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Clinical and immunological biomarkers can identify proliferative changes and predict renal flares in lupus nephritis

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Abstract

Background Kidney involvement is frequent in SLE, with proliferative lupus nephritis (LN) forms and nephritic flares being key predictors of poor outcomes. Conflicting results have been reported for anti-C1q antibodies among the serological markers. Our purpose was to assess the value of immunological tests (C3,C4 complement fractions, anti-DNA and antiC1q antibodies) in predicting histological classes and flares of lupus nephritis (LN).

Methods For histological class prediction, we evaluated the immunological tests performed on the day of kidney biopsy by linear and multiple regression analyses. For flare prediction, univariable and multivariable Cox analyses were made at baseline, 6, and 12 months.

Results Of 61 participants in the study, 47 had proliferative (III, IV) and 14 non-proliferative LN (II, V) at kidney biopsy. In proliferative LN, anti-DNA ($p=0.0186$) and anti-C1q antibodies (Ab) ($p=0.0050$) were significantly higher, and serum C3 and C4 lower ($p=0.0026$; $p=0.0212$) compared to non-proliferative LN. At multiple regression analysis, the best association to differentiate proliferative from non-proliferative LN was the number of urinary erythrocytes (OR 3.2292; CI 1.2585–8.2858; $p=0.0148$) and anti-C1qAb (OR 1.0288; CI 1.0016–1.0568; $p=0.0380$). Of 53 patients evaluated for flare predictions, followed for 60.69 (37.20-78.704) months, 10 (18.86%) had a renal flare at 28.19 months (24.84–39.38, range:16.3–55.8) from therapy initiation. At univariable analysis, anti-C1qAb ($p=0.0340$, $p=0.0005$) and no-use hydroxychloroquine ($p=0.0313$, $p=0.0276$) predicted flares at baseline and six months. Anti-C1qAb ($p=0.0047$), non-use hydroxychloroquine ($p=0.0252$), anti-C1qAb ≥ 40UA ($p=0.0047$), 24/h proteinuria ($p=0.0185$), and proteinuria ≥ 0.5 g/day ($p=0.0216$) predicted flares at 12 months. At multivariable analysis, anti-C1q > 40UA (OR 9.0721; CI 0.9146–42.9882; $p=0.0057$) and non-use of hydroxychloroquine (OR 0.1742 CI 0.0445–0.6823; $p=0.0126$) were the independent predictors of renal flares.

Conclusion Immunological tests can differentiate proliferative from non-proliferative LN, but anti-C1qAb and urinary erythrocytes had the best predictive power. Only persistent high anti-C1qAb at 1 year and non-use of hydroxychloroquine seem to predict renal flares.

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Keywords Lupus nephritis, Proliferative lupus nephritis, Membranous lupus nephritis, anti-DNA antibodies, anti-C1q antibodies, Complement fractions, renal flares

Introduction

Kidney involvement is frequent in systemic lupus erythematosus (SLE) and may show different clinical and histological features at presentation [1, 2, 3]. Although the short-term and long-term prognosis of lupus nephritis (LN) has considerably improved over the years [4], morbidity and mortality remain elevated in comparison to SLE without renal involvement and to the general population [5, 6]. In comparison to non-proliferative forms (class II and V LN), proliferative LN forms (class III, class IV and mixed forms) and delayed diagnosis of LN are frequently associated with challenging outcomes [3, 7], but kidney flares are probably the strongest clinical indicators of poor prognosis in LN [8]. Kidney flares are characterized by either a consistent increase in proteinuria (proteinuric flares) or a rapid increase in serum creatinine (nephritic flares) [9, 10]. The appearance of flares requires immediate diagnosis and management to avoid the transformation of inflammatory lesions into kidney scars [11]. In the search for noninvasive biomarkers, C3 and C4 complement fractions and anti-DNA antibodies have been tested for many years but the results were often disappointing because of false negative or positive data [12]. Conflicting results have also been reported with anti-C1q antibodies [13].

This retrospective study aims to assess the value of immunological tests (anti-C1qAb, anti-DNA antibodies, C3 and C4 complement fractions) in two different time points of LN: (a) on the day of the first kidney biopsy, to differentiate proliferative from non-proliferative LN (first endpoint); (b) in the first year after LN diagnosis, to predict the occurrence of kidney flares (second endpoint).

Methods

Ethical approval

The study was approved by the Ethics Committee of IRCCS Humanitas Rozzano, Milano, Italy (protocol code NEF0032023). All participants provided informed consent for the scientific use of their anonymized data. Patient or public involvement in the research was not applicable.

Inclusion and exclusion criteria

All patients older than 18 years with biopsy-proven lupus nephritis, complete immunological assessment (including anti-C1qAb, anti-DNA antibodies, C3 and C4 complement fractions), and a minimum follow-up of 2 years were considered for the study. Patients without a kidney biopsy or incomplete immunological tests were not admitted to the study. Patients with end-stage kidney disease (ESKD) requiring regular dialysis or kidney transplantation were also excluded. SLE was classified according to the American College of Rheumatology (ACR) criteria [14]. Kidney biopsy was classified according to the International Society of Nephrology/Renal Pathology Society criteria (ISN/RPS) and with evaluation of activity and chronicity indexes [1, 2].

Selection of participants

Among 128 patients with biopsy-proven LN in which we tested anti-C1qAb from March 2017 to March 2022 (Fig. 1), we selected patients with clinical and immunological tests measured (a) at the time of initial kidney biopsy (for the first endpoint) and (b) at the beginning of LN active therapy and 6 and 12 months (for the second endpoint). An electronic database was utilized to collect data on induction and maintenance therapy, including demographics, clinical, and laboratory features at baseline, at each clinical evaluation, and at the last observation.

Laboratory tests

Serum anti-DNA antibodies were measured on a random-access chemiluminescent analyzer (BIOFLASH, INOVA Diagnostics), with normal values < 27 IU/ml. C3 and C4 serum levels were expressed as mg/dl (normal values C3 80-120 mg/dl, C4 10-20 mg/dl). Serum anti-C1q antibodies were measured using an ELISA test (QUANTA Lite® Anti-C1q, INOVA Diagnostics) with normal values < 20 UA, medium values between 20 and 80 UA, and high values > 80 UA [15].

Proteinuria was measured by benzethonium chloride in the urine collected over 24 h. The value was expressed as g/24 hours. The estimated glomerular filtration rate (eGFR) was evaluated by the CKD EPI formula.

Events

Acute kidney injury (AKI) was defined by an absolute increase in serum creatinine of at least 0.3 mg/dL (26.5 µmol/L) within 48 h or by a 50% increase in serum creatinine from baseline within 7 days or a urine volume of less than 0.5 mL/kg/h for at least 6 h [16] along with hematuria [urinary red blood cells > 5/high power field (HPF)] and/or erythrocyte casts], proteinuria ≥ 0.5 g/day).

Nephrotic syndrome was defined by proteinuria ≥ 3.5 g/day, serum albumin ≤ 3 g/dL, and dyslipidemia.

Isolated urinary abnormalities: proteinuria > 0.3 g/day and/or microscopic hematuria (at least 3 dysmorphic erythrocytes per high-power field on urine microscopy).

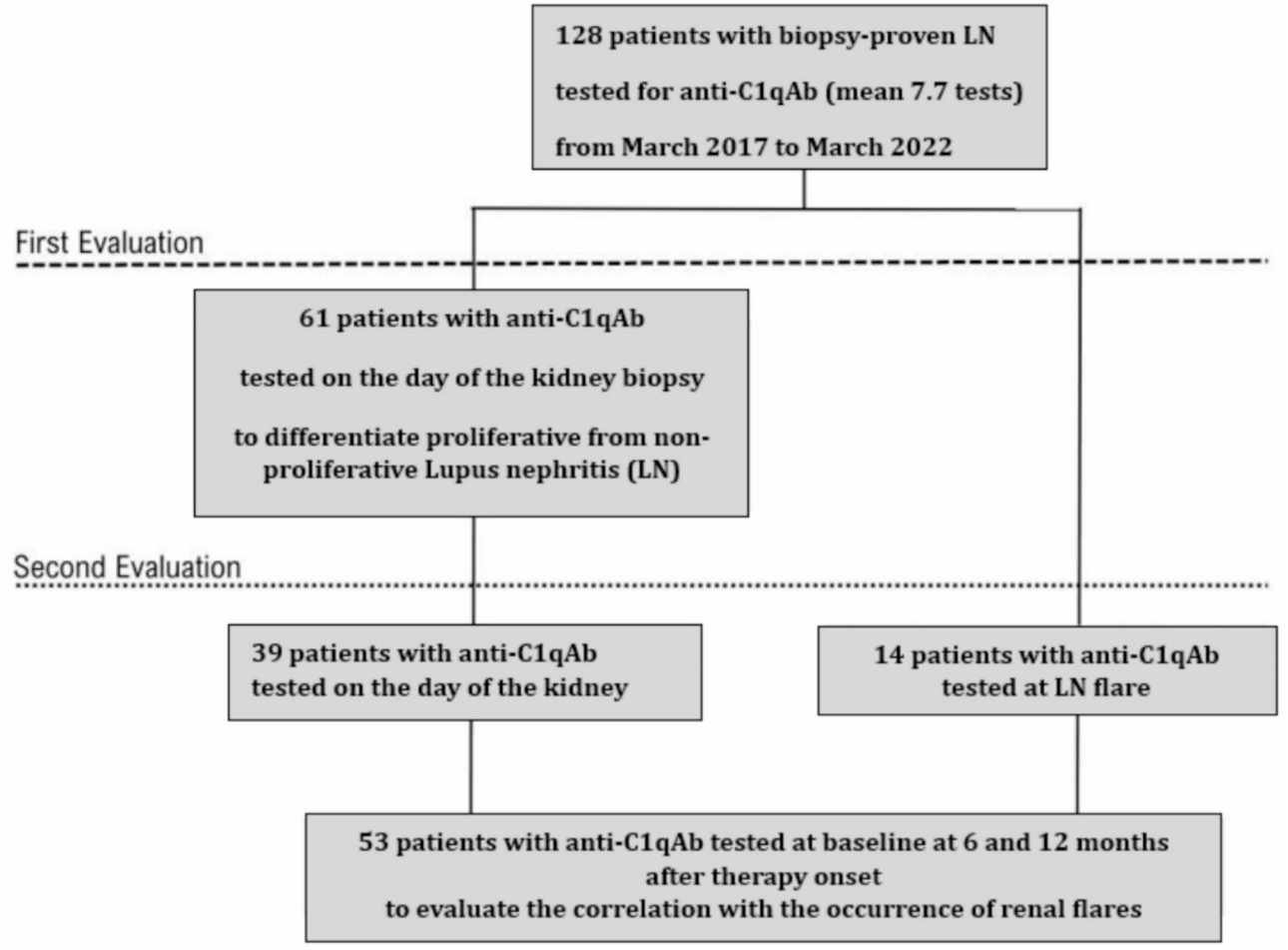

Fig. 1 Flowchart to identify patients selected for the first evaluation: to differentiate proliferative from non-proliferative LN (first endpoint); and for the second evaluation: to predict the occurrence of kidney flares (second endpoint)

Complete renal remission: eGFR ≥ 60 ml/min1.73 m^2, proteinuria < 0.3 g/day, and inactive urinary sediment (no dysmorphic erythrocytes, no pathologic casts).

Partial renal remission: eGFR ≥ 60 ml/min1.73 m^2, and > 50% reduction in peak proteinuria at subnephrotic levels < 3.5 g/day.

Chronic Kidney disease (CKD): eGFR < 60 ml/min/1.73 m^2 for at least 3 months and inactive urinary sediment. CKD was classified into different stages according to KDIGO definitions [16]).

Arterial hypertension: the mean of three consecutive measurements of systolic blood pressure > 140 mm/Hg and/or diastolic blood pressure > 90 mm/Hg in a sitting position.

Nephritic flares: increase in serum creatinine of at least 30% over the last value, associated with nephritic urinary sediment, with or without increased proteinuria [9].

Proteinuric flares: increase in proteinuria without modification of serum creatinine of at least 1 g/24 h if the previous proteinuria was < 0.5 g/24 h or doubling if the previous proteinuria was ≥ 3.5 g/24h [9].

Extrarenal flares were defined according to the revised Safety of Estrogens in Lupus Erythematosus National Assessment (SELENA)-SLEDAI criteria.

Statistical analysis

Demographic and clinical data were expressed as numbers or percentages for discrete variables and continuous variables as median and interquartile ranges. Comparison of continuous variables between groups was conducted using the non-parametric Mann-Whitney test or the Kruskal-Wallis H test for two or more independent samples, respectively. The chi-squared test was employed to compare categorical or dichotomized variables among groups of patients. The differences between proliferative (class III and IV) and non-proliferative (class II and V) LN were tested among the variables reported in Table 1 with linear and multiple regression analysis.

To evaluate the predictors of renal flares, the demographic, histological, clinical/immunological, and therapeutic features at baseline, six, and twelve months reported in Table 2 were analyzed using the Cox

Table 1 The value of clinical/immunological parameters at the time of the kidney biopsy in differentiating proliferative from non-proliferative forms of lupus nephritis at linear and multiple regression analysis

Parameters	Proliferative forms (class III + IV)	Non proliferative forms (class V)	OR	CI	*P*	OR	CI	*P*
			Univariable analysis			Multivariable analysis		
Number of patients	47	14	//	//	//			
Serum creat. mg/dl	0.88 (0.71–1.18)	0.7 (0.61–0.8)	0.9688	0.9324–1.0067	0.1051			
eGFR ml/min	84.8 (60.45-104.84)	112.3 (97.92-117.93)	0.9736	0.9497–0.9981	**0.0350**			
Proteinuria g/24 h	2.2 (1.33–3.46)	2.48 (1.63–3.96)	1.0022	0.8126–1.2360	0.9835			
RBC/HPF	10 (3-31.25)	0(0-0.25)	3.1694	1.3848–7.2537	**0.0063**	3.2292	1.2585–8.2858	0.0148
Arterial hyper								
ESR ml/h	56 (42–80)	54 (29.5–85)	0.9993	0.9774–1.0217	0.9523			
CRP mg/dl	0.3 (0.06–0.91)	0.06 (0.23–0.24)	1,1053	0.3302-3.7000	0.8717			
C3 mg/dl	59 (38.75–72.25)	84 (73–92)	0.9551	0.9269–0.9841	**0.0026**			
C4 mg/dl	7.5 (3.75-13)	9 (12.25–17.25)	0.8975	0.8186–0.9839	**0.0212**			
Anti-DNA AbIU/mL	263 (121–639)	44 (33-71.7)	1.0110	1.0018 1.0202	**0.0186**			
Anti-C1qAb UA	81 (44-143.5)	17.5 (9.25.53.5)	1.0361	1.0108 1.0621	**0.0050**	1.0288	1.0016–1.0568	0.0380

Legend: eGFR: glomerular filtration rate; RBC/HPF: red blood cells (high power field; hyper: hypertension, ESR: erythrocyte sedimentation rate, CPR: C reactive protein; Ab: antibodies

proportional hazard model. Both univariable and multivariable analyses were performed. Stepwise regression was utilized to identify variables retaining significance in the multivariable analysis. Kaplan-Meier estimates were employed to construct survival curves, and differences were assessed using the log-rank test. *P*-value < 0.05 was considered statistically significant.

Results

Comparison between proliferative and non-proliferative LN on the day of kidney biopsy (Table 1)

Sixty-one patients were included in this analysis. Forty-seven patients had proliferative LN; 14 patients were classified as class III (in 8 patients associated with class V), 33 patients were classified as class IV (in 9 patients associated with class V). Fourteen patients had non-proliferative LN; 2 class II, 12 class V. Among the clinical characteristics at kidney biopsy, eGFR was significantly lower [84.8 (60.45-104.84) vs. 112.3 (97.92-117.93)/ml/min; $p = 0.0350$], and the number of urinary erythrocytes higher [10 (3-31.25) vs. 0 (0-0.25)/HPF; $p = 0.0063$] in proliferative vs. non-proliferative forms. Serum C3 [59 (38.75–72.25) vs. 84 (73–92)mg/dl, $P = 0.0026$], and C4 [7.5 (3.75-13) vs. 9 (12.25–17.25) mg/dl, $p = 0.0212$] were significantly lower while anti-DNA antibodies [263(121–639) vs. 44 (33-71.7)IU/mL, $p = 0.0186$] and anti-C1qAb [81(44-143.5) vs. 17.5(9.25.53.5)UA, $p = 0.0050$] were significantly higher in the proliferative than in the no proliferative forms.

At multiple regression analysis, the number of red blood cell count in urinary sediment (OR 3.2292; CI 1.2585–8.2858; $p = 0.0148$) and the anti-C1qAb titers (OR 1.0288; CI.1.0016–1.0568; $p = 0.0380$) were the best tests to differentiate proliferative from no proliferative LN forms. At Roc curves the area under the curves for urinary red blood cells and for anti-C1qAb were respectively 0.926 and 0.820, their sensitivity 81.8% and 91.5%, specificity of 100% and 64.3%, negative predictive values 94.87% and 96.22%. After excluding from the analysis the 17 patients with mixed forms of LN, we found that the baseline parameters able to differentiate proliferative versus non-proliferative LN continued to be eGFR (p 0.0116), the number of red blood cell count in urinary sediment (p 0.0043), the title of antiC1qAb (p 0.0011) and of C3 complement fractions (p 0.0042) (Supplementary Table 1).

Clinical and immunological parameters at the beginning and after 6 and 12 months of therapy for active LN (Table 2)

Of 53 patients included in this second analysis, 90.7% were Caucasian. Thirty-nine patients (73.6%) were enrolled at LN diagnosis and 14 at diagnosis of a renal flare confirmed by a repeat kidney biopsy in seven patients. At the start of therapy, 18 patients (33.96%) had AKI, 20 (37.73%) had nephrotic syndrome and 35 (66.1%) had arterial hypertension. At kidney biopsy 10 patients had class III LN (8 with class V), 39 had class IV (10 with class V), and four patients had class V ISN/RPS LN [1, 2]. Induction treatment included three intravenous methylprednisolone pulses (MPP) in 77.3% of patients and oral prednisone 1 mg/kg/day for four weeks in 22.7% of patients, followed by oral prednisone progressively tapered to 7.5-5 mg/day. All patients received an immunosuppressive agent (mycophenolate mofetil in 73% of patients, cyclophosphamide, azathioprine, and

Table 2 Demographic, clinical, histologic, Immunologic and therapeutic characteristics of 53 patients at baseline at 6 and at 12 months after the start of therapy

	All 53 patients	43 Patients No renal flares	10 Patients Renal flares	43 Patients No renal flares	10 Patients Renal flares	43 Patients No renal flares	10 Patients Renal flares
	At the start of the study			At six months		At twelve months	
Male sex n. (%)	8 (15.09)	6 (13.95)	2 (20)				
Age at LN diagnosis Yrs	32 (24–40)	33 (24–41)	29 (24-37.25)				
Duration of LN at the start of the study (months)	6.88 (1.09–94.31)	6.15 (1.15–87.58)	71.92 (12.01–93.01)				
Histological classes n. (%): III /IV/ V/	10 (18.9), 39 (73.3), 4 (7.5)	7 (9.29), 33 (76.7), 3 (6.97)	3 (30), 6 (60), 1 (10)				
Activity Index	8 (4.25-13)	8 (4.25–13.75)	9 (6–12)				
Chronicity index	2 (1–3)	2 (1–3)	3 (1.75–3.25)				
Serum creatinine (mg/dL)	0.9 (0.67–1.15)	0.86 (0.67–1.21)	0.93 (0.83–1.02)	0.77 (0.66–0.93)	0.94 (0.84–1.06)	0.79 (0.72–0.95)	0.86 (0.83–0.9)
eGFR ml/min/1.73/m^2	84.8 (60.6-110.59)	84.8 (60.86-107.59)	86.14(61.17-109.02)	102.90 (84.62-119.92)	82.42 (71.21-112.48)	95.16 (67.25-105.12)	95.52 (75.08–99.17)
Proteinuria (g/24 h)	2.25 (1.28–4.15)	2.40 (1.45–4.20)	1.74 (1.45–2.95)	0.39 (0.24–1.20)	0.41 (0.33–0.57)	0.33 (0.17–0.54)	0.56 (0.38–0.83)
Arterial hypertension n. (%)	35 (66.1)	29 (67.4)	6 (60)	//	//	//	//
Red Blood cells /ul	3940 (3500–4480)	3940 (3530–4520)	3865 (3217.5–4375)	4565 (4160–4902)	4410 (4040–4075)	4635 (3995–4807)	4155 (3882–4517)
Erythrocyte sedimentation rate (ml/h)	54 (31.5–76.5)	56 (35–74)	42 (26–78)	18 (13–33)	15 (10–35)	20 (11.7–35.2)	29 (25–38)
C-reactive protein (mg/dL)	0.1 (0.06–0.81)	0.21 (0.06–0.84)	0.61 (0.08–1.05)	0.08 (0.05–0.33)	0.12 (0.03–0.16)	0.14 (0.06–0.31)	0.09 (0.05–0.41)
Anti-C1q antibodies (UA)	86 (56.2-140.2)	82 (51–130)	150 (75–218)	22 (10-40.5)	76 (66.5–81.5)	24 (15-47.7)	80 (45-97.7)
C3 (mg/dl)	58 (40.75–72.75)	56 (39.5–72.5)	61.5 (46.2–79)	92.5 (63.75-102.75)	86 (69.5–91)	88.5 (73.5-101.75)	78.5 (67.7–87.7)
C4 (mg/dl)	7.5 (3.25–12.75)	7 (3.5–11)	9.5 (3.75–12.5)	16 (9-23.5)	12 (8.5–14.5)	14.5 (10.2–20.7)	11.5 (16.2–14)
Anti-DNA antibodies IU/mL	202 (112–525)	202 (121–600)	203.5 (93.7-436.5)	49.2 (22.3–139)	99 (38.75–378.5)	46.8 (25.7-192.5)	133 (85–502)
Hydroxychloroquine n. (%)	32 (60.37)	27 (62.79)	5 (50)	28 (65.11)	5 (50)	30 (67.92)	6 (60)
Methylprednisolone pulses n. (%)	41 (77.3)	33 (76.74)	8 (80)	//	//	//	//
Prednisone mg/day	30 (25–50)	35 (26.2–50)	27.5 (25-35.6)	10 (7.5–12.5)	10 (8.75–11.25)	5 (5-7.5)	7.45 (5-7.5)
Cy/Aza/Csa/MMF n. (%)	9 (17)/ 1 (1.9)/ 4 (7.5)/ 39 (73.5)	7 (16.5)/0/ 4 (9.3)/ 32 (74.4)	2 (20)/ 1 (10)/ 0/ 7 (70)	1 (2.3)/3 (6.9)/39 (90.7)	0 (0)/1 (810)/9 (90)	1 (2.3)/3 (6.9)/ 39 (90.7)	0 (0)/1 (810)/9 (90)
Percent of patients in complete remission	//	//	//	58%	42.85%	82%	60%

Legend: SLE: systemic lupus erythematosus, n.: number; eGFR: glomerular filtration rate; Cy: Cyclophosphamide; Aza: azathioprine; Csa: Cyclosporine; MMF: mycophenolate mofetil. If not differently specified numbers are expressed as median and interquartile ranges

cyclosporine in the remaining), with 60.37% receiving hydroxychloroquine (HCQ).

At 6- and 12-months after therapy initiation, complete renal remission was achieved in 55.3% and 77.2% of patients, respectively. After a median observation of 60.69 (37.20-78.704) months, 45 (84.9%) patients were in complete renal remission, four (7.5%) were in partial renal remission, one (1.9%) had no response (and eventually died of lung neoplasia) and three (5.7%) had chronic kidney dysfunction stage G3A [16] (Table 3).

Table 3 Clinical characteristics of the patients at last observation

Clinical characteristics at the last follow-up	All 53 patients	43 Patients who did not develop renal flare	10 Patients who developed renal flare
Follow-up months	60.69 (37.20–78.70	63.56 (44.13–80.87)	28.19(24.83–39.38)
Serum creatinine (mg/dL)	0.80 (0.68–0.95)	0.77 (0.66–0.89)	0.89 (0.81–0.95)
eGFR (mL/min/1.73 m^2)	93.52 (79.47-107.56)	95.94 (84.80-108.39)	89.39 (79.42–93.61)
Proteinuria (g/24 h)	0.12 (0.09–0.26)	0.12 (0.08–0.26)	0.22 (0.12–0.48)
Arterial hypertension	27 (50.9)	21 (39.6)	6 (11.3)
Red Blood cells /ul	4465 (4095–4782)	4535 (4130-4842.5)	4395 (4032.5–4570)
C3 (mg/dl)	86 (75–105)	86 (76–106)	78.50 (65-94.25)
C4 (mg/dl)	14 (10–21)	15 (11–21)	11 (8-15.50)
Anti-DNA antibodies IU/mL	35 (10-89.5)	28 (9.83–71.28)	74.90 (36.5–184)
Anti.C1q antibodies UA	19 (10–42)	17 (9.5-30.75)	33 (25.55–55.5)
Hydroxychloroquine n. (%)	46 (86.79)	40 (93.02)	6 (60%)
Prednisone mg/day	5(2.5-5)	5(2.5-5)	5(5–5)
NO IS/Aza/CsA/MMF/belimumab n. (%)	1 (1.9)/ 4 (7.5) /1 (1.9)/47* (88.8)	0 (2.3)/4 (9.3)/1 (2.3)/37 (86%)**	0/0/0/10 (100) ***
Complete/partial remission/CKD/ PALN	45 (84.9%)/4 (7.5%)/3 (5.7%)/ 1(1.9)	37 (86.04)/2 (4.6), 2(4.6)/1 (2.3)	7/2/1
Death	1	1	0

Legend: SLE: systemic lupus erythematosus, n.: number; eGFR: glomerular filtration rate; Aza: azathioprine; CsA: cyclosporine: MMF: mycophenolate Mofetil; CKd: chronic kidney dysfunction; PALN: persistent active LN

*MMF + Calcineurin inhibitors: 5 patients; ** MMF + Calcineurin inhibitors: 2 patients; *** MMF + Calcineurin inhibitors: 3 patients

During the first year of the study, none of the patients experienced renal or extrarenal flares. Then, 10 patients (18.86%) developed renal flares after a median of 28.19 months (24.84–39.38, range 16.3–55.8) after the start of therapy. Four out of these 10 patients (40%) who developed flares had mixed forms of lupus nephritis (Class III or IV plus V) in comparison to 14 out of the 43 patients (32.5%) who never developed flares (p 0.56). In Table 4 we reported the clinical and histological characteristics before flare, at flare, and after induction therapy of the 10 patients who developed renal flares. Two flares were nephritic, while eight were proteinuric. All flares were treated with further induction therapy. Complete renal remission was achieved in seven patients, mild proteinuria persisted in two, and the last patient had CKD, with an eGFR of 58.2 ml/min/1.73 m^2. In addition, three other patients (5.7%) had extrarenal flares (characterized by arthralgias) respectively between 55 and 91 months after the start of the study. Flares were responsive to oral prednisone increase associated with belimumab in two patients.

Features associated with renal flares occurrence at univariable and multivariable analysis

Among the clinical, histological, and therapeutic features tested, at the start of the study and six months later, anti-C1qAb titers (OR 1.01, CI 1.00-1.02, p 0.034 at start; OR 1.02, CI 1.01–1.04, p 0.0005 at six months) and no therapy with HCQ (OR 0.23, CI 0.06–0.87, p 0.0313 at start; OR 0.22, CI 0.06–0.84, p 0.0276 at six months) were significantly associated with the occurrence of renal flares at univariable analysis.

One year after therapy initiation, at univariable analysis, anti-C1qAb ≥ 40 UA, (OR 7.93, CI 1.69–37.09, p 0.0089), no HCQ therapy (OR 0.22 CI 0.06–0.82, p 0.0252), and proteinuria ≥ 0.5 g/day (OR 4.89, CI 1.27–18.81, p 0.0216) were associated with renal flares. The survival free of flare at five years was 86.39% in patients with anti-C1qAb < 40 at 12 months vs. 52.00% in those with anti-C1qAb ≥ 40 U; it was 79.40% and 46.88% in patients given vs. those not given HCQ, and 87.04% in patients with proteinuria < 0.5 g/day vs. 46.67% in those with proteinuria ≥ 0.5 g/day (Table 5). At multivariable analysis, anti-C1q > 40 UA (OR 9.07, CI 0.91–42.99, p 0.0057) and non-use of HCQ (OR 0.17, CI 0.04–0.68, p 0.0126) were the independent factors associated with renal flare development 12 months after therapy initiation.

When the mixed forms of lupus nephritis (class III or IV plus class V) were excluded from the search of the predictors of flare development, anti-C1qAb titers continued to be predictive of flares at six (OR 1.03, CI 1.00–1.053, p 0.0018) and twelve months (OR 1.02, CI 1.01–1.04, p 0.0029). Moreover, at twelve months, anti-C1qAb ≥ 40UA (OR 8.37, CI 0.99–70.96, p 0.0207), the amount of proteinuria (OR 2.13, CI 1.17–3.86, p 0.0135) and no use of HCQ (OR 0.12, CI 0.02–0.69, p 0.0180) were associated with the development of flares.

Table 4 Clinical characteristics of renal flares

Pts	Histological class	Type of flare Nephritic 1 Proteinuric 2	Therapy at flare	Time to renal flare months	Serum creatinine mg/dl			Proteinuria g/24H			Number of red blood cells /high power field at urinary sediment		
					Pre flare	At flare	At last observation	Pre flare	At flare	At last observation	Pre flare	At flare	At last observation
1	V	2	Pr. 5 mg, Tacr 3 mg, MMF 750 mg	24.0	0.68	0.71	0.80	0.34	1.6	0.13	0	0	0
2	IV	2	Pr.5 mg, MMF 1 gr	42.9	0.92	0.86	0.85	0.15	1.6	0.11	0	12	0
3	IV+V	1	Pr. 10 mg Neoral 75 mg	55.8	0.76	**1.23**	0.90	0.26	0.93	0.72	0	35	0
4	IV	2	Pr 6.25 mg MMF 3gr	28.7	0.85	0.9	0.95	0.24	2.3	1.20	2	7	4
5	IV	2	Pr 12.5 mg MMF 2gr	16.3	0.72	0.88	0.89	0.85	5.1	0.15	2	8	0
6	IV	1	Pr 5 mg MMF 2gr	28.1	0.65	**1.1**	0.70	0.18	0.9	0.05	5	20	0
7	IV+V	2	Pr 5 mg MMF 1.5gr	26.2	0.46	0.57	0.54	0.29	1.6	0.16	0	0	0
8	III+V	2	Pr 6.25 mg Tacr 3 mg	28.3	0.89	0.91	0.75	0.28	1.4	0.42	6	2	0
9	III+V	2	Pr 3.75 mg MMF 1gr	14.5	1.09	1.03	1.30	0.27	1.05	0.20	2	12	0
10	III	1	Pr 6.25 mg Neoral 100 mg	49.9	0.74	1.0	0.70	0.14	1.2	0.34	0	20	1

Legend: Pr: prednisone; MMF, Mycophenolate mofetil; Tacr: tacrolimus

Table 5 Survival free from renal flares (with Kaplan-Meier survival estimate) for patients with or without antiC1q < 40 UA; with or without HCQ therapy; with or without proteinuria < 0.5 g/day 12 months after the start of therapy

	Flare-free survival			*p*
	At 2 year	At 3 years	At 5 years	
AntiC1q < 40 UA after 12 months of therapy	92.15%	86.39%	86.39%	0.0182
AntiC1q ≥ 40 UA after 12 months of therapy	68.25%	60.57%	52.00%	
HCQ 12 months after the start of therapy	87.94%	83.94%	79.40%	0.0245
No HCQ 12 months after the start of therapy	62.50%	46.88%	46.88%	
Proteinuria < 0.5 g/day 12 months after the star of therapy	92.16%	87.04%	87.04%	0.0057
Proteinuria ≥ 0.5 g/day 12 months after the star of therapy	66.67%	58.33%	46.67%	

Discussion

The first goal of this study was to understand the actual role of clinical and immunological tests in differentiating proliferative from non-proliferative lupus nephritis. The tests were performed on the day of the diagnostic kidney biopsy. At univariable analysis, acute kidney injury and hematuria were the clinical signs significantly associated with proliferative LN. All four immunological tests were significantly different between the two forms of LN. However, at multiple regression analysis, higher titers of anti-C1qAb and a higher number of erythrocytes at urinary sediment were the only variables associated with proliferative LN. Both variables had high negative predictive values, suggesting that a proliferative LN is very unlikely to occur in patients without erythrocyturia and with low anti-C1qAb titers. Considering the high probability of histological transformation from one class to another [10, 17], these results can help the clinicians in case of a renal flare to suggest or not a change in the histological class. Although urine sediment examination is operator-dependent, it was demonstrated that glomerular hematuria had specificities and positive predictive values between 90 and 100% for diagnosing proliferative glomerular diseases, with a variable sensitivity [18]. Martínez-Martínez MU et al. [19] demonstrated a positive correlation between the amount of hematuria and acanthocyturia with the severity of NIH Activity and Chronicity Index scores at kidney biopsy. In keeping with our results, they found a good discriminatory ability of hematuria for detecting proliferative (sensitivity and specificity of 0.83 and 0.81, respectively) vs. non-proliferative LN [19]. Moreover, our data confirm the importance of anti-C1qAb in monitoring the activity of LN at LN diagnosis [20, 21]. In an Italian cohort of 107 SLE patients, all the autoantibodies evaluated had significantly higher titers in proliferative than in non-proliferative LN; however, at multivariate analysis, anti-C1qAb (alone or in association with anti-dsDNA) was the best test to differentiate the two histological forms [21]. Chen et al. [20] found anti-C1qAb in 75% of 52 patients with biopsy-proven LN. The anti-C1qAb titers were significantly higher in class IV than in class II and were positively associated with the glomerular deposition of C1q at immunofluorescence.

The second outcome of this study was to assess the role of regular monitoring of immunological tests in predicting the occurrence of renal flares. It has been reported that 25 to 66% [11, 22, 23] of LN patients develop renal flares. This wide range depends on the definition of flare, the duration of follow-up, and ethnicity. There is general agreement that renal flares, particularly nephritic flares, are associated with a poor renal prognosis [11, 12, 24] causing residual chronic kidney damage. Several predictors of renal flares have been identified among the baseline characteristics, such as young age, high serum creatinine, low complement levels, and high titers of anti-DNA antibodies at LN diagnosis [25, 26, 27]. In addition to features at baseline, low serum levels of C3 and C4 at 6 and 12 months, and high levels of anti-dsDNA antibodies at 12 months [28] have been associated with a higher risk of flares. None of the above-reported variables emerged as predictors of renal flares in our cohort, perhaps due to the low number of patients included in our study.

In 53 patients with active LN prospectively followed in our Unit, serum C3, serum C4, anti-DNA antibodies, and anti-C1qAb were regularly monitored during the first year of active LN therapy. After a follow-up of 60 months, 85% of patients were in complete remission, 7.5% were in partial remission, three patients had stage G3A CKD, and one patient died. During the follow-up, around 20% of patients developed a renal flare; all flares occurred after 12 months of observation and in the median two years after the beginning of therapy. At univariable analysis, among the clinical features analyzed at baseline, at six, and 12 months after induction therapy, non-use of HCQ was associated with renal flares at any of the three-time points considered. At 12 months, the persistence of proteinuria ≥ 05 g/day was the second clinical variable able to predict renal flares. Among the immunological tests considered, only anti-C1qAb was associated with renal flares, its predictive power was confirmed not only at baseline and six but also at 12 months when an anti-C1qAb titer > 40UI increased the risk of renal flares of 7.9 points. At multivariable analysis non-use of HCQ and anti-C1qAb > 40UI at 12 months were significantly associated with the risk of renal flares.

Residual proteinuria one year after the start of therapy was associated with poor renal outcomes in some studies [29, 30] but few data are available about its value in predicting renal flares [31]. Ligtenberg et al. [28] found that

persistent proteinuria at 12 months was associated with the occurrence of renal flares. Kapsia et al. [26] confirmed the value of a proteinuria > 0.8 g/day at 12 months as a predictor of renal flares. Our results stressed the importance of even a mild residual proteinuria. Survival free of flares at five years was 87% in patients with proteinuria < 0.5 g/day and 46.7% in those with proteinuria ≥ 0.5 g/die. Among the multifaced favorable effects of HCQ therapy [32], there is also the prevention of renal flares. Both in children and adults, reduced blood levels of HCQ were associated with an increased flare rate [33, 34], and even in a BLISS pooled data set analysis, its use was protective against renal flares [35]. Our results reinforce the importance of an early and continuous administration of HCQ, even when the other drugs are stopped [36].

During the last decades, many efforts have been made to identify biomarkers useful for monitoring LN activity, to predict or confirm a renal exacerbation avoiding a new kidney biopsy. Among all biomarkers tested, anti-C1qAb seems to better match the premises. Anti-C1qAb were demonstrated to be strongly associated with active proliferative LN [20, 21, 37]. Persistent high serum of anti-C1qAb three months after the start of therapy may predict failure to achieve complete renal remission [38]. In a longitudinal study on a subgroup of 16 LN patients, titers of anti-C1q IgG increased from 6 to 4 months before renal flare but only in patients who were anti-C3b positive [39]. After a thorough evaluation of the literature on the subject, we didn't find studies that indicate that elevated antiC1qAb titers at baseline and their failure to normalize one year after the start of induction therapy for active lupus nephritis were associated with the development of renal flares in subsequent follow-up.

This study has limitations. It is retrospective and includes a low number of patients. Most patients were Caucasians and for this reason, the results cannot be applied to other ethnicities. Moreover, the therapies were not standardized. Despite these limitations, our data underline the association of proliferative lupus nephritis with active urinary sediment and high titers of anti-C1qAb. The persistence of anti-C1qAb positivity after one year of therapy is significantly associated with the risk of renal flares together with the persistence of mild proteinuria. We confirm the importance of therapy with HCQ to prevent renal flares.

Altogether our results confirm our previous experiences about the importance of anti-C1qAb as a valuable marker for the diagnosis and for the management of LN.

Abbreviations

ACR	American College of Rheumatology
AKI	Acute kidney injury
CI	Confidence interval
CKD	Chronic kidney disease
CKD-EPI	Chronic Kidney Disease– Epidemiology Collaboration
CRP	C-reactive protein
CSA	Cyclosporine
CY	Cyclophosphamide
eGFR	Estimated Glomerular Filtration Rate
ELISA	Enzyme-linked immunosorbent assay
ESKD	End-stage kidney disease
ESR	Erythrocyte sedimentation rate
HCQ	Hydroxychloroquine
HPF	High power field
ISN	International Society of Nephrology
LN	Lupus nephritis
MMF	Mycophenolate mofetil
MPP	Methylprednisolone pulses
OR	Odds ratio
PALN	Persistent active lupus nephritis
RBC	Red blood cells
RPS	Renal Pathology Society
SELENA	Safety of Estrogens in Lupus Erythematosus National Assessment
SLE	Systemic lupus erythematosus
SLEDAI	Systemic Lupus Erythematosus Disease Activity Index

Supplementary Information

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Supplementary Material 1

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Author contributions
G.M. and M.C. conceived the study. F.D.L. performed the measurements of anti-C1qAb. E.C. collected the data. F.R. analyzed the data. G.M., M.C., and M.S. wrote the paper.

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Data availability
No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate
The study was approved by the Ethics Committee of IRCCS Humanitas Rozzano, Milano, Italy (protocol code NEF0032023).

Consent for publication
All participants provided informed consent for the scientific use of their anonymized data. Patient or public involvement in the research was not applicable.

Competing interests
The authors declare no competing interests.

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