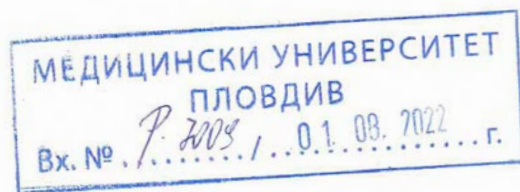


REVIEW

from

Prof. Dr. Raina Teodosieva Robeva

head of the nephrology clinic at the Military Medical
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according to the procedure for obtaining the educational and scientific degree "Doctor" in the scientific specialty "Nephrology" by Dr. Irina Yavorova Zdravkova, Second Department of Internal Medicine, Section "Nephrology" of MU-Plovdiv, according to the order of the Rector of MU-Plovdiv №1612/04.07.2022 and Protocol of FS №.6/15.06.2022 with a dissertation on the topic: "Specific serum and deposited autoantibodies and immunoglobulins in membranous nephropathy and their importance for the therapeutic approach" with scientific supervisors Assoc. Prof. Dr. Eduard Tilkiyan, m.d. and Prof. Dr. Ilian Doikov, m.d.

The submitted materials for the procedure meet the requirements of the Law on the Development of the Academic Staff of the Republic of Bulgaria and the Regulations for the Development of the Academic Staff of the MU - Plovdiv for awarding the scientific degree "Doctor".

Dr. Irina Yavorova Zdravkova graduated from secondary education in 2000 at the "Exarch Antim I" Science and Mathematics High School with intensive study of the English language, Vidin, with excellent results. Since 2001 until 2004 she studied medicine in Romania, the city of Cluj-Napoca at the MU Iuliu Hatcieganu. For family reasons, in 2004 she transferred to the MU Plovdiv, where she graduated in 2008 (registration number of diploma 13105/20.10.2008). In 2008-2009, she worked consecutively in CEMA-Plovdiv and as

an assistant personal physician in the Outpatient Clinic for Individual Practice for Primary Medical Assistance. From 10.2009 until now, she has been working at the Nephrology Clinic of UMBAL Kaspela. In 2018, she acquired the specialty of Nephrology (diploma registration № 021597/26.02.201).

Since 2015 is an assistant at the Department of Propedeutics of Internal Diseases, currently holding a teaching position. She has been teaching English-speaking students for four years. She is actively involved in teaching students during their summer internships.

For two years, he has also been leading an outpatient practice in II DCC Plovdiv.

Dr. Zdravkova has published in Bulgarian publications and presented reports and posters at Bulgarian scientific forums. She speaks English and Russian, has good communication and presentation skills, as well as the ability to work effectively in a team. Works freely with various software products.

The dissertation work of Dr. Irina Zdravkova examines a current problem of great scientific and practical importance in the field of nephrology - the importance of some specific serum and deposited autoantibodies and immunoglobulins in membranous nephropathy and their importance for the therapeutic approach. Membranous nephropathy is a glomerulonephritis with immunocomplex pathogenesis, in which deposits of IgG and complement fractions are deposited subepithelially on the surface of the glomerular basement membrane and gradually lead to its diffuse thickening. Primary membranous nephropathy accounts for about 75-80% of cases. Most patients with primary membranous nephropathy have circulating antibodies to phospholipase A2 receptor /PLA2R/, and the remaining cases of primary membranous nephropathy can be classified as idiopathic. Secondary membranous nephropathy accounts for 20-25% of cases, as the cause of its occurrence are other autoimmune

diseases, malignant tumors and drugs. It occurs with periods of flares and remissions. About 30% of cases do not respond to treatment and reach the end stage of kidney disease. In 2014, a new antigen was discovered - thrombospondin type-1 domain containing 7A (THSD7A) with localization on podocytes. The discovery of many new antigens and antibodies to them followed: Exostosin 1 (EXT1), Exostosin 2 (EXT2), NCAM1, NELL-1, Semaphorin 3B, etc. Evidence is accumulating for the role of complement and some immunoglobulins. Anti-PLA2R1 IgG4 autoantibodies were found to be capable of activating the lectin pathway and causing glycosylation-dependent damage to PLA2R1-expressing podocytes. Abnormalities in IgG4 glycosylation were found to correlate with disease severity. Chronic inflammatory processes in the body, against the background of diabetes mellitus, aging, chronic exposure to harmful factors from the environment, chronic autoimmune disease, lead to continuous formation of IgG4, in which changes in glycosylation occur, then damage occurs in the area to which this antibody is targeted. When the immune-mediated response fails to clear the target, this results in a broader immune response that extends to epitopes of glomerular antigens, such as PLA2R.

The dissertation is written on 163 pages. It is illustrated with 31 figures, 20 tables and 10 microscopic photographs, using 180 literary sources - 5 in cyrillic and 175 in latin.

Dr. Zdravkova has made a thorough review with presented basic and very up-to-date data from the medical literature, which shows a good knowledge of the problem, the ability to systematize the information and bring out the most important problems.

The aim of the dissertation is formulated briefly and clearly - to study the frequency of three types of deposits - PLA2R, IgG4, MBL on the surface of the basement membrane and the serum level of APLA2R antibodies in patients with

membranous nephropathy and their importance for refining the diagnosis, clinical course and the therapeutic effect. The doctoral student sets herself eight well-chosen tasks for her performance. 79 patients with membranous nephropathy, aged between 24 and 86 years, 47 men and 32 women, treated at the Nephrology Clinic of UMBAL "Kaspella" for the period April 2010 - December 2020 were examined. In all patients, the diagnosis was confirmed by puncture kidney biopsy, laboratory tests, including immunological, histopathological and immunohistochemical studies. Adequate statistical methods were applied.

Dr. Zdravkova found that the average age of patients with membranous nephropathy corresponds to the data described by other authors. In her study, PLA2R/IgG4/MBL triple positive, PLA2R/IgG4 double positive, and PLA2R positive only occurred in patients with primary membranous nephropathy. Double positives for IgG4/MBL are mainly found in idiopathic cases, which supports activation of the lectin pathway. The author concludes that the lack of double positivity for PLA2R/MBL indicates that IgG4 is required for activation of the lectin pathway of complement. The presence of MBL only in combination with IgG4 /without or with PLA2R/ supports the idea that IgG4 is required for the activation of the lectin pathway and is consistent with what was demonstrated by Haddad et al., as well as by Paolo Cravedi for the role of abnormally glycosylated IgG4 in the pathogenesis of the disease. What is new is that activation of the lectin pathway is present not only in antigen-associated membranous nephropathy, but also in idiopathic membranous nephropathy.

Dr. Zdravkova's conclusion that the presence of IgG4 alone cannot be used as a reliable marker to rule out secondary membranous nephropathy has important practical significance. In patients with secondary membranous nephropathy, MBL deposits are absent, complement activation is most likely not through the lectin pathway.

The doctoral student established 87.60% agreement between the diagnosis of positive and negative cases of PLA2R by immunohistochemistry and in serum with very high sensitivity and specificity, as well as the high diagnostic value of APLA2R in serum as a marker for the presence of primary membranous nephropathy (sensitivity 90% and specificity 100 %). When biopsying patients with type 2 diabetes mellitus, membranous nephropathy is often found, which in 50% of cases is idiopathic.

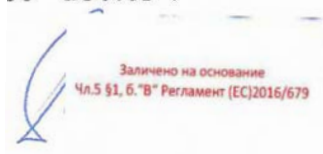
A very impressive result is the established relationship between disease stage and MBL deposition (decreasing with each subsequent stage), which is reported for the first time. Based on the accumulated experience, Dr. Zdravkova came to the conclusion that the level of APLA2R in serum shows a significant association with disease activity and can be used as a marker of primary membranous nephropathy activity, as well as to evaluate the results of therapy. In the dissertation, there are also valuable data on the treatment of studied glomerulonephritis. The highest percentage of complete remission has patients on therapy with the combination of corticosteroids, cyclophosphamide and azathioprine. As an alternative, the replacement of azathioprine with mycophenolate mofetil is indicated, and in the absence of effect, switching to corticosteroids and tacrolimus. APLA2R and MBL positives had higher rates of achieving complete and clinical remission than negatives, and IgG4 positivity showed no effect on rates of remission achieved in positives and negatives.

There are 12 scientific contributions - 7 original and 5 confirmatory.

The dissertation student attaches a list of 5 publications related to the dissertation work. The attached list meets the requirements for the acquisition of the ESD "Doctor".

The abstract is well-formed and illustrated, it reflects the essence of the dissertation work.

In conclusion, the dissertation of Dr. Irina Yavorova Zdravkova is an up-to-date scientific work of great theoretical and practical importance. Based on the above, I believe that the work presented meets the requirements of the law on the development of scientific staff and Dr. Irina Yavorova Zdravkova can be awarded the educational and scientific degree "doctor".



20.07.2022г.

Prof. R. Robeva