

# **REVIEW**

**By Prof. Dr Miglena Dimitrova Georgieva, PhD**

**Pediatrician, pediatric gastroenterologist**

**Head of the Second Children's Clinic, University Hospital "St. Marina"**

**Department of Pediatrics, Medical University of Varna**

appointed as an internal member of the Scientific Jury and for preparation of a review by Order № P -109-218 / 25.05.2022. of the Rector of the Medical University of Varna

Subject: Dissertation for awarding a scientific and educational degree "Doctor"

Author: Dr. Elena Panayotova Panayotova - Department of Propaedeutics of Internal Medicine, Faculty of Medicine, Medical University of Varna

Topic: "The role of necroptosis in inflammatory bowel disease" in the field of higher education 7. "Health and Sports" in professional direction 7.1. "Medicine" and the scientific specialty "Internal Medicine"

Research Supervisor: Assoc. Prof. Dr. Antonia Yordanova Atanassova, MD, PhD, Dsc

Dr. Elena Panayotova graduated with a degree in Medicine from the Medical University - Sofia in 2004, and in 2014 acquired a degree in "Internal Medicine" and in 2020 in "Gastroenterology". In 2005 she started working at the University Hospital "Alexandrovska" - Sofia, and since 2009 she has been a physician at the University Hospital "St. Marina" - Varna until present. Her career as an assistant at the Medical University - Varna began in 2013 as a part-time assistant at the Department of Propaedeutics of Internal Medicine, and since 2017 she has been a full-time assistant at the same department.

Inflammatory bowel disease (IBD), with Crohn's disease (CD) and ulcerative colitis (UC), is a group of idiopathic diseases characterized by chronic recurrent inflammation of the gastrointestinal tract. These are diseases that mainly affect young people and have a lifelong evolution. They alternate between periods of remission and relapse, with phases of activity varying in duration and often leading to local and systemic complications. This worsens the quality of life of patients, requires long-term treatment and is associated with significant socio-economic costs.

Despite intensive efforts over the years to understand the etiology and pathogenesis of IBD, they still remain unclear. This limits therapeutic options to the extent that symptoms are maintained, remission is maintained, and relapses are avoided, but not to definitively cure patients.

Evidence accumulated in recent years reveals that impaired intestinal 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 called necroptosis, which leads to inflammation, with characteristics similar to inflammatory bowel disease. This has suggested its involvement in the pathogenesis of these diseases.

The *structure* of the dissertation presented by Dr. Elena Panayotova fully corresponds to the presentation for awarding a scientific and educational degree "Doctor" - introduction, literature review, goal and tasks, methodology of the dissertation, results, discussion, conclusions and contributions. It is written on 166 pages and is illustrated with 16 tables, 90 figures and 2 photos.

The *bibliography* includes 283 literature sources, of which 9 are in Cyrillic and 274 in Latin. The bibliography is extensive and well-designed. Over 10% of the cited authors are from the last 5-6 years.

The *introduction* emphasizes that the data from the literature on the role of necroptosis in IBD in humans are scarce. Given this, the need to study the expression of RIPK3 in patients with IBD is a first step in a personalized approach to staging, follow-up and treatment.

The *literary review* presented in the dissertation is very in-depth and covers 5 sections in a logical sequence.

Dr. Panayotova concretely and analytically presents the most important highlights published in the literature in connection with necroptosis and inflammatory bowel disease. Necroptosis is a newly discovered inflammatory form of regulated cell death that depends on the activity of RIPK3 and MLKL.

Necroptosis in the intestinal epithelium and the subsequent loss of barrier integrity may explain the chronic intestinal inflammation that underlies the pathogenesis of IBD.

After an in-depth and analytical review of the literature and research on this issue, Dr. Panayotova concluded that inhibition of key molecules involved in the necrotic pathway may

provide new opportunities for the treatment of these diseases by not only reducing or suppresses the symptoms of intestinal inflammation, but also prevents major molecular processes. Therefore, it is important to clarify the role of necroptosis in patients with IBD as a first step in a personalized approach in patients with IBD.

The *study goal* of the dissertation is very clearly and specifically formulated - to study the presence of necroptosis in patients with inflammatory bowel disease (Crohn's disease and ulcerative colitis), determining the level of expression of the marker for necroptosis - RIPK3 in the intestinal mucosa and its relationship with clinical and pathological indicators in patients with inflammatory bowel disease.

To achieve this goal, she sets 5 *tasks*, which also correspond to the goal in a logical sequence.

The study included 170 patients over 18 years of age diagnosed with IBD (of which 85 patients with Crohn's disease and 85 with ulcerative colitis), as well as 30 healthy controls passed through the structures of the University Hospital "St. Marina - Varna for the period from 2011 - 06.2020. The study is retrospective, as the participants included in the study are patients diagnosed with CD and UC according to the criteria of the ECCO. Complete medical records as well as biopsy material are available for each patient. The medical documentation of each patient was examined in detail and anamnestic, clinical, laboratory, imaging, endoscopic and morphological data were collected and used in the analyzes.

The patients included in the control group have no history of disease and do not take any medication. Selected patients were diagnosed with functional gastrointestinal disorders in which there were no clinical, laboratory, imaging, endoscopic and histological data on gastrointestinal diseases, as well as data on inflammatory, oncological and severe comorbidities.

All clinical, laboratory, imaging, endoscopic (fibrogastroduodenoscopy, ileocolonoscopy) and morphological examinations, as well as surgical interventions with resections of segments of the gastrointestinal tract (incisional biopsies), which were used for immunohistochemical analysis were performed on the territory of the University Hospital "St. Marina" – Varna.

Patients were selected according to specific criteria for inclusion and exclusion in the study.

These data are indicative of the aspiration of Dr. Elena Panayotova not only to achieve new directions in diagnosis, treatment and follow-up, but also to derive reliable science-based results and conclusions to support and improve clinical practice and treatment approach in patients with inflammatory bowel disease.

The indicated statistical methods allow to illustrate in detail the set tasks.

The *results* obtained on the individual tasks confirm the in-depth analysis of the clinical material. Despite the specifics of processing this clinical material, Dr. Panayotova very clearly presents it in tables and figures. The PhD student reviews her results and discusses each individual task, which gives a very accurate assessment of the clinical material by comparing it with the available results of other authors who have performed similar studies among different groups of patients.

The *discussion* is focused and competent. The ability of Dr. Panayotova to analyze her own results in the context of those known in the literature is evident, comparing her results with the results of international and national databases.

The very scarce publications and information in the literature show that her research is timely, giving the opportunity to apply a new indicator for determining the diagnosis and prognosis of inflammatory bowel disease. There are few data in the literature on the role of RIPK3 in IBD, which is limited to single studies in patients with IBD, and the results of these studies are mainly aimed at demonstrating the relationship between necroptosis marker expression and the presence of inflammatory activity.

Due to the lack of validated values of the expression of the marker for necroptosis and better assessment of the prognostic role of the marker, cut-off levels were set to distinguish patients with IBD from healthy controls, as well as patients with CD and UC. These results serve as a guide for the direction and intensity of expression according to the considered characteristics and indicators in the studied groups. Thus, according to the established cut-off levels, it is proved that patients with IBD have increased expression compared to healthy controls, and patients with UC are characterized by overexpression of the marker compared to patients with CD. Thus, RIPK3 can be used as a marker to distinguish CD from UC and as a prognostic marker for the development of severe disease and progression.

Based on the obtained results, 11 *conclusions* are formulated, which follow the logically set goals and tasks of the study. Among them are the following:

1. The expression of RIPK3 differed significantly in patients with CD and UC and healthy controls, with the highest expression of the marker being found in patients with UC.

2. High expression of RIPK3 in CD patients is associated with age at debut up to 16 years (A1), increased number of hospitalizations, upper GIT involvement in combination with colonic (L2) or ileal involvement (L1), stricturing with fistulizing form of the disease (B2 + B3), accompanying perianal disease, clinical activity of the disease (CDAI > 220), frequent relapses, the presence of hematochezia, high endoscopic and histological activity, discontinuation of

conventional and biological treatment.

3. High expression of RIPK3 in UC patients is associated with debut, debut of disease over 40 years of age, rare relapses, hematochezia, severe disease activity as assessed by the Montreal Classification and the overall Mayo score, marked endoscopic and histological activity.

4. 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 future surgical interventions.

5. Increased expression of RIPK3 in UC patients is a prognostic factor that predicts clinical manifestations with hematochezia, increased incidence of diarrhea, future surgical interventions, high incidence of infections and high disease activity.

6. In IBD patients, high expression of the necroptosis marker (RIPK3) is associated with severe disease, marked clinical, endoscopic and histological activity, lack of response to treatment and poor prognosis associated with complications and surgical interventions.

The *contributions* presented by Dr. Panayotova are given very accurately and correctly and are defined as contributions of theoretical, practical-applied and original.

Dr. Elena Panayotova presents 5 *publications* in periodicals, 3 of which are full-text and 2 abstracts. She is the first author in all publications. The publications reflect various aspects of the results obtained and the reviews of the literature examining the association between necroptosis and inflammatory bowel disease.

The *abstract* is written on 86 pages and fully presents the dissertation

In conclusion, I would like to emphasize again my high appreciation for the clinical and research activities presented in the dissertation of Dr. Panayotova and I will confidently vote "YES" for the award of the scientific and educational degree "Doctor" of Dr. Elena Panayotova Panayotova.

2.06.2022.

Varna

Signature:

Prof. Dr Miglena Georgieva, PhD

