ATTH: THE CHAIRMAN OF THE SCIENTIFIC JURY
APPOINTED ACCORDING TO ORDER NO
R-109-83/MARCH 21, 2024
OF THE RECTOR OF THE MEDICAL UNIVERSITY
'PROF. PARASKEV STOYANOV' OF VARNA

STANDPOINT

by Assoc. Prof. Diana Todorova Gancheva-Tomova, MD, PhD
Medical University 'Prof. Dr. Paraskev Stoyanov' of Varna

Concerning: the dissertation work for the acquisition of the educational and scientific degree of 'doctor of philosophy' by Yoana Svetlozarova Stoyanova, MD, within the doctoral programme of 'Internal medicine', professional trend No 7.1 Medicine, field of higher education No 7 Public health and sports, and scientific speciality of 'Gastroenterology'.
Theme of the dissertation work: 'Antiviral therapy in chronic hepatitis B - viral markers' dynamics and long-term outcomes'.
Adviser: Assoc. Prof. Irina Ivanova Ivanova, MD, PhD

1. General presentation of the procedure

I have been elected member of the Scientific jury and according to the Protocol No 1/March 25, 2024, I have been assigned to prepare a standpoint.

The standpoint is prepared in concordance with the requirements of the Statute-book for structure and activity and the Statute-book for development of the academic staff of the Medical University "Prof. Dr. Paraskev Stoyanov" of Varna and with the Law for development of the academic staff in Republic of Bulgaria.

The set of materials submitted on paper and electronic carrier is in concordance with the procedure for the acquisition of the educational and scientific degree of 'doctor of philosophy' in the Medical University 'Prof. Dr. Paraskev Stoyanov' of Varna.

2. Brief biographical data

Yoana Svetlozarova Stoyanova was born on February 1, 1993 in Varna. She graduates with excellent grades at the First foreign language secondary school in Varna in 2012. In 2018, she graduates in the speciality of 'Medicine' from the Medical University "Prof. Paraskev Stoyanov" of Varna. She starts her professional development as physician in the Clinic of Internal Medicine at St. Marina University Hospital of Varna. In 2019, after a competition, she is appointed as physician-assistant and teaches students within the Bulgarian- and English-language syllabus. Since 2020, she is full-time doctoral student at the Second Department of Internal Medicine, Educational Sector of Gastroenterology, Hepatology, and Nutrition. Since April 2022, she works in the Clinic of Gastroenterology at

St. Marina University Hospital of Varna. In January 2023, she acquires the speciality of gastroenterology. She has a command of English, German, and Italian languages.

3. Actuality, significance and practical directedness of the theme

Chronic hepatitis virus B (HBV) infection is one of the most common reasons for the chronic liver disease and it is still a main problem for the public health in the world. According to WHO data of 2019, about 296 millions of individuals live with this chronic infection and 1,5 million are newly-infected every year. The high virus infectiousness determines its global dissemination of various incidence rates in different regions of the world, too.

During the last years, because of the wide application of the routine HBV vaccination in the early childhood, a considerable reduction of the chronic HBV infection is rendered an account. In spite of that, the dissemination remains high as 10 percent of the chronically infected are diagnosed and 2 percent only undergo antiviral therapy.

Contemporary therapy with HBV replication inhibitors improves survival and avoids and/or delays the progression of the liver disease towards liver cirrhosis and hepatocellular carcinoma (HCC). Medicines' intake is long-lasting and related to possible side effects and, sometimes, to difficult adherence to the therapy on the part of the patient.

The averting of the acute flares, the sustained viral suppression, the achievement of a non-detectable replication (HBV-DNA) and HBsAg negativity is an important target of the treatment. This determines the search for new markers reflecting HBV vital cycle with a view of the monitoring of the effect from the antiviral treatment such as the synthesis of virus proteins like HBsAg and HBcore-related antigens. The instrumental methods for the assessment of the stage of chronic hepatitis B infection are important, too. These topical and significant issues are a challenge in the clinical gastroenterology and they are profoundly investigated in the present dissertation work.

4. Structure and contents of the dissertation work

The dissertation work is elaborated in 106 pages and illustrated with 47 figures and 15 tables. The reference list includes 176 sources of which two in Cyrillic and 174 in Latin. The work is correctly structured and contains the following parts: literature survey, purpose, tasks, material and methods, results and discussion, conclusions, contributions, and references.

The literature survey is comprehensive and complete. It is entirely directed to the theme. It covers all the aspects of the problem and logically deduces the grounds for the accomplishment of this clinical elaboration. An analysis of a series of investigation on the theme is performed. It demonstrates doctoral student's good scientific preparation and excellent awareness of the problem elaborated as well as her skills to analyze and generalize literature data.

The purpose of the dissertation work is clearly formulated - to make a contemporary evaluation of the effectiveness of the antiviral treatment and the activity of the chronic hepatitis virus B infection in patients with a long-lasting intake of nucleotide/nucleoside analogues. Logically, eight concrete and feasible tasks which are directly related to the achievement of the purpose set are deduced.

Material and methods. Into the observation, 84 consecutive patients with chronic HBV infection from the registry of the Clinic of Gastroenterology at St. Marina University Hospital of Varna during the period from May 2022 to November 2023 are included. The patients selected meet the correctly defined including and excluding criteria. A clinical examination of the patients is carried out. Standard laboratory examinations are realized such as virus markers and virus loading, serum markers of fibrosis, abdominal echography, contrast enhanced echography, transient elastography and 2D-shear-wave elastography (in 68 patients) for the determination of the degree of the fibrosis and steatosis, liver biopsy (in 53 subjects), as well as fibrogastroduodenoscopy for the assessment of the signs of the portal hypertension. Adequate statistical methods ensuring the reliability of the results obtained are made use of. The analyses are performed by the doctoral student which additionally enhances the value of the elaboration.

Results and discussion. The demographic distribution, familial status, body mass index, course of the viral infection and accompanying diseases are analyzed in detail. A statistically significant difference in terms of patients' age in favour of the age of the younger patients with HBeAg(+) chronic hepatitis B is established. An assessment of the viral loading is done and a higher one prior to the onset of the therapy in the group of HBeAg(+) patients is found out. A relation between the initial ALAT value and HBV-DNA level is missing. The comparative analysis of the initial viral loading and the stage of the liver disease demonstrates a higher viral loading in the patients during the initial stage of the disease, while during the stage of hepatic cirrhosis, the initial HBV-DNA level is the lowest. The availability, benefit and information value of the non-invasive serum markers for fibrosis such as APRI and FIB-4 is evaluated.

Antiviral treatment is performed with a different duration by means of the nucleotide/nucleoside (NA) analogues available as more than one third of the patients are on suppression through these medicines since over ten years. Such an observation of a long-lasting therapy over five years until the present moment is missing in Bulgaria.

The virological and biochemical response of the persons examined is analyzed in detail. The frequency of HBV-DNA negativity during the last visit in the Clinic of Gastroenterology for evaluation of the antiviral treatment is 87%. An increased frequency of the biochemical response following the treatment with NA during a mean period of eight years is established. It reaches up to 91% and HBV-DNA negativity in HBeAg(-) patients is 87%. The reasons for the biochemical response absence such as liver steatosis in metabolic factors, MAFLD/MASLD, and HCC infiltration are commented. The HBeAg(+) patients

normalize ALAT in the course of a long-lasting treatment with NA. An analysis of HBsAg dynamics through a quantitative measurement (qHBsAg) as well as HBsAg seroconversion is made. The lowest qHBsAg is registered after a ten-year intake of these medicines.

The analysis of the virological response through an original examination of the serum HBcrAg level in all the 84 patients represents an interest. The important conclusion is that HBcrAg is established in 97% of the patients despite the non-detectable HBV-DNA in the course of this treatment. Its presence reflects the amount of the hepatocytic cccDNA as well as the serum HBV-DNA which can predict relapse or HCC development. There is an interesting observation that the patients with HBsAg seroconversion present with detectable serum HBcrAg levels, too. This interesting fact is explained with HBV vital cycle. HBcrAg are indicative of the on-going antigen expression and HBV replication and can be a reliable indicator for the evaluation of the chronic hepatitis virus B infection and of the further treatment. A critical detailed analysis of the relation between HBV and metabolic disturbances is done. The dynamics of the liver stiffness as a marker of fibrosis on the background of the treatment mentioned is assessed, too. The treatment of many years leads to hepatic fibrosis reduction as evaluated by means of the liver stiffness values. The upper endoscopy (fibrogastroduodenoscopy) registers a reduction of the signs of portal hypertension in 60% of the patients.

The presentation of the discussion at the end of every chapter is a merit of the dissertation work. This manner of the exposé makes the elaborated complex matter accessible and understandable. This as well as the juxtaposition with data from a series of investigations indicates the good familiarity with the problem examined, the excellent and competent clinical thinking on the theme of the doctoral student.

The results from the observation are well-illustrated with figures and tables.

The 13 conclusions deduced are correct and in concordance with the results obtained. They logically correspond to the purpose and tasks set.

The contributions are of scientific-applicable, practical, and original nature. For the first time in Bulgaria, an analysis of HBcore-related HBV antigens is performed.

The contents and quality of the author's dissertation summary completely conform on the work elaborated.

5. Evaluation of doctoral student's publications and personal contribution

Two participations in scientific forums and one publication related to the dissertation work of which Stoyanova, MD, is the first author, are presented.

6. Critical remarks and recommendations

I have no critical remarks to the precisely performed scientific investigation and the materials submitted. I can make the following recommendations:

It is logical to indicate the number of the patients examined already in the chapter of 'Patients and methods', too.

In the reference list, other Bulgarian authors presenting with publications in relation to the chronic hepatitis B could be cited, too.

It is pertinently to add in the first contribution that such an observation of a long-lasting therapy until this moment is realized for the first time in Bulgaria.

Having in mind the detailed and profoundly accomplished clinical investigation, it is desirable that Dr. Stoyanova enhances her publication activity and participation in scientific forums.

I recommend to Dr. Stoyanova to continue her investigations within a future research work with a view of the practical application of the new non-invasive serum markers for the evaluation of the chronic hepatitis virus B infection.

Conclusion

The elaboration submitted results from the joint work of the specialists in the Clinic of Gastroenterology and generalizes team's experience of many years in terms of the diagnosis, treatment, monitoring, and evolution of the chronic hepatitis virus B infection.

The dissertation work of Yoana Stoyanova, MD, is a well-planned and structured scientific investigation on a topical and significant health problem. It reflects profound knowledge and capacities for research and analytical presentation of the results and adequate argumentation of the corresponding conclusions. A profound and value work with adequate statistical processing is presented which is doctoral student's deed. The dissertation work meets the requirements of the Law for development of the academic staff in Republic of Bulgaria (LDASRB), the Statute-book for LDASRB application, and the Statute-book for development of the academic staff of the Medical University 'Prof. Dr. Paraskev Stoyanov' of Varna for the acquisition of the educational and scientific degree of 'doctor of philosophy'.

I convincingly give my **positive** evaluation and recommend to the honoured members of the Scientific jury to vote positively for awarding Yoana Svetlozarova Stoyanova, MD, the educational and scientific degree of 'doctor of philosophy'.

April 23, 2024 .....

city of Varna (Assoc. Prof. Diana Gancheva-Tomova, MD, PhD)