

STATEMENT

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On the dissertation for awarding of academic degree "Doctor" in Internal Medicine

Candidate: Dr. Rozalina Ivanova Balabanska, Clinic of Gastroenterology, Acibadem CityClinic Tokuda University Hospital, Sofia

Title of the thesis: „Liver steatosis and non-alcoholic steatohepatitis – clinical assessment and application of the transient elastography (Fibroscan)“

Scientific Supervisor: Prof. Dr. Simeon Georgiev Stoynov, DMSc

By order № 269/09.06.2021r. of the Executive Director of the Acibadem CityClinic Tokuda University Hospital, I have been appointed as a **member of the Academic jury**. In compliance with a Decision took at 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 Acibadem CityClinic Tokuda University Hospital.

1. Professional development

Dr. Rozalina Balabanska graduated in Medicine with honors from the Higher Medical University of Sofia in 1987. In 1987-1991 she worked as a GP in town of Pernik. Since March 1991 she has been appointed as a resident doctor in the leading gastroenterology clinics and departments at elite Institutions and hospitals in Sofia - Military Medical Academy (1991-1994); Fifth City Hospital (1994-2007) and Tokuda Hospital (since 2007). She acquired two clinical specialities - a major in **Internal Medicine in 1996** and in **Gastroenterology in 1999**. Dr. Rozalina Balabanska has been constantly developing her knowledge and clinical skills. She participated in numerous postgraduate courses and individual postgraduate trainings in Gastroenterology, abdominal ultrasound and transient elastography performed in the Netherlands, Italy, Spain and France.

Dr. Balabanska is an established specialist and expert in the diagnosis and treatment of gastrointestinal diseases, especially in the field of chronic liver injury. She has significant clinical experience and skills in performing abdominal ultrasound, as well as liver biopsy and other ultrasound guided invasive procedures. Dr. Balabanska in collaboration with Dr. Radin Tsonev and the team of the Clinic of Gastroenterology at Acibadem CityClinic Tokuda University Hospital implemented transient elastography with fibroscan in everyday clinical practice as a non-invasive method for assessment of liver damage. Her research interest is focused mainly in hepatology.

Dr. Rozalina Balabanska is a respected doctor. She has won twice the prestigious award "Researcher of the Year" at Tokuda Hospital. She is among the awarded "Doctors in whom we trust" from the newspaper "24-hours" and among the "Best doctors in Sofia" award of "Darik Radio". She is a member of the Bulgarian Society of Gastroenterology, Gastrointestinal Endoscopy and Abdominal Ultrasound; Bulgarian Association of Surgeons and Gastroenterologists; Bulgarian Association of Ultrasound in Medicine, European and World Federations of Ultrasound in Medicine; European Association for the Study of the Liver; American Association for the Study of Liver Disease. Dr. Balabanska speaks English and Russian languages.

2. Scientific relevance of the dissertation topic

Non-alcoholic steatohepatitis (NASH) is one of the scientific challenges of modern hepatology. The increasing incidence of obesity, metabolic syndrome and type-2 diabetes has made non-alcoholic fatty liver disease (NAFLD) and particularly NASH, the most common chronic liver disorder. Despite advances in knowledge, currently we still do not have widely accepted and effective treatment. A number of aspects related to the diagnosis of NASH are highly debatable. Liver biopsy is still the "gold standard" for assessment of liver damage, but it is associated with significant limitations due to the

probability of sample errors and risk of complications. Recently, there is a growing research interest in the study of non-invasive methods for the assessment of liver damage in patients with NASH. Of great clinical importance is early identification of NASH among subjects with NAFLD, as well as distinguishing patients with advanced liver fibrosis from those with mild disease. All of the above clearly demonstrates the relevance and clinical significance of the discussed problem.

3. **Dissertation structure**

The dissertation contains 145 pages in total. It is correctly and classically structured and includes the following parts: introduction; review of the literature; study goal and tasks; material and methods; 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 analyse non-invasive methods for assessment of hepatic steatosis and non-alcoholic steatohepatitis and to develop a clinical algorithm for liver examination and management of patients with metabolic syndrome, obesity, diabetes mellitus or elevated liver enzymes. The author logically has set 9 specific and feasible tasks that are directly related to the achievement of the study goal.

Material and Methods A total number of 170 subjects with metabolic syndrome were studied. They were investigated at the Clinic of Gastroenterology, Acibadem CityClinic Tokuda University Hospital during the following period: Dec 2016 – Mar 2020. In all patients, a transient elastography was performed with a Fibroscan 502 Touch device and steatosis and liver stiffness were measured simultaneously with an M or XL probe. In 135 of the patients, a parallel liver biopsy was performed for histological verification of the degree of steatosis and determination of hepatic necro- inflammatory activity as well as the stage of liver fibrosis by using the SAF scoring system, Metavir and Kleiner. In a group of 20 patients, a Liver Multiscan was also performed. By magnetic resonance spectroscopy the amount of liver fat as well as iron content were measured. LIF score was calculated to assess the degree of progression of liver damage. All 170 subjects included in the study were precisely characterized by using of modern and advanced diagnostic methods that included clinical examination, laboratory testing and imaging methods. Subjects with excessive alcohol consumption and those infected with HBV or HCV were excluded from the study. The presence of concomitant hypertension, DM, dyslipidemia and family history for metabolic disorders has been initially specified. A complete physical examination was performed, including measurement of height, weight, abdominal circumference and BMI calculation. The metabolic syndrome was confirmed in all patients by relevant testing of fasting glucose, insulin resistance and lipid status. In addition to transient elastography, FIB-4, NAFLD Fibrosis score and FAST score were used for non-invasive assessment of liver damage. 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 29 tables and 59 figures. A significant moderate correlation was found between the results of fibroscan and those obtained from histological analysis of liver biopsy both in terms of assessment of the degree of steatosis and staging of liver fibrosis. The cut-offs of CAP levels used in the present study (233 dB / m for steatosis S1, 270 dB / m for steatosis S2, over 300 dB / m for steatosis S3) distinguished with a high level of confidence the subgroups with mild, moderate and severe hepatic steatosis. The author confirms that the anthropometric indicators of height, weight, BMI, waist circumference are highly informative for the presence of metabolic disorders and liver steatosis and thus the above parameters should be measured in each patient. The results show that BMI distinguishes grade 3 from grade 1 steatosis as well as the group of patients without steatosis. It was confirmed also that normal values of liver enzymes do not rule out the presence of liver damage. In presence of liver injury, FIB-4 and

transient elastography are sufficient for determination of the stage of liver fibrosis. By using the adaptive LASSO method the following 11 factors that have a predictive value for non-invasive determination of liver fibrosis have been identified: liver stiffness, CAP, FIB-4, decreased platelet counts, elevated AST, age, elevated GGT, body weight, elevated triglycerides, elevated ALT and cholesterol. The cut-off values of liver stiffness used in the study (up to 5 - 5.5 kPa for FO; up to 7 kPa for F1; 7.5 - 10 kPa for F2; 10 - 14 kPa for F3; > 14 kPa for F4) distinguish with a high level of confidence all subgroups with fibrosis with the exception of F4 versus F3 and F1 vs. FO. It was found that non-invasive methods (Fibroscan, abdominal ultrasound and serum markers) may distinguish NAFLD from NASH, and FAST score based on Fibroscan and AST can identify patients with increased risk of NASH progression and complications.

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 9 conclusions that I fully agree with, as they are logical consequence of the obtained results and are in line with the main goal and tasks of the research. I accept also all contributions to medical science and practice that are precisely formulated by the author. Three of them are original author's contributions to medical science and clinical practice while in the rest 6 Dr. Balabanska has confirmed findings of other researchers by using her own results. The proposed original algorithm for liver examination and management of patients with metabolic syndrome, obesity, and diabetes mellitus is very useful and important for the clinical practice.

In relation to the present dissertation author has published 3 papers in Bulgarian Journals. Some of the results were presented also at 3 National and International scientific forums - National Conference on Gastroenterology with international participation, 2016, Varna; 62 Annual Meeting of the American Association for the Study of Liver Diseases AASLD, 2011, San Francisco, USA; 68 Annual Meeting of AASLD, 2017, Washington, USA. The abstracts of the last 2 scientific articles are published in Hepatology - an authoritative journal with a very high impact factor. In 4 of the considered 6 scientific works Dr. Balabanska is the first or 2nd author. This fact clearly shows her leading contribution in the research development and obtaining of the study results.


Conclusion

Dissertation of Dr. Rozalina Balabanska is well planned scientific study focused on the important problem of current hepatology. 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 study improves current knowledge in the field of non-invasive methods of diagnosis of liver damage in patients with NAFLD and NASH. 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 of Acibadem CityClinic Tokuda University Hospital.

I grant my positive assessment and I recommend that the academic jury awards Dr. Rozalina Ivanova Balabanska the academic degree "Doctor" in Internal Medicine.

17.07.2021r.

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/Professor Deian Jevlev, MD, PhD, DSc/