REVIEW

by Professor Dr. Krassimir Antonov Antonov, MD, PhD, DSc

appointed as an external member of the Scientific Jury and for the preparation of review with Order R-109-217 from 17.05.2021 of the Rector of the Medical University-Varna

Subject: Dissertation for obtaining the scientific degree "Doctor of Sciences"

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Topic: „Serum expression of microribonucleic acids in patients with chronic inflammatory bowel disease" in the field of higher education 7. „Healthcare and sports" in a professional direction 7.1. „Medicine" and scientific specialty „Gastroenterology" in the Department of Anatomy and Cell Biology at the Faculty of Medicine of the Medical University of Varna.

Inflammatory bowel diseases are immunomediated diseases in the pathogenesis of which lie chronic inflammation and altered immune responses. Globally and in our country, there is a tendency towards an increase in the incidence of these diseases, affecting mainly young patients. Early diagnosis, distinguishing Crohn's disease (CD) from ulcerative colitis (UC) at onset, controlling inflammation, and monitoring the effect of treatment to avoid disability in these patients are important challenges. Existing biomarkers for tracking inflammation in Crohn's disease and ulcerative colitis may not fully help to solve these tasks.

Numerous studies have shown that miRNAs play a significant role in each stage of inflammation. They are involved in the differentiation, regulation and cellular signalling of the innate and acquired immune system, the control of enterocyte barrier function, the negative feedback on various signalling pathways associated with inflammation, the control of apoptosis and autophagy processes, dysplasia and cell death.

The structure of the dissertation presented by Assoc. Prof. Dr. Antonia Atanassova fully corresponds to the presentation for defence - introduction, literature review, purpose and tasks, methodology of the dissertation, results, discussion, conclusions and contributions. It is written on 223 pages and is illustrated with 59 tables and 55 figures.

The bibliography includes 574 literature sources, 8 of which are in Cyrillic and 566 in English. The bibliography is extensive, well-formed, with very few Bulgarian authors present. Over 20% of the cited authors are from the last 5 years.

The introduction emphasizes that the data from the literature still remain quite contradictory due to the different sources of miRNAs (mucosa of the intestine and colon, peripheral blood, faeces, etc.) and the different patient populations (stage, disease duration and therapeutic regimens). Given the opportunities offered by research in the field of expression of miRNAs, their application among Bulgarian CD and UC patients 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 chronic inflammatory bowel disease.

The literary review presented in the dissertation is very in-depth and covers 15 sections in a logical sequence. In the first six sections, Assoc. Prof. Atanassova discusses the most important issues related to chronic inflammatory bowel disease, diagnosis, biomarkers of follow-up inflammation and the response to treatment.

Assoc. Prof. Atanassova specifically and analytically presents the most important approaches published in the literature in connection with the use of serum expression of certain microribonucleic acids to distinguish patients with Crohn's disease from those with ulcerative colitis, response to corticosteroid treatment, fibrosis formation, response of various therapies and progression of the inflammatory bowel disease to dysplasia and colorectal cancer. The indicated substantiation and conclusions from the literature review gives a full basis for the development of the dissertation.

The aim of the dissertation is very clearly and specifically formulated - to study and evaluate the serum expression of some miRNAs in patients with chronic inflammatory bowel diseases.

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

The following miRNAs were tested (*Hs_miR-28_1; *Hs_miR-29c_1; *Hs_miR-96_1; *Hs_miR-191_1; *Hs_miR-451_1; *Hs_miR-142-5p_1; *Hs_miR-199a_1; *Hs_miR-363_1; *Hs_miR-144_4; *Hs_miR-142-3p_2; *Hs_miR-155_2; *Hs_miR-16_2; *Hs_miR-1228-3p_1 и контролни: *Hs_ RNU6-2_11; Ce_miR-39_1) in serum in patients with proven IBD - CD and UC in the active stage of the disease and in the stage of remission achieved with medication.

Seventy patients with IBD, divided into 2 groups of 35 patients, respectively 35 CD (20 in the active stage and 15 in remission) and 35 UC (20 in the active stage and 15 in remission), who passed through the Clinic of Gastroenterology from 04.2019 to 10.2019.

The clinical trial also involved 30 healthy volunteers aged 18 to 42 years who had no history of disease, no medication, and after learning the conditions for participation in a clinical

trial, signed an informed consent to participate in the clinical trial.

In order to achieve the research goal and to solve the pre-formulated tasks, data from IBD patients was processed. They were examined according to the standard clinical approach: history of the disease and concomitant diseases, physical examination, abdominal ultrasound, CT enterography, or MR enterography (for patients with CD and at the start of IBD), ileocolonoscopy, assessment of endoscopic activity and morphological examination. Based on that data, the patients were diagnosed with CD or UC and they received therapy with mesalazine, corticosteroids, azathioprine or biological therapy.

This data is indicative of the desire of Assoc. Prof. Antonia Atanassova not only to achieve new dimensions in diagnosis, control and treatment outcome, but also to successfully derive scientifically sound results and conclusions that will support and improve clinical practice and treatment approach in CD and UC patients.

Quantitative measurement of serum expression level of a panel of microribonucleic acids was performed by real-time quantitative polymerase reaction (RealTime PCR).

These statistical methods allow to illustrate in detail the tasks. These are: Analysis of variance (ANOVA); Variation analysis; Correlation analysis; Regression analysis.

The results obtained for the individual tasks confirm the in-depth analysis of the clinical material. Despite the specifics of processing this clinical material, Assoc. Prof. Dr. Atanassova very clearly presents it in tables and figures. The author reviews her results and discusses each task, which gives a very accurate assessment of the clinical material by comparing it with the available results of other authors who performed similar studies among different groups of patients with chronic inflammatory bowel disease.

The discussion is focused and competent. The ability of Assoc. Prof. Dr. Atanassova to analyse her own results in the context of those known in the literature is evident. The author compares her results with the results of the international database.

Too scarce publications and information in the literature show that her study of the role of microribonucleic acids in patients with inflammatory bowel disease is timely, enabling them to be used as new non-invasive modern and complex biomarkers in these diseases.

Based on the obtained results, 11 conclusions are formulated, which follow the logically set goal and tasks of the research. Among them I would highlight the following:

1. The expression of the considered miRNAs differs in CD and UC patients. In CD patients, the expression of the studied miRNAs is significantly higher than in UC patients.

2. Examination of the expression of miRNAs in the two groups of patients according to the activity of the disease revealed a difference not only in the expression of the individual miRNAs, but also a disease-specific miRNA signature.
3. According to the localization and disease phenotype, different miRNAs were detected for the two diseases with different directions of expression relative to the threshold values.
4. Intestinal complications and extraintestinal manifestations correlate with different miRNAs in patients with CD and UC.
5. The duration of the disease correlates with the increased expression of various miRNAs in CD patients (miR-28 and miR-96) and UC patients (miR-144 and miR-155).
6. The expression of miR-28 in patients with CD is distinguished as a specific marker of achieved remission. Its increased expression correlates with decreased levels of CRP, FCP, remission achieved by CDAI, normal serum iron levels, normal values of vitamin B_{12} and vitamin D.
7. There is a relationship between the serum expression of miR-142-5p, miR-96, miR-199a and Vitamin D levels in IBD patients.

The contributions presented by Assoc. Prof. Atanassova are given very accurately and correctly. They are presented in three groups:

> Theoretical contributions;
> Practical contributions
> Original contributions.

The development of a microribonucleic profile of patients with Crohn's disease and ulcerative colitis among Bulgarian patients with inflammatory bowel disease can be defined as a contribution of international importance. This contribution has an important scientific and applied character, as it allows to introduce a new class of biomarkers, a new approach in characterizing patients with inflammatory diseases from the onset to different age, as well as a new approach in monitoring both diseases. Of no less high value are the contributions of original character for the country, and it is especially important to emphasize that for the first time in our country the connection between the expression of microribonucleic acids, serum levels of vitamin D and main characteristics of chronic inflammatory intestinal diseases.

Assoc. Prof. Antonia Atanassova presents 10 publications in Bulgarian and international periodicals. In all publications she is the first author, and in seven she is the only author. The publications reflect various aspects of the results obtained and the reviews of the literature in the field of application of microribonucleic acids.

The abstract is written on 80 pages and fully presents the dissertation.

In conclusion, I would like to emphasize again my very high appreciation for the great clinical and research activity presented in the dissertation of Assoc. Prof. Dr. Atanasova and I will confidently and categorically vote "YES" for the award of the degree of Doctor of Science. Assoc. Prof. Dr. Antonia Yordanova Atanassova, Ph.D.

14.06.2021

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