

S T A T E M E N T

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

Candidate: Dr. Antonia Yordanova Atanassova, PhD,
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Title of the thesis: „Serum expression of microribonucleic acids in patients with chronic inflammatory bowel diseases“

By order № P-109-217/17.05.2021 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 in 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

Assoc. Professor Antonia Atanassova graduated in Medicine from the Medical University of Varna in 1986. In 1986-1987 she worked as a physician in the Department of Internal Medicine at the District Hospital of Shumen. Consecutively she was appointed as an Assistant Professor (1987), Senior Assistant Professor (1994) and Chief Assistant Professor (1997) at the Clinic of Gastroenterology, University Hospital St. Marina, Medical University of Varna. She acquired two clinical specialities - a major in **Internal Medicine in 1994** and in **Gastroenterology in 1996**. In 2014 she obtained the **academic degree "Doctor" in Gastroenterology**, after successfully defending a dissertation on the following topic: "Clinical assessment of patients with ulcerative colitis – current approach". Since 2015 she is an Associate Professor in Gastroenterology at the Medical University of Varna. Dr. Antonia Atanassova has been constantly developing her knowledge and clinical skills. In 2008 she acquired qualification to perform abdominal ultrasound as well as diagnostic and therapeutic gastroscopy and colonoscopy. She has participated in numerous postgraduate educational gastroenterology courses conducted in Bulgaria, Czech Republic, Denmark, Greece, Hungary, Austria, Serbia, Spain, Belgium and United Kingdom.

The scientific interest of Assoc. Professor Atanassova is focused mainly on the inflammatory bowel diseases. In this direction are the majority of her scientific papers as well as the presented dissertation. She is leading investigator of scientific project funded by Medical University of Varna and participates in International Scientific project on Celiac disease in countries of Danube Region. Assoc. Prof. Atanassova is a supervisor of two PhD students. She is teaching medical students in Bulgarian and in English at the Medical University of Varna by lectures and practical exercises. She is also actively involved in the postgraduate training in Gastroenterology. Assoc. Prof. Atanassova is an established specialist and expert in the diagnosis and treatment of gastrointestinal diseases, especially in the field of intestinal pathology. She speaks French, English and Russian languages.

2. Scientific relevance of the dissertation topic

Inflammatory bowel diseases (IBD) are still a scientific challenge in current gastroenterology. Despite advances in our knowledge over the last decade, the aetiology and several aspects in the pathogenesis of IBD remain unknown. Monitoring of IBD patients is quite difficult due to the need of repeated invasive endoscopic biopsy procedures. This requires intensive search of non-invasive

methods for patients' assessment and follow-up. Recently, there is a growing research interest in miRNAs, especially after establishing of their essential role in each stage of the inflammation process. Most publications are focussed on the use of miRNAs for the diagnosis and treatment of various cancers. Research in IBD is still limited and the relevant results are very contradictory. The reason for this is the use of different panels and methods for extracting of miRNAs, the study of different populations of IBD patients with different disease duration, as well as with different treatment regimens. This significantly complicates data comparison from the available studies. It is very difficult to relay on the discussed results for selection of an appropriate panel of miRNAs in order to perform further validation of such a panel in Bulgarian subjects to distinguish IBD patients from healthy controls and to define the differences in miRNAs expression in subjects with Crohn's disease (CD) and ulcerative colitis (UC). The presented dissertation is the first, in-depth study on this topic in our country that includes Bulgarian patients with IBD.

3. Dissertation structure and topic

The dissertation contains 223 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 study and evaluate the serum expression of some miRNAs in patients with chronic inflammatory bowel disease. The author logically has set 5 specific and feasible tasks that are directly related to the achievement of the study goal.

The section „Methodology“ is very well presented. A total number of 100 subjects were studied - 30 healthy volunteers and 70 patients with IBD. All patients were prospectively studied. They were divided in 2 groups: 35 CD patients (20 with active disease and 15 in remission) and 35 subjects with UC (20 with active disease and 15 in remission). A complexed and precise patient characterization has been performed by using modern and advanced diagnostic methods that included clinical examination, laboratory testing and endoscopy, as well as morphological and imaging assessment. The specific laboratory protocols for measurement of miRNAs expression are precisely described and the type of kits used at any step of investigations 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 values, cut-off values of miRNAs were determined in healthy controls, which served as a guide in the direction of expression and based on these results it was noted that in CD patients there is overexpression of the considered miRNAs, while in UC patients' expression is reduced, approaching the cut-off values of the healthy controls. Different miRNA-signatures were observed for activity and remission of both diseases. According to the localisation and flow form a quite different miRNAs are detected for the two diseases. Intestinal complications and extra intestinal manifestations correlate with different miRNAs in patients with CD and UC. In patients with CD the duration of the disease correlates with the increased expression of miR-28 and miR-96, while in UC with - miR-144 and miR-155. Corticosteroids treatment is associated with increased expression of miR-96 for CD and miR-142-3p and miR-155 for UC, which are disease-specific. 5-ASA therapy in UC is associated with decreased expression of miR-16 and miR-142-5p, while in CD it is associated with increased expression of miR-144. Azathioprine treatment is associated with decreased expression of miR-28, miR-142-3p and miR-1228-3p in patients with CD, while the in UC patients the miR-96 expression is below the threshold determined in healthy subjects. Biologic therapy in patients with CD correlates with increased expression of miR-28, whereas in patients with UC the values of miR-1228-3p are approaching the

relevant threshold values in healthy individuals. Assoc. Prof. Atanassova clearly demonstrate that the increased expression of miR-28 in CD is a specific marker of induced remission and it correlates with decreased levels of CRP, FCP, CDAI, normal serum level of iron, vitamin B₁₂ and vitamin D. In addition there is a correlation between the serum expression of miR-142-5p, miR-96, miR-199a and vitamin D levels in patients with IBD.

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. There are significant author's contributions to medical science and clinical practice:

- **Theoretical contributions:** For the first time in Bulgaria the use of miRNAs in adult IBD patients has been studied in details. An accurate and precise description of the expression of miRNAs has been performed during active disease and in remission; the expression of miRNAs is described according to the characteristics of IBD patients and the ongoing treatment.
- **Practical contributions:** thresholds have been established to differentiate the expression of miRNAs; a specific profile of CD and UC patients is defined based on the expression of miRNAs; specific miRNAs have been identified for remission and disease activity, as well as localisation and treatment course; an in-depth analysis of miRNAs expression was performed according to Vitamin D levels.
- **Original contributions** (for the first time in Bulgaria): in adult patients a panel of miRNAs was studied to assess IBD; the role of miRNAs with proven effectiveness in characterising patients with oncological diseases (miR-16, miR-28, miR-96, miR-155, miR-199, miR-363 and miR-451) was applied and studied in adult patients with IBD; the expression of miRNAs has been described in accordance with the applied therapy in IBD patients; a correlation between the expression of certain miRNAs and Vitamin D deficiency in IBD patients has been demonstrated.

In relation to the present dissertation author has published 10 scientific papers – 8 in Bulgarian and 2 in English language. One of them is in the journal which is indexed and referred in the Web of Science database. One of the discussed papers is published in Journal with impact-factor. Dr. Atanassova is the first author of all 10 articles and in 7 of them she is the only author. This fact clearly shows her leading contribution in the research development and obtaining of the study results.

Conclusion

Dissertation of Assoc. Prof. Antonia Atanassova, MD, PhD is well planned scientific study that is focused on the important problem of current gastroenterology. A sufficient number of patients have been studied. Modern 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 with generalized observations and conclusions on the use of a panel of miRNAs for assessment of adult patients with IBD. The work answers a number of important scientific questions. I strongly believe that the discussed thesis meets all requirements of the Law 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 Associate Professor Antonia Yordanova Atanassova, MD, PhD the scientific degree "Doctor of Science" in Gastroenterology.

14.06.2021r.
S o f i a

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/Professor Dejan Jeleu, MD, PhD, DSc/