

REVIEW

by Prof. Dr. Boris Iliev Bogov, MD

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of a dissertation for awarding the educational and scientific degree "**doctor**", professional field 7.1. Medicine from the field of higher education 7. Health care and sports, doctoral program in "**NEPHROLOGY**". Appointed as a member of the Scientific Jury by order No. R-1091/08. 11. 2024 of Deputy The Rector of the NID of the MU-Plovdiv and the first meeting of the Scientific Jury on 12.11.2024 for the preparation of the "Review".

Author: Dr. Iskui Mnatsakan Erkanyan

Form of doctoral studies: independent preparation

Department: Second Department of Internal Medicine of the Medical University - Plovdiv

Topic: "IgA nephropathy - clinical, immunological and pathomorphological criteria for diagnosis and therapeutic approach"

Research supervisor: Assoc. Eduard Tilkiyan and Prof. Ilian Doikov, MU-Plovdiv

1. General presentation of the procedure and the doctoral student

The presented set of materials on paper and electronic media is in accordance with Art. 70 (1) of Section I. Acquisition of the educational and scientific degree "DOCTOR" at MU-Plovdiv.

2. Brief biographical data for the doctoral student

Dr. Iskui Mnatsakan Erkanyan graduated from Medical University - Plovdiv, with a master's degree in Medicine, in 2010. Since 2010, she has been appointed as a resident physician at UMBAL "Kaspela", and since 2012, she has been enrolled as a specialist in "Nephrology". Since 2016, he has a recognized specialty in clinical nephrology. After winning a competition, she was appointed as a doctor-assistant in nephrology at the Second Department of Internal Medicine of the Medical University - Plovdiv. Since October 2010, he has been working at UMBAL "Kaspela" until now.

Dr. Iskui Mnatsakan Erkanyan has 3 scientific publications related to the topic of the dissertation work, one of which is in print and 2 participations in scientific congresses and conferences. Fluent in written and spoken English, has good communication and presentation skills, as well as the ability to work effectively in a team.

3. Actuality of the topic and appropriateness of the set goals and tasks

The presented thesis is 84 standard computer pages (1800 characters) and is illustrated with 12 tables and 35 figures. The literary reference includes 178 literary sources, of which only 2 are in Cyrillic and 176 are in Latin. Includes literature review – 30 pages, aim and objectives of the

dissertation – 2 pages, patients and research methods – 9 pages, results – 34 pages, discussion – 5 pages, conclusions and contributions – 2 pages. The abstract is built according to the requirements, and it presents all the results and conclusions of the conducted research. The dissertation is written in literary Bulgarian.

The topic of the dissertation "IgA nephropathy - clinical, immunological and pathomorphological criteria for diagnosis and therapeutic approach" is current and significant, not only because IgA nephropathy is the most common glomerulonephritis, which mainly affects young people, but also because it is often associated with poor prognosis regarding the survival of the kidney and the selection of optimal behavior in this pathology is still discussed in the world literature.

4. Knowing the problem

The literature review written on 30 pages. contains general data on prevalence, pathogenesis as well as the role of micro RNA in disease pathogenesis. The extensive analysis shows that the author knows in detail the current state of the problem of "IgA nephropathy". It presents in detail the modern knowledge about the genetics, the pathophysiological aspects of the disease and the most modern hypotheses, the histopathological variants and the various classifications proposed so far, as well as the most optimal therapeutic models adopted in the world clinical practice. Dr. Erkanyan presents in the literature review the so-called multi-hit mechanism for the course of the disease, including the 4 main stages, from HIT 1 to HIT 4. He hints at the direct connection between the unregulated intestinal-associated lymphoid tissue and the manifestation of the disease, as and the relationship between different food antigens and the occurrence of IgA nephropathy. The pathogenetic mechanism of the occurrence of the disease is illustrated and schematic, which makes the problem even more understandable. The immune mechanism is presented in detail by describing the abnormal binding between circulating IgA1 and the soluble form of the myeloid IgA Fc α receptor CD89, leading to the formation of IgA1-sCD89 complexes, which, being deposited in the mesangium, lead to glomerular damage (hit 4). Special attention is also paid to the aberrant glycolysis of IgA, which is at the center of the pathogenesis of IgAN, and to the IgA complexes deposited in the glomeruli, which are predominantly of the IgA1 class and galactose-deficient IgA1 (Gd-IgA1). The review continues with the importance of the two pathways of complement activation – the alternative and the lectin pathway, and the importance of each on the prognosis and severity of IgA nephropathy. The role of genetic predisposition in the importance of one or the other activation pathway is also hinted at. Not omitted in the review and presentation of known facts to date, regarding the morphological diagnosis, classification, prognosis and treatment of IgA glomerulonephritis according to the severity of the course.

The literature review ends reasonably with basic conclusions from the analyzed scientific literature.

5. Research methodology

The goal is clearly and accurately formulated and fully corresponds to the thesis stated in the title of the dissertation, namely, to study the clinical, immunological and pathomorphological criteria for diagnosis and differential diagnosis of patients with IgA nephropathy and their importance for the therapeutic approach.

To fulfill the set goal, Dr. Erkanyan sets an optimal number of tasks in which the set problems are clearly and reasonably formulated. The tasks are specific, feasible and correspond to the defined goal in the dissertation work.

The study included a total of 110 who were followed up over a period of 13 years, all of whom were diagnosed histomorphologically by renal puncture biopsy (RPB). Dr. Erkanyan indicates the indications for conducting PBB in all the followed patients, grouping them by symptoms. An interesting detail is that the histological results of the followed 103 patients were compared with the histological conclusions of 1002 patients for the same period, who were hospitalized in "Kaspela" UMBAL - Plovdiv.

Methodologically, scientific research is well planned. Confirmed inclusion and exclusion criteria for the contingent of investigated persons are presented, which are sufficient in number to allow reliable conclusions to be drawn. Dr. Erkanyan presents a detailed description of all the methods used in the study, which shows his good knowledge of them. Along with standard laboratory tests, the doctoral student used in the study other state-of-the-art immunological tests to determine the serum concentration of the biomarkers ANA, dsDNA, anti-CSR, pANCA, cANCA, APLA2R, C3 and C4-complement, antiphospholipid and anticardiolipin antibodies, immunoglobulins, antigliadin At, light chains - kappa and lambda in serum and urine - done at the discretion of the treating team and are tracked accordingly. In 39 patients with a confirmed diagnosis of IgAGN and 29 healthy controls, the serum levels of IL-6 were also examined using the ELISA method. 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.

In the statistical processing of the obtained results, the doctoral student used a program for biostatistical data processing: IBM SPSS Statistics for Windows, Version IBM SPSS, version 27 (2020), Minitab version 19 (2020) and MedCalc, version 20.008 (2021). They include the most modern and used in biomedical research statistical analyses, through which the completely reliable objectification of the obtained results is possible. Most of the data were measured on a dichotomous (Yes/Yes-No/No), nominal or ordinal scale. These quantities are presented in numbers and percentages, and other known methods were used to establish statistically significant trends, such as:

- Fisher's exact test for dichotomous variables.
- Chi-square test if there are more than two categories.
- Results are illustrated with pie, bar and line graphs.

The Shapiro-Wilk test was also used, presenting the arithmetic mean value and the standard deviation (\pm SD); t-test for two independent samples (independent-samples t-test) when comparing two groups; Levine's test (Levine's test $p < 0.050$) one-way ANOVA; presentation of results and trends through graphs of the mean and individual values and 95% confidence interval (Individual value plots with the mean and 95% CI); the mean trend represented by the median and interquartile range (IQR).

All analyzes were conducted at an acceptable error level of $\alpha = 5\%$ ($p < 0.05$). Results are graded according to statistical significance as follows: * - $p < 0.05$; ** - $p < 0.01$; *** - $p < 0.001$.

6. Characterization and evaluation of the dissertation work

Dr. Erkanyan presents the results of the conducted research in detail on 38 pages. At the beginning, the doctoral student presents the epidemiological and clinical characteristics of patients with IgAGN, the distribution by gender and age, incl. the accompanying pathology, the stage of chronic kidney disease at diagnosis. When distributed in relation to the calculated glomerular filtration, the group of patients with III st. of HBZ. In the follow-up of glomerular filtration, an increase in the average values at the 6th and 12th months is reported, but a decrease in the values at the 5th and 10th year. This was more pronounced in the groups with lower clearance at disease onset.

The pathomorphological characteristics of the PBB results of the patients with IgAN were thoroughly analyzed. As the most common histological variant in his study, he points to OSGS, (58%). Mesangioproliferative - nearly 24%, with minimal changes - about 7% and semilunar - 4%.

In the conducted immunofluorescence examination of the histological material, deposition of C1q was observed in 15 of the patients (15.6%), and deposition of C4 in 2, and the doctoral student indicated that it was always in combination with C1q. C1q deposits prevail mainly in the focal-segmental variant - in 13 of the histologies.

An assessment of the factors affecting the severity of the disease at diagnosis was made, as well as an assessment of the factors affecting the progression of the disease. The presented evaluation of the biomarkers associated with the pathogenesis of the disease and their diagnostic and prognostic role is extremely modern and thorough. When analyzing the results obtained from the IL-6 study, Dr. Erkanyan found statistically significantly higher IL-6 serum levels among the patients with IgA GN compared to the control group with mean values of 15.99 pg/mL and 8.54 pg/mL, respectively. By comparing the data of IL-6 levels and some morphological signs of histologies in PBB, such as: number of sclerotic glomeruli, degree of mesangial proliferation, degree of increase in mesangial matrix substance, etc., Dr. Erkanyan proves that in 75% of patients with moderate and marked inflammatory infiltrate in the interstitium, higher levels of IL-6 were also observed. The results of the effectiveness of the therapeutic protocols used in patients with IgA GN were analyzed in detail. Critically and with a clinical point of view, the doctoral student presents the data of the treatment with the various therapeutic combinations used today in our country. An important conclusion he makes is that supportive treatment, beyond ongoing immunosuppression, plays an important role in the therapy of IgA GN and that RAAS inhibitors occupy a central place in the conservative treatment of IgA GN. The addition of a sodium-glucose cotransporter 2 (SGLT2) inhibitor has been shown to be effective in reducing glomerular hypertension and hyperfiltration, thereby reducing proteinuria and the rate of GFR decline.

7. Contributions and significance of the development for science and practice

In the "Discussion" chapter, Dr. Erkanyan, in a volume of 5 pages, makes a precise analysis of the obtained results, comparing them with modern literature data. The data from the obtained results regarding the age of the patients, the higher frequency among the male sex, the average value of proteinuria, the follow-up period, as well as the percentage of patients in whom the progression of the disease is observed, are of a confirmatory nature. Again, the data from the performed kidney biopsies are of a confirmatory nature, which show that IgA nephropathy is the second most common diagnosis in the 18-59 age group. The results in the followed patients showed a similar profile of clinical characteristics to those in most large studies in developed countries of Europe and the USA (VALIGA, OXFORD). The established trend regarding frequency of macroscopic hematuria, intermittent hematuria, frequency of OBU is confirmed.

The conclusions regarding the results of the IL-6 study, which show statistically significantly higher serum levels among patients compared to controls, are also of a scientific applied and confirmatory nature, with a tendency for the average levels in women to be higher than those of men. This is in confirmation of data reported so far in world sources - Rostoker G et al from 1998, Suzuki H et al, Yamada K, et al; Yuko Makita.

The view that the doctoral student presents is interesting regarding the progression of kidney damage in patients with IgAN, which varies significantly among different populations and is a huge challenge for the treating physician, as it is extremely difficult to predict in which patients a worsening of CKD is expected, as well as how quickly this will occur. This comment is present in distinguishing primary from secondary forms of IgA nephritis, presenting several clinical cases of combination of IgA H and psoriasis, PsA and RA. Of an applied nature is the conclusion that distinguishing primary from secondary IgAN plays an essential role in conducting treatment. For this purpose, some basic clinical and laboratory parameters and their relationship with the progression of

the disease are thoroughly analyzed. Again, the conclusion that concomitant therapy with RAAS and SGLT2 inhibitors contributes to prolongation of renal survival is of a confirmatory nature.

8. Evaluation of publications on the dissertation work

In connection with the dissertation work, Dr. Erkanyan has 3 scientific publications related to the topic of the dissertation work, one of which is in print and 2 participations in scientific congresses and conferences.

9. Personal participation of the doctoral student

The 8 conclusions drawn follow the previously set 8 tasks to be performed in order to achieve the goal of the dissertation work. From everything that has been presented, I could conclude that everything that was done in terms of recruitment, selection in groups and follow-up of patients during the study was the personal participation of the doctoral student, as well as the analyzes of the obtained results. The Conclusions presented by Dr. Erkanyan optimally reflect the main results of the study. The majority of them are of a confirmatory nature.

Dr. Erkanyan notes several contributions that outline the merits of the presented dissertation work. Two of them are particularly valuable, that for the first time in our country IL6 level was investigated in patients with IgA nephropathy and correlations with histological and laboratory changes were established, which are useful for refining the therapy and that, for the first time in our country results of a state-of-the-art therapeutic regimen with Budesonide are also presented.

10. Abstract

The abstract is written in literary Bulgarian language, clearly and accurately and fully reflects the main parameters of the dissertation work.

11. Critical remarks and recommendations

I have no critical remarks about the dissertation submitted for review, the abstract and publications related to the dissertation.

12. Recommendations for future use of dissertation contributions and results

My recommendations to the dissertation student are to continue working in the direction of searching for additional factors that would be related to the pathogenesis of the disease and to search for new possibilities for the therapeutic influence of the disease.

CONCLUSION

In conclusion, I can state that the presented dissertation fully meets all the requirements of the Law on the Development of the Academic Staff in the Republic of Bulgaria (LAD), the Regulations for the Implementation of the LAD and the relevant Regulations of the Ministry of Education - Plovdiv. The presented materials and dissertation results fully correspond to the specific requirements of the MU - Plovdiv. The dissertation work shows that Dr. Iskui Mnatsakan Erkanyan possesses in-depth theoretical knowledge and professional skills in the scientific specialty "NEPHROLOGY", demonstrating qualities and skills for independent conduct of scientific research.

Due to the above, I confidently give my positive assessment of the conducted research, presented by the above-reviewed dissertation work, abstract, achieved results and contributions and I propose to the honorable scientific jury to award the educational and scientific degree "DOCTOR" to Dr. Iskui Mnatsakan Erkanyan in PhD program in "NEPHROLOGY".

11/20/2024 Reviewer:

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


/Prof. Boris Bogov d.m./