STATEMENT OF OPINION

By Assoc. Prof. Sylvia Nikolaeva Genova, PhD, MD
of a dissertation for the acquisition of an educational and scientific degree
Doctor of Philosophy (PhD)
confirmed by order №3-1091/08.11.2024

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Doctoral student: Dr. Iskui Mnatsakan Erkanyan
Form of doctoral study: independent preparation
Department: Second Department of Internal Medicine; Nephrology Section
Clinic of Nephrology, UMBAL "Kaspela" - Plovdiv; MU Plovdiv;
Topic: IgA NEPHROPATHY - CLINICAL, IMMUNOLOGICAL AND PATHOMORPHOLOGICAL CRITERIA FOR DIAGNOSIS AND THERAPEUTIC APPROACH
Professional direction: 7. 1 "MEDICINE"
Doctoral program: "Nephrology"
Scientific supervisors: Assoc. Dr. Eduard Tilkiyan, MD
Prof. Dr. Iliyan Doikov, MD
The presented set of materials on paper and electronic media is in accordance with Art. 115 (1) of the Procedure for the acquisition of the doctorate in the MU - Plovdiv; Regulations of MU-Plovdiv from 06.01.2023 and includes all necessary documents.

The dissertation contains 113 pages and is illustrated with 10 tables, 35 figures, 8 microscopic photographs. The doctoral student has attached 3 publications referenced in an international database on the topic of the dissertation and two participations in scientific congresses and conferences.

Dr. Iskui Mnatsakan Erkanyan graduated as a Master of Medicine from MU-Plovdiv in 2010, acquired a specialty in nephrology in 2016. He has been working at the Nephrology Clinic of Kaspela Medical Center Plovdiv since 10.2010. Assistant in Nephrology at MU Plovdiv, Second Department of Internal Medicine from 01.2018. Good level of English.

2. Evaluation of the doctoral student's personal participation in the dissertation work:

The topic of the dissertation work is current and does not repeat other studies related to IgA NEPHROPATHY - CLINICAL, IMMUNOLOGICAL AND PATHOMORPHOLOGICAL CRITERIA FOR DIAGNOSIS AND THERAPEUTIC APPROACH.

IgA nephropathy is the most common form of primary glomerulopathy, which presents with different clinical and pathomorphological manifestations and leads to Chronic Kidney Disease (CKD) as well as to end-stage CKD. The incidence of the disease varies in different regions of the

world, and in Europe it is 2.53 per 10,000 people - from 1.14 in Spain to 5.98 in Lithuania. Light microscopy changes can be very variable, but the significance of the immunofluorescence finding is decisive. It affects more often young people, with great variability in the clinical course, as some patients may be asymptomatic and with only minor urinary abnormalities, which predisposes to their late diagnosis. In many cases, IgA nephropathy is a progressive disease associated with proteinuria, arterial hypertension, and renal damage, with about 30%–40% of patients progressing to renal failure over 20–30 years, regardless of treatment. Despite the new discoveries made in recent years, there are still unsolved questions regarding the pathogenesis of the disease and new therapeutic strategies are sought to optimize clinical outcomes.

In view of this, I believe that the topic of the dissertation work is extremely modern and relevant, with important scientific and practical significance, and the presented results will shed additional light on some of the still unsolved problems in IgA nephropathy.

The PhD student shows a thorough knowledge of the state of the problem and creatively uses the literature material by doing a literature review on 32 cells, which includes the epidemiology, pathogenesis, morphology, risk factors and genetic predisposition of IgA nephropathy (IgAN). IgAN is a mesangial proliferative glomerulonephritis and is characterized by diffuse mesangial deposits of IgA. It is also known as Berger's disease. IgA deposits are examined in detail - diagnosis by immunofluorescence examination of materials from a Pelvic Biopsy (BB). The pathogenetic mechanism is explained through the so-called multi-hit mechanism, including 4 main stages. For visual clarification of the pathogenesis of IgA nephropathy, the role of IL6 and T lymphocytes, 4 schemes and one photo material from immunofluorescence are attached. The clinical course and prognosis of the disease are described, and the broad differential diagnosis with other diseases having similar symptoms is discussed. IgA nephropathy has been linked as a manifestation of kidney damage in patients with psoriasis. The role of microRNAs in the pathogenesis of IgAN is discussed. The therapy for IgAN was reviewed, as well as the possible outcomes in transplanted patients. The treatment is systematized and illustrated in tabular form, and the new and experimental therapies for IgAN including selective inhibitors of factor B, monoclonal antibody against MBL-linked serine-protease 2, protease inhibitors, etc. are also presented, which shows that etc. Erkanyan thoroughly knows the problem in renal pathology, striving to apply novelties in the therapy of patients.

From the literature review, the doctoral student draws 5 conclusions, stating that there is a lack of sufficient clinical studies regarding the role of laboratory biomarkers as important predictors of the disease, increasing diagnostic reliability. There are no established schemes for an optimal therapeutic algorithm for individual histological variants, the level of glomerular filtration at diagnosis, interstitial changes, the rate of deterioration of renal function, etc.

Based on the overview and conclusions, the purpose of the research was formulated, as well as the tasks:

The goal is clearly and precisely formulated. The tasks are tailored to the objectives of the study.

Tasks 1 and 2 aim to study the frequency of patients with IgAN, and the frequency of secondary IgAN, which in this case could be combined in one task.

Task 3: to specify the possibilities for the use of biomarkers and genetic studies in the diagnosis and therapeutic approach of IgAN. The significance of serum and tissue IL-6 levels.

Tasks 5-8 aim to study the relationship of pathomorphological changes, the role of immunological studies and follow-up of therapy.

Of the methods:

The study included 110 patients, over the age of 18, treated at the Nephrology Clinic of Kaspela Medical Center for the period April 2010 - November 2023. The number of patients is sufficient for statistical data processing and for deriving statistically reliable results. In all patients, the diagnosis was confirmed by puncture kidney biopsy. Indications and contraindications for performing a Puncture Renal Biopsy (PBB) have been determined. The laboratory tests used are also analyzed in detail - hematological, biochemical and immunological. IL-6 was examined in the serum of 39 patients. Glomerular filtration was calculated according to the formula CKD-EPI / Chronic Kidney Disease Epidemiology Collaboration / for eGFR, developed in 2009. and recommended by KDIGO 2013.

Statistical methods

Statistical data processing was performed using the statistical data analysis package. The statistical programs IBM SPSS version 27 (2020), Minitab version 19 (2020) and MedCalc version 20.008 (2021) were used. The results are illustrated by scatter plots with fitted regression line and area under the curve (AUC) plots. Non-parametric tests, rank correlation, etc. were used. Values at the level of significance of the null hypothesis $p<0.05$ were accepted as statistically reliable.

It should be noted the extremely good and detailed statistical processing of the materials, which gives credibility to the results obtained. fully complying with the set purpose and enabling statistical processing. The results are summarized in tables and illustrated with appropriate graphs: boxplot type and bar graphs. The chosen research methodology allows achieving the set goal and obtaining an adequate answer to the tasks solved in the dissertation work.

Results:

Dr. Erkanyan presents the results of his own research very precisely, with clearly identified trends and main conclusions from it. The incidence of IgAN confirmed by PBB was 10% of all biopsies performed over the 10-year period, or a total of 110 patients. 100 patients were divided into 4 groups with respect to proteinuria. The group of older patients was also analyzed in detail, with 21 of them /20%/ being over 60 years of age. It is noteworthy that 54.3% of the patients had high creatinine at the time of diagnosis, and 3 of them started hemodialysis before conducting PBB. This once again confirms the thesis about the late diagnosis of IgA nephropathy, due to the variability and insufficient clinical manifestation, as well as the lack of clinical experience on the part of doctors in pre-hospital care.

The results of the presented study show that IgA nephropathy is the second most common diagnosis in the age group of 18-59 years, second only to Focal and Segmental Glomerulosclerosis (FSGS), but the latter group is quite heterogeneous and should not be considered as a separate nosological entity . The study shows that men are more affected 82 against 27 cases of women. In patients over 60 years of age, IgA nephropathy is only in 7th place among glomerular pathology, and the frequency decreases even more for cases of primary IgA glomerulonephritis. The results are described clearly and precisely, using graphical and tabular forms.

Dr. Erkanyan analyzes the biopsy results in detail and points out the reported differences compared to some of the generally accepted postulates, such as the significantly low frequency of IgG deposits and the C4 fraction of complement. Elevated levels of serum IgA were demonstrated in only two patients. The results with the most significant contribution to the study were those of IL-6, which had significantly higher serum levels among patients compared to healthy controls. Comparison with histological changes showed that patients with higher levels of IL-6 demonstrated more pronounced levels of interstitial infiltrates, mesangial hypercellularity, as well as higher levels of fibrosis compared to patients with lower levels. From the results, it was also concluded that the therapy for IgAN should be specified according to the leading immune cause, respectively primary or secondary. The photographic histological and immunofluorescent evidence is of very high quality.

The presented results reflect in detail the applied therapeutic approach for individual patients, depending on the severity of the pathology, the presence of a crescent, proteinuria and arterial hypertension, as well as the level of glomerular filtration, concomitant pathology, etc. For the first time in our country, Dr. Erkanyan applies modern treatment with Budezonid to patients with IgA nephropathy and analytically reports the obtained results. A follow-up of patients treated with Budesonide is presented in tabular form, which immediately gives clarity about the results achieved.

Discussion on each of the tasks:

In the Discussion chapter, the doctoral student critically analyzes the data from his research, comparing them with those of other authors from the available medical literature, using 176 sources in Latin and 2 sources in Bulgarian. The results reflect the set tasks and their implementation is correct and accurate, most of the discussion is presented in the results chapter. The presented Conclusions are 8 and optimally reflect the main results of the study.

Contributions:

As **contributions** to his dissertation work, Dr. I. Erkanyan notes that for the first time in our country an analytical assessment of the histological changes in a large group of patients with IgA nephropathy is made and established differences with the generally accepted data from the literature are highlighted. A significant contribution is also the application for the first time in our country of a state-of-the-art therapeutic regimen with Budezonid in these patients and the reported first results. For the first time in our country, the level of IL6 was investigated in patients with IgA

nephropathy, and correlations with histological and laboratory changes, which are essential for subsequent therapy, were established.

Important contributions of a scientific-applied nature are also the presented algorithm, regarding the possibilities for early diagnosis of patients with IgA nephropathy, the proposed different therapeutic approach for individual patients depending on the time of diagnosis of the disease, the features of the pathoanatomical findings and the accompanying pathology.

Abstract

The abstract is prepared on 67 pages according to the requirements and includes the main parts of the dissertation work, the necessary volume of tables, figures and photographs. The content of the abstract provides an excellent overview of the overall dissertation work and reflects the main results, conclusions and contributions.

IN CONCLUSION

In Conclusion, Dr. Iskui Erkanyan presents a dissertation on an extremely current and significant problem in the field of clinical nephrology. The scientific work is properly constructed, with a precisely selected methodology, completed tasks and credible scientific and applied results, which represent an original contribution and meet the requirements of the Law on the Development of the Academic Staff in the Republic of Bulgaria (ZRASRB), the Regulations for its Implementation (ZRASRB) and Rules of the MU - Plovdiv. The presented scientific work shows that Dr. I. Erkanyan possesses the necessary theoretical knowledge and professional skills in the scientific specialty "Nephrology", clearly demonstrating the qualities and abilities to independently conduct scientific research. The presented materials and dissertation results correspond to the specific requirements adopted in connection with the Regulations of the Ministry of Education - Plovdiv for the application of the ZRASRB.

Based on the above, I consider that the dissertation of Dr. Iskui Mnatsakan Erkanyan represents a fully completed scientific work and I give my positive assessment by voting 'Yes' awarding the ONS "Doctor" to Dr. Iskui Mnatsakan Erkanyan. I urge the other members of the esteemed scientific jury to also vote positively.

Заличено на основание
Чл.5 §1, б."В" Регламент (ЕС)2016/679

27.11.2024 Prepared the opinion: .

Plovdiv

Assoc. Prof. Sylvia Nikolaeva Genova, PhD, MD