



To the Chairman of the Scientific Jury,
Determined by Order No. 582 / 01.12.2023
of the Director of the National Center for
Infectious and Parasitic Diseases

STATEMENT OF OPINION

by

Prof. Dr. Dimitar Temelkov Kostadinov, MD, PhD
University Multi-Profile Hospital for Active Treatment "St. Ivan Rilski" - Sofia
Medical University – Sofia

Regarding: dissertation work for obtaining the educational and scientific degree "Doctor", field of higher education 4. "Natural sciences, mathematics and informatics", direction 4.3 "Biological sciences", doctoral program "Microbiology"

The author of the dissertation work is the PhD student at the National Center for Infectious and Parasitic Diseases - Borislava Ilieva Tsafarova.

Topic: "Microbiological, electron-microscopic and molecular-biological methods for studying the pathogenesis of sarcoidosis".

Scientific supervisor: Prof. Dr. Stefan Panayotov

I declare that I have no conflict of interest according to the Act for the Development of the Academic Staff in the Republic of Bulgaria.

The opinion has been prepared in accordance with the requirements of the Act for the Development of the Academic Staff in the Republic of Bulgaria and the regulations for the Terms and Conditions for Acquisition of Academic Degrees and Holding of Academic Positions in the National Center for Infectious and Parasitic Diseases.

The submitted documents are in accordance with the instructions published in the Regulations for the implementation of the Act for the Development of the Academic Staff in the Republic of Bulgaria and the internal Regulations of the National Center for Infectious and Parasitic Diseases.

I. Analysis of the candidate's career profile.

Borislava Ilieva Tsafarova has a bachelor's degree in "Molecular Biology", obtained at Sofia University „St. Kliment Ohridski” since 2009. In 2013, she obtained a master's degree in "Biology", specialty "Cell Biology and Pathology" at SU "St. Kliment Ohridski", and since 2015 she has a master's degree in "Molecular Biology", specialty "Physiology of animals and humans" at SU "St. Kliment Ohridski".

Her work experience began as a medical representative at Borola Ltd. from November 2015 to September 2020. From September 2018 to September 2020 she was on maternity leave, and from October 2020 until now she is a PhD student at the National Center for Infectious and Parasitic Diseases.

During her doctoral studies, Borislava Tsafarova showed an upward growth in the processing and presentation of scientific information. She has very good computer literacy. She

uses perfect English language. Her publications are in international and national journals. She presents the results at scientific forums at home and abroad.

II. Relevance of the topic.

As a granulomatous disease, sarcoidosis has immunopathological features and arises from the interaction of one or more triggers with an immunologically predisposed host. In support of this are the known granulomatous responses to a variety of infectious agents, including mycobacteria, parasites such as schistosoma, and fungi such as coccidiomycosis. Infectious triggers have always been the most favored and there have been numerous observations and studies over the years investigating this. More and more data point to microbial involvement in the development of the disease, through the influence of individual microbial species or as a result of dysbacteriosis of the microbiome. A number of researchers have demonstrated the presence of microorganisms in sarcoid samples, but so far no single etiologic agent of sarcoidosis has been determined. It is possible that different types of microorganisms are involved in the pathogenesis of the disease. The most evidence has been accumulated for representatives of the genus *Mycobacterium* (*Mycobacterium tuberculosis*) and for *Cutibacterium acnes*.

In her dissertation, Borislava Tsafarova focused on the search for evidence of microbial involvement in the etiology of sarcoidosis. The aim of her work is to study the microbiome in sarcoid patients and controls by molecular, cultural and microscopic analysis techniques.

III. Characteristics of the dissertation work.

The dissertation work is written on 169 pages. Of these, an introduction and a literature review occupy 27 pages and in one page is presented the aim of the study. Material and methods are described on 35 pages, and the results, discussion and conclusions occupy 67 pages. In the work 220 sources are cited - 8 in Cyrillic and 212 in Latin, occupying 18 pages. Of the cited publications, 120 are from the last 10 years, which is 54.6% (from the last five years there are 53 sources - 2018-2023). The dissertation work is very well illustrated with 42 figures and 11 tables.

In literature reviews, the doctoral student emphasizes the many factors that are responsible for the development of sarcoidosis (genetic predisposition, antigenic irritation, manifestation of Th 1 mediated immune response, gender, age, behavioural habits). The environmental dynamics likely also lead to changes in overall population morbidity. A relatively understudied area in sarcoidosis is the role of the microbiome in the pathogenesis of the disease. The blood microbiome in sarcoidosis has not yet been studied. It is possible that the combined study of the blood and tissue microbiome in sarcoidosis to contribute to a better understanding of the pathogenesis of the disease. This defines the purpose and main tasks in the dissertation work.

The "Materials and methods" section covers 35 pages, and here the doctoral student states that she is examining paired materials, such as blood and tissue biopsy material from patients admitted to a medical facility to confirm or reject the diagnosis of pulmonary sarcoidosis. The stages and methods in the research process are indicated: isolation of DNA from blood, biopsy material and bronchoalveolar lavage (BAL); examination of archival biopsy and operative material; culture methods and microscopic methods were used - for more detailed observation and characterization of the blood microbiome, as well as its mechanisms of proliferation; molecular methods for studying the pathogenesis of sarcoidosis.

The results and the discussion are combined in a general section occupying 63 pages. After the results of each method used, a discussion is made, which helps to draw seven conclusions. Each conclusion is of a scientific and applied nature.

IV. Evaluation of the scientific work of the candidate.

The doctoral student attached to the dissertation a total of 9 scientific publications (6 on the topic, 4 of which are in English and 2 in Bulgarian, and 3 publications are off the topic of the dissertation). In these publications, the PhD student is the first author in 4.

The bibliometric ranking indicator Q (quartile) of these 9 scientific publications reflects the highest rank (Q4) for three publications, for one it is Q2 and for two it is Q1.

The total impact factor of scientific publications is 16.359.

Borislava Tsafarova has 23 participations in national (out of 7 publications in 3 she is the lead author and in 4 is the second author) and international (out of 16 publications in 4 she is the lead and in 12 she is the second author) scientific forums with a poster or presentation on the topic. She took part in 6 national scientific forums off the topic of the dissertation and participated in 5 national research projects.

Publications, associated with the dissertation work are cited in 13 scientific articles.

The total number of credit points for the doctoral period (10.2020 - 10.2023) is 747 points.

The attached publications are directly related to the objectives and results of the dissertation work.

V. Comprehensive evaluation of the dissertation work.

The results achieved by the doctoral student provide an answer to research tasks that other researchers have been trying to implement for years. And in this regard, the conclusions and contributions are well formulated and give specific information about the qualities of the dissertation work.

It must be said that the results of Borislava Tsafarova's dissertation once again emphasize that despite numerous and thorough studies by specialists from various fields of medicine, sarcoidosis continues to be a mystery.

There are still unanswered questions, such as: why are there racial and geographic differences in the clinical forms and manifestations of sarcoidosis?; why is the symptomatology so non-specific and different?; why in some patients the disease is self-limiting with spontaneous remission, while in others it becomes chronic and leads to severe organ damage?; what does it depend on, in which organs the granulomatous process will develop?. And something important - is there a specific etiological cause of the disease? Perhaps the answer to the last question will help illuminate all the unknowns surrounding sarcoidosis. The dissertation is an excellent basis for building on the research topic.

The PhD student conducts scientific research, which has a significant contribution to personal and career development. The participation of the PhD student in independent and team work is evident from the list of publications and the presentation of the obtained results, through reports and posters on the topic, at scientific forums.

The abstract of the dissertation (on 68 pages) reflects the main goals, tasks and results of the dissertation, illustrated with 30 figures.

VI. Critical notes and recommendations.

I have no significant remarks and recommendations for the dissertation. The dissertation and all documents are prepared as required.

There are a few technical omissions that do not detract from the quality of the dissertation. These are: the plural of lung is used in the text, which is not correct, because it is not a paired organ. In the bibliography, the arrangement of the authors should start with those in Cyrillic and then in Latin.

It should be known that in the process of developing the dissertation from October 2022, the medical institution University Hospital "St. Sofia", by decision of the Minister of Health, with all assets and liabilities were taken over by University Hospital "St. Ivan Rilski", Sofia. This is rather for the reference, because in one text University Hospital "St. Sofia" is used, and in another UMBAL "St. Ivan Rilski", Sofia.

CONCLUSION

The documents submitted by the candidate cover all the criteria according to the Act for the Development of the Academic Staff in the Republic of Bulgaria and the National Center for Infectious and Parasitic Diseases requirements for the acquisition of the educational and scientific degree "Doctor".

Borislava Ilieva Tsafarova meets the mandatory and specific conditions and scientometric criteria for the scientific degree "Doctor" according to the Act for the Development of the Academic Staff in the Republic of Bulgaria and the National Center for Infectious and Parasitic Diseases requirements.

I confidently give my positive assessment and recommend to the respected Scientific Jury to award the educational scientific degree "Doctor" to Borislava Ilieva Tsafarova in the field of higher education 4. "Natural Sciences, Mathematics and Informatics", direction 4.3 "Biological Sciences", doctoral program "Microbiology".

With respect:

(Prof. Dr. D. Kostadinov, MD, PhD)