

## STATEMENT OF OPINION

By Prof. Dr. Milena Staneva Staneva, MD, PhD  
Head of Angiology Clinic,  
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of dissertation paper for acquisition of educational and scientific degree “**PhD**”  
in the field of higher education 7. Healthcare and sport, professional direction 7.1. Medicine, doctoral programme “Internal Medicine”

**Author:** *Rozalina Ivanova Balabanska*

**Form of doctoral programme:** self-tuition

**Research unit:** Clinic of Gastroenterology,

**Subject:** “*HEPATIC STEATOSIS AND NON-ALCOHOLIC STEATOHEPATITIS - CLINICAL EVALUATION AND APPLICATION OF TRANSIENT ELASTOGRAPHY (FIBROSCAN)*”

**Scientific supervisor:** Prof. Simeon Stoynov, MD, DSc

### 1. General presentation of the procedure and the doctoral student

The dissertation was discussed and proposed for defense by the extended Scientific Collegium of the Clinic of Gastroenterology at Acibadem City Clinic UMBAL Tokuda EAD. At a meeting of the Scientific Council of Acibadem City Clinic UMBAL Tokuda EAD (Minutes 37 / 20.05.2021) and by order № 269 / 09.06.2021 of the Executive Director and the Procurator of the hospital, I was appointed an internal member of the Scientific jury in connection with the dissertation of Rosalina Balabanska, MD. I am determined to present an Opinion. There are no omissions in the documentation attached by Dr. Balabanska, the requirements of LDASRB, RILDASRB and the Rules for the terms and procedure for acquisition of scientific degrees and holding of academic positions in Acibadem City Clinic UMBAL Tokuda EAD are met.

I declare that I have no conflict of interest with the author of the dissertation.

Rosalina Balabanska, MD graduated in medicine at the Higher Medical Institute in Sofia in 1987. In 1987 she started working at the District Hospital in Pernik, successively working at the Military Medical Academy and the 5th City Hospital in Sofia. Since March 2007 she has been a resident doctor in the Gastroenterology Department at Tokuda Hospital Sofia (Acibadem City Clinic, Tokuda University Hospital). In 1996 she acquired a specialization in "Internal Medicine", and in 1999 - a specialty in "Gastroenterology and Dietetics" from MU Sofia. Specializes in Bulgaria and abroad Abdominal ultrasound, Interventional ultrasound and liver biopsy, transient elastography.

### 2. Relevance of the topic

In recent years, the incidence of hepatic steatosis has increased, which is associated with an increasing incidence of obesity, diabetes and increased alcohol consumption. It is the hepatic manifestation of the metabolic syndrome. Non-alcoholic steatosis liver disease (NAFLD) is found in 70% of overweight or obese people, in 90% of those with morbid obesity, in 70% of diabetics. NAFLD is becoming the most common chronic liver disease in the world. The diagnosis is based on medical history, blood tests, imaging methods and a liver biopsy, which is still the gold standard for a comprehensive assessment of the disease. Hepatic steatosis is a

reversible process in eliminating the cause. The enormous social significance of this disease, as well as the limitations associated with liver biopsy, has led in recent years to work hard to find rapid, affordable non-invasive methods for diagnosing NAFLD and taking action to control it, which is a prerequisite for of the present study. The topic is relevant from a practical point of view.

### **3. Knowledge of the problem**

The doctoral student knows the state of the problem and creatively evaluates the literary material.

### **4. Research methodology**

The chosen research methodology allows achieving the set goal and obtaining an adequate answer to the tasks solved in the dissertation.

### **5. Characteristics and evaluation of the dissertation and contributions**

Balabanska's MD, dissertation is written on 145 standard pages and is illustrated with 59 figures and 29 tables. It is structured correctly and includes the following sections: Abbreviations and symbols used - 2 pages; Introduction - 3 pages; Literature review - 44 pages; Purpose and tasks - 1 page; Material and methods - 10 pages; Own results - 53 pages; Discussion - 15 pages; Conclusions and recommendations - 2 pages, Bibliography - 8 pages; Contributions - 2 pages. In accordance with the academic requirements, the contributions and publications related to the topic should be presented only in the abstract. The bibliography contains 133 literature sources, of which 11 in Cyrillic and 122 in Latin, 54.5% of which have been published in the last 5 years.

*In the introduction* the problem is well formulated and the purpose of the dissertation is substantiated.

*The literature review* is well structured in accordance with the developed topic. It is written competently and shows a good knowledge of the problem in its various aspects. The social significance, the main characteristics and the possibilities of the different methods for diagnosis and follow-up of the patients with the Non-alcoholic Steatotic Liver Disease (NAFLD) are considered. The latest expert proposal to replace NAFLD with a new term - MAFLD (Metabolic associated fatty liver disease), which more accurately reflects the pathogenesis of liver damage, as well as the criteria for its diagnosis.

*The aim* of the dissertation is well formulated in accordance with the topic of the dissertation and the presented literature review. The present work aims to analyze non-invasive methods for the assessment of hepatic steatosis and non-alcoholic steatohepatitis and to develop a clinical algorithm for liver examination in patients with metabolic syndrome, obesity, diabetes or elevated liver enzymes. The set tasks, a total of 9 are completely sufficient and are fully realized in the dissertation.

The section "*Materials and methods*" describes in detail 170 patients with metabolic syndrome, of which 90 women (52.9%) and 80 men (47.1%), aged 20 to 76 years, studied for the period 12.2016 - 03.2020 with Fibroscan 502 Touch (Echosens, France), M or XL probe for transient elastography with simultaneous measurement of density and hepatic steatosis. 135 of them underwent liver biopsy for histological evaluation of the degree of steatosis, inflammatory activity and fibrosis using the SAF scoring system, Metavir and Kleiner fibrosis evaluation. Liver Multiscan was performed on 20 patients - magnetic resonance spectroscopy with determination of the amount of fat in the liver, iron content, LIF score was determined for the degree of progression of liver damage. The following methods were applied to all patients: clinical examinations (history of subjective complaints, accompanying diseases and harmful habits and complete physical examination with anthropometric indicators (BMI, waist circumference, waist / hip ratio)); laboratory tests to assess liver function and the presence of metabolic syndrome (AST, ALT, GGT, AF, lipid profile, uric acid, blood sugar, HbA1c, serum

iron, ferritin, transferrin, highly sensitive C-reactive protein, CBC), calculated FIB-4 to assess the degree of liver fibrosis; standard ultrasound examination of the abdominal organs

3a statistical data processing, appropriate methods and a modern statistical program (STATISTICA version 13.0 and STATA version 16.1) have been used, which guarantees the reliability of the obtained results.

**Results:** The presented results are convincing and clear. The most important of these are the following. The doctoral student establishes a direct correlation between scientometric indicators - body weight, BMI and waist circumference with the degree of fat accumulation in the liver. The study demonstrated that Fibroscan results were moderately correlated with histologically established hepatic steatosis and fibrosis.

Own cut-off values for staging of liver steatosis based on Fibroscan SAR were proposed in the study: for S1 - 233 dB / m, S 2 - 270 dB / m and S3 - over 300 dB / m.

The study proposed intrinsic cut-off values of elastography (up to 5 - 5.5 kPa for norm; up to 7 kPa for stage F1; 7.5 - 10 kPa for stage F2; 10 - 14 kPa for stage F3;> 14 kPa for stage F4), which distinguish with a high level of confidence all subgroups with fibrosis, with the exception of stage 1 compared to patients without fibrosis and 4 versus stage 3.

The application of the adaptive LASSO method identified 11 factors that have a predictive value for non-invasive determination of liver fibrosis: liver density, CAP, FIB-4, decreased platelet counts, elevated AST, age, elevated GGT, body weight, elevated triglycerides, cholesterol. The PhD student applied the FAST score (Fibroscan and AST score), which distinguishes patients at increased risk of progression and complications from NASH. FIB-4 and transient elastography have been shown to be markers for non-invasive determination of the severity of liver fibrosis.

**Discussion:** The obtained results are discussed and compared with the known data in the medical literature. The doctoral student is free to handle scientific facts and demonstrates competence in the matter.

**Conclusions and contributions:** Based on the results obtained, Balabanska MD logically draws 9 conclusions that correspond to the tasks.

The contributions are 9, of which 3 are original and 6 - of a confirmatory nature. I accept the proposed contributions. Of great clinical importance is the proposed original clinical algorithm for studying the condition of the liver in patients with metabolic syndrome, diabetes or obesity.

**6. The abstract reflects** what is written in the dissertation. In accordance with the academic requirements, the contributions are presented in it and a list of the publications in connection with the dissertation is attached.

#### **7. Assessment of the publications and personal contribution of the doctoral student**

The doctoral student has submitted 3 publications in national journals and 3 scientific communications, 2 of which in international scientific forums published in a journal with IF. In two publications Balabanska MD is an independent author. This scientific activity is enough to say that the doctoral student is consistent in his scientific activity and exceeds the national minimum requirements for the educational and scientific degree "PhD".

### **CONCLUSION**

The dissertation contains scientific, applied science and applied results, which represent an original contribution to science, expanding our knowledge in the field of hepatology. It is written in a strictly scientific style. It meets all the requirements of **complies to all requirements** of the Law on the Development of the Academic Staff in the Republic of Bulgaria (LDASRB), the Rules for Implementation of the LDASRB and the Rules of Acibadem City Clinic Tokuda UM-HAT EAD for implementation of the LDASRB.

Dr. Rosalina Ivanova Balabanska is an established doctor - clinician, specialist in gastroenterology, hepatology, abdominal ultrasound and transient elastography with extensive experience. The dissertation shows that the doctoral student has in-depth theoretical knowledge and professional skills in the scientific specialty "Internal Medicine", demonstrating qualities and skills for independent research.

**In view of the above I give my *positive assessment* and recommend in full confidence to the members of the scientific jury to grant positive evaluation to the dissertation paper "Liver steatosis and non-alcoholic steatohepatitis - clinical evaluation and application of transient elastography (Fibroscan)" and to award Rosalina Ivanova Bavalabanska MD, "PhD" educational degree in the field of Higher Education 7. Healthcare and sports, professional field 7.1. Medicine, doctoral program "Internal Medicine"**

26.07.2021

Sofia

Statement of Opinion elaborated by:

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