-of-data hypotheses, which resulted to direct consequences - high mortality and severe infections with frequent occurrence of post-covid syndrome. Therefore, a systematic and more in-depth study of memory immunity in the Bulgarian population and characterization of developed collective immunity to viral infections is particularly significant, as it can provide important information and methodological preparation for a new epidemic/pandemic and for rapid assessment of the immune response to vaccination. The introduction of mRNA vaccines provides an opportunity for a new and innovative approach to induce antigen-specific B and T cell immune responses. However, it is still unclear whether vaccination and recovery from infection sufficiently expand B and T cell heterogeneity simultaneously with the B and T cell repertoire, whether there is plasticity in the memory B cell response and whether this would be reflected in hybrid immunity, when vaccinating recovered individuals, or whether this is relevant to the emergence of post-covid syndrome. In this aspect, the presented dissertation is extremely relevant and significant.

4. State-of-the art

The literature review describing the state of the problem includes 2 figures summarizing the phases of B-lymphocyte development and presenting the antigen-dependent phase of B-lymphocyte development in the germinal centers of the lymph follicles and 3 informative tables for the phenotypic characteristics of the main B-cell subpopulations, the advantages and disadvantages of various techniques for studying antigen-specific B lymphocytes and immune deficiencies associated with impaired formation of memory B cells. Dr. Martina Bozhkova thoroughly systematizes the latest research on immunological memory and in particular memory B lymphocytes, using over 80% of published sources in 2025. The inclusion of new concept on trained innate immunity makes a great impression. The review shows excellent knowledge of the state of the art.

5. Methodology

In Dr. Martina Bozhkova's dissertation, the precise time points selection and design of the prospective longitudinal study of the two cohorts—infected with the virus and vaccinated—is noteworthy. For the vaccinated individuals, the time before injection and at the second boost (T0) are correctly included to determine the baseline, followed by time points at 1, 6, and 12 months (after the second dose/after the onset of clinical symptoms). The number of participants in each group is also noted. The study defines 4 groups: group 1: vaccinated with BNT162b2 mRNA (Pfizer-BioNTech), group 2: vaccinated with mRNA-1273 (Moderna), group 3: recovered from SARS-CoV-2 infection (positive RT-PCR), and group 4: healthy donors.

To determine anti-SARS-CoV-2-specific RBD-IgG via ELFA, a reliable kit with good sensitivity for PCR-positive patients with clinical symptoms of SARS-CoV-2 was used. It is

considered that the kit has low cross-reactivity due to low homology and structural differences in the RBD with other coronaviruses (J Clin Microbiol 59: e02292-20. <https://doi.org/10.1128/JCM.02292-20>). To determine neutralizing antibodies against SARS-CoV-2, the reliable GenScript cPass™ kit was also used, which simulates virus-host interaction and is antibody isotype independent.

The techniques used, T- or B-spot, are well described, and the necessary controls are included in the experiments. Dr. Martina Bozhkova made considerable efforts to perform precise multiparametric flow cytometric analysis—the antibodies used were titrated before the main experiment, and the conditions for using tetramers in flow cytometry were optimized. The gating strategy in the flow cytometric analysis is appropriate, with differentiation of plasma and memory B lymphocytes, followed by the distinction of transitional cells and the identification of IgG⁺ S1-specific memory B lymphocytes.

The statistical analyses is adequate, precise, and demonstrates integrity.

6. Characterization and Evaluation of the Dissertation

The dissertation is structured with the typical chapters for a dissertation - introduction, description of the state of the art, aim and objectives, results, discussion, conclusions, and impact.

The dissertation aims to study the dynamics and duration of the humoral and memory B cell immune response following recovery and vaccination with mRNA vaccines against SARS-CoV-2. Five tasks are clearly formulated.

In the Materials and Methods chapter, the consumables and methodologies used are well described, allowing experimental reproducibility.

The discussion of the results demonstrates maturity and depth, with a critical view of the obtained results.

The conclusions are clearly and correctly formulated based on the results obtained.

7. Contributions and significance of the study

In my opinion, the most significant contribution of the dissertation is the long-term monitoring of specific memory B cells following the administration of two different mRNA vaccines (BNT162b2 and mRNA-1273) and the comparison with SARS-CoV-2 infection in the Bulgarian population, demonstrating that BNT162b2 and convalescent individuals exhibit more dynamics and variable long-term memory, whereas mRNA-1273 maintains a more homogeneous memory response. The study also includes data on T cell reactivity in the Bulgarian population among vaccinated and infected individuals.

In Dr. Martina Bozhkova's dissertation, a modern method for data processing is applied, which ensures data reproducibility and guarantees that the proposed platform for assessing B cell heterogeneity and antigen-specific memory B cell response has more versatile application:

- Adequate assessment of B cell heterogeneity and memory B cell immune response in the population during a new epidemic/pandemic.
- Optimization of vaccination strategy for different patient groups and its personal approach according to age, immune status, and previous infections.

- Analysis of B cell heterogeneity and memory B cells in different subtypes of patients with sepsis, defined according to the degree and profile of innate immune activation. Study of post-COVID syndrome.
- Broader screening of infections with simultaneous monitoring of heterogeneity in the B cell pool and B cell repertoire.
- Application of this analysis to assess population immunity when administering new mRNA vaccines including AI modeling of immune response and algorithm design
- Adaptation of this analysis to evaluate hybrid immunity and in studies of post-COVID syndrome.

7. Assessment of publications related to the Doctoral Thesis

Dr. Martina Bozhkova has 3 publications on the topic, in which she is the first author. The main part of the data is published in the indexed and peer-reviewed journal *Vaccine*, in Q1 with an impact factor of 5.5, supporting my opinion that the PhD thesis shows the research excellence. The research methodology is published in the peer-reviewed journal *Biotechnology & Biotechnological Equipment* with Q2, and a clinical case description is published in the journal *Cureus*.

8. Personal contribution of the PhD Candidate

In the PhD thesis, the obtained results are original and demonstrate the personal contribution of the PhD candidate to the presented work. In the publications related to the dissertation, Dr. Martina Bozhkova is the first author, and her role in the research and her personal contribution – in conceptualization, data collection, and processing – is described in the Author Contributions section of each article.

10. Short-version of the PhD thesis

The Short-version of the PhD thesis is prepared according to the requirements and concisely presents the main results, conclusions, and contributions of the dissertation. The English version is professionally prepared.

11. Critical Remarks and Recommendations

The critical notes are related to the use of certain terms.

12. Personal Impressions

None.

13. Future recommendations

In the future, it would be interesting to evaluate B-cell heterogeneity and the memory B-cell immune response in the context of the B-cell repertoire and increased antibody polyreactivity during infections.

CONCLUSION


The dissertation contains fundamental and scientific-applied results that represent **an original contribution to the research field and meet all the requirements** of the Law on the Development of the Academic Staff in the Republic of Bulgaria (ZRASRB), the Regulations for the Implementation of the ZRASRB and the Regulations for the Implementation of the ZRASRB

of the Medical University-Plovdiv. The presented dissertation results fully comply with the specific requirements of the Rules the Medical University-Plovdiv for the application of ZRASRB.

Martina Radoslavova Bozhkova's PhD thesis demonstrates skills and characteristics **required for the independent conduct of scientific research**, proving that she possesses in-depth theoretical knowledge and professional skills.

Due to the aforementioned, **I can confidently state my positive assessment** of the research that was done and **I strongly recommend to award** Martina Radoslavova Bozhkova the educational and scientific degree of "Doctor" in the field of 7.1 Medicine (Immunology).

20.01.2026 r.

Reviewer: 
Assoc. Prof. Petya Dimitrova

Залчено на основание
Чл.5 §1, 6."В" Регламент (ЕС)2016/679