

STATEMENT ON PhD THESIS

of Dr Pavlina Georgieva Boykova-Vylcheva, entitled: „Screening, diagnosis and clinical assessment of the patients with Non-Alcoholic Fatty Liver Disease“ for obtaining the educational and scientific degree “PhD” – higher education field 7. “Healthcare and sport”, professional field 7.1 “Medicine”, scientific specialty 01.03.14. “Gastroenterology”.

Scientific supervisor: Prof. D-r Iskren Andreev Kotzev, MD, PhD, Doctor of medical sciences

REVIEWER

Assoc. prof. Irina Ivanova Ivanova, MD, PhD

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I have been entitled to present the current statement in accordance with a decision of the head of the Scientific jury and Medical University Rector’s order P-109-326/1.08.22 and Protocol N1/10.08.22.

1. Brief biographic and professional notes:

Dr. Pavlina Boykova-Valcheva is a former student of MU - Varna, where she graduated with honors and the "Golden Hippocrates" award. From his graduation and the beginning of 1998 until now, he has been working at the Gastroenterology Clinic at St. Marina University Hospital. Since 2004, she has been a specialist in internal medicine, and since 2011 she has acquired the specialty "Gastroenterology", with interests in hepatology, in the diagnosis and follow-up of patients with non-viral chronic hepatitis and inflammatory bowel diseases. Dr. Boykova has over 23 years of clinical experience, a recognized specialist at St. Marina UMBAL and is a favorite teacher of students and trainee doctors in "Medicine", with practice as an assistant at the Department of Internal Medicine since 2015. With this structure, Dr. Pavlina Boykova completed her doctoral studies in an independent form of preparation.

2. Relevance of the thesis’ topic

The topic of steatosis of the liver has attracted many clinicians and researchers due to the increasing importance of obesity and obesity-associated diseases, as well as the justified need to screen patients with components of the metabolic syndrome for liver disease. The detection of non-alcoholic fatty liver disease (NAFLD) requires further differentiation of cases with a high risk of progression to complications of chronic liver disease - cirrhosis and hepatocellular carcinoma. It is expected that in the coming years, the burden of patients with steatosis liver disease will increase, and this requires the development of an adequate and tailored approach to the specificities of the country, training of doctors and the health system. Therefore, the present work is relevant and necessary to meet the needs posed by clinical practice.

3. Characteristics, volume, structure and evaluation of the thesis

The dissertation of Dr. Pavlina Boykova consists of 158 pages and follows the traditional structure: introductory words - 2 pages; literature review – 45 pages; purpose and tasks – 1 page; research methods – 10 pages; results – 48 pages; discussion and summary with proposed screening algorithm - 16 pages; conclusion – 2 pages; conclusions – 1 page; contributions - 1 page and bibliography of 172 sources. The dissertation has a balanced content and is written in a clear, understandable and comprehensive style.

The literature review competently and comprehensibly sets out both the main and the most recent publications, in consideration of the topic. Dr. Boykova reviews all developments and dissertations on the problems of the NAFLD carried out so far in our country. The presentation begins with definitions of NAFLD and the metabolic syndrome and nomenclature issues related to the introduction of the term metabolic-associated fatty liver disease (MAFLD). Risk factors for NAFLD have been consistently described, including genetic polymorphisms predisposing to ectopic adipose tissue deposition and progressive tissue damage and inflammation. An important place in the overview is occupied by diagnostic methods, divided into invasive methods, mainly histological analysis of liver biopsy material and non-invasive tests in two categories - elastography and serum markers. Dr. Boykova correctly points out the biomarkers indicating steatosis, inflammation and fibrosis. The new indicators and point systems are interpreted with the necessary criticality and the need for subsequent clinical validation. Their general importance for the stratification of patients, according to the individual risk of development and possible mortality from the complications of liver disease, is highlighted. 5 figures and 13 tables help to systematize the information in the overview.

The aim of the dissertation work is ambitious and clinically oriented: to evaluate the options for screening, diagnosis and behavior in patients with NAFLD. The tasks are seven - clearly stated and define the steps for carrying out the study.

The "Methods" section begins with the definition of the study population: 148 patients with NAFLD were prospectively studied, 38 of them with non-alcoholic steatohepatitis and 10 with a compensated stage of liver cirrhosis. The inclusion and exclusion criteria for the selection of the studied group are indicated. Patients were examined with clinical examination and abdominal ultrasound; routine laboratory tests were performed; HOMA defined insulin resistance and multiple scoring systems aimed at assessing steatosis, inflammation and fibrosis in chronic liver disease and NAFLD; transient elastography and determination of cytokeratin-18 (CK-18) levels were performed in 91 and 61 patients, respectively. In most cases, these are outpatient cases investigated by the doctoral student for the purposes of the study. Dr. Boykova independently performed all the indicated clinical examinations and the ultrasound examination, including the transient elastography. An innovative immune enzymatic determination of CK-18 was organized, financed within the framework of a scientific project at MU-Varna. The results are summarized in a database that serves the doctoral student for further analysis. Statistical methods correspond to the purpose and tasks of the study and are competently specified and used.

The studied group of patients has in 100% of cases an increased abdominal circumference, covering one of the criteria for metabolic syndrome, and the registration of other metabolic components is lower: overweight and obesity in 95%; hypertensive disease in 81%; diabetes mellitus (DM) or increased fasting glycemia in 63%; hypertriglyceridemia in 47% and decreased HDL cholesterol in 60%. Thus, 88% of the examined have a defined metabolic syndrome when 3 criteria are present. Insulin resistance according to the HOMA indicator was determined in 72%. A regression model in the present study showed that waist circumference was the most predictive of steatosis severity of all metabolic components. Dr. Boykova defines that every increase in abdominal girth by 1.6 cm corresponds to a higher CAP index by 2 dB/m. There was a significant correlation ($r=0.463$) between waist circumference and liver density determined with FibroScan.

Patients have no or mild biochemical activity, with a predominance of an increase in GGT followed by an increase in ALT. It should be noted that in 35% of patients the AST/ALT ratio is above 1. Steatosis, determined by conventional and/or transient elastography with FibroScan with calculation of the parameter for attenuation of the US wave CAP, was present in all patients. Variation analysis defines the difference in the CAP-value in the first vs. II and I vs. III B-mode degree of steatosis. It would be advisable to statistically analyze the concordance of degrees of steatosis, according to B-mode US and according to the accepted thresholds for CAP. For the prediction of hepatic steatosis with non-invasive scoring systems with a good role in the present study is hepatic steatosis index (HSI) and NAFLD liver fat score, determined by the correct diagnosis of steatosis in 96% and 85%, respectively, and a low proportion of cases in the "gray zone". The FLI index is informative about the severity of steatosis, because of the statistically significant difference in the values of this non-invasive indicator between the three main degrees of steatosis.

Based on transient elastography (FibroScan), 16% of patients were staged with significant fibrosis, 7% with advanced fibrosis and 11% with compensated cirrhosis. Analysis of the diagnostic role of serum markers for fibrosis assessed the NAFLD fibrosis score in the first place over FIB-4. The present study shows that both indices can distinguish absent and mild fibrosis from advanced liver disease. However, diagnostic accuracy with the necessary statistical analysis has not been investigated.

Innovative testing of the serum level of cytokeratin 18 fragments (CK-18) found an increase above 5 ng/ml in 6 of 55 patients, in 10%. If this threshold for establishing non-alcoholic steatohepatitis (NASH) is accepted, none of the subjects had evidence of NASH with advanced fibrosis. This fact is in some contradiction with the staging of patients with US elastography, which is taken as a reference in the present study. An explanation can be found in the relatively small number of subjects and/or the lack of current inflammatory activity, despite advanced fibrosis.

The own results are well illustrated by 41 figures and 42 tables and Appendices 1 and 2. The lack of morphological examination of the liver to assess the histological stage, degree of steatosis and neuroinflammatory activity could be pointed out as a drawback of the study. On the other hand, the design of the study is properly conditioned by the predominantly outpatient source of the patient's problem and the worldwide trend for non-invasive

assessment of liver disease in NAFLD. The title of the dissertation describes "clinical evaluation of NAMCHB", which fully corresponds and is covered by the work done and the provided data.

The dissertation analyzes the results in the separate "Discussion" section, which can be characterized as pleasant to read and understand, with a full comparison of the data with those published in the Bulgarian scientific and foreign language literature. A strength of the dissertation work is a well-explained and illustrated algorithm for screening, diagnosis and follow-up of patients with NAFLD. Methods are graded from readily available to tests requiring competency in a hepatology center. The indicated algorithm can be introduced into clinical practice and later be more widely validated in the approach in people with metabolic factors and risk of NAFLD.

A logical consequence of the stated results, discussion and conclusion are 9 clearly formulated and informative conclusions.

The literature reference of 172 sources, 9 of which are in Bulgarian with authors leading researchers of the NAFLD in our country. The bibliographic reference meets the requirements for completeness, providing all important current works.

Consistent to the dissertation work, Dr. Pavlina Boykova provides 4 full-text publications and 1 scientific communication in an authoritative international forum, and Dr. Boykova is the lead author of all publications.

4. Evaluation of the conclusions of the thesis

I fully accept the stated conclusions, conclusions and contributions of the dissertation.

5. Critical notes

No critical notes applicable.

6. Conclusion

The review of the dissertation work and proposed publications, as well as my impressions of Dr. Boykova's thorough and competent clinical work prove my positive opinion. I would like to recommend to the respected Scientific Jury to award Dr. Pavlina Georgieva Boykova-Valcheva the educational and scientific degree "Doctor" in the scientific specialty "Gastroenterology".

X

Irina Ivanova
MD, PhD

Date: 22 Sep 2022

Varna

/Assoc. Prof. Irina Ivanova, MD, PhD/