To the members of the scientific jury
Defined by Order No. P-1247/ 02.12.2020
of the Rector of MU-Plovdiv

RECENSION

Of Prof. Dr. Iva Stefanova Christova, MD, Ph.D., D.Sc.
National Center of Infectious and Parasitic Diseases

Concerning Dissertation thesis for the award of the educational and scientific degree "DOCTOR" in the scientific specialty "Microbiology"

Author of the thesis: Yordan Ivanov Kalchev, MD
Form of doctoral studies: independent preparation
Department: "Microbiology and Immunology", PhF, MU-Plovdiv

Topic: "Microbiological and immunological approaches for rapid etiological diagnosis of acute infections of the central nervous system"
Scientific adviser: Prof. Mariana Murdzheva, MD, PhD, MHM

General presentation of procedure and doctoral student

A set of materials on electronic media is presented, which is in accordance with Art. 115 (1) of the Procedure for acquisition of ONS "Doctor" in MU-Plovdiv and the Regulations of MU-Plovdiv from 06.11.2014 and includes the necessary list of documents.

Brief biographical data about the doctoral student

Dr. Yordan Ivanov Kalchev graduated in medicine at Medical Faculty of MU-Plovdiv in 2014 with excellent score in the course and full honors from the state exams. He began his doctoral internship as a doctor in the Department of Nervous Diseases at

the University Hospital "St. Georgi "in 2015, since 2016 he has been an assistant at the Department of Microbiology and Immunology, MU-Plovdiv, and since 2017 - a specialist in microbiology. Thus, combining his interests in neurology and microbiology, the doctoral student logically chooses a topic for a dissertation on approaches to the diagnosis of acute CNS infections. A large number of internships and courses in cell biology, encephalitis, CNS infections and their molecular diagnostics at prestigious European universities followed. They all are the valuable foundation on which Dr. Kalchev builds in order to achieve impressive theoretical and practical training.

Dr. Yordan Kalchev speaks English and German. He is a member of prestigious scientific organizations: European Society of Clinical Microbiology and Infectious Diseases, Bulgarian Association of Microbiologists, Bulgarian Microbiological Society. He supervises practical exercises in microbiology and immunology in English and Bulgarian.

Relevance of the topic and expediency of the set goals and objectives

The dissertation of Dr. Kalchev is extremely relevant. CNS infections are severe, disabling, and often fatal. They require reliable and rapid diagnosis in order to quickly begin adequate etiological treatment. The mediators of inflammation in them are still insufficiently studied. The targeted study of the main biomarkers in CNS infections is strategic in order to use them in practice in a differential diagnostic plan.

My general impression of the dissertation is that it is an excellently conceived, precisely executed at a high methodological level and perfectly formed work, written very intelligently, with comprehensive information in all its sections, with many merits and contributions. It contains a wealth of evidence and is very well illustrated with 29 tables, 89 figures and 14 attached material from his own research.

The paper contains 181 pages. The main part of the experimental work was performed in the Department of Microbiology and Immunology at MU-Plovdiv, University Hospital "St. George" - Plovdiv and Research Institute of MU-Plovdiv, and part of the microbiological tests were performed or confirmed in NCIPD and MU-Sofia.

Based on 213 literature sources, the doctoral student makes an in-depth literature review of 35 pages. The review is one of the great merits of the work, it was done

excellently and I would recommend it to be published separately to help the specialists. It makes a very good impression that there is no redundant information, only that which concerns his research is included. The information is described in detail and at the same time concise.

The literature review ends with a summary of the unsolved problems, which logically leads to the goal of the dissertation: to analyze comparative microbiological and immunological methods for rapid etiological diagnosis in patients with acute CNS infections. The tasks for achieving this goal are 5, are clearly formulated and outline the specific areas of research.

Research methodology

The materials and methods of the dissertation are presented comprehensively and concisely. They are a total of 19 pages and include a description of the studied huge number of clinical materials cerebrospinal fluid (1775), bacterial and fungal pathogens (211), patients (114 for task 3 and 80 for task 4), presentation of signs of observation: clinical data, results of clinical-laboratory and instrumental research. Attention is paid to the selection of patients for whom all inclusion and exclusion criteria are described, which is the basis for obtaining reliable results. The methods used for conventional microbiological diagnostics are presented in a concise way: direct microscopy, cultivation and determination of susceptibility of isolates to antimicrobial agents. The film array molecular genetic approach used for rapid detection of pathogens in cerebrospinal fluid is presented in detail. Additionally, the PhD student has mastered and used a wide range of immunological techniques, including latex agglutination enzyme-linked immunosorbent assay (ELISA) to determine serum and cerebrospinal fluid cytokine levels, enzyme-linked fluorescence test (ELFA) in serum and an immunoturbidimetric method for measuring serum C-reactive protein values. The huge set of statistical methods used for evaluation and analysis of the obtained results is also impressive.

The way of presenting the various techniques used speaks of the excellent methodological readiness of Dr. Kalchev.

Characteristics and evaluation of the dissertation

The results of his research are presented and discussed on 73 pages. Description of the results is accurate and informative. The results are presented in 5 sections, following the logical sequence of the set tasks.

Task 1 presents current data on the etiological structure and epidemiological features of cerebrospinal fluid isolates, including 24 species of bacteria and fungi, a total of 211 isolates from the territory of Plovdiv district for a period of 7 years (2013-2019). It is noteworthy that in addition to bacteria, pathogenic fungi are also in demand, so the results are interesting. Representatives of *Candida* spp., *Cryptococcus* spp. and *Aspergillus* spp. A demographic characterization of the patients was made depending on the pathogens isolated from their cerebrospinal fluid. Results were analyzed by sex and age for the most common causes of infections acquired in society - pneumococcal and listeriosis neuroinfections.

Task 2 analyzes antimicrobial susceptibility of successfully cultured cerebrospinal fluid isolates - both bacterial and fungal. Important data have been established regarding trends in the development of antimicrobial sensitivity in our country.

Task 3 provides a comparative assessment of the diagnostic value of the most commonly used methods for rapid etiological diagnosis in patients with acute CNS infections. 114 cerebrospinal fluid samples were examined simultaneously with direct microscopy, latex agglutination, cultivation and multiplex PCR. In 42 of the patients, etiological diagnosis was established microbiologically: 23 bacterial pathogens, 17 viral and 2 cases of pathogenic fungi. Sensitivity and specificity, positive and negative predictive value of the used microbiological methods were calculated. Multiplex PCR possessed the highest sensitivity.

Under task 4, a pioneering study was made for our country on the role of cytokines IL-6, IL-8, IL-10, IL-12, TNF-α and levels of serum CRP and procalcitonin in the etiological diagnosis of acute CNS infections. Demographic characteristics of 80 studied patients and etiology of the detected pathogens are presented. Cytokine levels were tested in parallel in serum and cerebrospinal fluid. Higher levels of IL-6 and IL-8 in the cerebrospinal fluid than in serum were found in viral, bacterial and etiologically unexplained acute neuroinfections, as well as IL-10 and TNF-α only in bacterial neuroinfections. Only IL-12 levels are higher in serum than in cerebrospinal fluid in

viral neuroinfections. Significantly higher levels of CRP and procalcitonin in serum were found in bacterial neuroinfections compared to viral, etiologically unclear and control groups. Using ROC curves for each cytokine tested, its significance was analyzed. Similarly, a ROC assay was performed for serum concentrations of CRP and procalcitonin. Serum IL-12 and serum CRP levels were found to have the highest discriminant value to distinguish bacterial from viral etiology.

In Task 5, an exemplary diagnostic algorithm has been developed to support rapid etiological diagnosis of patients with acute CNS infections. The algorithm is prepared on the basis of the analysis of the results obtained in the dissertation. It is centered on the combination of levels of IL-12 in the cerebrospinal fluid and serum CRP, indicating possible viral or bacterial infection of the CNS, for cerebrospinal fluid samples in which standard microbiological and molecular methods have not detected a pathogen.

Contributions and significance of development for science and practice

A large volume of valuable experimental data was obtained. Significant scientific-theoretical and applied contributions have been achieved. Many original contributions are essential, among which are the high diagnostic value of multiplex PCR in the diagnosis of acute CNS infections; the country's pioneering studies on the discriminant value of cytokines, levels of CRP and procalcitonin to differentiate bacterial from viral neuroinfection, on the basis of which importance of the combined study of cerebrospinal fluid IL-12 and serum CRP was derived.

I also appreciate the contributions of confirmatory and scientifically applied nature, namely: establishing discriminatory role of cytokines to differentiate bacterial from viral CNS infection, determining diagnostic value of standard microbiological methods and multiplex PCR, as well as proposed practical algorithm to help rapid etiological diagnosis of acute neuroinfections.

Evaluation of dissertation publications

A list of 6 publications and 12 participations in scientific forums (7 national and 5 abroad) related to the dissertation is attached. The fact that in 4 out of 6 presented publications, as well as in 7 out of 12 presented participations in scientific forums, Dr.

Kalchev is the first author, is indicative and I accept it as proof of his active leading role in scientific development. Moreover, the PhD student is the lead author in a publication on serum procalcitonin levels in patients with acute CNS infections, accepted for publication in the prestigious journal Cytokones with high IF (2,952 for 2019).

Personal participation of the doctoral student

The doctoral student has an independent merit in development of the entire study, in obtaining and processing the data, in design of the research and analysis of the results, in derivation of the conclusions and scientific contributions.

Author's summary

The summary is written on 52 pages and summarizes the structure and content of the dissertation. The results are illustrated with 57 figures and 9 tables. The summary presents the purpose, tasks, main research, results, conclusions and contributions of the dissertation.

Critical remarks and recommendations

I have no critical remarks on the dissertation. I think it was planned, developed and written perfectly. My recommendation is Dr. Yordan Kalchev to promote the results of his dissertation in order to optimize the rapid diagnosis of acute CNS infections in the country.

Personal impressions

I know Dr. Kalchev from his participations in the national congresses of clinical microbiology and infections and have excellent impressions of him. Intelligent, motivated to build himself as a clinical microbiologist, always ready to respond where needed. He was the first volunteer to work in the COVID laboratory at MU-Plovdiv.

Conclusion

The dissertation of Dr. Yordan Kalchev is at very high methodological level, modern techniques are used for etiological detection of pathogens in cerebrospinal fluid and to monitor levels of major cytokines in serum and cerebrospinal fluid. Many

valuable results have been obtained, the relevant analyzes have been made for a very important problem related to the need to modernize the methods and speed up the diagnosis of acute CNS infections. The topic is dissertable, the set goals and objectives have been achieved and very well reflected in the conclusions and scientific contributions. The dissertation contains scientific and applied scientific results, which represent an original contribution to science.

I believe that the peer-reviewed dissertation fully meets the requirements of the Law for the development of the academic staff in the Republic of Bulgaria, the Regulations for its application and the Regulations of MU-Plovdiv. Giving my positive assessment, I strongly suggest to the members of the scientific jury to award Dr. Yordan Ivanov Kalchev the educational and scientific degree "Doctor" in the doctoral program "Microbiology".

Sofia

December 26, 2020

Reviewer:

Prof. Iva Christova, MD, DSc.