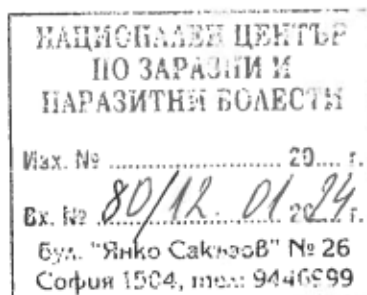


To the Chairman
of the Scientific Jury appointed by Order
No 582/01.12.2023 of the Director of the NCIPD



REVIEW

by Assoc. Prof. Iva Petrova Trifonova, PhD, NCIPD, Sofia

of a dissertation for acquiring the educational and scientific degree "Doctor"

to Borislava Ilieva Tsafarova,

doctoral student at the National Center of Infectious and Parasitic Diseases

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

Field of higher education: 4. Natural Sciences, Mathematics and Informatics, Professional field: 4.3. Biological sciences, Scientific specialty: Microbiology

Scientific supervisor: Prof. Stefan Panayotov, PhD, DSc

Relevance and significance of the thesis: Sarcoidosis is a multisystem granulomatous disease with nonspecific and diverse clinical manifestations. Despite many studies and accumulated scientific data, the etiology of the disease remains unknown. One of the main hypotheses is related to microbial involvement in the development of the disease, through the impact of individual microbial species or a consequence of microbiome dysbiosis. Multiple investigators have demonstrated the presence of microorganisms in sarcoid samples. To date, no single etiological agent of sarcoidosis has been identified. Different types of microorganisms may be involved in the pathogenesis of the disease. Most evidence has accumulated for representatives of the genus *Mycobacterium* (*Mycobacterium tuberculosis*) and *Cutibacterium acnes*.

This dissertation focuses on an important and interesting problem - studies to elucidate the etiology of sarcoidosis by searching for evidence of microbial involvement. The main objective is to study the microbiome in patients with sarcoidosis using molecular, culture, and microscopic analysis techniques.

The presented dissertation of Borislava Tsafarova is relevant and significant research in the field of a still unsolved medical problem. Probably many factors are responsible for the development of sarcoidosis - genetic predisposition, antigenic irritation, manifestation of Th 1 mediated immune response, gender, age, and behavioral habits. All of these are the subject of active research. Environmental dynamics are also likely to lead to changes in overall population morbidity. A relatively understudied area is the role of the blood and tissue microbiome in disease pathogenesis. The blood microbiome in sarcoidosis has not yet been investigated.

Structure of the thesis: The thesis consists of 169 standard pages. It is illustrated with 42 figures and 11 tables. It contains all the required sections, which are structured as follows: introduction, literature review, aim and objectives, materials and methods, results and discussion, conclusions and bibliographic reference, publications and contributions to scientific forums related to the topic are presented.

The literature review presents a systematic introduction to the problem, describing the epidemiology, clinical forms of the disease, diagnostic approaches and therapy. Emphasis is placed on the various hypotheses regarding the etiology of sarcoidosis and the current scientific status of the problem. That sarcoidosis can be detected in different organs suggests that the causative agent is transmitted from one bodily location to another, via the blood or lymph. The main thrust of Borislava Tshafarova's research is outlined here, namely the combined study of the blood and tissue microbiome in sarcoidosis to contribute to a better understanding of the pathogenesis of the disease. The doctoral student's good literary awareness and in-depth knowledge of the topic can be judged from the 220 references cited (8 of them, in Bulgarian) in the dissertation.

The goal is clearly and specifically formulated, and for its implementation are set two main tasks, each with two sub-tasks: 1. Analysis of blood and tissue microbiome of patients with sarcoidosis and 2. Investigation of the role of *Cutibacterium acnes*, *Mycobacterium tuberculosis* and other mycobacteria and microorganisms to elucidate their potential involvement in the pathogenesis of sarcoidosis.

The Materials and Methods section comprehensively describes the clinical materials used in the course of their experimental work, as well as the groups of subjects, which total 44 in number. A control group of healthy subjects (7 in number) is also included, which is of great importance in the analysis and interpretation of the results obtained. For her thesis, the doctoral student examined blood and tissue biopsy material from patients with sarcoidosis, as well as blood, biopsy material and bronchoalveolar lavage fluid from patients susceptible to sarcoidosis. An interesting approach is the study of archival tissue biopsies from patients with pulmonary sarcoidosis and surgical material from patients with pulmonary tuberculosis. The wide range of research techniques - culture, molecular, genetic and immunohistological methods, various types of microscopy, as well as sequence and bioinformatics analysis - is noteworthy.

The results are presented sequentially for each of the applied research methods, followed by a discussion. Borislava Tsafarova critically analyses her results, comparing them with the results of other researchers.

Based on the results obtained, Borislava Tsafarova draws seven conclusions that reflect the results obtained and their discussion.

Publications and other scientific activities related to the dissertation. Two of the publications are review papers published in Bulgarian journals. The total impact factor of the scientific papers is 20.9 and three of them are in Q1 journals. The PhD student is the first author of four of the attached publications, which is indicative of her contribution to the research topic. So far, 13 citations of the research papers have been noted. The results of her work have also been

presented at 23 scientific forums - 16 international and 7 national. From the summary presented by the PhD student of the total of 747 credits accumulated during the three-year term (2020-2023) of her full-time PhD, it is evident that she exceeds the minimum required number of 200 credits according to the Law on the Development of the Academic Staff in the Republic of Bulgaria and the Regulations for its implementation in the Republic of Bulgaria and the NCIPD by many times.

The abstract consists of 68 pages and is formatted as required. It includes the original contributions of the dissertation work of Borislava Tsfarova, which are formulated based on the conclusions and are divided into contributions of a fundamental scientific and theoretical nature. 2. Demonstration of several mechanisms of multiplication of blood microbiota, by budding, chain formation, or detachment (extrusion). 3. Demonstration of a new mechanism of multiplication called "cell within a cell". 4. The microbiome profile of blood is specific and different from that of lung tissue in patients with sarcoidosis. 5. Microbiome analysis indicates that other microbial species could also be involved in the pathogenesis of sarcoidosis.

Contributions of scientific and applied nature: 1. A method for DNA extraction from blood microbiota in cultured and uncultured blood was developed. 2. It has been established that formalin-fixed and paraffin-embedded tissue samples are not a suitable model for molecular biological study of the pathogenesis of sarcoidosis. 3. Mycobacteria, *Cutibacterium acnes* and *Toxoplasma gondii* are relevant to the pathogenesis of sarcoidosis. 4. The combination of whole genome sequencing, bioinformatics analysis and immunohistochemistry methods is suitable for the study of pulmonary multisystem granulomatous diseases.

I have no critical remarks regarding the structure of the thesis, the presentation of the results and the conclusions drawn. The text is written in a highly scientific style and is very well laid out. Some minor technical inaccuracies do not affect the overall value of the thesis.

The submitted dissertation of Borislava Ilieva Tsfarova fully complies with the requirements of the Law on the Development of the Academic Staff in the Republic of Bulgaria and the Regulations for its implementation in the Republic of Bulgaria and the NCIPD.

On the basis of the above, I give my positive opinion on the dissertation and recommend to the esteemed Scientific Jury to award the degree of Doctor of Education and Science to Borislava Ilieva Tsfarova in the scientific field of Natural Sciences, Mathematics and Informatics; professional field: biological sciences; scientific specialty: microbiology.

Sofia, 12.01.2024

Assoc. Prof. Iya Trifonova, PhD