STATEMENT OF OPINION

by Prof. Maria Petrova Georgieva - Sredkova, M.D., Ph.D.

Research Institute

Medical University - Pleven

of a Ph.D. thesis for awarding the educational and scientific degree 'Doctor of Philosophy'
Professional field: 4.3. "Biological sciences"
Doctoral program: Microbiology
Author: Yordan Ivanov Kalchev, M.D.
Form of doctoral studies: independent
Department: Microbiology and Immunology
Topic: "Microbiological and immunological approaches for rapid etiological diagnosis of acute central nervous system infections"
Scientific Supervisor: Prof. Marianna Atanasova Murdjeva, M.D., Ph.D., M.H.M, Medical University - Plovdiv

1. Overview of the procedure and the Ph.D. candidate

The presented set of paper/electronic documents is in accordance with Art. 115 of the Procedure for obtaining scientific and educational degree "Doctor of Philosophy" in Medical University – Plovdiv; 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 public defense of the doctoral thesis
- European format of CV signed by the Ph.D. candidate
- A notarized copy of the Diploma for Higher Education
- Orders for enrollment in doctoral studies; for completion with the right of public defense
- Order for conducting an exam from the individual plan and a respective protocol for passing an exam on 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 Ph.D. 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 declaration of originality and authenticity of the attached documents
- A list of the attended educational courses associated with the topic of the thesis

The Ph.D. candidate has attached 6 publications and 12 scientific reports in association with the thesis.

The set of materials is arranged sequentially and is very well presented.

Introducing the Ph.D. candidate

Yordan Ivanov Kalchev, M.D. has acquired a Master's degree in Medicine in 2014 from the Medical University - Plovdiv. Since May 2015 he has been working as a doctor in the Clinic of Neurology of the St. George University Hospital - Plovdiv. Since March 2016 he has been an assistant in the Department of Microbiology and Immunology at MU-Plovdiv, and since 2017 he has been a resident in Microbiology.

In July 2019, Kalchev, M.D. was enrolled in a doctoral program in Microbiology as an independent Ph.D. student. He completed the Ph.D. program by Ordinance of the Rector of the Medical University of Plovdiv (R-2714/05.10.2020). The thesis was discussed, accepted, and directed for defense on 31.07.2020 by the Extended Department Council of the Department of Microbiology and Immunology, MU-Plovdiv.

As part of the ERASMUS + exchange program, the Ph.D. candidate has completed one-week internships in microbiology at Julius-Maximilians-Universitat (Würzburg, Germany), University of Latvia (Riga, Latvia), and University of Rijeka, Croatia. He attended also a one-week educational course on vector-transmitted infections in Salzburg, Austria, two three-day courses on CNS infections in Munich, Germany and Grenoble, France, and a two-day course on molecular diagnostics at the National Center of Infectious and Parasitic Diseases (NCIPD)-Sofia.

He has participated in the National Project for Doctoral Studies at MU-Plovdiv, in the National Program "Young Scientists and Post-Doctoral Students" and was a principal researcher in two research projects funded by MU-Plovdiv.

2. Actuality of the topic

The accurate etiological diagnosis of neuroinfections is essential for the selection of adequate antibacterial therapy and the outcome of the disease. Despite the great achievements in the microbiological diagnosis of infections of the central nervous system, a significant proportion of them still remain of unknown etiology. It imposes the need for searching new diagnostic approaches, including molecular biological methods to identify the potential causes and also the use of inflammatory biomarkers for early differentiation of bacterial from viral neuroinfections. The introduction of new methods and approaches will contribute to a more favorable outcome of these infections in individual patients and will have great health and social effect. This makes the presented doctoral thesis very relevant in scientific and scientific-applied aspect.

3. Knowledge of the problem

The Ph.D. candidate is well acquainted with the traditional and modern microbiological and immunological approaches for rapid diagnosis of acute neuroinfections, which allows him to analyze the results of his own research very well.

4. Research methodology

In order to achieve the set aim and solve the related objectives in the thesis, a modern methodological approach has been used, which allows for obtaining and analyzing correct results.

5. Characteristics and evaluation of the doctoral thesis

The thesis is written on 181 standard typewritten pages and is structured according to the generally accepted rules. It includes: title page; abbreviations - 3 pages; introduction - 3 pages; literature review - 24 pages; purpose and objectives - 1 page; material and methods - 19 pages; results - 54 pages; discussion - 19 pages; conclusions - 3 pages and contributions - 2 pages. It is illustrated with 29 tables, 89 figures and 14 appendices.

The *bibliography* includes 213 literature sources, of which 77 (36.15%) are from the last 5 years.

The *literature review* presents and thoroughly analyzes data on the etiology and methods for diagnosing infections of the central nervous system. Special attention is paid to molecular biological methods for rapid diagnosis of acute meningitis and meningoencephalitis, and the possibilities of using some biological markers for distinguishing bacterial from viral neuroinfections. The review ends with a summary and conclusions that reflect the essence of the developed work.

The *purpose* of the thesis is formulated precisely. The 5 objectives arising from it correspond to the topic and their implementation leads to the fulfillment of the set aim.

The *material and methods* section describes the object and the observation units, including 1,775 cerebrospinal fluid samples, 211 microbial isolates, and 194 patients were selected based on well-formulated inclusion and exclusion criteria. An informative scheme of the study design was presented. Selected methods for laboratory analysis (direct microscopy and culture of cerebrospinal fluid, testing of susceptibility to antimicrobial agents, multiplex PCR and immunological methods for rapid detection of pathogenic microorganisms in cerebrospinal fluid, determination of the levels of certain cytokines in serum and cerebrospinal fluid, determination of the levels of procalcitonin and C-reactive protein in serum) are described accurately and in detail. The statistical analysis of the results was performed with very well selected modern methods, which allows to correctly formulate the conclusions and contributions of the study.

The *results* of Kalchev, M.D.'s research are presented and illustrated very well and their description corresponds to the objective set. *S. pneumoniae* is the leading cause of bacterial meningitis with a predominance of non-vaccinal serotypes 3 and 19a and relatively high resistance to penicillin. Fungi of the genus *Candida* and *Cryptococcus* are the main causes of mycotic meningitis with a pronounced predominance of *non-albicans* species, and enteroviruses are the leading causes of viral neuroinfections. The use of multiplex PCR in the early stages of infection increases the relative proportion of etiologically proven bacterial and viral neuroinfections. Higher values of IL-6, IL-8, IL-10 and IL-12 in the cerebrospinal fluid of patients with bacterial meningitis have been found, but only the values of IL-6 in the serum and IL-12 in the cerebrospinal fluid have a discriminative effect on differentiation of bacterial from viral neuroinfections. The combination of elevated levels of IL-12 in the cerebrospinal fluid and C-reactive protein in the serum has the highest discriminative power.

The *discussion* and interpretation of the obtained results were performed thoroughly and professionally.

The *conclusions* of the study reliably reflect the results obtained and fully comply with the objectives.

A total of 9 *contributions* were formulated, four of which were of an original nature, two of a confirmatory nature, and three of an applied nature. The main practical contribution to the country is the proposed algorithm for optimizing the rapid etiological diagnosis in patients with acute neuroinfection.

6. Evaluation of the scientific publications and the personal contribution of the Ph.D. candidate

In association with the thesis, 5 publications were published, four of which in journals referenced in world databases (one in a journal with SJR-0,26) and one in a journal with a scientific review. There is one article in press in a refereed foreign journal with IF-2,952. In four of the publications Kalchev, M.D. is the first author, which shows his leading role and is proof that the thesis is his personal act. The Ph.D. candidate has 7 attendances in scientific forums in the country, 5

abroad, and 2 participations in research projects. This high scientific activity fully meets the criteria of MU-Plovdiv for the educational and scientific degree "Doctor of Philosophy".

7. Author's summary

The author's summary is written on 51 pages and meets the requirements for the layout and content. It correctly presents the methodology, the main results of the study and their discussion, the conclusions, and contributions. It is illustrated very well with a sufficient number of tables and graphics.

CONCLUSION

The Ph.D. thesis entitled "Microbiological and immunological approaches for rapid etiological diagnosis of acute central nervous system infections meets 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 results of the Ph.D. thesis fully comply with the specific requirements of MU - Plovdiv.

The doctoral thesis demonstrates that the Ph.D. candidate Yordan Ivanov Kalchev, M.D. has profound theoretical knowledge and professional skills in the scientific specialty of Microbiology, and is capable of independent research.

Given the above, I **give my positive assessment** of the Ph.D. thesis, 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, M.D. in a doctoral program in Microbiology.**

11.01.2021 г. The statement is prepared by: .....

Prof. Maria Petrova Georgieva - Sredkova, M.D., Ph.D.