POSITION

ON A DISSERTATION

of **Dr. Deyan Georgiev Radev**, on the topic: **"Spleen density measured by pSW elastography as a predictor of high-risk esophageal varices in patients with hepatic cirrhosis"** for awarding the educational and scientific degree "Doctor" in the field of higher education 7. "Health and Sports", professional field 7.1 "Medicine" in scientific specialty 01.03.14. "Gastroenterology".

Supervisors: Prof. Dr. Vladimir Andonov, MD, PhD and Assoc. Prof. Katya Doykova, MD, PhD

REVIEWER

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By decision of the Chairman of the Scientific Jury and in accordance with the order

R-1344/20.11.2024 of the Rector of MU-Plovdiv, I am appointed to present this opinion.

1. **Brief biographical and professional data of the PhD student:**

Dr. Deyan Georgiev Radev is a graduate of the Medical University of Plovdiv. Since graduation in 2017, until now, he works at the Gastroenterology Clinic at Kaspela University Hospital. Since 2022, he has been a specialist gastroenterologist with interests in interventional procedures in gastroenterology and liver pathology, in particular liver cirrhosis. Dr. Radev has been a teacher of medical students for 6 years, and since 2018 he has been part of the Second Department of Internal Medicine, Department of Gastroenterology of the Faculty of Medicine, Medical University of Plovdiv. At this structure, Dr. Deyan Radev completed his doctoral studies in an independent form of PhD training.

2. Relevance of the dissertation topic

The involvement of the spleen in the pathophysiology of portal hypertension syndrome (PH) is the subject of intriguing studies. Undoubtedly, the spleen is more than a passive receiver of congestion. This largest lymphatic organ is a modulator of local and systemic inflammatory processes, and the general circulation in the portal system makes the liver and spleen respond to common signaling molecules of inflammation. Splenomegaly is a characteristic feature of advanced chronic liver diseases and is part of the clinical syndrome PH, along with the establishment of ascites and porto-systemic shunts. It is known that the size of the spleen is not necessarily proportional to the portal pressure and the severity of the liver damage. In the pathophysiology of changes in the spleen in PH, the following are discussed: passive

congestion; increased blood flow in the lienal artery; hyperplasia of reticuloendothelial cells by immunological stimuli; induction of neoangiogenesis and fibrosis; resistance to venous edema through the lienal vein. The most accurate method, noted as the "gold standard" for assessing PH, is the measurement of the hepatic vein pressure gradient (HVPG), but the study is not a routine part of the clinical evaluation of patients with chronic advanced compensated liver disease (cACLD). At the same time, abdominal ultrasound today is a multi-parametric examination, informative of topographic and anatomical features through B-mode, of the details and dynamics of tissue macro and microcirculation through Doppler modalities and contrast-enhanced ultrasound, as well as density, compression and loss of elasticity through ultrasound elastography. Measurement of liver stiffness (LSM) by any method of ultrasound shear-wave elastography (SWE) is a well-established approach to assessing the stage of liver disease. The Baveno VI consensus proposed, for the first time, patients with viral etiology and compensated stage cirrhosis to receive a risk assessment for clinically significant PH (CSPH) through the combination of LS and platelet count. The concept was expanded after the Baveno VII meeting with the recommendation that CSPH can be excluded in patients with LS ≤15 kPa and platelet count ≥ $150x10^9$/l. With the combination of LS < 20 kPa and a platelet count > 150, there is a very low risk of high-risk esophageal varices (HRV), therefore upper endoscopy may not be performed (ANTICIPATE algorithm). A spleen stiffness measurement (SSM) can be proceeded to a second stage, as a supplement after LS measurement and cACLD observation. Most studies on the role of SSM are based on the method of transient elastography (TE or VCTE). The thresholds and models for the PH and CSPH estimation algorithm are based on values obtained with TE. In 2019, the team of the Clinic of Gastroenterology at the University Hospital "St. Marina", Varna published in the journal "Diagnostic and Therapeutic Ultrasound" a summary of the report "Ultrasound parameters of the spleen in portal hypertension". In a prospective study (2016 to 2018), we investigated the clinical significance of LS and SS measured by TE in 103 patients with the need to specify the severity of PH and found that a threshold of 25 kPa for SSM defines in 100% the presence of esophageal varices. At the Baveno VII meeting, the role of the model combining LSM + SSM for non-invasive PH assessment is defined, applicable in cases of cACLD based on viral hepatitis, alcohol-related impairment and "lean NASH", according to which a threshold of SSM < 21 kPa (up to 30 kPa) and SSM > 50 kPa can exclude CSPH with a sensitivity > 90% and correspondingly confirm CSPH with specificity > 90%. An SSM limit < 40-46 kPa can be used to shut down HRV with a sensitivity > 90%. Therefore, an SSM ≤ 40 kPa, as a low-probability parameter for HRV, allows optimal non-invasive evaluation in patients who do not fall into the category of the Baveno VI criteria for "avoiding" upper endoscopy. **The SSM thresholds are to be specified using the new methods for ultrasound elastography. At this stage, it is assumed that SSM (via pSWE) < 2.5 m/sec excludes CSPH and HRV; An SSM > 3.5 m/sec implies the presence of HRV. Therefore, the dissertation topic is relevant and meets the scientific interests and the need for research in modern hepatology.**

3. Characteristics, structure and evaluation of the dissertation

The dissertation of Dr. Deyan Georgiev Radev consists of 154 pages and follows the traditional structure: introduction - 3 pages, literature review - 50 pages; goal and objectives - 1 page; research methods - 11 pages; results – 62 pages; summary of the results with discussion - 10 pages; conclusions – 2 pages; Contributions – 2 pages and a literature review from 135 sources. The dissertation has a balanced content and an orderly, consistent and clear style in presenting the data. An indisputable quality of the work is the excellent illustration, with a total of 22 tables and 44 figures. The literature reference includes 135 sources, the first 5 of which are by Bulgarian researchers.

In the literature review, the dissertation consistently discusses the pathophysiological mechanisms of hepatic fibrosis and portal hypertension. The visualization methods and the systems for classification and gradation of esophageal varices are excellently presented. In subsection 2.2 of the review, Dr. Radev well describes the "gold standard" for invasive assessment of portal hypertension – hepatic vein pressure gradient (HVPG) measurement and points out the main criticism of the methodology related to the suboptimal assessment of the presinusoidal component of PH. A gap in the literature review is the lack of comment regarding the direct measurement of portal pressure gradient in endo-ultrasound navigation (EUS-PPG), which has become a modern alternative to HVPG for PH estimation. As endoscopic ultrasound (EUS) is increasingly entering hepatology, its role in the complex assessment of changes in the vascular network: presence, size, structure and flow in collateral vessels, mainly in terms of esophageal and gastric varices, should also be discussed. In Section 3 of the literature review, Dr. Deyan Radev fully and informatively examines the physical principles and types of ultrasound elastography, competently comparing the variants of shear-wave elastography (SWE): transient; pSWE; 2D-SWE. In my opinion, the literature data on the applicability of spleen density evaluated by ultrasound elastography for the diagnosis of CSPH and HRV are insufficiently presented, with some repetition in sections 2.4.2.2 and 3.3. The review ends with well-summarized conclusions that justify the need for additional research in the field of portal hypertension syndrome and the choice of ultrasound elastography method applied in the dissertation – pSWE.

The purpose of the dissertation is clinically oriented: to evaluate the possibilities of point swear wave elastography (pSWE) as a predictor of high-risk esophageal varices in patients with liver cirrhosis. There are 6 tasks, clearly stated and defining the steps for carrying out the study. Methodologically, I assess its design as adequate for the hypothesis. The dissertation and the supervisors correctly compared patients without data for liver disease with the main group of the study – liver cirrhosis. The approach to separating patients with cirrhosis, according to the leading etiology, corresponds to preliminary literature data on differences in threshold values defining CSPH according to the cause of liver damage. A sufficient number of 184 patients were included to provide credibility in the statistical analysis. Logically, for each of the sub-groups of patients, inclusion and exclusion criteria are clearly stated. The study was conducted at the Reference Center for Diagnosis, Follow-up and Treatment of Patients with Liver Diseases (University Hospital "Kaspela") with the ability to provide all modern methods.

The elastographic measurements were carried out with ultrasound equipment of a "high class": Esaote MyLab 9. The protocol for performing pSWE is comprehensively explained, with 10 measurements of spleen density performed under the validity rule if IQR/M <30. The data is derived as the average wave velocity (SWV; shear wave velocity) in m/s, i.e. the characteristic unit of measurement for pSWE. It is likely that the software of ultrasound equipment does not recalculate the calculated average density in kPa. However, it would be an advantage to look for a way to present one's own data in the standardized metric unit kPa in order to fully discuss and compare with the results of other clinical trials. The dissertation does not offer sample images from the practical application of SSM via pSWE. A protocol for performing upper endoscopy follows, and the gradation of esophageal varices follows a classification system from the Baveno VI consensus. Thus, in general, all patients with cirrhosis are further categorized: without varices; with small, medium and large varices; with HRV in the presence of medium and large esophageal varices. The statistical methods are applied adequately and competently. I consider as a major drawback in the study methodology the lack of simultaneous assessment of liver density (LS) in the control group and in each of the patients involved. LS by ultrasound elastography is a generally accepted stage of the study of each patient with chronic liver disease and part of the CSPH assessment algorithm proposed in the Baveno VII Consensus.

Own results are excellently arranged and illustrated by 38 figures and 19 tables. The discussion of the data follows the sequence of tasks and follows the analysis and summary of the results on each of the scientific problems posed. Under "Task 1", Dr. Deyan Radev derives reference values for the group of healthy controls, with a lower limit of SWV set at 1.76 m/sec; upper limit of 2.60 m/sec and an average value of 2.26 m/sec. This task is essential for the subsequent comparison with the values in patients with present liver cirrhosis. "Task 2" analyzed the mean and reference values for the wave velocity in the spleen in patients with alcoholic etiology. Significantly higher SWV scores were observed in this group, with the mean being defined as 3.23 m/sec. It is important to mention that a high positive association was found between the presence of varices and the values of the wave speed (rs= 0.734). This trend is also maintained in "tasks 3 and 4", and in all patient groups the SWV values show a high association with the degrees of varices, with the highest being in patients with liver cirrhosis of alcoholic etiology. "Task 5" derives threshold values defining patients as high-risk for varicose bleeding - 3.17 m/sec for alcoholic cirrhosis, 2.94 m/sec and 2.64 m/sec, respectively for HBV and HCV etiology. On the other hand, there is no clear statement of the baseline clinical and laboratory characteristics of each of the three groups of patients with cirrhosis of alcoholic and viral origin, respectively data on the severity and presence of complications, the degree of decompensation of cases with cirrhosis, the intake of medications that affect portal pressure (non-selective beta-blockers, antiviral therapy). Under "Task 6", I would expect a more in-depth and complex analysis of non-invasive indicators for fibrosis and PH, combining laboratory tests, ultrasound and ultrasound elastography indicators with endoscopic data on the degree of esophageal varices. It is known that at this stage in hepatology there is no single non-invasive

marker for the severity of PH and the risk of bleeding from GHT due to PH, so algorithms and step-by-step combinations of indicators are developed and validated in clinical practice.

Throughout the dissertation, the excellent presentation of the data through the full use of the possibilities of graphical analysis is impressive. The positive opinion is confirmed by the clarity in displaying the results of each of the tasks of the dissertation in a framework with generalized trends. The discussion of the results follows the sequence of tasks, clearly points again to the importance of the data obtained for SSM, but I would criticize the completeness of the analyzed information published so far concerning the main topic of the dissertation in the "discussion" sections of each task. Practically, the comparison of one's own with literary data is expanded and brought out in "Chapter 5" – a summary.

A logical consequence of these results and discussion are 10 clearly formulated and informative conclusions. I accept the conclusions made as justified, based on the collected clinical facts and the correct statistical analysis. The dissertation of Dr. Deyan Radev has contributions of an original and confirmatory nature. The applicability of ultrasound elastography in assessing the density of the spleen as a non-invasive indicator of the presence of esophageal varices, risky for bleeding, was shown. The study shows that the gastroenterology referent center of the University Hospital "Kaspela", Plovdiv is proactive and ambitious in promoting multiparametric ultrasound as the main method for assessing patients with chronic liver diseases.

In connection with the dissertation, Dr. Deyan Radev offers 3 full-text publications as the first author, one of them in a refereed journal, as well as a poster on the topic of the dissertation, presented at a prestigious European forum. I wish Dr. Radev to continue his clinical work with success and to expand the prognostic markers in the field of hepatology with new data.

4. Conclusion

The dissertation of Dr. Deyan Georgiev Radev on the topic: "Splenic density measured by pSW elastography as a predictor of high-risk esophageal varices in patients with liver cirrhosis" presents results and conclusions with an original contribution to science and meets the requirements of the Law on the Development of the Academic Staff of the Republic of Bulgaria and the Regulations for the Implementation of the Law on the Development of the Academic Staff of the Republic of Bulgaria and the Regulations for the Implementation of the Law on the Development of the Academic Staff of the Republic of Bulgaria.

The dissertation shows that Dr. Deyan Radev has the qualities and skills to independently conduct scientific research on the basis of in-depth theoretical knowledge and professional skills in the scientific specialty "Gastroenterology".

In this regard, I give my positive assessment of the work of Dr. Deyan Radev and propose to the Scientific Jury to award Dr. Deyan Georgiev Radev the educational and scientific degree of "Doctor".

Date: 31.12.2024 /Assoc. Prof. Dr. Irina Ivanova/