

REVIEW

by Prof. Iskren Kotzev, MD, DSc, professor of internal medicine and gastroenterology

of the dissertation work on the topic "Antiviral therapy in chronic hepatitis B - dynamics of viral markers and long-term results" of Dr. Joana Svetlozarova Stoyanova, assistant and physician at the Gastroenterology Clinic of UMBAL "St. Marina" at the Medical University, Varna, doctoral student in an independent form of study at the 2nd Department of Internal Medicine, Department of Gastroenterology, Hepatology and Nutrition, Faculty of Medicine at the Medical University, Varna, for the award of the scientific and educational degree "Doctor" in the field of higher education 7. health care and sports, professional direction 7.1. "Medicine", doctoral program "Gastroenterology"

By order No. P-109-83, city of Varna, dated 21.03.2024 of Prof. Dr. Dimitar Raykov, MD. - Rector of the Medical University, city of Varna, I was confirmed as a member of the Scientific Jury in the procedure for public defense of the dissertation work of Dr. Ioana Svetlozarova Stoyanova on the topic "Antiviral therapy in chronic hepatitis B - dynamics of viral markers and long-term results". At the first meeting of the Scientific Jury on March 25, 2024, I was assigned to prepare a review. I was provided with all the materials, which I examined in detail, and I confirm that the procedure for disclosure, development and admission to defense was carried out in accordance with the requirements of the Law on the Development of the Academic Staff in the Republic of Bulgaria and the Rules for its Application from 2010, as well as in accordance with the requirements of the Regulations for the Development of the Academic Staff at the Medical University, Varna. I was guided by the same documents when preparing this review.

Since the introduction of mass vaccination against HBV infection at the end of the last century, the incidence of chronic hepatitis B has been steadily decreasing. In our country, we have experienced a decline in 50 years from 7% HBsAg positive among the general population to below 1% among the young generation of the 21st century. However, radical treatment in the sense of HBsAg seroconversion remains an unresolved issue. The incidence of chronic HBV infection in the elderly population remains higher. Intensive studies at the end of the last century and the beginning of the present century led to the creation of nucleoside analogs, providing in an acceptable time complete suppression of viral replication, but without affecting the viral reservoir of HBV - cccDNA of the infected hepatocytes. The hepatitis C RNA virus was successfully defeated, but the DNA virus of HBV is significantly more resistant and continues to be the subject of intense research today.

I consider the present work as a successful continuation of this antiviral fight, which the Clinic of Gastroenterology led in the years of modern hepatology. Colleagues from the clinic participated in this multi-year struggle, but at its core was the scientific supervisor of the doctoral student - Associate Professor Irina Ivanova, MD. This gives the necessary scientific depth, modern continuation and development

of the great problem of chronic hepatitis B virus infection.

Modern maintenance nucleoside therapy interrupts the progressive liver fibrosis, even leads to a certain regression of it, but the carcinogenic virus remains, minimal inflammation persists, the unwanted manifestations of long-term therapy accumulate. In recent years, new methods for evaluating therapy and fibrosis, new predictors of successful functional healing, new treatment options have been sought.

Today, Dr. Ioana Stoyanova presents to us her excellently structured dissertation work "Antiviral therapy in chronic hepatitis B - dynamics of viral markers and long-term results", in which she develops an extremely actual topic, which is currently the subject of numerous studies, scientific disputes, with a dynamically changing research consensus or lack thereof. The dissertation student sets herself the lofty goal of making a modern assessment of today's antiviral treatment and the activity of chronic HBV infection in patients with long-term use of nucleotide/nucleoside analogues. She boldly ventures into new and unknown areas regarding the role and importance of quantitative measure of HBsAg, HBcrAg, RT-PCR HBV DNA, and the interrelationship between all biomarkers of infection.

In recent years, chronic HBV infection has again become the subject of new extensive and consistent studies. Dr. Ioana Stoyanova's scientific work is well motivated in both scientific and practical terms. Her in-depth research is unique for Bulgaria and carries a pioneering spirit.

The clinic where Dr. Stoyanova works has an indisputable merit (along with other Bulgarian university clinics) for the introduction into clinical practice in our country of modern treatment for patients with chronic hepatitis B. In this sense, she has a sufficient number of the necessary contingent for the study. To achieve her ambitious goal, Dr. Stoyanova set herself 8 main tasks, which, in my opinion, she fulfilled excellently. The most important tasks concern the virological and biochemical response during long-term treatment with nucleoside analogues, monitoring the dynamics and level of HBsAg and HBcrAg. The PhD student monitors liver disease with laboratory indicators of liver synthetic function, indirect fibrotic serum markers, abdominal ultrasound, elastography and endoscopy. Dr. Stoyanova evaluates the role of metabolic disorders in the dynamics of liver disease in patients with chronic hepatitis B who are treated with nucleoside analogues.

Dr. Stoyanova also evaluates the differences in the dynamics of viral markers in the classic division of those infected with HBV, namely HBeAg-positive and HBeAg-negative. With the application of nucleoside/nucleotide analogs (NAs), indisputable successes were achieved in the treatment of HBV-infection. NAs inhibit the DNA polymerase created by reverse transcription during HBV replication and stop the synthesis of HBV plus and minus chains, but do not destroy mRNA and covalently closed circular DNA (cccDNA) in the hepatocyte nucleus, which serve as a template for viral synthesis. Different ways to eliminate the virus have been tried - a combination of NAs, stopping therapy with the hope of restoring elimination immunity, T-cell therapy, therapeutic transgenic HBV vaccines, cell entry inhibitors (Bulevirtide, Hepcludex), affecting the HBV polymerase, anti-sense probe (Bepirovirsen), HBc-inhibitors, immunotherapy, etc. So far, Bulevirtide has been

approved for the treatment of HDV-infection, and Bepirovirsen has so far shown very good results – up to 10% loss of HBsAg and HBV DNA. It is assumed that a combination of the NAs circuit, Bepirovirsen, pegylated IFN, therapeutic vaccines, polymerase PAPD5 and PAPD7 enzyme inhibitors, will be able to eliminate the virus. New biomarkers and indicators are needed to assess the course and clearance of the virus, as well as predictors of good response. The efforts of the doctoral student fit into the general trend of global scientific and applied research in this field.

Dr. Stoyanova examined 84 consecutive patients with chronic HBV infection from the register of the Gastroenterology Clinic, 90% with HBeAg-negative status and 10% with HBeAg-positive status.

All examined patients were undergoing antiviral treatment in the Gastroenterology Clinic and met adequate and correctly observed inclusion and exclusion criteria. Dr. Stoyanova's dissertation is a large and representative study for Bulgaria in this field, confirmed by clinical, laboratory, virological and imaging verification, using all modern possibilities for diagnosis and treatment.

The dissertation student uses modern research methods - clinical, laboratory, virological (HBV DNA, HBeAg, anti Hbe, quantitative HbsAg, HbcrAg), serum fibrosis markers APRI and FIB-4, abdominal ultrasound, liver biopsy with METAVIR staging, shear-wave and vibrational transient elastography (fibroscan) and endoscopy. The doctoral student uses an adequate statistical apparatus, which allows her to draw valid conclusions. The dissertation work is richly illustrated with 47 figures and 15 tables.

The scientific work is presented on 106 pages, structured in 10 parts, the most important of which are: literature review, research methodology, results with discussion and conclusions. The ratio between the individual parts is correct, the language is clear and precise, the author presents the complex matter in an accessible and understandable way. The bibliography presents practically all contemporary publications related to the topic and consists of 176 author publications (2 in Cyrillic and 174 in Latin).

It is now understood that the goals of therapy in chronic HBV infection include not only the interruption of viral replication, but also the eradication of the viral matrix in the hepatocyte nucleus. 6 NAs were introduced into clinical practice, 3 of which are still actively used today. Although NAs maintenance therapy slows the progression of HBV-hepatitis, low-grade intrahepatic HBV activity and procarcinogenic risk persist. The sword of Damocles for reactivation and resistance is also present. Clearly, in addition to the level of HBV DNA, it is necessary to look for new indicators of the effectiveness of antiviral treatment. Therefore, the doctoral student correctly tracks the dynamics of qHBsAg, HBcrAg and HBV DNA. It is expected that HBcrAg may prove to be a suitable surrogate marker, along with other HBV biomarkers, to predict progression and treatment response in patients with chronic HBV hepatitis.

HBcore-related antigens may persist in patients with HBsAg seroconversion, and in fact unite HBeAg, HBcAg, and the 22-kDa precore protein p22cr, which are encoded by the precore/core region of the viral genome and therefore can be co-

quantified in the serums of HBV patients. These 3 proteins are translation products of mRNA transcribed from cccDNA and therefore can be taken as their indirect indicator.

Dr. Stoyanova in her study confirms that the administration of nucleotide/nucleoside analogs in chronic hepatitis B achieves highly effective inhibition of HBV replication and provides an initial virological response in 63% while maintaining viral suppression - HBV DNA <10 IU/ml in 90.4% and undetectable HBV DNA in 84.5% of those treated. The doctoral student recorded the difference in the virological response in HBeAg negative and HBeAg positive chronic hepatitis – 87% and 66%, respectively, which coincides with previous literature data. The achieved sustained biochemical response of 91.6% in treatment with nucleoside/nucleotide analogues is optimal. The doctoral student correctly notes the suboptimal response to Lamivudin, where viral suppression is not achieved in 19% and there is a high risk of drug resistance.

Dr. Stoyanova conclusively shows that long-term NAs therapy leads to improvement in indirect serum markers of fibrosis, with a significant improvement in APRI in most cases and a reduction in FIB-4 in 56% of those treated. She points out through her study that long-term treatment with nucleoside/nucleotide analogues reduced liver stiffness assessed by ultrasound elastography by an average of 4 kPa in 64% of patients. This is convincing evidence for the reversible course of fibrosis with long-term maintenance treatment with NAs.

Dr. Stoyanova found a significantly lower concentration of HbsAg in patients with liver cirrhosis, which can be explained by the 5-phase classification of chronic HBV-infection, namely, as fibrosis progresses, the move is towards the HbsAg-negative phase. But she recorded the fact that the level of HB core-related antigens did not decrease in cirrhosis.

The PhD student established the relationship between HBV DNA and the level of HbsAg. By regression analysis, it was estimated that a 1 IU/ml increase in HBV DNA resulted in a 30.5 IU/ml increase in qHBsAg.

Through her research, Dr. Stoyanova states that the modern follow-up of HBV-hepatitis patients treated with NAs requires dynamic follow-up of the HbsAg level. She confirms in her observation that long-term maintenance therapy with nucleoside/nucleotide analogues (over 10 years) leads to a progressive decrease in the level of HbsAg and favorable dynamics in 90% of those treated with the lowest concentration of HBsAg of an average of 898 IU/ml.

Dr. Stoyanova established in the observed group of patients a functional cure with complete seroconversion of HbsAg and appearance of anti-HBs-antibodies in 4.8%. The low rate of seroconversion confirmed by the doctoral student is the basis of the ongoing research on new therapeutic approaches.

The role of abdominal ultrasound in the follow-up of patients with HBV-cirrhosis has been confirmed. The doctoral student found the development of hepatocellular carcinoma in 3 cases (12.5%) among patients with cirrhosis.

The data on the regression of portal hypertension found in 3 patients, with reverse development of esophageal varices and portal hypertensive gastropathy, are

interesting.

Of considerable interest are the pioneering studies of HB core-related proteins, which are detected in the blood of patients with long-term antiviral therapy and successful suppression of viral replication, representing an indirect marker for persistent cccDNA.

The doctoral student found that 67% of the examined patients had an HBcrAg level between 3 and 4 log10. Also of interest is the proven detectable level of HBcrAg in patients with HbsAg clearance. The doctoral student found that in cases with progression of liver disease, assessed with indirect fibrotic markers and with high liver density, other pathogenetic factors, such as alcohol consumption and metabolic disorders, should be sought. This is a very common picture in practice, and the conclusion of the doctoral student speaks of her clinical maturity.

Dr. Stoyanova's recommendation is reasonable that the follow-up of patients with chronic hepatitis B during long-term therapy with NAs should necessarily include a complex assessment of new viral markers, non-invasive fibrotic indicators and data from abdominal ultrasound and gastrointestinal endoscopy.

Dr. Stoyanova confirms the high efficiency of modern treatment of HBV infection and brings new emphasis to the follow-up of these patients. It clearly demonstrates the need for dynamic monitoring of qHBsAg, HbcrAg, HBV RNA, non-invasive fibrotic markers and elastographic providers. The data reported by the author coincide with those of leading European hepatology centers.

With her scientific work, Dr. Ioana Stoyanova made original contributions:

- A comprehensive evaluation of the results of treatment with nucleoside/nucleotide analogues was carried out for a mean period of 8.2 years
- For the first time in Bulgaria, an original HBcrAg analysis was performed
- The quantitative level of HBsAg was evaluated in relation to disease stages and viral load
- Initial clinical experience of the application of the new viral markers of HBV activity in patients with medically inhibited viral replication has been gathered.
- The analysis and conclusions of the long-term results of the current treatment of hepatitis B is a prerequisite for setting new goals to optimize the therapeutic behavior in these patients.

Her contributions are of a scientific applied and confirmatory nature and are directly applicable in clinical practice. With her dissertation, Dr. Stoyanova confirms the high informative value of non-invasive methods for monitoring patients. Dr. Stoyanova has 3 publications and participation in scientific forums related to her dissertation work. She is the first author.

The following recommendations can be made:

- Given her extensive research with original results, it is desirable that Dr. Stoyanova has a higher publication activity
- To increase his scientific activity in national and international scientific forums with scientific announcements, reports or posters

The notes made do not reduce the value of the dissertation work. We allow ourselves to do them for the purpose of future scientific and clinical development of

the doctoral student.

The achieved results and conclusions are a valuable contribution to Bulgarian hepatology. Dr. Yoana Stoyanova is a young doctor with ambitions and opportunities for development. During the time she worked at the Gastroenterology Clinic, she established herself as a knowledgeable and capable gastroenterologist with a valuable contribution to the general activity of the Clinic.

She works in a university gastroenterology clinic headed by Associate Professor Irina Ivanova, a leading expert in Bulgaria in the field of hepatology. In the Gastroenterology Clinic, Dr. Stoyanova is fortunate to count on the expert presence and assistance of Associate Professor Antonia Atanasova, MD, with whose personal and active participation the hepatological infrastructure of the Clinic was developed, as well as the active contribution to the development of hepatology by Dr. Pavlina Boykova, MD, and Associate Professor Diana Gancheva, MD, as well as in cooperation with all other colleagues from the clinic and the hospital.

Dr. Ioana Stoyanova graduated with honors from her higher medical education in 2018 and has 6 years of medical experience. She worked as a specialist doctor at the Gastroenterology Clinic, and since 2019 she is an assistant doctor at the Medical Universities, Varna. Since 01.01.2023, she has a recognized specialty in gastroenterology and works as a gastroenterology specialist in the Gastroenterology Clinic.

Dr. Ioana Stoyanova's study is a thorough and scientifically sound study with a modern complex assessment of the effectiveness of antiviral treatment and the activity of chronic HBV infection in patients with long-term use of nucleotide/nucleoside analogues. The study has its remarkable scientific and practical contributions. I believe that this work has high scientific value and useful practical applicability.

Evaluating the dissertation work of Dr. Stoyanova in its entirety with its complex studies, achieved results, original contributions, scientific and practical significance, I believe that the presented dissertation work of Dr. Ioana Stoyanova fully meets the requirements for awarding the scientific and educational degree "Doctor " and I give my positive vote.

I take the liberty of recommending the same to the esteemed members of the Scientific Jury.

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city of Varna

**Prof. Dr. Iskren Kotzev,
MD, DSc, VMA, MBAL-Varna
Clinic of Gastroenterology**