S T A T E M E N T

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On the dissertation for awarding of scientific degree "Doctor" in the field of higher education 7-Healthcare and sport, professional direction 7.1 - Medicine, Scientific Speciality – "Internal Medicine"

Candidate: Dr. Elena Panayotova Panayotova
Assistant Professor at the Department of "Propaedeutics of Internal Diseases",
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Title of the thesis: „The role of necroptosis in inflammatory bowel diseases"

By order № P-109-208/25.05.2022 of the Rector of the Medical University of Varna, I have been appointed as a **member of the Academic jury**. In compliance with a Decision took at the first meeting of the jury, I have been elected to prepare a **statement** on the aforementioned dissertation.
The submitted documents for review meet all requirements of the Law for development of the academic staff in the Republic of Bulgaria as well as the Regulations for development of the academic staff in the Medical University of Varna.

1. **Professional development**

Dr. Elena Panayotova graduated in Medicine from the Medical University of Sofia in 2004. Dr. Panayotova started her professional development as a physician in 2005 at the University Hospital "Alexandrovska" in Sofia. She has been appointed as a resident at the Clinic of Internal Medicine, University Hospital "St. Marina" in Varna, since 2009. She acquired two clinical specialities - a major in **Internal Medicine in 2014** and in **Gastroenterology in 2020.** Her teaching career at the Medical University of Varna started in 2013, as a part-time Assistant Professor at the Department of Propaedeutics of Internal Medicine. She is a full-time Assistant Professor at the same Department since 2017. Dr. Panayotova has been constantly developing her knowledge and clinical skills. Consecutively she acquired qualification to perform conventional abdominal ultrasound (first level of competence), Doppler ultrasound (second level of competence) as well as the right to perform conventional endoscopy (first level of competence) and interventional endoscopy (second level of competence). Dr. Panayotova is a co-author of more than 10 publications and has participated in several scientific events in the field of Internal Medicine and Gastroenterology. She is a member of the Bulgarian Society of Gastroenterology, Gastrointestinal Endoscopy and Abdominal Ultrasound. Dr. Panayotova speaks English and Russian languages.

2. **Scientific relevance of the dissertation topic**

Inflammatory bowel diseases (IBD) are still a scientific challenge in current gastroenterology. Clinical course of IBD includes alternating periods of remission and exacerbation of active inflammation of the intestinal mucosa, which can affect different parts of the gastrointestinal tract. Severe local and systemic complications often occur during disease activity. The significant social and economic importance of the problem is determined by affecting of young people and causing a significant decrease of patient's quality of life, as well as development of disability in active age. There is a need of long-term treatment, frequent and prolonged hospitalizations, which is associated with the high costs and need of significant public resources. Despite advances in our knowledge over the last decade, the aetiology and several aspects in the pathogenesis of IBD remain unknown. The accumulated evidence in recent years has shown that impaired barrier function, as a primary defect, is a key factor in the development of IBD. In order to ensure the structural integrity and stability of the barrier, the cell death of intestinal epithelial cells needs to be strictly regulated. Recent experimental studies have identified a new type of cell death in the intestinal epithelium known as necroptosis. Necroptosis is associated with inflammation, which characteristics are quite similar to IBD. This suggests its potential involvement in the pathogenesis of IBD. The presented dissertation is dedicated to this important topic

that is not fully elucidated. Actually, the discussed PhD thesis is the first in-depth study on this issue in our country, which evaluated Bulgarian IBD patients.

3. **Dissertation structure and topic**

The dissertation contains 166 pages in total. It is correctly and classically structured and includes the following parts: introduction; review of the literature; goal, tasks and research hypothesis; methodology; results; discussion; conclusions and references.

The literature review is comprehensive and professionally written. It provides a complete description of the scientific problem under consideration. The content is well focused on the purpose and objectives of the dissertation. An in-depth analysis of the most significant current studies focused on the scientific topic has been performed. The leading tendencies of the discussed problems are clearly outlined with special attention of the contradictory data that have motivated the author to conduct her research and to develop the presented thesis.

The study goal is correctly and clearly stated: to investigate the presence of necroptosis in patients with inflammatory bowel disease (Crohn's disease and ulcerative colitis), determining the level of RIPK3 expression in the intestinal mucosa as a marker of necroptosis and to assess its relationship with clinical and pathological disease indicators in patients with IBD. The author logically has set 5 specific and feasible tasks that are directly related to the achievement of the study goal.

Methodes: A total number of 200 subjects were studied - 30 healthy volunteers and 170 patients with IBD. They were divided in 2 groups: 85 patients with Crohn's disease (CD) and 85 subjects with ulcerative colitis (UC). The study is retrospective. Complete medical records, as well as available biopsy samples were found for each participant. The selected patients and healthy controls meet strict and well-defined inclusion and exclusion criteria. A complexed and precise patient characterization has been performed by using current and advanced diagnostic methods that included clinical examination, laboratory testing and endoscopy, as well as morphological and imaging assessment. For the histological examinations, paraffin blocks with biopsy samples from the studied subjects stored at the Clinic of General and Clinical Pathology of the University Hospital "St. Marina" were used. The expression of RIPK3 as a marker for necroptosis was determined by immunohistochemistry. The specific laboratory protocol used for measurement of RIPK3 expression is precisely described. The types of kits used are correctly provided with exact citation of their catalogue numbers. A wide range of adequate and reliable statistical analyses have been used for data processing, guaranteeing the reliability of the obtained results and the conclusions made.

The results are presented in details and are appropriately illustrated with tables and figures. Due to the lack of validated cut-off values of RIPK3 expression the author determined the threshold values for distinguishing IBD patients from healthy controls, as well as patients with CD from UC. Dr. Panayotova found a higher RIPK3 expression in inflamed tissues of patients with IBD compared to healthy controls. The highest expression of the studied marker was found in patients with UC. Different patients' profiles were found in CD and UC in terms of RIPK3 expression. In CD patients, the high expression of the marker for necroptosis correlates with the young age of the disease onset (<16 years), the increased number of hospitalizations, the extensive spread of the disease (upper GIT with ileum / colon), stricturing with fistulising form of the disease (B2 + B3), the presence of perianal disease, moderate and severe clinical activity (CDAI> 220), frequent relapses, presence of endoscopic and histological activity. In patients with UC, elevated RIPK3 expression correlates with disease onset over 40 years, severe clinical and histological activity, and haematochezia. The increased expression of RIPK3 in CD patients is a prognostic factor for predicting the frequency of relapses, clinical manifestations with hematochezia, the occurrence of intestinal complications, accompanying diseases and need of future surgical interventions. In UC patients the increased expression of RIPK3 is a prognostic factor that predicts clinical manifestations with hematochezia, increased incidence of diarrhoea, future surgical interventions, high incidence of infections and high disease activity. Dr. Panayotova shows that in patients with IBD high RIPK3 expression is associated with severe disease, pronounced clinical, endoscopic and histological activity, lack of response to treatment and poor prognosis associated with complications and need of surgical interventions.

In the section "Discussion" the obtained results are compared and creatively discussed in the light of currently published data.

The essential part of dissertation ends with ***11 conclusions*** precisely formulated by the author that I fully agree with, as they are logical consequence of the obtained results and are fully in line with the main goal and tasks of the research. I also acknowledge the full list of author's contributions. The most significant are four of them that are original: for the first time in Bulgaria and globally the role of RIPK3 expression as a marker for differentiating patients with IBD has been studied; a relationship between the expression of this marker and the clinical and endoscopic activity of patients with IBD has been demonstrated; RIPK3 expression was assessed as a prognostic marker for the development of severe and progressive disease. For the first time in our country the nuclear expression of RIPK3 was studied in patients with IBD.

In relation to the present dissertation author has published 5 scientific papers – 4 in Bulgarian and 1 in English language. Some of the results were also presented at 2 national scientific events, one of them were with international participation. Dr. Panayotova is the first author in all 7 articles. This fact clearly shows her leading contribution in the research development and obtaining of the study results.

Conclusion

Dissertation of Dr. Elena Panayotova is a well-planned scientific study that is focused on an important problem of current gastroenterology. A sufficient number of patients have been studied. Current and advanced methods of research were used to achieve the study goal. The obtained results are well summarized and analyzed. They lead to important conclusions and significant contributions. This is the first study in Bulgaria on the role of necroptosis in IBD, assessed by measurement of RIPK3 expression in patients with IBD. Some of the obtained results and conclusions are original and are pioneering not only for our country, but also from the international perspective. They definitely enrich our current knowledge in this interesting and still insufficiently studied field of medical science.

I strongly believe that the discussed thesis meets all requirements of the Low for development of the academic staff in the Republic of Bulgaria, as well as the relevant inner Regulation for development of the academic staff in the Medical University of Varna.

I grant my positive assessment and I recommend that the academic jury awards Dr. Elena Panayotova Panayotova the academic degree "Doctor" in Internal Medicine.

30/May/2022.
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/Professor Deian Jelev, MD, PhD, DSc/