

REVIEW

from Professor Dr. Iskren Kotzev, MD, PhD, Professor of Internal Medicine and Gastroenterology

on the dissertation work on the topic "Fatty Liver and Non-alcoholic steatohepatitis - clinical assessment and application of transient elastography (Fibroscan)" of Dr Rosalina Ivanova Balabanska, resident at the Gastroenterology Clinic in "Ajubadem City clinic MHAT Tokuda", Sofia, for awarding the scientific and educational degree "Doctor" in the field of higher education 7. healthcare and sports, professional direction 7.1. "Medicine", doctoral program "Internal diseases"

By order 269/09.06.2021, the Executive Director of the "Ajubadem City clinic MHAT Tokuda", Sofia, I was recognized as a member of the Scientific Jury for the procedures of public defend for a dissertation of Dr. Rosalina Ivanova Balabanska on the topic "Fatty Liver and Non-alcoholic Steatohepatitis – clinical assessment and application of transient elastography (Fibroscan)". At the first meeting of the scientific jury, I was appointed to make up a review. I have been provided with all the materials that I have considered in detail and confirm that the procedure for disclosure, development and admission to defend was carried out in accordance with the requirements of the Law on the Development of Academic Fellow Members in the Republic of Bulgaria and the rules for its application since 2010, as well as in accordance with the requirements of the Regulations for the development of academic staff in the "Ajubadem City Clinic MHAT Tokuda", Sofia. I led from the same documents in the composition of this review.

For many years, a quiet "epidemic" has been unfolding in the world of the so-called Non-alcoholic fatty liver diseases (NAFLD) based on metabolic dysfunction and determined by insulin resistance, clinically performed as a multi-system disease, with possible complications associated with obesity, diabetes mellitus 2T, liver disorders with possible progress to liver cirrhosis, cardiovascular pathology, chronic kidney diseases, neoplasia (of the liver, breast and colon) and polycystic ovaries. The multi-system nature of the pathological condition in internal medicine led to the idea to change its name from "negative" nonalcoholic, to metabolic associated fatty liver disease (MAFLD).

The noninvasive method of diagnosing and quantifying hepatic steatosis in NAFLD, as well as noninvasive staging of liver fibrosis, is an actual and important topic in modern hepatology. Today, ultrasound methods are widely used for this purpose – strain elastography (SE and strain rate imaging), acoustic radiation force impulse (ARFI), transient elastography (fibroscan), controlled attenuation parameter (CAP), point shear wave elastography (pSWE) and multidimensional shear wave elastography (2D-SWE and 3D-SWE).

In her dissertation work "Fatty Liver and Non-alcoholic Steatohepatitis – clinical assessment and application of transient elastography (Fibroscan)". Dr. Rosalina Ivanova Balabanska has developed a very relevant topic, which is currently object to a lot of research and controversy, with a dynamic diagnostic consensus and without a golden standard for the treatment. The scientific work is well motivated in a scientific and practical attitude. There is not so much researches in debt on this topic and those are mainly in the academic centers.

Dr. Rosalina Balabanska, and before her, Dr. Radin Tsonev, D.M. have indisputable merit for entering in the clinical practice in Bulgaria, the first and only, **routine** investigation of liver stiffness with a world acknowledged method of transient elastography with fibroscan, despite the resistance of some hepatologists in our country. The dissertant deserves praise for her pioneer work in the introducing and approving of this method in Bulgaria, where "Ajbadem City Clinic MHAT Tokuda" is undisputed leader in this attitude.

Dr. Balabanska determines the ambiguous purpose to investigate the possibility of a non-invasive methods for evaluation of fatty liver and nonalcoholic steatohepatitis and on the basis of this her experience, to suggest clinical and practical diagnostic algorithm in the cases with metabolic syndrome, obesity, diabetes 2T, or in the presence of elevated liver enzymes. For the implementation of the purpose, Dr. Balabanska set 9 main tasks, which in my opinion, she explained brilliantly.

Dr. Balabanska investigates retrospectively the indicators on 170 patients with metabolic syndrome and fatty liver, who were admitted to the clinic for 4 years, all of them were examined with fibroscan, and 135 patients with liver biopsy. This study is the most representstive Bulgarian investigation on this topic, approved by histological verification. In her study, she uses all modern clinical, laboratory, instrumental and histological methods of researche for full assessment of the patients with a metabolic associated fatty liver disease – anthropometry, laboratory indicators, indices FIB-4 (Fibrosis-4 Index for liver fibrosis – age, AST, platelet number, ALT), NFS (NAFLD fibrosis score), FAST (Fibroscan and AST), ultrasound, elastography, histology of SAF (Steatosis, Activity, Fibrosis) and NAS (NAFLD Activity Score), as well as Magnetic Resonance Spectroscopy. The dissertant uses a very complex and statistically adequate apparatus, which allows her to make valid conclusions. The dissertation work is performed perfectly with 59 figures and 29 tables.

The scientific work is presented on 145 pages, structured in 8 chapters, the most important are: a bibliography review, methodics of the study, results and discussion, conclusions and recommendations. The relationship between the separated parts is correct, the text is clear and accurate, the author presents a complex matter by accessible and understandable way. Bibliography presented practicaly all up to date publications, associated with the topic and consists of 133 author`s publications (11 in Cyrillic and 122 in Latin).

A classic concept is that liver biopsy gave a very accurate diagnostic assessment, as well as reliable information about the activity of the process and the stage of the liver fibrosis. Natural aspiration of the clinicians is to search for replacing the biopsy with non-invasive diagnostic methods. Dr. Balabanska used elastography through vibrating fibroscan for a quantitative assessment of fatty liver and also a quantitative measurement of the liver fibrosis, comparing the results with the data from histology and other non-invasive methods. The correlation between the data for liver steatosis and fibrosis was found to be statistically significant, measured with fibroscan and with histological investigation. Correlation coefficient is positive - for steatosis ($r=0.37$, $p<0.0001$; Spearman $p=0.3000$, $p=0.0005$) and for fibrosis ($p=0.3779$, $p<0.0001$).

In the chapter of own results, she describes in detail the data on the anthropometry of the investigated persons, steatosis and fibrosis, assessed by CAP and fibroscan, comparing with histology, laboratory data and the FIB-4 index. On the basis of detailed investigations and statistical processing of the data is seen, that the fibroscan can use as a routine non-invasive method for quantitative assessment of hepatic steatosis and liver stiffness in all patients with metabolic syndrome, obesity, diabetes and patients with elevated liver enzymes. The expanded girth of nonalcoholic fatty liver, as the possibility for favorable therapeutic intervention with diet, aerobic exercises and pharmacotherapy, in conjunction with the possibility for follow-up and for accounting the therapeutic effect, define the fibroscan as mean of choice for this goal.

Dr. Balabanska convincingly shows that the assessment of liver fibrosis is possible with a non-invasive method, namely with fibroscan and with the FIB-4 index. The important conclusion of her study is that normal values of liver enzymes did not exclude liver disorder of this type. The dissertant shows us that with the help of fibroscan, liver ultrasonography and serum markers is possible to distinguish fatty liver from nonalcoholic steatohepatitis. With the help of the FAST index, depending on the values of controlled attenuation parameter (CAP) for steatosis, the stiffness in kPa and ASAT, it is possible successfully determine the risk of progression and complication of non-alcoholic steatohepatitis.

Despite the complex research methodology, Dr. Balabanska successfully uses anthropometric indicators – height, weight, BMI, waist circumference and confirms that they are highly informative indicators for metabolic disorders with liver steatosis and that they should be used in all patients.

The author found no changes in the iron metabolism of patients with fatty liver and /or NASH.

The dissertant, based on the studied patients with severe steatosis (S3) and with normal values of aminotransferases unequivocally confirms that normal values of liver enzymes do not exclude progressive damage of the liver cells and prove the need not only in laboratory studies, but also in imaging methods for evaluating the liver. Dr. Balabanska established original cut-off values of the CAP for S1 (liver steatosis of the 1st degree) over 250 db/m (231-281), which

are closed to the proposed by EASL cut-off values of more than 275 db/m, with a sensitivity of more than 90% for the detection of steatosis.

Based on a sufficient number of studied individuals with histology and non-invasive methods, she gives recommendations to pathologists on the use of the point system of **SAF** (the size of **steatosis**, the degree of **activity** and the stage of **fibrosis**) as the best complex indicator of histological changes in steatosis.

Dr. Balabanska has original contributions with her dissertation work. Based on its rich clinical material, she offers us a convenient and modern algorithm for studying the liver in patients with metabolic syndrome, diabetes mellitus or obesity. In addition, an original clinical algorithm of behavior in patients with fatty liver was proposed. Based on the studied patients, Dr. Balabanska has created her own cut-off values of CAP (controlled attenuation parameter) to measure the degree of steatosis, which is an undeniable contribution to hepatology.

From her dissertation, Dr. Balabanska confirms the high informative value of non-invasive methods for assessing liver steatosis and the need for the introduction of elastography with simultaneous measurement of steatosis and fibrosis as a mandatory routine method in the management of patients with liver disorder, as well as in all patients with diabetes and obesity.

Dr. Balabanska's research and conclusions are in harmony with this year's updated EASL recommendations for fibroscan as a cornerstone in non-invasive tests to determine further treatment of liver diseases, as well all along the liver care continuum and across all nosological groups - NAFLD/NASH, alcoholic liver disease, HCV, autoimmune liver diseases and in patients with metabolic risk. After FIB-4 was established, fibroscan is recommended for use in primary medical care, in diabetology clinics and in hepatology clinics. I am pleased to note that Dr. Balabanska, with her current work, has demonstrated in advance the benefits and full implementation of these latest recommendations.

Dr. Balabanska has published part of her research in medical journals - 6 publications are presented (4 in Bulgarian and 2 in English). Dr. Balabanska is the first author in 2 publications, the second author in 2, the third and subsequent author in 2 publications. Both scientific reports in English were published in the famous referred scientific journal "Hepatology" (organ of AASLD). The rest are published in scientific journals "Medical Journal", "GP news", "Diagnostic and therapeutic ultrasound". Dr. Balabanska is a co-author of the national rules and recommendations on ultrasound elastography of the liver.

Given her thorough research with original results, we can recommend a higher publication activity to Dr. Balabanska.

The achieved results and conclusions are a valuable contribution to Bulgarian hepatology. Dr. Rosalina Balabanska is a prominent doctor, well-known in the circles of the Scientific Society of Gastroenterology and the Bulgarian Association of Ultrasound in Medicine, where she is actively involved

in scientific life. She works in a leading gastroenterology clinic under the guidance of professor Simeon Stoinov, MD, PhD, who is one of the leading doctors of Internal Medicine and Gastroenterology in our country and in cooperation with all other colleagues from the clinic.

Dr. Rosalina Balabanska graduated her medical education with excellent success, she has 2 recognized specialties - in internal medicine and gastroenterology and a 30-year medical internship. She is an active participant in all significant Bulgarian and international scientific forums on gastroenterology and hepatology. She is a member of the national societies of gastroenterology and ultrasonography, as well as the European and American associations of hepatology. She has extensive research experience as a principal investigator in the clinical trials at all stages. She enjoys the respect and recognition of her patients, colleagues and society. He was awarded as a researcher in 2016 and 2018 from the Tokuda Hospital, and according to the survey "24 Hours" she has been a constant among "doctors in whom we trust" since 2012 till now

From our joint work on some clinical cases and based on my long-standing view to the development of gastroenterology in our country, I know Dr. Balabanska as a conscientious researcher and as a scientist with an original creative manner, a respected and highly valued clinical doctor.

In accordance to the minimal points requirements of group's indicators, Dr. Balabanska has a sufficient number of points - 50 points for an indicator 1, 40 points for an indicator 7 and 20 points for an indicator 8, or a total sum of 110 points, with a minimum number for points of 80.

Dr. Balabanska's research is a thorough and scientifically based study of the role and place of vibrational elastography of the fibroscan type in modern medicine in patients with fatty liver and non-alcoholic steatohepatitis. She has his own remarkable scientific and practical contributions. My opinion is that this work has a high scientific value and useful practical applicability.

Evaluating the dissertation work of Dr. Balabanska as a whole with its innovative research, impressive results, original contributions, scientific and practical significance, I consider that the presented dissertation work of Dr. Balabanska fully meets the requirements for awarding the scientific and educational degree "Doctor" and I give my positive vote.

Allow yourself to recommend the same to the respected members of the scientific jury.

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