



**TO
THE CHAIRMAN OF THE SCIENTIFIC JURY,
DETERMINED BY ORDER no 251/12.06.2023 OF
THE DIRECTOR OF NCIPD.**

Review

By Prof. Dr. Hristo Borisov Taskov, DSc

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***Regarding:** Competition for the occupation of the academic position "Associate Professor" in the scientific specialty "Immunology" in the field of higher education: 4. "Natural sciences, mathematics and informatics", direction 4.3. "Biological sciences", for the needs of department "Immunology" of the NCIPDs with a decision of the Scientific Council, promulgated in SG, no. 54/23.06.2023*

In the current competition for the academic position "Associate Professor", one candidate submitted documents within the legal term and was allowed to participate: head assistant Radoslava Emilova Grozdanova PhD. The set of documents complies with all the requirements of the Law on the Development of the Academic Staff in the Republic of Bulgaria and the Regulations for its application in the National Center of Infectious and Parasitic Diseases (NCIPD).

Biographical data, professional development

Radoslava Emilova Grozdanova has completed her higher education and acquired master's degree in biology in 2003 at SU "Sveti Kliment Ohridski" - Sofia. In the period 2010 - 2014, she was a full-time doctoral student at the Faculty of Biology, Department "Physiology of animals and man". She defends a dissertation on the topic: "Role of mediators of perivascular adipose tissue for arterial contraction". From 2023 until now, he is a specialist in "Biochemistry" at the Medical University - Sofia.

She started working in the specialty in 2003 in the Clinical Laboratory at the Medical Center "Saint Magdalena" - Pernik. In the period 2005 - 2013, she worked at "Professor Ivan Mitev" hospital - Sofia for National screening programs and functional endocrine diagnostics. Engaged in hormone analyzes of serum and plasma by means of ELISA and

DELFA, as well as by molecular genetic methods (DNA isolation, PCR, gel electrophoresis, RT-PCR). In 2011 - 2023 works as a biologist in the Cytogenetic Laboratory at SBALDB-EAD "Professor Ivan Mitev" - Sofia. From 2016 to now he is the chief assistant at the National Reference Laboratory of Immunology - (NRLI) at the National Center for Infectious and Parasitic Diseases - city Sofia. From the above can be concluded that after completing her higher education, Radoslava Grozdanova continued to work in her specialty, having qualified in modern immunological methods for studies such as flow cytometric immunophenotyping, determination of the antigen-specific response in acute and chronic infections and functional studies of lymphocytes, basophilic degranulation test, ELISA, CLIA; EliSpot, Lensinex and others. Her participation in a number of qualification courses at home and abroad also contributed to this: *"Not at all Population and Evolutionary Genetics"*, Pasteur Institute, MOOC: Massive Open Online Course; *"Modeling of Infectious Diseases"*, Institut Pasteur, Ministry of Education and Culture: Massive Open Online Course; *"Viral fusion and molecular-epidemiological methods for surveillance and control of viral diseases"* - NCIPD; *"Introduction of statistical methods for processing and analysis of data from medico-biological studies"*–NCIPD; *"NIV Science"*, Institut Pasteur, MOOC: Massive Open Online Course; *"Tuberculosis"*, Institut Pasteur, MOOC: Massive Open Online Course; *"Innate Ittunity"*, Institut Pasteur, MOOC: Massive Open Online Course; *"Diagnosis and prognosis Blotarkers in global health"*, Institut Pasteur, MOOC.

Radoslava Grozdanova speaks English and Russian.

Research activity

1. Scientific indicators

In the competition, Radoslava Grozdanova presented herself with 53 scientific works in scientific and medical publications and participation in 173 scientific forums, at which an equivalent number of reports, presentations, posters were presented. Of these, 4 scientific works represent the dissertation and its related publications. I accept 49 scientific publications for review. They are distributed as follows:

1. Scientific publications	
• In foreign editions and editions with a certain quartile (Q)	29
• In foreign and national editions without a defined quartile	18

2. Chapters of monograph in Bulgaria	2
Total scientific publications	49

In 44% of the mentioned publications Assistant Professor Radoslava Emilova Grozdanova, PhD is first (7 articles), second (4 articles) or last author (12 articles):

The observed citations in Scopus are on 11 scientific works. They have been cited 164 times, of which 74 citations are from the last five years. The Hirsch index (h) is 5.

The total impact factor of the publications submitted in connection with this competition is 31,688.

A significant part of the scientific results and developments were reported as oral communications or were presented in the form of poster presentations in a total of 173 scientific forums. The total number of reports or posters in international forums is 63, with 34 abstracts published in impact factor journals (23 published in Q1 journals, 6 in Q2 and 5 in Q3 journals). The total number of participations with reports or posters in national forums is 110.

3. Participation in congresses	
Abroad in magazine supplements with a certain quartile (Q)	34
Abroad in anthologies and magazines without specified quartile	29
In Bulgaria	110
Total participations in congresses	173

Main scientific directions and contributions:

The analysis of scientific publications outlines the following scientific, scientific and applied directions of research and contributions of Radoslava Grozdanova. In the period 2005-2015 she has made a significant contribution to mass neonatal screening programs for congenital hypothyroidism and congenital adrenal hyperplasia (CAH) of all newborns in Bulgaria, as well as in the development and implementation of methods for the determination of various hormones and for the optimization of functional endocrine diagnostics. (Publications №. 2, 3, 4, 5 (Q4) as well as №34, 35 and 44).

After the acquisition of her PhD, the main scientific, scientific-applied and methodical contributions of Radoslava Emilova Grozdanova are in the field of the cellular immune response against *Mycobacteria tuberculosis*, HIV and SARS-CoV-2 infection, cytokine

production, small signaling molecules and intercellular signaling. oxidative stress and iron homeostasis in various diseases.

1. Contributions to studies of iron homeostasis in norm and in various pathological conditions.

In this direction, Radoslava introduced a method for determining serum hepcidin and its significance in the treatment of diseases with disturbed iron homeostasis. It validates the ELISA method for determining erythroferon in serum and determining the reference limits of the indicator for the Bulgarian population. This allows to assess the relationship between hepcidin and ferroportin with indicators of cerebrovascular atherosclerotic changes in patients with chronic kidney disease. Radoslava made significant contributions to the study of the correlation dependence between indicators of iron metabolism and IMT in patients with sleep apnea, as well as to the role of the haptoglobin polymorphism on iron metabolism in a healthy group of volunteers from the Bulgarian population. (Publications no 9, (Q1), 14 (Q4) and 37, 38, 39, 40, 41, 42, 43, 46, 47, 48, 49, 51)

2. Contributions to the field of infectious immunology. In this area, Grozdanova's research is focused on characterizing the immune response to infections with SARS-COV-2, Mycobacterium tuberculosis and HIV.

a) *Investigation of the immune response against SARS-COV-2 in recovered and vaccinated individuals.*

Grozdanova conducted one of the first studies in Bulgaria of the immunological parameters in SARS-COV-2 infection. A connection was made between the values of the specific antibodies of class IgG and IgA depending on viral load and severity of infection, as well as in vaccinated individuals. Correlational dependences were established between the severity of the cytokine secretion and between the Th1 mediators IL-18 and IL-10 and the pro-inflammatory IL-6. Investigation of the role of induced Treg (CD39+) and the expression of the activation marker CD38 by CD4 and CD8 T lymphocytes in the course of infection. By analyzing plasma cytokine concentrations using a Luminex™ 200X detection system (Invitrogen) and a 25-plex panel (Cytokine 25-Plex Human ProcartaPlex™ Panel 1B), a relationship was made between pro-inflammatory and anti-inflammatory cytokines (GM-CSF, IFN- γ , IL-1/3, IL-2, IL-4, IL-5, IL-6, IL-12p70, IL-13, IL-18, TNF- α ; IL-9, IL-10, IL-17A, (CTLA-8), IL-21, IL-22, IL-23, IL-27, IFN- α , IL-1 α IL-1 RA, IL-7, IL-15, IL-31, TNF- β), disease course and immunization status. Of particular interest are studies on virus-specific stem-like memory T lymphocytes as a stable marker of protection.

In view of the immune background of the population, important information was

obtained on the cross-reactivity of SARS-CoV-2 with non-SARS-CoV-2 alpha- (HKU-1 and 229E) and beta-coronaviruses (OC43 and NL63). (*Publications Nos. 22, (Q1), 26, 27 (Q2), 24, 25, 29 (Q4), as well as №. 32, 33 and 52*).

b) Examination of the immune response against *Mycobacterium tuberculosis*. An advanced flow cytometric immunophenotypic panel was applied to analyze the cellular a response that differentiates patients with active and latent tuberculosis. This has been combined with functional studies of lymphocytes to secrete IFN γ , TNF α , IL-1, IL-17 and IL-22 after specific stimulation to determine in individuals with active, latent tuberculosis and healthcare workers at risk. The results of the research of pro/anti-inflammatory effects eicosanoids, PGE2/LXA4 during the stages of MTB infection.

An interferon-gamma-based test (IGRA) was implemented for the first time to screen healthcare workers for tuberculosis infection. This screening was carried out in implementation of the Program "Improving the sustainability of the National tuberculosis program - Tuberculosis Care and Prevention Module" (*Posters № 11, 15, 31 (Q4)*)

(c) Contributions to HIV infection studies

A detailed genetic analysis of the diversity of HIV-1 subtypes and recombinant forms in Bulgaria was made. A country-specific landscape of strains has been identified, which is determined by the country's location at the crossroads between east-west and north-south.

When assessing T-cell subpopulations, it was found that the percentage and absolute number of double-negative (CD4-CD8-) and double-positive (CD4+CD8+) T cells could serve as a prognostic marker of immune recovery in HIV+ patients on long-term antiretroviral therapy.

Studies on the regulation of intracellular iron and hepcidin in patients with HIV infection represent an indisputable scientific contribution. For this purpose, two new methods for flow cytometric analysis have been introduced - for the determination of intracellular labile bound iron and for membrane ferroportin. The relationship between the serum levels of hepcidin, the distribution of labile bound iron in lymphocyte subpopulations, which are related to the activity of the HIV virus, was established.

Of epidemiological interest are the results of studies on the seroprevalence of HEV among people living with HIV and the role of HCV co-infection on the cellular immune response in people living with HIV. (*Publications №. 30 (Q1), 17, 21, (Q2), 16, 23, 29 (Q4), and 45*)

3. Contributions in the area on oxidative stress and intercellular signaling

Original protocols for the determination of ROS in the cytoplasm of lymphocytes by

means of flow cytometry have been developed and implemented. In parallel spectrophotometric determination of SOD in plasma and cell lysate from peripheral blood mononuclear cells (PBMCs) was developed and implemented.

These protocols have revealed the possibility of determining intracellular free oxygen radicals in CD4+ and CD8+ cell subpopulations from HIV+ individuals on long-term viral therapy and with a persistently suppressed viral load. Correlational dependences between residual viral activity, the combined effects of aging, long-term therapy, and free oxygen radicals in T cell populations. (Publications №. 8, (Q2), 10, 28 (Q3), as well as № 14 (Q4).)

4. Contributions to the field of allergology

Of particular scientific interest is the determination of T-regulatory cells (nTreg; Tr1 and Th1 7/Treg cells) in patients with clinically manifested inhalant allergy, as well as the analysis of the percentage of CD8+CD25+CD103- and CD8+ CD25+ CD122+ T- subpopulations regulatory cells and evaluation of their role in the successful clinical effect of immunotherapy with allergens.

A significant scientific contribution is the demonstration of the sIgE/sIgG4 ratio, as an objective marker for the immunological efficiency of SCIT and an expression of the immunomodulatory potential for restoring peripheral immune tolerance to environmental allergens. (Publications No. 18, (Q2), 13 (Q4), and 50.)

5. Applied contributions in the field of introducing non-invasive and gentle methods in various pathological conditions: To this section you should attribute the development of the following original methods:

- 1) Development of a method for the determination of cortisol in saliva.
- 2) Development of a method for determining immunoglobulins in saliva.
- 3) Development on chemiluminescent method for determination on antimicrobial peptides in feces.
- 4) Development of a method for the determination of anti-CCP and RF in crevicular fluid in patients with rheumatoid arthritis (RA) and periodontitis in order to establish a relationship between the two diseases.

(Publications № 20,(QZ),4, 12(Q4),and № 44.)

Participation in scientific projects

In connection with the research activity, Radoslava Grozdanova actively participates in three research projects financed by the Scientific Research Fund:

The participation in scientific projects is a total of 18, as follows:

- Management of a scientific research project funded by the Scientific Research

Fund (FNI)

- Participation in the scientific team of six research projects funded by the National Institute of Scientific Research.
- Participation in five scientific research projects financed by MU-Sofia.
- Participation in four research projects financed by "St. Kliment Ohridski".
- One project with international participation.
- Leading researcher under work package 3 "Investigation of the immune response against microorganisms, under Project "Fundamental, translational and clinical research in the field of infections and infectious immunology" - №. BG05M20POO 1-1.002-0001.

Teaching and learning activity

In the period 2022-2023. Radoslava Grozdanova conducts a full cycle of exercises according to "Medical genetics" with students majoring in "Medicine" at the Faculty of Biology of the University "Prof. Dr. Asen Zlatarov" - 70 hour course.

In the period 2016-2023. conducts exercises and seminars from SDO courses in a department "Immunology", NCIPD - hours 107 hours.

In the period 2012-2013 Conducting a full cycle of "Animal and Human Physiology" exercises with undergraduate students from various majors at the Faculty of Biology of the University of St. Kliment Ohridski" - 105 hours.

Radoslava Grozdanova is the second academic supervisor of a graduate for the acquisition of the educational qualification degree "Master, who defended with distinction in March 2013".

Conclusion

I know Radoslava Grozdanova personally and I have witnessed her upward scientific and professional development. She is a built scientist and specialist in the field of immunology. The analysis of the scientific and teaching activities of Radoslava Grozdanova shows that she fully meets the conditions and requirements under Art. 24 of the Law for the Development of the Academic Staff, the qualitative and quantitative criteria for the development of the academic staff, specified in the Regulations for the Low application in the National Center for Infectious and Parasitic Diseases (NCIPD) for the acquisition of the academic position "Associate Professor".

Based on this, I confidently suggest to the honorable members of the scientific jury to award **Assistant Professor Radoslava Emilova Grozdanova, PhD** the academic position "ASSOCIATE PROFESSOR" in the scientific specialty "Immunology" in the field of higher

education: 4. "Natural sciences, mathematics and informatics", direction 4.3. "Biological sciences", for the needs of the "Immunology" department of the NCIPD.

Sofia, 9/10/2023

A handwritten signature in black ink, appearing to read 'Hristo Taskov', written in a cursive style.

/Prof. Dr. Hristo Taskov, DSc/