

PEER REVIEW

by

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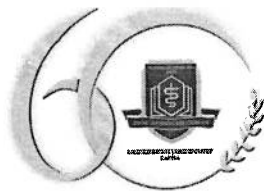
Regarding:

Defense of Dissertation Thesis „Serum expression of micro ribonucleic acids in patients with chronic inflammatory bowel diseases” with author Assoc. Prof. DR. ANTONIA JORDANOVA ATANASSOVA, MD, PHD, Department of Anatomy and Cell biology, Medical University “Prof. Dr. Paraskev Stoyanov “Varna for awarding the degree of Doctor of Medical Sciences, in the specialty "Gastroenterology", professional field 7.1 "Medicine".

In accordance with **Order No P-109-217** of the **Rector** of MU “Prof. Dr. Paraskev Stoyanov“ Varna, dated **17.05.2021** I was elected as an internal member of the Scientific Jury (SJ) under the procedure for public defense of the dissertation for the degree of “ Doctor of Science” with the candidate **assoc. Prof. Dr. Antonia Jordanova Atanassova, MD, PhD**. Protocol N 1 / 31.05.2021 of a sitting of the Scientific Jury designated me as a reviewer on the procedure.

The documents submitted to me comply with the Law for Development of the Academic Staff of Republic of Bulgaria (LDASRB) and the regulations for implementation of the Law for development of academic staff in the Republic of Bulgaria (RILDASRB), as well as Art. 89, para 4 of the Rules for the Development of the Academic Staff at the Medical University – Varna (RDAS-MU-Varna). They meet the requirements of the “Doctor of Science” degree procedure. I have no comment on the documents submitted.

The evaluation of the doctoral thesis is complex and includes the research and practical activity, as well as the structure, scientific characteristics and the scientific-applied results of the dissertation, evaluation of the abstract, according to the RDAS-MU-Varna. The dissertation is discussed and directed to the defense of the Department Council of the Department of Anatomy and Cell Biology at MU "Prof. Dr. Paraskev Stoyanov" Varna on 21.04.2021.



I. Brief biographical data and career development

Assoc. Prof. Dr. Antonia Atanasova, MD was born in 1961 in Varna. In 1980 she graduated from high school at French language school and in 1986 she graduated in medicine from the Medical University of Varna (Certificate №001249). She has worked as a therapist at the District Hospital of Shumen (12.1986- 06.1987) and since June 1987 until now she has been working as a gastroenterologist at the Gastroenterology Clinic of the University hospital "Saint Marina" Varna. From the same year 1987, she is a full-time assistant, in 1994, she became a senior assistant and since 1997 – 2015, she has been a chief assistant at the Department of endocrinology and gastroenterology, Medical University – Varna. In 2015 she was habilitated – an academic position "Associate Professor" and appointed at the Department of anatomy and cell biology, Medical University – Varna.

Scientific development

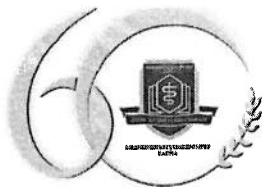
Over the years, the dr Atanasova goes through the following stages of scientific development:

- In 1986 she obtained a master`s degree in Medicine;
- In 1994 she acquired the medical specialty "Internal medicine" and in 1996 – the medical specialty "Gastroenterology"
- In 2014 defended her dissertation on the topic „Clinical evaluation of patients with Ulcerative colitis – contemporary approach”, obtaining the educational and scientific degree "Doctor" in the specialty "Gastroenterology"
- In 2015 she was elected as an Associate Professor at the Medical University - Varna

Scientific and practical activities

Assoc. Prof Atanasova`s main scientific research is studies of the chronic inflammatory bowel disease. It is noteworthy that many of the courses taught by Assoc. prof. A. Atanasova are in the field of abdominal ultrasound, with training and certificates for "Diagnostic and therapeutic fibro-gastro-scopy and fibro-colon-scopy" - level II and I. Over the years, she has been systematically and purposefully upgrading her professional qualifications and receiving extensive theoretical and practical training. She has participated in numerous international training courses, in Bulgaria (2008) and abroad (2014, Copenhagen, Denmark; 2016 Amsterdam, The Netherlands; 2016, Belgrade, Serbia; 2017 Barcelona, Spain; 2018, Vienne, Austria; 2019, Copenhagen, Denmark etc.).

Assoc. Prof Atanasova is a scientific adviser of two PhD students (2018, 2020) for the acquisition of Ph.D. and head of one national scientific project on one of the thesis. She has been teaching students (lectures and seminars) from different faculties of the University of Varna for almost 34 years. She is a member of Bulgarian Society for gastroenterology and endoscopy, Bulgarian association in ultrasound in medicine, European Association for gastroenterology EAGE, European Crohn's disease ulcerative colitis organization ECCO.



II.Characteristics and evaluation of the thesis

1. Content and structure of the thesis

The dissertation submitted to me for evaluation of the topic „Serum expression of micro ribonucleic acids in patients with chronic inflammatory bowel diseases” with author Assoc. prof. dr. Antonia Jordanova Atanasova, MD, PhD, structured into 223 pages (59 tables and 55 figures included) as follows:

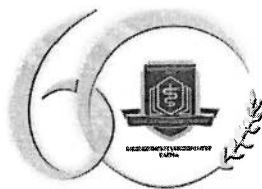
- Abbreviations – 3 pages
- Introduction – 1 page
- Literary Review – 46 pages
- Purpose, tasks and hypotheses – 2 pages
- Materials and Methods – 5 pages
- Own results – 75 pages
- Research discussion – 37 pages
- Conclusions – 2 pages
- General conclusions – 3 pages
- Contributions – 1 page
- Author`s publications on the topic of thesis – 1 page
- Bibliography – 43 pages – it lists 574 titles, of which 8 are in Cyrillic and 566 are in Latin, mostly from the last 5 years (25,4%) or 10 years (52%).

Conclusion: The structure of the dissertation work meets the requirements of the legal framework (too many pages of abbreviations).

2. The topicality and necessity of developing the thesis.

The problem developed is mainly focused to two major chronic inflammatory bowel diseases (Crohn's disease, CD and Ulcerative Colitis, UC). As a doctor, dr. Atanasova defended 2 dissertations in the field contemporary personalized approach to affected patients in her medical practice (laboratory diagnostic investigations, stratification and prognosis and treatment). Inflammatory bowel diseases (IBD) are common and complex diseases with unclear pathogenesis. IBD are based on chronic inflammation and altered immune responses. Traditionally, the monitoring of patients with IBD has been somewhat complicated by the need for repeated invasive methods (endoscopic procedures with biopsies).

Contemporary knowledge in the field of *molecular medicine*, gives significant role to Microribonucleic acids (miRNAs) expression. These small nucleotide double-stranded RNA molecules can cause cleavage of certain mRNAs molecules that are their targets. The disclosure of these miRNAs gave rise to a new conception and definition of the hereditary entity – gene as both protein - coding and protein *non-coding*



(*ncRNA* sequences like *miRNA*, *lncRNA*, *circRNA*) of DNA. This process caused RNA silencing of the relevant genes. They are involved in the differentiation, regulation and cellular signaling of the innate and acquired immune system, the control of enterocyte barrier function, the negative feedback on various signaling pathways associated with inflammation, the control of apoptosis and autophagy processes, dysplasia, in cell death, division and angiogenesis. Numerous studies have shown that miRNAs play a significant role in each stage of inflammation.

There are ongoing discussions and controversies in literature data connected to the characteristics (sources of miRNAs, phase of disease – active or remission - duration, therapeutic regimens) and the diagnostic and prognostic possibilities offered by studies in the field of miRNAs expression in patients with IBD. Significant problems have been properly identified and addressed in her thesis. She emphasizes the role of miRNAs as possible biomarkers in diagnosis, follow-up and treatment in a study group of patients with Crohn's disease and ulcerative colitis.

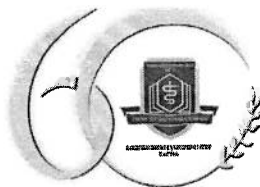
Conclusion: The problem developed in the dissertation is topical and would help to individualize the approach in patients with Crohn's disease and Ulcerative Colitis. No Bulgarian data exist for application of contemporary miRNAs studies to patients with chronic inflammatory bowel diseases of any type.

3. Literature review

Based on her fluent French and English languages and analysis of 574 papers, the review gives good cognitive value of information „tsunami“ not only in clinical manifestation of IBD, but also in the field of molecular medicine – genetics and immunology *iyi*.

There appears to be genetic components in lots of disorders of altered epithelial barrier function according to whole genome sequence database more obvious in CD to UC. There seems to be a primary genetic component since the most important independent risk factor is a family history of the UC disease (8% to 14% of patients as a first-degree relative of a patient with ulcerative colitis has a four times higher risk of developing the disease). More than 240 loci of particular single genes and more than 40 000 miRNAs in complex regulatory gene nets are involved in the development of IBD, but a few of the general characteristics of CD and UC correlate to genetic factors beyond inflammatory and immune mechanisms. In Crohn's disease particularly, there appears to be a genetic association with the phenotypes, specifically (*NOD2/CARD15* mutations in those diagnosed at a younger age, ileac involvement, increased severity, requiring surgical intervention), some single-nucleotide variants (SNV) and miRNAs regulating autophagy process (*ATG16L1*).

Some of the literature scientific papers could be defined as key research studies provoked the author's interest (Wu et al., 2011; Luo et al., 2018; Zeng et al., 2019). These data suggest that IBD may develop because of the complex interaction between genetic predisposition, environmental factors, and altered immune response. At the end of the review, the author draws conclusions about contemporary medicine and the refinement of diagnosis and personalized approach in the follow-up and treatment of a disease as a major goal in clinical practice, based



on which, she formulates the purpose of the dissertation:

Conclusion: The review of the literature clearly shows that the author has thoroughly studied a large number of contemporary literary sources in details of world trends and results in this field.

4. Purpose, tasks and hypothesis

The research hypothesis states, “As miRNAs are involved in all signaling pathways of inflammation and control of the acquired and innate immune system, it is assumed that serum expression of certain miRNAs could distinguish between the inflammatory bowel diseases - CD and UC. It could serve as a non-invasive biomarker to assess the activity of each respective disease that monitors the inflammation and the effectiveness of the chosen therapeutic approach”.

The scientific *aim of the dissertation* “to study and evaluate the serum expression of some miRNAs in patients with chronic inflammatory bowel disease” is correct in the general context of IBD, though it would be more precise to name the identified subjective state - it addresses 2 groups of patients - CD and UC).

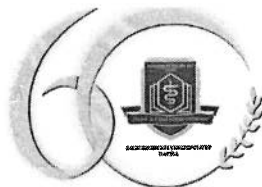
Five main *tasks* have been identified, the solution of which is the basis for the fulfillment of the set goal. They make it possible to cover all the main aspects of the topic under consideration.

1. To examine the serum expression of the relevant miRNAs: * 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 and control: * Hs_RNU6-2_11; Ce_miR-39_1 in CD patients.
2. To examine the serum expression of the relevant miRNAs (the same type and number) in UC patients.
3. To compare the expression data of the respective miRNAs (the same type and number) in CD patients and in UC patients at different stages of the disease.
4. To develop a profile of IBD patients that reflects the main characteristics of the disease according to the serum expression of the respective miRNAs (the same type and number).
5. To examine the serum expression of the relevant miRNAs (the same type and number) in IBD patients and compare with serum levels of Vitamin D.

Conclusion: Having in mind the research methods applied I consider the aim is feasible. It would be more accurate the author use the term “miRNA panel of 13 target miRNAs” (instead of “relevant miRNAs” for no difference in them). Task N 5 could pass a better edition „To examine the serum level of Vitamin D and consider it in the context of serum expression of the target miRNAs in the patients of both groups”.

5. Material and methods

The section covers five pages. The conducted clinical study is cross-sectional prospective trial. It involves 70 patients (30 with CD and 30 with UC) passed through the Clinic of Gastroenterology for the



period 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 research.

Patients are examined according to the standard clinical approach. The disease assessment follows clinical, endoscopic, transverse imaging approaches (abdominal ultrasound and/or CT enterography, MR-enterography) and morphological criteria. The distribution according to localization (L/E) - the Montreal classification; the activity of the disease was assessed in CD patients using the Crohn's Disease Activity Index (CDAI), and in UC patients using the Montreal Severity (S) and Partial Mayo score (Endoscopic Mayo score) .

The subject of the study is focused on molecular – biological (genetic) laboratory studies on levels of serum expression of miRNAs. The methods are *reverse transcription* and *quantitative real time polymerase chain reaction (q-PCR)* for a first time applied to patients of these clinical disorders

Dr. Atanassova skillfully uses various statistical methods (Dispersion analysis (ANOVA), Variational analysis , Correlation analysis, Regression analysis, risk assessment analysis, ROC curve analysis, Prognostic analysis –prognostic value, Comparative analysis (hypothesis evaluation), Graphical and tabular method of displaying results). The statistical software package IBM SPSS Statistics version 25.0) is the heart of the results obtained which give the author grounds for interpretations and analyzes.

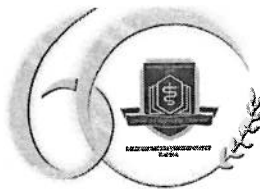
Conclusion: *The applied methods and analytical tools are aimed at the accomplishment of the set scientific tasks. Clinical methods, personally applied by the author should state first. In this short chapter, additional information on data processing is consequently needed (questionnaire (in an application) for gathering the necessary primary information, clinical hospital structure - why not on put on the top of title page, target panel miRNA choice. They would better clarify the complex use of all the information to study the object in its entirety. The $\Delta\Delta C_t$ control method of serum expression is not a statistical method but last step of Laboratory protocol.*

6. Assessment of the doctoral own research – Results

Undoubtedly the greatest weight in the dissertation is the own research – results and discussion of Assoc. Prof, Atanassova. They are organized in groups. Each of these studies is well structured, indicating the goals, objectives, each of itself a separate scientific problem. The results are well described in 75 pages and richly illustrated with numerous figures (50) and tables (55). In this chapter, we find a very important methodical explanation of validation cut off values of the expression of the studied miRNAs calculated in healthy controls for the purposes of the present study to distinguish patients with IBD from healthy individuals. We can see the ROC curve analysis for each of them.

This table also presents the sensitivity and specificity of the respective miRNAs, as well as the positive predictive value (PPV) and the negative predictive value (NPV). This is a reason to be convincing.

The essence of the doctoral thesis is the involvement and the evaluation of serum expression of target



miRNAs in the inflammatory bowel diseases - CD and UC. Is it possible they be used as non-invasive biomarkers to assess the *course*, the *activity*, the *localization*, the effectiveness of the *therapeutic* approach of each disease for the purpose of inflammation monitoring? The molecular – biological investigations give a systematic solution to the assignments and bring the contributions of the dissertation. A significant summary from the results obtained from this study the overexpression of target miRNA in patients with CD and decreased expression close to threshold values of normal controls in patients with UC. It can be concluded that the serum expression of miR-191 can be used as a non-invasive biomarker to distinguish CD from UC. The results concerning a correlation between vitamin D levels as immune modulator and serum expression of miR-142-5p, miR-96, and miR-199a are of specific interest in IBD patients - to date, a limited number of reports have documented the effects of serum concentrations of 25 (OH) D on miRNAs expression and the presence of inflammation. This study is the first clinical trial in patients with UC that evaluates the effect of Vitamin D serum concentrations on the expression of some circulating miRNAs.

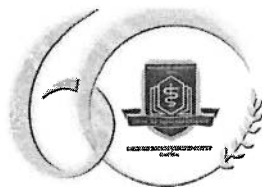
Conclusion: The results are well described and statistically processed. They are relevant to the scientific tasks.

7. Assessment of the doctoral own research – Discussion

In this chapter of 37 pages, the author analyses in details the levels of serum expression of each target miRNA in both diseases in her attempt to evaluate the different *profiles* in context of diagnostic monitoring biomarkers in patients CD and UC, I consider it of great value. The clinical practice of the author directs the discussion in the field of progression and activity (shortly presented different stages) of the diseases. The discussion summarize the findings, highlighting the dependences, correlations and trends obtained from the clinical, laboratory and statistical data. Each study leads to the definition of specific well-formulated conclusion.

A special place is reserved for discussion of the author's idea to study of the correlation between vitamin D and some miRNAs in patients with CD. The vitamin D deficiency in IBD patients demonstrates direct-proportional correlation to relapse and hospitalization of these patients (Atanassova et al., 2019). She considers small number of scientific reports in the global database on this issue as a limiting factor for assessing the reliability of their results and comparability with others. The study is conducted for the first time in Bulgaria. In order to overcome the limitations, it is appropriate to conduct new intelligent researches among larger cohorts of IBD patients in order to prove the results obtained with high reliability.

Conclusion: The discussion is indicating the goals of the thesis in seeking contemporary approach for validation of biomarkers to contribute to diagnostics prognosis and monitoring of IBD.

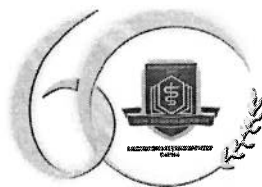


8. General conclusions of the thesis

Based on the obtained results and discussion of the survey area, Assoc. Prof. Atanassova formulates 11 conclusions that answer 5 tasks:

1. The expression of the considered miRNAs differs in CD and UC patients. In CD patients, the expression of 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 (miR-199a, miR-96 and miR-155 for UC and miR-28, miR-142-5p, miR-191, miR-199a, miR-155, miR-1228-3p and miR-96 for CD) and disease phenotype (miR-155, miR-191 and miR-199a for UC and miR-16, miR-28, miR-142-3p, miR-142-5p and miR-1228-3p for CD), different miRNAs were detected for the two diseases with different directions of expression relative to the threshold values.
4. Intestinal complications and extra intestinal 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. Corticosteroids are associated with increased expression of miR-96 for CD patients, miR-142-3p, and miR-155 for UC patients, which are disease-specific.
7. 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.
8. Azathioprine treatment results in decreased expression of specific miRNAs, such as miR-28, miR-142-3p and miR-1228-3p in CD patients, and miR-96 expression below the threshold in UC patients, determined in healthy individuals.
9. Biologic therapy in CD patients correlates with increased expression of miR-28, whereas in UC patients the values of miR-1228-3p approach the threshold values in healthy individuals.
10. 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 B12 and vitamin D.
11. There is a relationship between the serum expression of miR-142-5p, miR-96, and miR-199a and Vitamin D levels in IBD patients. It is not yet clear whether vitamin D deficiency is associated with the onset of IBD or it is a consequence of IBD, but vitamin D deficiency is high in patients with IBD.

Conclusion: *I completely agree with all the conclusions; however, I pay particular attention to the significance and relevance of serum expression of miR-28 from various applied miRNAs as potential biomarker in patients with CD and UC. In my opinion, conclusions could be stated separately for both diseases.*



9. Scientific contributions of the dissertation

The contributions of the dissertation are 11. The author classifies them in the following consequence: theoretical (3), practical (4) and original (4), no confirmatory group.

Theoretical contributions

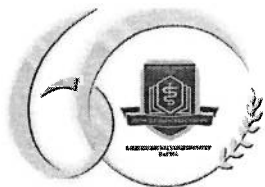
1. For the first time in Bulgaria, the use of miRNAs in adult patients with IBD has been reported in detail and thoroughly.
2. A decisive, accurate and detailed description of the expression of miRNAs in IBD patients in active disease and remission has been performed.
3. The expression of miRNAs is thoroughly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

Practical contributions

1. Thresholds have been established to differentiate the expression of miRNAs.
2. A specific profile of patients with CD and UC was prepared based on the expression of miRNAs.
3. Specific miRNAs for remission and activity, localization, 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 patients with IBD, the role of miRNAs was studied, which has proven its effectiveness in characterizing 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 patients with IBD has been described.
 4. For the first time in Bulgaria, a relationship between the expression of certain miRNAs and Vitamin D deficiency in patients with IBD has been demonstrated.
- I consider the doctoral thesis as an example of clinic-laboratory collaboration in which the author demonstrates very good ability to formulate, summarize and analyze significant information, adapting it to meet the goal and objectives of the thesis. This study is the only one applied to patients with IBD (with the exception of 7 target miRNAs studied in oncological patients) in our country and the first trial of associating micro RNAs panel with vitamin D serum level.
 - I highly appreciate (combined 2nd and 3rd practical contributions) the implementation of molecular-biology approach for development a profile that reflects the main characteristics of the diseases and distinguishes IBD patients according to the level of serum expression of target miRNAs.
 - As for theoretical contributions, *in my opinion* the first one is generally overlapping the next two.



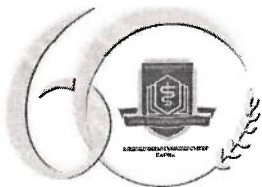
As a specialist in medical genetics, I could not evaluate the clinical significance of this scientific study on 70 patients in terms of practical application from diagnostic and therapeutic point of view (no doubt about valuable data for gastroenterologists). I agree with the author's statement that "the limitations in this study are the small number of patients, the absence of CD patients with high disease activity, the different duration of the disease, and the fact that the study showed the instantaneous expression of miRNAs. Therefore, extensive cohort studies are needed to validate the established results and to consider the changes in the respective miRNAs in dynamics". I completely support and encourage Dr. Atanassova to extend her research with some new target miRNAs (miRNA93-5p, miR-31, miR-224 – GC resistance, miR-15b, miR-17, miR-21, miR-26b и miR-145 – complications, miR-346, miR-637 експресия на рецептора на vitamin D receptor expression in epithelial cells), familial screening of first degree relatives, DNA sequence of NOD2 gene in UC patient as described in the literature review.

***Conclusion:** I agree with the contributions of the dissertation and would like to make some comments. In my opinion, the most significant contribution of this work comes from the author's idea and skills in discussion of disease phenotype, progress and treatment in the context of the scientific aspect of molecular medicine. The laboratory approach, applied in the research process, is required by the specific nature of the subject and the phenomena studied in the dissertation. A brief note must be taken into consideration to the laboratory investigation – the threshold values distinguishing expression of target miRNAs are variable and serve the present study. The very RNA micro molecules are stable markers; the method of reverse transcription and quantitative PCR is reproducible, but levels of expression would be different in next examination.*

10. Dissertation-related publications an abstract.

The author presents 10 real full-text publications related to the thesis work with requirement of 10 – according to Art.89, para1, item 3 of the Rules for development of the Academic Staff of medical University – Varna. They have been published in Bulgarian (9) and foreign (1) journals. One of the Bulgarian papers is published in a Web of Science indexed journal (Bulgarian Medical Journal) admitted by the National Centre for Information and Documentation. Dr Atanassova is the sole author in 7 of them and first of two co-authors in 2 papers and have not been cited yet.

The academic certificate from the Medical library of MU Varna declares the total scientific activity of Assoc. Prof Atanasova`s (according to RILDASRB professional field 7.1 "Medicine") as follows: 285 points (minimum level of 100) from 1 monograph with a volume of 223 pages; 105 points from citations in indexed medical Journals by scientific database (Web of Science and Scopus). These points completely satisfy the required criteria.



I have no comment on the abstract. It logically follows the development of the dissertation and shows the most characteristic moments, main conclusions and suggestions. It has been developed over 80 pages and is illustrated with 10 figures and 57 tables that represent the most important results obtained from the own studies. Upon careful reading of all the proceedings, I did not find any signs of plagiarism or incorrect citation of sources.

Conclusion: *The publications are related to the topic of the dissertation or present a stage in its development.*

11. Critical comments and recommendations

My critical comments regarding the thesis work are summarized at the end of the each review point; more substantive in „Materials and methods“, less-significant re-edition in “Aim and tasks”, some recommendations to “Contributions”.

From the documents presented to me on the procedure, I become convinced that the deep knowledge on the issue, the clinical dissertation work, the results discussion and the contributions made were the result of Dr. Atanassova`s independent focused and in-depth research activity.

III. Conclusion

The dissertation work of Assoc. Prof. Dr. Antonia Atanassova, MD, PhD represents contemporary study on a relevant clinical problem associated with scientific search for independent biomarkers in diagnosis, follow-up and treatment of IBD, mainly Crohn's disease (CD) and ulcerative colitis (UC). The author shows remarkable theoretical background and clinical practical experience to identify, formulate, analyze and properly discuss molecular-medical database in the field of gastroenterology. I know the author as an extremely conscientious, hardworking and innovative colleague and believe she is an established specialist, researcher and teacher with high academic potential.

The thesis meets the scientific criteria, as well as the rules for academic development of MU – Varna. Based on the analysis of the overall documentation of the procedure and its compliance with the LDASRB, I have a “**positive**” opinion on the qualities of the candidate and in this regard propose to the Honorable Jury to award to **Ass. Prof. A. Atanassova a scientific degree “Doctor of Medical Sciences”** in the area of higher education 7.Health and sports, professional field 7.1. Medicine, specialty “**Gastroenterology**”.

18.06.2021

Varna

Reviewer:

Prof. Lyudmila Angelova, MD, PhD