REVIEW

By Professor Mariyana Stoycheva Vartigova, MD, PhD, DSc

Specialist in Infectious Diseases, External Member of the Scientific Jury

of the Dissertation

for awarding the educational and scientific degree "Doctor"

of Dr. Ekaterina Lyutsova, Assistant Professor at the Department of Infectious Diseases, Parasitology and Dermatovenereology, Faculty of Medicine, Medical University of Varna

Field of Higher Education 7. Health & Sport

Professional field: 7.1. Medicine

Doctoral Program – Infectious Diseases

Form of doctoral studies – full-time form of study

Department of Infectious Diseases, Parasitology and Dermatovenereology, Faculty of Medicine, Medical University Varna

Topic: **Fecal calprotectin as a diagnostic and prognostic marker in acute intestinal infections in children**

Supervisor: Professor Margarita Gospodinova, MD, PhD

General presentation of documents

The set of documents **provided to me** on electronic media includes 14 components, in full compliance with the requirements of the the Law on the Development of Academic staff in the Republic of Bulgaria, and the Rules of for its implementation, and the Regulations of the Medical University, Varna.

1. Dissertation.
2. Abstract.
3. Creative autobiography.
4. Master's degree – notarized copy.
5. Certificate of Recognized Specialty
6. Enrollment order
7. Protocol of completed doctoral studies in the specialty
8. List of publications related to the topic of the dissertation.
9. Copies of publications on the topic of the dissertation.
10. Protocol of the Departmental Council for Readiness for Defense.
11. Statement of assurance
12. Declaration of originality
13. Other documents related to the course of the procedure.

Dissertation and Abstract – **on paper

Analysis of the candidate's career profile

Dr. Ekaterina Lyutsova is a graduate of the Yaroslavl State Medical Academy, Russian Federation (RF), graduating with honors in 2006, acquiring the qualification of "doctor". In 2015 she successfully passed the state exams at the Medical University - Varna for the recognition of her professional qualification in the Republic of Bulgaria.

In the period 2006-2009 she worked as an internist in the city of Vologda, Vologda region, Russia, where she acquired the specialty "Therapy", and in the period 2009-2013 - as a general practitioner in a city polyclinic in the Moscow region. Since January 2016 she has been working at the First Clinic of Infectious Diseases at the University Hospital "St. Marina" – Varna and was elected as an assistant professor at the Department of Infectious Diseases, Parasitology and Dermatovenereology at the Medical University – Varna. In 2019 she was enrolled as a PhD student in the specialty of infectious diseases, full-time at the Department of Infectious Diseases, Parasitology and Dermatovenereology, Medical University of Varna (Rector's Order No R-109-37, 01.02.2019).

Acquired specialties and educational degrees: (1) *Infectious diseases* (Certificate No. 024494/12.02.2021, series MUV-2021, No. 4498 issued by the Medical University – Varna. (2) Master of Health Management (Burgas Free

University, electronic diploma number 43263WW, registration No 2520542227/23.05.2025, with excellent grades); (3) Master of Artificial Intelligence in Healthcare (Diploma series MUV, No 013406, registration No 013646/27.10.2025, Medical University – Varna, with excellent grades).

Dr. Ekaterina Lyutsova leads practical classes in infectious diseases with students from the specialties of medicine - BLE and ELE, dentistry, public health, medical laboratory assistants, nurses and midwives. She actively participates in the seminars held within the framework of the state internship in infectious diseases, as well as in the course of "Tropical Medicine". She actively participates in the scientific activities of the Department, and annually presents her scientific reports at the conferences of the Bulgarian Society of Infectious Diseases, as well as at interdisciplinary scientific forums, including various medical specialties and computer science.

Dr. Ekaterina Lyutsova is fluent in written and spoken Russian, Bulgarian and English. She is a member of the Bulgarian Medical Union, Bulgarian Society of Infectious Diseases, and European Society of Clinical Microbiology and Infectious Diseases (ESCMID).

The dissertation work provided to me is dedicated to acute intestinal infections in childhood and the possibilities for the application of a less well-known biomarker in infectology, fecal calprotectin (FC) in the diagnosis and prediction of the course of these diseases. Acute infectious diarrhea (AID) is the second most common cause of childhood morbidity and mortality globally, after pneumonia. According to the WHO, about 1.7 billion diarrhoeal diseases are registered annually in children under 5 years of age, and 443,832 end fatally, mainly in developing countries. In economically developed countries, AID is rarely fatal, with the exception of terminal age groups and immunocompromised people, but it is the cause of significant morbidity and financial losses for society. Persistent or recurrent diarrhea causes malnutrition, and has an adverse effect on the physical and psycho-motor development of children.
The topic is extremely actual and of significant clinical significance, for which I would especially like to congratulate the scientific supervisor of the doctoral student.
Dr. Ekaterina Lyutsova's dissertation includes 183 standard pages. It is structured according to the accepted requirements for scientific work and the Rules of Procedure of the MU - Varna, and contains: a literary review, purpose

and tasks, materials and methods, results and discussion, conclusions and bibliography. The author has noted the contributions and publications related to the dissertation, which is illustrated with 23 figures and 26 tables.

The literature review (44 pages) includes several main areas: the significance of AID for public health; a modern view of the etiology, epidemiology and pathophysiological characteristics of these diseases in children, in the global world and in our country; the clinical assessment of AID; their etiological verification and the role of FC in their diagnosis and prognosis.
Contemporary data on the structure of FC, its expression and functional characteristics are presented. I would especially like to note that already at this stage, summarized data on its clinical value have been presented. Data are presented on the widespread use of FC as a biomarker in gastroenterology, especially in patients with inflammatory bowel diseases, in surgical practice, in patients with enteropathy induced by long-term use of non-steroidal anti-inflammatory drugs, i.e. diseases in which FC has a leading pathogenetic role. There is a growing interest in the potential role of FC in infectology, as a non-invasive marker and reference point for the etiology and severity of AID. Data from studies on Clostridioides difficile, other acute bacterial and viral intestinal infections are indicated. The advantages of determining FC - non-invasiveness, accessibility and speed, and the ability to use it both for early diagnostic orientation and for dynamic monitoring of the intestinal inflammatory process are highlighted.
The Literature Review presents the up-to-date scientific achievements on the problems under consideration, noting the ambiguity and incompleteness in a number of areas. It is written intelligently and skillfully, based on the 159 literary sources used. It is illustrated with 4 tables and 9 figures that facilitate the perception of information.
Purpose and objectives. The goal and specific tasks are clearly and precisely defined. The goal corresponds to the topic, and the six main tasks ensure its implementation.
Materials and methods. The dissertation work includes 137 children aged 1-5 years, treated at the First Clinic of Infectious Diseases, University Hospital "St. Marina" Varna, in the period June 2024 - February 2025. Of these, (1) 107 are patients in the acute stage of AID, selected according to predefined inclusion criteria, based on clinical, epidemiological data and verified diagnosis, and (2) a control group of 30 clinically healthy individuals, comparable in age, who came

for a follow-up examination one month after de-hospitalization on the occasion of AID.
Including/excluding criteria are very correctly selected, in order to exclude the influence of side factors on the deviations of the studied indicators and to maximize the reliability of the results.

The methods of clinical and epidemiological analysis, clinical-laboratory, microbiological (virological), serological and statistical methods are used. The main place is given to the determination of FC by immunoturbodimetric method with latex-enhanced agglutination, by automatic biochemical analyzer A15 (Biosistems). The selection of the methods is made with skill and makes it possible to solve the assigned tasks. I admire the testing the FC in dynamics, during the course of the disease, which allows for prognostic assessment, as well as determining the FC values in a control group, with the establishment of reference limits for the indicator, for the purposes of the study.

A set of modern statistical methods using Microsoft Excel (descriptive statistics, variation, correlation and graphical analysis) is used, which guarantees the reliability of the analyses.

Definitions used in the dissertation are included in this section, which brings additional precision to the work.

Results and discussion
The results are divided into several sections.
Demographic, epidemiological and nosological distribution of patients. Distribution by nosological units shows the highest incidence of Rota-virus gastroenteritis (RV) 63.64% in the group of viral intestinal infections (VII, n=55) and Salmonella spp 40.38% followed by *Campylobacter spp.* 28.85% in the group of bacterial intestinal infections (BII, n=52).
A different age model was reported in the two groups. The distribution by age illustrates a higher incidence of VII in the earlier age groups (children 1–2 years old is 36.36%) and a relative shift in BII cases to older groups (30.77% are children 3–4 years old). The distribution by gender showed a slight predominance of the boys. The high share of children attending organized children's groups is taken into account and the importance of preventive measures in this environment is emphasized.

Distribution and characteristics of patients according to the clinical course and severity of the disease.

The assessment of the course and severity of AID was carried out by analyzing the leading clinical symptoms, the degree of dehydration and applying the modified Vesikari scale. A more unfavorable clinical course in BII has been established, often accompanied by prolonged diarrhea, persistent fever and a noticeably higher incidence of severe clinical forms.

The comparative analysis of the values of FC in patients with viral, bacterial intestinal infections and control group found the highest levels in bacterial, followed by viral infection, and the differences between them, as well as with the levels in the control group, were statistically significant.

Intragroup analysis did not reveal significant differences between FC values in patients with different etiological agents. The highest FC values were found in BII caused by *Shigella spp.* (821.53±105.23 μg/g), followed by *Y. enterocolitica* (765.5±140.94 μg/g), and in VII the highest values (~111 μg/g) were in Noro and RV gastroenteritis.

The **levels of** FC **in AID with different severity were determined** and significantly higher levels were found in the severe forms in the two compared groups, which supports the thesis that FC is a sensitive marker of the intensity of intestinal inflammation and has the potential to assess the severity of the diseases.

The values of the FC are tracked in dynamics, in the course of the disease and in hospital treatment. According to the data of the doctoral student, the level of FC increases significantly in the acute period of the disease. During the repeated measurement, in the early convalescence, in children with viral intestinal infections, the values are close to the reference values, and in the case of bacterial intestinal infections, the levels remain significantly elevated, which is an indicator of a longer inflammatory process.

The comparative analysis between FC and other inflammatory laboratory markers presents data that FC is a higher informative indicator compared to CRP, ESR and leukocytes, taking into account the severity of the infectious process, the effect of the applied treatment and the course of the disease. FC has the highest prognostic utility in viral intestinal infections and remains clinically relevant for initial assessment and risk stratification in acute infectious diarrhea.

Higher baseline values of FC are associated with a longer and/or complicated clinical course, which supports its prognostic potential and highlights its importance to provide additional information on the likely course and severity of the disease in the early stages of the diseases.

In order to quantify the ability of the FC to distinguish between patients with a favorable and unfavorable clinical course ROC analysis was carried out. Cut-off values of FC in bacterial and viral intestinal infections have been determined, which allows for timely recognition of patients with an increased likelihood of a longer or more complicated course and is important for clinical decision-making, including the need for hospitalization, intensification of treatment or stricter monitoring.

The conclusions are entirely derived from the results of the dissertation and correspond to the goals and objectives set.

I agree with **the Contributions** in their two sections: original for the Republic of Bulgaria and those of a confirmatory nature.

The bibliography includes 159 titles, of which 25 in Cyrillic and 134 in Latin, and 92 (58%) are from the last 10 years.

Publications related to the dissertation - two articles in peer-reviewed international journals, and 1 scientific report presented as a poster and awarded at the *VII National Conference on Epidemiology with international participation*. In all presented publications, Dr. Ekaterina Lyutsova is the first author.

The abstract is written on 64 pages. It is structured according to the accepted norms and reflects the essence of the dissertation. It is illustrated with 23 tables and 14 figures of high technical quality. The research was done in the Central Clinical Laboratory, at the University Hospital "St. Marina" in Varna. The interpretation of the data is undoubtedly the work of the author and deserves admiration for the thoroughness and striving for maximum accuracy.

Critical Notes.

1. With regard to the structure of the dissertation, there is much to be desired in order to achieve an optimal balance in the voluminous presentation of the individual components. *The literature review* is longer than is generally accepted, and the explanations in the chapter *Results and* Discussion of places are unnecessarily detailed with frequently repetitive judgments.

2. Some of the figures/tables do not have the necessary requisites.

3. The statement does not always comply with the accepted *Definitions* in the Materials and Methods chapter.

Critical notes do not in the least reduce the value of the dissertation. I hope they will help Dr. Ekaterina Lyutsova in her future scientific work.

Conclusion

The dissertation is a well-thought-out, carefully constructed and wonderfully executed scientific development. It complies with the requirements of the Law on the Development of Academic staff in the Republic of Bulgaria, and the Rules for its implementation, and the Regulations of the Medical University, Varna. It is the result of hard work, a lot of effort on the part of the PhD student and the supervisor. It is a personal file of Dr. Ekaterina Lyutsova and shows that the PhD student has:

(1) in-depth theoretical knowledge of the problems of acute diarrheal diseases in childhood and the participation, diagnostic, and prognostic value of FC in the course of these diseases;

(2) ability to independently develop scientific research.

I give a positive assessment of the dissertation, I evaluate as positive the overall scientific-educational and medical-diagnostic activities of Dr. Ekaterina Lyutsova, and propose to the honorable scientific jury to award Dr. Ekaterina Lyutsova the educational and scientific degree "DOCTOR"

Professor, Mariana Stoycheva, MD, PhD, DSc

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