

REVIEW

for the competition for the academic position of 'Associate Professor' in the professional field 4.3
Biological Sciences, with a scientific specialty in 'Immunology', sh. 01.06.23,
As announced in the State Gazette, No. 38 from 28.04.2023, for the requirements of the Pathology
Section at IEMPAM - BAS.

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I. Analysis of the candidate's career profile.

Chief Assist. Rositsa Svetolik Milcheva is the only candidate in the competition. She has a Master's degree programme in Molecular Biology with a specialization in Clinical Chemistry at the Bulgarian Faculty of Sofia University "St. Kliment Ohridski". Kliment Ohridski" in 2002. In 2002 She was appointed as a specialist at the Institute of Experimental Pathology and Parasitology, Department of Biochemistry – Bulgarian Academy of Sciences. In the period 2007- 2011 year She was a PhD student in Pathological Anatomy and Forensic Medicine at Comenius University in Bratislava, Slovakia, Faculty of Medicine, Department of Pathology. Since 2010. until 2014. has held the academic position of Assistant Professor at the Institute of Experimental Morphology, Pathology and Anthropology with Museum, Department of Pathology – Bulgarian Academy of Sciences. In 2011. Rositsa has defended a dissertation on "*Mechanisms of apoptosis in striated muscle fiber after invasion by Trichinella spiralis*". Since 2014, the candidate in the competition has held the academic position of Chief Assistant at the same institute. Chief Assist. Prof. Rositsa Milcheva has undergone specializations at the Comenius Universities in Bratislava, Imperial College London, the University of Wales in Aberystwyth, and the Karl Franz University in Graz.

II. General description of the submitted materials for the competition.

The submitted materials under the competition are well arranged and reflect the requirements of the Law and the Regulations for the Implementation of the Law on the Development of Academic Staff in the Republic of Bulgaria, and the materials are presented both on paper and electronically. The overall story and production of Ch. Assist. Prof. Rositsa Svetolik Milcheva, both through CV, lists of publications and citations, as well as through the presented scientific contributions, and a reference-declaration for compliance with the minimum national requirements and the requirements of BAS for participation in a competition for the academic position "Associate Professor" under field 4. Natural sciences, mathematics and informatics, Professional field 4.3. Biological Sciences. The presented data show according to a preliminary assessment coverage of the minimum scientific and other criteria of the Law on Combating Organized Crime and the Law on the Development of the Academic Staff, which allows subsequent detailed assessment of the application.

III. Evaluation of the candidate's scientific works for the overall academic development.

✓ *General characteristics of scientific production and publication activity;*

For participation in the competition are presented 1 dissertation and 19 scientific articles, all of which are published in English, all of which have an impact factor or rank. Of the refereed and peer-reviewed publications 4 are in journals with rank Q1; 3 are of rank Q2; 10 are of rank Q3 and 2 are of rank Q4. The publications are in a number of prestigious for thematic fields journals such as "Materials Science and Engineering", "Parasitology Research", "Gels" and others.

✓ *Scientific activity – dissemination and application of the candidate's scientific and practical achievements among the scientific community*

The scientific production is very good and is well reflected in the scientific community through publications, citations and by presenting it in a number of scientific forums. Ch. Assist. Prof. Rositsa Milcheva is a participant in 29 scientific publications, of which in 12 she is the first author, with a total impact factor of 24.609. Data on participation in 22 scientific forums in the country and abroad are presented, with 16 oral papers and 6 posters. Data are presented for 32 citations of 9 literary sources, of which Dr. R. Milcheva is an author.

The high scientific activity of Dr. R. Milcheva in the last 5 years is highlighted, which is demonstrated by the publication of almost half of the articles with which she applied for the competition. Also, these articles have an impact factor and rank.

Ch. Assist. Prof. R. Milcheva is also successful in the field of project financing, having participated in 4 projects and is the manager of another 3 scientific projects funded by NSF. She has won three awards and two certificates.

✓ *Scientific and creative achievements (scientific authority);*

Contributions from the thesis

The candidate found that after invasion with *Trichinella spiralis*, *the onset of transformation of a cross-striated muscle cell into a Support Cell was associated with the initiation of nuclear apoptosis preceding cytoplasmic apoptosis. The main contribution of the candidate is the disclosure of the key role of Trichinella spiralis in preventing necrotic cell death by inducing apoptosis.*

Contributions after the dissertation

The contributions of Dr. R. Milcheva could be classified in related to three main directions – studies of the biology and the parasite-host interaction of the nematode *Trichinella spiralis*; research related to the development of new biomedical materials and technologies with application in infectious medicine; and improvement of scientific analytical methods:

Studies of the biology and parasite-host interaction of the nematode *Trichinella spiralis*:

1. The candidate studies changes in glycosylation during the different stages of development of nematodes of the genus *Trichinella*. Particularly interesting is the establishment of ways in which *Trichinella spiralis* can modify the immune response, altering the dynamics between the parasite and the host. This unique mechanism has the potential to provide new perspectives and methods in the treatment of allergic, autoimmune and oncological diseases.

2. Dr. R. Milcheva considers apoptosis as a method of adaptation of *Trichinella*, which allows the parasite to create an effective environment for habitation in muscle cells. This clarifies the ways in which *Trichinella* manages to suppress and avoid the immune response of the infected organism.

3. During the intestinal phase of *Trichinella* infection, the candidate found that invasion of the gastrointestinal tract did not lead to apoptosis. Subsequently, it was revealed that this invasion acted as a modulator of inflammation, altering the host's immune response.

4. Chief Assistant R. Milcheva found that serum sialic acid levels can serve as a reliable marker of inflammation in *Trichinella spiralis* invasion of rats. This discovery provides opportunities to use this marker in other types of infections, such as skin infections.

5. In the transformation of an invaded muscle cell into a *T. spiralis* support cell, Dr. R. Milcheva identified the increased activity of two key nuclear proteins – poly-(ADP-ribose) polymerase 1 (PARP-1) and nuclear cell proliferation antigen (PCNA). Her research found that the transformation process is associated with nuclear translocation of pro-apoptotic proteins from the cytoplasm, such as apoptosis-inducing factor (AIF), Bcl-2 associated protein X (BAX) and caspase-3, as well as with nuclear localization of secretory proteins of the nematode itself. The observed phenomenon of nuclear translocation of the indicated pro-apoptotic proteins is usually related to the executive phase of programmed cell death, but may also be part of certain forms of necrosis. The studies allow to better characterize the complex way in which *T. spiralis* modifies host cells and the changes it causes at the molecular level.

6. Chief Assist. Prof. R. Milcheva revealed an increased biosynthesis of certain sial glycoproteins in the invasion of *T. spiralis*. Particularly interesting is the discovery about the alteration of glycosylation and the associated adaptive abilities of muscle tissue, which has an innovative and original character, which may be important for understanding various myopathies and disorders in the glycosylation apparatus.

7. The candidate presents a detailed and updated characterization of gene expressions of enzymes from three different families of sialyltransferases in the muscle tissue of *Mus musculus* and the cell line C2C12. The aim of this study is to determine the differences in expression of these enzymes and the possibility that the C2C12 cell line could replace the use of animal models in similar studies. This work also has relevance in the context of oncology studies.

8. Dr. R. Milcheva found that the infection of muscle tissue with *T. spiralis* and the subsequent transformation of the affected cells into Support Cells is associated with a significantly increased expression of the dystrophin protein. This protein is key to the functioning of muscle cells, and its abnormal biosynthesis can lead to serious diseases such as muscular dystrophies. This research provides a new and unique insight into the role of *T. spiralis* in modulating the genetic activity of infected cells.

Part of the research has contributed to the field of innovative developments in biomedical materials and technologies:

1. Ch. Assist. Prof.R. Milcheva has also developed in the field of improving experimental analytical approaches. It demonstrates the effectiveness of various alcohol fixatives in molecular biology and histology. Especially emphasized is the advantage of the metakarn fixative for the preservation of morphology and recognizable epitopes, in immunohistochemistry, which makes it particularly suitable for various studies.

2. Catheter-associated infections are extremely prevalent in medical practice and pose a challenge due to the persistent biofilms that form on catheters. With this problem in mind, the research focused on the development of novel coatings based on zinc oxide nanoparticles in combination with the enzyme amylase, for silicone urinary catheters. This innovative approach was tested preclinically using an experimental model with rabbits, and the results showed a significant improvement in the ability of catheters to prevent biofilm formation and associated infections. This approach has the potential to change the way catheter-associated infections are addressed, offering a new perspective in infectious medicine.

3. Ch. Assist. Prof.Rositsa Milcheva has participated in a study aimed at creating a new type of polymer material called interpenetrating polymer network - hydrogel. This hydrogel, based on components poly (carboxybetaine) methacrylate and poly (sulfobetaine) methacrylate, showed the unique ability to respond to various external stimuli, such as temperature change, pH and salt concentration. Additionally, the hydrogel has shown excellent antibacterial properties and high biocompatibility, making it attractive for applications in medicine and pharmacy. This material could represent a significant advance in the development of new and innovative medical instruments and applications.

Other studies of a contributory nature:

1. Dr. R. Milcheva studied the influence of the feed mycotoxins Fumonisin B1 (FB1) and deoxynivalenol (DON) on the immune system of chickens, as well as their action on different cell lines in *in vitro* conditions. These mycotoxins were found to negatively affect the lymphocytes of chickens and have an antiproliferative and cytotoxic effect. These results illuminate the influence of certain toxins on the health of poultry.

In summary:

Dr. Rositsa Milcheva studied the interaction between the nematode *Trichinella spiralis* and cross-striated muscle tissue. She found that the invasion by the parasite causes morphological and functional changes in muscle cells by transforming them into a "Support Cell". The main role of apoptosis has been studied, finding that it is initiated in the nucleus before affecting the cytoplasm. Specific proteins such as PARP-1, PCNA, AIF, BAX and caspase-3 have been identified as key activators or regulators of this process. The findings suggest that *T. spiralis* is capable of manipulating these molecular pathways, which prevents necrotic cell death. These findings evolved from basically morphological observations to detailed molecular analyses that reveal host-parasite interaction at the cellular and gene levels. Such studies have the potential to discover new therapeutic strategies for the treatment of a variety of diseases related to the immune system and muscular dystrophy.

The research of Ch. Assist. Prof. Rositsa Milcheva is characterized by improving analytical approaches in medicine, preventing infectious diseases through innovative materials, and developing new biocompatible materials. It demonstrates the unique properties of alcohol fixatives in molecular biology, presents solutions to combat biofilms on medical catheters, and develops a new type of hydrogel with a wide range of applications.

IV. Evaluation of the monographic work or equivalent publications submitted for participation in the competition for "ASSOCIATE PROFESSOR" by the candidate.

Ch. Assist. Prof. Rositsa Milcheva has presented 5 publications that correspond in weight to monographic work. My personal opinion is in favor of the application of original scientific works, as modern trends in scientometrics give weight to original research.

V. Overall assessment of the candidate's compliance with mandatory conditions and mandatory quantitative criteria and scientific indicators.

Under the procedure, scientific data according to the Law on Combating Organized Crime and the Law on Combating Organized Crime are presented, as well as the minimum criteria of BAS in District 4. Natural sciences, mathematics, and informatics Professional field 4.3. Biological Sciences (Immunology), in sub-categories, as follows:

1. By group of indicators "A" – 1. Successfully defended dissertation for awarding NSA "Doctor" – submitted Autoreferate **(50 out of 50 points)**
2. By group of indicators "C" – Habilitation work or scientific publications in publications – publications with rank Q1 – 2 pcs. are presented; Q2 – 1 pcs.; Q3 – 2 pcs. **(100 out of 100 points)**
3. By indicator group "D" – Scientific publication in publications that are referenced and indexed in world-famous databases – 14 publications (with rank Q1 – 2 pcs., with Q2 – 2 pcs., with Q3 – 8 pcs. and with Q4 – 2 pcs.) **(234 points out of 220 points)**
4. By group of indicators "D" - Citations in scientific publications, monographs, collective volumes, and patents, referenced and indexed in world-famous databases with scientific information – 32 citations **(64 points from 60 points)**

This application exceeds the required points under the criteria **(448 points out of 430 points required)**.

VI. Conclusion

Dr. Rositsa Milcheva studies the complex interactions between the nematode *Trichinella spiralis* with muscle cells, focusing on apoptosis and the key molecular pathways associated with it and modulating the host's immune response. It introduces innovations in analytical approaches in medicine, develops methods to combat nosocomial infectious agents by creating new biofilms. Participates in the development and validation of new biocompatible materials.

This application fully meets the mandatory and specific conditions and scientific criteria – for the academic position of "Associate Professor". Ch. Assist. Prof. Rositsa Milcheva has 29 scientific papers, 22 participations in scientific forums. For the competition she has submitted 19 publications, a self-report and 32 citations. All submitted publications have an impact factor and/or rank. She has been a project manager and has participated in many projects. She has several specializations at the prestigious universities Comenius (Bratislava), Imperial College (London), the University of Wales and Carl Franz (Graz). She has won three awards and two certificates.

In conclusion, I firmly vote "YES" and recommend to the Scientific Jury in this competition the awarding of the academic position "Associate Professor" to Dr. Rositsa Milcheva, and I believe that her professional qualities and long-term achievements make her suitable for a leading researcher and future scientific supervisor of graduates and PhD students.

Sofia
04 September 2023

Reviewer:



/Prof. K. Todorova-Hayrabyan, DSc/