OPINION

By Professor Dr. Iskra Georgieva Raynova, "Doctor of Sciences",

National Center of Infectious and Parasitic Diseases (NCIPD)

About: Dissertation work for the aquisition of the educational and scientific degree

"Doctor of Philosophy"

Professional field: 4.3 "Biological Sciences",

Scientific specialty: "Microbiology"

Author: Yordan Krasimirov Hodzhev

Department: Microbiology, NCIPD

entitled:**"Microbiome and bioinformatics analysis to study the pathogenesis of sarcoidosis"**

Scientific supervisor: Prof. Stefan Vachev Panayotov, DsC

1. General presentation of the procedure and the doctoral student

The presented set of materials on paper and electronic media is by Art. 31 (2) of the regulations for the application of the Law on the Development of the Academic Staff in the Republic of Bulgaria in the NCIPD and includes all the required documents. The preparation and arrangement of the necessary documents are correct and precise.

2. Relevance of the topic

Sarcoidosis is a rare inflammatory disease, with the development of granulomas in various organs, and they are mainly found in the lungs and lymph nodes. Over the past two decades, significant progress has been made in the diagnosis, clinical evaluation, and treatment of sarcoidosis, but the exact etiology of the disease remains unknown.

The role of the microbiome in various autoimmune and inflammatory diseases is an active area of research. There is scientific evidence for the interaction of microbes with immune cells that may be involved in the immunopathogenesis of sarcoidosis. New studies demonstrate the immunomodulatory role of the lung microbiome in sarcoidosis. This new study's condition and the topicality of the topic are due to the need to clarify the relationship between the blood microbiome and sarcoidosis. The main working hypothesis of the dissertation is that microbial imbalance or quantitative changes of specific representatives of the blood microbiome can contribute to the development of pulmonary sarcoidosis or even induce it. The doctoral student's thesis is that this one new and under-researched scientific field can be developed using the methods of band informatics and modern microbiome analysis techniques.

3. Knowing the problem

In the dissertation work of Yordan Hodzhev, a total of 121 author titles in Latin are referenced, with 42% of the referenced publications being from the last six years. The Ph.D. student is very well-versed in the achievements of world science and practice on the various issues concerning the microbiome. The purpose of the dissertation work is clearly and precisely formulated and for its implementation four tasks related to the test of a bioinformatics approach to establish the composition of the blood microbiome in normal healthy individuals, visualization and evaluation of the quantitative and qualitative composition of the blood microbiome in individual patients with sarcoidosis, elucidating the potential causal relationships between blood microbiome dysbiosis and the pathogenesis of pulmonary sarcoidosis and applying machine learning algorithms to identify key microbial taxa with potential contributions to the pathology of pulmonary sarcoidosis. The content of the goal and tasks indicate a well-reasoned approach to the implementationof the doctoral thesis.

4. Research methodology

The dissertation included seven patients aged 48.33 (± 19.14) years, diagnosed with pulmonary sarcoidosis between 2020-2022. To study the taxonomic composition of the blood microbiome, 28 healthy adults were selected - 14 men and 14 women, mean age of 41 ±12 years. The blood group of the healthy persons is also correctly indicated, and according to this indicator, their distribution is balanced. The criteria for the selection of patients and healthy individuals are described in detail and clearly. For microbiome study, clinical samples from patients include venous blood, BAL, biopsy, wash, and water and reagent controls. Blood samples collected from the healthy subjects were examined without pre-culture and after-culture. The general design of the study includes several stages that are tailored to the tasks set. In the dissertation, the DNA sequencing methods are used, which are modern, as can be seen from the literature used. The machine learning analysis developed to enrich the information on changes in the microbiome in sarcoid patients is original and innovative.

Statistical software R version 3.6.0 was applied for statistical processing of the results, and with its help, a comparison of microbial species between cultured and non-cultured samples was made. In addition, a comparison between samples was performed by principal coordinate analysis (PCoA)-based cluster analysis, as well as using the Jensen-Shannon divergence method for distance determination and permutation ANOVA (PERMANOVA) for statistical validation. Microbiome data analysis and visualization were performed using the web-based Microbiome Analyst platform. The platform enables profiling of amplicon sequencing data by

providing an online graphical user interface application, R version 3.5.1, and provides numerous algorithms for the analysis of metagenomic information.

5. Characterization and evaluation of the dissertation work and contributions

Own data from the conducted studies are presented in a summary section "Results and Discussion" and occupies 30% of its volume. The results of the implementation of each of the 4 tasks set out in Yordan Hodzhev's thesis are described in an excellently designed tabular form and figures.

On the first task, results show the presence of well-cultured bacteria and fungi as part of normal blood microflora in healthy individuals. By combining the operational taxonomic units (OTUs) from all cultured and uncultured samples, a total of 105 bacterial and 133 fungal genera were identified.

In the second task 937 sequences per sarcoid patient sample using a 16S metagenomic approach. Multi-method microbiome analysis showed a significant increase in five bacterial genera in patients with sarcoidosis: *Veillonella, Prevotella, Cutibacterium, Corynebacterium, and Streptococcus.*

In the third task, the differentiation of the potential causal relationship between dysbiosis of the blood microbiome and the pathogenesis of pulmonary sarcoidosis was realized through PCA analysis. Its data show a significant increase in the richness of bacterial diversity in patients with sarcoidosis, compared to healthy controls

On the fourth task, the application of a machine learning classification model identified the taxa with the greatest possible contribution to the pathogenesis of sarcoidosis. On the basis of amplicon metagenomic analysis, bacterial types and genera were identified with the highest estimated contribution coefficient in sarcoid patients, so that patients could be differentiated from clinically healthy individuals.

The studies of the doctoral students that the blood microflora is a viable ecological niche with hidden microbial diversity (Proteobacteria, Actinobacteria, Firmicutes, and Bacteroidetes) identified by culture. The results described in the dissertation work on the fungal microbiome in the blood of healthy persons are also to the credit of Yordan Hodzhev.

I fully approve the eight contributions proposed by the dissertation, four of which are of a fundamental nature and four of which are of an applied nature. In my opinion, a significant scientific contribution is made by: a) The hypothesis of interaction between the blood and lung microbiome in the pathogenesis of sarcoidosis; b) The cultural and metagenomic proof of the presence of a fungal microbiome in the blood of healthy individuals; c) The development of an effective bioinformatics method for the comparative analysis of the taxonomic composition of

cultured blood samples for testing different nutritional environments and stressful conditions for resuscitation of the latent blood microbiota; d) The applied classification model for machine learning.

6. Evaluation of the publications and personal contribution of the doctoral student

In connection with her dissertation work, Yordan Hodzhev has attached six articles, in journals with an impact factor and an impact rank, of which she is the first author of four. The total impact factor of the PhD student is 22.559. During the development of the dissertation, the doctoral student collected 1,239 credit points, which many times exceeded the requirements of the NACID for PhD students. In addition, he has also presented 22 summaries of participation with reports and posters in scientific forums on the subject, of which 15 were exported abroad.

The abstract is structured in 41 pages, includes all the results of the dissertation work and their discussion.

CONCLUSION

The dissertation contains scientific and scientific-applied results, which represent an original contribution to science, meeting and exceeding the requirements of the Law on the Development of the Academic Staff in the Republic of Bulgaria, the Regulations for the Implementation of the Law, and the Regulations of the NCIPD. The dissertation shows that the doctoral student Yordan Krasimirov Hodzhev possesses in-depth theoretical knowledge and professional skills in the scientific specialty "Microbiology", demonstrating qualities and skills for the independent conduct of scientific research.

Due to the above, I confidently give my positive assessment for the awarding of the educational and scientific degree "Dooctor of Philosophy" to Yordan Krasimirov Hodzhev in the PhD program in "Microbiology".

11/01/2024   **Prepared the opinion:**

Professor Dr. Iskra Georgieva Raynova, DsC