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ABSTRACT

On a dissertation for the award of an educational and scientific degree "doctor"

IgA NEPHROPATHY – CLINICAL, IMMUNOLOGICAL AND PATHOMORPHOLOGICAL CRITERIA FOR DIAGNOSIS AND THERAPEUTIC APPROACH

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The dissertation contains 113 pages and is illustrated with 10 tables, 35 figures, 8 microscopic photographs. 179 literary sources were used, in Cyrillic and Latin.

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The public defense of the dissertation work will be held on 06.01.2025. from 11:00 a.m. in the 2nd auditorium of the Medical University - Plovdiv, 15A "Vasil Aprilov" Blvd.

The defense materials are available at the Dean's Office of the Faculty of Medicine at the Medical University - Plovdiv, Vasil Aprilov Blvd. No. 15A and are published on the website of the Medical University of Plovdiv

CONTENTS

I.Brief introduction and epidemiology

IgA nephropathy (IgAN) is a mesangial proliferative glomerulonephritis characterized by diffuse mesangial deposits of IgA. Also known as Berger's disease, IgAN was first demonstrated in 1968 by Jean Berger when immunofluorescence was introduced as an adjunctive technique to the examination of renal biopsy material. IgAN is unique among glomerular diseases in that it is evidenced by the presence of immune deposits and not so much by morphologic changes in renal biopsy, as light microscopic changes can be highly variable.

IgA nephropathy is the most common form of primary glomerulopathy in Europe and Asia and the second most common in the USA and Latin America, presenting with different clinical and pathomorphological manifestations and leading to CKD as well as end-stage CKD. This is the most common pattern of glomerular disease found in most Western countries and Asia, where PBB is widespread. Frequency of IgA nephropathy - 2.5/year. per 100,000 people Significant geographic variation has been found to be associated with certain gene alleles that protect against the development of IgA nephropathy or other factors. There is also a known racial predisposition - higher incidence in Asia. In the US, IgAN is less common in blacks than in whites of European descent. Systematic analysis of 1,619 publications to establish the incidence of IgAN in different parts of the world and analysis of factors responsible for geographical differences. IgAN is most common in Asia - up to 45 cases/million (Japan) compared to the Caucasian race - 31 cases/million. in France; 200,000 – 350,000 cases/year (R. Glassock, May, 2019).

IgA nephropathy has a clinical association with a wide range of inflammatory and infectious diseases. Bacterial (Campylobacter, Yersinia, Mycoplasma, Haemophilus), viral (cytomegalovirus, adenovirus, coxsackievirus, Epstein-Barr virus) and fungal (aspergilous) agents are isolated as causative agents. However, no single microbial agent has been consistently isolated in glomerulin deposits in typical cases of IgAN. Adding to this is the fact that mucosal hypersensitivity to various food antigens exists in many of the IgAN patients. This indicates that mesangial IgA may represent a

common immune response to various foreign antigens, and that the disease has a direct relationship with mucosal immunity.

The pathogenesis of the disease involves the deposition of polymeric and defectively glycosylated IgA1 (Gd-IgA1) in the mesangium originating from the mucosa of the upper respiratory tract or gut-associated lymphoid tissue. Until now, it is clear that this is an autoimmune disease that proceeds through the so-called multi-hit mechanism, including 4 main stages:

*Hit 1 starts with increased production of Gd-IgA1.

*Hit 2 involves formation of anti-glycan antibodies that recognize Gd-IgA1.

*Hit 3 is the formation of immune complexes between Gd-IgA1 and glycan-specific antibodies, which leads to complement activation via the alternative pathway.

*Hit 4 is deposition of these immune complexes in the mesangium, with subsequent proliferation and release of extracellular matrix, cytokines, and chemokines, ultimately leading to renal injury.

Many mediators are involved at each stage of pathogenesis – APRIL, BAFF, IL, TNF-α, TGF-β, TLR9, tTG, MBL, etc. Their role is still not fully understood, and their study enables the validation of new diagnostic and prognostic markers, as well as the search for new targets for IgAN therapy.

IgA nephropathy as a manifestation of kidney damage in patients with psoriasis

Psoriasis is a common chronic inflammatory disease that affects 0.33%-0.6% in different races or a total of up to 125 million people worldwide. It is now known that this autoimmune disease has a significant impact on other organs and systems, not just skin manifestations. Psoriasis is a multisystem disease associated with cardiovascular disorders, metabolic syndrome and diabetes mellitus, neurological disorders. Renal involvement has so far not been sufficiently well studied and still remains unclear.

The interaction between TNF-α, IL-17 and IL-23 is at the heart of the pathogenesis of psoriasis and, accordingly, the target of its therapy. In this connection, the correlation between the manifestation of psoriasis and other immune diseases - psoriatic arthritis, RA, IBD, glomerulopathies - is sought. PsA, like psoriasis, is considered an immune-mediated inflammatory disease with autoimmune genesis. Many researchers are looking for immune markers to demonstrate a link between Ps/PsA and other immune diseases. A 2022 study investigated AGA-IgA/IgG tTG-IgA/IgG levels as a possible

link between CD and psoriasis, but showed that they were not significantly elevated in psoriatic patients. This confirms other studies conducted to date.

The relationship between psoriasis and CKD has been investigated in several large studies, which show that psoriatic patients are at higher risk of developing CKD and reaching end-stage CKD, demonstrating a relationship between the severity of psoriatic lesions and the extent of CKD, as well as , that patients with PsA are at higher risk for CKD.

The specific relationship between the pathogenesis of psoriasis and the development of CKD is not known, but it is known that the immune response to skin damage leads to immune irritation of the renal structures. The presence of inflammatory IL-17A-expressing cells leads to increased increased production of proinflammatory cytokines and increases the risk of kidney damage. Blockers of IL-17A improve renal function. Another short retrospective study showed that biologics did not affect renal survival in patients with psoriasis.

To date, there are no definitive data on specific kidney damage in psoriasis, but cases have been reported mostly of IgAN and less of another type of glomerular damage in patients with already diagnosed psoriasis.

IgAN AS A RESULT OF THERAPY WITH A BIOLOGICAL MEDICATION

Adalimumab (fully humanized) and infliximab (chimeric) are monoclonal antibodies against tumor necrosis factor alpha (TNFα) that are approved for the treatment of several chronic inflammatory diseases, including Crohn's disease, ulcerative colitis, ankylosing spondylitis, and others. These preparations are widely used and their effectiveness has been proven, both for induction and maintenance of remission. These biologic agents are generally used in patients unresponsive to standard immunosuppressive drug therapy. TNFα inhibitors are potent immunomodulators and have been associated with the development of autoimmunity. Both agents have been reported to cause IgAN among patients with CD and other autoimmune conditions. However, infliximab has also been documented to successfully treat a patient with IgAN secondary to CD.

There have been cases of IgAN nephropathy occurring in patients treated with anti-TNFα medications since 2009. Bhagat Singh AK et al reported in 2019 a case of adalimumab-induced IgAN that went into complete clinical and paraclinical remission after stopping adalimumab and remained in remission despite initiation of another TNFα inhibitor (infliximab). Adalimumab-induced IgAN has been described in patients with psoriasis and Crohn's disease. All reported cases, except one patient who developed lunate glomerulonephritis, improved renal function after discontinuation of adalimumab and initiation of alternative immunosuppressive therapy. Despite the

pronounced association between IgAN and IBD, PS, AS, the underlying mechanism contributing to this association has not yet been determined.

Numerous case reports have been published documenting IgAN as a complication of Crohn's disease, but in each of these reports, IgAN occurred only in the context of active bowel disease. With regard to infliximab, there are case reports presenting both the onset of IgAN after its use and the achievement of remission in patients with IgAN secondary to autoimmune conditions. Why one TNFα inhibitor causes IgAN and another does not is unclear. Adalimumab is a fully humanized monoclonal antibody, while infliximab is a chimeric monoclonal antibody that consists of both human and murine sequences. It is possible that the difference in monoclonal structures led to the formation of autoantibodies against adalimumab alone. Alternatively, it may also represent an idiosyncratic drug reaction. Unlike other glomerular diseases, IgAN is defined by the presence of an immune reagent rather than specific morphologic features, with the pathognomonic finding of mesangial IgA deposits on immunofluorescence. IgAN is also associated with a number of inflammatory diseases. It is likely that IgAN is not an independent entity, but a common immune response to various inflammatory mechanisms. With their immunomodulatory effect, anti-TNFα blockers can modulate this response and simultaneously induce and support the treatment of IgAN.

The role of immunosuppressive therapy in primary IgAN is controversial with recent clinical trials showing limited benefit and significant harm associated with steroid therapy. This is in contrast to case reports of adalimumab-induced IgAN in which renal recovery was observed after discontinuation of adalimumab and initiation of an alternative immunosuppressive regimen. This finding suggests that patients with adalimumab-induced IgAN may be a distinct subgroup of IgAN, and these patients may have a markedly different response to immunosuppression compared with idiopathic IgAN. The case described by Bangh et al demonstrates that while TNFα inhibitors can induce IgAN, this is not always a class effect, and the use of an alternative TNFα inhibitor can be considered in patients with TNFα inhibitor-induced IgAN and refractory autoimmune diseases with careful monitoring.

PATHANATOMY

Pathoanatomically, the histological diagnosis of IgAN is definitive, as it is determined by the presence of IgA-dominated deposits – alone or in combination with other deposits in the mesangium. There may also be complement components - C3, but not

C1q and C4; IgG in 50% - 60%; IgM in 40-80%; However, light microscopic histological material shows a wide range of histological changes that may affect the clinical course. Changes can range from minor glomerular damage to severe necrotizing glomerulonephritis, advanced glomerular sclerosis, or tubular atrophy. Typical cases present with mesangial cell proliferation and enlargement of the mesangial matrix with normal-appearing capillary loops, but endocapillary hypercellularity may also be present. Because of this variability in histology, numerous studies have been conducted that aim to find a relationship between histological changes and clinical presentation. Some of the histological lesions are considered to have a high prognostic value. In 2005, a meeting of pathologists was held in Oxford, UK, which determined the morphological variations in biopsies of

The Oxford classification of IgA nephropathy: pathology definitions, correlations, and reproducibility

A Working Group of the International IgA Nephropathy Network and the Renal Pathology Society:

initial (2009) 76, 546–556

1.Mesangial hypercellularity - in > or <50% of glomeruli M0 or M1

2.Endocapillary hypercellularity – present/absent E0 E1

3.Segmental sclerosis/adhesions – present/absent S0 or S1

4.Tubular atrophy/interstitial fibrosis – 0-25%, 26-50%, >50% T0,T1,T2

5.C – Presence of crescents /MEST + C/

The clinical picture, as well as the histology of the disease, has a wide range of manifestations, which differ both in age and in the severity of clinical symptoms. There is no typical clinical symptom for IgAN. In the European race, the male:female ratio is 3:1, while in the Asian population this ratio reaches 1:1.

Clinical manifestations can be:

1. Macroscopic hematuria - 40% - 50% present with episodes of macroscopic hematuria, most often in the second decade of life. intercurrent infection follows, most often of GDP (synpharyngitic hematuria) and less often GIT. No clots.

2. Asymptomatic hematuria and proteinuria - 30% - 40% present with asymptomatic erythrocyturia with or without proteinuria (usually < 2 g/24h).

3. Nephrotic syndrome - only in 5% of all patients with IgAN.

4. Some patients present with CKD and AH at the time of diagnosis of IgAN – usually older people who have had the disease for a long time and it remains undiagnosed due to lack of macroscopic hematuria and routine urinalysis.

5. OBU is not characteristic of IgAN (<5%), but is possible, especially in older people. Three OBU mechanisms:

--- Crescent IgAN – about 7% of IgAN

--- Tubular occlusion from high-grade hematuria.

--- Chronic IgAN predisposes to OBU

Arterial hypertension – relatively rare at first

It often appears during the course of the disease, before the increase in serum creatinine. When performing PBB, AH is found in 52.5% of Australians, 24.7% of Europeans, 24.2% of North Americans and 18.6% of Asians.

AH is more uncommon in children (5%) and is more common in patients with disease onset after 35 years.

IgA – clinical course and prognosis:

1. 20% - progressive loss of kidney function

2. After 20 years, ¼ of the patients reach THBZ

3. Rapidly progressive lunate IgAN / poor prognosis /

4.AH, proteinuria, reduced GFR, and histological evidence of glomerular and interstitial fibrosis are associated with a worse prognosis.

5. Episodes of macroscopic hematuria are not associated with poor prognosis

6. The risk of progression is minimal in the absence of AH and proteinuria < 0.2 g/24h.

The diagnosis of IgAN is made by immunofluorescence positivity of IgA in the mesangium, and this can only be done by PBB, a diagnosis can hardly be made by the clinical picture alone. None of the light microscopic findings alone can establish the diagnosis.

A differential diagnosis can also be made with extrarenal diseases:

1. Chronic liver diseases /cirrhosis, chronic hepatitis/ - impaired clearance of IgA from Kupffer cells. They rarely reach CSBN.
2. Infections /HIV, HBV/
3. Immune and inflammatory diseases / Ankylosing spondylitis, Rheumatoid arthritis, Reiter syndrome, Psoriasis, Behçet syndrome, Takayasu arteritis, Myasthenia gravis /
3. Neoplasias / especially in patients >60 years old/ - GDP, lungs, kidney.
3. Diseases of GIT - Celiac disease, IBD.
4. Familial IgAN /most often associated with 6q 22-23 locus/.

In addition to PBB, as an addition to diagnostic methods, markers characteristic of IgAN can be used:

1. Gd-IgA1 - serum level
2. Gd-IgA1 - specific autoantibody /serum level/
3. IgA – IgG - immune complexes in serum
4. Gd-IgA1 - level in urine
5. CD89, CD71, CD80
6. Podocyte urokinase-type plasminogen activator receptor
7. TLR9
8. APRIL

THERAPY OF IGA NEPHROPATHY

There is a disease therapy algorithm that divides patients into 3 groups according to proteinuria, eGFR, presence or absence of elevated BP.

1. Good prognosis – minimal urinary abnormalities, normal GFR and normotensive. Only monitoring 1-2 times a year for at least 10 years.
2. Intermediate prognosis – significant proteinuria />1.0/, AH and slowly decreasing GF. ACE inhibitors and strict control of AH. The risk of progression greatly decreases when proteinuria drops to <1 g/d, with preserved renal function. Omega 3 MK.
3. Poor prognosis – rapidly deteriorating kidney function.

At levels of proteinuria of 1 g/d, RAAS inhibitor therapy is carried out

CS is started when proteinuria persists > 1 g/d after optimal ACE inhibition for 3 - 6 months. VALIGA (1147 pts.) CS + RAASi v/s RAASi/

Prednisolone 1 mg/kg/d for 2 months with gradual tapering for a total of 6 months course.

Pulse MP - 7mg/kg 3 consecutive days on I, II and V months or I, II, III and VI months with a maintenance dose of MP 12-16mg per day in the interpulse intervals.

Budesonide – in 2021, the FDA officially included it as a therapy for IgAn, with local action on the mucosal lymphoid tissues of the distal ileum and the proximal part of the colon, modulating IgA production. Dose – 9 or 12 mg/day. It is possible to start therapy or as a follow-up therapy after Methylprednisolone.

More aggressive immunosuppression - Cyclophosphamide, Azathioprine, MMF is recommended if crescents are present.

SGLT2 inhibitors – in combination with RAAS have been shown to reduce the progression of CKD (DAPA-CKD)

Sparsentan is a non-immunosuppressive selective antagonist of endothelin type A and angiotensin II subtype 1 receptors. The combination of inhibition of these two receptors helps to improve hemodynamics, better anti-inflammatory and anti-fibrotic effect, as well as podocyte protection.

GOAL AND OBJECTIVES

1. PURPOSE:

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.

2. TASKS:

1. To study the frequency of patients with IgAN in whom the diagnosis is confirmed by puncture renal biopsy.

2. To specify the frequency of secondary IgAN
3. To specify possibilities for the use of other biomarkers and the diagnosis and therapeutic approach of IgAN.
4. To study the diagnostic significance of serum and tissue IL-6 levels, as well as the difference in primary and secondary IL-6
5. To study the relationship of pathomorphological changes with clinical presentation.
6. To explore the role of immunological studies in diagnosis, follow-up of activity and therapy.
7. To follow the clinical course of IgAN in different age groups.
8. To give recommendations for therapeutic behavior in patients depending on the clinical presentation, immunological and pathomorphological changes.

PATIENTS AND METHODS

In the period April 2010 - November 2023, 110 patients between the ages of 18 and 78 with biopsy-proven IgA glomerulonephritis were followed in Kaspela UMBAL. Of them, 84 are men and 26 are women.

Table 1. Characteristics of patients by gender and age.

men	women	total
84	26	110
75,2	24,7	100

1.2 Inclusive criteria for patient selection:

All patients over the age of 18, treated in the Clinic of Nephrology, who underwent a puncture kidney biopsy and were diagnosed with IgA nephropathy during the period 2010-2023.

2. Puncture kidney biopsy

The histological examination of material from a kidney taken through PBB occupies a central place in the diagnosis, determination of therapy and prognosis of primary and secondary glomerulopathies.

2.1 Indications for performing a puncture kidney biopsy

Indications for BB in older patients and in younger patients should not differ, but there are still some peculiarities.

1. Nephrotic syndrome
2. Acute kidney injury of unclear origin / after exclusion of prerenal and postrenal causes /
3. Non-nephrotic proteinuria
4. Systemic disease with clinical and paraclinical evidence of renal involvement
5. Chronic kidney disease with proteinuria and/or erythrocyturia
6. Unspecified erythrocyturia

2.2 Contraindications for performing a kidney biopsy:

2.2.1 Absolute

*Patient's refusal to consent

*Impaired coagulation

* Purulent processes in the kidney or perirenal space

*Kidney tumors

2.2.1 Relative

*Cystic kidneys

*Advanced atherosclerosis

*Malignant or resistant arterial hypertension

*Acute myocardial infarction and unstable angina pectoris

*Acute pulmonary embolism

*Single functioning kidney

*Reduced kidney sizes

При проведеното изследване, пациентите са имали следните показания за извършване на бъбречна биопсия:

1.Хематурия: макроскопска или интермитентна

2.Протеинурия: ненефротична

3.Бъбречна недостатъчност, неуточнена диагностично при запазени размери на бъбреците

4.Нефротичен синдром

5. Proteinuria, erythrocyturia and CKD in patients with diabetes mellitus without diabetic retinopathy

6. Kidney damage in the presence of systemic manifestations: rashes, joint manifestations, consumptive syndrome

The puncture kidney biopsy was performed in the kidney biopsy room at the Nephrology Clinic of the Kaspela UMBAL after preliminary preparation of the patients, which included signing an informed consent for the procedure, urine test, FBP, SR, blood sugar, urea, creatinine, uric acid, total protein, albumin, electrolytes, coagulogram, hepatitis "B" and "C" and HIV, sensitivity tests to local anesthetic / lidocaine / and antibiotic. Discontinuation of concomitant therapy with anticoagulants and antiaggregants 5 days before the manipulation and switch to therapy with low molecular weight heparin with the last application 24 hours. before KB. KB was performed under ultrasound guidance with an automatic biopsy gun "Galini" or "Möller" with disposable needles with a lumen of 16G after layer-by-layer infiltration of the tissues with Lidocaine 2%. The left kidney is biopsied, but in some patients the right kidney is preferred, due to contraindications for the left and more convenient location of the right. After the manipulation, the patient is required to observe bed rest for 24 hours with monitoring of heart rate and arterial pressure and triple examination of urine and full blood picture. Hemostatic agents / calcium gluconate, Dicinon / and antibiotic are prescribed at the discretion of the treating team. The next day, an ultrasound of the kidneys was performed to detect post-biopsy complications.

In the absence of indications for continued hospital stay, the patients were discharged 2 days after the kidney biopsy.

The material is placed in NaCL and sent for immunofluorescence and histological examination.

Immunofluorescence study: performed on cryostat sections with a thickness of 4 microns with a standard package of fluorochromic anti-human rabbit antisera against

IgG, IgA, IgM and three complement fractions – C1, C3 and C4, as well as against human fibrinogen. If necessary, kappa and lambda light chains are also examined.
Histological and histochemical examination: the material is brought to a paraffin block and the following stains are routinely applied to 2 micron sections: hematoxylin/eosin, PAS, Masson's trichrome, silver impregnation/JMS/, congo-rot for amyloid.
The pathoanatomical processing and the corresponding histological result were done in the "Department of Clinical Pathology" of UMHAT Kaspela, in the Department of "General and Clinical Pathology" of the Sofia Academy of Medical Sciences, Lora laboratory, Sofia. Five of the biopsies were performed in other hospitals.
The histological results were compared with the diagnoses of 1002 patients over 18 years of age who underwent kidney biopsy in the Nephrology Clinic of UMHAT "Kaspela" during the same period.

1.2. LABORATORY PARAMETERS

In all patients, the following were examined and monitored in order to objectify the course of the disease and the results of the treatment:
1. Full blood picture, ESR, sugar, total protein, albumin, electrolytes, cholesterol, triglycerides, transaminases
2. Urine analysis - relative weight, pH, protein, sediment; Proteinuria for 24 hours; Urina according to Amburger; Uro cultures
3. Immunological tests: 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 - performed at the discretion of the treating team and are followed up accordingly.
4. 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. It is considered more reliable than the Cockcroft-Gault formula and is preferred for older patients over the MDRD /Modification of Diet in Renal Disease / equation.

All biochemical and immunological tests were carried out in the Clinical Laboratory at UMHAT Kaspela

1.3. LEVELS OF IL-6 IN SERUM IN PATIENTS WITH IgAN

Serum levels of IL-6 were measured in 39 patients with a confirmed diagnosis of IgAN and 29 healthy controls by ELISA method. Serum samples were collected, aliquoted and stored at -80°C until assayed by a commercial IL-6 assay kit (Elabscience, Houston, TX, USA). Control and patient samples were analyzed on the same plate by measuring absorbance at ʎ=450 nm using a microplate reader (Biochrom EZ Read, Fisher Scientific, Denmark). IL-6 concentrations were calculated according to a standard curve and are presented in pg/mL.

1.4. STATISTICAL RESEARCH METHODS

The statistical methods were determined according to the goals and tasks of the dissertation work and the type of quantities (metric, rank, nominal, dichotomous). 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 the following methods were used to establish statistically significant trends:

• Fisher's exact test for dichotomous variables.

• Chi-square test if there are more than two categories.

• 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 (±SD). To establish statistically significant trends, the following statistical methods were used depending on the number of groups:

When comparing two groups, an independent-samples t-test was performed. In certain comparisons, a lack of homogeneous variability (homogeneity of variances) was found according to Levine's test (Levine's test $p < 0.05$). In such cases, the value of p was reported under the condition equal variances not assumed.

• One-way ANOVA was performed for more than two groups.

• The graphical representation of the results includes graphs of the mean and individual values and a 95% confidence interval (Individual value plots with the mean and 95% CI).

In the absence of a normal distribution (Shapiro-Wilk $p < 0.05$), the mean trend is represented by the median and interquartile range (IQR). The following methods were used to establish statistically significant trends:

• The Kruskal-Wallis non-parametric test for comparison of more than two groups/categories.

• Spearman rank-order correlation for analyzing the relationship between two quantities.

• Analysis with ROC curve (Receiver operating characteristic curve/ROC curve) to investigate the diagnostic ability of APLA2R in serum as a marker for the presence of pMN. Statistics include area under the curve (AUC), sensitivity, specificity, and cutoff criterion value.

• Results are illustrated by scatter plots with fitted regression line and area under the curve (AUC) plots. All analyzes were conducted at an acceptable error level of alpha =5% ($p < 0.05$). Results are graded according to statistical significance as follows: * - $p < 0.05$; : ** -$p < 0.01$; *** - $p < 0.001$.

The statistical programs IBM SPSS version 27 (2020), Minitab version 19 (2020) and MedCalc version 20.008 (2021) were used for data analysis.

IV. RESULTS:

In the period April 2010 - November 2023, 110 patients over 18 years of age with biopsy-proven IgA glomerulonephritis were followed up in Kaspela UMBAL. Of them, 84 are men and 26 women, aged between 18 and 78 years.

Table 2. Characteristics of patients by gender.

GENDER

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	F	27	24,8	24,8	24,8
	M	82	75,2	75,2	100,0
	Total	109	100,0	100,0	

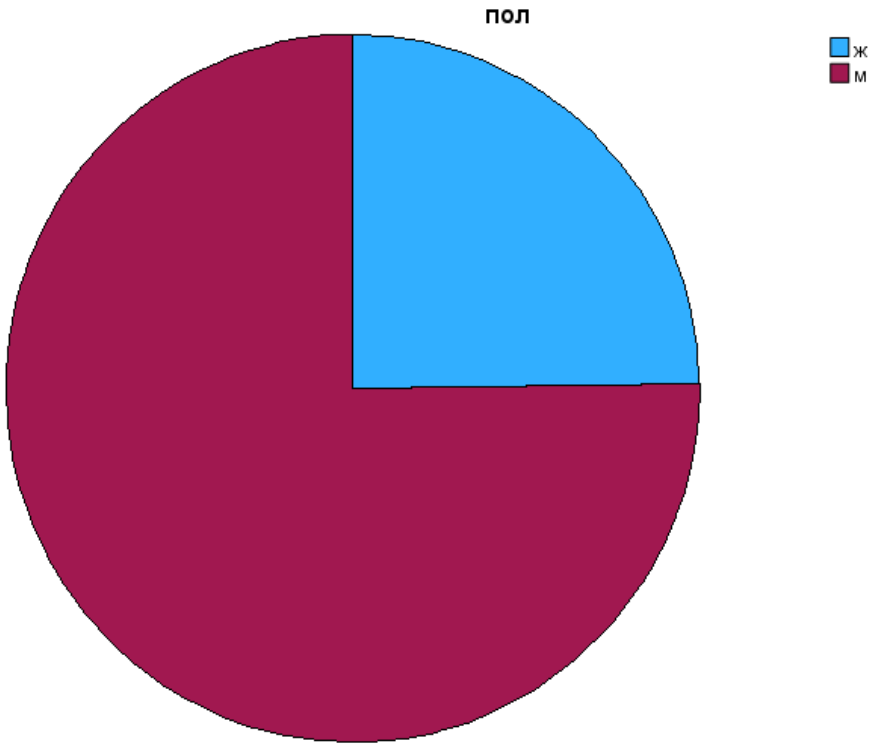


Fig 1 Distribution of patients by gender

Table 2 Clinical characteristics of patients with IgAN

	Total number of patients (n=110)
Age (years) (Mean±SD) (Range)	46,68 ± 15,220
Gender (M: Ж) (n)	75,2:24,7 %
Gross haematuria (n)	11
Erithricyteuria (n)	66
Proteinuria (mg/day) (Mean±SD)	1472,2 ± 1536
eGFR (ml/min/1.73 m^2) (Mean±SD)	57,25 ± 31

1. The frequency of PBB-proven IgAN cases in the Nephrology Clinic of UMBAL Kaspela:

- 110 patients with IgAN, or 10% of biopsies performed over a 10-year follow-up period.

- Of these, 21 people / 7 women, 14 men /, or 20%, are patients over 60 years of age.

- The distribution of men/women is in favor of men – 82/27

- With eGFR < 60 ml at the time of diagnosis, 54.3% of the patients were

- Of these patients, 3 started hemodialysis before conducting PBB.

2. Clinical characteristics of the patients:

2.1. At the onset of the disease, 77 patients had microscopic (66 or 54%) or macroscopic hematuria (11 or 10%). The presence of episodes of macroscopic hematuria was not associated with lower eGFR at diagnosis.

2.2. The distribution regarding proteinuria – 100 people were followed

	<500 мг/24h	500-1000мг/24h	1000-3000 мг/24h	>3000 мг/24h
n	25	25	37	13
%	25	25	37	13

Table 3 Distribution of patients regarding proteinuria

The distribution of patients with respect to proteinuria at disease diagnosis shows that the majority of cases present with protein loss levels between 500 and 3000 mg. Although high-grade proteinuria is not characteristic of IgAN, it is observed in 13% of cases. In the follow-up of these patients at 3, 6, 12 months, no significant difference was observed in the prognosis regarding renal function based on estimated glomerular filtration. The histological distribution shows a slight preponderance in favor of sclerotic lesions – 10 of the patients, the remaining 3 patients have crescents.

At the 3rd, 6th, 12th month, 5th and 10th year proteinuria follow-up, there was a trend towards retention and a slight decrease in the values at 6th and 12th month, and at 5th and 10th year a decrease in proteinuria levels was observed.

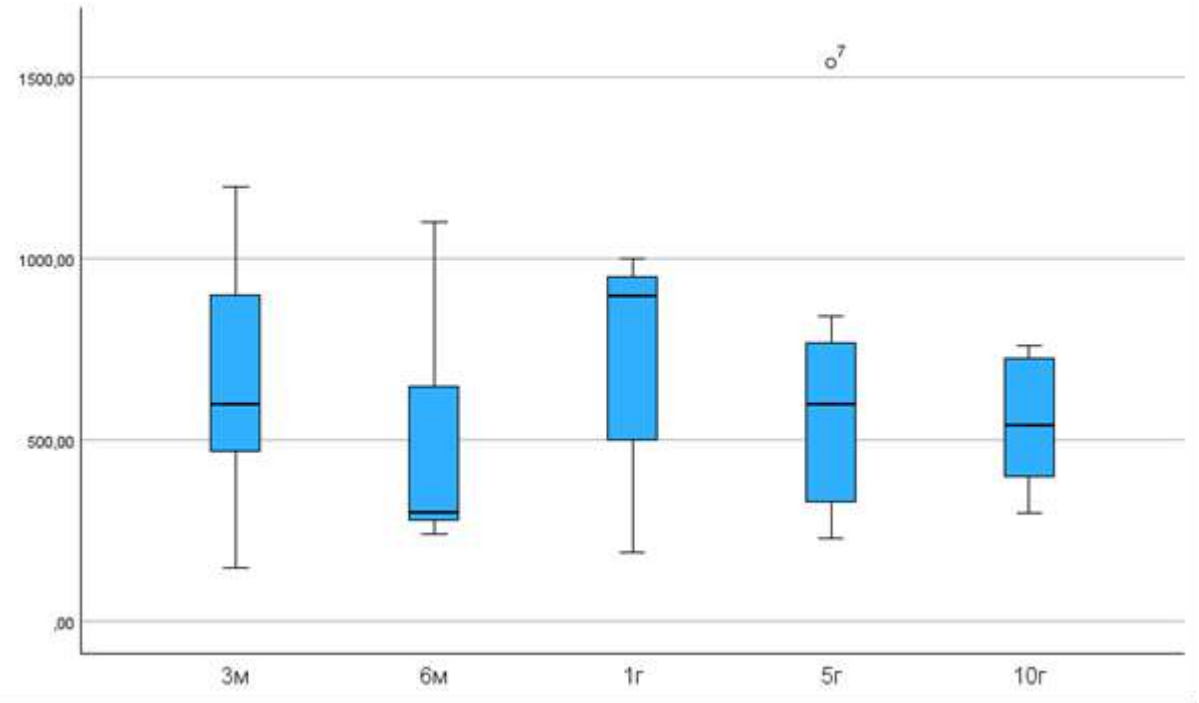


Fig 2 Box plot chart follow-up of average values of proteinuria at 3m, 6m, 1y, 5y and 10 years

2.3. Distribution by degree of CKD – 100 people followed:

	I gr	IIgr	IIIgr	IVgr	Vgr
n	18	27	33	16	6
%	18	27	33	16	6

Table 4 Distribution of patients according to the stage of CKD, based on eGFR

When distributed in relation to the calculated glomerular filtration, the group of patients in the III stage of CKD is the largest. Six of the patients had a clearance of less than 15 mL/min, and three of them were on hemodialysis when PBB was performed. In these patients, the histological distribution is as follows: 2 have the sclerotic variant, and 4 have crescentic glomerulonephritis.

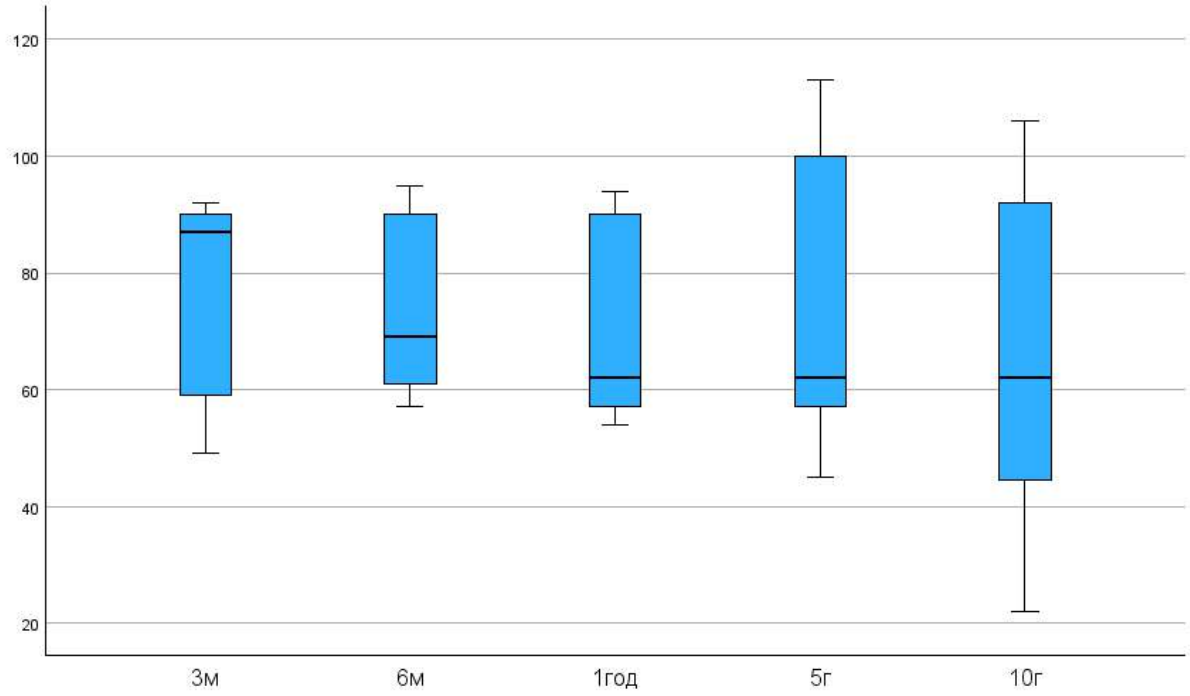


Fig 3 Box plot chart showing follow-up of mean eGFR values at 3m, 6m, 1y, 5y and 10y

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.

4. Antigliadin antibodies were tested in 25 of the patients, and in all of them they were within the normal range. The study was conducted after pathogenetic treatment was started in all of them.

5. Elevated levels of total IgA were present in 2 patients, levels of C3 and C4 were normal in all examined patients.

3. Comorbidities:

*86 of the patients had hypertension at baseline.

*ICS - 6

*Diabetes – 10

*6 patients had thyroid pathology at diagnosis, of which 4 had clinically evident hypothyroidism, 1 with thyrotoxicosis, 1 with Hashimoto's in the euthyroid phase

*There are 3 patients with liver cirrhosis, and it is on an ethylic basis

*Virus hepatitis B – 3

*Gout, gouty arthritis – 8

*Rheumatoid arthritis – 6, of which 4 were on biological therapy before a kidney diagnosis

*Psoriasis/Psoriatic arthritis – 10, of which 4 have proven Ps/PsA before PBB, 1 has started biological treatment.

*No patients with proven IBD and biopsy-proven IgAN.

*Eight of the patients had a clinical diagnosis of IgA vasculitis (Henoch-Schonlein purpura).

4. SECONDARY IGAN

As a secondary IgAN was accepted in 6 patients with Rheumatoid Arthritis, 4 of them were on biological therapy before setting a renal diagnosis. There are 10 with Psoriasis/Psoriatic Arthritis, 4 of them have proven Ps/PsA before PBB, 1 has started biological treatment. Only 3 of these patients were considered to have developed IgAN as a consequence of the ongoing therapy with the biological agent. In the others, the therapy with a biological agent was continued, and as a new one, it was started in one.

Table 5, presenting paraclinical data at diagnosis and histological data in patients with proven Ps/PsA before the onset of renal disease,

Three of the patients – 1, 2, 3 – were treated with a biological agent after being diagnosed with PsA. The duration of treatment varies from 3-6 years.

In patients 2, 3, 4 pulses with Methylprednisolone and Cyclophosphamide were conducted

In patients 2, 3, 4, progressive deterioration of renal function was observed

All patients had hypertensive disease, patient 3 had chronic hepatitis B and primary hyperaldosteronism

In patient 1, it is assumed that the treatment was the cause of the manifestation of CKD

Table 5 Paraclinical data in patients with proven Ps/PsA before the appearance of kidney disease - follow-up

	Пациент 1	Пациент 2	Пациент 3	Пациент 4
Креатинин	283	183	229	150
Протеинурия	0,51	2,6	8,78	1,13
Еритроцитуия	+	+	+	+
% Склеротични гломерули	1/13	2/7	4/13	6/30
Туб/инт засягане	+	+/-	+/-	+
полулуния	-	-	-	+

Table 6 presenting paraclinical data at diagnosis and histological data in patients with proven Ps/PsA after the onset of renal disease,

The period of onset of PsA after performing kidney biopsy in patients was immediately after PBB or up to 2 years

All patients were treated with Methylprednisolon and cyclophosphamide

Three of the patients have Budesonide in their therapy - 2,3,6

One patient was treated with Mycophenolate - 6

Two of the patients started biological treatment for PsA (2,4)

All patients have Hypertensive disease

Two of the patients maintained serum urea and creatinine levels

One patient experienced worsening

Renal function improved in one patient

Table 7 Paraclinical data in patients with proven Ps/PsA before the appearance of kidney disease - follow-up

The trend that IgAN is the leading renal pathology in Ps/PsA is IgAN is confirmed

In only one of the patients, the treatment with a biological preparation was accepted as the cause of the appearance of IgAN

Therapy for IgAN should be specific according to the underlying cause – in patients with Ps/PsA these should mainly be biological agents.

Budesonide as the therapy of choice remains the priority for primary IgAN

5. ONSET OF IgAN AFTER TREATMENT WITH A BIOLOGICAL MEDICATION

The increasingly widespread use of biological medicines leads to more common complications. In the Clinic of Nephrology of UMHAT Kaspela, one case of a patient

with pronounced IgAN after therapy with an anti-TNF-a preparation has been proven so far. This is a 66-year-old woman with proven seropositive rheumatoid arthritis for 3 years, for which she is being treated with Adalimumab /anti-TNF-α/. Nephrotic syndrome was found during the tests. A kidney biopsy was performed with a diagnosis of IgAN. Adalimumab therapy was stopped, treatment with Methylprednisolone pulse 500 mg on three consecutive days, Cyclophosphamide 500 mg was remembered, and this scheme was repeated on the 2nd and 3rd months. Corticosteroids were continued orally at a dose of 0.5 mg/kg, and the dose was gradually reduced. During the patient's follow-up, an improvement in renal function was noted - an increase in eGFR, as well as a decrease in proteinuria levels.

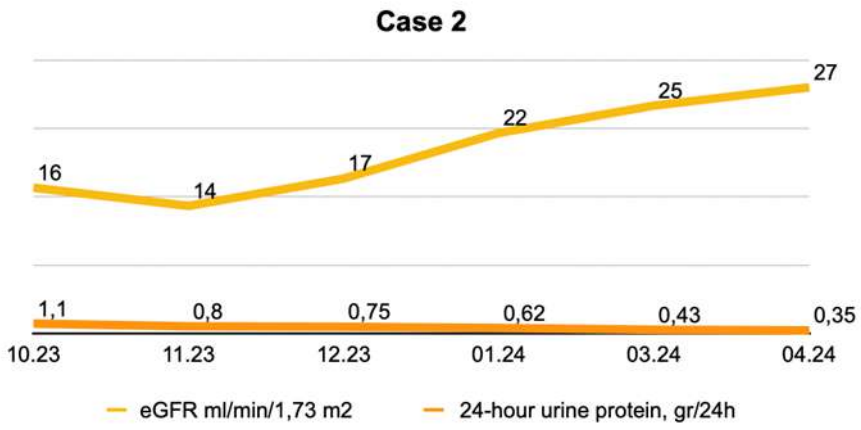


Fig 4 Follow-up of proteinuria and GFR for a 6-month period in a patient with IgAN after therapy with an anti-TNF-α drug

6. HISTOLOGICAL CHANGES

Хистологични изменения	Честота	Прогресия на болестта на базата на eGFR на 1 год		P
		Не	Да	
Незначителни хистологични промени	n	4	1	
	%	80%	20%	
Огнищно-сегментни Склеротични лезии	n	28	17	
	%	62,2%	37,7%	
Мезангиопролиферативен	n	14	2	
	%	87,5%	12,5	
С полулуния/Полулунен	n	12	8	
	%	60%	40%	
ИгА васкулит	n	4	1	
	%	80%	20%	

Стадий на ПББ по Haas	Честота	Прогресия на болестта на базата на eGFR през годините		P
		Не	Да	
Незначителни хистологични промени	n	9	5	
	%	14,8	9,6	
Огнищно-сегментен гломерулонефрит	n	23	20	8
	%	37,7	38,5	
Огнищен пролиферативен	n	16	10	0,217
	%	26,2	19,2	1
Дифузен пролиферативен	n	13	13	
	%	21,3	25,0	
Склеротичен	n	0	4	
	%	0,0	7,7	

Таблица 8 Прогресия на бъбречното заболяване на основата на изчислената гломерулна филтрация на първа година

Хистологични изменения	Честота	Прогресия на болестта на базата на eGFR на 5 год		P
		Не	Да	
Незначителни хистологични промени	n	0	0	
	%			
Огнищно-сегментни Склеротични лезии	n	12	13	
	%	48%	52%	
Мезангиопролиферативен	n	2	3	
	%	40%	60%	
С полулуния/Полулунен	n	3	8	
	%	27,27%	72,72%	
ИгА васкулит	n	3	3	
	%	50%	50%	

Таблица 9 Прогресия на бъбречното заболяване на основата на изчислената гломерулна филтрация на пета година

1. 1. Focal segmental sclerotic – 64 patients. This is the most common histological variant. At diagnosis, mean estimated glomerular filtration was 51.19 mL/min, and mean proteinuria levels were 1684.7 mg/24h. Follow-up of renal function based on estimated creatinine clearance showed a good response during the first year and a trend towards maintenance of levels at year 5. Followed-up patients at the tenth year / 6 patients / have an increase in the average calculated value of eGFR. Regarding proteinuria, the trend is towards a decrease in the levels at the 3rd, 6th, 12th month, 5th and 10th year. All patients received corticosteroid therapy - initiating therapy with pulse doses of Methylprednisolone, followed by tapering doses orally. 40 of the

patients were treated with cyclophosphamide in pulse doses. Budesonide therapy was prescribed in 26 of the patients.

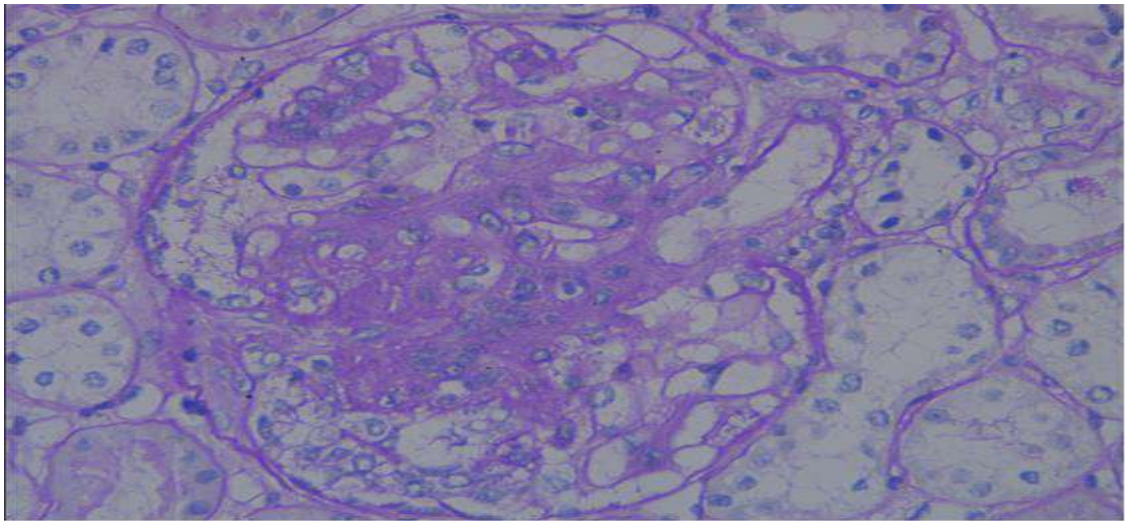


Fig 5 Segmental sclerosis and adhesion, PAS stain, own archive

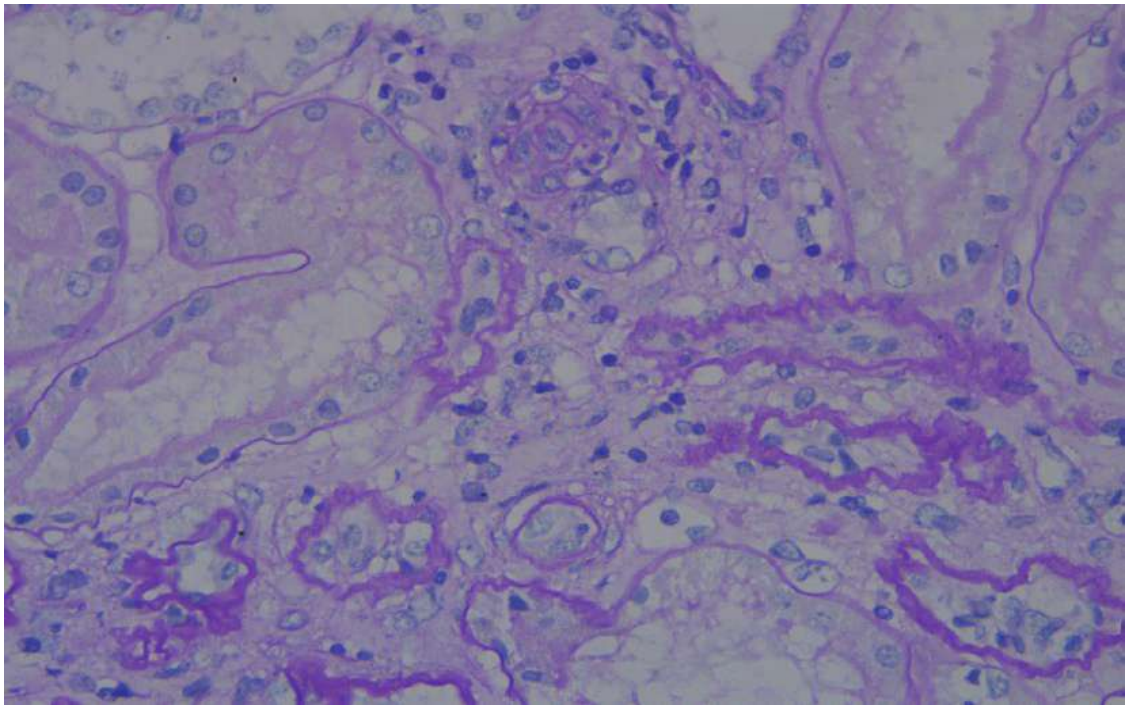


Fig 6 Tube atrophy in PAS cells, own archive

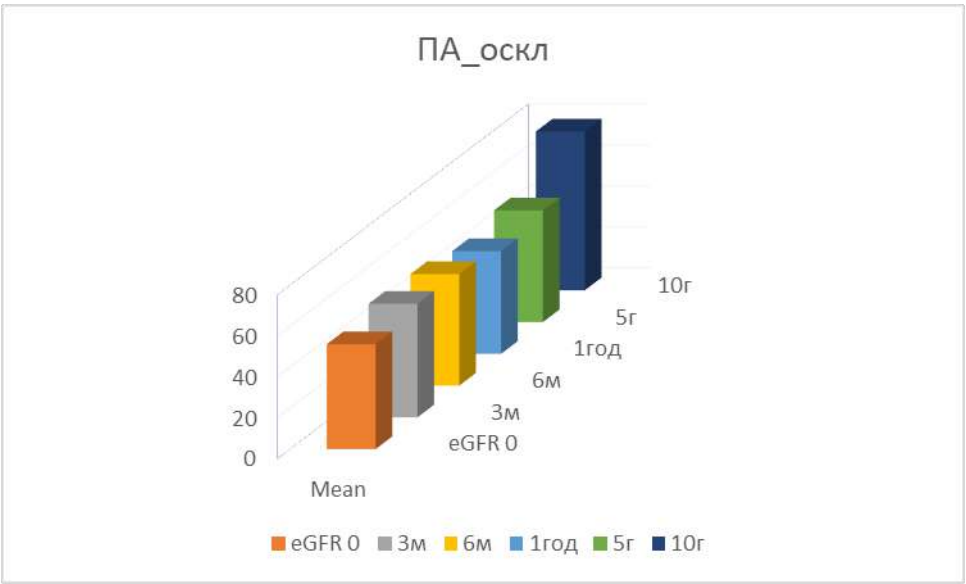
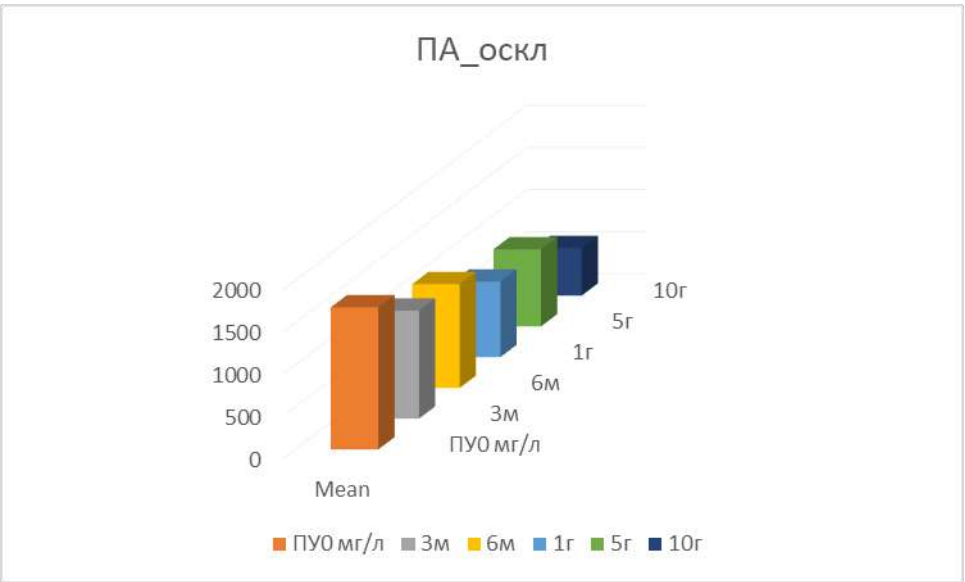


Fig 7 Follow-up mean eGFR values at 3 months, 6 months, 1 year, 5 years and 10 years



Фиг8 Проследяване на средните стойности на протеинурия на 3ти, 6ти мес, 1ва, 5та и 10 год

2. 2. With mesangial proliferation - 26. This is the second most frequent histological finding. Mean eGFR at diagnosis was 68.6 with a standard deviation of 38.3, and mean proteinuria levels were Follow-up during the first year of estimated glomerular filtration showed a slight increase and retention of

values. One patient was followed up at year 10, so no comparison could be made. Proteinuria at diagnosis was 1094 mg/24h. At follow-up, there was a retention of the 3rd and 6th month values and a decline in the 1st and 5th year mean values. In these patients, therapy with RAAS inhibitor only was carried out in 4 of the cases, in all the others treatment was carried out with Methylprednisolone, in 3 cases in combination with Cyclophosphamide, but in them crescents were proven. Budesonide therapy was initiated in 7 of these patients.

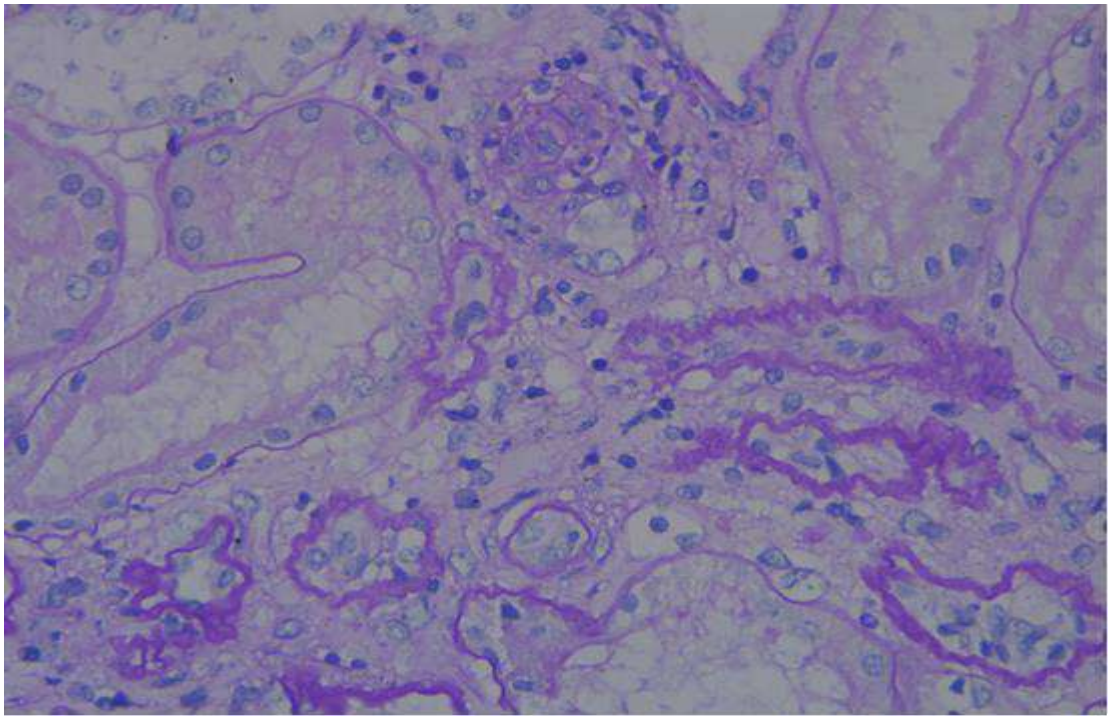


Fig 9 Mesangial hypercellularity and cell crescent, HE, own archive

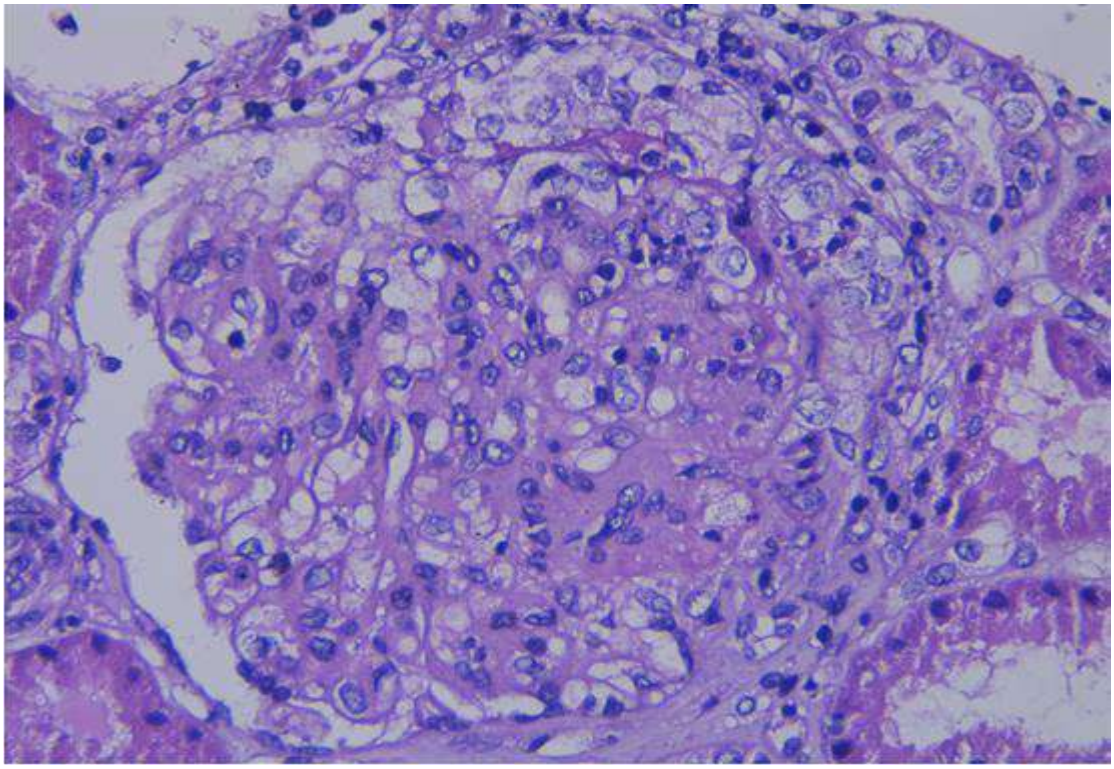


Fig 10 Mesangial hypercellularity and crescent, own archive

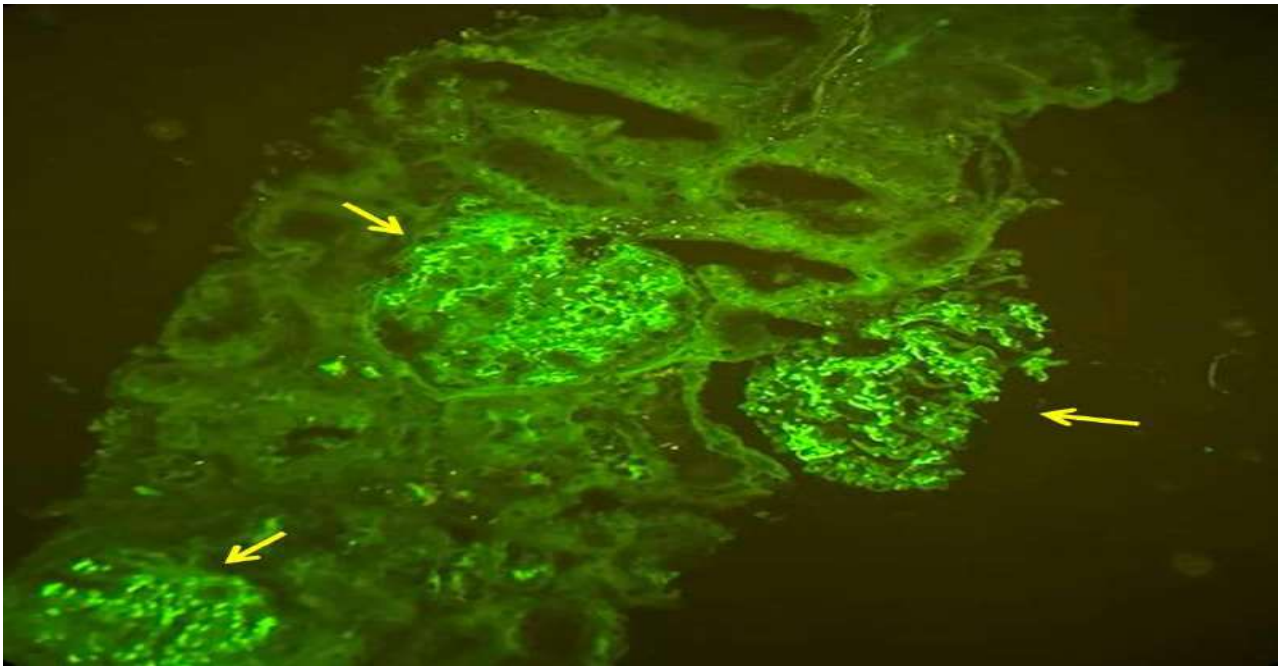


Fig 11 Illumination of IgA

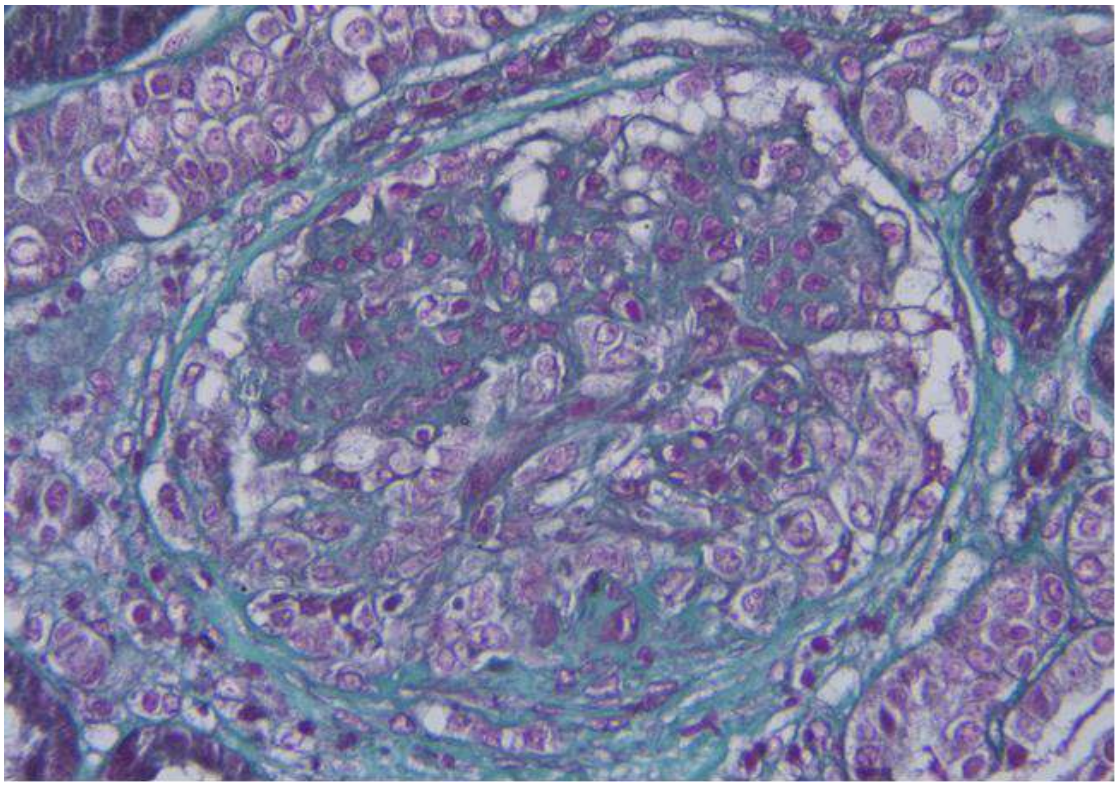


Fig 12 Mesangial hypercelularity PAS

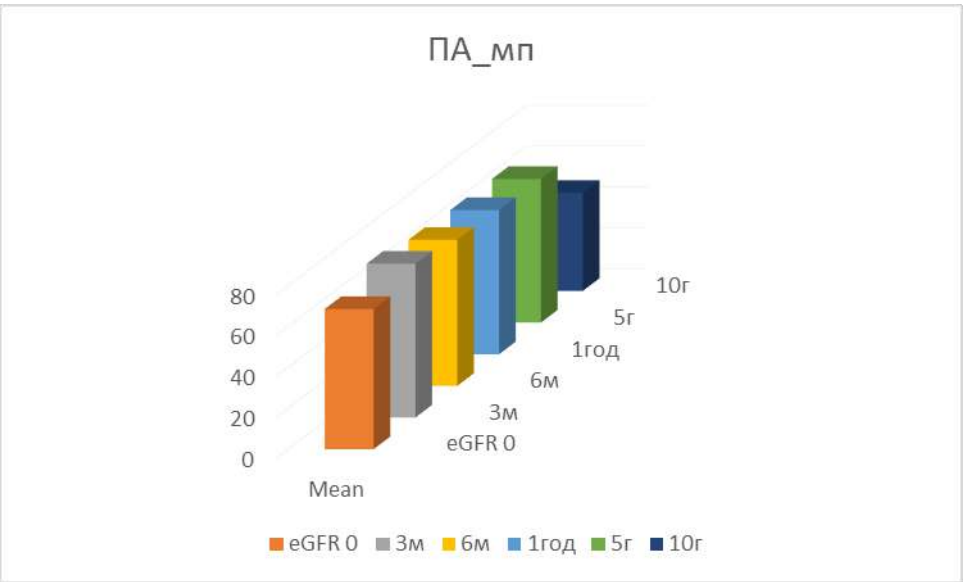


Fig 13 Follow-up mean eGFR values at 3, 6, 12 months, 5 and 10 years

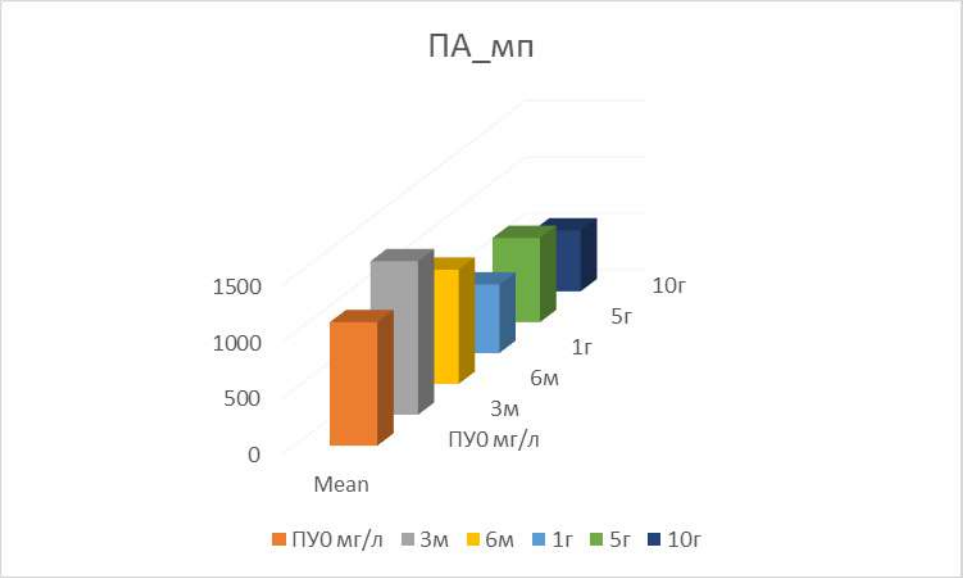


Fig 14 Follow-up mean values of proteinuria at 3, 6, 12 months, 5 and 10 years

3. . Minimal changes – 8 patients were diagnosed with this histological variant and were followed up to the first year. In those, mean eGFR levels at diagnosis were 67 ml/min. An increase in the levels of estimated glomerular filtration was observed at the 3rd, 6th month and at the 1st year. Regarding proteinuria, the mean values at diagnosis were 713 mg/24h, with a decrease in the levels seen at the 3rd and 6th month and an increase at the 1st year. RAAS inhibitor therapy alone was administered in two of these patients. In the rest, it was administered with Methylprednisolone - pulse, then with decreasing doses orally, in 3 cases

Cyclophosphamide, MMF or Azathioprine was added, one of them took Budesonide.

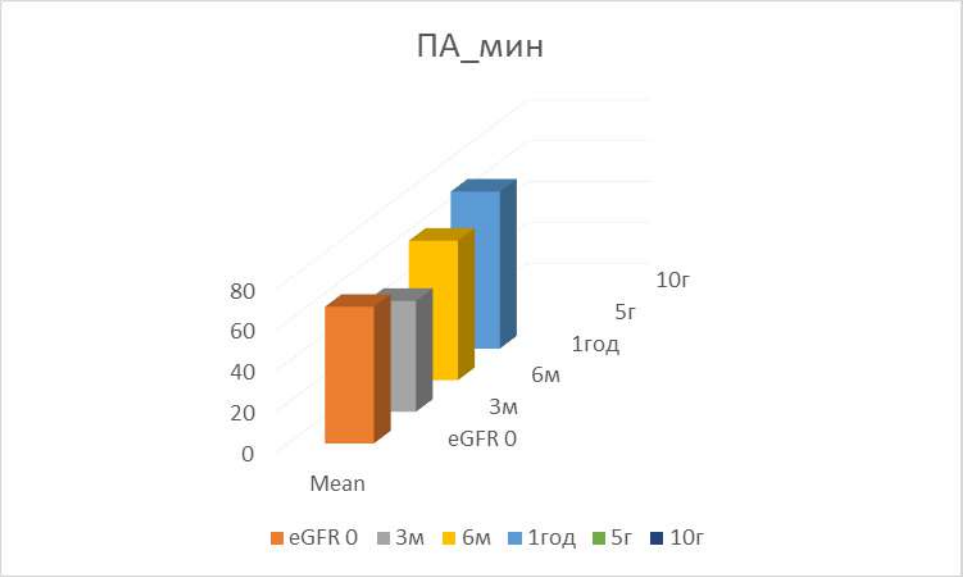


Fig 15 Follow-up mean eGFR values at 3, 6, 12 months, 5 and 10 years

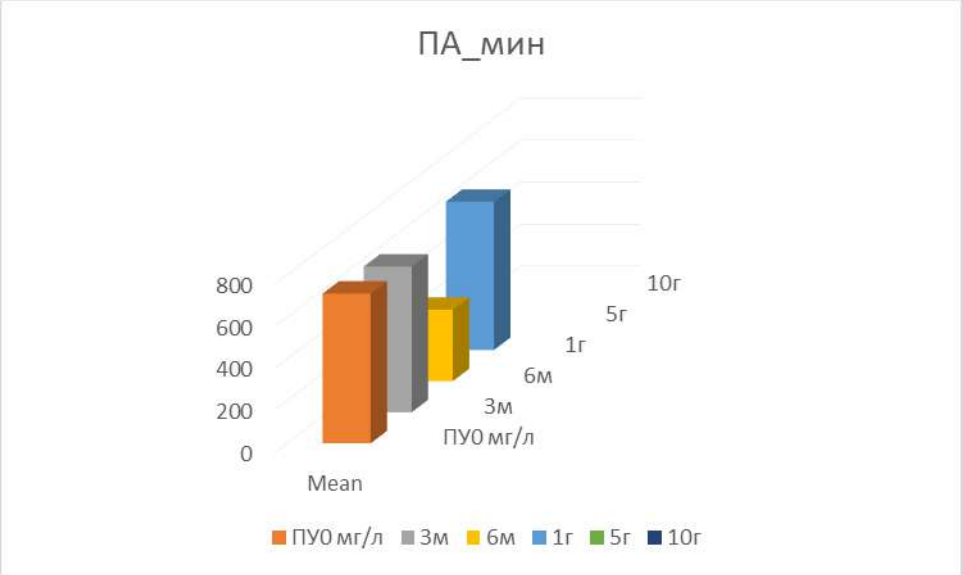


Table 16 Follow-up mean values of proteinuria at 3, 6, 12 months, 5 and 10 years

4. Crescentic - in this group, the patients who meet the histological criteria for semilunar glomerulonephritis were followed, i.e. with the presence of more than 50% crescents in the histological material. 4 people fall here, 1 was followed up

to the 10th year. Mean levels of the calculated glomerular filtration at diagnosis was 44 ml/min, there was an increase in the values at the 3rd month, a decrease at the 6th and 12th, an increase at the 5th year and a decrease again at the 10th year, but eGFR levels were not lower than at disease onset. Mean levels of proteinuria at diagnosis were 2570 mg/24h, with levels falling at 3rd, 6th month, 1st, 5th and 10th year. These patients were treated with pulses of Methyprednisolone, pulses of Cyclophosphamide and/or Mycophenolate or Azathioprine.

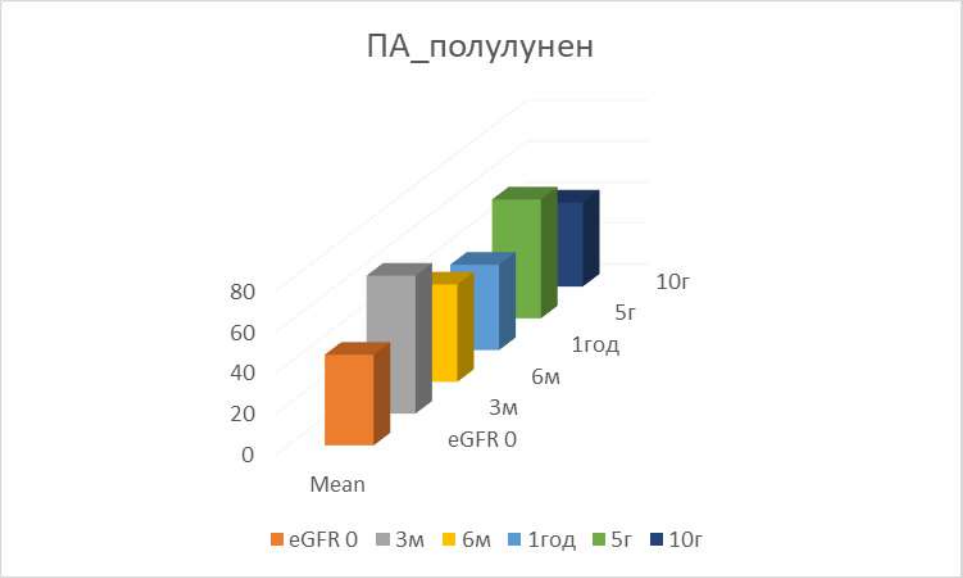


Fig 17 Follow-up mean eGFR values at 3, 6, 12 months, 5 and 10 years

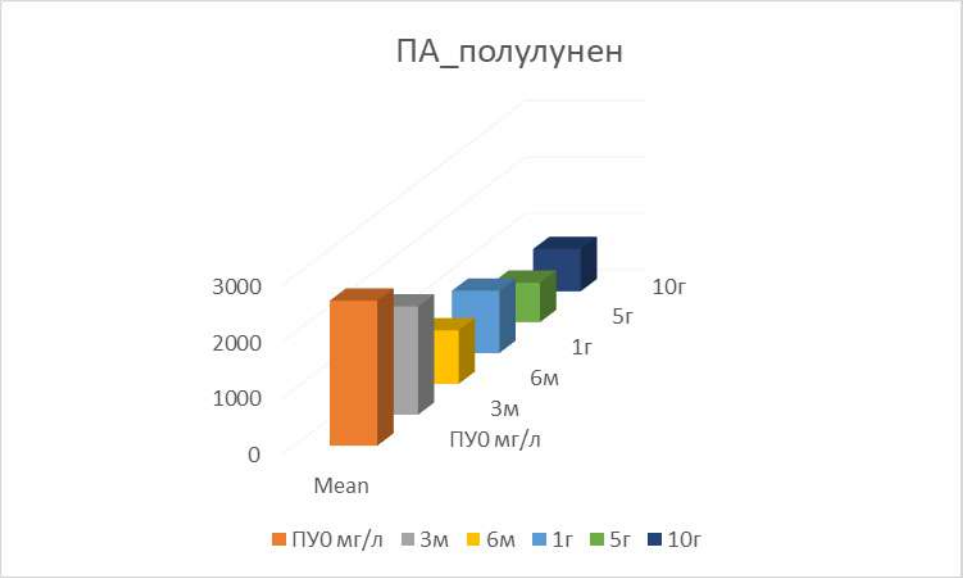


Table 18 Follow-up mean values of proteinuria at 3, 6, 12 months, 5 and 10 years

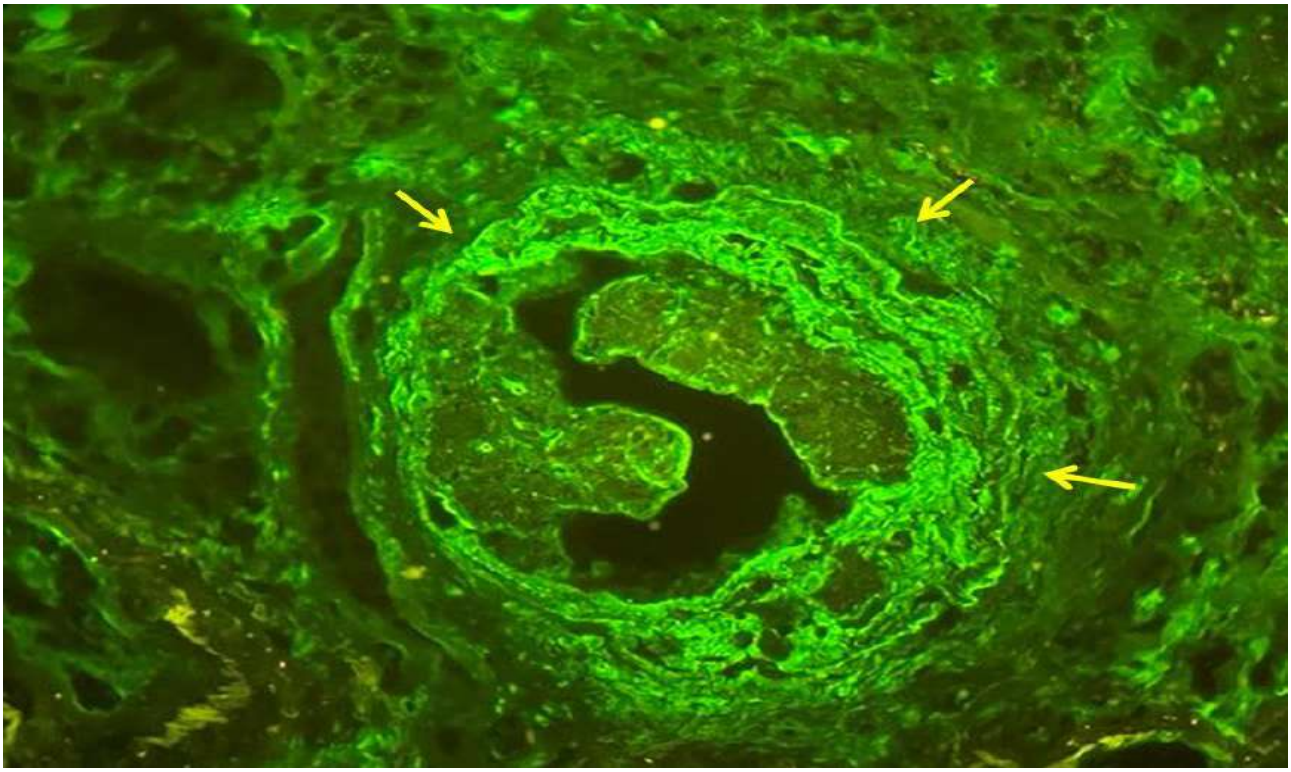


Fig 19 IgA crescent, own archive

5. IgA vasculitis – 8 patients. Mean eGFR levels were 69 ml/min, a slight decline and retention of values was reported in the first year, rising levels in the 5th year. With regard to proteinuria - average values at diagnosis 1255 mg/ml, at the 3rd, 6th month and 1st year there was a decrease in the level of proteinuria, at the 5th year there was an increase and again at the 10th year there was a

decrease. In one of these patients therapy was carried out with RAAS inhibitor only, one patient received CS monotherapy, the others received CS, Cyclophosphamide and MMF or Azathioprine therapy.

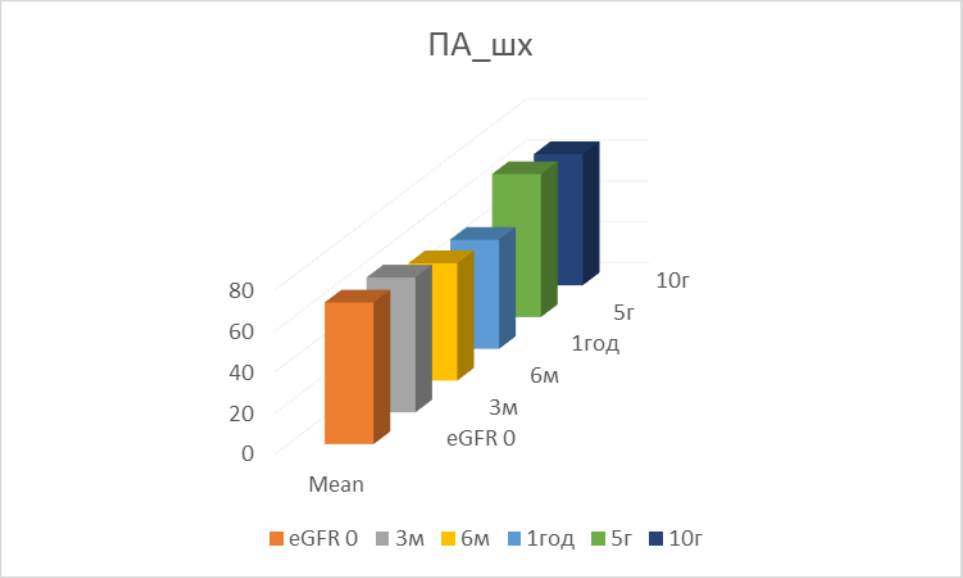


Fig 20 Follow-up mean eGFR values at 3, 6, 12 months, 5 and 10 years

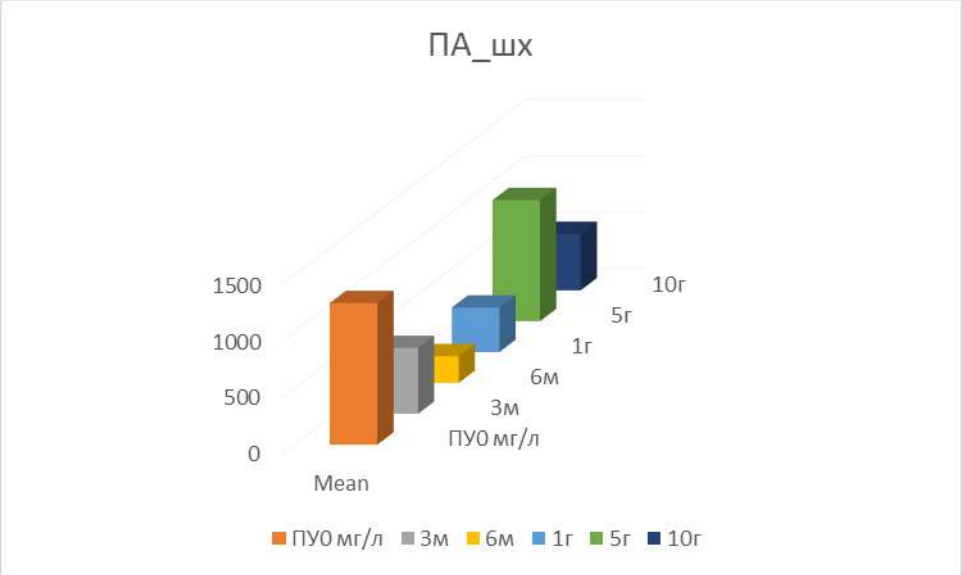


Table 21 Follow-up mean values of proteinuria at 3, 6, 12 months, 5 and 10 years

IMMUNOFLUORESCENCE STUDY

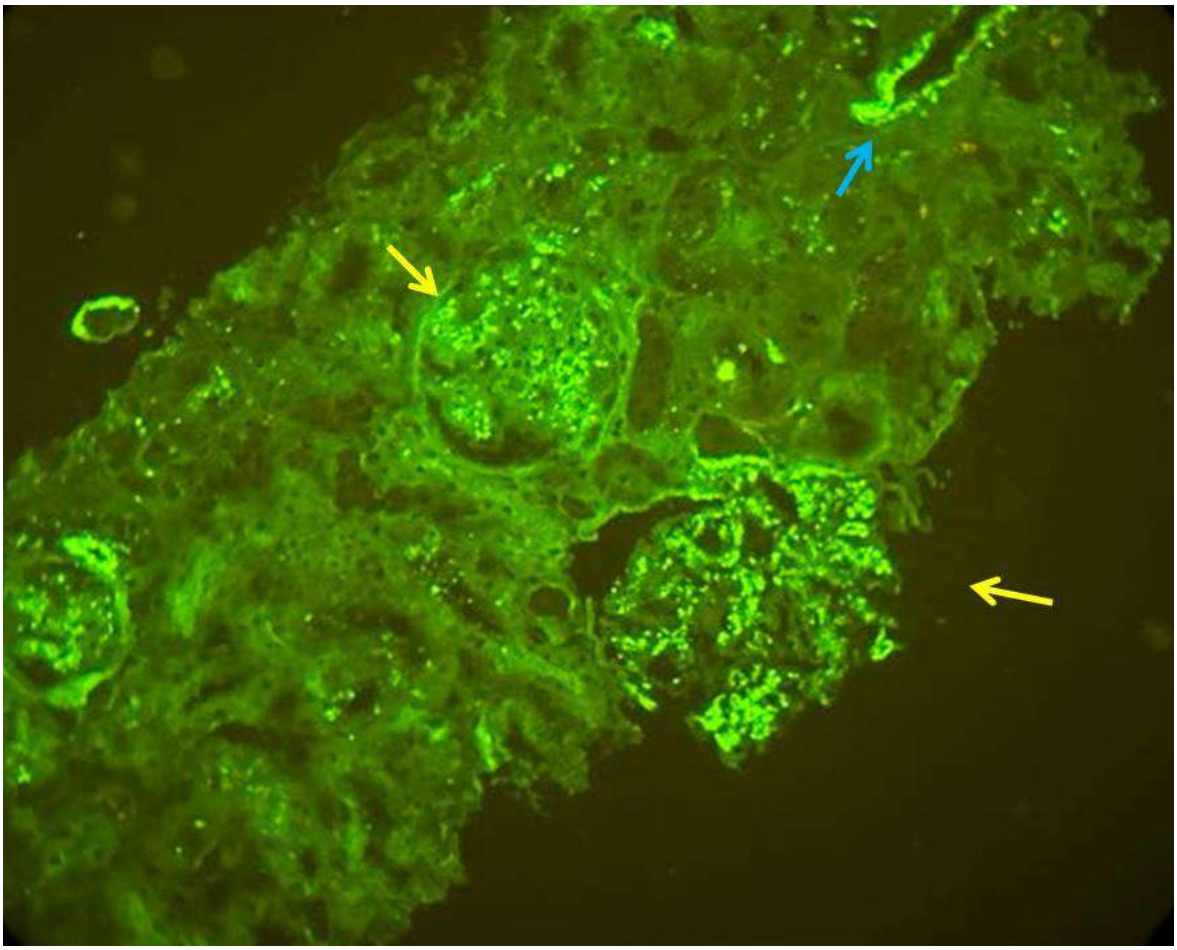


Fig 22 deposition of C3, own archive

In the performed immunofluorescence examination of the histological material, IgG deposition was demonstrated in only two patients.

Deposition of C1q was observed in 15 of the patients / 15.6% of the conducted kidney biopsies/, and deposition of C4 – in 2, and it was always in combination with C1q. C1q deposits mainly prevail in focal-segmental variant - 13, in 1 a mesangial proliferative variant with a crescent is observed, 1 has IgA vasculitis.

Mean level of proteinuria at diagnosis was 2042 mg/24h, and mean eGFR level was 84 ml/min

Only two of these patients had macroscopic hematuria. 10 of the patients have arterial hypertension.

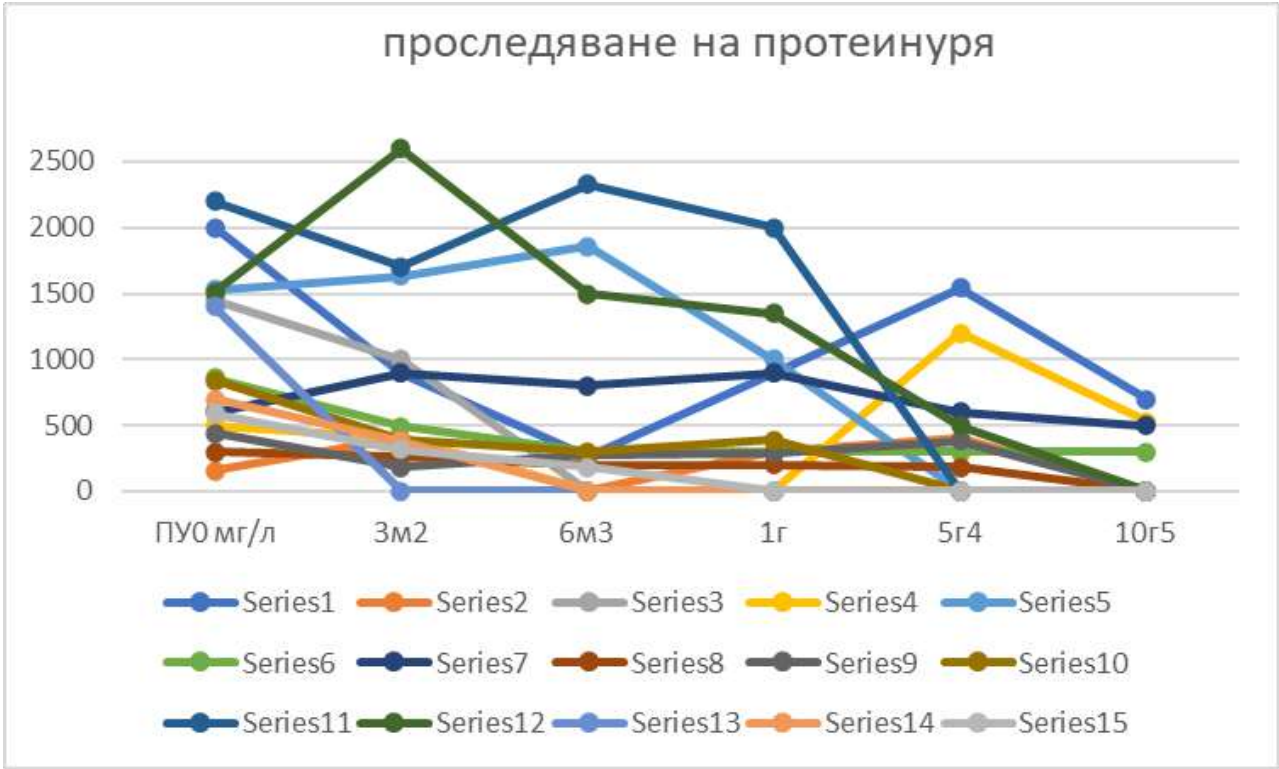


Fig 23 Follow-up of proteinuria in C1q (+) patients

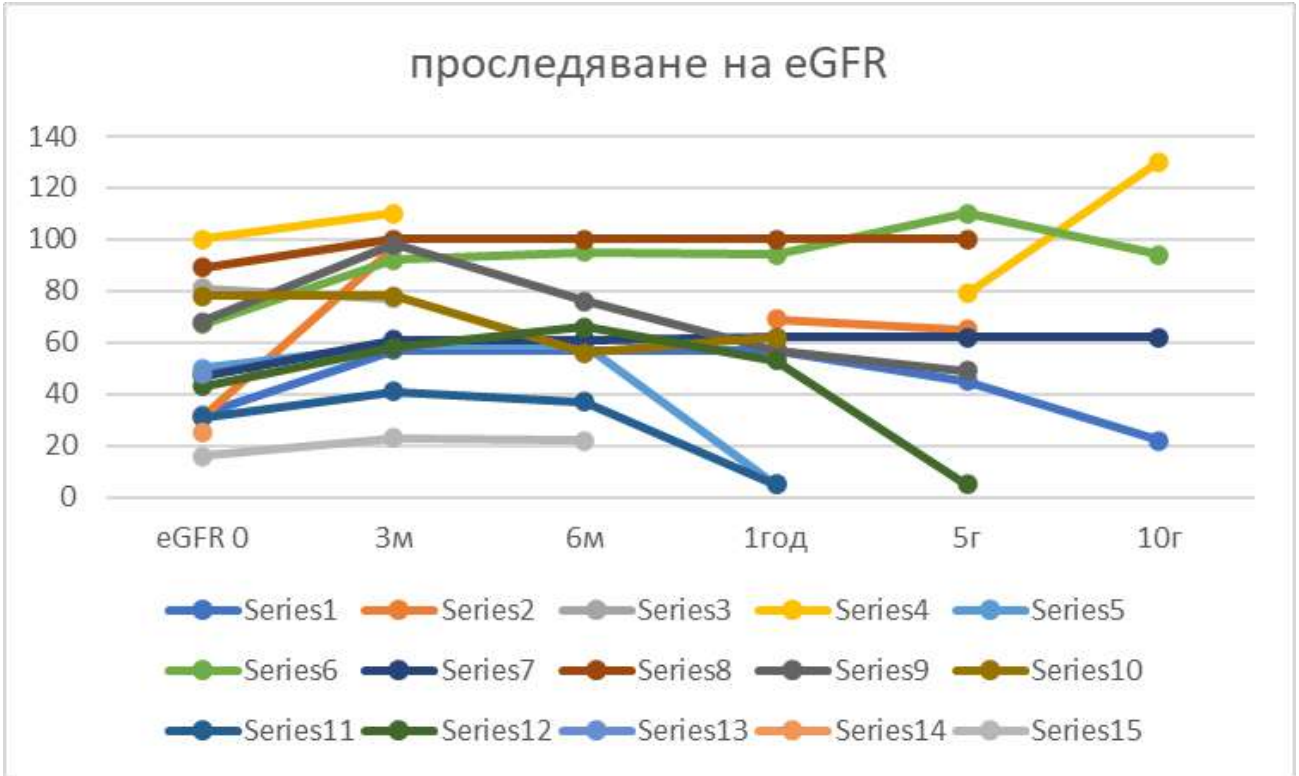


fig 24 Follow-up of eGFR in C1q (+) patients

The clinical, paraclinical and histological manifestations of patients with deposited C1q and the possible correlation with the progression of CKD were followed. A decline in proteinuria was observed after the first year and retention of the values at the 5th and 10th year. Estimated glomerular filtration rates tended to hold values in the first year, with a slight decline in the 5th and 10th years. The follow-up of these values coincided with the follow-up of patients in the corresponding histological groups, confirming that C1q deposits alone cannot be a predictive factor for the progression of renal disease.

7. Measurement of IL-6 serum concentration and comparison of IL-6 levels with some morphological parameters

When analyzing the obtained results, statistically significantly higher IL-6 serum levels were found among the patients compared to the control individuals with mean values of 15.99 pg/mL and 8.54 pg/mL, respectively (p=0.005), fig. 1. In addition, it was found that among the patient group, women (22.37 pg/mL) had higher IL-6 serum levels than men (13.79 pg/mL), without reaching statistical significance (p=0.297).

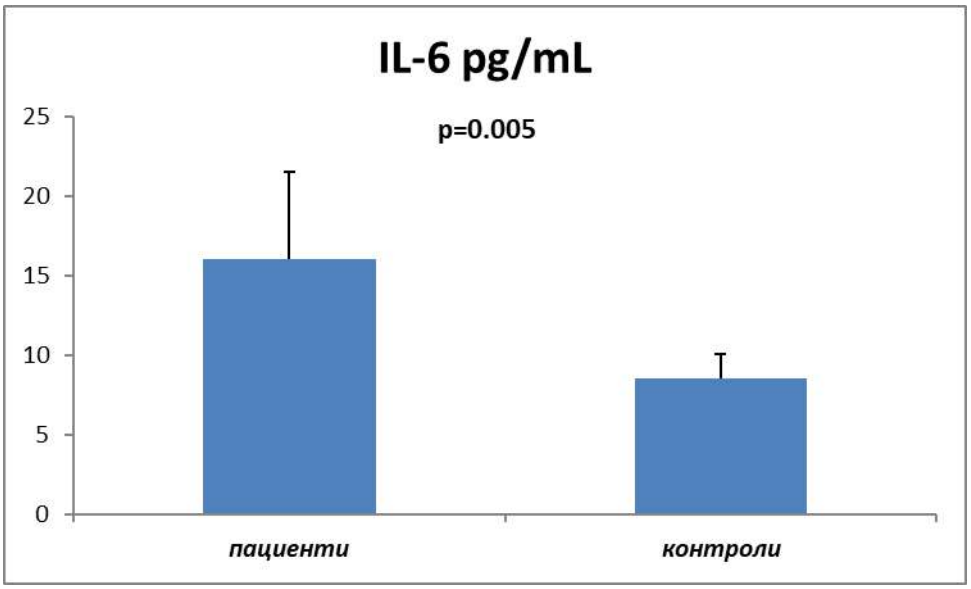


Fig 31 Plot of IL-6 serum levels in IgAN patients and healthy controls

We performed an analysis comparing the data of IL-6 levels and some morphological markers, such as: number of sclerotic glomeruli, degree of mesangial proliferation,

degree of increase in mesangial matrix substance, presence of segmental sclerosis, presence of atrophic tubules and tubular damage, presence of interstitial inflammatory infiltrate and interstitial fibrosis, vascular damage and degree of expression of – IgA, IgG, IgM, C3, C1q and fibrinogen.

After analysis, we found that in 75% of patients with moderate and marked inflammatory infiltrate in the interstitium, higher levels of IL-6 were also observed, while in patients with milder or absent infiltrate, IL-6 levels were also lower. low (p=0.048).

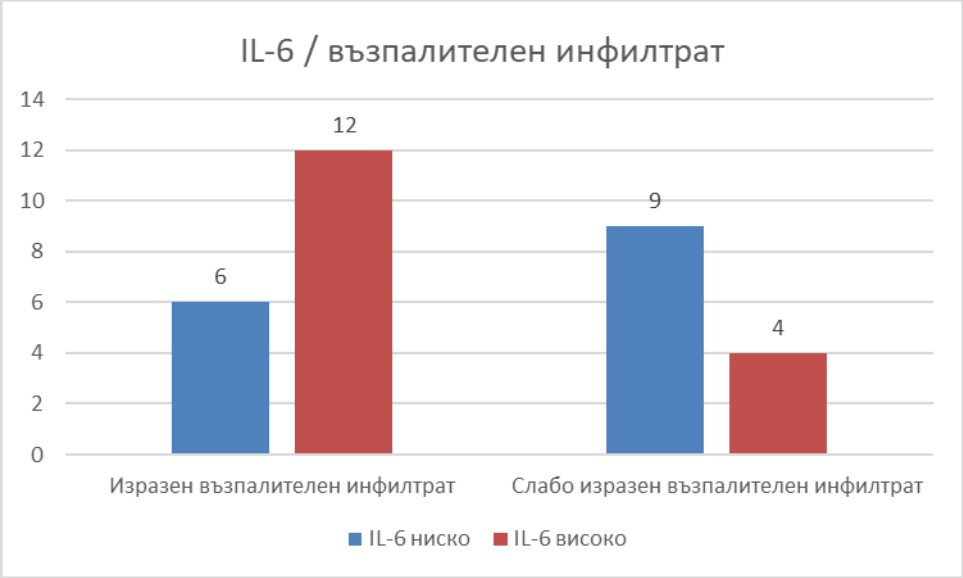


Fig 32 Correlation plot between serum levels of IL-6 and inflammatory infiltrate in kidney tissue

Without statistical certainty, but with a trend, we found that in 81.25% of patients with pronounced segmental sclerosis in glomeruli, IL-6 levels were higher, while only 40% of patients without segmental sclerotic changes or discrete ones had high IL-6 levels (p=0.193).

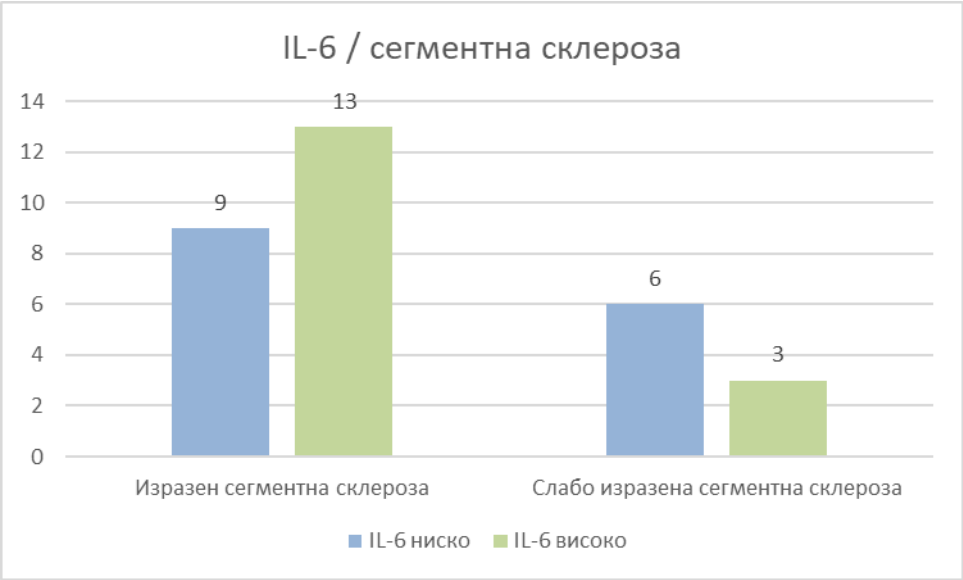


Fig33 Correlation plot between serum levels of IL-6 and segmental sclerosis in kidney tissue

In 80% of patients with an increase in mesangial matrix substance, IL-6 levels were low compared to patients with no change in matrix substance (p=0.081, trend).

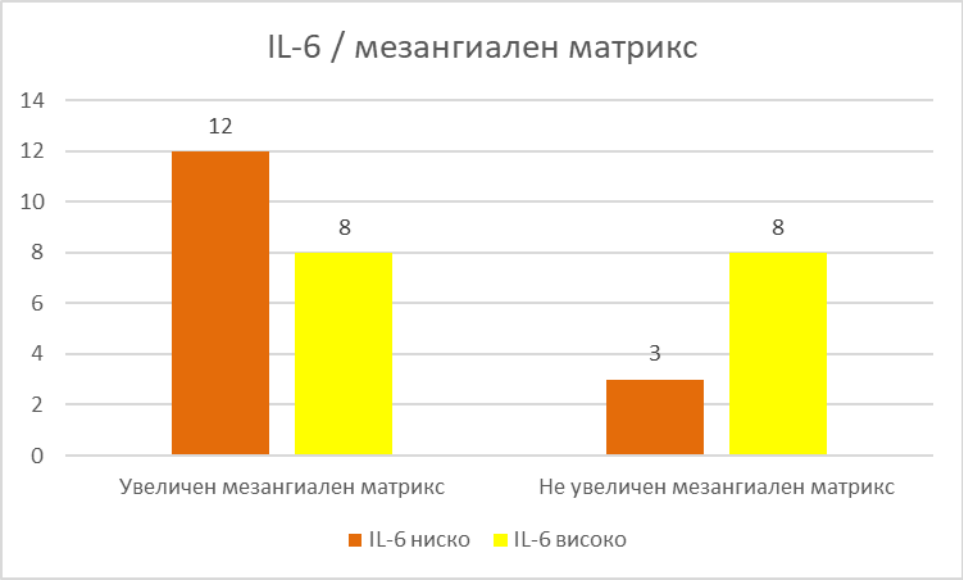


Fig 34 Correlation plot between serum levels of IL-6 and increase in mesangial matrix in kidney tissue

8. Evaluation of therapy:

1. Therapy with only RAAS inhibitor is available in 11 patients, all of them have a proteinuria level below 500 mg/24h; 1 on HD

2. Methylprednisolone - pulse therapy was carried out in 82 patients
3. Cyclophosphamide - 54
4. IMF – 11
5. Azathioprine – 10
6. Advagraph – 1
7. Budesonide – 24, of them only 1 as initiating therapy
8. 17 patients received dapagliflozin.

For the first time in Bulgaria, treatment of patients with IgAN with Budesonide is being introduced at the Nephrology Clinic UMBAL Kaspela. Until December 2023, 24 patients were treated with this medication, and the duration of therapy varied between 3 and 20 months. Of them, 19 are men and 6 are women, aged between 18 and 74. Patients showed good tolerance to the preparation, with no reported side effects.

15 patients were treated with Methylprednisolone and Cyclophosphamide until the start of therapy, 8 had only MP pulses, 1 had only Cyclophosphamide therapy, and in the same patient immunosuppression was started with Budesonide, 7 of them had another immunosuppressive medication added / MMF or AZA/, 4 of them have a crescent in the PBB, 1 has IgA vasculitis. In 1 of the patients, the medication was stopped and treatment with a biological agent was started because of a diagnosis of Ps/PsA. A combination with a RAAS blocker was present in 15 patients, and a combination with an SGLT2 inhibitor in 10 patients, and therapy with these medications was tailored to eGFR.

Table 8. Follow-up of patients treated with Budesonide

N	Name	eGFR 0 M	6M	1г	ПУ 0м	6м	1г2	Хист вариант	Терапия ММФ/АЗА	дапагл	РААС
1	Р.К	43	-	44,2	0,5	-	2,2	Скл с пол	ммф	+	+
2	Н.Н	9,5	-	8,2	0,25	-	0,57	?	-	-	-
3	М.К.	56,4	57,8	70	1	0,64	1,25	?	-	-	+
4	Б.Г.	93,5	-	80,5	0,18	-	0,3	осгс	-	-	+
5	И. Л.							осгс	-	-	+
6	Х.И.	27,7	26,6	-	0,95	-	-	осгс	-	-	+
7	Ш.Х	70	110,2	-	0,78	1	-	осгс	аза	-	+
8	Й.Г.	45	-	48,3	0,38	-	0,11	Мп с пол	аза	+	-
9	И.Б.	37,1	-	-	3,4	-	-	осгс	-	-	-
10	В.С.	26,8	-	-	0,2	-	-	Мин?	-	-	-
11	М.Н.	12,7	-	-	0,6	-	-	Осгс	-	-	-
12	Н.Р.	31	24	27,7	7,56	2,7	3,2	Осгс	-	+	+
13	А.З.	29,1			1,7			осгс	-	+	-
14	Р.Я.	49	-	-	0,42	-	-	Осгс с пл	-	+	-
15	Г.Х.	33,8	36	-	1,6	-	-	мин	хум	+	+
16	Р.М.	34	-	-	0,66	-	-	осгс	-	-	-
17	С.М.	34,6	32,9	-	0,24	0,65	-	?	-	+	+
18	Г.Р.	90	90	90	1,3	0,19	-	осгс	-	-	+
19	П.Б.	20,1	-	-	0,3	-	-	шх	ммф	+	-
20	А.Г.	31,8	-	-	0,72	-	-	осгс	-	+	+
21	Д.Д.	28	-	26,4	0,46	-	1,5	осгс	-	+	+
22	Е.С.	26	26	-	0,12	0,08	-	Осгс с пл	аза	+	+
23	А. Д.							осгс	аза	+	+
24	Р.П.	26	-	23	0,6	-	1,16	Осгс с пл	ммф	+	+

Supportive treatment, beyond ongoing immunosuppression, plays an important role in the therapy of IgAN. Regardless of the initial damaging factor leading to the development of CKD, glomerular hypertension and subsequent hyperfiltration occur. Given the proven effects on glomerular hemodynamics, RAAS inhibitors occupy a central place in the conservative treatment of IgAN and CKD in general. In addition to inhibiting the RAAS system, dapagliflozin (Forxiga), 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. At the Nephrology Clinic of Kaspela UMBAL, we conducted a retrospective study over a period of 6-12 months (approximately 9 months) of the effects of dapagliflozin added to standard immunosuppressive and conservative therapy with RAAS inhibition in patients with histologically proven IgAN. The study cohort included 17 patients - 13 men and 4 women. Only 2 of them had type 2 diabetes. None of the patients reported any side effects from the treatment. Results: During the study period, there was a trend towards a decrease in proteinuria, as well as a stabilization of GFR after a slight decrease in the third month of treatment. In addition to them, there was a drop in arterial blood pressure, a decrease in serum uric acid levels, and an increase in hematocrit. Based on RAAS inhibitor intake, patients were divided into 2 groups - users (12 patients) and non-users (5 patients).

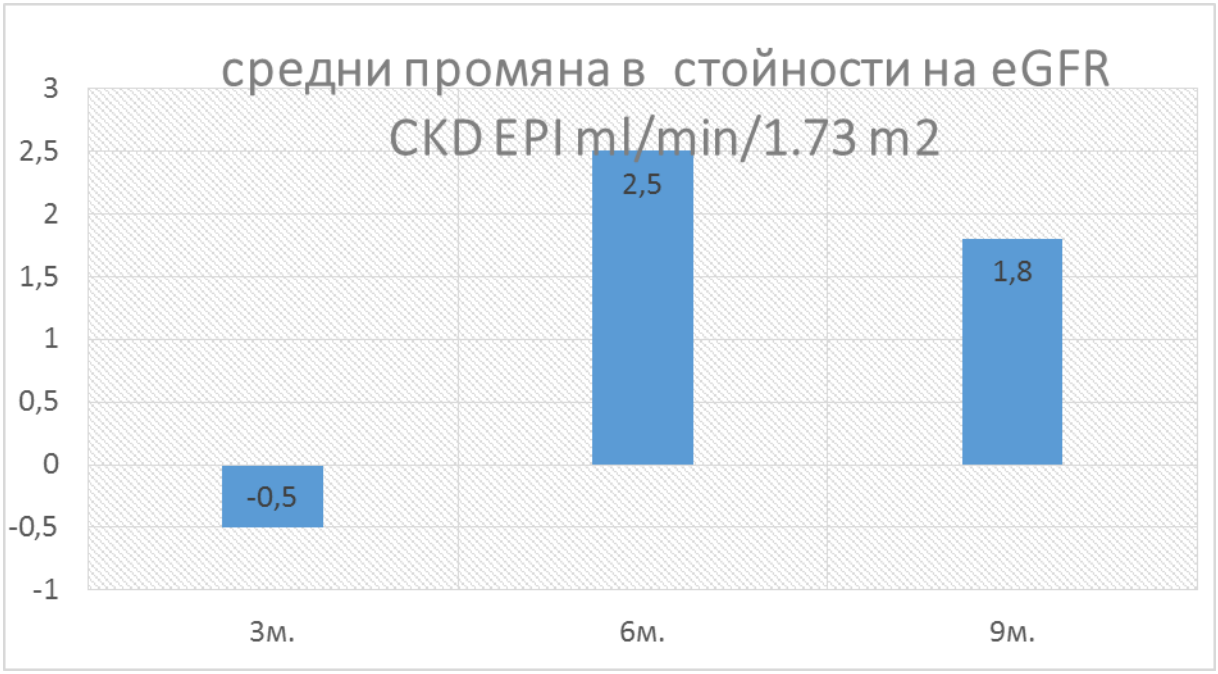


Fig 25 Graphic of changes in mean GFR on the 3rd, 6th and 9th months.

сец.

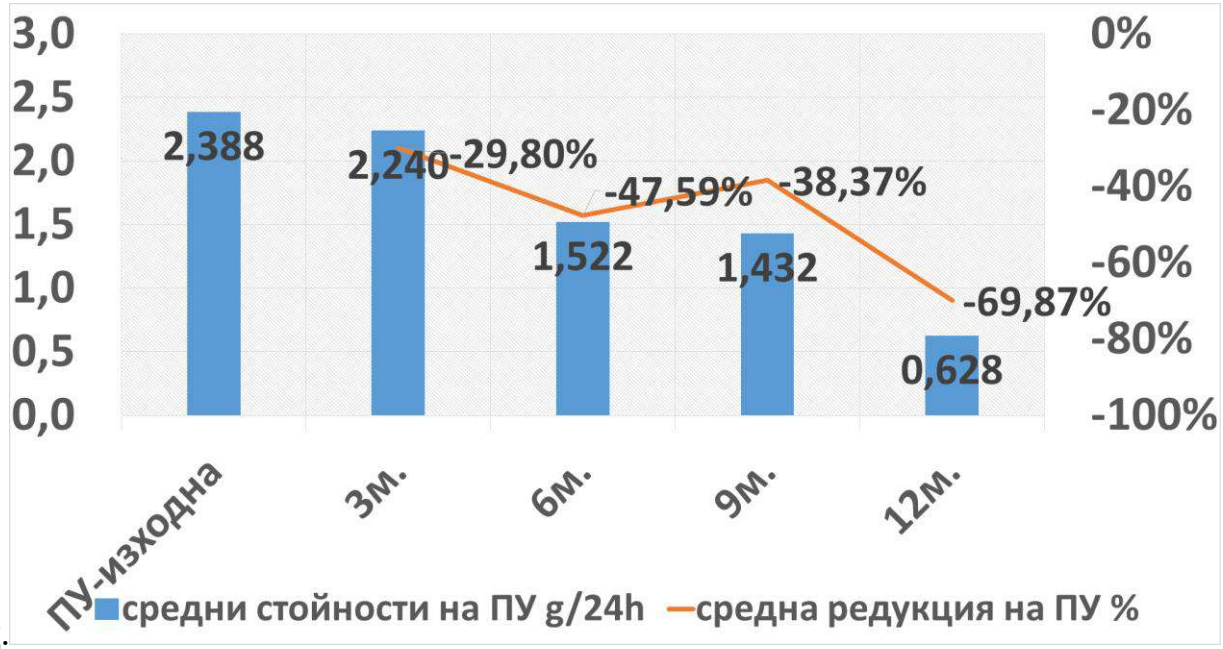


Fig 26 Graphically presented follow-up of proteinuria in IgAN patients treated with dapagliflozin. Blue bars indicate the absolute value of proteinuria in g/L; The orange line indicates the percent reduction in proteinuria compared to control at the beginning of the period

All patients were followed up for a period of 18m+-m to 3 years.

In the first group, divided according to proteinuria, improvement was observed in 16 of the patients, only two of whom received only RAAS inhibition, one received treatment for RA, and all others received pathogenetic treatment with MR. In 7 of the patients after follow-up there was a deterioration in PU/eGFR values.

In the patients of the 2nd group, 16 have improved indicators, 8 worsened

Among patients from the 3rd group, 15 have improved, 19 have worsened values,

In the patients of the 4th group, improvement was observed in 5 and deterioration in 6 of the patients

GLUTEN-FREE DIET

In one of the patients, immunosuppressive treatment was stopped at the first year, after which nephroprotection with RAAS in combination with a gluten-free diet was continued. At year 10, an SGLT2 inhibitor is also added.

We present a case of a woman with biopsy-proven IgAN in 2014 with the result of IgA nephropathy - focal and segmental sclerotic histological variant.

The patient underwent three pulses of CS 250 mg and Endoxan in a total dose of 900 mg immediately after PBB, and since September 2014 she has not taken immunosuppressive and antihypertensive treatment. Since then, he has only followed a gluten-free diet.

Date/labratory	Proteinuria	Spot urine protein and sediment	Urea	Creatinine	HgB
01.2014	0,6 0,54	2+ 10-12 Еритр, 3-4 Левк	5,2	105	123
04.2014	0,87; 0,9	2+ 2-3Е; 4-5Л	4,3	85	135
01.2015	0,24	+/- следи 2-3Л, ед еп кл	7,8	107	137
06.2020	0,34	2+ 1-2 Л и Е	6,9	95	143
04.2022	0,3	+/- следи 1-2Л, пл еп кл	6,8	100	145

Table 9. Follow-up of paraclinical indicators when conducting a gluten-free diet as fat from the therapeutic plan of a patient with IgAN

In this case, we observe a good influence on the clinical and paraclinical indicators of the gluten-free regimen in confirmation of the above-mentioned experimental data. However, the absence of high-grade proteinuria, renal failure and systemic manifestations at the onset of the disease should be considered. This gives us the possibility, when choosing therapy for the milder variants of IgAN, to prefer a gluten-free regimen as an option to maintain proteinuria levels in combination with RAAS blockers before carrying out systemic immunosuppressive therapy.

V. DISCUSSION

IgAN is the most common glomerulopathy among the young population worldwide and therefore has serious social significance. It is one of the leading nosological entities that are the cause of CKD and the end stage of CKD. IgAN is a heterogeneous disease with a wide clinical and histological presentation. The cases of secondary IgAN associated with other immune diseases - rheumatological, of the gastrointestinal tract, after therapy with a biological preparation - are increasingly common. Therefore, more and more global studies are directed to this glomerulopathy and research is being conducted in search of new therapeutic regimens targeting different stages of its pathogenesis. This dissertation evaluates the epidemiology, clinical, histological, and therapeutic outcomes of patients diagnosed and treated at Kaspela UMBAL in the period 2010-2023.

The results of the kidney biopsies performed at the Nephrology Clinic of the "Kaspela" UMBAL Plovdiv show IgAN as the second most common diagnosis in the age group of 18-59 years, second only to OSGS / this group is quite heterogeneous and should not be considered as a separate nosological group unit/, while among the over 60s it is only in 7th place. This frequency decreases even more for cases of primary IgA glomerulonephritis. 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 intra-infectious hematuria, frequency of OBU is confirmed.

The results show a high frequency of immune glomerulopathies in elderly and elderly patients. An active approach is required in the diagnosis of kidney diseases in these patients. IgAN is a relatively rare histological diagnosis in older patients. The clinical picture is presented with leading renal failure with relatively poor urine findings - low to moderate proteinuria and erythrocyturia. Arterial hypertension is almost always present, but is not severe and can easily be classified as essential hypertension. In the diagnosis of IgAN, a thorough investigation for systemic disease, infection, and neoplasia is appropriate, the presence of which is critical to the treatment and outcome

of the disease. Adequate therapy, including corticosteroids, and at discretion immunosuppressants also has a beneficial effect in the presence of renal failure.

The histological examination confirms the distribution of patients according to the light microscopic finding - the most common variant is focal-segmental sclerotic 58%, followed by mesangioproliferative 23.6% and minor damage - 3.6%. Presence of crescent/semilunar glomerulonephritis was observed in 20.9% of cases. In the immunofluorescence study, it is confirmed that the most frequent deposits are from IgA and C3 - 100%, but there is a significant discrepancy in the deposits of IgG - only in 2 of the monitored patients there is a deposit of the same immunoglobulin, in contrast to the reported up to 50% in the foreign literature. C1q is present in 15.6% of patients, which is in confirmation of data from the world literature. They are found only in the sclerotic areas, with the highest percentage being mainly associated with the sclerotic histological variant, again confirming Western studies. C4 is present in 2 patients, never alone, but always in combination with C1q. This incidence of C4 deposits differs from the incidence reported by Segarra A et al in a large retrospective study from Spain. They reported that in 20.4% of IgAN biopsies, deposition of this component of complement was present, accepting C4 deposits as a predictive factor for disease progression. Due to the small number of our patients, it is not possible to draw conclusions about the importance of these deposits in the progression of the disease. Follow-up of eGFR and proteinuria values in these patients at 3m, 6m, 1y, 5y and 10y coincided with the follow-up of patients in the respective histological groups, confirming that C1q deposits alone cannot be a predictive factor for progression of kidney disease.

Examination of IL-6 showed statistically significantly higher serum levels among patients compared to controls, with mean levels in females tending to be higher than in males. 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. Understanding elevated levels of IL-6 and its involvement in the pathogenesis of IgAN may be key to implementing new therapeutic regimens involving IL-6 inhibitors. This is in keeping with the trend for therapy to be based on targeting specific levels of the pathogenesis of the disease, which in turn will enable the selection of specialized medications for individual glomerulopathies.

Furthermore, a trend was found that in 81.25% of patients with marked segmental sclerosis in glomeruli, IL-6 levels were higher, while only 40% of patients without segmental sclerotic changes or discrete ones had high IL-6 levels. Patients with high levels of IL-6 have a marked inflammatory infiltrate in the interstitium.

Differentiation of primary from secondary IgAN plays an essential role in conducting therapy. A good knowledge of the pathogenesis of the disease reveals the connection of IgAN with other immune diseases and, accordingly, the treatment of any of the cases as secondary. We have described several cases of IgAN associated with Ps/PsA, with RA, or secondary to biologic therapy. Psoriasis is a widespread immune disease, affecting a large percentage of the population. The pathogenesis of both diseases have many common elements – involvement of TNF-a, IL-6, 17, 23, etc. Therefore, IgA has been reported as the most common renal involvement in patients with Ps/PsA in most global scientific reports. Our evaluation of these patients confirmed this trend. The behavior in these cases requires a strictly individual approach, an accurate assessment of the activity of the underlying disease and the adequacy of the current therapy. Careful consideration is needed in patients already on some type of therapy, especially a biologic. Depending on these factors, the need to carry out additional immunosuppressive therapy, the choice of medication and whether the current treatment will not be discontinued or replaced with another preparation is assessed.

The increasingly frequent use of biological medicines also leads to a more frequent occurrence of unwanted side effects. The possible appearance of IgAN after therapy with such a preparation should not be ignored. A histological distinction is not possible, therefore this possibility must be considered and therapy reassessed.

It is known that GALT plays an essential role in the pathogenesis of IgAN, mucosal immunity disorders are the basis of the disease. The modern therapy of IgAN is based on a preparation that acts precisely at the level of intestinal mucosal immunity. In the world literature, many common stages in the pathogenesis have been reported, as well as similar molecules - cytokines, receptors, etc., the same for IgAN and chronic inflammatory bowel diseases. (33-35 , 178) We therefore investigated antigliadin antibody levels in relation to the reported similar pathogenetic profiles of celiac disease and IgAN. There was no positive result in any of the examined patients. In

addition, none of the patients with biopsy-proven IgA had inflammatory bowel disease.

The follow-up of the effect of the ongoing treatment shows very good results in the groups of patients at medium and high risk, with the achievement of clinical and complete or partial paraclinical remission and improvement or long-term delay of the progression of CKD in most patients. In patients with semilunar GNs, who are assumed to have a very poor prognosis in terms of renal function and mortality, we achieve good results, especially with early diagnosis and initiation of therapy. With a later start of therapy, the outcome of the disease is unfavorable for renal and overall survival. Concomitant therapy with RAAS and SGLT2 inhibitors contributes to prolongation of renal survival. The follow-up of patients to whose immunosuppressive therapy an SGLT2 inhibitor was added shows very good results. An increase in the values of glomerular filtration, an improvement in the values of proteinuria was reported. These results once again prove the importance of complex nephroprotection in immune nephropathies. Conducting therapy with Budesonide reports a good therapeutic effect with lowering of proteinuria levels and improvement of glomerular filtration. The preparation is well tolerated by patients, which facilitates its long-term use. The ability to deliver targeted oral treatment specific to IgAN, in turn, leads to a lower number of hospitalizations related to the need for intravenous treatment or deterioration of indicators.

Tracking the effect of a gluten-free diet in a patient with IgAN confirms the data on a close relationship between the antigenic irritation of some food allergens and the manifestation of IgAN. Although a dietary regimen alone is not sufficient to achieve remission, it can be added as maintenance once remission is achieved.

Accurate diagnosis by kidney biposy spares patients the potentially toxic and associated with many side effects of corticosteroid and immunosuppressive therapy conducted "blindly"/without histological diagnosis/.

VI. CONCLUSIONS:

1. The conducted research confirms the data that IgAN is the most common glomerulopathy in patients up to 60 years of age with predominantly male involvement.

2. The results in the followed patients show a similar profile of light microscopic diagnoses to those in most large studies in the developed countries of Europe and the USA, but also show some differences in the immunofluorescence study - a low percentage of IgG deposition.

3. Elevated levels of serum IgA were demonstrated only in 2 patients and cannot be a diagnostic criterion

4. The established trend regarding the clinical picture is confirmed - frequency of erythrocyturia, intermittent intra-infectious hematuria, frequency of ACI

5. The follow-up of the effect of the treatment carried out shows very good results in the groups of patients at medium and high risk, and in those with semilunar GN, for whom a very poor prognosis is assumed in terms of renal function and mortality, good results have been achieved, especially in early diagnosis and initiation of therapy.

6. In the patients treated with Budesonide, the data from the studies carried out so far worldwide for a good effect on kidney function are confirmed

7. The trend that the leading renal pathology in Ps/PsA is IgAN is confirmed

8. None of the patients had proven IBD and no positive antigliadin antibodies were detected

9. Therapy for IgAN should be specified according to the leading immune cause, respectively primary or secondary

VII. CONTRIBUTIONS:

1. An analytical evaluation of the histological changes in a large group of patients with IgA nephropathy was made, in which pronounced differences in the immunofluorescent findings with generally accepted data from the literature were established.

2. For the first time in our country, the level of IL6 in patients with IgA nephropathy was investigated and correlations with histological and laboratory changes were established, which are useful for refining the therapy.

3. For the first time in our country, the results of a state-of-the-art therapeutic regimen with Budezonid are presented.

4. A detailed analysis of histological and laboratory parameters of patients with Ps/PsA with IgAn was carried out and the therapeutic approach was evaluated

5. A contribution of a scientific-applied nature is 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 peculiarities of the pathoanatomical finding and accompanying pathology.

ABBREVIATIONS:

ACE – angiotensin converting enzyme

AGA – antigliadin antibodies

APRIL – A proliferation-inducing ligand

BAFF – B-cell activating factor

CIC – circulating immune complexes

eGFR – estimated glomerular filtration rate

FDA – American Federation of Medicines

GALT – gut-associated lymphoid tissue

Gd-IgA1 - defectively glycosylated IgA1

HIV- HIV

HBV – chronic hepatitis B viremia

IBD – immune diseases of the intestine

IgAN – Immunoglobulin A nephropathy

IgA – immunoglobulin A

IgA1 – immunoglobulin A subclass 1

IgA2 - immunoglobulin A subclass 2

IgAV – IgA vasculitis

IL – interleukin

MBL – mannose-binding lectin

MMF – mycophenolate mofetil

Ps - psoriasis

PsA - psoriatic arthritis

RAAS - RAAS inhibitors

TGF-β - transforming growth factor beta

TLR9 - toll-like receptor 9

tTG - tissue transglutaminase

TNF-α - tumor necrosis alpha

SigA - secretory IgA

SGLT2 - sodium-glucose transporter protein 2