REVIEW

Of Dissertation on:

Screening, diagnostics and clinical evaluation of the patients with non-alcoholic fatty hepatic disease.

For acquiring educational and scientific degree "Doctor"

of scientific specialty "Gastroenterology"

in professional filed: Medicine

Author: Dr. Pavlina Georgieva Boykova-Valcheva

Reviewer: Professor Dr. Ivaylo Petrov Vazharov, MD, PhD, professor of gastroenterology, specialist of internal diseases and gastroenterology

I. Procedure of defense

With order No R-109-326 / 1.08.2022 of Rector of Medical University " Prof. Dr. Paraskev Stoyanov" – Varna on the basis of a decision of the Faculty Council of faculty "Medicine" of Medical university " Prof. Dr. Paraskev Stoyanov" – Varna according to protocol No 69 / 26.07.2022 I am assigned a review of the dissertation of Dr. Pavlina Georgieva Boykova-Valcheva – physician – gastroenterologist at the Clinic of gastroenterology at MHAT " Sveta Marina" , city of Varna on "Screening, diagnostics and clinical evaluation of the patients with non-alcoholic fatty hepatic disease" for acquiring educational and scientific degree "Doctor" of scientific specialty "Gastroenterology" in professional filed: Medicine.

My review is complaying with the requirements of drafting a review for acquiring educational and scientific degree "Doctor".

II. Brief biographical data

Dr. Pavlina Boykova was born in Varna on 27.09.1972. She graduated medicine at Medical university, city of Varna in 1997 with honors. She acquired first specialty of Internal diseases in 2004 and in 2011 – second specialty of Gastroenterology. She has certificates of professional qualification of abdominal ultrasound examination first, second and third level MU – Varna. She acquired certificates of professional qualification for upper and lower endoscopy – first and second level. Dr. Boykova works at the Clinic of Gastroenterology of MHAT " Sveta Marina" from 1998 and from 2015 she is assistant in Second department of Internal diseases of MU – Varna

and conducts classes of the subject discipline gastroenterology. She is fluent in English and has a very good computer literacy.

III.   Structure of the dissertation

The dissertation is 157 written pages: introduction 2 pages, literature overview 46 pages, objective and tasks 1 page, material and methods used 11 pages, results 49 pages, discussion of the results – 12 pages, conclusion 2 pages, bibliography 172 literature sources, 9 of them in Cyrillic alphabet and 163 in Roman alphabet, appendix 4 pages, conclusion – 9, contribution – 8. The work is illustrated with 50 figures and 59 tables.

IV.   Actuality and significance of the dissertation

The topic of the dissertation is distinguished by significant actuality in the world, as well as in our country.

The non-alcoholic fatty hepatic disease (NAFHD) is a common healthy problem with a global significance affecting approximately one fourth part of the population in the world. The incidence of NAFHD increases worldwide together with the increase of the incidence of the components of the metabolic syndrome. NAFHD includes a wide specter of conditions with a different risk of progression, as well as the presence of necrotic inflammation and stage of fibrosis are predictors of progressive course of the disease. The hepatic biopsy as a "Golden standard" does not allow screening for NAFHD and can't be used as a diagnostic method amongst the population. Easy accessible, highly sensitive and specific tests are necessary to allow not only identification of the patients with high risk of unfavorable outcome of the disease, but to give an opportunity for monitoring the course of the disease and the therapeutic response among the population. FibroScan elastography is a recently introduced method for evaluation the stage of the hepatic fibrosis and the degree of steatosis, but more data are necessary for validation of this method in the daily clinical practice in patients with NAFHD. There is no enough clinical practice of non-invasive biomarkers' use for estimation of the fibrosis, inflammation and steatosis in NAFHD, especially among the Bulgarian population. The adequate staging of the patients with NAFHD and stratification of the risk groups provides guidance for adequate therapeutic behavior. Increase of the knowledge of non-alcoholic fatty hepatic disease and the its clinical consideration leads to timely diagnosing

of the disease in the early stages with the purpose of managing and opportunity of reverse development of the hepatic changes.

The dissertation is one of the first detail studies in the country regarding the screening, diagnostics and clinical evaluation of the patients with NAFHD.

V. Literature overview

In the literature overview the risk factors leading to the occurrence and progress of steatosis are discussed, because they are the main comorbidities important for the clinical assessment of the patients with NAFHD. The data of the worldwide experience regarding the invasive and non-invasive serum and imaging diagnostic methods for defining the presence of steatohepatitis and staging of the hepatic fibrosis are analyzed. The world data proving the possible progressive course of the disease are presented. The actual world tendencies for development of screening programs for diagnosing of NAFHD are reviewed.

The literature overview ends with a conclusion that in view of the wide distribution of the disease worldwide and its increasing occurrence, new easily accessible, highly sensitive and specific tests are necessary, which would allow not only identification of the patients with high risk of progressive disease, but an opportunity for monitoring the course of the disease and the therapeutic response.

The objective is clearly formulated – to study the screening, diagnostics and the clinical evaluation of the patients with NAFHD.

The relevant tasks are 7 and they are concrete and realistic. They are well defined and adequate for the allocated objective.

VI. Clinical material used in the dissertation.

Totally 148 patients are examined for the period of October 2016 to May 2022. All the patients have evidence of non-alcoholic fatty hepatic disease, 38 patients of them have non-alcoholic steatotic hepatitis, 100 patients with non-alcoholic hepatic steatosis and 10 with compensated hepatic cirrhosis. The including and excluding criteria are clearly formulated. Full clinical, laboratory and ultrasound examinations are performed in all patients. Elastography with defining of CAP (FibroScan, Echosence) is performed in 91, and serum cytokeratin 18 is examined in 61 patients as a marker of hepatocytic apoptosis. The obtained material is enough for analysis and statistical processing.

VII. Methods

Complete clinical examination was performed in all patients, including detailed history of concomitant diseases, medicines taken, harmful habits as well as a detailed physical status, including anthropometric indicators. Complete clinical examination is performed in all patients, including a detailed history of the concomitant diseases, medicines taken and harmful habits, as well as a detailed physical status with anthropometric indicators. The data of the measured height, weight and BMI are consistent with the classification of the body weight defined by WHO and its association with the disease prevalence and death-rate. The data of the measured abdominal girth as an anthropometric indicator for visceral fat accumulation are stratified according to the defined levels of the abdominal obesity, connected with the degree of risk of metabolic disorders and health problems. The criteria adopted by International diabetic federation were used for defining the diagnosis metabolic syndrome.

In 61 patients the levels of the total cytokeratin 18 in serum were measured quantitatively by "sandwich" ELISA method as a marker of hepatocyte apoptosis and a predictor of presence of steatohepatitis. Specific antibodies – anti Human Cytokeratin 18 are used. According to the world literature Cytokeratin 18 is a validated biomarker of verification of inflammation and underlying steatohepatitis in patients with NAFHD, but yet there are no accessible clinical tests for routine application and experience in general practice. Dr. Boykova's study is the first study among the Bulgarian population examining the connection between CK 18 and NAFHD.

The point systems (scores) are calculated for non-invasive evaluation of the fibrosis – APRI, NAFLD fibrosis score, FIB-4 , correlation ASAT/ALAT, as well as score systems for assessment of steatosis – hepatic steatosis index, lipid accumulation product, fatty liver index, NAFLD liver fat score. The used mathematical models are exactly defined with formulas and the set laboratory and clinical indicators and the accepted border values are described.

The main imaging method used in the dissertation is described in detail – ultrasound examination of organs with ultrasound apparatus with convex transducer with frequency 2.2 – 2.5 MHz, with complete inspection of the abdominal organs performed. The criteria of ultrasound grading of steatosis of the liver: degree of increase of echogenicity of the hepatic parenchyma, compared to the parenchyma of the right kidney, visualization of the diaphragm,

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intra-hepatic vessels and the wall of the gall bladder, the dimensions of the liver and the presence of focal absence of steatosis.

In 91 patients with NAFHD the apparatus FibroScan, Echosens is used for defining the hepatic density and quantitative measurement of steatosis by defining of CAP (Controlled Attenuation Parameter – parameter of ultrasound amplification). The way study is conducted is described in detail. The evaluation of the stage of fibrosis and the degree of steatosis according to CAP is realized on the basis of the accepted border values of meta-analyses of studies in patients with NAFLD and NASH and literature data from world and Bulgarian studies.

VIII. Results, algorithms of behavior, discussion, conclusions and contributions of the dissertation

The patients were analyzed according to the demographic indicators gender and age, clinical anamnestic, laboratory and imaging evidence. Complete clinical evaluation of the patients with defining the presence of components of metabolic syndrome is performed. The results were presented regarding the distribution of glycemic disorders – Diabetes mellitus type 2 and pre-diabetes, hypertonic disease, obesity and abdominal girth among the examined group of patients with non-alcoholic fatty liver disease. It was found that 63% of the patients have glycemic disorders – 47.5 % with known diabetes mellitus type 2 and 15.5 % with pre diabetes. In 81 % hypertonic disease was found and the degree of steatosis increases in parallel with the increase of the stage of hypertonic disease and its longevity. The proportion of the patients with high values of ASAT, ALAT, GGT, AP and the proportion of the patients with changes of the lipid profile – triglycerides, HDL cholesterol, total cholesterol was determined.

The results of the distribution by seriousness of steatosis according to the ultrasound examination and the evidence of the parameter of ultrasound attenuation by FibroScan among the examined group of patients with NAFHD are presented. Statistical analysis is performed regarding the results of measurement of steatosis by FibroScan CAP and its connection to the clinical characteristics of the patients and the determined indexes of steatosis. High association is found between BMI and the abdominal girth with the degree of steatosis, moderately high positive connection between hypertonic disease and the degree of steatosis and low relation between the presence of diabetes and high triglycerides and the degree of steatosis. The analysis of the data

demonstrated that all components of the metabolic syndrome are important for determining the degree of steatosis. but most predictive of the occurrence and degree of the steatosis defined with CAP is the increase of the abdominal girth, the latter being the main factor presenting the degree of visceral obesity.

The data of ultrasound examination are compared to the ones of the degree of steatosis by CAP, establishing a high degree of relation between the different degrees. The data proved undoubtedly the importance of the ultrasound examination as an imaging method of first line for screening and verification of NAFHD but for more precisely defining the steatosis found by ultrasound and differentiation of moderate from severe steatosis, FibroScan with determining of CAP is recommended. The analysis of the results of the examined indexes of steatosis found moderately high statistical relation between the values of CAP and the indexes of steatosis (FLI and HIS). showing parallel growth of CAP and the values of FLI and HIS. On account of these data the steatosis indexes are recommended as an easy screening method of prediction of the presence of steatosis.

The results of the staging of fibrosis are presented, defined by measuring the hepatic density with FibroScan and correlated to the results of the indexes determined for fibrosis. Analysis of the assessment of the relations between the clinical indicators and their effect on fibrosis was performed. A moderately strong relation is found between the hepatic density and body mass index, as well as a high correlation with the increase of the waist circumference. Moderately high relation between the stage of fibrosis and the presence of Diabetes mellitus type 2, and the correlation to hypertonic disease is low. These data give reason to the dissertant to develop a strategy for screening and intensive observation among the target groups with a probable aggressive course of the disease, like the patients with high BMI, large abdominal girth and Diabetes mellitus type 2.

The comparison between the results of the scores for fibrosis with the results of hepatic density from FibroScan found a significant relation for the three scores – NAFLD fibrosis score, APRI and FIB-4, the highest being the association with NAFLD fibrosis score.

The results of the examination of serum cytokeratin 18 are presented as it is the single independent marker of hepatic apoptosis and detecting steatohepatitis. The patients are analyzed according to the demographic

indicators, clinical anamnestic and laboratory data. The relation between the increased levels of CK-18 and the clinical characteristics of the patients and the indexes of fibrosis defined is analyzed, and correlated to the degree of steatosis and the changes of the laboratory indicators. Increase of CK-18 is found in 10 % of the examined group with NAFLD. A moderately high positive relation is found between the level of triglycerides and CK 18, but there is no such connection with the increase of the abdominal circumference, BMI, presence of hypertonic disease and Diabetes mellitus. No relation was found between the increased values of CK 18 and the degree of the steatosis verified by ultrasound examination. Statistically significant differences were found between the group of patients with increased and normal CK 18 compared to the average values of ASAT, ALAT, GGT, with significantly higher values in the patients with increased CK 18.

The data are presented in well-illustrated table and graphic manner / 59 tables and 50 figures/. Own photo material of the different clinical cases is attached.

Algorithm is suggested for screening and diagnostics of NAFLD with a high practical value, based on the evidence of the study performed.

The discussion comprises of 12 pages. The dissertant compares her own results to other authors' results.

The conclusions are 9, having predominantly practical importance.

The most significant results of the study are summarized in the conclusion.

The dissertation ends with six concrete, well formulated scientific-applicable contributions with confirmative character and 2 contributions with original character.

The autoreferate is structured according to the requirements. The contents comply with the dissertation.

IX. Publications and scientific activity

8 scientific publications are included in the dissertation of Dr. Boykova, related to the subject of the dissertation – 4 in national magazines as first author, 1 in international scientific forum and 3 in national forum. This certifies of her leading role in conducting the examinations and preparation of publications. The

publications are from 2018, 2019, 2020 and 2022 and reflect the relevance of the theme.

X. Conclusion

As a conclusion Dr. Pavlina Georgieva Boykova-Valcheva managed to create a thorough and comprehensive scientific report on "Screening, diagnostics and clinical evaluation of patients with non-alcoholic fatty liver disease". The topic is relevant for our country; the examination is well organized and conducted. She managed to achieve this because of her brilliant literary knowledge and very well combined competent knowledge and qualification.

All that gives me the reason to suggest to the scientific jury members at Medical University " Prof. Dr. Paraskev Stoyanov" – Varna, to award Dr. Pavlina Georgieva Boykova-Valcheva an educational and scientific degree " Doctor" of scientific specialty " Gastroenterology".

21.09.2022

City of Varna

Reviewer: _____

Prof. Dr. Ivaylo Vazharov, MD