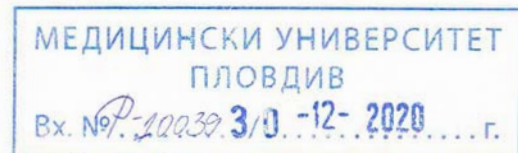


TO

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



REVIEW

By: Prof. Todor Veselov Kantardjiev, MD, DSci, MHM

Director of the National Center of Infectious and Parasitic Diseases, Sofia

Of: 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 of Microbiology

Form of doctoral study: independent

Department: Microbiology and Immunology, Medical University - Plovdiv

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

To participate in the announced competition, the PhD candidate Yordan Ivanov Kalchev, MD **has submitted the relevant** set of documents and materials on electronic media 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, which includes the following documents:

- Application to the Rector of Medical University – Plovdiv to open a procedure of the public defense of the doctoral thesis
- European format of CV signed by the PhD candidate
- A notarized copy of the Diploma for Higher Education
- Orders for enrollment in doctoral studies, interruption of studies (due to maternity) and for a continuation of studies; for completion with the right of defense
- Order for conducting an exam from the individual plan and a respective protocol for passing an exam or a doctoral minimum in the specialty

- Minutes from the department council for a preliminary discussion of the doctoral thesis and the decisions taken for opening the procedure and for the members of the scientific assessment committee
- A PhD thesis
- An Author's summary
- A list of the scientific publications associated with the topic of the thesis
- Copies of the scientific publications
- A list of the attended scientific forums
- A list of citations
- declaration of originality and authenticity of the attached documents
- other documents, associated with the procedure

The list of scientific publications of Kalchev, MD includes a total of 6 scientific articles. In addition to the mentioned scientific papers, the candidate has also submitted a list of abstracts, including 7 participations in scientific forums in the country and 5 participations in scientific forums abroad. These report mainly scientific results. The PhD candidate has also taken part in 2 national and 2 intra-university projects.

The analysis of the scientific production and the publishing activity of Kalchev, MD shows that he is an active and productive researcher.

The correct and precise preparation and arrangement of the materials and documents for the competition makes a very good impression.

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

Infections affecting the central nervous system (CNS) are a current health problem of great importance worldwide. In Bulgaria, according to the National Center for Infectious and Parasitic Diseases, neuroinfections are the main cause of mortality among acute infectious diseases and account for 40% of all deaths in the country.

In recent years, there has been an increase in their frequency, despite significant advances in infection control and public health. This is largely dependent on the continuous increase in antibiotic resistance. This severely limits the treatment options and the choice of empirical antibiotic therapy. Another factor in increasing the incidence of CNS infections is the increase in the number of immunocompromised individuals due to the increase in autoimmune and oncological diseases and in connection with this the widespread use of immunosuppressive drugs.

The high mortality rate and the frequency of subsequent permanent neurological damage among patients who have survived neuroinfection determine the social significance of this disease.

Meningitis is one of the most common acute diseases of the central nervous system and is among the 10 leading infectious causes of death in the world. Over 1.2 million cases of bacterial

meningitis occur annually. Various infectious agents can lead to infection of the CNS - bacteria, fungi, viruses, and parasites. They differ greatly in the geographical region, country, age, and immunological reactivity of the macro-organism. This etiological diversity makes it very difficult both to identify the specific cause and to choose the appropriate therapy. Therefore, rapid and accurate detection of the infectious agent is essential for the outcome and prognosis of CNS disease. As the treatment varies according to the etiological agent, early detection of the etiology is vital. This directs the attending physician to choose the most appropriate therapy, and hence directly affects the outcome of the disease.

Timely and rapid up-to-date etiological diagnosis in patients with acute CNS infections would lead to a reduction in hospital stays, reduction of death in some of the cases, reduction of treatment costs due to the use of inadequate antimicrobial therapy.

Knowledge of the problem

CNS infections are caused by a large number of different pathogens. Of particular importance for clinical practice is the early differentiation of bacterial from viral neuroinfections, due to the fact that viral diseases as the most common cause of aseptic meningitis have a better course and do not require antibiotic treatment. And in turn, bacterial infections require antibiotic treatment. Determining the etiology of CNS infections is a process that requires a comprehensive approach.

I believe that the thesis of the PhD candidate Yordan Kalchev, MD is very timely and up-to-date, given the **large-scale study** of current data on the etiological structure and epidemiological features of non-viral cerebrospinal fluid isolates in Plovdiv for the period 2013-2019. In his work the PhD candidate has evaluated the comparative diagnostic value of the microbiological methods applied by him - direct microscopy, latex - agglutination test, and mPCR; He also analyzed the antimicrobial susceptibility of cerebrospinal fluid non-viral isolates. From an immunological point of view, he studied the role of the cytokines IL-6, IL-8, IL-10, IL-12, TNF- α , classical cerebrospinal fluid laboratory parameters, as well as serum CRP and PCT for rapid etiological diagnosis in patients with acute CNS infections.

Based on the results obtained, he developed a diagnostic algorithm to support rapid etiological diagnosis in patients with acute CNS infections.

All this speaks of the excellent knowledge of the problem which is considered in the doctoral thesis of Kalchev, MD, as well as his personal involvement in the work on the experimental and diagnostic part.

Research methodology

The selected research methods allow to achieve the set aim and to obtain an adequate answer to the objectives formulated in the thesis.

Characteristics and evaluation of the doctoral thesis

The thesis is properly structured and is written on 181 standard typewritten pages, and is formed in 11 chapters. It is illustrated with 29 tables, 89 figures, and 14 appendices.

All necessary sections of the PhD thesis are well designed: introduction, literature review, aim and objectives, materials and methods, results and discussion, conclusions, contributions, used literature, publications related to the topic of the thesis, and attended scientific forums. The bibliographic reference covers 213 literature sources.

The introduction summarizes the topicality of the problem in the world and our country, and the need to elaborate on the subject by the PhD candidate.

The literature review presents an analysis of the anatomical features of the central nervous system and its location. The spectrum, etiology, and symptomatology of acute CNS infections are also presented in detail. A thorough analysis of the course of CNS infections was performed depending on the etiological cause.

The etiological diagnosis and treatment of patients with neuroinfections is a big challenge for medical practice. The wide variety of pathogens associated with CNS disease sometimes makes it difficult to identify the specific cause and select the appropriate antimicrobial therapy. Of great importance is the timely and proper therapy. All this is directly related to mortality and subsequent neurological complications.

The review also focuses on the types of laboratory diagnostics in acute CNS infections, such as clinical-laboratory and microbiological examination of cerebrospinal fluid, direct microscopy, cerebrospinal culturing, latex agglutination test, molecular genetic analysis. Historical data in connection with some of the diagnostic methods are also presented.

Changes in biological markers - cytokines, C-reactive protein (CRP), procalcitonin - are also considered.

The various etiology is a prerequisite for the need to use different classes of antimicrobial drugs - antibiotics, antifungals, antivirals, or antiparasitic drugs. In the literature review, Kalchev, MD has also touched on the empirical anti-microbial chemotherapy in the different etiological causes.

In conclusion, emphasizing the importance of the problem, the PhD candidate logically reaches **the aim of the thesis, namely** to compare the microbiological and immunological methods for rapid etiological diagnosis in patients with acute CNS infections. The aim and the five objectives for its implementation are formulated correctly and reflect the main elements of the doctoral thesis.

In the section on materials and methods, the PhD candidate has presented in detail the design of the study, spanning a 7-year period from January 2013 until December 2019;

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

On the first objective for the period 2013-2019 were studied 1775 clinical samples of cerebrospinal fluid, corresponding to 1258 patients hospitalized in clinics of the “St. George” University Hospital Plovdiv.

On the second objective, 211 bacterial and fungal pathogens were isolated for the same period.

On objective 3, Kalchev, MD selected 114 patients on the basis of inclusion and exclusion criteria for the study.

On the fourth objective - to determine the concentrations of the cytokines IL-6, IL-8, IL-10, IL-12, TNF- α , and the biomarkers CRP and PCT, the PhD candidate selected 80 patients, according to some additional criteria for the prospective period studied.

I believe that the volume and diversity of the research materials are quite sufficient to guarantee their representative nature. The section materials and methods present schematically and very clearly the design of the study.

The subsection methods are much more extensive. The routine methods for microbiological diagnostics are described briefly, but very precisely and clearly - direct microscopy, culture methods, determination of the antimicrobial susceptibility of isolates. Kalchev, MD also described the molecular genetic methods based on two-step multiplex PCR. The test is qualitative and simultaneously detects specific genes of 14 of the most common bacteria, fungi, and viruses associated with central nervous system infections. The immunological methods used in the thesis, which are the basis of the dissertation, are described in detail, namely LAT for rapid identification of cerebrospinal fluid pathogens, ELISA, Enzyme-linked fluorescence test (ELFA) for measuring procalcitonin in serum, and immunoturbidimetry to measure serum CRP. The statistical methods used are also presented in detail.

The own research corresponds to the set objectives. The obtained results represent an essential section - 54 pages, from the doctoral thesis of the PhD candidate. They are very well illustrated with 11 tables and 88 figures. This section describes in detail the results of the 5 tasks. After each completed task, an in-depth discussion of the result is made, which aids the perception of the large factual material.

After the section of own results, a thorough general discussion of all the obtained results was made, which clearly demonstrates the issues. It clarifies the problems and identifies and proposes concrete measures. The thesis summarizes well the obtained results, proposing an exemplary algorithm for optimizing the rapid etiological diagnosis in patients with acute neuroinfections.

Based on this discussion, the PhD candidate presents ten conclusions that have theoretical and practical value.

I fully approve of the specific conclusions.

Based on the completed work, the author defines 6 contributions of scientific and theoretical nature, 4 of which are original and 2 contributions of a confirmatory nature, as well as 3 contributions of mainly - applied nature, with which I agree.

Bibliography. The thesis includes 213 references with over 70% of the literary sources are from the last 10 years.

Publications and other scientific activities related to the dissertation.

Yordan Kalchev, MD presents the results of his thesis in a total of 6 publications in renowned scientific journals, and he is the first author in 4 of the publications. He has participated in 12 scientific forums: 5 international and 7 national congresses.

All this proves the personal participation of Kalchev, MD in the doctoral research, and that the formulated contributions and results obtained are his personal merit.

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

Reviewer:

Prof. Todor Kantardjiev, MD, DSci, MHM