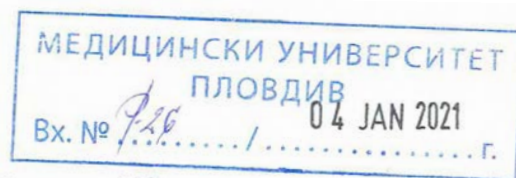


STATEMENT



By Professor Dr. Anastasyia Georgieva Trenova, DSc

Department of Neurology, Medical University of Plovdiv

On dissertational thesis for awarding educational and scientific degree '**Doctor of Philosophy**'

Professional field *4.3 Biological sciences*

doctoral program *MICROBIOLOGY*

Author of the thesis: Dr. Yordan Ivanov Kalchev

Department of Microbiology and Immunology, Medical University of Plovdiv

Topic: „Microbiological and immunological approaches for rapid etiological diagnosis of acute central nervous system infections”

Supervisor: Prof. Dr. Marianna Atanasova Murdjeva, PhD, HMM, Medical University of Plovdiv

1. General presentation of the procedure and the doctoral student

The presented set of materials is in accordance with the requirements of the Procedure for acquisition of "Doctor of Philosophy" in MU - Plovdiv and Regulations of MU – Plovdiv. The doctoral student has 6 publications.

Dr. Yordan Ivanov Kalchev was born in 1989. He graduated in medicine in 2014 with honors. Since 2016 Dr. Kalchev is an assistant in the Department of Microbiology and Immunology at MU-Plovdiv and began developing his dissertation as a doctoral student in independent training. The specialization in Neurological Diseases between May 2015 and February 2016 significantly expanded and enriched the professional skills of Dr. Kalchev and contributed to the in-depth approach in the study of diagnostic aspects of neuroinfections. He has participated in 7 training courses, mainly abroad, during which he got acquainted with the world's achievements in the field. Dr. Kalchev presented scientific abstracts on seven scientific forums in Bulgaria and five abroad. He is a leading researcher in two university projects.

2. Actuality of the topic

The dissertation of Dr. Kalchev is focused on the methods for etiological diagnosis of acute infections of the nervous system. In recent years, there has been a tendency to increase the incidence of infectious diseases, including neuroinfections worldwide, and also the increasing antibiotic resistance makes them more difficult to be controlled. High mortality and residual neurological deficits, which significantly debilitate the sufferers, are other factors determining the social burden of nervous system infections. Early application of adequate etiological therapy plays a key role in the development and outcome of the disease. From this aspect, the dissertation of Dr. Kalchev, which is focused on developing an approach for rapid identification of the cause of neuroinfection is extremely relevant and necessary. The establishment of the etiological structure and epidemiological features of non-viral cerebrospinal fluid isolates in the Plovdiv region for the period 2013-2019 provides up-to-date information to be used for developing strategies for control of acute infections of the nervous system.

3. Knowledge of the problem

The literature review, presented in 25 standard pages, summarizes the existing data on the clinical and epidemiological spectrum of acute infections of the nervous system and their etiology depending on the age of the patients. The modern methods for etiological diagnosis are critically analyzed, emphasizing the advantages and limitations of each of them. The data from the research in the investigated area are presented in a logical sequence, the main contradictions and the unclear aspects of the problem are well outlined, which shows an in-depth knowledge of the topic and skills for creative evaluation of scientific publications. The conclusions made from the literature review adequately motivate the working hypothesis of the study and the need to conduct it.

4. Study design

The aim of the study is clear and precisely formulated - to compare the microbiological and immunological methods for rapid etiological diagnosis in patients with acute infections of the CNS. To achieve it, five tasks are derived, which determine in a logical sequence the individual stages of the research.

The study design is adequate to the objectives. To assess the etiological structure and epidemiological features of acute neuroinfections, 1775 cerebrospinal fluid samples were analyzed, the antimicrobial susceptibility of non-viral isolates was studied in 211 bacterial and fungal pathogens, in 114 cases the microbiological methods for rapid etiological diagnosis were compared – direct microscopy, latex-agglutination test, and mPCR, and in 80 patients serum and cerebrospinal fluid concentrations of IL-6, IL-8, IL-10, IL-12 (p40), TNF- α , serum levels of C-reactive protein and procalcitonin were determined. The clinical and laboratory material is completely sufficient to obtain valid results and achieve the aim of the study. Precise including and excluding criteria for patient selection are set, which is also a prerequisite for reliability and authenticity of the results. Selected laboratory methods - direct microscopy, cerebrospinal fluid (CSF) culturing methods, Bauer-Kirby disk diffusion method for fast-growing bacteria, E-test for determination of minimum inhibitory concentrations in slow-growing microorganisms and automated methods for determination of antimicrobial susceptibility of the isolates, multiplex polymerase chain reaction (mPCR) for rapid in-vitro molecular genetic diagnostics, latex agglutination, enzyme-linked immunosorbent assay (ELISA) and enzyme-linked fluorescence assay are fully adequate and allow the tasks to be solved. The collected data are analyzed with modern and correctly applied statistical methods.

5. Characteristics and evaluation of the dissertation and contributions

The dissertation is presented on 181 standard typewritten pages, contains 11 chapters, and is sufficient in volume. It is illustrated very well with 29 tables, 89 figures, and 14 appendixes. The bibliography includes 213 literature references, 12 of which in Cyrillic and 201 in Latin. The obtained results, which occupy the largest relative share in the whole work, are presented in a logical sequence, corresponding to the defined tasks. The following evidence was obtained in the main directions of the research activity:

- The most common bacterial causes of acute neuroinfections in the Plovdiv region are *Coagulase-negative staphylococci* (which role is uncertain), *Streptococcus pneumoniae*, and *Listeria monocytogenes*, *Enteroviruses* are most often found among the viruses, and among the fungal isolates, the genera of *Candida* and *Cryptococcus* are equally represented.

- A specific age characteristic of the affected contingent was found for pneumococcal and listerial meningoencephalitis, and seasonality in *N. meningitidis*, coagulase-negative staphylococci, and the group of viridans streptococci.
- The use of multiplex PCR significantly increases the frequency of etiological agent detection.
- Bacterial neuroinfections show significantly higher levels of CSF IL-6, IL-8, IL-10, IL-12 (p40), TNF- α , and serum CRP, and procalcitonin when compared to viral.
- Combined testing of cerebrospinal fluid IL-12 (p40) and serum CRP showed the highest sensitivity in distinguishing bacterial from viral neuroinfections and a combined optimal absolute discriminative value of 144 was determined.

In the "Discussion" section the obtained results are thoroughly and consistently analyzed, and the own data are compared with the achievements of other authors. The differences are clearly outlined and scientifically substantiated hypotheses for their existence are proposed. The conclusions are clearly and precisely formulated and logically follow from the obtained results and their adequate interpretation. An original scientific contribution of mainly theoretical significance is the study of the discriminant value of some cytokines, CRP, and procalcitonin to distinguish bacterial from non-bacterial acute CNS infections. Of particular value is the determined combined absolute value 144, obtained from the summation of the cerebrospinal fluid concentration of IL-12 (p40) and the serum level of CRP, which can be used in clinical practice. A very significant practical contribution is the introduction of multiplex PCR in the rapid microbiological diagnosis in patients with acute neuroinfections, supported by the established significantly higher frequency of detection of the microbial agent compared to routine methods. Valuable for the clinical practice is the optimized algorithm for rapid etiological diagnosis of acute neuroinfections derived on the basis of the obtained data. Dr. Kalchev's dissertation outlines guidelines for future research to determine reference levels of cytokines and their diagnostic potential in acute socially significant infectious diseases, and thus to optimize the diagnostic and treatment process and reduce the frequency of complications in these patients.

6. Assessment of the publications and personal contribution

Dr. Kalchev has six publications related to the topic of the dissertation, one of which is review in a journal with IF. He is the first author of 4 of the articles. The results obtained from the study were presented at 7 scientific forums in Bulgaria and 5 abroad. The comparison with the requirements of the current Law on the Development of the Academic Staff in the Republic of Bulgaria and the Regulations of MU-Plovdiv shows that **Dr. Kalchev fulfills and even exceeds the set minimum scientometric indicators for "PhD"**.

After getting acquainted in detail with the dissertation and the presented documents, I am convinced that the research, the results obtained and the formulated conclusions and contributions are the personal merit of the doctoral student.

I have no critical remarks and recommendations.

7. Author's summary of the dissertation

The presented summary of the dissertation has 52 pages and is structured according to the requirements. Its content sufficiently reflects the main results of the dissertation.

CONCLUSION

The dissertation contains scientific and practical research results, which represent an original contribution to science and meet all the requirements of the Law for Development of the Academic Staff in the Republic of Bulgaria (LDASRB), the Regulations for application of LDASRB, and the Regulations of the Medical University of Plovdiv. The dissertation shows that the doctoral student Dr. Yordan Kalchev has in-depth theoretical knowledge and professional skills in the scientific specialty of Microbiology, demonstrating qualities and skills for independent research. Based on this, I confidently give my **positive assessment** for the research presented by the above peer-reviewed dissertation, author's summary, results, and contributions, and I invite the esteemed scientific jury to award the educational and scientific degree "DOCTOR OF PHILOSOPHY" to Dr. Yordan Ivanov Kalchev in a doctoral program "Microbiology".

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