UNIVERSITÀ DEGLI STUDI DI GENOVA

Dipartimento di neuroscienze, riabilitazione, oftalmologia, genetica e scienze materno-infantili (DINOGMI)

TESI DI DOTTORATO DI RICERCA IN SCIENZE PEDIATRICHE (XXXIV CICLO)

"Il protocollo EUROlupus nel trattamento della nefrite lupica in età pediatrica: 20 anni di esperienza nei centri italiani di reumatologia e nefrologia"

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A.A. 2020-2021

INTRODUCTION

Overview

Systemic Lupus Erythematosus (SLE) is a multi-systemic autoimmune disorder usually associated with typical autoantibodies. Clinical presentation is widely unpredictable ranging from mild disease (i.e. mostly cutaneous manifestations) to life-threatening conditions, such as renal or cardiac disease, or combined to severe complications (i.e. infections). It consists of a relapsing-remitting disorder: acute flares alternating with disease-free periods are very common [1].

Epidemiological and pathological aspects

A large amount of studies has been conducted to find out the causes of such a complex disease as well as to understand its pathogenesis: although most of these studies enrolled adult patients, it is fair to transfer this knowledge to the pediatric population. The etiology of SLE has to be found in a variable combination of genetic susceptibility and exposure to environmental factors, such as solar radiation, infections and drugs. The reported prevalence of SLE in the adult population varies between 20 and 150 per 100.000 inhabitants with a strong shift towards female sex; 20% of SLE onset is before the age of 16. Among the pediatric population, the prevalence is 6 to 8 times lower and the female-to-male ratio ranges from 3:1 to 15:1, depending on sex hormones (lower before school age, higher in puberty)[2,3,4]. SLE is more prevalent and much more aggressive between African Americans and Hispanic ethnicity[5].
The clinical presentation of extrarenal lupus disease is widely variable: any organ can be involved in this disease, even at the same time, but the systematic discussion of these features is beyond the scope of this thesis.

Genetic and acquired causes play a pivotal role, probably in association, in the onset of renal involvement within SLE. New recent theories have been developed about the key role that innate immunity would have, together with adaptive immunity, in the pathogenesis of lupus nephritis (LN). In the last ten years, it has been demonstrated that neutrophil granulocytes release

extracellular traps (Neutrophil Extracellular Traps, NETs) to fight against pathogens, together with phagocytosis and degranulation: this process is called NETosis. The composition of these NETs is extremely heterogeneous but one of the main components is nuclear DNA: it is released in the extracellular space after a decondensation process and the enzymatic addition of citrulline residues in specific parts of the protein. The NETosis generates a proteic tight mesh network to trap pathogens facilitating the activity of defensive enzymes[6]. The presence of DNA and other proteins that have undergone post-transcriptional modifications make the NETs potentially a source of autoantigens. This hypothesis is the base of studies aimed at finding a relationship between NETosis and the genesis of some autoimmune diseases through the presentation of self-proteins that have been modified in their structure, thus escaping tolerance mechanism and becoming potentially antigenic[7]. Another key aspect is the removal of residual products (remnants) derived from the NETosis process: if the production/clearance rate is imbalanced they will accumulate in the bloodstream increasing the probability to become self-antigens. The negative alteration of remnants removal in SLE patients is mainly due to the reduced activity of DNases, enzymes with the aim of cutting the NETs. It has also been hypothesized that the presence of serum-circulating DNase inhibitors could explain such disorder, as found in LN patients[6].

Clinical and histological features of lupus nephritis

LN has a major (the greatest) impact on the prognosis, among SLE patients, notably in the pediatric age; therefore, a considerable amount of literature has been produced to fully understand the mechanism underlying its pathogenesis. As for all autoimmune diseases, circulating autoantibodies against autoantigens are involved: IgG_2 class autoantibodies directed towards specific renal epitopes have been detected as well as circulating immune complexes not involving renal antigens have been demonstrated in kidney biopsies as deposit. Both of the above mechanisms have a key role in the pathogenesis of LN: in the first case with the kidney as a target of immune response and in the latter as a matrix for immune deposition and subsequent inflammation[8].

Signs and symptoms of renal involvement are clinically relevant in about half of patients with LN; their severity can be extremely variable ranging from occasional findings of slight abnormalities in urine specimens up to a severe nephrotic syndrome with marked hypertension and rapid progression to end-stage renal disease (ESRD).

Hematuria, proteinuria and cell casts are variably associated with renal disease; overall up to 50% of renal function impairment has been demonstrated among pediatric patients[8]. In the past years, it has been clarified that the long-term prognosis of SLE patients (notably pediatric patients) is closely related to the course of renal disease and infectious complications, if they occur. Considering the extreme variability of renal involvement in LN and the wide range of immunosuppressive therapies available at present, it is obvious that a prognostic stratification system of LN patients at their onset is essential[8]. Unfortunately, there is no deep correlation between urinary or hematological abnormalities and severity of renal involvement, therefore histological staging through kidney biopsy plays a key role in understanding the right prognosis. The international classification currently recognized as the most complete is shown in the table below (Table I).

ISN/RPS 2003 Classification of Lupus Nephritis	
Class I	Minimal mesangial Lupus Nephritis Only mesangial immune deposits that are identified by immunofluorescence alone or by both immunofluorescence and electron microscopy, but such patients do not have light microscopic abnormalities
Class II	Mesangial proliferative Lupus Nephritis Light microscopy reveals mesangial hypercellularity or mesangial matrix expansion. A few isolated subepithelial or subendothelial deposits may be seen on immunofluorescence or electron microscopy
Class III	Focal Lupus Nephritis Although less than 50 percent of glomeruli are affected on light microscopy, immunofluorescence microscopy (for IgG and C3) reveals almost uniform involvement. Endocapillary or extracapillary glomerulonephritis is almost always segmental (ie, involves less than 50 percent of the glomerular tuft). Electron microscopy usually reveals immune deposits in the subendothelial space of the glomerular capillary wall as well as the mesangium
Class III (A)	Active lesions: focal proliferative Lupus Nephritis
Class III (A/C)	Active and chronic lesions: proliferative and sclerosing Lupus Nephritis
Class III (C)	Inactive chronic lesions with scars: sclerosing Lupus Nephritis
Class IV	Diffuse Lupus Nephritis More than 50% of glomeruli are affected by light microscopy. Affected glomeruli display endocapillary with or without extracapillary glomerulonephritis. Mesangial abnormalities may also be observed. Electron microscopy reveals subendothelial deposits, at least during the active phase. The presence of diffuse wire loop deposits, but with little or no glomerular proliferation, is also considered class IV disease. With active disease, hypercellular, necrotizing lesions, and

	crescent formation may all be present, affecting more than 50 percent of glomeruli on light microscopy. The marked deposition of immunoglobulins (especially IgG) and complement (especially C3) in this setting results in thickening of the glomerular capillary wall and a pattern on light microscopy that is similar to that in membranoproliferative glomerulonephritis. These lesions are characterized by the marked influx of proinflammatory cells (monocytes, suppressor/cytotoxic T cells), sometimes resulting in cellular crescents
Class IV-S (A)	Diffuse segmental proliferative Lupus Nephritis
Class IV-G (A)	Diffuse global proliferative Lupus Nephritis
Class IV-S (A/C)	Diffuse segmental proliferative and sclerosing Lupus Nephritis
Class IV-S (C)	Diffuse segmental sclerosing Lupus Nephritis
Class IV-G (C)	Diffuse global sclerosing Lupus Nephritis
Class V	Membranous Lupus Nephritis Characterized by diffuse thickening of the glomerular capillary wall on light microscopy and by subepithelial immune deposits (either global or segmental involvement) on immunofluorescence or electron microscopy. Mesangial involvement may also be seen. Although sparse subendothelial deposits can be seen by immunofluorescence or electron microscopy with class V disease alone, the presence of such deposits as detected by light microscopy warrants a combined diagnosis of classes III and V disease or of classes IV and V disease. In this setting, the additional designation of class III or IV is based upon the distribution of the deposits.
Class VI	Advanced sclerosing Lupus Nephritis Most of glomerular tufts have undergone sclerosis process

Table I ISN/RPS 2003 Classification of Lupus Nephritis

Class I LN is rarely diagnosed due to its weak association with clinical signs; therefore, it is unlikely that a kidney biopsy will be proposed. Class II is usually associated with microhematuria or mild proteinuria, largely under nephrotic range. The renal prognosis of these two classes is therefore excellent. Patients who receive a diagnosis of Class III LN may have hematuria, proteinuria even in the nephrotic range, a variable degree of renal function impairment and associated hypertension. The prognosis of Class III LN can be undermined because the natural history of this class is mainly related to the extension of glomerular damage compared to the total number of glomeruli in the biopsy sample; therefore, underrating or overrating of the disease class can be a risk in unrepresentative samples. Class IV LN represents the most severe form of renal involvement: in addition to the previously reported signs and symptoms, the patients affected are often hypocomplementemic and have a high anti-ds-DNA antibody titer. Patients with membranous lupus nephritis (Class V) usually come to clinical evaluation due to a variable degree of nephrotic range proteinuria. The prognosis of this class is uncertain and related to the association with typical lesions of other classes (class III and class IV). Finally, class VI is associated with advanced renal disease, with renal function heavily compromised: these patients will soon need renal replacement treatment.

Diagnostic considerations

The diagnosis of SLE is troublesome due to the very heterogeneous features of the disease. Ten years ago, the diagnostic criteria were updated to simplify the clinician's work but the detailed discussion is beyond the scope of this thesis. The diagnosis of LN is made by renal biopsy in patients who have already received the diagnosis of SLE or not; in the latter the finding of such histological abnormalities together with the presence of autoantibodies in the serum is pathognomonic.

Treatment of Lupus Nephritis

Pediatric onset SLE has specific features that make the therapeutic pathway somewhat different from that indicated for adult-onset disease. Nevertheless, therapeutic goals are similar in both ages and every clinician should aim to[9]:

- Ensure long term survival of the patient
- Achieve the lowest degree of disease activity
- Avoid severe flares as much as possible
- Prevent organ damage
- Minimize the adverse effect of therapies
- Improve patient's quality of life
- Train the patient and the family about managing the disease in all its aspect

Pediatric data regarding the treatment of lupus nephritis are quite incomplete and predominantly derived from adult-based studies. Although adult and pediatric patients are quite similar regarding treatment efficacy, this is not true if we consider drug toxicity, quality of life and life expectancy. However, the fundamentals of pediatric LN management are not dissimilar to adults and include aggressive and early treatment of the acute phase and maintenance of remission with the lowest possible dose of steroid and immunosuppressive drugs. Class I and II LN patients seldom require therapies, however, if needed, it should be an angiotensin-converting enzyme I (ACE-I) inhibitor or a sartanic (ARB), as a first-line. Close clinical and biohumoral monitoring is recommended in any case for these patients[8]. Class III nephritis with less than 20% of the glomeruli involved has a favorable long-term prognosis, so the use of steroid and non-steroidal immunosuppressive drugs is debated and usually depends on the patient's characteristics besides extrarenal symptoms that are usually associated. The remaining patients have a kidney prognosis similar to those in Class IV and should therefore receive adequate immunosuppressive therapy. There is substantial agreement between the various international guidelines (KDIGO, ACR, EULAR / ERA-EDTA) regarding the need to use a steroid-based remission-inducing therapy in addition to other immunosuppressive drugs. Administration of the steroid should be done

initially by intravenous bolus over 3 days although there is no data on the superiority of one steroid tapering regimen over another. Cyclophosphamide (CYC) and mycophenolate (MMF) are often used as immunosuppressive drugs for LN; the first has certainly been used for a longer time and therefore is a better-known drug; on the other hand, MMF could be preferred in patients of childbearing age, due to better compliance (no toxic effects on fertility).

The use of CYC in the therapy of LN was evaluated by the National Institute of Health (NIH) with several studies that still represent the cornerstone of the therapy of this disease. The use of intravenous (IV) CYC was postulated as more effective and affected by fewer adverse effects compared to oral administration.

In addition, the need for therapeutic regimens providing long-term immunosuppression for patients with proliferative LN has been demonstrated[10-12]. Since IV CYC has been consolidated as a therapeutic standard for lupus nephritis, further aspects became relevant, such as the reduction of toxicity, especially for ethnic groups with a significantly better prognosis for renal disease (i.e. Caucasian Europeans).

The EUROlupus trial

For this purpose the EUROlupus trial[13] was designed, collecting a population of 90 adults mainly of Caucasian European race: the aim of the study was the evaluation of non-inferiority in inducing remission of a low-dose CYC protocol (low-dose, LD) versus the standard dose used in previous studies conducted at the NIH (high-dose, HD). All patients received 3 daily pulses of 750 mg of IV methylprednisolone followed by oral glucocorticoid therapy as standard of care. Patients were also randomized to receive HD IV CYC protocol (8 pulses, 6 monthly followed by 2 quarterly, 500 mg/m^2) *versus* LD IV CYC protocol (6 fortnightly pulses at 500 mg fixed dose). In the HD schedule, the dose could be increased up to 1500 mg per pulse, depending on neutrophil count on day 14 of the protocol. For every patient, long-term maintenance therapy with azathioprine was started two weeks after the last administration of CYC. The results were as follows:

- the cumulative dose of CYC administered in the LD protocol was approximately one third compared to the HD protocol
- the treatment failure rate (primary end-point) was 16% in the LD protocol and 20% in the HD protocol; however, the significance of this data has not been demonstrated
- both regimens showed great improvement in 24-hour proteinuria, serum creatinine, albumin, C3 levels and ECLAM score at one year after treatment, with similar kinetics, including the subgroup of patients with renal insufficiency
- the overall follow-up period was over 40 months for both groups with no significant difference in the probability of developing renal flare, which was just under 30%
- the adverse events recorded were small in number for both groups with no significant difference, except for severe infections, where enrollment in the LD protocol was found to be protective

The Authors extended the follow-up of EUROlupus trial up to 10 years overall in subsequent studies[14,15]. While the preliminary data obtained with the first study were confirmed, it appeared that the most accurate predictor of the best outcome is the reduction of 24-hour proteinuria at 6 months after CYC below 1 gr/day. A further finding emerged from these studies is the high rate of renal flares during drug tapering or drug-free periods (up to 50% of patients). It is therefore essential to maintain an immunosuppressive state for a long time, burdened by the side effects as few as possible. The best known drugs for this purpose are certainly azathioprine (AZT) and MMF: while the latter has shown a lower rate of renal recurrence than AZT, especially in patients with a worse prognosis, azathioprine is more advisable in the childbearing age for female patients due to negligible teratogenic risk[16]. Among the negative prognostic factors at LN onset, the main ones are acute kidney injury, hypertension, nephrotic range proteinuria, anemia and African American race. Among the ones developed during follow-up, the main factors are frequency and severity of renal flares, in addition to the degree of disease remission achievable with therapy.

Class V LN treatment is an ongoing debate: clinically patients can vary from microhematuria up to severe proteinuria and hypertension, along with kidney failure. As aforementioned, renal prognosis is mainly related to histological markers of other classes (class III or class IV) that can be found associated with class V features in kidney biopsy; therefore, immunosuppressive therapy is mainly related to the first. Studies including patients diagnosed with class V LN alone showed a 10-year kidney survival of more than 80%. However, these patients have an estimated 35% risk of developing proliferative lesions within 10 years from diagnosis. Moreover, class V LN may be associated with signs and symptoms of extra-renal disease, although not invariably, as for other classes of LN. Taking into account these data, there is substantial agreement on the need for immunosuppressive therapy for these patients, which is generally represented by MMF or calcineurin inhibitors (cyclosporine or tacrolimus).

Overall and renal prognosis of SLE patients

The overall prognosis of children and adolescents with SLE receiving appropriate immunosuppressive therapy is generally good. The main negative determinants are:

- poor adherence to therapeutic protocols by the patient/family
- neurologic disease
- recurrent infections
- African-American race
- Renal disease, notably for proliferative glomerulonephritis
- Delay in referring patient to pediatric rheumatology centers experienced in the treatment of SLE

As mentioned, renal disease plays a pivotal role in formulating the prognosis for these patients; in a study of 66 Canadian children with proliferative glomerulonephritis (class IV) mortality at 10 and 20 years was 9 and 12% respectively and there was an ESRD in 25% and 40% respectively. The subgroup of Caucasian patients, as expected, had an overall better prognosis than non-Caucasian patients. Furthermore, patients with a type II and III class

of lupus nephritis did not develop ESRD at the end of follow-up (21 years) and there were no deaths among them[17].

Major negative prognostic factors regarding LN are the occurrence of renal flares, the achievement of sustained remission and blood pressure control[8].

AIM OF THE STUDY

Our study aims to collect and analyze data derived from a 20-year experience in managing pediatric patients affected by SLE and LN, from a multicenter network of Italian Lupus Clinics.

PATIENTS AND METHODS

We collected data from pediatric onset LN (pLN) in Italian patients in the previous 20 years; unfortunately, the spreading of COVID-19 has severely affected the number of participating centers, decreased from ten to three. In addition to our Institute, three other pediatric Lupuc Clinic collaborated to our study:

- Presidio Ospedaliero Vito Fazzi, Lecce (Dr. Adele Civino)
- Ospedale Santa Maria Goretti, polo pontino Sapienza, Latina (Prof. Riccardo Lubrano, Dott. Emanuela del Giudice)
- Fondazione Policlinico Universitario Gemelli, Roma (Dott. Rigante)

A total of 28 patients were enrolled in this study and met the following criteria (all met):

- A diagnosis of SLE according to ACR/SLICC criteria, depending on the time of diagnosis
- Age < 18 years at the time of SLE diagnosis
- Biopsy-proven LN as follows:
    - Class III or Class IV LN *or*
    - Class V LN associated with nephrotic range proteinuria or
    - Acute kidney injury (KDIGO criteria) at LN onset
- All patients were treated according to EUROlupus protocol
- A signed informed consent, where feasible

Exclusion criteria were similar to those of the original study:

- Renal thrombotic microangiopathy
- Preexisting chronic renal failure
- Pregnancy
- Previous malignancy
- Diabetes mellitus

- Previously documented severe toxicity to immunosuppressive drugs

The study was approved by the ethics committees of all participating hospitals. Once the indication to administer EUROlupus protocol was taken, patients received 3 daily pulses of IV steroid, then 6 fortnightly pulses at 500 mg fixed dose of CYC, followed by long-term oral immunosuppressive therapy (according to centers' protocol, mainly MMF or AZT).

Patients enrolled in our study have the following features: SLE diagnosis was formulated following ACR/SLICC criteria (depending on the time of the diagnosis); all patients but one (96.4%) are Caucasian Europeans and the female-to-male ratio is 23/5 (82.1%). The mean age of SLE onset was 12.1 years with a standard deviation (SD) of 3.2 years and a median of 13.5 years; the mean age of LN onset was 14.1 years, with a SD of 3.8 years and a median of 15.2 years. LN was diagnosed before 18 years of age in all patients but one: however, we decided to consider this case as part of our study because SLE started 8 years before and we took care of the patient since then. A kidney biopsy was performed in 27/28 patients: one patient didn't receive this examination due to his critical clinical condition at that time; however, we decided to enroll this case due to severe kidney involvement at diagnosis which led us to administer EUROlupus protocol. The mean delay between SLE onset and LN onset was 2 years; 15/28 patients (53.6%) developed kidney disease within 6 months of SLE onset.

Description of study patient and clinical manifestations are reported in the following tables (Table II and Table III).

Table II. Description of the study patients [N = 28]

	n./N. (%)	
Sex: Male, n. (%)	5/28 (17.9)	
Female	23/28 (82.1)	
Ethnicity: Caucasian	27/28 (96.4)	
Afro-American	1/28 (3.6)	
	Mean (SD)	**Median (1st - 3rd q)**
Age at onset (years)	12.1 (3.2)	13.5 (9.6 - 14.6)
Age at onset (LN) (years)	14.1 (3.8)	15.2 (13 - 15.9)
Extrarenal manifestations: yes	23/28 (82.1)	
no	5/28 (17.9)	
Histological class‡: III	9/27 (33.3)	
IV	12/27 (44.4)	
V	8/27 (29.6)	
VI	1/27 (3.7)	
HTN during FU¶: yes	14/27 (51.9)	
no	13/27 (48.1)	
HTN at end of FU: yes	2/27 (7.7)	
no	25/27 (92.3)	

‡ one patient didn't perform kidney biopsy (see text) ¶Hypertension recorded any time during FU visits

Table III. Description of clinical manifestation

		n./N. (%)
Cutaneous manifestations[Δ]:	yes, alone	18/23 (78.3)
	yes, associated	2/23 (8.7)
	no	3/23 (13)
Arthritis[Δ]:	yes	17/23 (73.9)
	no	6/23 (26.1)
Serositis[Δ]:	yes	4/23 (17.4)
	no	19/23 (82.6)
Neurological manifestations[Δ]:	yes	3/23 (13.0)
	no	20/23 (87.0)
Hematological manifestations[Δ]:	yes	15/23 (65.2)
	no	8/23 (34.8)
RAASI usage rate[ǂ]:	yes	20/28 (71.4)
	no	8/28 (28.6)
aPL[ǂ]:	yes	10/28 (35.7)
	no	18/28 (64.3)
Nephrotic syndrome at onset[ǂ]:	yes	10/28 (35.7)
	no	18/28 (64.3)
AKI at onset[ǂ]:	yes	2/28 (7.1)
	no	26/28 (92.9)

[Δ]these data refer only to patients with extrarenal manifestations (N=23); [ǂ]these data refer to the total amount of patients (N=28).

Patients were evaluated every 6 to 12 months as outpatients or during short hospitalization, with a shared protocol between pediatric Rheumatologists and Nephrologists. More than half of them completed the 5-year FU (15/28 corresponding to 53.6%); only one patient with recent onset didn't reach the one-year visit. Mean FU time was 43.5 months per patient and we recorded a total of 1,218 months of FU.

Descriptive statistics of the study patients were firstly performed and reported.

Categorical variables were reported in terms of absolute frequencies (N) and percentages (%).

Quantitative variables were described in terms of means and Standard Deviations (SD), median with first and third quartiles and minimum and maximum values. Quantitative variables were reported for each time point during follow-up time (from baseline, T0, to the last follow-up). Analysis of the change over time was performed by the non-parametric Analysis of Variance (Friedman's test); this test was chosen since the distribution of these variables was not normal. The normality of the quantitative parameters was evaluated by means of the Shapiro-Wilk test.

Finally, survival curves were drawn; the event of interest was the occurrence of renal flare and time was measured starting from EUROlupus protocol administration to the event (in case of renal flare) or the last follow-up visit available for the patients. Two patients were not followed for a reasonable time and therefore were excluded from the analysis of survival curves. The Log-rank test was used to compare survival curves between different categories of patients (example: males *vs* females).

A p value < 0.05 was considered statistically significant. The software Stata (StatSoft Corporation) was used for all the statistical analyses.

RESULTS

At LN onset we recorded nephrotic syndrome in 10/28 patients (35.7%) and acute kidney injury (AKI) in 2/28 patients (7.1%) (Table III). Treatment evaluation was made following the criteria used by EUROlupus original article[13] (Table IV). The primary endpoint of our study was the evaluation of treatment failure: 6/27 patients (22.2%) were classified as below (one patient missed the 6-month FU visit and was not included).

Table IV. Definition of treatment failure

Treatment failure was defined as either of the following 3 features:

1. Absence of a primary response (applicable only to patients presenting with severe renal disease), which was defined as renal impairment and/or nephrotic syndrome)
    a. For patients with a baseline serum creatinine level > 1.3 mg/dl but < 2.6 mg/dl, absence of primary response was defined as failure of the serum creatinine level to decrease to < 1.3 mg/dl (*kidney failure criterion*)
    b. For patients with a baseline serum creatinine level > 2.6 mg/dl, absence of primary response was defined as failure of the serum creatinine level to improve by 50% at 6 months (*kidney failure criterion*)
    c. For patients with nephrotic syndrome at baseline (serum albumin level < 3.5 gr/dl and 24-hour urinary protein level ≥ 3 gr/day) but without renal impairment, absence of a primary response was defined as the persistence of nephrotic syndrome at 6 months (*nephrotic syndrome criterion*)
2. A glucocorticoid-resistant flare (defined as a severe flare that did not respond to a 1-month increase in the glucocorticoid dosage) (*glucocorticoid resistant flare criterion*)
3. A doubling of the serum creatinine level over the lowest value reached at any time during the follow-up and confirmed on 2 consecutive visits 1 month apart (*doubling of serum creatinine criterion*)

These patients were classified as treatment failure as follows:

- Kidney failure criterion (1/6)
- Nephrotic syndrome criterion (3/6)
- Glucocorticoid resistant flare criterion (1/6)
- Doubling of serum creatinine criterion (1/6)

We recorded 6 severe renal flares in 5 patients (1 patient experienced 2 renal flares during FU period); severe renal flare was defined according to Table V.

Table V. Definition of severe renal flare

A severe renal flare was defined as 1 of the following 3 features: 1. 3-fold increase in 24-hour proteinuria from last visit 2. Relapse of nephrotic syndrome 3. A doubling of the serum creatinine level over the lowest value reached at any time during the follow-up and confirmed on 2 consecutive visits 1 month apart

We recorded 3 severe infections in 3 patients during FU period: these were defined as infections leading to hospital admission. Moreover, we documented 1 case of cancer (cervical intraepithelial neoplasia treated with local surgical therapy and optimal outcome) and 1 case of kidney transplant, after the FU period (Table VI).

Table VI. Description of outcome and adverse events

	n./N. (%)
Treatment failure [N=27]: yes	6/27 (22.2)
no	21/27 (77.8)
Renal Flare: yes	5/28 (17.9)
no	23/28 (82.1)
Severe infections: yes	3/28 (10.7)
no	25/28 (89.3)
Cancer: yes	1/28 (3.6)
no	27/28 (96.4)
Transplant: yes	1/28 (3.6)
no	27/28 (96.4)

Severe infections: 2 Herpes Zoster and 1 mycotic esophagitis. Type of cancer: Cervical Intraepithelial Neoplasia (CIN); 1 treatment failure is missing

Later we evaluated the relationship between variables, such as socio-demographic features, extrarenal manifestations and clinical variables, with primary endpoint (treatment failure). Results are reported in the following tables (Table VIIa, VIIb, VIIc): the absence of extrarenal manifestations is linked to a more probable treatment failure.

Table VII/A. Relationship between socio-demographic and clinical variables and **treatment failure (primary endpoint)** [N = 27]**.**

		Treatment failure		
		Yes	**No**	**P**
Center: Gaslini	[n= 17]	3/17 (17.6 %)	14/17 (82.4 %)	0.64[#]
Other	[n= 10]	3/10 (30.0 %)	7/10 (70.0 %)	
Sex: Male	[n= 5]	1/5 (20 %)	4/5 (80 %)	1.00[#]
Female	[n= 22]	5/22 (22.7 %)	17/22 (77.3 %)	
Age at onset: < 10 years	[n= 8]	1/8 (12.5 %)	7/8 (87.5 %)	0.63[#]
≥ 10 years	[n= 19]	5/19 (26.3 %)	14/19 (73.7 %)	
Age at onset LN: < 10 years	[n= 4]	1/4 (25 %)	3/4 (75 %)	1.00[#]
≥ 10 years	[n= 23]	5/23 (21.7 %)	18/23 (78.3 %)	
Histological class: III	[n= 8]	1/8 (12.5 %)	7/8 (87.5 %)	0.85[#]
IV	[n= 11]	3/11 (27.3 %)	8/11 (72.7 %)	
V	[n= 8]	2/8 (25 %)	6/8 (75 %)	
Extra-renal manifestations: yes	[n= 22]	3/22 (13.6 %)	19/22 (86.4 %)	0.06[#]
no	[n= 5]	3/5 (60 %)	2/5 (40 %)	

[#]P: Fisher's Exact test

Table VII/B Relationship between extrarenal manifestations and **treatment failure (primary endpoint)** [N = 22]**.**

		Treatment failure		
		Yes	**No**	**P**
Cutaneous: yes	[n= 20]	3/20 (15 %)	17/20 (85 %)	1.00[#]
no	[n= 2]	0/2 (0 %)	2/2 (100 %)	
Arthritis: yes	[n= 16]	1/16 (6.3 %)	15/16 (93.8 %)	0.17[#]
no	[n= 6]	2/6 (33.3 %)	4/6 (66.7 %)	
Serositis: yes	[n= 3]	0/3 (0 %)	3/3 (100 %)	1.00[#]
no	[n= 19]	3/19 (15.8 %)	16/19 (84.2 %)	
Neurologic manifestations: yes	[n= 3]	0/3 (0 %)	3/3 (100 %)	1.00[#]
no	[n= 19]	3/19 (15.8 %)	16/19 (84.2 %)	
Haematological manifestations: yes	[n= 14]	2/14 (14.3 %)	12/14 (85.7 %)	1.00[#]
no	[n= 8]	1/8 (12.5 %)	7/8 (87.5 %)	
Number of manifestations: 1 - 2	[n= 10]	2/10 (20 %)	8/10 (80 %)	0.57[#]
3 - 4	[n= 12]	1/12 (8.3 %)	11/12 (91.7 %)	

[#]P: Fisher's Exact test.

There was only 1 patient with mucosal manifestations (associated with serositis, neurologic and hematologic manifestations) but his outcome was missing. No patients had alopecia. Only 22/28 patients have treatment failure and extrarenal manifestations data recorded.

Table VII/C Relationship between clinical variables and **treatment failure (primary endpoint)** [N = 27]**.**

		Treatment failure		
		Yes	**No**	**P**
HTN before LN: yes	[n= 4]	2/4 (50 %)	2/4 (50 %)	0.20[#]
no	[n= 23]	4/23 (17.4 %)	19/23 (82.6 %)	
AKI at onset: yes	[n= 2]	0/2 (0 %)	2/2 (100 %)	1.00[#]
no	[n= 25]	6/25 (24 %)	19/25 (76 %)	
NS at onset: yes	[n= 10]	4/10 (40 %)	6/10 (60 %)	0.15[#]
no	[n= 17]	2/17 (11.8 %)	15/17 (88.2 %)	

[#]P: Fisher's Exact test [¶]Hypertension recorded any time during FU visits

Then, we collected data on secondary endpoint: renal remission (Table IX). This was defined according to urine protein (UPr) criterion or hypertension (HTN) criterion, as reported in Table VIII. Moreover, we investigated the possible relationship between variables and renal remission (Table Xa, Xb, Xc, XIa, XIb, XIc, XIIa, XIIb, XIIc, XIIIa, XIIIb, XIIIc). The absence of extrarenal manifestation was linked to a less probable renal remission at 1 year (UPr criterion), as Gaslini patients were linked to a more probable renal remission at the last FU visit (UPr criterion). Furthermore, patients with elevated blood pressure at the end of FU period are linked to the absence of renal remission (UPr criterion) at the same visit.

Table VIII. Definition of renal remission

A renal remission was defined following 2 criteria: 1. Renal remission following Urine Protein (UPr) criterion: urine protein < 1 gr/day on that visit 2. Renal remission following Hypertension (HTN) criterion: no hypertension detected at follow-up visit 3. Renal remission following combined criteria: urine protein below 1 gr/day and no hypertension detected at follow-up visit

Table IX. Secondary endpoint

	n./N. (%)
Renal remission UPr criterion[ǂ] 1y [N=25]: yes	19/25 (76)
no	6/25 (24)
Renal remission UPr criterion last FU [N=26]: yes	21/26 (80.8)
no	5/26 (19.2)
Renal remission HTN criterion[Δ] 1y [N=26]: yes	23/26 (88.5)
no	3/26 (11.5)
Renal remission HTN criterion last FU [N=26]: yes	22/26 (84.6)
no	4/26 (15.4)
Renal remission combined criteria[¥] 1y [N=25]: yes	19/25 (76)
no	6/25 (24)
Renal remission combined criteria last FU [N=25]: yes	20/25 (80)
no	5/25 (20)

UPr: Urine Protein; HTN: hypertension; FU: follow-up

[ǂ]Urine Protein < 1 gr/day; [Δ]no hypertension detected at follow-up visit;

[¥]Urine Protein < 1 gr/day *and* no hypertension detected at follow-up visit

Total number of patients is less than 28 because some data are missing

Table X/A. Relationship between socio-demographic and clinical variables and **Renal remission <u>at 1 year</u> of Follow-up defined according to UPR criterion (secondary endpoint)** [N = 25]

		Renal remission at 1 year (UPR criteria)		
		Yes	**No**	**P**
Center: Gaslini	[n= 16]	14/16 (87.5 %)	2/16 (12.5 %)	0.14[#]
Other	[n= 9]	5/9 (55.6 %)	4/9 (44.4 %)	
Sex: Male	[n= 3]	2/3 (66.7 %)	1/3 (33.3 %)	1.00[#]
Female	[n= 22]	17/22 (77.3 %)	5/22 (22.7 %)	
Age at onset: < 10 years	[n= 6]	5/6 (83.3 %)	1/6 (16.7 %)	1.00[#]
≥ 10 years	[n= 19]	14/19 (73.7 %)	5/19 (26.3 %)	
Age at onset LN: < 10 years	[n= 2]	1/2 (50 %)	1/2 (50 %)	0.43[#]
≥ 10 years	[n= 23]	18/23 (78.3 %)	5/23 (21.7 %)	
Histological class: III	[n= 7]	6/7 (85.7 %)	1/7 (14.3 %)	0.72[#]
IV	[n= 9]	7/9 (77.8 %)	2/9 (22.2 %)	
V	[n= 8]	5/8 (62.5 %)	3/8 (37.5 %)	
Extra-renal manifestations: yes	[n= 21]	18/21 (85.7 %)	3/21 (14.3 %)	0.031[#]
no	[n= 4]	1/4 (25 %)	3/4 (75 %)	

[#]P: Fisher's Exact test.

Table X/B Relationship between type and number of extra-renal manifestations and **Renal remission <u>at 1 year</u> of Follow-up defined according to UPR criterion (secondary endpoint)** [N = 21]

		Renal remission at 1 year (UPR criteria)		
		Yes	**No**	**P**
Cutaneous: yes	[n= 18]	15/18 (83.3 %)	3/18 (16.7 %)	1.00[#]
No	[n= 3]	3/3 (100 %)	0/3 (0 %)	
Arthritis: yes	[n= 15]	13/15 (86.7 %)	2/15 (13.3 %)	1.00[#]
no	[n= 6]	5/6 (83.3 %)	1/6 (16.7 %)	
Serositis: yes	[n= 4]	3/4 (75 %)	1/4 (25 %)	0.49[#]
no	[n= 17]	15/17 (88.2 %)	2/17 (11.8 %)	
Neurologic manifestations: yes	[n= 3]	3/3 (100 %)	0/3 (0 %)	1.00[#]
no	[n= 18]	15/18 (83.3 %)	3/18 (16.7 %)	
Haematological manifestations: yes	[n= 14]	12/14 (85.7 %)	2/14 (14.3 %)	1.00[#]
no	[n= 7]	6/7 (85.7 %)	1/7 (14.3 %)	
Number of manifestations: 1 - 2	[n= 9]	8/9 (88.9 %)	1/9 (11.1 %)	1.00[#]
3 - 4	[n= 12]	10/12 (83.3 %)	2/12 (16.7 %)	

There was only 1 patient with mucosal manifestations (associated with serositis, neurologic and hematologic manifestations) but his outcome was missing. No patients had alopecia. [#]P: Fisher's Exact test.

Table X/C Relationship between clinical variables and **Renal remission <u>at 1 year</u> of follow-up defined according to UPR criterion (secondary endpoint)** [N = 25]

		Renal remission at 1 year (UPR criteria)		
		Yes	**No**	**P**
HTN before LN: yes	[n= 3]	1/3 (33.3 %)	2/3 (66.7 %)	0.13[#]
no	[n= 22]	18/22 (81.8 %)	4/22 (18.2 %)	
AKI at onset: yes	[n= 1]	1/1 (100 %)	0/1 (0 %)	1.00[#]
no	[n= 24]	18/24 (75 %)	6/24 (25 %)	
NS at onset: yes	[n= 9]	5/9 (55.6 %)	4/9 (44.4 %)	1.00[#]
no	[n= 16]	14/16 (87.5 %)	2/16 (12.5 %)	

[#]P: Fisher's Exact test.

Table XI/A. Relationship between socio-demographic and clinical variables and **Renal remission at <u>1 year</u> of Follow-up defined according to HTN criterion (secondary endpoint)** [N = 26]

		Renal remission at 1 year (HTN criteria)		
		Yes	**No**	**P**
Center: Gaslini	[n= 16]	15/16 (93.8 %)	1/16 (6.3 %)	0.54[#]
Other	[n= 10]	8/10 (80 %)	2/10 (20 %)	
Sex: Male	[n= 4]	3/4 (75 %)	1/4 (25 %)	0.41[#]
Female	[n= 22]	20/22 (90.9 %)	2/22 (9.1 %)	
Age at onset: < 10 years	[n= 7]	6/7 (85.7 %)	1/7 (14.3 %)	1.00[#]
≥ 10 years	[n= 19]	17/19 (89.5 %)	2/19 (10.5 %)	
Age at onset LN: < 10 years	[n= 3]	2/3 (66.7 %)	1/3 (33.3 %)	0.32[#]
≥ 10 years	[n= 23]	21/23 (91.3 %)	2/23 (8.7 %)	
Histological class: III	[n= 8]	7/8 (87.5 %)	1/8 (12.5 %)	1.00[#]
IV	[n= 9]	8/9 (88.9 %)	1/9 (11.1 %)	
V	[n= 8]	7/8 (87.5 %)	1/8 (12.5 %)	
Extra-renal manifestations: yes	[n= 22]	19/22 (86.4 %)	3/22 (13.6 %)	1.00[#]
no	[n= 4]	4/4 (100 %)	0/4 (0 %)	

[#]P: Fisher's Exact test.

Table XI/B Relationship between type and number of extra-renal manifestations and **Renal remission <u>at 1 year</u> of Follow-up defined according to HTN criterion (secondary endpoint)** [N = 22]

		Renal remission at 1 year (HTN criteria)		
		Yes	**No**	**P**
Cutaneous: yes	[n= 19]	16/19 (84.2 %)	3/19 (15.8 %)	1.00[#]
no	[n= 3]	3/3 (100 %)	0/3 (0 %)	
Arthritis: yes	[n= 16]	13/16 (81.3 %)	3/16 (18.8 %)	0.53[#]
no	[n= 6]	6/6 (100 %)	0/6 (0 %)	
Serositis: yes	[n= 4]	3/4 (75 %)	1/4 (25 %)	0.47[#]
no	[n= 18]	16/18 (88.9 %)	2/18 (11.1 %)	
Neurologic manifestations: yes	[n= 3]	3/3 (100 %)	0/3 (0 %)	1.00[#]
no	[n= 19]	16/19 (84.2 %)	3/19 (15.8 %)	
Haematological manifestations: yes	[n= 15]	12/15 (80 %)	3/15 (20 %)	0.52[#]
no	[n= 7]	7/7 (100 %)	0/7 (0 %)	
Number of manifestations: 1 - 2	[n= 9]	9/9 (100 %)	0/9 (0 %)	0.24[#]
3 - 4	[n= 13]	10/13 (76.9 %)	3/13 (23.1 %)	

There was only 1 patient with mucosal manifestations (associated with serositis, neurologic and hematologic manifestations) but his outcome was missing. No patients had alopecia. [#]P: Fisher's Exact test.

Table XI/C Relationship between clinical variables and **Renal remission <u>at 1 year</u> of follow-up defined according to HTN criterion (secondary endpoint)** [N = 26]

		Renal remission at 1 year (HTN criteria)		
		Yes	**No**	**P**
AKI at onset: yes	[n= 1]	1/1 (100 %)	0/1 (0 %)	1.00[#]
no	[n= 25]	22/25 (88 %)	3/25 (12 %)	
NS at onset: yes	[n= 9]	8/9 (88.9 %)	1/9 (11.1 %)	1.00[#]
no	[n= 17]	15/17 (88.2 %)	2/17 (11.8 %)	

[#]P: Fisher's Exact test.

Table XII/A. Relationship between socio-demographic and clinical variables and **Renal remission at 5 years of Follow-up defined according to UPR criterion (secondary endpoint)** [N = 26]

		Renal remission at 5 years (UPR criteria)		
	N.	**Yes**	**No**	**P**
Center: Gaslini	[n= 16]	15/16 (93.8 %)	1/16 (6.3 %)	0.06#
Other	[n= 10]	6/10 (60 %)	4/10 (40 %)	
Sex: Male	[n= 4]	2/4 (50 %)	2/4 (50 %)	0.16#
Female	[n= 22]	19/22 (86.4 %)	3/22 (13.6 %)	
Age at onset: < 10 years	[n= 7]	5/7 (71.4 %)	2/7 (28.6 %)	0.59#
≥ 10 years	[n= 19]	16/19 (84.2 %)	3/19 (15.8 %)	
Age at onset LN: < 10 years	[n= 3]	1/3 (33.3 %)	2/3 (66.7 %)	0.09#
≥ 10 years	[n= 23]	20/23 (87.0 %)	3/23 (13.0 %)	
Histological class: III	[n= 8]	6/8 (75.0 %)	2/8 (25 %)	1.00#
IV	[n= 9]	7/9 (77.8 %)	2/9 (22.2 %)	
V	[n= 8]	7/8 (87.5 %)	1/8 (12.5 %)	
Extra-renal manifestations: yes	[n= 22]	19/22 (86.4 %)	3/22 (13.6 %)	0.16#
no	[n= 4]	2/4 (50 %)	2/4 (50 %)	

#P: Fisher's Exact test.

Table XII/B Relationship between type and number of extra-renal manifestations and **Renal remission at <u>5 years</u> of Follow-up defined according to UPR criterion (secondary endpoint)** [N = 22]

		Renal remission at 5 years (UPR criteria)		
	N.	**Yes**	**No**	**P**
Cutaneous: yes	[n= 19]	16/19 (84.2 %)	3/19 (15.8 %)	1.00[#]
no	[n= 3]	3/3 (100 %)	0/3 (0 %)	
Arthritis: yes	[n= 16]	14/16 (87.5 %)	2/16 (12.5 %)	1.00[#]
no	[n= 6]	5/6 (83.3 %)	1/6 (16.7 %)	
Serositis: yes	[n= 4]	4/4 (100 %)	0/4 (0 %)	1.00[#]
no	[n= 18]	15/18 (83.3 %)	3/18 (16.7 %)	
Neurologic manifestations: yes	[n= 3]	3/3 (100 %)	0/3 (0 %)	1.00[#]
no	[n= 19]	16/19 (84.2 %)	3/19 (15.8 %)	
Haematological manifestations: yes	[n= 15]	12/15 (80 %)	3/15 (20 %)	0.52[#]
no	[n= 7]	7/7 (100 %)	0/7 (0 %)	
Number of manifestations: 1 - 2	[n= 9]	8/9 (88.9 %)	1/9 (11.1 %)	1.00[#]
3 - 4	[n= 13]	11/13 (84.6 %)	2/13 (15.4 %)	

No patients had alopecia. [#]P: Fisher's Exact test.

Table XII/C Relationship between clinical variables and **Renal remission at <u>5 years</u> of follow-up defined according to UPR criterion (secondary endpoint)** [N = 26]

		Renal remission at 5 years (UPR criteria)		
	N.	**Yes**	**No**	**P**
HTN before LN: yes	[n= 3]	2/3 (66.7 %)	1/3 (33.3 %)	0.49[#]
no	[n= 23]	19/23 (82.6 %)	4/23 (17.4 %)	
HTN at the end of FU: yes	[n= 2]	0/2 (0 %)	2/2 (100 %)	0.031[#]
no	[n= 24]	21/24 (87.5 %)	3/24 (12.5 %)	
AKI at onset: yes	[n= 1]	1/1 (100 %)	0/1 (0 %)	1.00[#]
no	[n= 25]	20/25 (80 %)	5/25 (20 %)	
NS at onset: yes	[n= 9]	6/9 (66.7 %)	3/9 (33.3 %)	0.30[#]
no	[n= 17]	15/17 (88.2 %)	2/17 (11.8 %)	

[#]P: Fisher's Exact test.

Table XIII/A. Relationship between socio-demographic and clinical variables and **Renal remission at 5 years of Follow-up defined according to HTN criterion (secondary endpoint)** [N = 26]

		Renal remission at 5 years (HTN criteria)		
	N.	**Yes**	**No**	**P**
Center: Gaslini	[n= 16]	15/16 (93.8 %)	1/16 (6.3 %)	0.26#
Other	[n= 10]	7/10 (70 %)	3/10 (30 %)	
Sex: Male	[n= 4]	2/4 (50 %)	2/4 (50 %)	0.10#
Female	[n= 22]	20/22 (90.9 %)	2/22 (9.1 %)	
Age at onset: < 10 years	[n= 7]	5/7 (71.4 %)	2/7 (28.6 %)	0.28#
≥ 10 years	[n= 19]	17/19 (89.5 %)	2/19 (10.5 %)	
Age at onset LN: < 10 years	[n= 3]	1/3 (33.3 %)	2/3 (66.7 %)	0.052#
≥ 10 years	[n= 23]	21/23 (91.3 %)	2/23 (8.7 %)	
Histological class: III	[n= 8]	6/8 (75 %)	2/8 (25 %)	0.82#
IV	[n= 9]	8/9 (88.9 %)	1/9 (11.1 %)	
V	[n= 8]	7/8 (87.5 %)	1/8 (12.5 %)	
Extra-renal manifestations: yes	[n= 22]	19/22 (86.4 %)	3/22 (13.6 %)	0.51#
no	[n= 4]	3/4 (75 %)	1/4 (25 %)	

#P: Fisher's Exact test.

Table XIII/B Relationship between type and number of extra-renal manifestations and **Renal remission at <u>5 years</u> of Follow-up defined according to HTN criterion (secondary endpoint)** [N = 22]

		Renal remission at 5 years (HTN criteria)		
	N.	**Yes**	**No**	**P**
Cutaneous: yes	[n= 19]	16/19 (84.2 %)	3/19 (15.8 %)	1.00[#]
no	[n= 3]	3/3 (100 %)	0/3 (0 %)	
Arthritis: yes	[n= 16]	13/16 (81.3 %)	3/16 (18.8 %)	0.53[#]
no	[n= 6]	6/6 (100 %)	0/6 (0 %)	
Serositis: yes	[n= 4]	3/4 (75 %)	1/4 (25 %)	0.47[#]
no	[n= 18]	16/18 (88.9 %)	2/18 (11.1 %)	
Neurologic manifestations: yes	[n= 3]	3/3 (100 %)	0/3 (0 %)	1.00[#]
no	[n= 19]	16/19 (84.2 %)	3/19 (15.8 %)	
Haematological manifestations: yes	[n= 15]	12/15 (80 %)	3/15 (20 %)	0.52[#]
no	[n= 7]	7/7 (100 %)	0/7 (0 %)	
Number of manifestations: 1 - 2	[n= 9]	9/9 (100 %)	0/9 (0 %)	0.24[#]
3 - 4	[n= 13]	10/13 (76.9 %)	3/13 (23.1 %)	

No patients had alopecia. [#]P: Fisher's Exact test.

Table XIII/C Relationship between clinical variables and **Renal remission at <u>5 years</u> of follow-up defined according to HTN criterion (secondary endpoint)** [N = 26]

		Renal remission at 5 years (HTN criteria)		
	N.	**Yes**	**No**	**P**
no	[n= 24]	22/24 (91.7 %)	2/24 (8.3 %)	
AKI at onset: yes	[n= 1]	1/1 (100 %)	0/1 (0 %)	1.00[#]
No	[n= 25]	21/25 (84 %)	4/25 (16 %)	
NS at onset: yes	[n= 9]	7/9 (77.8 %)	2/9 (22.2 %)	0.59[#]
no	[n= 17]	15/17 (88.2 %)	2/17 (11.8 %)	

[#]P: Fisher's Exact test.

Moreover, we analyzed collected data regarding plasma creatinine, 24H urine protein, plasma albumin, C3 and C4 level and ECLAM score at follow-up visits, drawing response kinetics (Table XIVa, XIVb, XIVc, XIVd; Figure I, II, III, IV). For every variable analyzed over time, we demonstrate statistically significant improvement from T_0 baseline to 1-year visit and from T_0 baseline and last FU visit.

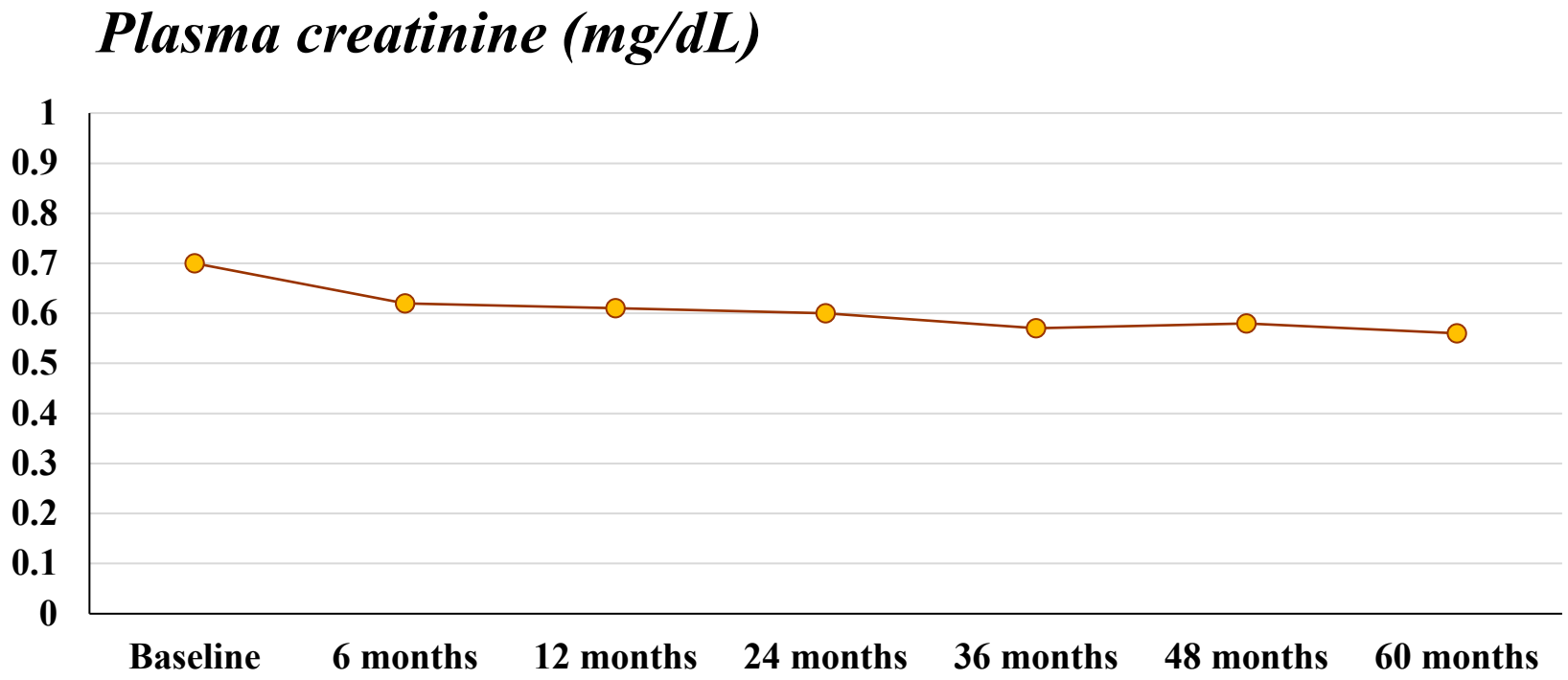
Finally, we compared our main data with those recorded from the original article, limited to the low-dose group patients (Table XV).

Table XIV / A. Quantitative parameters over time from baseline to last follow-up

			Median	P			
	N.	**Mean (SD)**	**[1st – 3rd quartile]**	**(To - T12)**	**(To - T24)**	**(To - T36)**	**(To - T60)**
Plasma creatinine (mg/dL)							
Baseline (T_0)	28	0.82 (0.4)	0.7 [0.52 - 0.98]				
6th month	28	0.68 (0.26)	0.62 [0.49 - 0.8]				
12th month	28	0.68 (0.22)	0.61 [0.59 - 0.75]	0.049			
24th month	21	0.68 (0.33)	0.6 [0.5 - 0.72]		0.25		
36th month	18	0.59 (0.14)	0.57 [0.5 - 0.7]			0.30	
48th month	14	0.69 (0.44)	0.58 [0.5 - 0.64]				
60th month	14	0.55 (0.07)	0.56 [0.5 - 0.6]				0.051 [N=13]
24 h Urine protein (g/24 h)							
Baseline (T_0)	28	2.77 (1.84)	2.16 [1.45 - 3.8]				
6th month	27	0.94 (1.39)	0.4 [0.2 - 1]				
12th month	27	0.75 (1)	0.3 [0.19 - 1]	< 0.0001			
24th month	20	1.03 (1.31)	0.39 [0.12 - 1.44]		< 0.0001 [N=19]		
36th month	18	0.58 (0.68)	0.31 [0.14 - 0.5]			< 0.0001	
48th month	14	1.18 (1.99)	0.25 [0.2 - 1]				
60th month	14	0.32 (0.31)	0.2 [0.12 - 0.33]				< 0.0001 [N=13]

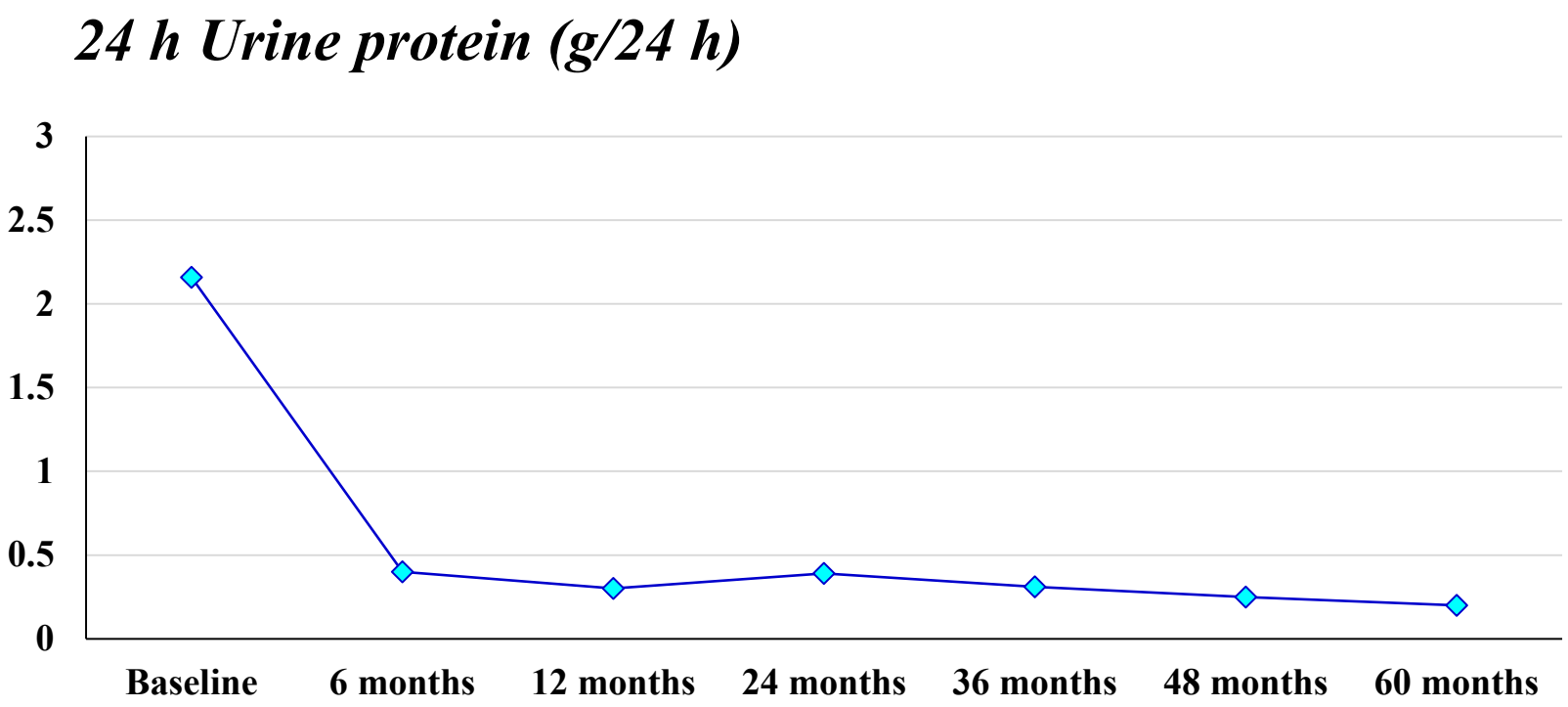
P value refers to the Friedman's test (Non-parametric Analysis of variance).

Figure I. Plasma creatinine (mg/dL) over time from baseline to last follow-up



Small orange circles represent median values.

Figure II. Urine protein (g/24 h) over time from baseline to last follow-up**.**



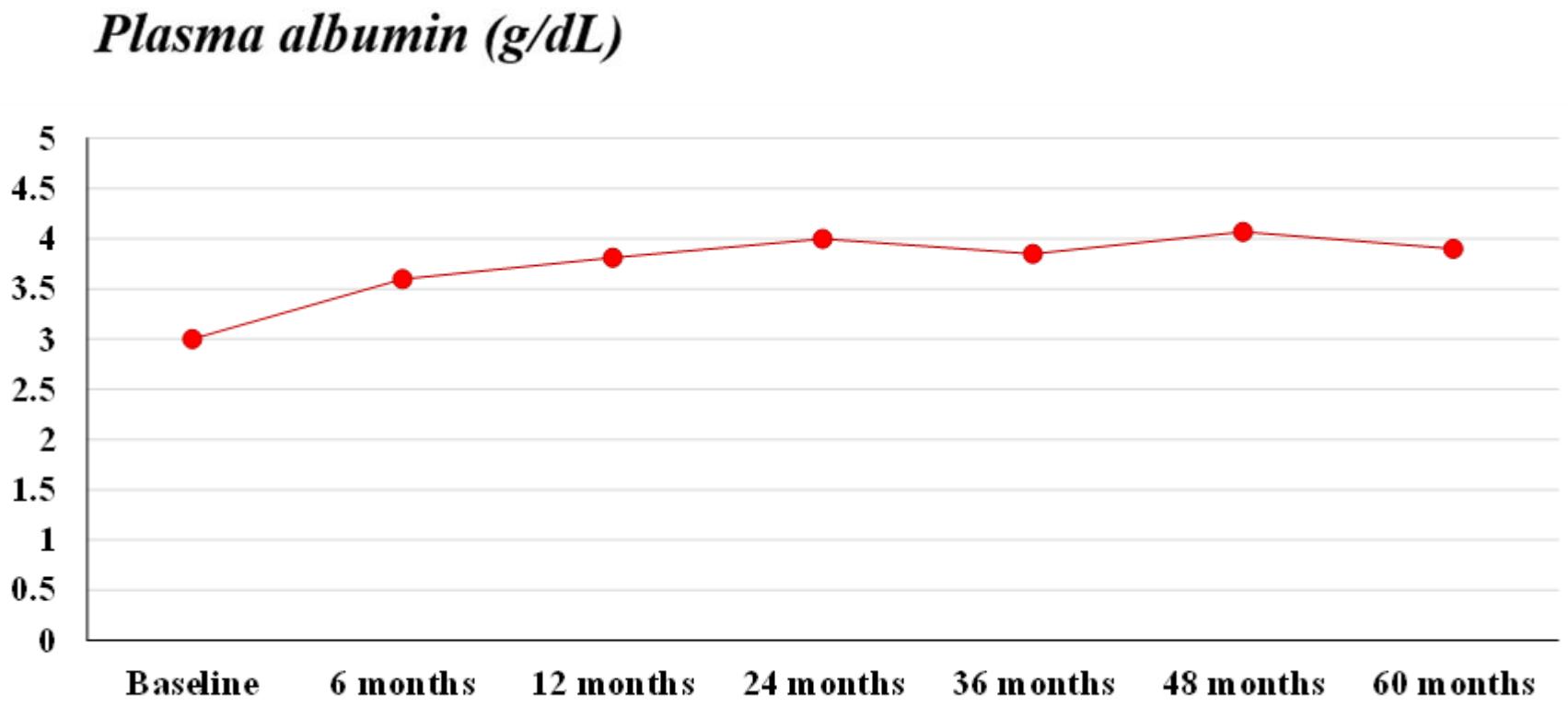
Small light blue diamonds represent median values.

Table XIV / B. Plasma albumin (g/dL) over time from baseline to last follow-up

			Median	P			
	N.	**Mean (SD)**	**[1st – 3rd quartile]**	**(To - T_{12})**	**(To - T_{24})**	**(To - T_{36})**	**(To - T_{60})**
Plasma albumin (g/dL)							
Baseline (T_0)	26	3.06 (0.56)	3 [2.8 - 3.51]				
6th month	26	3.53 (0.48)	3.6 [3.3 - 3.82]				
12th month	26	3.75 (0.47)	3.81 [3.6 - 4.01]	< 0.0001			
24th month	21	3.72 (0.7)	4 [3.2 - 4.1]		< 0.0001		
36th month	18	3.76 (0.49)	3.85 [3.35 - 4.21]			0.0001	
48th month	14	3.75 (0.67)	4.07 [3.2 - 4.23]				
60th month	13	3.87 (0.47)	3.9 [3.5 - 4.13]				< 0.0001

P value refers to the Friedman's test (Non-parametric Analysis of variance).

Figure III. Plasma albumin (g/dL) over time from baseline to last follow-up.



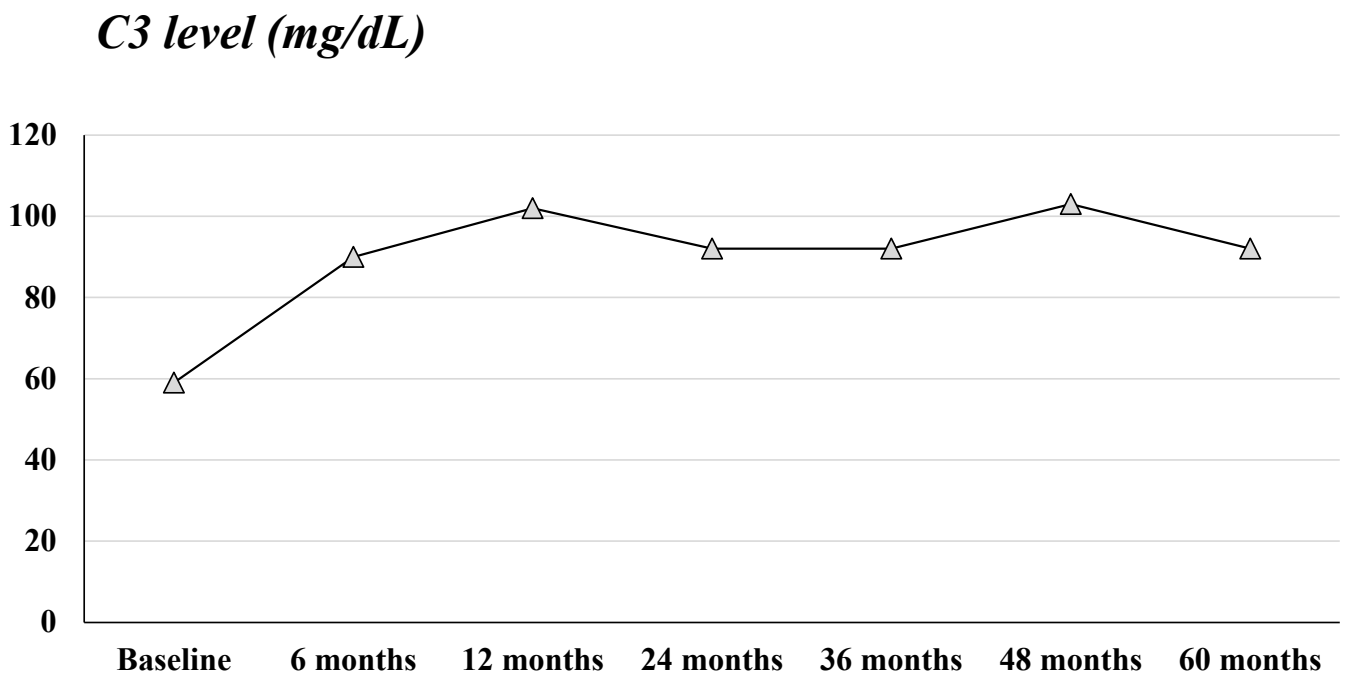
Small red circles represent median values

Table XIV / C. C3 and C4 levels (mg/dL) over time from baseline to last follow-up

	N.	Mean (SD)	Median [1st – 3rd quartile]	P (To - T12)	P (To - T24)	P (To - T36)	P (To - T60)
C3 level (mg/dL)							
Baseline (T_0)	23	58.74 (25.51)	59 [36 - 74]				
6th month	23	90.76 (21.91)	90 [81 - 105]				
12th month	23	98.52 (27.31)	102 [79 - 113]	< 0.0001			
24th month	16	90.69 (40.68)	92 [58.5 - 108]		0.004		
36th month	13	91 (26.47)	92 [79 - 103]			0.045	
48th month	9	89.44 (34.45)	103 [90 - 108]				
60th month	9	97.44 (20.83)	92 [88 - 105]				0.10 [N=8]
C4 level (mg/dL)							
Baseline (T_0)	23	7.13 (5.65)	6 [3 - 9]				
6th month	23	15.65 (7.58)	15 [11 - 19]				
12th month	23	18.35 (8.31)	17 [13 - 27]	< 0.0001			
24th month	16	14.75 (8.92)	14.5 [8 - 22]		0.0004		
36th month	13	16.15 (8.14)	16 [11 - 21]			0.001	
48th month	9	14.33 (7.76)	16 [11 - 18]				
60th month	9	16.78 (12.85)	13 [12 - 16]				0.035 [N=8]

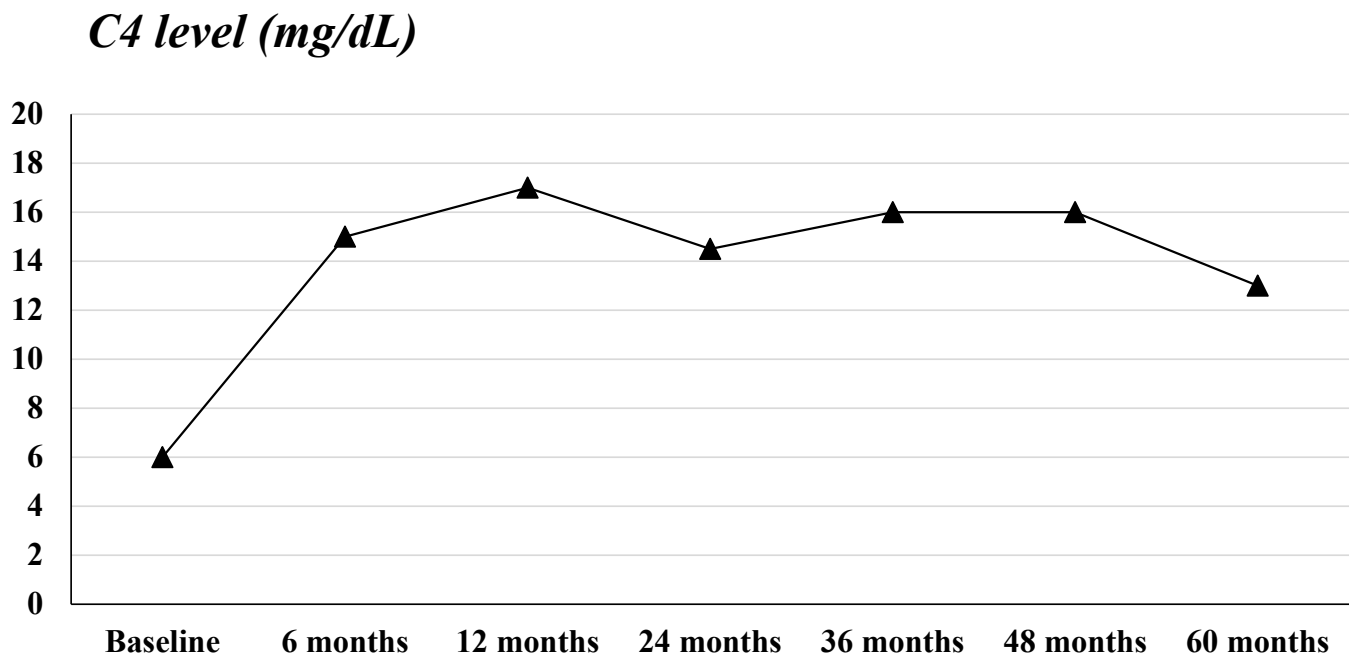
P value refers to the Friedman's test (Non-parametric Analysis of variance).

Figure III. C3 levels (mg/dL) over time from baseline to last follow-up



Small grey triangles represent median values.

Figure IV. C4 levels (mg/dL) over time from baseline to last follow-up



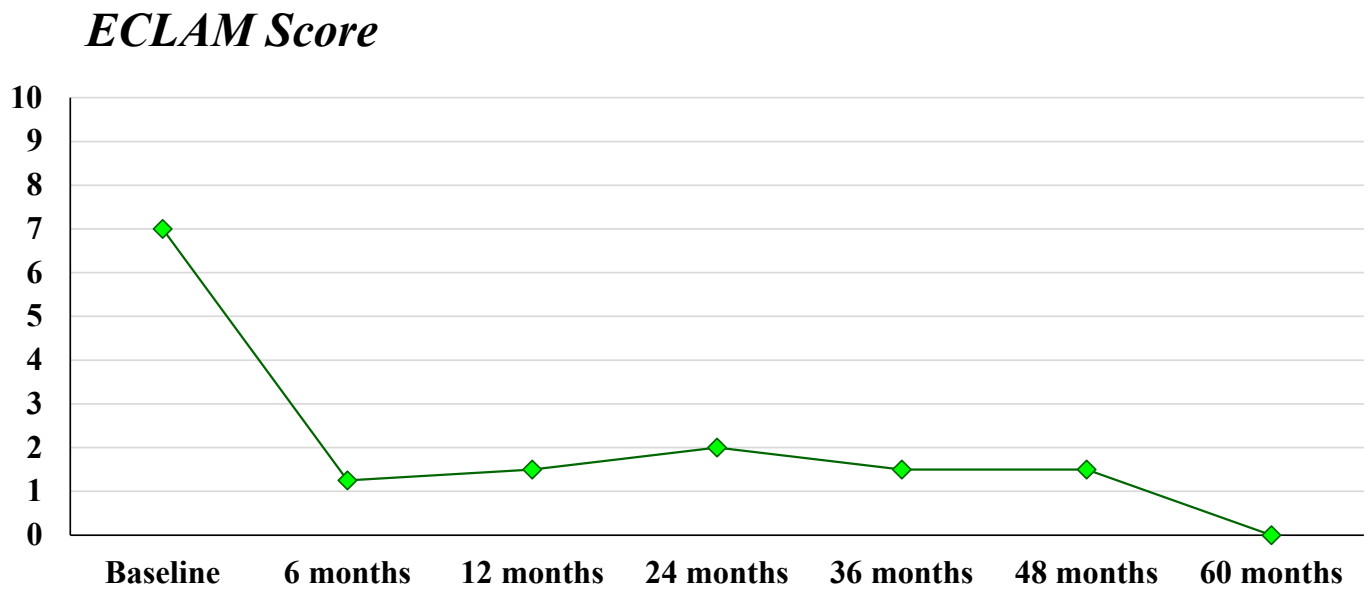
Small black triangles represent median values.

Table XIV / D. ECLAM score over time from baseline to last follow-up

			Median	P			
	N.	**Mean (SD)**	**[1st – 3rd quartile]**	**(To - T_{12})**	**(To - T_{24})**	**(To - T_{36})**	**(To - T_{60})**
ECLAM score							
Baseline (T_0)	27	6.44 (3.15)	7 [4 - 10]				
6th month	28	1.79 (1.91)	1.25 [0 - 3]				
12th month	27	1.85 (1.99)	1.5 [0 - 3]	< 0.0001 [N=27]			
24th month	21	1.9 (1.78)	2 [0 - 3]		< 0.0001		
36th month	18	1.78 (1.73)	1.5 [0 - 3]			< 0.0001	
48th month	14	2.14 (2.68)	1.5 [0 - 3]				
60th month	14	1.07 (1.38)	0 [0 - 3]				< 0.0001 [N=13]

P value refers to the Friedman's test (Non-parametric Analysis of variance).

Figure V. ECLAM score over time from baseline to last follow-up



Small green diamonds represent median values.

Table XV. Comparison between our data (left) and low-dose group or total data (right), where available, derived from the original article

		n./N. (%)	**n./N. (%)**
Nephrotic syndrome at onset:	yes	10/28 (35.7)	25/90[¥] (27.8)
	no	18/28 (64.3)	65/90[¥] (72.8)
AKI at onset:	yes	2/28 (7.1)	20/90[¥] (22.2)
	no	26/28 (92.9)	70/90[¥] (77.8)
RAASI usage rate:	yes	20/28 (71.4)	(59)[ǂ]
	no	8/28 (28.6)	(41)[ǂ]
Completed FU (60 months)[¶]:	yes	15/28 (53.6)	38/44 (86.4)
	no	13/28 (46.4)	6/44 (13.6)
Histological class:	Class III	9/27[Δ] (33.3)	11/44 (25)
	Class IV	12/27[Δ] (44.4)	31/44 (70.5)
	Class V	8/27[Δ] (29.6)	2/44 (4.5)
Treatment failure:	yes	6/27[€] (22.2)	7/44 (15.9)
	no	21/27[€] (77.8)	37/44 (84.1)
Renal remission[§]:	yes	19/25[£] (76)	30/42[£] (71.4)
	no	6/25[£] (24)	12/42[£] (28.6)

[¥]only data regarding the total amount of patients were available; [ǂ]only percentage data were available; [¶]if we consider a 24-month FU period, 82.1% of patients in our study reached this minimum FU period; [Δ]one patient didn't perform renal biopsy (see text); [€]for one patient data regarding treatment failure at 6 months were missing; [§]we used combined criteria (UPr and HTN) at 1 year for comparison; [£]for some patients data regarding renal remission were missing

Considering renal flare as the event, we draw a Kaplan-Meier analysis of the event-free survival estimates: time was measured starting from EUROlupus protocol administration to the event (in case of renal flare) or the last follow-up visit available for the patients. Two patients were not followed for a reasonable time and therefore were excluded from the analysis of survival curves (Table XVI, Figures S1, S2, S3, S4). No data reaches statistical significance in the analysis.

Table XVI Relationship between demographic and clinical variables and **Renal flare evaluated both as categorical data or as survival data (considering the time of events)** [N = 26]

		Renal Flare		P	P	see
		Yes	**No**	**(Fisher's Exact test)**	**(Log-Rank test)**	**Figure**
Sex: Males	[n= 4]	0/4 (0 %)	4/4 (100 %)	0.56	0.33	S1
Females	[n= 22]	5/22 (22.7 %)	17/22 (77.3 %)			
Age: ≤ 10 years	[n= 10]	1/10 (10.0 %)	9/10 (90.0 %)	0.62	0.34	S2
≥ 11 years	[n= 16]	4/16 (25 %)	12/16 (75.0 %)			
Histological class: III	[n= 8]	2/8 (25.0 %)	6/8 (75.0 %)	0.70	0.78	S3
IV	[n= 9]	1/9 (11.1 %)	8/9 (88.9 %)			
V	[n= 8]	2/8 (25.0 %)	6/8 (75.0 %)			
Extra-renal manif.: yes	[n= 22]	5/22 (22.7 %)	17/22 (77.3 %)	0.56	0.25	S4
no	[n= 4]	0/4 (0 %)	4/4 (100 %)			

Log-Rank test refers to the comparison of survival curves; this test takes into account not only the number of events but also the time at which the event occurred.

Figure S1. Survival curve by sex (Male; Female). Event of interest Renal flare; observation time from EUROlupus administration to the event or last follow-up visit

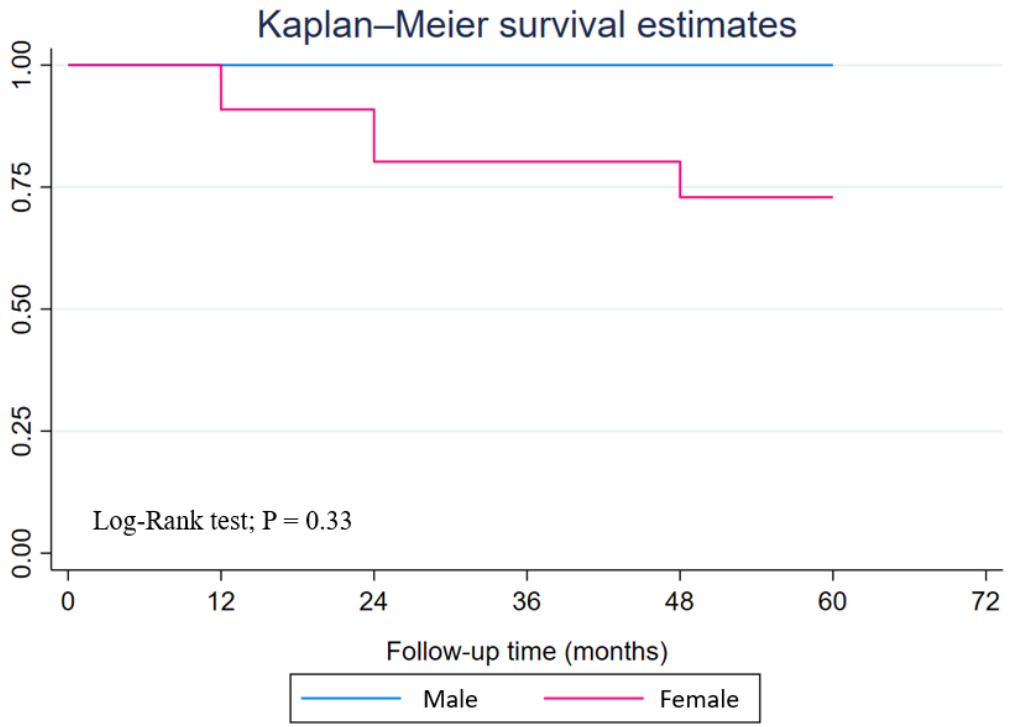


Figure S2. Survival curve by age category (1 ≤ 10 years; 2 ≥ 11 years). Event of interest Renal flare; observation time from EUROlupus administration to the event or last follow-up visit.

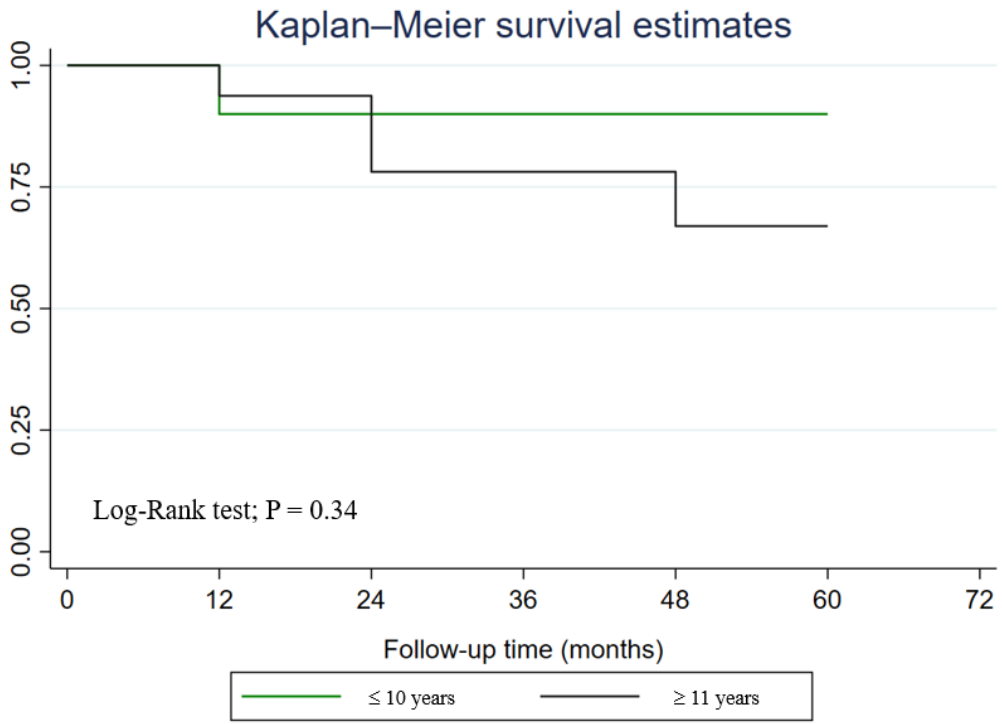


Figure S3. Survival curve by histological class (Class V contains also V + II, V + III and V + IV). Event of interest Renal flare; observation time from EUROlupus administration to the event or last follow-up visit.

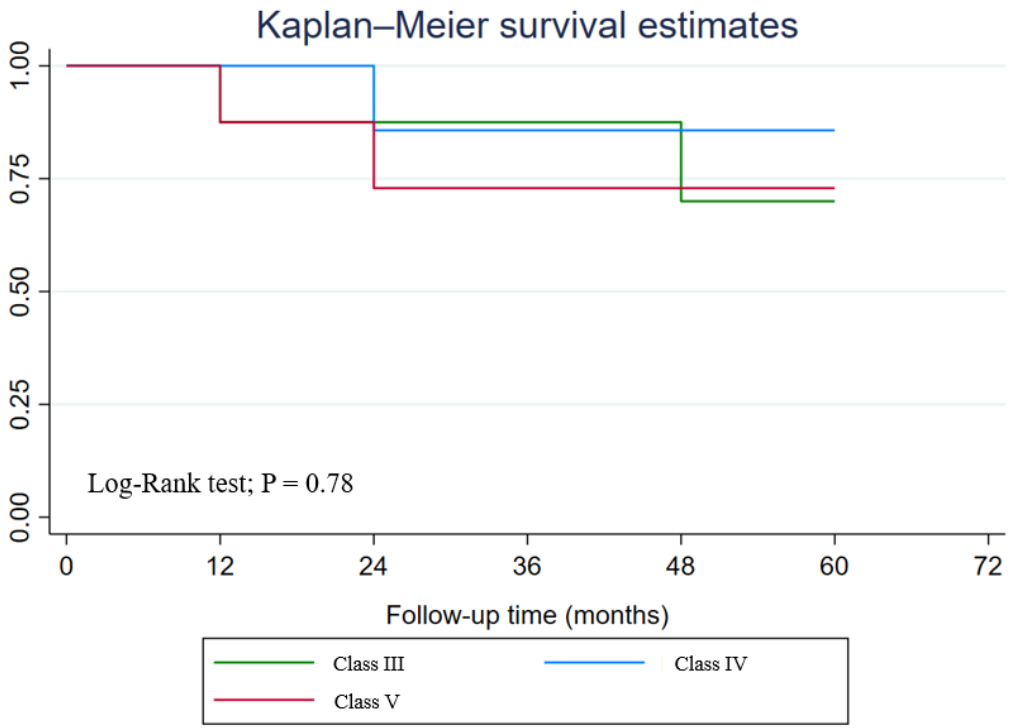
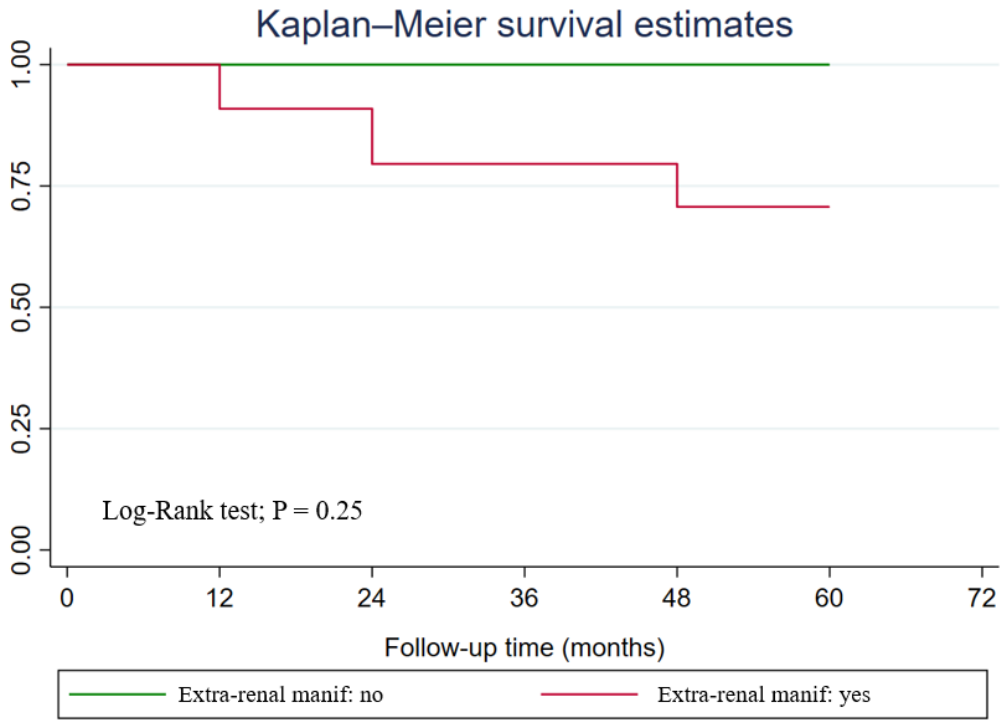


Figure S4. Survival curve by the presence of extra-renal manifestations (yes; no). Event of interest Renal flare; observation time from EUROlupus administration to the event or last follow-up visit



DISCUSSION AND CONCLUSION

Although our data derive from an observational longitudinal study, it is still useful to highlight some aspects. Our group of patients holds more Class V LN and less Class IV than in the original article (29.6 % vs 4.5% and 44.4% vs 70.5% respectively): this is reflected in more nephrotic syndrome and less AKI at LN onset.

The efficacy of EUROlupus protocol is high regarding the primary outcome, both when we consider patients with renal impairment and patients with nephrotic syndrome at the time of protocol administration. Indeed, all patients with AKI reach a normal renal function at 6 months whereas 4/10 patients with NS at onset were still heavy proteinuric at T_6, but only one of them with impaired renal function. Globally we had a slightly elevated treatment failure rate compared with the original article (22.2% vs 15.9%). The rate or renal remission (combined criteria) at 1 year of follow-up was almost equivalent (76% vs 71%); if we focus on 5 year/last FU visit the renal remission rate reaches 80%: these data show great efficacy over time of EUROlupus protocol combined with immunosuppressive therapies. Moreover, figures from I to V show the kinetics of the initial response to therapy: plasma creatinine and albumin, 24H urine protein, C3 and C4 level and the ECLAM score significantly improved during the first year of follow-up, as in the original article.

5/28 patients experienced renal flares during the FU period, slightly less than in the original study (17.9% vs 27%): this could be related to a global shorter observational period. Indeed only 42.9% of patients reached 60 months term, whereas in the original article the drop off rate was 13.6%. However, 82.1% of patients reached at least 24 months of FU in our study. No

relationship between variables (such as sex, age at LN onset, histological class, extrarenal manifestations) and the event "renal flare" was found. We also searched for a relationship between AKI or NS at onset with the event "renal flare" but this was unsuccessful (data not shown).

As in the original article, the probability of treatment failure (primary endpoint) and renal remission at 1 year or 5 year/last FU visit (secondary endpoints) was not related to histological class found in kidney biopsy. Moreover, we performed statistical analysis regarding a possible relationship between all socio-demographic and clinical variables recorded in our study with the primary and secondary endpoints: we found that the absence of extrarenal manifestations is linked to treatment failure and absence of renal remission (UPr criterion) at 1 year of FU. This is probably in agreement with the poor outcome related to so called "full-house" lupus nephropathy, whose main features are the absence of extra-renal disease associated with severe kidney involvement.

Furthermore, patients with elevated blood pressure at the end of FU period are linked to the absence of renal remission at the same visit (UPr criterion): this is probably related to the well-known detrimental effect of poor blood pressure control on renal outcome in LN. We also found that patients derived from other Centers than our Institute were at major risk for absence of renal remission at the last FU visit (UPr criterion): unfortunately, this data is of poor interest due to the lack of homogeneity between patients group from every center; moreover, there could be another bias related to the fact that the majority of Gaslini's patients derived from 2015-2022 years, when new immunosuppressive protocols were adopted (mofetil mycophenolate vs azathioprine and cyclosporine). Finally, age at onset > 10 years is linked to renal remission at last visit/5 year of FU following HTN criterion.

Later, we evaluated the safety of the EUROlupus protocol in terms of severe infections and cancers that occurred during FU period. 3 cases of infections were reported, requiring hospitalization for treatment: 2 herpes zoster reactivations treated with iv antiviral therapy and 1 mycotic esophagitis treated with iv antifungal therapy, all the patients reached complete remission and were discharged soon after hospitalization. Moreover, 1 female patient received a diagnosis of cervical intraepithelial neoplasia derived from a routine screening schedule, was treated locally and continued FU visits to our Center without complications. The high occurrence of these lesions during the normal screening schedule of childbearing age women doesn't allow us to consider this cancer as a direct consequence of our protocol.

In conclusion, our study has two main strong points: it is indeed a database filled with Italian pediatric-onset SLE and notably, pediatric-onset LN; for this reason, its implementation is of particular interest for efficacy and safety studies regarding this disease in such a special subset of patients. Moreover, this database includes patients derived both from the first and second decade of the 21st century: during these years several changes occurred mainly in immunosuppressive therapies started after EUROlupus administration, where mofetil mycophenolate deserved the first choice against some old drugs (azathioprine and cyclosporine). Hence, increasing the number of patients included in this database could lead to data of great interest, such as life-long immunosuppressive therapies with the lowest side effects and the greatest efficacy. However, this study reproduced the good results in terms of efficacy and safety obtained in the original article among Italian pediatric patients affected by proliferative LN. This data is of particular interest since the EUROlupus protocol was originally meant to reduce the cumulative dose of cyclophosphamide among adult patients

with less aggressive disease (Caucasian European): our study, therefore, encourages clinicians to use EUROlupus protocol for pLN treatment.

The weak points of our study are the small number of patients included and the high rate of drop off before 60 months of FU. This is mainly related to the pandemic situation at the time of Centers enrolling and the low rate of incidence among pediatric patients of LN. Finally, patients reaching 16 to 18 years old (depending on Centers protocol) usually were moving from pediatric to adult care. For all these reasons, it is desirable to increase the number of centers participating in this project.

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