A prospective observational cohort study highlights kidney biopsy findings of lupus nephritis patients in remission who flare following withdrawal of maintenance therapy

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One of the most difficult management issues in lupus nephritis (LN) is the optimal duration of maintenance immunosuppression after patients are in clinical remission. Most patients receive immunosuppression for years, based mainly on expert opinion. Prospective data are unavailable. Complicating this issue are data that patients in clinical remission can still have histologically active LN; however, the implications of this are unknown. To study this, the Lupus Flares and Histological Renal Activity at the end of Treatment study (ClinicalTrial.gov, NCT02313974) was designed to examine whether residual histologic activity predisposes to LN flares in class III and IV LN. Patients in complete clinical remission for at least 12 months who had received at least 36 months of immunosuppression were eligible. Patients consented to a second kidney biopsy, were tapered off maintenance immunosuppression and were then followed prospectively for LN flares over 24 months. Forty-four patients were enrolled, and 36 completed the study. LN flares occurred in 11 patients, and ten of these had residual histologic activity on the second biopsy. All patients with an NIH activity index over two flared. The activity index and duration of systemic lupus erythematosus at the second biopsy were independent predictors of flare. A predictive equation based on these variables discriminated between flare and no flare with a sensitivity of 100%, specificity of 88%, and a misclassification rate of 8.3%. Thus, a repeat kidney biopsy may be useful in managing maintenance immunosuppression in LN, and patients in histologic remission may be candidates for withdrawal of therapy.

Kidney International (2018) ■, ■–■; https://doi.org/10.1016/j.kint.2018.05.021

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Received 18 February 2018; revised 12 May 2018; accepted 17 May 2018

KEYWORDS: histologic activity; kidney biopsy; lupus nephritis; renal flare; systemic lupus erythematosus

Systemic lupus erythematosus (SLE) has a relapsing and remitting course, with patients experiencing episodic disease activity (flares) over time. Kidney biopsy plays an important role in the initial diagnosis and staging of lupus nephritis (LN). It also guides the appropriate selection of treatment, especially for high-risk patients.[1,2]

The duration of maintenance treatment for International Society of Nephrology/Renal Pathology Society (ISN/RPS) class III and IV LN is currently based on the clinical evolution of the LN flare. Many guidelines recommend at least 3 years of total treatment and 1 year of complete remission before withdrawing treatment. These recommendations are based mainly on expert opinion, as there are few data to develop an evidence-based guideline. Protocol repeat biopsy studies after complete remission show continuing histologic activity in a significant number of patients.[3–7] Stopping maintenance immunosuppression in such patients may theoretically put them at risk of renal flare. Management of maintenance therapy is even more uncertain in patients with stable partial renal remission and no extrarenal lupus activity. Such patients often have ongoing proteinuria, and thus continue to receive immunosuppression indefinitely. Repeat biopsies in such patients[3,4,8] have shown that many have no histologic activity and are in histologic remission. Persistent proteinuria may be from past injury and scarring, so continuing treatment may put such patients at risk for infectious morbidity with little benefit for the LN.[2,9]

We suggest that a repeat biopsy in LN patients on long-term maintenance therapy who have been in complete renal remission for at least 1 year may help guide the withdrawal of maintenance immunosuppression. We postulated that patients with remaining histologic activity will have a greater tendency toward LN flares than those with no remaining activity, and tested this hypothesis prospectively in the Lupus Flares and Histological Renal Activity at the End of the Treatment (LuFla) study (ClinicalTrial.gov identifier: NCT02313974).

RESULTS

The overall study design and patient flow of LuFla is shown in Figure 1. LuFla recruited 44 patients, and 36 completed the study. All patients were Hispanic and white, 25 (83%) were female, and the average age of the cohort at biopsy 1 was 31.6 ± 11.3 years. Table 1 presents the demographic, clinical, and histologic characteristics of the patients at biopsy 1 segregated by renal flare status after stopping immunosuppression.[10] Patients who experienced flare were comparable to patients who did not in all respects except for having a longer overall duration of SLE. There were fewer males in the flare group, but this did not reach significance. LN histologic classes were distributed as follows: IV A ($n = 7$), IV A/C ($n = 16$), III A ($n = 7$), III A/C ($n = 6$), and 4 of the class III A patients had concomitant class V LN.

After a minimum of 36 months of immunosuppression and at least 12 months of clinical renal remission, a repeat kidney biopsy (biopsy 2) was performed. The clinical and histologic findings at biopsy 2 are summarized in Table 2. Overall, 20 patients (55.6%) achieved complete histologic remission with an activity index (AI) of 0. Nine patients (25%) had an AI of 1 or 2. The remaining 7 patients (19.4%) had an AI between 3 and 5. Despite complete clinical renal remission, persistent histologic activity was present in 16 patients (44.4%). The histologic components of the AI that were found in these patients were endocapillary proliferation in 13 (81%), subendothelial deposits in 14 (88%), and interstitial inflammation in 4 (25%). No patient had persistent glomerular crescents or necrosis.

After maintenance therapy was tapered and discontinued, LN flared in 11 patients (30.5%). The clinical findings at flare for these patients are shown in Table 3. All but 1 flare (91%) occurred in patients who had active histology at biopsy 2, and everyone with an AI > 2 experienced flare (Figure 2). Among the no-flare group, 6 patients (24%) had an AI of 1 to 2 on biopsy 2. In the entire cohort, the incidence of renal flare in patients who had an AI ≤ 2 at biopsy 2 was 13.8%.

Clinical, serologic, and histologic findings at biopsy 2 are provided in Table 2. Although proteinuria decreased below 500 mg/d in all patients, the flare patients showed a trend ($P = 0.06$) toward more proteinuria than the no-flare patients in remission. Additionally, while not statistically significant, more patients who experienced flare were positive for anti–double-stranded DNA antibodies, had low C3 and C4 levels at biopsy 2, and showed a decline in C3 in the 6 months preceding biopsy 2 ($P = 0.06$). Proteinuria, change in C4, and anti–double-stranded DNA antibody status at biopsy 2 did not correlate with the presence or absence of persistent histologic activity in biopsy 2 (AI $= 0$ vs. AI > 0). The decline in C3 in the 6 months preceding biopsy 2 showed a tendency to associate with the AI ($P = 0.073$ by logistic regression), but its correlation was not strong (Spearman $r = –0.20$; $P = 0.23$).

Chronicity at biopsy 2, measured by the chronicity index (CI), did not correlate with proteinuria at biopsy 2 (Spearman

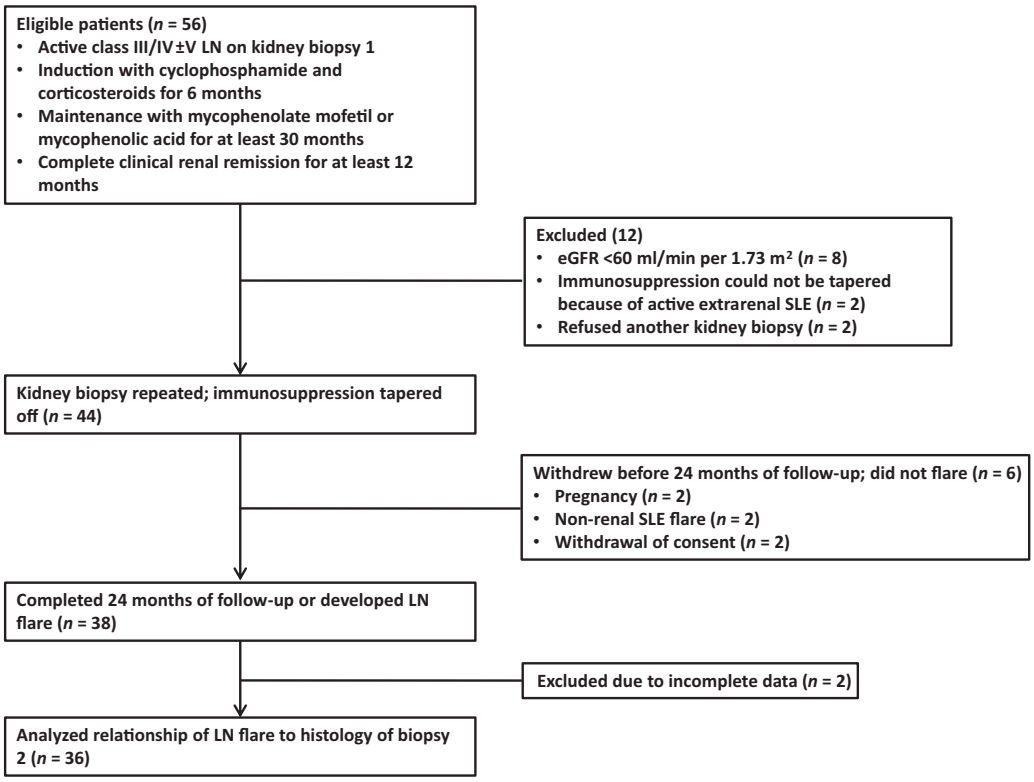

Figure 1 | Lupus Flares and Histological Renal Activity at the End of the Treatment (LuFla) study design and patient flow. eGFR, estimated glomerular filtration rate; LN, lupus nephritis; SLE, systemic lupus erythematosus.

Table 1 | Clinical and histologic findings at biopsy 1

Variable	Entire cohort (n = 36)	Flare group (n = 11)	No-flare group (n = 25)	P value[a]
Age (yr)	31.6 ± 11.3	30.0 ± 6.5	32.0 ± 13	0.92
% male	16.7	9.1	20	0.64
Duration of SLE (mo)	54 (1-240)	120 (12–240)	48 (1–240)	0.03
% with prior history of renal flare	33.3	55	24	0.12
Proteinuria (g/d)	2.1 (0.2–20)	2.1 (0.17–4.6)	2.0 (0.3–20)	0.66
SCr (mg/dl)[3]	0.78 (0.46–2.80)	0.74 (0.55–1.00)	0.80 (0.46–2.80)	0.51
eGFR (ml/min per 1.73 m^2)[b]	103 (20–143)	109 (86–122)	99 (20–143)	0.39
C3 (mg/dl)	81 (25–140)	89 (40–118)	68 (25–140)	0.36
C4 (mg/dl)	12 (0–32)	12 (3–23)	11 (0–32)	0.78
% low C3	52.8	27.3	64.0	0.07
% low C4	69.4	72.7	68.0	1.00
% anti-dsDNA–positive	80.6	81.8	80.0	1.00
Activity index	8 (3–16)	11 (4–16)	7 (3–13)	0.23
Chronicity index	3 (0–6)	2 (0–4)	3 (0–6)	0.24

C3, complement component 3; C4, complement component 4; dsDNA, double-stranded DNA autoantibody; eGFR, estimated glomerular filtration rate; SCr, serum creatinine concentration; SLE, systemic lupus erythematosus.
Data are presented as mean ± SD, proportion (%) of patients, or median (range).
[a]Calculated between the flare and nonflare groups by the nonparametric Mann-Whitney test for continuous data and Fisher's exact test for proportions.
[b]Calculated by the CKD-EPI formula (Levey *et al.*[10]).

r = 0.003; P = 0.99). This lack of association was also observed when proteinuria was correlated with chronicity in the glomerular compartment (glomerulosclerosis plus fibrous crescents) or chronicity in the tubulointerstitial compartment (tubular atrophy plus interstitial fibrosis) (data not shown).

By logistic regression, the AI at biopsy 2 was significantly associated with the odds of an LN flare within 2 years of stopping maintenance immunosuppression ($P < 0.0001$). If the AI was at least 1, the odds ratio for LN flare was 31.7 (95% confidence interval 3.3–300). For flare prediction an AI cutoff of 1 had a sensitivity of 91%, a specificity of 76%, and a misclassification rate of 16.7%. Similarly, when other variables were tested as potential predictors, only the duration of SLE (P = 0.0297) and the decline in C3 in the 6 months preceding biopsy 2 (P = 0.0386) were significant. Univariate predictors that were not significant are provided in Supplementary Table S2.

Multivariable logistic regression models were developed for predicting future LN flare using the predictors found to be significant on univariate analysis (Table 4). The table contains the intercepts and slope coefficients for the presented models. Of these, model 4 is parsimonious and has the lowest misclassification rate and the highest area under the receiver operating characteristic curve. Using Model 4, flare is predicted if Y is greater than cutoff value C, where Y = 3.48 x (AI biopsy 2) + 4.58 x natural log (duration of SLE in months)

Table 2 | Clinical and histologic findings at biopsy 2

Variable	Entire cohort (n = 36)	Flare group (n = 11)	No-flare group (n = 25)	P value[a]
Duration of treatment (mo)	38 (36–54)	38 (36–48)	38 (36–54)	0.61
Time to remission (mo)	24 (12–40)	24 (16–36)	24 (12–40)	0.75
Duration of remission (mo)	12 (12–30)	12 (12–20)	13 (12–30)	0.43
Proteinuria (g/d)	0.11 (0.03–0.48)	0.16 (0.06–0.48)	0.07 (0.03–0.48)	0.06
SCr (mg/dl)[b]	0.70 (0.50–1.12)	0.66 (0.60–0.90)	0.70 (0.50–1.12)	0.70
eGFR (ml/min per 1.73 m^2)	114 (81–135)	114 (95–127)	114 (81–135)	0.85
C3 (mg/dl)	112 (55–188)	100 (55–170)	116 (64–188)	0.19
C4 (mg/dl)	19 (3–51)	15 (3–28)	20 (6–51)	0.20
% low C3	13.9	27.3	8.0	0.15
% low C4	36.1	45.5	32.0	0.47
ΔC3[c]	1 (–36 to 77)	–7 (–30 to 26)	10 (–36 to 77)	0.07
ΔC4[c]	0 (–15 to 15)	–3 (–13 to 11)	0 (–15 to 15)	0.42
% anti-dsDNA–positive	22.2	36.3	16.0	0.21
Activity index	0 (0–5)	3 (0–5)	0 (0–2)	<0.0001
% endocapillary proliferation[d]	30.6	90.9	4	<0.0001
% subendothelial deposits[d]	38.9	90.9	16	<0.0001
% glomerular leukocytes[d]	25	45.5	16	0.075
Chronicity index	3 (0–5)	3 (0–4)	2 (0–5)	0.13

C3, complement component 3; C4, complement component 4; dsDNA, double-stranded DNA autoantibody; eGFR, estimated glomerular filtration rate; SCr, serum creatinine concentration.
Data are expressed as proportion (%) of patients, or median (range).
[a]Calculated between the flare and nonflare groups by the nonparametric Mann-Whitney test for continuous data and Fisher's exact test for proportions.
[b]Calculated by the CKD-EPI formula.
[c]The decline in complement C3 and C4 from 6 months before biopsy 2 to biopsy 2 (value at biopsy 2 minus value 6 months before biopsy 2).
[d]Histologic components of the activity index expressed as percentage of patients positive for the finding at biopsy 2.

Table 3 | Clinical findings of patients who experienced flare

		Proteinuria (g/d)		SCr (mg/dl)		eGFR[b] (ml/min per 1.73 m^2)		C3 (mg/dl)		C4 (mg/dl)		Anti-dsDNA antibodies (present/absent)		Urinalysis (RBC/hpf)[c]
Patient	Time to renal flare (mo)[a]	Biopsy 2	Flare	Biopsy 2	Flare	Biopsy 2	Flare	Biopsy 2	Flare	Biopsy 2	Flare	Biopsy 2	Flare	Flare
1	18	0.43	1.40	0.66	0.50	117	129	170	146	24	17	A	A	4–8
2	6	0.12	1.12	0.80	0.85	97	90	82	46	14	2	P	P	12–14
3	6	0.10	1.02	0.72	0.74	116	111	107	81	14	7	P	P	4–8
4	15	0.07	0.65	0.60	0.80	127	102	66	67	5	6	A	P	8–12
5	12	0.06	1.06	0.90	1.00	118	94	95	78	19	12	A	P	10–14
6	18	0.48	2.00	0.84	0.60	95	95	134	73	11	5	A	A	2–4
7	12	0.426	0.54	0.60	0.55	110	114	124	110	24	12	A	A	14–20
8	21	0.16	0.33	0.61	0.68	119	114	100	107	15	12	P	P	30–50
9	21	0.08	2.59	0.77	1.40	107	52	83	26	18	5.7	P	P	70–80
10	21	0.30	1.47	0.60	0.67	119	114	122	128	28	34	A	A	8–10
11	6	0.40	2.06	0.60	0.60	114	113	55	55	3	3	A	A	10–20

dsDNA, double-stranded DNA autoantibody; eGFR, estimated glomerular filtration rate; hpf, high-power field; RBC, red blood cells; SCr, serum creatinine concentration.
[a]Time from stopping maintenance immunosuppression.
[b]Calculated by the CKD-EPI formula.
[c]All samples contained acanthocytes.

– 24.83. A C value of 0 gave the maximum sum of sensitivity (100%) and specificity (88%) to discriminate between flare and no flare. The odds ratios for LN flare per unit change of AI and log (duration SLE) were 32.5 (95% confidence interval 4.4–1912) and 97 (95% confidence interval 3.8–5049), respectively. There was no relationship between AI and duration of SLE (Spearman *correlation* $r = 0.023$, $P = 0.89$), indicating that they can be viewed as independent predictors.

Individual components of the AI listed in Table 2 were examined as possible independent predictors of LN flare. In univariate analysis, endocapillary proliferation and subendothelial deposits were both significantly associated with the odds of an LN flare, but endocapillary proliferation was more robust (Table 4). Combining endocapillary proliferation and duration of SLE led to a flare prediction model (model 11, Table 4) that reduced the misclassification rate and increased the AUC compared with model 4. Using model 11, flare is predicted if Y is greater than the cutoff value C, where Y = 9.64 x (endocapillary proliferation at biopsy 2) + 5.07 x natural log (duration of SLE in months) – 27.53. A C value of 0 gave the maximum sum of sensitivity (100%) and specificity (92%) to discriminate between flare and no flare.

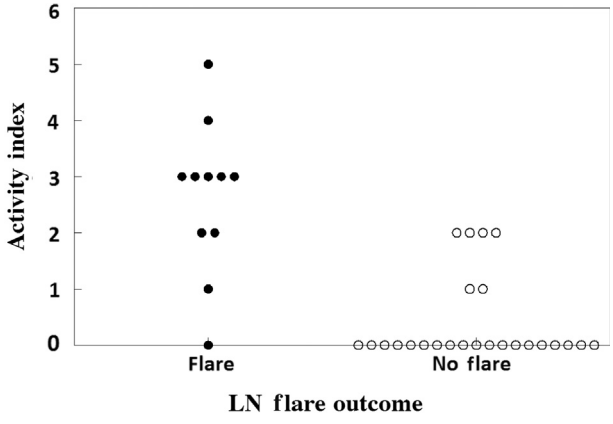

Figure 2 | Activity indices of patients who experienced lupus nephritis (LN) flare and patients who did not after withdrawing maintenance immunosuppression. Each dot represents an individual patient.

DISCUSSION

These data demonstrate that despite extensive and long-term immunosuppression, patients with LN who enter a complete clinical renal remission still have a high rate of relapse following withdrawal of maintenance immunosuppression. Patients prone to relapse cannot be identified *a priori* by clinical or demographic variables that are commonly collected during standard office visits. However, examination of kidney histology during treatment and after clinical remission provides information that can be used to predict who is likely to relapse and who is likely to remain in remission after immunosuppression is stopped. Almost every patient who developed an LN flare had persistent histologic LN activity, and every patient in this cohort with an activity index above 2 had an LN flare. These data suggest that a kidney biopsy should be considered when withdrawal of maintenance therapy is contemplated. The activity index of this biopsy, and more specifically the extent of endocapillary proliferation present in this biopsy, combined with the duration of a patient's SLE may be used to predict who is or is not likely to experience a flare. Patients predicted not to experience a flare may be candidates for stopping treatment.

Persistent histologic activity was found in 44% of this cohort. Previous studies have also shown that about 30% to 60% of LN patients have residual evidence of active inflammation on protocol biopsies performed during maintenance therapy after complete clinical remission.[5,11–13] The predominant persisting lesions found in our patients' biopsies were endocapillary proliferation and subendothelial immune complexes. Cellular crescents and glomerular necrosis were

Table 4 | Performance characteristics of logistic regression models to predict future LN flare

						Predictor coefficient (*P* value)					
Model	Predictor[a]	Model *P* value[b]	Misclassification rate[c]	AUC	Intercept	AI at biopsy 2	Duration of SLE[d]	ΔC3[e]	Endocap at biopsy 2	Subendo	Glom leuk at biopsy 2
1	1	<0.0001	0.17	0.91	−3.12	1.66 (<0.0001)					
2	2	0.030	0.22	0.72	−6.84		1.28 (0.030)				
3	3	0.039	0.33	0.69	−0.74			−0.035 (0.039)			
4	1, 2	<0.0001	0.083	0.98	−26.50	3.48 (<0.0001)	4.58 (0.0014)				
5	1, 3	<0.0001	0.083	0.92	−3.22	1.74 (<0.0001)		−0.053 (0.078)			
6	2, 3	0.006	0.25	0.81	−8.25		1.60 (0.015)	−0.043 (0.019)			
7	1, 2, 3	<0.0001	0.083	0.99	−43.91	5.07 (<0.0001)	8.01 (0.0013)	−0.081 (0.07)			
8	4	<0.0001	0.056	0.94	−3.18				5.26 (<0.0001)		
9	5	<0.0001	0.14	0.90	−3.09					3.68 (<0.0001)	
10	6	0.067	0.28	0.65	−1.25						1.48 (0.067)
11	2,4	<0.0001	0.056	0.99	−29.07		5.07 (0.031)		9.64 (<0.0001)		

AI, activity index; AUC, area under the receiver operating characteristic curve; Endocap, endocapillary proliferation on biopsy 2; Glom leuk, glomerular leukocyte infiltration at biopsy 2; SLE, systemic lupus erythematosus; Subendo, subendothelial deposits on biopsy 2.
[a]Predictors are defined as follows: 1 = AI at biopsy 2, 2 = log (duration of SLE), 3 = decline in complement C3 from 6 months before biopsy 2 to biopsy 2, 4 = endocapillary proliferation, 5 = subendothelial deposits, and 6 = glomerular leukocytes.
[b]Based on the likelihood ratio test.
[c]Using predicted probability of 0.5 as the cutoff.
[d]Natural log duration (mo).
[e]Decline in complement C3 from 6 months before biopsy 2 to biopsy 2 (value at biopsy 2 minus value 6 months before biopsy 2).

not found, similar to in other repeat biopsy cohorts.[4] The endocapillary proliferation component of the AI predicted flare better than did the combined AI.

At biopsy 1 the flare and no-flare patients were similar clinically and histologically, except the no-flare group had more patients with a low C3 level. Interestingly, 2 of the 3 flare patients who had low C3 at biopsy 1 continued to have a low C3 at biopsy 2, while all but 2 of the 16 no flare patients with low C3 at biopsy 1 had normal levels by biopsy 2.

At biopsy 2 the flare group had somewhat more proteinuria than the no-flare group, and more patients in the flare group had a positive double-stranded DNA titer, low C3, and low C4. Additionally, in the 6 months preceding biopsy 2, the patients who experienced flare tended to show a decrease in C3, and the patients who did not tended to show an increase in C3. Taken together, and despite individual measurements not reaching statistical significance, these findings suggest that a low level of clinical activity persisted in the flare group. Despite this, no clinical measurement obtained around biopsy 2 was significantly associated with, or could robustly predict, the AI of biopsy 2, but this needs to be examined in a larger cohort.

In our cohort, anyone with an AI of 1 or more on biopsy 2 had a high risk of future LN flare, and everyone with an AI of >2 experienced flare. Interestingly, a Middle Eastern cohort of LN patients who had protocol biopsies during maintenance therapy was found to have a poor (44%) 10-year kidney survival rate if the AI was >2 at re-biopsy, fair (80%) survival if the AI was 1 or 2, and 100% survival if the AI was 0.[5] These findings suggest that complete histologic remission may be a target treatment goal for LN.

This study had several limitations. The cohort was a relatively small and ethnically homogeneous population, and results may differ in an ethnically and racially diverse LN population. No sample size calculations were done, but the small *P* values for the slopes associated with AI and log (duration of SLE) and the lower confidence limit for the odds ratios for predicting future flare being away from 1 suggest the data are robust. The finding that duration of SLE was associated with future relapse may be due to the fact that patients with persistent disease activity have a longer follow-up duration. However, even among those with long disease duration, all but 1 patient who relapsed had persistent histologic activity. The study was under-powered to draw firm conclusions regarding proteinuria and serologic markers at biopsy 2. The duration of follow-up was limited. Patients with no histologic activity may flare, but it may take longer than 2 years. The principal investigator was not blinded to the results of the second biopsy, and this could have influenced flare diagnosis; however, for LN flares objective data were required, mitigating bias. The most important limitation was that LuFla did not demonstrate that continuation of maintenance therapy in patients with persistent histologic activity and complete clinical remission would have prevented future LN flares. This is a critical question and will need to be examined in a follow-up study, powered by the LuFla outcomes.

In summary, the LuFla investigation supports the use of a kidney biopsy to help manage the duration of maintenance therapy for LN patients who have been in complete renal remission for at least 1 year and have had at least 3 years of immunosuppression. Persistent histologic activity on this biopsy, especially if sufficient to be labeled as a National Institutes of Health AI of >2, is associated with LN relapse. If patients have an activity index of 0, indicating complete histologic remission, withdrawal of immunosuppression may be reasonable.

METHODS

Study design

LuFla was a prospective observational cohort study of adult patients (ages 18–70) with class III/IV±V LN to assess the relationship of

post-therapy LN flares to kidney histology found on biopsies performed immediately before maintenance therapy was tapered off. Written informed consent was obtained from all participants. The study was approved by the University Hospital Ethics Committee. The investigators and hospital ethics committee judged that clinical equipoise existed for LuFla because the consequences of stopping immunosuppression for patients lacking clinical evidence of LN activity but with persistent histologic activity are unknown.[14–16] Patients with an estimated glomerular filtration rate $<$ 60 ml/min per 1.73 m^2 were excluded at the request of the ethics committee because of concern that patients who already had impaired kidney function and less renal reserve may have been at risk for kidney failure if LN flared. The investigators and ethics committee were less confident that equipoise extended to such patients. Patients who consented to enroll in the trial understood that the second biopsy findings would not impact treatment decisions.

From February 2014 to October 2015, 56 consecutive SLE patients who had biopsy-proven class III/IV±V LN followed up at the University of Buenos Aires Hospital were recruited. These patients had been treated with cyclophosphamide during induction and had been given mycophenolate mofetil or mycophenolic acid as maintenance therapy for a minimum of 3 years (Figure 1). Patients who had been in complete renal remission for at least the last 12 months of maintenance and consented to a repeat kidney biopsy were enrolled in LuFla ($n = 44$). Before the second biopsy, 12 patients were excluded. Active extrarenal SLE prevented tapering of immunosuppression in 2 patients, 2 patients did not want to have another kidney biopsy, and 8 patients had an estimated glomerular filtration rate of $<$60 ml/min per 1.73 m^2 (Figure 1). The investigators were not blinded to the biopsy results, but the pathologists reading the kidney biopsies were blinded to patients' remission status. After the second biopsy, maintenance immunosuppression was tapered off by 6 months regardless of the biopsy findings. Patients were followed up prospectively for 2 years after the kidney biopsy. Urine sediment was evaluated every 45 days, and blood and urine testing were performed quarterly. At each visit patients were assessed for renal flare. Participants who became pregnant or developed an extrarenal flare that required re-initiation of immunosuppression, infection, or other serious medical complications during follow-up after biopsy 2 were withdrawn from the study.

The primary end point of this investigation was the difference in incidence of LN flare after the withdrawal of maintenance immunosuppression in patients with complete histologic remission and patients with persistent histologic activity.

Kidney biopsy

Kidney tissue was obtained through percutaneous needle biopsies. One core of tissue was frozen and sectioned for direct immunofluorescence (IF), and a second core was fixed in 10% formalin and processed for light microscopy. The latter was stained with hematoxylin and eosin, periodic acid–Schiff, Masson's trichrome, or methenamine silver. Kidney biopsies were classified according to the ISN/RPS system,[1] and activity and chronicity indexes were calculated as previously described.[17] In determining the AI, endocapillary proliferation was defined as profusion of cells internal to the glomerular basement membrane in the capillary loops. Glomerular subendothelial deposits were identified by light microscopy as glassy or hypereosinophilic deposits on the internal side of the glomerular basement membrane with the hematoxylin-eosin stain, red or fuchsinophilic with the trichrome stain, and pink or red with the Jones methenamine silver stain. Complete histologic remission was defined as AI $= 0$.

Treatment

Patients were diagnosed clinically with active LN, and the diagnosis was confirmed by kidney biopsy (biopsy 1). Patients with class III/IV±V LN were treated initially with 3 pulses of methylprednisolone 1 g/d for 3 consecutive days, followed by oral prednisolone at a starting dose of 60 mg/d, tapered by 10 mg/mo until the dose was 10 mg/d (month 6). Patients were also treated with i.v. cyclophosphamide, 1 g/month for 6 months. After cyclophosphamide induction, maintenance therapy consisted of mycophenolate mofetil 2000 mg/d or mycophenolic acid 1440 mg/d. At biopsy 2 all patients were taking either mycophenolate mofetil 2000 mg/d or mycophenolic acid 1440 mg/d, and the median dose of prednisone was 8 mg/d (range: 2–10 mg/d). Additionally, all patients were administered a low-sodium diet, hydroxychloroquine 400 mg/d, and renin-angiotensin system (RAS) blockers titrated to a blood pressure target of 130/80 mm Hg or lower. Medication adherence was assessed at each clinic visit by talking with the patients, talking with family members, and monitoring the need for prescription refills.

After at least 36 months of immunosuppression and 12 months of complete renal remission, patients had a repeat kidney biopsy (biopsy 2), and then mycophenolate was tapered off by reducing the dose 50% during the first 3 months after biopsy 2, 50% during the next 3 months, and then stopping. Prednisolone was weaned below 10 mg/d as dictated by extrarenal symptoms. One year after biopsy 2, the median dose of prednisone for the cohort was 4 mg/d (range: 0–10 mg/d). There was no difference in the median dose of prednisone in the flare and no-flare groups after biopsy 2 (Mann-Whitney test, $P = 0.88$). Antimalarial medication use was not discontinued.

Definition of remission and flare

Complete renal remission was defined as proteinuria <0.5 g/d, inactive urinary sediment, and normal serum creatinine concentration.

Urine sediment was considered inactive in the absence of red blood cell casts, white blood cell casts, and glomerular hematuria ($<$5% dysmorphic red blood cells per high power field). To obtain a concentrated urine for sediment analysis, patients were advised to restrict fluids starting at 10 p.m. and bring their first morning void urine to the clinic.

LN flare was defined as an increase in disease activity that required restarting immunosuppression. This included new glomerular hematuria, an increase in serum creatinine level of $\geq$0.3 mg/dl, and/or an increase in proteinuria to over 500 mg/d. These signs of active disease had to be persistent and present for at least 2 follow-up visits 1 week apart.

Data analysis

Patients were divided into those who had an LN flare and those who did not have an LN flare during the 2-year prospective observation period. The decline in complement C3 and C4 was taken as the value at biopsy 2 minus the value 6 months before biopsy 2. Summary statistics were calculated to compare clinical and histologic variables between these 2 groups. Data that were not normally distributed were analyzed using the Mann-Whitney test, and proportions were analyzed by Fisher's exact test. The Spearman correlation was used to test associations. A 2-tailed P value of <0.05 was considered significant.

Univariate and multivariable logistic regression modeling was performed to identify predictors of LN flare after withdrawal of maintenance immunosuppression using JMP v.12 statistical software (SAS Institute, Cary, NC). For the final model, a receiver operating characteristic curve was generated (data not shown). The linear equation for the logarithm of the odds ratio generated by the coefficients of the logistic model (reported in Table 4) was used for the predictive equation, and the cutoff value C for predicting LN flare was chosen to maximize the sum of sensitivity and specificity. With limited sample size and availability of cases with renal flare, statistical cross-validation of models was not performed.

DISCLOSURE

All the authors declared no competing interests.

ACKNOWLEDGMENTS

MDR would like to acknowledge Dr. Javier Robaina who helped with this study, Silvina Katz who helped with his English, and Drs. Doval and Tajer from the University's Study, Teaching and Clinical Research Group (GEDIC), who helped with the design of the study.

SUPPLEMENTARY MATERIAL

Table S1. Histologic findings at kidney biopsies 1 and 2.

Table S2. Lack of fit of various logistic regression models to predict future LN flare.

Supplementary material is linked to the online version of the paper at www.kidney-international.org.

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