Original Paper

The Clinical Significance of Uric Acid and Complement Activation in the Progression of IgA Nephropathy

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Key Words

Biomarkers • Complement • End stage renal disease • IgA Nephropathy • Uric acid

Abstract

Background/Aims: The aim of this study is to investigate the utility of clinical [age, gender, mean arterial pressure (MAP)] and laboratory parameters [eGFR, hemoglobin (Hgb), serum levels of creatinine, uric acid, albumin, proteinuria, hematuria] and also histopathological lesions (Oxford classification parameters, crescents, intensity and pattern of staining for C3, C1Q, IgA, IgG, IgM) as progression markers in patients with IgA Nephropathy (IgAN).

Methods: A total of 111 IgAN patients with a follow-up period >1 year or who reached kidney failure [GFR category G5 chronic kidney disease (CKD)] <1 year were investigated. Primary endpoint was the development of kidney failure or eGFR decline ≥50% from the baseline. Kaplan–Meier and Cox proportional hazards analyses were performed. **Results:** Mean follow-up period was 33±29 months. Thirty-seven (33.3%) patients progressed to kidney failure and 4 (3.6%) patients developed eGFR decline ≥50% from the baseline after a median of 23 and 65 months, respectively. In multivariate Cox regression analysis, baseline levels of Hgb (HR:0.782, 95% CI 0.559-0.973, p=0.037), serum uric acid (HR:1.293, 95% CI 1.023-1.621, p=0.046), eGFR (HR:0.966, 95% CI 0.947-0.984, p=0.004) and intensity of C3 staining (HR:1.550, 95% CI 1.198-1.976, p=0.049) predicted primary endpoint. Serum uric acid level was associated independently with T score (β=0.303, p=0.005) in patients with eGFR>30 ml/min/m². **Conclusions:** Hyperuricemia and the deposition of C3 are independent risk factors for IgAN progression.

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Introduction

IgA nephropathy (IgAN) is the most frequent primary glomerular disease worldwide and an important cause of end stage renal disease (ESRD) [1-3]. Several studies have been performed

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to evaluate the risk factors for progression of renal insufficiency in IgAN. Hypertension, elevated serum creatinine concentration, proteinuria, old age, male sex and the absence of macroscopic hematuria have been found to be independent predictors of an unfavorable outcome [4-7]. Hyperuricemia and a number of proteins related to the complement system also emerged as independent risk factors for progression of IgAN [8-12]. Previous studies suggest the crucial role of the complement system in the pathogenesis of IgAN [8, 9, 12-17]; therefore, quantification of complement factors in serum, urine or renal tissue can be a good marker for disease activity and prognosis.

Recently described Oxford histopathological classification of IgAN identified four parameters that were predictive for progression, namely mesangial or endocapillary hypercellularity as well as renal scarring both in the form of glomerulosclerosis and tubulointerstitial fibrosis [18, 19]. Various studies suggested the significance of some other morphological lesions to complete this classification as well [8, 9]. However, it is still unclear whether the immunostaining features are of predictive value.

The aim of this observational cohort study is to investigate the utility of clinical [age, gender, mean arterial pressure (MAP)] and laboratory markers [estimated glomerular filtration rate (eGFR), hemoglobin (Hgb), serum levels of creatinine, uric acid, albumin, proteinuria, hematuria] as well as histopathological lesions (mesangial and endocapillary hypercellularity, segmental glomerulosclerosis, tubular atrophy/interstitial fibrosis, crescents, intensity and pattern of staining for C3, C1Q, IgA, IgG, IgM) as tools for prediction of progression in patients with IgAN.

Materials and Methods

Patients

A total of 158 patients (86 males, 72 females) with biopsy-proven primary IgAN between ages of 16 and 75 (mean age: 36 ± 12 years) with a follow-up period >1 year or who reached kidney failure [category G5 chronic kidney disease (CKD)] in less than 1 year were evaluated [20]. Their medical records were reviewed and the following information at the time of the renal biopsy was recorded: Patient age, sex, body mass index (BMI), presence or absence of macroscopic hematuria, proteinuria, hypertension, serum HBsAg, anti-HCV, anti-HIV, antinuclear antibodies (ANA), anti-double-stranded DNA (anti-dsDNA) and serum complement levels. Exclusion criteria were patients with diabetes at the time of kidney biopsy ($n=8$), treatment with allopurinol therapy ($n=8$), history of gout disease ($n=3$), solid organ, bone marrow transplant at the time of biopsy, other pre-existing parenchymal kidney disease ($n=3$), diagnosis of Henoch-Schonlein purpura (HSP) ($n=5$), systemic lupus erythematosus, hepatitis B or C infection ($n=7$), HIV infection, malignancy, patients in whom serum complement levels were not available at the time of renal biopsy ($n=4$) and patients with an inadequate biopsy sample with the number of glomeruli ≤ 7 ($n=9$). In this study, in order to include the whole spectrum of cases encountered in clinical practice, patients with a baseline eGFR value of < 15 ml/min per 1.73 m² were also included and this enabled us to test the independent value of pathology and treatment response in cases with most acute course or advanced disease. After exclusions, a total of 111 patients were included in this study.

Body mass index (BMI) was calculated by using the formula of weight/height² (kg/m²). Blood pressure (BP) was measured in the sitting position after 5 min of rest with an ERKA sphygmomanometer (PMS Instruments Ltd., Berkshire, UK) with appropriately sized cuff on the right upper arm. Patients with systolic BP ≥ 140 mm Hg or diastolic BP ≥ 90 mm Hg or those on antihypertensive drugs were considered to be hypertensive. Mean arterial pressure (MAP) was diastolic BP plus a third of systolic BP. Glomerular filtration rate (GFR) was predicted by using Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation [21]. Kidney failure (GFR category G5 CKD) was defined as eGFR < 15 ml/min per 1.73 m² according to KDIGO 2012 Clinical Practice Guideline for the Evaluation and Management of CKD [20]. Hyperuricemia was defined as serum uric acid levels equal to or more than 6.5 mg/dL among women and equal to or more than 7.0 mg/dL among men. Normal serum levels of C3 were defined as 88 to 252 mg/dL for males and 88 to 206 mg/dL for females. Normal serum levels of C4 were defined as 12 to 72 mg/dL for males and 13 to 75 mg/dL for females.

Fasting serum samples for biochemical studies had been obtained between 8.00 a.m. and 8.30 a.m. on the day of kidney biopsy in all cases. Laboratory values including complete blood cell count and serum levels of creatinine, uric acid, albumin, C3 and C4 were measured by standard enzymatic procedures. Urinary protein-to-creatinine ratio (Up/c) in spot urine specimen was used to measure level of proteinuria.

Examinations of the patients were performed in accordance with the good medical and laboratory practices and the recommendations of the Declaration of Helsinki on Biomedical Research involving Human Subjects.

Pathology review

Adequate renal biopsy specimen was defined as having 8 or more glomeruli. All histochemical and immunohistochemical stains were prepared using 3–4 micrometer sections. 0.4–0.6 cm unfixed tissue was frozen with liquid nitrogen for immunofluorescent staining (IgG, IgM, IgA, C1q, C3 and fibrinogen). Immunofluorescent staining findings were graded by using a scale of 0 to 3, including 0, trace, 1+, 2+, 3+. Remaining tissues were fixed in Hollande's fixative, embedded in paraffin, and processed routinely for light microscopic evaluation (hematoxylin and eosin, periodic acid-Schiff, methenamine silver-periodic acid, Masson trichrome, Congo red). Each renal biopsy was scored by the local pathologist (YO) according to the Oxford classification: Mesangial hypercellularity, M0/M1 (< or equal to >50% of glomeruli with >4 mesangial cells/area); endocapillary hypercellularity, E0/E1 (present/absent); segmental glomerulosclerosis, S0/S1 (present/absent); tubular atrophy/interstitial fibrosis, T0/T1/T2 (<25%, 25–50%, >50%). In addition, arterial intimal thickening and extracapillary proliferation with cellular or fibrocellular crescents were categorized as present/absent according to the Oxford report [18, 19]. The pathologist was blinded to clinical data.

Study Endpoints

The primary endpoint of the study was the development of composite kidney failure events defined as: 1) kidney failure (category G5 CKD) (eGFR<15mL/min/1.73 m²); 2) eGFR decline ≥50% from the baseline value as compared to baseline. Follow-up period was considered as the time interval between renal biopsy and the last outpatient visit, death, or kidney failure. The impact of clinical markers (age, gender, MAP) laboratory markers (eGFR, Hgb, serum levels of creatinine, uric acid, albumin; proteinuria and hematuria) and histopathological lesions (mesangial and endocapillary hypercellularity, segmental glomerulosclerosis, tubular atrophy/interstitial fibrosis, crescents, intensity and pattern of staining for C3, C1Q, IgA, IgG, IgM) on primary endpoint were analyzed.

Statistical Analyses

Results are reported as the mean±SD when normally distributed or as the median (interquartile range [IQR]) otherwise. Comparisons of continuous variables between two groups were assessed using the unpaired t test or the Mann–Whitney U test where appropriate. The differences in the proportions of different patient groups were compared by the Fisher's exact test. Univariate survival comparisons were made using the log-rank test. Renal survival times were analyzed with the Kaplan–Meier method and the renal survival time for each patient was computed from baseline evaluation to the last follow-up or the primary endpoint. Variables previously found to affect renal survival were included in the multivariate Cox proportional hazards model. Variables were selected by backward elimination using likelihood ratio tests. Receiver operating characteristic (ROC) analysis was used and the area under curve (AUC) was calculated to assess the predictive power of clinical, laboratory and histopathological markers for the primary endpoint. Calculations were performed using SPSS statistical software (version 15.0; SPSS, Chicago, IL). A p value < 0.05 was considered to be statistically significant. This study was approved by the Istanbul Faculty of Medicine Clinical Studies Board. Patient information was managed according to applicable data protection regulations. This study is registered with ClinicalTrials.gov, number NCT02529722 (August 7, 2015).

Results

Baseline Clinical, Histological and Treatment Features

The baseline clinical and laboratory features are shown in Table 1. History of hypertension was noted in 10 (9%) patients. Histologic findings, according to the Oxford classification are shown in Table 2. Treatment characteristics of the patients are also shown in Table 2. Age, BMI, MAP, Hgb, serum creatinine, uric acid, albumin and eGFR levels, Oxford classification E, S and T scores were similar among the different treatment groups. ACEI/ARB had been used for treating high BP in all hypertensive patients. Only the baseline proteinuria levels of patients given steroid treatment was significantly higher as compared to patients who were administered only ACEI or ARB therapy (p=0.015). The Oxford classification M scores

Table 1. The demographic, clinical and laboratory characteristics of the patients at baseline

Demographic / Clinical Characteristics	(n=111)
Male/Female, n (%)	69 (62.2%) / 42 (37.8%)
Age (years)	35 (16-75)
BMI (kg/m ²)	25.5 (17.3-38.3)
Macroscopic hematuria, n (%)	24 (21.6%)
Systolic BP (mmHg)	130 (90-200)
Diastolic BP (mmHg)	80 (60-130)
MAP (mmHg)	97 (70-147)
Hypertension on admission, n (%)	83 (73.9%)
Laboratory data	
Hemoglobin (g/dL)	12.5 ± 0.17
Serum creatinine (mg/dL)	1.4 (0.5-7.9)
Serum uric acid (mg/dL)	6.47 ± 0.15
Serum albumin (g/dL)	3.94 ± 0.06
eGFR (ml/min per 1.73 m ²)	56 (8-139)
Stages of CKD (ml/min per 1.73 m ²), n (%)	
1 (≥ 90)	24 (21.6%)
2 (60-89)	28 (25.2%)
3 (30-59)	46 (41.4%)
4 (15-29)	10 (9%)
5 (<15)	3 (2.7%)
Proteinuria (Up/c) (g/day)	1.8 (0.17-11)
Urinary protein excretion (g/d), n (%)	
< 0.30	7 (6.3%)
0.30-0.99	15 (13.5%)
1-2.99	64 (57.7%)
≥ 3	25 (22.5%)
Serum complement levels	
Low C3 levels (<88 mg/dL), n (%)	14 (12.6%)
Low C4 levels (<12 mg/dL), n (%)	3 (2.7%)
Abbreviations: BMI: body mass index, BP: blood pressure, MAP: mean arterial pressure, eGFR: estimated glomerular filtration rate, CKD: chronic kidney disease, Up/c: Urinary protein-to-creatinine ratio.	

Table 2. The histologic and treatment characteristics of the patients

Histologic / Treatment Characteristics	(n=111)
Oxford Classification (ref 18,19)	n (%)
M1	48 (43.2%)
E1	24 (21.6%)
S1	70 (63.1%)
Tubular atrophy/interstitial fibrosis	
T0 (<25%)	66 (59.5%)
T1 (25-50%)	31 (27.9%)
T2 (>50%)	14 (12.6%)
Immunohistochemical Staining	
IgG (≥1+)	14 (12.6%)
C3 (1+)	25 (22.5%)
C3 (2+)	36 (32.4%)
C3 (3+)	41 (36.9%)
Treatment	
Fish oil	27 (24.3%)
ACEI/ARB	59 (53.2%)
Only prednisolone	25 (22.5%)
Prednisolone + other immunosuppressive	14 (12.6%)
Other immunosuppressive ^a without prednisolone	13 (11.7%)
Abbreviations: M: mesangial hypercellularity, E: endocapillary hypercellularity, S: segmental glomerulosclerosis, T: tubular atrophy/ interstitial fibrosis, ACEI: angiotensin-converting enzyme inhibitor, ARB: angiotensin receptor blocker. ^a Immunosuppressive agents include cyclophosphamide, mycophenolate mofetil, or others.	

of patients given immunosuppressive treatment were significantly higher as compared to patients who were administered only ACEI or ARB therapy (p=0.031).

Primary Endpoint

Forty-one patients (36.9%) reached the primary endpoint after a median of 46 months (IQR, 23-74). 37 (33.3%) of them progressed to kidney failure after a median of 23 months (IQR, 9-51). 4 (3.6%) of them developed eGFR decline ≥50% from the baseline after a median of 65 months (IQR, 44-145). Renal survival by Kaplan-Meier analysis at 5 and 10 years was 74% and 38%, respectively. In multivariate Cox regression analysis, baseline levels of Hgb (HR:0.782, 95% CI 0.559-0.973, p=0.037), serum uric acid (HR:1.293, 95% CI 1.023-1.621, p=0.046), eGFR (HR:0.966, 95% CI 0.947-0.984, p=0.004) as laboratory markers and intensity of C3 staining (HR:1.550, 95% CI 1.198-1.976, p=0.049) (Figure 1a) as a histopathological marker predicted primary endpoint in IgAN patients. However age, sex, BMI, systolic and diastolic BP levels, serum albumin, proteinuria levels and MEST scores were not found as the predictors of IgAN progression in multivariate Cox regression analysis. Kaplan-Meier analysis of primary endpoint according to serum uric acid levels of patients is shown in Figure 1b. The AUC for Hgb, uric acid, eGFR, proteinuria and intensity of C3 staining were 0.641, 0.686, 0.804, 0.611 and 0.617, respectively (Figure 2). There were no significant differences regarding to primary endpoints between the patients treated only with ACEI or ARB and patients received immunosuppressive treatment.

Characteristics of the patients with normouricemia and hyperuricemia

Overall, 50 patients (45%) presented with hyperuricemia. There were no differences in gender, age, type, BMI, Hgb, serum albumin, C3, C4 and proteinuria levels between normouricemic and hyperuricemic groups (Table 3). The MAP was higher in hyperuricemic patients ($p=0.004$). Hyperuricemic patients had lower mean eGFR levels compared to normouricemic patients ($p<0.001$). There was no difference in use of ACEI/ARB, steroid and other immunosuppressive treatment in hyperuricemic patients compared with normouricemic patients, respectively. The percentage of hyperuricemic patients reaching to primary endpoint ($n=24$, 48%) was significantly higher than the number of patients with normal uric acid levels ($n=17$, 27.8%) ($p=0.029$). Progression to kidney failure was also significantly more common among hyperuricemic patients ($n=24$, 48%) than patients with normal uric acid levels ($n=13$, 21.3%) ($p=0.003$). Regarding histopathological Oxford classification, hyperuricemic patients had higher S and T scores than the normouricemic patients ($p=0.011$ and $p<0.001$, respectively) (Table 3).

Associations among serum uric acid, clinical, laboratory and histopathologic features

The simple correlation analysis revealed that serum uric acid was significantly correlated with age ($r=0.280$, $p=0.001$), male sex ($r=0.312$, $p<0.001$), MAP ($r=0.215$, $p=0.024$), eGFR ($r=-0.524$, $p<0.001$), Oxford classification T score ($r=0.336$, $p<0.001$) (Figure 3) and S score ($r=0.232$, $p=0.014$). Multiple regression analysis with uric acid as the outcome variable, and age, sex, MAP, eGFR, Oxford classification T score and S score as possible predictors was performed and revealed that serum uric acid level was independently associated with sex ($\beta=-0.248$, $p=0.002$) and eGFR levels ($\beta=-0.504$, $p<0.001$). Multiple regression analysis was also performed to understand the association between tubulointerstitial damage and uric acid in patients with $eGFR>30$ ml/min/m². Only serum uric acid level was found to be associated independently with the presence of T scores ($\beta=0.303$, $p=0.005$) when studied in a model with age, gender, BMI, MAP, proteinuria and intensity of C3 staining and age. Serum uric acid levels of patients according to T scores are shown in Figure 3.

Fig. 1. A: Cox-regression analysis of overall renal survival according to the intensity of C3 staining (HR:1.550, 95% CI 1.198-1.976, $p=0.049$). B: Kaplan-Meier analysis of renal survival according to the serum uric acid levels ($p=0.003$).

Fig. 2. ROC Curve. Receiver operating characteristic (ROC) analysis was used to assess the predictive power of baseline uric acid and intensity of C3 staining (Area under the curve; 0.686 and 0.617, respectively).

Characteristics of the patients according to intensity of C3-staining

Table 4 shows the clinical data of the patients at the time of renal biopsy according to the intensity of staining for C3. Patients with higher intensity ($\geq +2$) of C3 staining significantly have lower C3 levels ($p=0.04$). During the follow up period, the proportion of patients reaching to primary endpoint was also significantly higher in this group compared to lower intensity ($\leq +1$) C3 staining group (44% versus 21%; $p=0.04$). The patients either in higher or lower intensity C3 staining groups also received similar treatments which consisted of ACEI/ARB, prednisolone, or other immunosuppressive agents. Regarding histopathological Oxford classification, the M, E, S and T scores were similar between groups.

Associations between C3 staining, clinical and laboratory features

The simple correlation analysis between clinical variables and intensity of C3 staining revealed that intensity of C3 staining was significantly correlated with age ($r=-0.25$, $p=0.009$) and serum C3 levels ($r=-0.326$, $p<0.001$). Multiple linear regression analysis with intensity of C3 staining as the outcome variable, age and lower C3 levels as possible predictors was performed and revealed that intensity of C3 staining was independently associated only with serum C3 levels ($\beta=-0.25$, $p=0.009$).

Discussion

In this study, we identified higher serum uric acid levels and moderate-severe mesangial C3 deposition as independent predictors of IgAN progression. ROC curve analysis demonstrated that high levels of serum uric acid and C3 staining had a better predictive value for renal outcome compared to proteinuria.

Hyperuricemia appears to be a common manifestation in IgAN patients, even when their GFR are normal and has been considered as a risk factor for IgAN progression [10, 11, 22, 23]. However, a clear evaluation of the role of uric acid with histopathological Oxford classification on IgAN progression is still lacking and the causative role of uric acid in IgAN progression

Table 3. Characteristics of the patients with normal uric acid levels or hyperuricemia

Demographic / Clinical Characteristics	Hyperuricemia (n=50)	Normouricemia (n=61)	p value
Male/female	32 (64%) / 18 (36%)	37 (61%) / 24 (39%)	0.72
Age (years)	38 $\pm$ 12	37 $\pm$ 12	0.61
BMI (kg/m ²)	26.5 $\pm$ 3.2	25.3 $\pm$ 3.3	0.06
Macroscopic hematuria, n (%)	9 (18%)	15 (25%)	0.40
MAP (mmHg)	105 $\pm$ 18	96 $\pm$ 13	0.004
Laboratory data			
Hemoglobin (g/dL)	12.3 $\pm$ 1.9	12.6 $\pm$ 1.8	0.30
Serum creatinine (mg/dL)	2.0 $\pm$ 1.2	1.4 $\pm$ 0.8	0.001
Serum uric acid (mg/dL)	7.8 $\pm$ 0.9	5.4 $\pm$ 1.1	<0.001
Serum albumin (g/dL)	3.9 $\pm$ 0.5	4.0 $\pm$ 0.7	0.40
eGFR (ml/min per 1.73 m ²)	47 $\pm$ 22	76 $\pm$ 33	<0.001
Proteinuria (Up/c) (g/day)	2.2 $\pm$ 1.3	2.2 $\pm$ 2.1	0.97
Serum complement levels n (%)			
Low C3 levels (<88 mg/dL)	7 (14%)	7 (12%)	0.69
Low C4 levels (<12 mg/dL)	-	3 (5%)	0.11
Oxford Classification (ref 18,19)			
M1	25 (50%)	23 (38%)	0.19
E1	11 (22%)	13 (21%)	0.93
S1	38 (76%)	32 (53%)	0.011
Tubular atrophy/interstitial fibrosis n (%)			
T0 (<25%)	21 (42%)	45 (74%)	<0.001
T1 (25-50%)	17 (34%)	14 (23%)	<0.001
T2 (>50%)	12 (24%)	2 (3.3%)	<0.001
Primary endpoints n (%)			
*Event	24 (48%)	17 (28%)	0.029
Kidney failure	24 (48%)	13 (21%)	0.003
eGFR decline $\geq$ 50% from baseline	-	4 (7%)	

*Composite kidney failure events defined as: 1) Kidney failure (eGFR<15mL/min/ 1.73 m²); 2) eGFR decline $\geq$ 50% from the baseline value as compared to baseline. Abbreviations: BMI: body mass index, MAP: mean arterial pressure, eGFR: estimated glomerular filtration rate, M: mesangial hypercellularity, E: endocapillary hypercellularity, S: segmental glomerulosclerosis, T: tubular atrophy/interstitial, Up/c: Urinary protein-to-creatinine ratio.

remains to be established. Hyperuricemia is also well known to be associated with hypertension, endothelial dysfunction and development of cardiovascular and kidney diseases [24-27]. Previous studies suggested that uric acid induces proliferation of vascular smooth muscle cells, activates the renin-angiotensin-aldosterone system and also reduces the synthesis of nitric oxide; while at the same time increases inflammation and oxidative stress [26, 28, 29]. In animal studies, hyperuricemia was demonstrated to cause renal injury by leading to Th1/Th2 cell polarization and extracellular matrix gene expression [30]. All of these uric acid related changes might also have a role in the pathogenesis and progression of IgAN.

IgAN is characterized by a variation of pathological features, especially variable tubulointerstitial lesions from almost normal to diffuse tubular atrophy and interstitial fibrosis [18, 19]. Tubulointerstitial damage has been reported to be an important risk factor on progression of IgAN [18, 19]. In the present study, we systematically evaluated the pathological changes of 111 individuals with IgAN by Oxford classification system [18, 19], and found that tubular atrophy/interstitial fibrosis were associated with serum uric acid levels. Higher serum uric acid levels indicated higher tubulointerstitial scores, even in patients with eGFR > 30 ml/min/m². The present study confirms the findings of previous studies that hyperuricemia could be a marker to predict tubular atrophy/interstitial fibrosis in IgAN [11, 23]. Tubular excretion of uric acid plays a major role in the removal of uric acid from the body [31, 32]. Hyperuricemia could be considered a secondary consequence of the tubular damage and thus a biomarker of disease severity.

The contribution of the complement system to augment the inflammatory cascade and potentiate tissue injury in IgAN has been suggested by several studies [5, 8, 9, 12-17], but the precise pathways of complement activation remain largely unknown. The present study showed that intensity of mesangial C3 staining in the baseline histopathological assessment, a correlate of the complement system activation, is a predictor for IgAN progression. Deposition of IgA1 in mesangial areas is usually accompanied by complements including C3, C4d, C4-binding protein, mannose-binding lectin (MBL), C5b-9, and properdin [12-16, 19, 33-35]. The deposited immune complexes can activate alternative and lectin pathways [13-16], and initiate the inflammatory process. Similarly, a recent study also found that decreased circulating C3 levels and mesangial C3 deposition was associated with renal outcome in patients with IgAN [9]. In another study by Roos et al. [35], glomerular deposition and urinary levels of MBL has been suggested as a reliable non-invasive biomarker for evaluation of IgAN severity. In the same study, C4d deposition and urinary MBL were also suggested as biomarkers for prediction of the prognosis of IgAN [35]. These studies supported the crucial roles of complements during the disease process, and the assessment of complement factors in serum, urine or renal tissue could be a good marker for disease activity, prognosis, and even inform treatment with immunosuppressive agents. In fact, C3 staining is routinely performed in renal biopsies and the interpretation of C3 staining is simple and relatively specific, thus intensity of C3 staining may be a good marker for prediction of IgAN prognosis.

Although proteinuria is a known risk factor for the progression of IgAN [4-7], after a multivariate Cox regression analysis, the baseline proteinuria was not associated with renal outcomes. There are several important questions regarding the role of proteinuria at the time of biopsy in the prognosis of IgAN. Some studies have proven that proteinuria levels at diagnosis is often not a predictor of the outcome according to a Cox regression analysis [36, 37]; instead, these studies suggested that time average proteinuria levels that represents the average level of proteinuria during the follow-up and proteinuria levels at 1 year or later may better indicate the prognosis [36, 37]. In the present study, multivariate Cox analysis also showed no predictive value of baseline hypertension and MEST scores. Although this result was in contrast to

Fig. 3. Serum uric acid levels of patients according to Oxford classification T scores ($p=0.004$).

previous reports, in the European VALIGA and the Korean cohorts the MEST scores were not found associated with outcome in patients who received steroid/immunosuppressive drugs [19, 38]. The predictive effect of MEST and hypertension may be blunted in the present cohort in which 46.3% of the patients received steroid/immunosuppressive treatment. In the present study, the patients with more severe proteinuria were more likely to be treated with immunosuppressive therapy, which may also impact the predictive value of baseline proteinuria levels in renal outcomes. Post-biopsy therapeutic maneuvers are likely to modulate the predictive value of some pathological and clinical risk factors.

In the present study, the outcome of patients with IgAN seems to be worse than the previously published large multicenter study cohorts [18, 19]. However, as one of the most significant predictor of renal survival is baseline eGFR, the lower baseline eGFR value in the present cohort compared to these multicenter study cohorts may have contributed to this poor outcome [18, 19].

The present study has some limitations. Firstly, the relationship between C3 staining and progression of renal damage must be interpreted cautiously in terms of association rather than causality. Second, only baseline serum C3 and C4 levels were measured in this study, however monitoring serum C3 and C4 levels at different time points can be more useful to evaluate complement system activity and disease prognosis.

Conclusion

In conclusion, this study revealed that hyperuricemia and the deposition of C3 are independent risk factors for renal survival in IgAN. These findings are consistent with the possibility that complement activation and uric acid may be involved in the pathogenesis of IgAN. The level of serum uric acid is a hopeful clinical marker indicating tubulointerstitial lesions and predicting progression in patients with IgAN. It appears possible that high serum uric acid may independently cause progression in IgAN by contributing tubulointerstitial damage.

Table 4. Characteristics of the patients according to histopathological intensity of C3 staining

Demographic / Clinical Characteristics	C3 staining (≥2+) (n=77)	C3 staining (≤1+) (n=34)	p value
Male/Female	47 (61%) / 30 (39%)	22 (65%) / 12 (35%)	0.71
Age (years)	36 ± 12	39 ± 13	0.20
BMI (kg/m ²)	25.5 ± 3.1	26.5 ± 3.7	0.11
Macroscopic hematuria, n (%)	19 (25%)	5 (15%)	0.24
MAP (mmHg)	99 ± 15	102 ± 17	0.34
Laboratory data			
Haemoglobin (g/dL)	12.3 ± 1.8	12.7 ± 1.9	0.29
Serum creatinine (mg/dL)	1.7 ± 1.1	1.6 ± 0.9	0.58
Serum uric acid (mg/dL)	6.5 ± 1.8	6.5 ± 1.5	0.92
Serum albumin (g/dL)	4.0 ± 0.5	3.8 ± 0.7	0.20
eGFR (ml/min per 1.73 m ²)	62 ± 32	65 ± 34	0.68
Proteinuria (Up/c) (g/day)	2.2 ± 1.9	2.3 ± 1.5	0.80
Serum complement levels n (%)			
Low C3 levels (<88 mg/dL)	13 (17%)	1 (3%)	0.04
Low C4 levels (<12 mg/dL)	4 (3%)	-	0.24
Oxford Classification (ref 18,19)			
M1	35 (46%)	13 (38%)	0.48
E1	19 (25%)	5 (15%)	0.24
S1	50 (65%)	20 (59%)	0.54
Tubular atrophy/interstitial fibrosis n (%)			
T0 (<25%)	45 (58%)	21 (62%)	0.37
T1 (25-50%)	24 (31%)	7 (21%)	0.37
T2 (>50%)	8 (10%)	6 (18%)	0.37
Primary endpoints n (%)			
*Event	34 (44%)	7 (21%)	0.02
Kidney failure	30 (39%)	7 (21%)	0.05
eGFR decline ≥ 50% from the baseline	4 (5%)	-	

*Composite kidney failure events defined as: 1) Kidney failure (eGFR<15mL/min/ 1.73 m²); 2) eGFR decline ≥ 50% from the baseline value as compared to baseline. Abbreviations: BMI: body mass index, MAP: mean arterial pressure, eGFR: estimated glomerular filtration rate, M: mesangial hypercellularity, E: endocapillary hypercellularity, S: segmental glomerulosclerosis, T: tubular atrophy/interstitial, Up/c: Urinary protein-to-creatinine ratio.

Disclosure Statement

All authors declare no conflicts of interest.

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