

**STATEMENT
ON PhD THESIS**

of Dr. Yoana Svetlozarova Stoyanova, entitled: „Antiviral therapy of hepatitis B – dynamics of viral markers and long-term results“ 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: Assoc. Prof. Dr. Irina Ivanova Ivanova, MD, PhD

REVIEWER

Col. Prof. Dr. Ivaylo Petrov Vazharov, MD, PhD

Specialty of Internal diseases, Gastroenterology and dietetics

Head of MHAT-Varna, Military Medical Academy

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-109-83/21.03.24 and Protocol N1 of 25.03.

Brief biographic and professional notes:

Dr. Yoana Svetlozarova Stoyanova graduated from the University of Varna with honors in 2018. Since her graduation, she has been working at the University Hospital "St. Marina" - Varna, at first as a resident at the Clinic for Propaedeutics of Internal Diseases, then from 2019 she is an assistant at the Faculty of Dental Medicine of the Medical University of Varna, and from April 2022 she is a physician assistant at the Clinic for gastroenterology. In December 2022, she acquired the specialty "Gastroenterology", with interests in hepatology, in the diagnosis and follow-up of patients with viral chronic hepatitis and inflammatory bowel diseases. At the Second Department of Internal Medicine, Dr. Stoyanova begun her education as a PhD student in the specialty of "Gastroenterology" in January 2020.

Relevance of the thesis topic

Worldwide, approximately 316 million are infected with HBV, resulting in a high mortality rate of 800,000 people in 2019. Treatment with nucleoside/nucleotide

analogues (NUCs) is effective in suppressing viral replication, reversing liver disease, and reducing the risk of hepatocellular carcinoma, but clearance of HBsAg is rarely achieved (<5%). Therefore, therapy in most patients is long-term and associated with problems of potential side effects, increasing costs, and suboptimal outcome in the part of treated patients. Thus, the present work is relevant and necessary to meet the needs posed by clinical practice.

Characteristics, volume, structure, and evaluation of the thesis

Dr. Yoana Stoyanova's dissertation consists of 106 pages and follows the traditional structure: introduction - 1 page; literature review – 37 pages; purpose and tasks – 1 page; patients and methods – 11 pages; results and discussion in a general structure of 47 pages; conclusions – 2 pages; contributions - 1 page and a literature reference of 176 sources, arranged in order of citation, 6 of which are by Bulgarian authors. The dissertation has balanced good content in accurate and correct language.

The literature review is an extensive presentation of the current epidemiological data on the spread of HBV, containing the concept of phases of chronic infection and characteristics of the viral cycle. The dissertation reveals in detail markers for HBV diagnosis and well presents studies on the role of quantitative HBsAg (qHBsAg), as a relatively new biomarker in clinical practice. Thus, the qHBsAg test can be an indicator of HBV activity even in patients with an undetectable viral load, due to the synthesis of this viral protein from the HBV sequences integrated into the hepatocyte genome and from the "template or mini chromosome" - cccDNA. The conclusion of this part of the review is that qHBsAg surpasses the informativeness of the qualitative test and is a major marker of HBV activity in NUC-suppressed patients. The detailed presentation of goals, advantages, and disadvantages of current hepatitis B therapy, the future of HBV drug treatment, and patient follow-up strategies during long-term treatment is accomplished. The review concludes that new markers of HBV activity, such as qHBsAg and HB-core-associated antigens (HBcrAg) are highly desirable to be used in not only clinical trials but also to therapeutic decisions.

Dr. Yoana Stoyanova's research has a clear goal and well-formulated 8 tasks. 84 consecutive patients from the register of the Clinic of Gastroenterology, undergoing antiviral treatment against HBV with the standard indications, who met the inclusion and exclusion criteria specified by the dissertation were studied

prospectively. Most of the patients are with HBeAg-negative HBV infection, chronic hepatitis B and HBV-related cirrhosis (89.3%) and are compared with the group of patients with HBeAg-positive chronic hepatitis B. Retrospective data on baseline characteristics of included patients (before initiation of etiological therapy) are also discussed. Research methods correspond to the modern approach to evaluate the evolution of chronic liver diseases and the effectiveness of antiviral treatment, with an emphasis on non-invasive markers - transaminase activity, non-invasive indirect markers of fibrosis (FIB-4, APRI), abdominal ultrasound, viral load and HBeAg status. Mainly, the treatment was carried out with lamivudine and tenofovir, and the group treated with entecavir was only 2 patients. The dissertation includes an innovative study of the serum level of HBcrAg, for which there is no published experience in our country. qHBsAg was monitored in two measurements during therapy with an interval of at least 6 months between them. It should be noted that patients were monitored with multiparametric ultrasonography including assessment of liver stiffness measurement. Statistical methods are correctly used. Graphical analysis is well applied to visualize the obtained data. The statistical power of the subgroup analysis was restricted by the small number of patients falling into distinct categories, such as HBeAg (+) chronic hepatitis B (n=9), baseline cirrhosis (n=24), particularly with baseline decompensation (n=8).

Dr. Yoana Stoyanova's dissertation combines the chapter of achieved results with discussion. The study follows the order of the tasks. 15 tables and 47 figures help illustrate proprietary data.

The baseline, pre-treatment characteristics of the included patients elucidated useful information about the role of indirect serum markers for fibrosis (although not formulated as a separate task), mainly in relation to the fact that 53 of 84 patients had a baseline liver biopsy with histological analysis of METAVIR disease stage. Thus, Dr. Stoyanova's study found that FIB-4 is a useful marker for differentiating patients with early-stage liver disease from cases with advanced fibrosis and cirrhosis, and to some extent is more informative than APRI. The result of task 1 is also interesting, showing that in baseline HBeAg (+) chronic hepatitis B, the concentration of HBsAg is higher than in cases with negative HBeAg. Literature data on qHBsAg in the stage of cirrhosis are conflicting. The present thesis strongly shows that the baseline qHBsAg is lower in advanced stage of the disease, probably also corresponding to a longer duration of chronic HBV infection. Does qHBsAg

decrease in the natural course of HBV infection, especially in cirrhosis? This should be a future goal of monitoring at the clinical center, and by focusing on this goal, I believe that the Gastroenterology Clinic team can achieve interesting results.

Results regarding the efficacy of nucleoside analogue therapy, in particular biochemical response rates and viral suppression, were consistent with those of international studies. Dr. Stoyanova correctly compares the obtained data with those from the dissertation work of Dr. Klimentina Gerdzhikova, as one of the contemporary publications in our country on the effects of antiviral treatment in hepatitis B. It is of interest to observe that patients who achieve permanent inhibition of HBV replication (HBV DNA < 10 IU/ml) with long-term use of NUCs have a lower concentration of HBsAg compared to cases with low-level but detectable viremia.

Undoubtedly, an important part of the Results and Discussion section is the analysis of HBcrAg concentration in NUC-suppressed patients. The thesis did not show a relationship between the level of HBcrAg and the duration of treatment, the stage of liver disease, as well as the baseline HBeAg status (probably due to the small number of patients part of the relevant subgroups). Patients with HBsAg seroconversion also have detectable serum levels of HBcrAg with an average value of 3.8 log 10 IU/ml, and this result should be described in a full-text publication by Dr. Stoyanova and the team of the Gastroenterology Clinic at MBAL St. Marina - Varna. Also, the dissertation's initial data on the worsening of hepatic steatosis during the long-term treatment and follow-up of patients with hepatitis B should be further developed and become the target of new prospective studies. The Gastroenterology clinic is one of the "pioneers" in our country for the application of US elastography in the evaluation of chronic liver diseases. Thus, in continuation of the center's traditions, Dr. Stoyanova suggests monitoring the evolution of liver disease during antiviral therapy according to assessment of liver stiffness. Although viral suppression is a fact in more than 90% of treated patients, a favorable dynamic in liver stiffness, with an average of 4 kPa, is established in 64% of patients, while taking NUCs for more than 1 year.

Part of the cases with decompensated cirrhosis in the present dissertation are a good example of achieving recompensation of the disease as a result of the etiological therapy. The interesting, even to a certain extent, provocative results of

Dr. Stoyanova's work should receive extensive discussion and more in-depth further studies in the future.

A logical consequence of the stated results, discussion and conclusion are 13 clearly formulated and informative conclusions.

The literature reference contains 176 citations, 6 of which are by leading Bulgarian authors in the field of chronic viral hepatitis. The bibliography meets the requirements for completeness, providing all important current works.

Consistent to the dissertation work, Dr. Yoana Stoyanova provides 3 publications all with the participation of the PhD student as a leading author. I would like to recommend future publication activities to illustrate the conclusive data of the current study.

Evaluation of the conclusions of the thesis

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

Critical notes

No critical notes applicable.

Conclusion

The dissertation work of Dr. Yoana Svetlozarova Stoyanova is a thorough and significant scientific study in the field of hepatology. The clinical training and scientific activity of Dr. Yoana Stoyanova justify my positive opinion and I consider that the PhD student meets the criteria for obtaining the educational and scientific degree "Doctor" in the scientific specialty "Gastroenterology".

April 19, 2024

Varna

Заличено на основание чл. 5,
§1, б. „В“ от Регламент (ЕС)
2016/679

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