

STATEMENT

by Prof. Dr. Valeria Ignatova Kaleva, MD, PhD

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On: Dissertation for obtaining scientific degree "Doctor of Science" for Higher Education in the field 7. Health and Sport, Professional Field 7.1. Medicine, Scientific specialty: "Gastroenterology", on the topic "Serum expression of microribonucleic acids in patients with chronic inflammatory bowel disease"

Candidate: Assoc. Prof. Dr. Antonia Yordanova Atanassova, MD, PhD, Department of Anatomy and Cell Biology, Faculty of Medicine, Medical University "Prof. Dr. Paraskev Stoyanov" – Varna

With a Decision of the Faculty Council of the Faculty of Medicine No. 43/ 11.05.21 and an Executive Order of the Rector of the Medical University "Prof. Dr. Paraskev Stoyanov" - Varna No. P-109-217 from 17.05.2021, I have been appointed as a member of the Scientific Jury evaluating the dissertation of Assoc. Prof. Dr. Antonia Yordanova Atanassova, MD, PhD, for acquiring the scientific degree "Doctor of Science". With Protocol 1/31.05.2021 from a meeting of the Scientific Jury, I was assigned to prepare a Statement of the thesis.

1. Biographical data and professional development

In 1980, Assoc. Prof. Dr. Antonia Atanassova, MD, graduated with honours IV EG "Fr. Joliot-Curie", in 1986 she graduated with honours from the Higher Medical Institute - Varna. After obtaining a master's degree in Medicine, from 01.12.1986 to 16.06.1987 she worked as a resident doctor in a hospital in Shumen. After successfully passing the exam for assistant physician, Dr. Atanassova began working at HMI- Varna and at the Clinic of Gastroenterology at MHAT "St. Marina" in the period 1987-1994. From 1994 to 2015, Dr. Atanassova worked as a senior and chief assistant at the same Clinic. In 1994, Dr. Atanassova acquired the specialisation "Internal Medicine", and in 1996, she acquired the specialisation "Gastroenterology". Antonia Atanassova speaks Russian, English and French.

In 2014, she defended a dissertation entitled Clinical Evaluation of Patients with Ulcerative

Colitis-Modern Approach. With this, Dr. Antonia Atanassova acquired a PhD in the scientific specialty "Gastroenterology", and since 2015 she has been an associate professor at the Department of Anatomy and Cell Biology, Faculty of Medicine at MU - Varna.

Assoc. Prof. Atanasova has participated in numerous postgraduate courses in various fields of gastroenterology, held in Bulgaria, the Czech Republic, Denmark, Greece, Hungary, Austria, Serbia, Spain, Belgium and the United Kingdom.

The research interests of Assoc. Prof. Atanassova are mainly in the field of inflammatory bowel diseases. Most of the scientific publications are in this field, including the dissertation submitted for review. She manages a project under the Science Fund at the Medical University of Varna. She also participates in an international research project on Celiac disease in the Danube countries. Assoc. Prof. Atanassova is a research supervisor of two PhD- students. At MU Varna, she gives lectures in Bulgarian and English and practical exercises to students, trainee doctors, postgraduates and general practitioners. Assoc. Prof. Atanassova is an established specialist in the field of gastroenterology.

2. Evaluation of the significance of the topic of the dissertation

Chronic inflammatory bowel diseases (IBD) are diseases with an increasing frequency worldwide and in our country. They are associated with large direct and indirect financial costs for diagnosis and treatment, and in scientific terms are one of the biggest challenges in modern gastroenterology. Despite numerous studies over the last twenty years, the etiology and pathogenesis of IBD remain poorly understood. Modern treatment is associated with insufficiently effective response, loss of response and a number of adverse events. Follow-up of patients is associated with invasive endoscopic procedures and insufficiently effective surrogate biomarkers. This necessitates the search for new non-invasive methods for assessment and monitoring. The use of miRNAs in cancer patients to stratify the risk of onset, progression, and treatment response has recently been established. Given the link between inflammation and the occurrence of many cancers, it is logical that Assoc. Prof. Atanassova is interested in establishing whether it is possible through serum expression of certain miRNAs to diagnose and monitor patients with immunomediated diseases such as Crohn's disease (CD) and ulcerative colitis (UC). Publications in the global database in the field of IBD and miRNAs are limited, and data is very contradictory because the sources of miRNAs are different tissues and secretions, and the patients studied have

different disease characteristics and treatment. This significantly complicates the comparison of the data and therefore the selection of a panel of miRNAs with which to characterize these patients and to validate the data among the Bulgarian population is an important task. Using the opportunities provided by research in the field of miRNAs expression, their application among Bulgarian patients with CD and UC is a new modern approach that provides a different view and current solution in the field of diagnosis, follow-up and treatment of patients with IBD.

The presented dissertation is the first systematic analytical study on the issue in our country among Bulgarian patients with IBD.

3. Structure and content of the dissertation

Assoc. Antonia Atanassova's dissertation is spread over 179 standard printed pages (excluding the pages of the bibliography). The dissertation has the following chapters: 1. Abbreviations; 2. Introduction; 3. Literature review; 4. Aim, objectives and hypothesis; 5. Methodology; 6. Results; 7. Discussion; 8. Implications; 9. Conclusion; 10. Original contributions of the dissertation; 11. Publications related to the dissertation; 13. Bibliography

The **literature review** is comprehensive and written in correct Bulgarian and is easy to read. It gives an in-depth analysis of the problem in the light of the latest research. Its content is entirely focused on the purpose and objectives of the dissertation. The modern tendencies, difficulties and contradictory data, which are the main motive for the development of the dissertation, are clearly outlined.

The **goal** is clearly stated: to study and evaluate the serum expression of some miRNAs in patients with chronic inflammatory bowel disease. It follows logically and reasonably 5 specific and logical tasks directly related to the achievement of the set goal.

The **Material Methodology** chapter is presented very accurately. A total of 100 subjects were studied - 30 healthy volunteers and 70 patients with IBD. Patients were examined prospectively. They are divided into two groups: with CD - 35 patients (20 with active CD and 15 in remission) and UC - 35 patients (20 with active UC and 15 in remission).

Accurate characterization of patients was performed using classical and state-of-the-art examination methods - clinical, laboratory, endoscopic, morphological and imaging. The steps of

testing the serum expression of miRNAs are described in detail, presenting the type of kits used, catalogue numbers and the process of obtaining the results. A wide range of reliable and up-to-date statistical analyses have been applied for data processing, guaranteeing the reliability of the obtained results and the conclusions made. The research results are presented in detail and appropriately illustrated with 59 tables and 55 figures. The author begins by calculating the threshold values of the selected panel of miRNAs in healthy controls due to the lack of validated values and compares their expression with that in patients with IBD. The positive and negative predictive values of this panel of miRNAs were established. Assoc. prof. Atanassova discovered that the expression of the considered miRNAs differs in CD and UC patients. In CD, there is overexpression, while in UC the expression is significantly lower. In the two groups of patients, not only was there a difference in the expression of the individual miRNAs according to the disease activity, but a disease-specific miRNA- signature was also found. Depending on the location and form of the disease, different miRNAs have been identified for the two diseases. It has been observed that in patients with CD and UC intestinal complications and extraintestinal manifestations correlate with different miRNAs. In CD, the disease duration is associated with increased expression of miR-28 and miR-96, and in UC, the expression of miR-144 and miR-155 is increased. Increased expression of miR-96 (CD) was observed during treatment with corticosteroids, and miR-142-3p and miR-155 were expressed in UC patients. It has been observed that 5-ASA therapy in UC patients is associated with decreased expression of miR-16 and miR-142-5p, whereas in CD patients there is increased expression of miR-144. Decreased expression of specific miRNAs (miR-28, miR-142-3p and miR-1228-3p) was observed during treatment with azathioprine in CD patients, while in UC patients the expression of miR-96 was below the threshold value determined in healthy individuals. Biologic therapy correlated with increased expression of miR-28 in CD patients, while in UC patients the values of miR-1228-3p approached those of controls. Assoc. Prof. Atanassova shows that the increased expression of miR-28 in CD patients is a specific marker for achieved remission and correlates with decreased levels of CRP, FCP, CDAI, normal serum Fe, vitamin B12 and vitamin D. It also demonstrates a correlation between serum expression of miR-142-5p, miR-96, miR-199a and vitamin D levels in patients with IBD.

In the "**Discussion**" chapter, the results are explained in detail and systematically compared with the data available in the medical literature. Assoc. Prof. Atanassova shows that she has

significant awareness and competence, which allow her to compare the data from the literature with the results achieved.

The dissertation presents eleven conclusions. Significant contributions to medical science and practice stand out:

Theoretical contributions

1. For the first time in Bulgaria, the use of miRNAs in adult IBD patients has been reported in detail and thoroughly.
2. A decisive, accurate and detailed description of the expression of miRNAs in IBD patients during active disease and remission has been performed.
3. The expression of miRNAs is thoroughly reflected according to the characteristics of IBD patients and the ongoing treatment.

Practical contributions

1. Thresholds have been established to differentiate the expression of miRNAs.
2. A specific profile of CD and UC patients was prepared based on the expression of miRNAs.
3. Specific miRNAs for remission and activity, localisation, course and treatment have been identified.
4. An in-depth analysis of miRNAs expression was performed according to Vitamin D levels.

Original contributions

1. For the first time in Bulgaria, a panel of miRNAs was studied to assess IBD in adult patients.
2. For the first time in Bulgaria, in adult IBD patients, the role of miRNAs was studied, which has proven its effectiveness in characterising patients with oncological diseases (miR-16, miR-28, miR-96, miR-155, miR-199, miR-363 and miR-451).
3. For the first time in Bulgaria, the expression of the studied miRNAs in relation to the applied therapy in IBD patients has been described.
4. For the first time in Bulgaria, a correlation between the expression of certain miRNAs and Vitamin D deficiency in IBD patients has been demonstrated.

In connection with the dissertation, 10 published articles were submitted - 8 in Bulgarian and 2 in English. There is one publication printed in a publication indexed and referenced in the Web of Science and one in a journal with an impact factor. In all 10 articles, Assoc. prof. Atanassova is the

first author and in 7 of them she is the only author. This shows her indisputable leading role in the research and the results obtained, which are the work of the dissertation.

The **abstract** is written on 80 pages and precisely and accurately presents the dissertation.

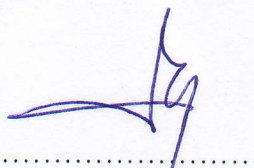
5. Conclusion

The dissertation of Assoc. Prof. Antonia Atanassova is dedicated to an extremely important and modern subject in gastroenterology. A sufficient number of patients have been studied. Modern research methods adequate to the purpose and tasks are used. The obtained results are well summarized and analyzed and lead to important conclusions and significant contributions.

I conclude that the presented dissertation completely fulfils the Bulgarian Law on the Career Development of the Academic Staff and local regulations of MU-Varna in this respect for acquiring the scientific degree "Doctor of Science". That is why I will vote in favour for awarding her with the Degree and recommend the same to the other members of the honoured Scientific Jury, appointed with an Executive Order No R-109-217 from 17.05.2021 of the Rector at Medical University-Varna.

14.06.2021 r.

Varna



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