

REVIEW

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on the dissertation for the award of educational and scientific degree "Doctor" of Dr. Rozalina Ivanova Balabanska, PhD student, on "Liver steatosis and non-alcoholic steatohepatitis - clinical evaluation and application of transient elastography (Fibroscan)" with a supervisor Prof. Simeon Stoynov, DSc.

The dissertation of Dr. Rozalina Balabanska is dedicated to a current problem in modern hepatology, namely the clinical evaluation of non-alcoholic fatty liver disease (NAFLD) using non-invasive methods for liver examination.

The dissertation is presented in a fully completed form. It is structured correctly and complies with the generally accepted requirements - introduction, literature review, goals and objectives, materials and methods, results and discussion, main conclusions and contributions, bibliography. It is written in clear scientific Bulgarian language on 143 pages.

The introduction is 3 pages long and in a concentrated form overlaps the literature review. The literature review, written on 44 pages (31% of the volume of the dissertation) is completely up-to-date. It discusses the basic characteristics of Non-Alcoholic Fatty Liver Disease (NAFLD), defining liver steatosis, historical data on the development of understandings of this disease state, as well as the histological characteristics of steatosis and steatohepatitis. The epidemiology and social significance of liver steatosis are shown, as well as the reasons for its occurrence. The pathogenesis of this disease state is studied in detail, namely its manifestation as part of the metabolic syndrome, the role of insulin resistance in its occurrence, the importance of leptin and adiponectin, the intestinal microbion, genetic factors. An important focus of the literature review is the diagnosis of NAFLD. The possibilities of various diagnostic methods such as laboratory (blood) tests, imaging tests (ultrasound, CT, MRI), liver biopsy, transient elastography are shown. Prevention, treatment and follow-up of NAFLD patients are discussed. The latest expert proposal to replace NAFLD with a new term - MAFLD (Metabolic associated fatty liver disease) has been given. The diagnosis of MAFLD will be based on the presence of hepatic steatosis and one of three indicators - overweight or obesity, type II diabetes mellitus and metabolic dysregulation. It should be noted that the literature review contains a lot of data from Bulgarian authors who worked on the same issue, which shows the great awareness of the dissertation.

The bibliography contains 133 literature sources, of which 72 (54.5%) have been published in the last 5 years. This is a very high percentage, which confirms the relevance of the issues discussed in the dissertation. The sources in Cyrillic are 11 (8.3%), which shows a very good knowledge of the scientific developments of Bulgarian scientists who have worked on this issue.

The goal is clear - to analyze non-invasive methods for the assessment of liver steatosis and



non-alcoholic steatohepatitis and to develop a clinical algorithm for liver examination in patients with metabolic syndrome, diabetes mellitus or elevated liver enzymes.

The nine tasks are adequately selected, and their implementation guarantees the achievement of the set goal.

The studied groups of patients and the methods used are written in detail on 10 pages. For the period December 2016 - until March 2020, 170 patients with metabolic syndrome were studied. All patients underwent transient elastography with Fibroscan 502 Touch with simultaneous measurement of liver stiffness and steatosis.

135 of them underwent liver biopsy for histological assessment of the degree of steatosis, inflammatory activity and fibrosis using the SAF scoring system, fibrosis assessment by Metavir and Kleiner.

Liver Multiscan was performed in a group of 20 patients - magnetic resonance spectroscopy with determination of the amount of fat in the liver, iron content, together with calculation of LIF score to assess the degree of progression of liver damage.

Clinical studies (history and physical status, BMI, waist circumference, waist / hip ratio), laboratory tests (liver enzymes, lipid status, uric acid, blood sugar, HbA1c, serum iron, ferritin, transferrin, highly sensitive CRP, blood count, HOMA-IR, fasting glucose, impaired glucose tolerance, FIB-4, NAFLD Fibrosis score, FAST score), abdominal ultrasound, transient elastography, liver biopsy, nuclear magnetic resonance.

The software products - STATISTICA version 13.0 and STATA version 16.1 were used for the statistical analysis. The results in all patients were analyzed by the methods of descriptive statistics, nonparametric test, correlation analyzes, LASSO method. The results obtained are original, reflected on 53 pages. The most significant of them are:

Metabolic syndrome and liver steatosis are most commonly found between the ages of 40 and 70 in both males and females.

The calculated BMI distinguishes grade 3 steatosis from grade 1 steatosis, as well as the group without steatosis.

Serum iron and ALT levels distinguish grade 3 steatosis from grade 1 steatosis.

HOMA-IR distinguishes grade 3 steatosis from grade 1 steatosis, and ASAT shows no statistically significant differences between subgroups.

The cut-off values of CAP (233 dB / m for steatosis S1, 270 dB / m for steatosis S2, over 300 dB / m for steatosis S3) proposed in the study distinguish the subgroups with hepatic steatosis with a high level of reliability.

In the study group with metabolic syndrome 77.6% (132 patients) were diagnosed with severe third degree steatosis (CAP 300 - 400 dB / m), 12.4% (21 patients) with second degree (S2) and 8.8 % (15 patients) with first degree (S1).

The study group is dominated by patients who meet the criteria for grade F2 (72 of 170 patients, 42.4%, 31 men and 41 women), while patients with grade F1 and F3 are, respectively, 37 (21.8%, 20 men and 17 women) and 39 (22.9%, 22 men and 17 women). In 4 of 170 patients (2.3%, 2 men and 2 women) there was evidence of fibrosis grade F4



(cirrhosis), and in 18 of 170 patients (10.6%, 5 men and 13 women) with metabolic syndrome no fibrosis was detected by examination with Fibroscan.

The cut-off values of elastography proposed in the study (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) distinguish with a high level of confidence all subgroups with fibrosis with the exception of 4 versus 3 stages and 1 stage compared to patients without fibrosis.

The presence of a moderate correlation between Fibroscan steatosis data and liver biopsy data was confirmed.

The adaptive LASSO method reveals 11 factors that have a predictive value for non-invasive determination of liver fibrosis, namely: liver density, CAP, FIB-4, decreased platelet counts, elevated AST, age, elevated GGT, body weight, elevated triglycerides ALT, cholesterol.

The discussion of the obtained results covers 14 pages. Here is shown their place in relation to modern knowledge of NAFLD. An original diagnostic algorithm for NAFLD in the presence of metabolic syndrome has been proposed. It includes sequentially: anamnesis; anthropometric indicators; laboratory tests; abdominal ultrasound; Fibroscan; FIB-4 and FAST Score.

The 9 conclusions made correspond to the obtained results. The contributions are 9 - 3 with original character, 6 with confirmatory character.

I accept the above conclusions and contributions to the dissertation. Especially valuable for clinical practice is the proposed original clinical algorithm for studying the condition of the liver in patients with metabolic syndrome, diabetes or obesity. Own cut offs (limit values) have been developed to stage hepatic steatosis based on Fibroscan measurements with CAP. The importance of non-invasive assessment of liver fibrosis as an indicator of progression of liver damage, determining the prognosis and risk of complications, is confirmed. Elastography of the liver, FIB-4, NFS (NAFLD Fibrosis score) are accepted as the main methods for its assessment. The use of the FAST score is introduced to rapidly differentiate cases of advanced fibrosis and NASH activity.

The abstract is written on 80 pages and fully presents the dissertation. In summary, the results of the dissertation of Dr. Rozalina Balabanska are original and are the work of the dissertant. They have scientific and practical significance and lead to the expansion of our scientific knowledge in the field of non-invasive diagnosis of liver damage in patients with NAFLD, and further reveals the importance of many additional factors that help predict significant liver disease without performing invasive research. Dr. Rozalina Balabanska presents a list of 6 publications and participations in national and international scientific forums related to the topic of the dissertation.

Based on the above, I believe that the dissertation of Dr. Rozalina Balabanska is fully completed with a clear goal and objectives, original results, correct conclusions and important scientific and applied contributions. It fully meets the requirements of the Law for the Development of the Academic Staff in our country and the terms and conditions for obtaining scientific degrees in Acibadem City Clinic - Tokuda University Hospital, EAD Sofia and I will confidently and categorically vote "YES" for the award of scientific and educational



degree "Doctor" (PhD) of Dr. Rozalina Balabanska.

04/Jul/2021

Prof. Dr. K. Antonov, DSc
/ Prof. Dr. K. Antonov, MD /

A handwritten signature in blue ink, appearing to be 'K. Antonov', written over a horizontal line.