OPINION

by Assoc. Prof. Georgi Stoyanov Nikolov, MD, PhD
National Center for Infectious and Parasitic Diseases, Sofia,
Department of Immunology,

Subject: awarding the Doctor of Philosophy (PhD) degree at Acibadem City Clinic Tokuda University Hospital JSC, Clinic of Dermatology and Venereology, Sofia,

Field of higher education: 7. Health and Sport
Professional Direction: 7.1. Medicine
Scientific specialty: Dermatology and Venereology

PhD student: **Zlatko Dobrev Dimitrov, MD**

Topic: **"Diagnostic Value of Specific Allergy Tests in Patients with Cow's Milk Protein Allergy (CMPA)"**

General Presentation of the Procedure:

By Order No. 15-05-78/19.03.2025, issued by Prof. Dr. Milena Staneva, MD, Head of Research and Accreditation Activities at Acibadem City Clinic Tokuda University Hospital JSC, and based on the decision of the Scientific Council under Protocol No. 66/27.01.2025, I have been elected as external member of the Scientific Jury for awarding the Doctor of Philosophy (PhD) degree of PhD student Zlatko Dobrev Dimitrov, MD. Based on the decision taken during the first meeting of the Scientific jury I am assigned to prepare an opinion on the procedure for acquiring the educational and scientific degree Doctor of Philosophy.

The set of materials, provided to me on paper/electronical media, fully complies with the requirements of the Law For The Development Of Academic Staff In The Republic Of Bulgaria, as well as the Rules On The Conditions And Procedure For Acquiring Science Degrees And Holding Academic Positions in Acibadem City Clinic Tokuda University Hospital JSC.

Zlatko Dimitrov was born on June 1, 1977. In 2003, he graduated from the Faculty of Medicine at Trakia University, Stara Zagora, with a medical degree.

Between 2005 and 2009, he was a resident physician specializing in Clinical Allergology at the Department of Pediatrics, Faculty of Medicine, Trakia University, Stara Zagora. In 2009, he obtained a specialty in Pediatrics.

From 2010 to 2013, he continued his residency in Clinical Allergology at the Clinic of Clinical Allergology at "Alexandrovska" University Hospital, Sofia. In 2013, he acquired a specialty in Clinical Allergology.

Since 2022, he has been enrolled as an independent PhD student at Acibadem City Clinic Tokuda University Hospital, Sofia.

Since 2014, he has been working as an allergologist at the Allergology Outpatient Clinic at Acibadem City Clinic Tokuda University Hospital, Sofia.

He is a member of several Bulgarian and international scientific societies, including the Bulgarian Society of Allergology, the European Academy of Allergy and Clinical Immunology (EAACI), and the World Allergy Organization (WAO).

Relevance of the Dissertation Topic

Cow's milk protein allergy (CMPA) is the most common food allergy observed during the first months of life, with a reported prevalence of 2–3% in the first year. The condition arises as a result of an abnormal immune response to ingested cow's milk proteins, mediated through IgE, non-IgE, or mixed mechanisms.

In clinical practice, the diagnosis of CMPA can be particularly challenging, as its symptoms often mimic those of other conditions such as lactose intolerance or food protein-induced enterocolitis syndrome (FPIES). This diagnostic complexity necessitates the use of precise and reliable diagnostic methods.

The oral food challenge is currently regarded as the "gold standard" for diagnosing food allergies and evaluating the development of tolerance. However, this method is time-consuming, difficult to implement, and carries a significant risk of inducing severe allergic reactions. Consequently, in recent years, considerable efforts have been made to identify alternative diagnostic tools that are less invasive and pose fewer risks. Despite these efforts, the international scientific literature still lacks sufficient data on the diagnostic value of specific allergological tests in patients with cow's milk protein allergy.

In this context, the topic of Dr. Dimitrov's doctorate is highly relevant and addresses a pressing clinical need. The PhD thesis provides a comprehensive and detailed account of his clinical experience in the diagnostic process of patients with CMPA.

General characteristics and structure of the dissertation

The dissertation is well-structured and written in a scientific and academic style. It comprises 146 standard pages, illustrated with 21 figures, 7 graphs, 31 tables, and includes 1 appendix. The work contains all the mandatory components of a doctoral dissertation: table of contents, list of abbreviations, introduction, literature review, objectives and tasks, materials and methods, results and discussion, and conclusion. A total of 12 conclusions have been formulated, along with 5 original contributions, 4 scientific-theoretical contributions, and 3 practical contributions. The bibliographic reference list consists of 307 sources, of which 4 are in Bulgarian and 303 are in English, reflecting a comprehensive review of the relevant international scientific literature.

Evaluation of the structural parts of the dissertation

The literature review spans 40 pages and provides a comprehensive overview of current data on the epidemiology, pathogenesis, and clinical presentation of IgE-mediated cow's milk protein allergy. It offers a detailed description of the various diagnostic approaches used to identify sensitization to cow's milk proteins. The review also analyzes contemporary methods for the prevention and treatment of food allergies, with particular emphasis on the latest developments in biological therapies.

However, the literature review concludes without a summarizing section that would clearly establish the rationale for the dissertation's objective - namely:

"to investigate the level of tolerance to cow's milk in children based on various allergy testing methods (skin prick tests, patch tests, in vitro tests for specific IgE, and oral food challenges)."

To achieve the dissertation's primary objective, Dr. Dimitrov focused his efforts on addressing five practical tasks, which structure a well-reasoned approach to the study. The chosen study design allows for the successful execution of these specific tasks and ensures the achievement of the main goal of the dissertation.

The key research framework and stages of the study are clearly described. However, the methods used in the investigation are presented somewhat schematically, and could benefit from a more detailed explanation.

The retrospective study included 248 children aged between 3 months and 9 years, with 96 girls and 151 boys. All children had confirmed cow's milk protein allergy and were referred to the outpatient clinics of Acibadem City Clinic Tokuda and Tokuda University Hospital, Sofia, during the period from 2016 to 2022.

The patient cohort is large enough to provide reliable results, which have been analyzed using appropriate clinical, laboratory, and statistical methods that align with the objectives of the study. The results are adequately illustrated with a sufficient number of figures and tables. The obtained data are sufficient to draw valid conclusions.

In the Materials and Methods chapter, a considerable amount of theoretical material (12 pages) is included, which I would recommend relocating to the literature review section.

The results from each task of the study are presented in comprehensive detail and are appropriately illustrated.

The age range of the studied patient group is from 5 to 28 months. Among children under 24 months, the most common manifestations are allergic proctocolitis (24.4%) and urticaria (24.4%), followed by atopic dermatitis with sensitization to cow's milk (17.8%) and maculopapular rash (16.3%). Among children older than 24 months, the most pronounced clinical manifestation is maculopapular rash (30.4%), followed by atopic dermatitis with cow's milk sensitization (19.6%) and angioedema (18.8%).

In the oral food challenge with cow's milk, 28 positive results (8.6%) were observed. 41.4% of the examined patients had elevated allergen-specific IgE levels for cow's milk (>0.35 kU/L). Significantly higher levels of allergen-specific IgE were found in patients with angioedema, compared to the overall population. When using the skin prick test with cow's milk allergen, 18.5% of patients tested positive. On the other hand, the patch test with cow's milk allergen showed a positive result in only 5.0% of cases.

A correlation analysis was conducted between the results of the oral food challenge with cow's milk and the determination of allergen-specific IgE levels. The analysis revealed that the relationship was statistically insignificant, with low levels of accuracy, specificity, and sensitivity. Similarly, the relationship between the oral food challenge and the skin prick test with cow's milk was also statistically insignificant. Here, higher levels of specificity were observed, but very low sensitivity. A comparison with the patch test for cow's milk yielded similar results, showing no significant relationship. While specificity increased, the sensitivity dropped further to 11.1%, with the majority of positive cases being misclassified as negative.

The relationship between the combined results of the three tests and the results of the oral food challenge with cow's milk was also analyzed. A statistically significant relationship was confirmed in this case. 100% of negative results were correctly classified, indicating a calculated 100% sensitivity.

The influence of nosological classification on the above results was analyzed using logistic models. It was found that nosological units had no influence on the confirmed findings: no relationship was observed between any of the three tests and the results of the oral food challenge with cow's milk, while a significant relationship was confirmed between the combination of the three tests and the oral food challenge results.

Here, I would like to note that the presented original results and corresponding analyses would have greater credibility and weight if they were competently discussed and a more in-depth analysis had been made in the Discussion section."

The concluding findings of the dissertation are well-formulated and reflect the essence of the results obtained. Dr. Dimitrov has made 12 final conclusions, which succinctly present the most significant aspects of the study. These conclusions emphasize the relevance of the research and its practical significance.

The dissertation work is designed precisely with the exception that some figures are presented in English. I have no remarks on the methodology, the presentation of the results, or their analysis.

Assessment of contributions, publications and personal contribution of the PhD student

I accept the formulated contributions and believe that they objectively reflect the real results of the conducted studies. The following contributions are considered particularly significant:

- For the first time in Bulgaria, the diagnostic value of each method for diagnosing cow's milk protein allergy, referenced to an oral food challenge with cow's milk, has been analyzed.
- For the first time, the diagnostic value of the combined use of various diagnostic tests to determine allergy and tolerance to cow's milk has been investigated.
- A diagnostic algorithm for comprehensive diagnosis and determination of tolerance levels to cow's milk has been developed, along with guidelines for the treatment of cow's milk protein allergy.

The author's summary includes a list of 4 publications in Bulgarian scientific journals and 1 participation in a scientific forum on the topic of the dissertation. In terms of quantity and quality, the scientific papers meet the minimum requirements for the academic degree of Doctor.

Abstract

The abstract is written on 46 pages and is prepared according to the requirements, accurately reflecting the dissertation research, results and conclusions.

CONCLUSION

In conclusion, the doctoral thesis of Zlatko Dobrev Dimitrov, MD meets the regulatory requirements and is the result of the doctoral student's own development. It addresses a significant issue in modern medicine related to the diagnosis of one of the most widespread food allergies – cow's milk protein allergy. The specific tasks ensuring the achievement of the main goal of the dissertation have been successfully completed, and the obtained results lead to the formulation of conclusions and contributions that I accept.

The dissertation shows that Dr. Dimitrov possesses in-depth theoretical knowledge and professional skills in the scientific field, demonstrating the qualities and abilities necessary for conducting independent scientific research.

The dissertation fully complies with all the requirements of the Law on the Development of the Academic Staff in the Republic of Bulgaria and the respective Regulations of Acibadem City Clinic Tokuda University Hospital JSC, Sofia.

Everything outlined here gives me grounds to give a positive assessment of the conducted research and I propose to the members of the esteemed Scientific jury to award Zlatko Dobrev Dimitrov, MD the academic degree of " Doctor of Philosophy" in the scientific field of "Dermatology and Venereology", Professional field: Medicine."

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28. 04. 2025

Assoc. Prof. Georgi Nikolov, MD, PhD