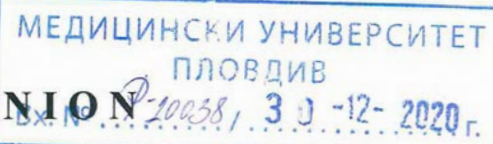


TO

THE CHAIR OF THE SCIENTIFIC ASSESSMENT COMMITTEE
ASSIGNED UNDER ORDINANCE № P-1247/2.12.2020
OF THE RECTOR OF MEDICAL UNIVERSITY - PLOVDIV

STATEMENT OF OPINION



By Assoc. Prof. Victoriya Levterova, PhD
National Reference Laboratory of Molecular Microbiology
National Center of Infectious and Parasitic Diseases

On: PhD Thesis of Yordan Ivanov Kalchev, MD

On topic: „MICROBIOLOGICAL AND IMMUNOLOGICAL APPROACHES FOR RAPID ETIOLOGICAL DIAGNOSIS OF ACUTE CENTRAL NERVOUS SYSTEM INFECTIONS”

For awarding educational and scientific degree “**Doctor of Philosophy**”,

In the area of higher education: 4. „Natural sciences, mathematics and informatics”,

Professional field: 4.3. „Biological sciences”,

A doctoral program in the scientific specialty: Microbiology

Form of doctoral study: independent

Department: Microbiology and Immunology, Medical University - Plovdiv

Scientific supervisor: Prof. Marianna Atanasova Murdjeva, MD, PhD, MHM

The PhD candidate Yordan Ivanov Kalchev, MD **has submitted the relevant set** of documents and materials on electronic media to participate in the announced competition in accordance with the Law for the development of the academic staff in the Republic of Bulgaria, Art. 115 of the Procedure for obtaining scientific and educational degree “Doctor of Philosophy” in Medical University - Plovdiv and the Regulations of Medical University – Plovdiv from 06.11.2014.

The scientific publications of Kalchev, MD associated with the PhD thesis are a total of 6 titles of scientific journal articles. In addition, the candidate submitted a list of reports, including 7 participations in scientific forums in the country and 5 participations in scientific forums abroad. These report mainly the scientific results related to the thesis. The doctoral student has also **taken part** in 2 national and 2 intra-university projects.

From the scientific production and publication activity of Kalchev, MD it can be seen that he is an active, productive, and in-depth researcher.

The doctoral student has very correctly and precisely prepared and arranged the materials and documents for the competition, which makes a very good impression.

The actuality of the topic and relevance of the aim and the objectives

Central nervous system (CNS) infections, like medical emergencies requiring immediate diagnosis and immediate treatment, continue to be a current problem of the 21st century. Despite the use of increasingly modern antibiotics and efforts to improve patient care, this disease is still an unresolved issue in clinical medicine with over 40% mortality. It should be added that the diagnosis in these cases is extremely difficult, especially due to the fact that they can be caused by various bacterial pathogens. Therefore, the development of increasingly rapid and accurate diagnostic methods remains a constant task for microbiologists worldwide. In 2015, the WHO announced a strategy to deal with meningitis, including 5 axes, one of which concerns the development of rapid diagnostic tests. Identifying the cause in a short time not only ensures the proper treatment of patients but in most cases, it is a life-saving factor.

Recently, there has been an increase in the incidence of CNS infections, despite significant advances in infection control and public health. We believe that this is largely due to the increased antibiotic resistance, which severely limits the treatment options and the choice of empirical antibiotic therapy. A significant factor is also the growing number of immunocompromised individuals due to the increase in autoimmune and oncological diseases.

Given the high mortality and subsequent neurological impairments among patients with neuroinfection, the social significance of this disease is defined as extremely high.

It is known that many different infectious agents can lead to infection of CNS - bacteria, fungi, viruses, and parasites. This is the reason for the difficulties in identifying the specific cause and the choice of appropriate therapy. As the treatment varies according to the etiological agent, early detection of the etiology is vital.

The main focus of the work is to create a scientific basis for rapid diagnosis and improvement of the control of CNS infections.

Knowledge of the problem

Specifying the etiology of CNS infections is a process requiring a comprehensive approach and high competence.

I would like to note that this is a PhD thesis that provides diagnostic laboratories with up-to-date solutions and approaches for the timely diagnosis of CNS infections. The PhD candidate had the chance to work under the supervision of Prof. Mariana Murdjeva, a recognized authority in the country and abroad in the field of Clinical Microbiology and Immunology.

I believe that the PhD thesis of Kalchev, MD is very relevant, given the **large-scale study** for the Plovdiv region over the period 2013-2019. The PhD candidate evaluates the diagnostic value of the microbiological methods used by him - direct microscopy, latex agglutination test, as well as the multiplex polymerase chain reaction. It also analyzes the antimicrobial susceptibility of cerebrospinal fluid non-viral isolates and studies the role of cytokines IL-6, IL-8, IL-10, IL-12, TNF- α , the classical cerebrospinal fluid clinical-laboratory parameters, as well as serum CRP and PCT for the rapid etiological diagnosis of acute CNS infections. The PhD candidate develops a diagnostic algorithm to support the rapid etiological diagnosis in patients with acute CNS infections.

These facts themselves show that Kalchev, MD is well acquainted with the problems considered in his thesis. His personal participation in the work on the experimental and diagnostic part is also evident.

Research methodology

The research methods that are chosen by the PhD candidate and his scientific supervisor allow it to achieve the set aim and to obtain an adequate answer to the objectives solved in the doctoral thesis.

Characteristics and evaluation of the doctoral thesis

The thesis is constructed in a traditional form with the relevant sections. It is written on 181 pages. It is illustrated with 29 tables, 89 figures, and 14 appendices.

All necessary sections of the dissertation are well designed. The bibliographic reference covers 213 literature sources.

The introduction summarizes the topicality and the need to develop the theme by the PhD candidate.

The literature review presents the spectrum and etiology of acute CNS infections. The course of CNS infections was analyzed depending on the etiological cause. The PhD candidate has thoroughly examined the types of laboratory diagnostics for acute CNS infections and has also presented historical facts in connection with some of the diagnostic methods. Changes in biological markers - cytokines, C-reactive protein (CRP), and procalcitonin - are also considered. The empirical antimicrobial chemotherapy for various etiological agents is discussed.

Based on this in-depth analysis, the aim of the present doctoral thesis is brought - to compare the microbiological and immunological methods for rapid etiological diagnosis in patients with acute CNS infections.

I believe that the goal and the five tasks for its implementation are formulated correctly and reflect the main elements of the dissertation.

This includes improving the control of these diseases, which increases the level of significance of the dissertation.

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In the section **materials and methods**, the design of the study is presented, which covers a period of seven years (from January 2013 to December 2019).

The object of observation for the doctoral thesis is the results of clinical - laboratory examination of cerebrospinal fluid and serum, microbiological analysis, levels of cytokines determined in serum and cerebrospinal fluid, and CRP and PCT values in patients with suspected central nervous system infection.

Over the period of 2013-2019, 1775 clinical samples of cerebrospinal fluid were examined, corresponding to 1258 patients hospitalized in clinics of the "St. George" University Hospital Plovdiv. During the same period, 211 bacterial and fungal pathogens were isolated from the collected clinical specimens.

114 patients were selected based on inclusion and exclusion criteria for the study and 80 patients were selected from some additional inclusion criteria for determining the concentrations of cytokines IL-6, IL-8, IL-10, IL-12, TNF- α , and the biomarkers CRP and PCT.

It is obvious that the volume and diversity of the research materials are completely sufficient. The subsection methods briefly, but very accurately and clearly describes the routine methods for microbiological and immunological diagnostics used by Kalchev, MD. Molecular genetic methods based on multiplex PCR are also described. The method simultaneously detects 14 specific genes of the most common pathogens associated with central nervous system infections. The PhD candidate also described in detail the statistical methods used in the doctoral thesis.

The own research results correspond to the set objectives and are described on 54 pages. They are perfectly illustrated with 11 tables and 88 figures. The results of the set 5 objectives are described in detail. After the section with the own results, an in-depth summary discussion of all obtained results was made. The extremely valuable merit of the PhD candidate is that he also offers an exemplary algorithm for optimizing the rapid etiological diagnosis in patients with acute neuroinfections.

The conclusions made by the PhD candidate are a logical consequence of the experimental data and provide the necessary information about the value of the research.

I fully accept the ten conclusions made by Yordan Kalchev, MD.

Based on the completed work, the PhD candidate makes 6 contributions of scientific and theoretical nature, 4 of which are of original character and 2 contributions of confirmatory nature, as well as 3 contributions of mainly applied nature. I also accept the wording of the contributions.

Bibliography. A total of 213 literature sources are reflected in the review. All of them fully correspond to the set objectives. Over 70% of the articles are from the last 10 years, and over 20% from the last 5, which emphasizes once again its relevance.

Publications and other scientific activities related to the thesis. The PhD candidate has presented 6 publications in renowned scientific journals. He is the first author in 4 of the articles, which gives me the reason to believe that the main contribution to the doctoral thesis is attributed to the PhD candidate. He has participated in 12 scientific forums - 5 international and 7 national congresses.

The personal involvement of Kalchev, MD in the thesis is evident.

The author's summary **is designed according to the requirements and reflects the main results achieved in the thesis.**

CONCLUSION The PhD thesis **contains scientific, scientific-applied, and applied results, which represent an original contribution to science and meet all the requirements** of the Law for the development of the academic staff in the Republic of Bulgaria, The Rules of its application, and the Regulations of Medical University – Plovdiv. The presented materials and PhD thesis **fully comply** with the specific requirements of MU - Plovdiv.

The doctoral thesis demonstrates that the PhD Candidate Yordan Ivanov Kalchev, MD **has** profound theoretical knowledge and professional skills in the scientific specialty of Microbiology, demonstrating qualities and skills for independent research.

Given the above, I confidently give my **positive assessment** of the conducted study, presented to me by the above-reviewed PhD thesis, author's summary, results, and contributions, and **I propose to the esteemed members of the scientific assessment committee to award the educational and scientific degree "Doctor of Philosophy"** to Yordan Ivanov Kalchev, MD in a doctoral program in Microbiology.

20.12.2020

The statement is prepared by:

Assoc.Prof. Victoriya Levterova, PhD