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ORIGINAL ARTICLE

Predictors of poor kidney outcome in children with C3 glomerulopathy

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Abstract

Background C3 glomerulopathy (C3G) is characterized by heterogeneous clinical presentation, outcome, and predominant C3 accumulation in glomeruli without significant IgG. There is scarce outcome data regarding childhood C3G. We describe clinical and pathological features, treatment and outcomes, and risk factors for progression to chronic kidney disease stage 5 (CKD5) in the largest pediatric series with biopsy-proven C3G.

Methods Sixty pediatric patients with C3G from 21 referral centers in Turkey were included in this retrospective study. Patients were categorized according to CKD stage at last visit as CKD5 or non-CKD5. Demographic data, clinicopathologic findings, treatment, and outcome data were compared and possible risk factors for CKD5 progression determined using Cox proportional hazards model.

Results Mean age at diagnosis was 10.6 ± 3.0 years and follow-up time 48.3 ± 36.3 months. Almost half the patients had gross hematuria and hypertension at diagnosis. Nephritic-nephrotic syndrome was the commonest presenting feature (41.6%) and 1/5 of patients presented with nephrotic syndrome. Membranoproliferative glomerulonephritis was the leading injury pattern, while 40 patients had only C3 staining. Patients with DDD had significantly lower baseline serum albumin compared with C3GN. Eighteen patients received eculizumab. Clinical remission was achieved in 68.3%. At last follow-up, 10 patients (16.6%) developed CKD5: they had lower baseline eGFR and albumin and higher frequency of nephrotic syndrome and dialysis requirement than non-CKD5 patients. Lower serum albumin and eGFR at diagnosis were independent predictors for CKD5 development.

Conclusions Children with C3G who have impaired kidney function and hypoalbuminemia at diagnosis should be carefully monitored for risk of progression to CKD5.

Keywords Complement · CKD stage 5 · Children · C3 glomerulopathy · Predictors

Introduction

C3 glomerulopathy (C3G) is a rare disease characterized by the accumulation of complement factors in glomeruli, due to abnormalities in the alternative pathway of complement, which leads to dominant glomerular C3 staining with slight or without deposition of immunoglobulins on immunofluorescence (IF) microscopy, as well as a membranoproliferative pattern of injury on light microscopy (LM). It is divided into dense deposit disease (DDD) and C3 glomerulonephritis (C3GN) according to electron microscopic (EM) findings

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[1–3]. In C3GN, C3 deposits are scattered in the mesangium and capillary walls, whereas in DDD, C3 deposits are found more intensely in the mesangium and glomerular basement membrane and form a unique ribbon-shaped band [4]. C3G presents with various symptoms ranging from a mild disease, such as asymptomatic microhematuria and/or mild proteinuria, to a serious disease such as nephritic/nephrotic syndrome and severe acute kidney injury requiring dialysis [4].

C3G has a chronic and progressive course resulting in stage 5 chronic kidney disease (CKD 5) in 10–29% of patients [4–6]. Besides variable clinical symptoms and poor outcome, there is no consensus on the treatment of C3G. In recent years, complement targeting agents are increasingly being used [7]. Compared with adults, children with C3G show higher rates of hypertension, nephrotic syndrome at presentation, lower degrees of tubulointerstitial fibrosis, and better response to immunosuppressive (IS) treatment [4, 8–10]. However, we have recently shown that the treatment response and prognosis of C3G remain unsatisfactory [11]. As with our recent study, most of the data in previous studies were obtained from small series of patients. This retrospective multicenter study was conducted to investigate clinical disease course and to define predictive factors of poor kidney outcome in the largest pediatric series with biopsy-proven C3G.

Methods

Study design and study population

The Turkish C3G working group in children was created under the Turkish Society of Pediatric Nephrology in 2017, and all data were entered into a password-restricted web database (www.e-cnbs.com). Details of study design, data collection, and study populations are described in the Supplementary data. C3G was defined based on the 2013 C3G consensus guidelines [1]. Twelve patients from our previous cohort [11] were included in the recent data. The study protocol was approved by the local institutional Ethics Committee (2017/209).

Definitions

Details of the definitions used in the present study are given in the Supplementary data. Given the lack of definition of clinical remission in children with C3G, we used modified criteria described by Rabasco et al. [4]. Complete remission (CR) was defined as normal serum albumin (> 3 g/dL) and eGFR (> 90 mL/min/1.73 m^2) without proteinuria (< 4 mg/m^2/h or PCR < 0.2 mg/mg), and partial remission (PR) was defined as stabilized or increased eGFR, plus a proteinuria reduction of 50% at last follow-up. Children who had CR or PR were categorized as "responders." Children with neither complete nor partial remission were considered as "non-responders." CKD 5 was defined as having eGFR below 15 mL/min/1.73 m^2 or requiring chronic dialysis or kidney transplantation.

Outcomes

The primary outcome was the development of CKD 5. Secondary outcomes were CKD 5-free kidney survival and clinical remission, either partial or complete. The effects of demographic features (age, gender), history of upper respiratory tract infection (URTI), clinical findings (blood pressure, clinical presentation such as nephrotic syndrome and gross hematuria), laboratory markers (baseline eGFR, serum levels of creatinine, albumin), and histopathologic lesions on LM, on primary outcome, were also analyzed.

Genetic and autoantibody testing

Due to the retrospective nature of the study, only 19 patients could be tested for mutations in genes regulating the alternative pathway of complement. Details are given in the Supplementary data. Only 7 patients were tested for C3 nephritic factor (C3NeF).

Statistical analysis

Analyses were conducted using TURCOSA Cloud (Turcosa Analytics Ltd. Co, Turkey, www.turcosa.com.tr) software and IBM SPSS 20 (Statistical Package for Social Sciences, SPSS Inc., Chicago, IL, USA). Normality of the data was checked using the Shapiro–Wilk test. Data with normal distribution are expressed as mean ± standard deviation (SD), and parameters with non-normal distribution are expressed as median [interquartile range (IQR)]. Differences between the means of continuous variables in two groups were evaluated by the Student's *t* test or the Mann–Whitney *U* test. Categorical variables are expressed as percentages and were tested using the Pearson chi-square test or Fisher's exact *t* test.

Variables compared between patients with non-CKD 5 and CKD 5 were clinical presentation (pure nephrotic syndrome, nephritic syndrome, urinary abnormalities alone, nephritic-nephrotic syndrome ± hypoalbuminemia, blood pressure), serum creatinine and eGFR at diagnosis, serum complement levels (C3 and C4), and histological findings, including injury pattern on LM, immunostaining (C3 alone versus C3 dominant), and location of deposits on EM, where available.

Univariate and multiple binary logistic regression analyses (method: Backward Wald) were performed to examine risk factors for the development of CKD 5. Variables with low frequency values were not analyzed by multiple logistic regression. Predicted probabilities of the development of CKD 5 were computed from the univariate logistic model using eGFR at baseline. Kaplan–Meier curves were used to determine

cumulative kidney survival. The kidney survival time for each patient was calculated from the time of kidney biopsy to the last follow-up time or to the beginning of kidney replacement therapy (dialysis) or to the time to reach eGFR less than 15 mL/min/1.73 m^2. A Univariate Cox proportional hazard regression model was used to assess the association between the baseline variables (eGFR, serum albumin, and requirement for dialysis) and the primary outcome, CKD 5. To identify independent predictors of CKD 5 development, we performed a multivariate Cox regression analysis (method: Backward Wald) with a selection of variables. A p value less than 0.05 was considered as statistically significant.

Results

A total of 60 patients with C3G were collected from 21 nephrology centers (Supplementary data, Figure 1). Supplementary Table 1 shows demographic features and clinical and kidney biopsy findings of patients at the time of diagnosis. The mean age at diagnosis was 10.6 ± 3.0 years and follow-up duration 48.3 ± 36.3 months (min 6, max 140). At the time of diagnosis, gross hematuria and hypertension were found in 30 (50%) and 27 (45%) children with C3G, respectively, and 61.6% of the patients had nephrotic range proteinuria. Twelve patients (20%) presented with nephrotic syndrome and 25 patients (41.6%) presented with nephritic syndrome in addition to nephrotic proteinuria with or without hypoalbuminemia (nephritic-nephrotic syndrome). Eighteen patients had asymptomatic urinary abnormalities. The mean eGFR at diagnosis was 108 ± 68 mL/min/1.73 m^2, and 78.3% of the patients had low C3 level (Supplementary Table 1).

Histopathological findings are shown in Supplementary Table 1. The median time from admission to biopsy was 15 days (IQR, 10–60). Membranoproliferative glomerulonephritis (MPGN) was the leading pattern of injury (63.3%). On IF, 40 patients (66.6%) had only C3 staining, and the rest of the patients had both C3 staining and a small amount of IgG, IgA, C4, or IgM staining. Thirteen patients had re-biopsy. Five of them had dominant C3 staining with trace amounts of IgG, IgM, and C4 on IF at the first biopsy sample. None of them had IgG staining on repeat kidney biopsy sample.

In 6 patients, 7 variants in complement genes were detected (Supplementary Table 2). Only two variants in the *CFH* gene (p.Arg127Leu) were previously reported to be pathogenic [12]. None of these patients reached CKD 5, 3 had very low levels of C3, and 5 had proteinuria (nephrotic or non-nephrotic) at last follow-up. C3Nef was positive in 4 patients, two of them reached CKD 5, three had proteinuria, and all but one had very low levels of C3.

The treatment details are listed in Supplementary Table 3. There was no statistically significant difference between patients treated with or without mycophenolate mofetil/azathioprine (MMF/AZA) in terms of baseline clinical and laboratory findings (data not shown). Eighteen of the 31 patients on MMF/AZA responded to treatment (CR in 4 and PR in 14) (Fig. 1). Eculizumab (Ecu) was given in 7 patients after non-MMF-based immunosuppressive treatment and in 11 patients after MMF-based immunosuppressive treatment. Six patients (33.3%) were unresponsive to Ecu and experienced CKD 5. Eight patients (25.8%) treated with MMF/AZA, including 5 patients who also did not respond to Ecu, developed CKD 5. The mean eGFR at last follow-up was 87.8 ± 34.5 mL/min/1.73 m^2 in 12 patients who responded to Ecu.

Outcomes

Ten patients (16.6%) progressed to CKD 5 at last follow-up, 3 of who had DDD (half of the DDD patients). Changes in eGFR and proteinuria according to response to immunosuppressive therapy are shown in Fig. 2. Clinical remission was achieved in 41 patients (68.3%). Of these, 12 had CR and 29 had PR. At last follow-up, 9 patients still had active disease without any remission.

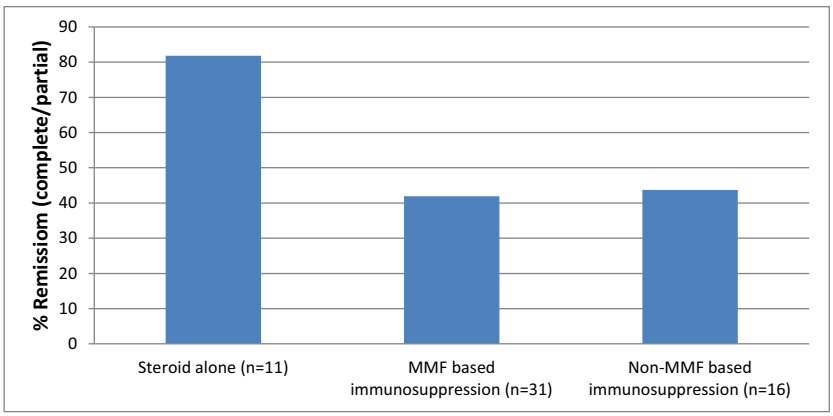

Fig. 1 Remission rate of patients with C3G based on immunosuppressive therapy

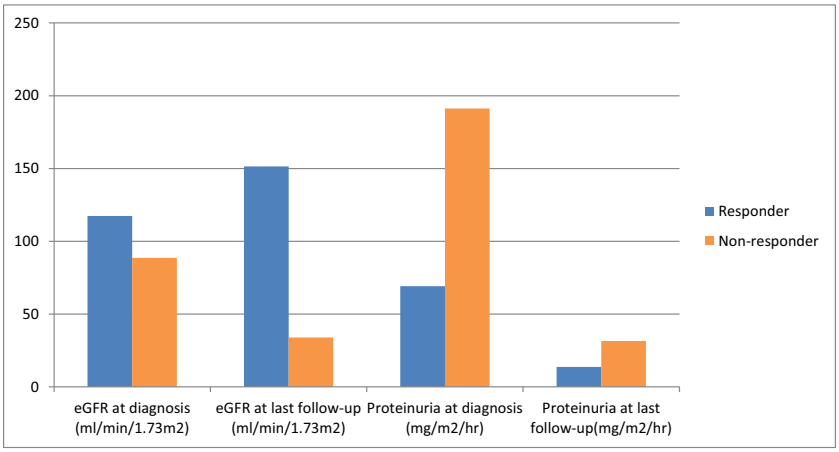

Fig. 2 Changes in eGFR and proteinuria in patients with C3 glomerulopathy according to response to immunosuppressive therapy

Low C3 level persisted in 50% of the patients at last follow-up (Supplementary Table 1). Six of 13 patients who required dialysis at baseline developed CKD 5. There was no difference between patients who were diagnosed with MPGN or PIGD before the 2013 C3 consensus criteria and those who were diagnosed with C3G after 2013 in terms of eGFR and reaching CKD 5 at last follow-up (Supplementary Table 3).

Comparison between CKD5 and non-CKD5 patients in terms of clinical and laboratory parameters at baseline and last follow-up

Comparisons are given in Table 1. The proportion of patients having nephrotic range proteinuria, nephrotic syndrome, and requirement for dialysis at baseline was significantly higher in the CKD 5 group. In 6 of 13 patients who required dialysis at baseline, kidney replacement therapy was required at last follow-up. Baseline blood urea nitrogen and creatinine levels were significantly higher in CKD 5 patients, and eGFR and albumin levels were significantly lower in CKD 5 compared with non-CKD 5 patients. Although baseline complement levels were not different between groups, C3 levels were significantly lower at last follow-up in CKD 5 patients. There was no significant difference between groups in terms of gender, gross hematuria, preceding URTI, and ASO titers. CKD 5 was more common in steroid-resistant patients who needed additional IS drugs (Table 1). There was also no difference between groups regarding hypertension and histological patterns of kidney injury (data not shown).

Electron microscopy findings

Based on EM findings, there were 18 (75%) C3GN and 6 (25%) DDD patients (Table 2). The mean serum albumin level at diagnosis was significantly lower in DDD patients as compared with C3GN patients ($p = 0.002$) (Table 2). There was a preceding URTI in 5 patients.

Predictors of CKD 5

The predictors of CKD 5 in univariate binary logistic regression were eGFR, serum albumin, and requirement for dialysis at the time of diagnosis (Table 1). By multivariable logistic regression model, initial eGFR (OR 0.98; 95% CI 0.96–0.99) and serum albumin (OR 0.19; 95% CI 0.05–0.832) were independently associated with CKD 5 (Table 3). Every 0.01 decrease in initial GFR increases the risk of CKD 5 0.98 times. Every 0.01 decrease in albumin increases the risk of CKD 5 0.19 times. The predicted probability of CKD 5 was approximately 27% in patients with an eGFR of 50 ml/min/1.73 m^2 at the time of diagnosis (Supplementary Figure 2). Kaplan-Meier analysis revealing kidney survival during follow-up is shown in Fig. 3a, with an overall mean kidney survival of patients with C3G of 105.8 months (95%CI, 89.8–121.8).

Univariate Cox regression analysis of the significant variables determined by the log-rank test demonstrated that eGFR (HR 8.034; 95% CI 1.435–44.988, $p = 0.018$), serum albumin (HR 10.046: 95% CI 1.233–81.828, $p = 0.031$), and requirement of dialysis (HR 12.460; 95% CI 2.490–62.334, $p = 0.002$) at baseline were associated with CKD 5 (Table 3). In multivariate Cox regression analyses, initial eGFR (HR 0.956; 95% CI 0.928–0.984, $p = 0.003$) and initial serum albumin (HR 0.116; 95% CI 0.025–0.549, $p = 0.007$) were independent predictors of CKD 5 (Table 3). While patients with serum albumin higher than 3 g/dL reached CKD 5 in 133.3 months, patients with serum albumin less than 3 g/dL reached CKD 5 in an average of 82.7 months. Besides, time to CKD 5 is shorter in patients with an initial eGFR less than 60 ml/min/1.73 m^2 compared with those with eGFR higher than 60 ml/min/1.73 m^2 (57 months vs. 114 months), and time to CKD 5 was shorter in patients who required dialysis at diagnosis compared with those without requirement for dialysis (65.4 months

Table 1 Comparison between CKD 5 and non-CKD 5 patients for clinical and laboratory parameters at baseline and last follow-up, and univariate binary logistic regression

Variables	CKD 5		*p*	Univariate binary logistic regression	
	CKD 5 Ø (*n* = 50)	CKD 5 + (*n* = 10)		OR (%95 CI)	*p*
Gender					
Female	24 (48.0)	6 (40.0)	0.488	1.00	–
Male	26 (52.0)	6 (60.0)		1.62 (0.40–6.46)	0.491
Age of diagnosis (years)	10.71 ± 3.14	10 ± 2.49	0.505	0.92 (0.73–1.16)	0.499
Follow-up time (months)	43.94 ± 34.48	62.5 ± 36.61	0.129	1.01 (0.99–1.03)	0.136
History of URTI					
No	28 (56.0)	5 (50.0)	0.728	1.00	–
Yes	22 (44.0)	5 (50.0)		0.78 (0.20–3.06)	0.728
Nephrotic syndrome					
No	25 (50.0)	1 (10.0)	0.020*	1.00	–
Yes	25 (50.0)	9 (90.0)		9.00 (1.06–76.42)	0.044*
Nephrotic range proteinuria(*n* = 49)					
No	29 (59.18)	0 (0.0)	0.013*	1.00	–
Yes	10 (40.82)	10 (100.0)		–	
Gross hematuria					
No	25 (50.0)	4 (40.0)	0.563	1.00	–
Yes	25 (50.0)	6 (60.0)		−1.50 (0.37–5.97)	0.565
Requirement of dialysis					
No	43 (86.0)	4 (40.0)	0.001*	1.00	–
Yes	7 (14.0)	6 (60.0)		9.21 (2.06–41.14)	0.004*
Medical treatment					
Steroid	16 (32.0)	0 (0.0)	0.054	1.00	–
Steroid + other immunosuppressive	34 (68.0)	10 (100.0)		–	
Pulse methyl prednisolone					
No	37 (74.0)	1 (10.0)	0.275	1.00	–
Yes	13 (26.0)	9 (90.0)		3.16 (0.36–27.43)	0.296
MMF/AZA					
No	27 (54.0)	2 (20.0)	0.050*	1.00	–
Yes	23 (46.0)	8 (80.0)		4.69 (0.90–24.35)	0.066
CNIs (*n* = 58)					
No	36 (73.5)	1 (11.1)	<0.001*	1.00	–
Yes	13 (26.5)	8 (88.9)		22.15 (2.52–194.69)	0.005*
CYP (*n* = 59)					
No	39 (79.6)	5 (50.0)	0.050*	1.00	–
Yes	10 (20.4)	5 (50.0)		3.90 (0.94–16.15)	0.060
Eculizumab					
No	38 (76.0)	4 (40.0)	0.023*	1.00	–
Yes	12 (24.0)	6 (60.0)		4.75 (1.14–19.68)	0.032*
Laboratory parameters at baseline					
BUN (*n* = 58), median (IQR)	16.3 (12.0–32.5)	37.5 (25.50–70.50)	0.004*	1.02 (1.00–1.05)	0.051*
Creatinine, median (IQR)	0.69 (0.47–1.42)	1.15 (0.95–1.81)	0.019*	1.16 (0.82–1.63)	0.392
eGFR	117.40 ± 69.93	62.69 ± 24.62	0.018*	0.98 (0.96–0.99)	0.027*
Albumin	3.04 ± 0.99	2.26 ± 0.55	0.020*	0.36 (0.14–0.90)	0.030*
Complement C3	53.9 ± 39.2	42.1 ± 43.0	0.396	0.99 (0.97–1.01)	0.393
Complement C4 (n = 38)	20.3 ± 5.9	21.1 ± 5.6	0.748	1.02 (0.88–1.19)	0.740
ASO (*n* = 23)	334 ± 308	122 ± 73	0.257	0.99 (0.98–1.00)	0.311
Laboratory parameters at last visit					
Complement C3 (*n* = 57)	82.7 ± 45.1	43.8 ± 31.6	0.023*	0.97 (0.95–0.99)	0.034*
Complement C4 (*n* = 20)	23.1 ± 8.5	25.62 ± 5.9	0.587	1.04 (0.90–1.20)	0.566

IQR interquartile range, *CKD 5* chronic kidney disease stage 5, *URTI* upper respiratory tract infection, *eGFR* estimated glomerular filtration rate, *MPGN* membranoproliferative glomerulonephritis, *MesPGN* mesangial proliferative glomerulonephritis, *MMF/AZA* mycophenolate mofetil/azathioprine, *CNI* calcineurin inhibitors, *CYP* cyclophosphamide, *ASO* antistreptolysin O

*$p < 0.05$

Table 2 Clinical and laboratory characteristics of patients with DDD and C3GN based on EM findings at baseline and last follow-up

Variables	C3GN ($n = 18$)	DDD ($n = 6$)	p value
At baseline			
Gender (female/male)	6/12	6/0	0.005*
Age (years)	10.2 ± 3.1	10.5 ± 4.3	0.866
eGFR (ml/min/1.73 m^2)	94 ± 75	105 ± 45	0.742
Albumin (g/dl)	3.1 ± 0.8	2.2 ± 0.8	0.002*
Nephrotic syndrome, n (%)	10 (55.5)	5 (83.3)	0.233
Low C3 level, n (%)	14 (77.8)	6 (100)	1.000
At last follow-up			
eGFR (ml/min/1.73 m^2), median (IQR)	127.5 (48.2–169.5)	43.5 (9.0–109.7)	0.06
CKD 5, n (%)	3 (16.6)	3 (50)	0.102

eGFR estimated glomerular filtration rate, *CKD 5* chronic kidney disease stage 5

*$p < 0.05$

vs. 121.6 months) (Table 4; Fig. 3b, c, d). Patients with hypoalbuminemia (< 3 g/dL) and low eGFR (< 60 ml/min/1.73 m^2) were 10.4 and 8.03 times more likely to develop CKD 5 than patients with normoalbuminemia (> 3 g/dL) and eGFR > 60 ml/min/1.73 m^2, respectively (Table 3).

Discussion

This study presents the first largest multicenter C3G series of pediatric patients (age ≤ 18 years old) and demonstrates that most of the children with C3G present with nephrotic range proteinuria, nephrotic-nephritic syndrome, low complement levels, and MPGN pattern of injury. This study also shows that impaired kidney function and hypoalbuminemia at diagnosis are predictors of CKD 5 development. Moreover, patients with DDD are more likely to have impaired kidney function at last follow-up.

In line with our previous study [11], a heterogeneous clinical presentation and outcome of C3G had been demonstrated. It may present with mild to severe kidney disease [4]. In some studies, nephritic-nephrotic syndrome was the most common clinical presentation [9, 13], whereas in other studies, nephritic syndrome [4, 14] and asymptomatic urinary abnormalities [15] were more common. In our study, almost half of the patients (41.6%) presented with nephrotic-nephritic syndrome, and 61.6% of patients had nephrotic range proteinuria at the time of diagnosis.

The most common pattern of injury in the present study was MPGN (63.3%) which was almost similar to that of other groups [4–6, 13, 14, 16]. In addition to MPGN, other histopathological findings such as MesPGN, DPGN, and crescentic GN can be seen on kidney biopsy specimens of patients with C3G [5, 6, 17]. In our patients, MesPGN, DPGN, and crescentic GN were observed in 53.3%, 26.7%, and 11.7% of patients, respectively. Electron microscopy is a routine diagnostic tool in the evaluation of kidney biopsies and is crucial in the differentiation of DDD, atypical post-infectious glomerulonephritis (aPIGN), and C3GN [18]. However, we could only perform EM in 24 patients (40%) because of non-availability in every hospital in Turkey. With EM, 6 patients were diagnosed as DDD and 18 patients as C3GN. As in previous studies [5, 6, 15], subepithelial deposits and humps were detected in some of C3G biopsies in our cohort. Due to

Table 3 Risk factors and predictors of CKD 5 in C3 glomerulopathy

Variables at baseline	Multiple binary logistic regression		Univariate Cox regression		Multiple Cox regression	
	OR (%95 CI)	p	HR (%95 CI)	p	HR (%95 CI)	p
eGFR (mL/min/1.73 m^2) categorical (< 60) Quantitative	− 0.98 (0.96–0.99)	0.017*	8.034 (1.435–44.988) 0.975 (0.954–0.996)	0.018* 0.018*	0.956 (0.928–0.984)	0.003*
Serum albumin categorical (< 3 g/dL) Quantitative	− 0.19 (0.05–0.832)	0.027*	10.046 (1.233–81.828) 0.550 (0.311–0.974)	0.031* 0.040*	0.116 (0.025–0.549)	0.007*
Requirement of dialysis	–	–	12.46 (2.49–62.334)	0.002*	–	–

Multiple binary logistic regression: eGFR and serum albumin, requirement of dialysis at baseline were analyzed using backward regression analysis

eGFR estimated glomerular filtration rate, *CKD 5* chronic kidney disease stage 5

*$p < 0.05$

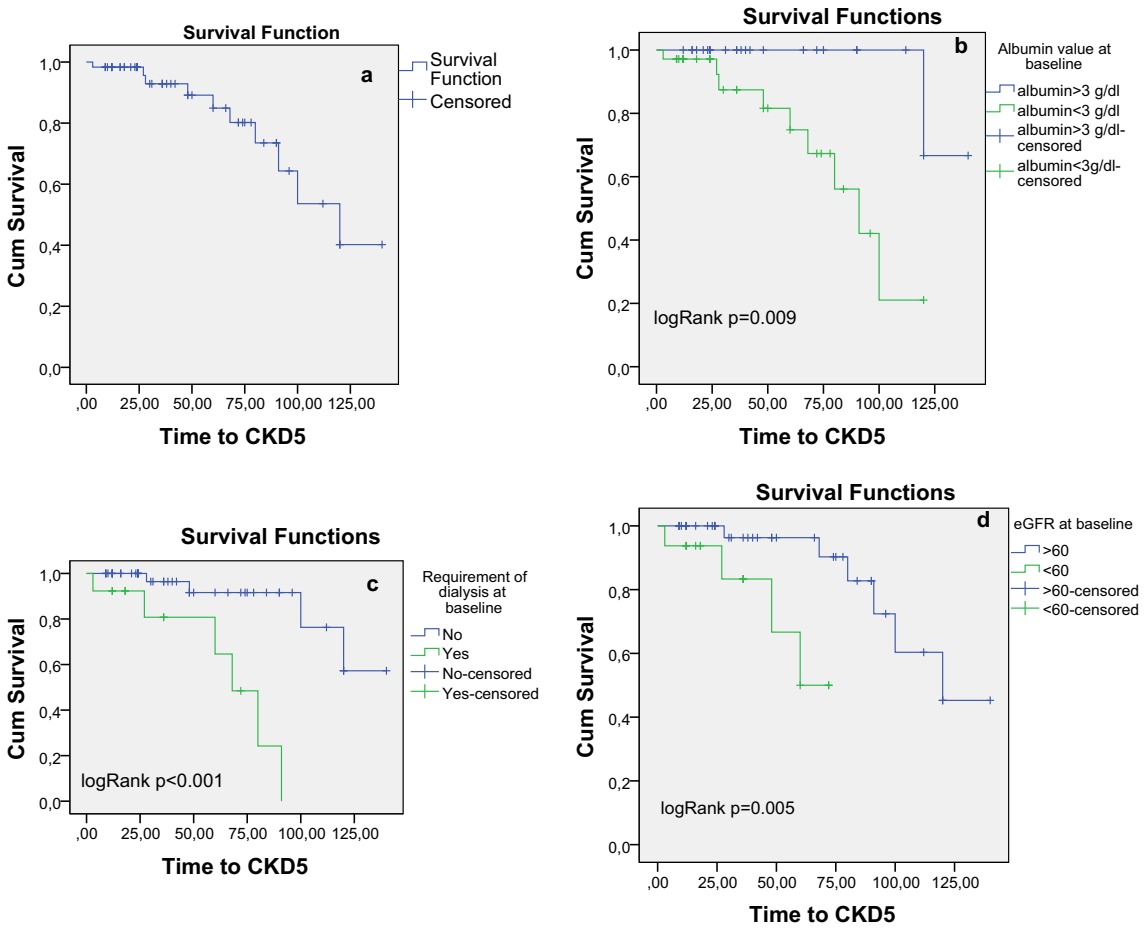

Fig. 3 **a** Kaplan-Meier analysis of overall kidney survival of patients with C3 glomerulopathy. **b** Cox proportional hazards regression curves defining long-term risk of chronic kidney disease stage 5 for albumin. **c** For requirement of dialysis. **d** For eGFR < 60 ml/min/1.73 m^2

the fact that we could not perform EM in the remaining patients, it is possible that some of the patients in our cohort may have aPIGN, which is characterized clinically by nephritic-nephrotic syndrome, history of preceding URTI, and by characteristic histopathologic findings of PIGN [19]. As in patients with aPSGN, patients with C3G may have few intramembranous deposits and occasional sub-epithelial humps on EM [19]. Therefore, biopsy findings of patients with aPIGN and C3G may demonstrate striking similarities [20], and both diseases are located at two different ends of a glomerular disease [8], possibly due to a defect of alternative pathway of complement [19, 20].

The pathological findings of C3GN and DDD show some differences, and the clinical signs and outcomes are still controversial. Medjerel-Thomas et al. [5] evaluated 80 patients, including 32 children with C3G (C3GN in 18, DDD in 14) and found no statistical difference between patients with C3GN and DDD in terms of baseline clinical and laboratory features, except age and C3 level. Patients with DDD were younger, and their complement levels were lower than those

Table 4 Predicted time to CKD 5 using Kaplan-Meier (log-rank)

Variables at diagnosis		Time to CKD 5 (months)	95% Confidence Interval	*p*
eGFR (ml/min/1.73 m^2)	< 60	57.00 ± 6.23	44.80–69.19	0.005*
	> 60	114.09 ± 8.09	98.22–129.96	
Albumin (g/dL)	< 3	82.74 ± 7.87	67.30–98.17	0.009*
	> 3	133.33 ± 5.44	122.66–144.00	
Requirement of dialysis	Yes	65.45 ± 9.35	47.12–83.78	< 0.001*
	No	121.64 ± 7.8	106.34–136.93	

CKD 5 chronic kidney disease stage 5, *eGFR* estimated glomerular filtration rate

*$p < 0.05$

with C3GN. Crescentic GN was more common in patients with DDD. On the contrary, patients with C3GN had more severe arteriolar sclerosis, glomerular sclerosis, and interstitial fibrosis on kidney biopsy than patients with DDD. This difference was not detected in the study of Bomback et al. [13], who described 111 patients (C3GN in 87, DDD in 24) including 35 pediatric cases (C3GN in 32, DDD in 3) and showed that patients with C3GN were younger than patients with DDD, and histopathologic findings at baseline were similar between C3GN and DDD groups. In our study, the pattern of glomerular injury was similar among the two sub-groups of C3G, and albumin level at baseline and eGFR at last follow-up were lower in children with DDD than children with C3GN.

To the best of our knowledge, there is no consensus on the treatment of C3G patients even in adults. Current treatment strategies based on adult studies consist of case series, observational studies, and expert opinions. They include antiproteinuric therapy with ACEIs or ARBs, IS, and complement targeting therapy [21]. Although PR or CR was achieved with MMF in 62–67% of the patients with MPGN, there are few studies about the effects of MMF in children with C3G [22, 23]. Although there are controversial results [14], it has recently been shown that the treatment of C3G with corticosteroids plus MMF in adults caused better kidney survival as compared with patients treated with other IS regimens and untreated patients [4, 15]. Ravindran et al. [6] evaluated the treatment response of patients with C3G (C3GN in 70, DDD in 8). Immunosuppressive and conservative treatment was given in 42 and 34 patients, respectively. Median serum creatinine level and proteinuria value were higher in patients treated with IS medications than patients who received conservative management (1.4 mg/dl vs. 1.1 mg/dl for creatinine and 3100 mg/day vs.1600 mg/day for proteinuria). At last follow-up, CKD 5 or doubling of serum creatinine developed in 10 patients (23.8%) in the IS treatment group and 6 patients (17.6%) in the conservative treatment group. Until now, the largest study including 35 pediatric cases (C3GN in 32 and DDD in 3) with C3G was performed by Bomback et al. [13]. However, they did not evaluate pediatric patients separately. Recently, a few pediatric studies including small numbers of children with C3G [9, 11, 16, 23] have reported outcomes with different IS drugs (corticosteroids in 39, MMF in 22, and CNIs in 7 patients) and with Ecu (9 patients). At last follow-up, clinical remission rates were 75–94.4% in these case series. Kojc et al. [16] reported the clinical outcomes of 11 children with C3G and mentioned more favorable response to IS therapy. Only 2 patients in their cohort did not respond to steroids and/or other IS treatments and achieved remission with Ecu. In our study, most of the patients were treated with corticosteroids with or without ACEIs, or corticosteroids plus other IS medications. CKD 5 was more common in patients who needed additional IS medications. Contrary to previous studies by Avasare et al. [15] and Rabasco et al. [4], the response to MMF/AZA treatment in our patients was not impressive. In our cohort, 18 of the 31 patients responded to MMF/AZA (CR in 4 and PR in 14). However, 8 patients (25.8%) progressed to CKD 5. We could not explain this discrepancy with impaired kidney function with a median creatinine level of 0.9 mg/dl at baseline and interstitial fibrosis on kidney biopsy at diagnosis in our patients who received MMF. Similar [15] and higher creatinine levels [4, 6] were reported in different pediatric cohorts. The number of patients who had mild to moderate interstitial fibrosis was 15 (48.3%) in our cohort which is much lower compared with those reported by Avasare et al. (70%) [15]. This discrepancy may be due to the retrospective design of our study. We did not receive any information about MMF dosage. It is known that low MMF exposure is associated with more relapse compared with high MMF exposure in children with frequently relapsing nephrotic syndrome, and the effective dose to achieve target levels of MMF may show considerable intra-individual variability [24].

Complement targeting therapy looks reasonable since the data demonstrate a central role of abnormalities in the alternative pathway of complement in C3G [2]. Eculizumab, which is a humanized monoclonal antibody that interferes with membrane attack complex (MAC) assembly [25], is thought to be a rescue therapy for C3G [26, 27]. Quintrec et al. [28] described clinical remission in 46% of patients using Ecu in their cohort including 26 children and adults with C3G. In line with this, Bomback et al. [29] and Labrenon et al. [30] reported promising results in their cohorts. Bomback et al. described 6 C3G patients treated with Ecu: while 4 of them had high C5–9 levels and responded to Ecu, 2 patients with normal C5–9 levels did not respond to Ecu treatment. Therefore, it is suggested to give Ecu therapy early in patients with high levels of C5–9 [29]. In our cohort, 12 of 18 patients who received Ecu responded to treatment, with eGFR of 87.8 ± 34.5 ml/min/1.73 m^2.

Kidney outcome in patients with C3G varies between studies. CKD 5 has been reported in 10–23% of patients with C3GN and in 20.8–47% of patients with DDD [5, 6, 13]. In our cohort, we found that 16.6% of patients with C3G developed CKD 5 at last follow-up.

Understanding clinical, laboratory, and histopathological risk factors for poor outcome in patients with C3G at the time of diagnosis may provide important advantages to clinicians to guide treatment and follow-up. However, predictors of CKD 5 in patients with C3G are different due to differences in study designs [5, 6, 13, 14]. Serum creatinine level [6], eGFR [13, 14], heavy proteinuria [6, 14], older age [5], younger age [14], severity of glomerulosclerosis and degree of tubular atrophy and interstitial fibrosis [6, 14], crescentic GN [5, 14], isolated C3 staining [13], and DDD subtype [5] at the time of biopsy have been reported to be independent predictors for poor kidney outcome. In this study, in the multivariate

logistic regression model, eGFR and serum albumin as clinical markers at baseline independently predicted CKD 5. Our study results are consistent with some previous studies that patients with C3G with decreased eGFR had poor kidney prognosis [13, 14]. To date, there is no large pure pediatric series; therefore, we compared our results with adult/pediatric combined studies showing that impaired kidney function at the time of diagnosis is a risk factor for CKD 5 [5, 13]. Additionally, we demonstrate that hypoalbuminemia at baseline, but not proteinuria, was a strong predictor of CKD 5. The only previous study to evaluate the relationship between hypoalbuminemia and CKD 5 or eGFR decline ≥ 50% from the baseline value in patients with C3G found no relationship [14]. Since 61.6% of the children in our cohort had nephrotic range proteinuria, we do not know exactly whether hypoalbuminemia is a contributing factor for progression to CKD 5, independently from proteinuria.

The strengths of this study include the following: (1) number of patients in our cohort, representing the largest pediatric series in the literature; (2) data were collected from 21 pediatric nephrology centers from 12 cities in different regions; (3) it is the first multicentric study showing baseline hypoalbuminemia as a significant risk factor for progression to CKD 5 in children with C3G.

Our study also has limitations:

1. We were unable to evaluate the findings of EM to differentiate DDD, aPIGN, and C3GN in all patients. Therefore, except for 24 patients in our cohort, we could not rule-out patients with aPIGN from the cohort and evaluate the effect of subtype of disease (C3GN vs. DDD) on kidney survival.
2. The absence of a standard treatment regimen may influence the outcome, i.e., dose information for MMF or blood level for CNI were not available. A difference in MMF dose may reduce the positive effect of MMF treatment on kidney survival seen in previous studies [4, 15].
3. Kidney biopsy samples were evaluated by the pathologist at the treating institution, instead of a single center.
4. We were unable to perform genetic and serological tests in all patients, and therefore, evaluation of the possible effects of specific genetic or serological markers on kidney survival was not possible. Even in patients with aPIGN, Sethi et al. [19] detected mutations in genes regulating alternative pathway of complement in 4 of 11 patients and autoantibodies (C3Nef) against C3 convertase in 7 of 11 children. The screening of C3Nef and autoantibodies to alternative complement pathway proteins and genetic testing of alternative pathway of complement genes were suggested to distinguish autoimmune and genetic forms of C3G and optimize treatment strategy [31].
5. Since Ecu was given as a salvage therapy in our MMF resistant patients, we could not make a clear conclusion on potential early beneficial effect of Ecu on kidney survival, as previously shown [26–28].
6. The retrospective nature of this study.

Despite all these limitations, we still think that this paper is a valuable guide for pediatric nephrologists in the management of children with C3G.

In conclusion, C3G represents a heterogeneous clinical presentation, commonly with MPGN pattern of injury and unfavorable outcome, and therefore, all patients should be monitored for CKD progression. Impaired kidney function and hypoalbuminemia are significant predictors of CKD 5. Additional larger prospective studies, including genetic testing of the alternative pathway of complement components and screening for autoantibodies, are needed to evaluate the effect of different treatment strategies on long-term kidney survival.

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Authors' contributions A.S.P, I.D, and R.D designed the study. A.S.P, I.D, I.G, E.Ç, S.S, M.T.B, O.D, E.M, D.T, N.Ç. D.Y, S.A.B, Y.T, Z.Y.Y, E.B, M.K, A.S, N.C, B.A, M.E.Ç, M.T, M.B, G.Ö, and RD carried out the recruitment of patients into the study. I.D and A.S.P analyzed, interpreted the data, and wrote the article. All the authors reviewed and revised the article and approved the final version.

Compliance with ethical standards

The study protocol was approved by the local institutional Ethics Committee (2017/209).

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