



**MEDICAL UNIVERSITY - SOFIA
FACULTY OF MEDICINE**

DEPARTMENT OF INTERNAL DISEASES

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REVIEW

**by Prof. Dr. Boris Iliev Bogov, m.d.,
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Appointed as a member of the Scientific Jury by order No. R-1612/04. 07. 2022 of the Rector of the MU-Plovdiv, for the preparation of a "Review" on the dissertation work of **Dr. Irina Yavorova Zdravkova**, on the topic "**Specific serum and deposited autoantibodies and immunoglobulins in membranous nephropathy and their significance for the therapeutic approach**", for the awarding of the educational and scientific degree "Doctor", in the doctoral program "Nephrology", in the professional direction 7.1. Medicine from the field of higher education 7. Health care and sports

I. Presentation of the dissertation student.

Dr. Irina Yavorova Zdravkova is born on September 19, 1982 in Odessa, Ukraine, where she studied until the 5th grade. He completed her secondary education at the "Exarch Antim I" Science and Mathematics High School with intensive study of the English language, the city of Vidin in 2000, with excellent success. In 2001 studied medicine in Romania, the city of Cluj-Napoca at the Medical University Iuliu Hatcieganu, until the 3rd year - 2004. In 2004, she continued her studies at the Medical University of Plovdiv and graduated in medicine in 2008. From 11. 2008 to 01. 2009 she worked at CEMA-Plovdiv as a resident doctor. From 01. 2009 to 10. 2009 she worked as an assistant personal physician in the Outpatient Clinic for Individual Practice for Primary Medical Care, and from 10. 2009 until now she worked in the Nephrology Clinic of UMBAL "Kaspela". In 2018, she acquired the clinical specialty "Nephrology". Since 2015 after a competition, she was appointed to the position of assistant at the Department of Propedeutics of Internal Medicine.

As a doctoral student on independent preparation, she developed a dissertation on the topic: "**Specific serum and deposited autoantibodies and immunoglobulins in membranous nephropathy and their importance for the therapeutic approach**", with scientific supervisor Assoc. Dr. Eduard

Tilkiyan and Prof. Dr. Ilian Doikov, for acquisition of the educational degree "Doctor", in the doctoral program "Nephrology".

Dr. Zdravkova has 15 publications in Bulgarian collections and refereed journals, 3 of which she is the first author and participation in 21 reports and posters at Bulgarian scientific forums. She is a member of the Bulgarian Nephrology Society and BLS. She is fluent in Russian, English and Romanian, has good communication and presentation skills, as well as the ability to work effectively in a team.

II. For the dissertation.

The presented dissertation has a volume of 163 standard pages and is illustrated with 20 tables, 31 figures and 10 photographs. The literary reference includes 180 literary sources, of which 5 are in cyrillic and 175 are in latin. Includes introduction - 1 page, literature review - 53 pages, with general and special part, purpose and tasks of the dissertation work - 2 pages, research material and methods - 8 pages, results - 41 pages, discussion - 14 pages. Conclusions and contributions follow - a total of 3 pages. The abstract is built according to the requirements, presenting all the results and conclusions of the conducted research.

1. Relevance of the topic and appropriateness of the set goals and tasks.

The topic of the dissertation work "**Specific serum and deposited autoantibodies and immunoglobulins in membranous nephropathy and their importance for the therapeutic approach**" is current and significant both in a scientific and purely practical aspect. The fact is that membranous glomerulonephritis (MGN) is the most common glomerular disease in adults, leading to the development of nephrotic syndrome, in about 25%. Despite multiple therapeutic agents and treatment regimens, about 30% of patients with MGN progress to end-stage renal disease. Also, the medications used in the treatment of MGN often give many side effects, including complications such as diabetes mellitus, sterility, neoplasms, etc.

The literature review on the subject is presented on 53 pages, and the bibliography contains 180 titles, of which 5 are by Bulgarian authors. Contains two main parts - general and special - which follow logically one after the other, presenting the epidemiology and pathogenesis of membranous glomerulonephritis, the evolution in unraveling the pathogenetic mechanisms, the importance of megalin, antimegalin and Heymann antigen in experimental models and in humans, the main mechanisms about the formation of immune complexes, as well as the role of M-type A2 phospholipase receptors (PLA2R) in the pathological process of MbGN. Dr. Zdravkova cites that antibodies against PLA2R1 are detected in approximately 70-80% of cases with membranous nephropathy, which are primary and more often in men, and are a reliable prognostic factor that may change therapeutic behavior in the long term as well to improve treatment outcome. The dissertation presents in historical terms the scientific achievements regarding the discovery of various antibodies related to the pathogenesis and development of MbGN, with the emphasis being the discovery of new

antigens, by laser microdissection of glomeruli from PLA2R - negative biopsies, followed by mass spectrometric identification of trypsin-digested proteins. This approach has proved extremely successful, contributing to the discovery of four new antigens: exostosins 1 and 2 (EXT1/2), neural epidermal growth factor-like 1 protein (NELL-1), semaphorin 3B (Sema3B) and neural cell adhesion molecule 1 (NCAM1).

Dr. Zdravkova examines and presents in great detail the role of complement in the pathogenesis of podocyte damage, the different pathways of activation and the possibilities for cooperation between the alternative, classical and lectin pathways of complement activation, as well as their role in the pathogenesis of immune deposits in MbGN. Represents a putative model of complement-mediated podocyte injury induced by anti-PLA2R antibodies. She has examined in detail the various pathogenetic mechanisms and clinical course of patients with diabetes mellitus and membranous nephropathy. The following is a detailed presentation of the four histomorphological stages of MbGN, depending on the deposition of immune complexes and the clinical course according to the histological stage, as well as histomorphological differences between primary and secondary membranous glomerulonephritis.

At the end of the literature review, Dr. Zdravkova makes a detailed and generalizing analysis, drawing her own conclusions that progress in the precise identification of target antigens and the role of complement will most likely provide new insights into the pathogenesis of this disease, which in turn will lead to new approaches in the therapy and diagnosis of the disease.

The *set goal is clearly formulated, and the 8 tasks to be performed* are specific and clear to solve the set goal. Based on the literature review and extensive literature reference, the dissertation aimed to investigate the frequency of three of the many and different 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 in refining diagnosis, clinical course and therapeutic impact.

In Bulgaria, so far, there are not many such in-depth studies on this problem. **The methodological part** is presented on 8 pages. Applied *research methods* include: history and physical status, clinical and laboratory studies, abdominal ultrasound. In all patients, a percutaneous puncture kidney biopsy (PBB) was performed, under ultrasound control, with an automatic GUN "Galini" or "Möller" system. Each kidney tissue material was stained with hematoxylin-eosin and immunofluorescence examination was performed on cryostat sections with a standard package of fluorochromic anti-human rabbit antisera against IgG, IgA, IgM and three complement fractions. If necessary, tests were also done to rule out monoclonal gammopathy. An immunohistochemical study followed to demonstrate a recombinant Anti-PLA2R antibody; Anti IgG4 antibody and Anti-Mannan Binding Lectin/MBL antibody. That is, in all patients, membranous glomerulonephritis was histologically and immunohistochemically proven.

To assess renal function, serum creatinine is examined according to the Jaffe method, which is based on a non-specific color reaction, and glomerular filtration rate (eGFR) is calculated according to the formula of Modification of Diet in Renal Disease Study Equation for Estimating Glomerular Filtration Rate with Standardized Serum Creatinine Values (MDRD) in mL/min/1.73 m².

The "Material" part includes a total of 79 patients, all histomorphologically diagnosed, treated and followed up at the Nephrology Clinic of the Kaspela UMBAL, for a period of 10 years. It is important to note that Dr. Zdravkova specified inclusion and exclusion criteria, with which the patients participating in the study met precise and clear criteria.

The statistical analysis is determined according to the goals and tasks of the dissertation and the type of quantities (metric, rank, nominal, dichotomous). Most of the data were measured on a dichotomous (Present/Yes-Absent/No), nominal or ordinal scale. These quantities are presented in numbers and percentages, and to establish statistically significant trends, Fisher's exact test was used for dichotomous quantities; the Chi-square test in the presence of more than two categories, and the results are illustrated with pie charts, bar charts and line charts. Metric (continuous) values were checked for normal distribution using the Shapiro–Wilk test. Accordingly, values with a normal distribution (Shapiro-Wilk $p > 0.05$) are presented with the arithmetic mean and standard deviation (\pm SD). To establish statistically significant trends, statistical methods were used depending on the number of groups: t-test for two independent samples (independent-samples t-test); one-way ANOVA – for more than two groups. To establish statistically significant trends, the non-parametric Kruskal-Wallis test was used to compare more than two groups/categories; the Spearman rank-order correlation for analyzing the relationship between two quantities; receiver operating characteristic curve (ROC curve) analysis to investigate the diagnostic ability of APLA2R in serum as a marker for the presence of pMN. The results are illustrated by scatter plots with fitted regression line and area under the curve (AUC) plots. The statistical programs IBM SPSS, version 27 (2020), Minitab version 19 (2020) and MedCalc, version 20.008 (2021) /169-171/ were used for data analysis.

The results of the conducted studies, presented after the completion of each task, are presented on 41 pages, 20 tables, 31 figures and 10 photographs. Analyzing the data gives visibility and better receptivity. The first task, in relation to distribution by gender, Dr. Zdravkova shows a higher relative share of men, who make up 59.50% of the entire group compared to 40.50% women. An important conclusion from the performance of task 3 is that APLA2R positivity in serum showed a high degree of precision of 95% in the diagnosis of primary MH, with a sensitivity of 90% and a specificity of 100%, and that the frequency of deposits PLA2R, IgG4, MBL did not show a significant difference between patients with and without DM. An important prognostic conclusion made by the doctoral student is that the highest relative proportion with positive results for MBL is found in patients in the I and II stages and gradually decreases in the following stages, but without significance of the difference. The relative proportion of MBL positivity was 10% higher in patients with diabetes

mellitus compared to those without diabetes mellitus, although the difference did not reach statistical significance. One of the important findings is related to the level of APLA2R, glomerular filtration, disease activity and relationship with proteinuria and serum albumin, namely: an increase in APLA2R in serum is associated with an increase in proteinuria and a decrease in serum albumin.

The result of the last task, concerning the outcome of the treatment, Dr. Zdravkova accepts as "complete remission" - the absence of nephrotic syndrome /absence of edema, normal values of total protein, albumin, cholesterol and triglycerides/ and negative values of APLA2R. For "clinical remission" - accept reduction/absence of edema, improvement but not reaching normal values of total protein, albumin, cholesterol and triglycerides, as well as reduction but not normalization of APLA2R values. Results indicated that 82% of patients achieved complete remission, including 61% with complete remission, 16% with clinical remission, and 45% with spontaneous remission. According to the type of MH, the relative proportion of patients in remission was significantly higher in patients with pMN and iMN compared to those with sMN. In the sMN group, a significantly higher relative proportion of patients with no effect of the therapy was found.

The discussion is presented on 14 pages, where Dr. Zdravkova analyzes the obtained results in detail. From the analysis of the own data and the comparison with the literature, it is clear that the obtained results are original, with a scientific and practical focus. Dr. Zdravkova analyzes every single result for every single task. She points to the diagnostic ability of APLA2R in serum as a marker for the presence of pMN. APLA2R positivity in serum showed a high accuracy rate of 95% in the diagnosis of primary MN, with a sensitivity of 90% and a specificity of 100%. Comparing the results of a study by Sanjeet Roy and colleagues in 2017 [127], on 153 patients with MH, they found that the study of PLA2R immunohistochemically had a sensitivity of 70.2% and a specificity of 96.6% in the differentiation of primary from secondary MN.

The presence of +/- APLA2R in serum is a reliable and precise marker for the diagnosis of MN, but it is the clinician who determines the type of MN/ pMN, iMN or sMN.

The conclusions summarized by Dr. Zdravkova are twelve, but the most significant and important of them are that the level of APLA2R in serum showed a significant association with the activity of the disease. APLA2R can be used as a marker of disease activity, early diagnosis of relapse and one of the markers of complete disease remission. It is a cornerstone in the diagnosis, differentiation of pMN and in the therapy of the disease, without which the treatment of these patients would be tantamount to blind treatment.

In her dissertation, Dr. Zdravkova divides the **contributions** into contributions with an original nature and contributions with a confirmatory nature. One of the most important contributions of an original nature is that for the first time in Bulgaria a study of a large group of patients with MN, examined for APLA2R in serum, which has high sensitivity and high specificity for the diagnosis of

MN, is presented. For the first time in Bulgaria, an immunohistochemical test for the presence of PLA2R antigen in kidney tissue is performed, a comparison is made with the presence of antibodies in serum and their role in establishing the diagnosis of MN and differentiating the type of MN. For the first time in Bulgaria, the deposition of IgG4, MBL was investigated by IHC in kidney biopsy tissue and it was found that it has a role in the pathogenesis of the disease not only in antigen-associated MN, but also in iMN.

Of the contributions of a confirmatory nature, the importance of APLA2R in serum for diagnosis, treatment and monitoring of disease activity is confirmed, and it is confirmed that there is activation of the lectin pathway and its activation requires IgG4.

Dr. Zdravkova has 7 actual publications on the topic of her dissertation, all in Bulgarian journals. In 3 of the publications, Dr. Zdravkova is the first author.

In conclusion, I can state that the presented dissertation work fully meets the qualitative and quantitative criteria of the requirements of the ZRASRB and Annexes 3 and 4 of the PURPNSZAD of MU-Plovdiv for awarding the scientific and educational degree "Doctor" in the field of higher education 7 "Health and Sports", in professional field 7.1. "Medicine" and scientific specialty "Nephrology" and I recommend to the respected Scientific Jury to award Dr. Irina Zdravkova the scientific and educational degree "Doctor".

18. 07. 2022

Prepared by:

/ Prof. B. Bogov M.D./

Заличено на основание
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