TO

THE CHAIRMAN OF THE SCIENTIFIC JURY,

DETERMINED BY ORDER No.-15-05-78/19.03.2025

TO THE EXECUTIVE DIRECTOR AND PROCURATOR

OF "ASK UMBAL TOKUDA" EAD

To your protocol No. 1

I present herewith:

REVIEW

By Assoc. Prof. Dr. Kalin Yordanov Lisichki, MD, PhD

ASK UMBAL Tokuda – Sofia, Clinic of Pediatrics

Specialty – pediatric diseases, pediatric rheumatology

Internal member

On a dissertation for awarding the ONS "doctor"

Field of higher education: 7 "Healthcare and sports"

Professional field: 7. 1 "Medicine"

Doctoral program: "Dermatology and venereology"

Author: Dr. Zdravko Dobrev Dimitrov

ASK UMBAL Tokuda – Sofia, Clinic of Skin and Venereological Diseases

Topic: DIAGNOSTIC VALUE OF SPECIFIC ALLERGIC TESTS IN DETERMINING TOLERANCE TO COW'S MILK IN PATIENTS WITH APCM

Scientific supervisor: Prof. Dr. Nikolay Konstantinov Tsankov, MD, DSc

Form of doctoral studies: independent preparation

I declare that I have no common publications with the doctoral student.

Presentation of the doctoral student

Biographical data

Dr. Zlatko Dobrev Dimitrov was born on 1. 06. 1977 in the town of Nebit - Dag, Turkmenistan.

He graduated from the Faculty of Medicine at Thrace University - Stara Zagora in 2003, after which he started working at the General Hospital - Galabovo. From 2005 to 2010 he was an assistant at the Children's Clinic of the Thrace University. He obtained specialties in "pediatrics" in 2010 and in "allergology" in 2014. Since 2011 he has been working at the ASK UMHAL Tokuda - Sofia.

He is a doctoral student in independent preparation with the topic of his dissertation "Diagnostic value of specific allergological tests in patients with APKM".

He is fluent in written and spoken English and Russian.

He has participated in international and national scientific forums.

He is a member of the Bulgarian Society of Allergology, the Bulgarian Pediatric Association, and the European Association of Immunology and Clinical Immunology (EAACI).

1. Relevance of the topic, justification of the goal and tasks

Introduction to the problem.

In recent decades, allergic diseases have increased exponentially, and while the prevalence of bronchial asthma in childhood has stabilized at around 10%, food allergies and anaphylaxis are clearly increasing and are becoming a significant public health problem. Taking into account epidemiological trends, the European Academy of Allergy and Clinical Immunology estimates that in less than 15 years more than half of the European population will suffer from some type of allergic disease.

Allergy and severe allergic reactions are increasing worldwide. APKM is the most common cause of food allergy in the first months of life. There are many theories about this change in the global epidemiology of allergic diseases, the most frequently commented on being the hygiene hypothesis and the theory of microbiome depletion. These theories relate to the impact that modern lifestyles have on the microbial flora (the human microbiome) as well as reduced exposure to environmental allergens. Research has shown that multiple changes (molecular, genetic, manipulation of the external environment in which humans exist) have an impact on the human microbiome. A link has been established between reduced exposure to infectious organisms and an "ultra-clean" environment (increased use of antibiotics, reduced parasitic diseases) and changes in human immune tolerance, leading to allergy or autoimmune disease.

The topic of the dissertation "Diagnostic value of specific allergy tests in patients with APKM" is dedicated to a current problem for childhood. Although allergy to cow's milk proteins has long been known, its exact frequency is not known to date, since there is no generally valid diagnostic marker. Its frequency varies in different sources from 3% -7% -10%, and in breastfed children - about 0.5%.

Food allergy is defined as an adverse immune response to food proteins. Food-related reactions are manifested by a wide range of signs and symptoms that can affect any system, including the skin, gastrointestinal and respiratory tracts, and the cardiovascular system. The development of allergy to cow's milk proteins depends on the interaction between the genetic predisposition of the individual and environmental factors, especially the load with food proteins.

Immunoglobulin E (IgE)-mediated food allergy is the leading cause of anaphylaxis. Diagnosis involves a careful history and diagnostic tests, such as skin prick tests, serum-specific IgE, and, if indicated, oral food challenge. After confirming the diagnosis of food allergy, strict elimination of the relevant allergen from the diet is necessary.

The doctoral candidate draws attention to the need to conduct the necessary allergological tests and compare them with the results of the provocation tests and the relevant elimination diet.

The overall excellent knowledge of the problem - clinical picture, diagnosis, tests, their interpretation and ways to solve the problems of the child with APCM justifies the goal, respectively the tasks that the doctoral candidate sets for himself.

The goal is clearly defined, with 8 main tasks set, which allow for good implementation of the planned study.

The tasks cover the design of the study, which is appropriately selected.

2. General characteristics of the dissertation

The dissertation is developed on 150 standard typewritten pages and includes 19 figures, 7 graphs, 31 tables and one appendix. It is structured in 11 parts – introduction, literature review, methodological framework of the study, results of the retrospective study conducted to determine the tolerance to cow's milk in patients with APCM, diagnostic algorithm for determining the level of tolerance to cow's milk, discussion, conclusion, inferences, scientific contributions, bibliography and one appendix. The bibliography includes 308 sources, of which 4 in Cyrillic and 304 in Latin, 148 are from the last 10 years.

The dissertation is in form and volume in accordance with generally accepted requirements. The abstract is also structured according to the adopted scheme.

Four articles related to the topic of the dissertation are presented.

The submitted scientific publications are sufficient for admission to defense.

3. Literature review, knowledge of the problem, choice of goal and tasks

Literature review

The literature review is written on 46 pages, well-structured, purposeful, with a good and understandable presentation of the problem – APCM and its features in childhood. The clinical picture of food allergy in childhood is presented, including allergy to cow's milk, allergic rhinitis, allergy to eggs and the role of the oral food provocation test. The epidemiology, pathogenesis, pathophysiology and clinical picture of IgE-mediated APCM are examined. The main diagnostic tests, elimination diet, as well as justification for why these are the methods used are indicated. The fact that tests without diagnostic value are also given is also interesting, which shows a thorough knowledge of the problem by the dissertationist and a correct assessment of the results. Separate chapters are devoted to the prevention and possibilities for modern treatment of APCM.

The literature review is sufficiently informative and up-to-date.

The doctoral student has a comprehensive and in-depth knowledge of the problem. He has the ability to creatively evaluate literary material, evidence of which is the excellently presented and commented literature review.

Without a good knowledge of the problems in diagnostics, monitoring and treatment, of world experience and innovations, it is not possible to correctly determine the topic, goal and tasks of the dissertation work. Therefore, the literature review is a starting point for the entire work.

The review presented by the doctoral student Dr. Zlatko Dimitrov is sufficiently informative, shows knowledge of the problems of the disease, his ability to select and cite. He

objectively and critically analyzes foreign scientific experience. The problems are presented with the necessary clarity, scientific reference and ways to solve them.

Goal and tasks.

The goal is formulated precisely and clearly in 1 point, namely − 1. The goal of the study is to establish, based on various allergy tests (prick tests, epicutaneous tests, in vitro tests for specific IgE, provocation tests) the level of tolerance to cow's milk in children. The goal is well justified by the presented literature review, interesting, well-conceived and implemented. It shows knowledge of the problem and a vision for presenting and extracting the necessary evidence and facts to achieve the necessary results by the doctoral student. This is also clearly evident from the well-formed and precise tasks.

The tasks are structured in two groups: theoretical and scientific tasks and applied tasks. There are 8 in total, and with them the goal of the work is achieved, namely: research and analysis of patients with APCM.

A correlation between the individual factors - allergy tests and provocation tests was sought.

The goal, as well as the tasks, are clear, requiring serious and in-depth coverage of the studied groups, accurate analysis and establishment of interrelationships, requiring good knowledge of the characteristics of the disease - such as clinical picture, course and evolution. Also technical skills acquired by the doctoral student and applied in practice. The doctoral student coped with these tasks excellently.

4. Material and methods

The study is a retrospective study of the indicated indicators in patients with APCM.

To prepare the study for the dissertation, the analysis of data of patients with established allergy to cow's milk proteins, referred to the DCC of ASK "Tokuda" and ASK UMBAL "Tokuda", Sofia, for the period from 2016–2022, was applied. The retrospective study included 248 children aged 3 months to 9 years, of which 96 were girls and 151 boys. There is no information on gender for one of the children.

Inclusion criteria were developed, as well as an algorithm for conducting provocative tests and excluding infective infection.

The systematic grouping of data is based on the following standard information sources:

1) History of the disease - covers the symptoms of the disease, the patient's complaints, family history.

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2) Diagnostic protocol sheet – a standard document with data on the time, type and team that performed the patient's diagnosis.

3) A summarized sheet in a computer version with all the data necessary for the study from the remaining documentation.

Prick tests (prick test), epicutaneous tests were conducted using IQ Ultra patch test units and in vitro tests, which were performed in certified clinical laboratories

The data from the study were processed with statistical software packages SPSS (Statistical Package for the Social Sciences) version 13.0. The results are described using tables, graphs and numerical indicators for structure, frequency, average values, correlation coefficients. The following statistical methods/tests were applied in the analysis of the results: z-test for the difference between two calculated proportions and Clopper-Pearson method for alternative estimates of the empirical significance levels, correlation analysis, using the so-called. exact indicator for the empirical significance level (p) of Cramer.

Appropriate clinical, laboratory and statistical methods were used that were appropriate and in line with the purpose and objectives.

The number of patients included was sufficient to obtain reliable results, which were analyzed with appropriately selected statistical methods and illustrated with tables and figures.

The chosen approach achieved an effect – sufficiency and reliability, based on a number of patients sufficient to derive reliable results.

The study design, the stages of the study and the research methods used were well described.

5. Results and discussion

The results and analyses are presented in a clear form, corresponding to the assigned tasks and subtasks, well presented in tables and figures, well analyzed.

The study indicates the most common allergic diseases in APCM, as well as the dependencies between their frequency, the three tests and the provocation tests. No statistically significant differences were found. There is no difference between the diagnostic reliability of the allergy tests and the provocation tests.

The presented results point to the important practical value of the development, namely the need to conduct tests and provocation tests in order to confirm, respectively. reject the diagnosis of APCM.

The detailed presentation of the results is impressive, as the discussion is focused and competent, following the basis of the obtained results point by point. Dr. Zlatko Dimitrov's

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ability to analyze his own results in the context of the known scientific data in the literature is evident.

I accept the achieved results and the conclusions drawn.

6. Conclusions and contributions and significance of the dissertation work

Based on the study, the doctoral student has formulated 12 conclusions that logically follow the goals and objectives of the study.

The contributions of the dissertation are also presented: of an original, scientific-theoretical, scientific-practical and confirmatory nature.

Based on his own research and the available scientific literature, the doctoral student has developed a diagnostic algorithm for determining the level of tolerance to CM. Guidelines for the treatment of CMPA are listed, with one of the most frequently recommended being a strict diet.

7. Publications related to the dissertation work, evaluation

The publications and scientific events related to the dissertation work are also presented.

In connection with the dissertation work, 4 articles and one participation in a scientific forum on the topic of the dissertation are presented. In terms of number and quality, the scientific works meet the minimum requirements for the ONS "Doctor" according to the "Law on the Development of the Academic Staff in the Republic of Bulgaria, the Regulations for its Implementation and the Regulations of ASK UMBAL Tokuda - Sofia.

8. Abstract

The abstract is written in 46 pages. It corresponds to the materials included in the dissertation. The main results and discussions, conclusions, contributions and publications are presented concisely and clearly and gives a visual idea of the nature and contributions of the dissertation.

In the discussion, the author objectively and critically analyzes and discusses his results and compares them with the results of leading foreign authors.

The abstract reflects the content of the dissertation and meets the requirements.

9. Remarks and recommendations

I have no critical remarks. The goal of the dissertation has been achieved.

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CONCLUSION:

The dissertation contains scientific, scientifically applied and practical results. It presents original scientific contributions. The dissertation meets the requirements of the Act on the Development of the Academic Staff in the Republic of Bulgaria, the Regulations for its implementation and the Regulations of MU, Sofia.

Dr. Zlatko Dobrev Dimitrov shows profound theoretical knowledge and professional skills, is precise and critical in his work, searching and analyzing. Demonstrates qualities and abilities for independent scientific research.

As a scientific reviewer, I give my positive assessment of the dissertation work "Diagnostic value of specific allergological tests in patients with APCM" of the achieved results and contributions and I propose to the esteemed members of the Scientific Jury to award the scientific and educational degree "Doctor" to Dr. Zlatko Dobrev Dimitrov.

19.02.2024.

City of Sofia

Signature:

Assoc. Prof. Dr. Kalin Yordanov Lisichki, PhD

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