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Beyond ISN/RPS lupus nephritis classification: adding chronicity index to clinical variables predicts kidney survival

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Key Points:
*Older age and delay between clinical onset of LN and kidney biopsy were significantly correlated with baseline chronicity index.

*Chronicity index and its components, but not activity index, were significantly associated with long-term impairment of kidney function.

*Baseline serum creatinine, arterial hypertension, chronic glomerular lesions, delay in kidney biopsy predicted Kidney Function Impairment.

Abstract:
Background. A renewed interest for activity and chronicity indices as predictors of lupus nephritis (LN) outcome has emerged. Revised National Institutes of Health (NIH) activity and chronicity indices have been proposed to classify LN lesions but should be validated by future studies. Aims of this study: i) to detect the histological features associated with the development of Kidney Function Impairment (KFI); ii) to identify the best clinical-histological model to predict KFI at time of kidney biopsy. Methods. LN patients with kidney biopsy containing >10 glomeruli per specimen were admitted to the study. Univariate and multivariate logistic regression and Cox proportional hazards model were used to investigate whether activity and chronicity indices could predict KFI development. Results. Among 203 LN participants followed for 14 years, correlations were found between activity index and its components and clinical-laboratory signs of active LN at baseline. Chronicity index was correlated with serum creatinine. Thus, serum creatinine was significantly and directly correlated with both activity and chronicity indexes. At multivariate analysis glomerular sclerosis (OR:3.0478, CI:1.173-7.91, P=0.022) and fibrous crescents (OR:6.8352, CI:3.218-14.519, P<0.001) associated with either moderate/severe tubular atrophy (OR:3.1697, CI:1.042-9.643, P=0.0421), or with interstitial fibrosis (OR:2.361, CI:1.047-5.322, P=0.0383) predicted KFI. Considering both clinical and histological features, serum creatinine (OR:1.677; 1.311-2.145; P<0.001), arterial hypertension (OR:4.641, CI: 1.902-11.324, P<0.001), glomerular sclerosis (OR:2.123, CI:1.001-4.503, P=0.049), and fibrous crescents (OR:5.182, CI: 2.433-11.037, P<0.001) independently predicted KFI. Older age (P<0.001) and longer delay between clinical onset of LN and kidney biopsy (P<0.001) were significantly correlated with baseline chronicity index. Conclusions. Chronicity index and its components, but not activity index, were significantly associated with an impairment of kidney function. The Cox model showed that serum creatinine, arterial hypertension, chronic glomerular lesions and delay in kidney biopsy predicted KFI. These data reinforce the importance of timely kidney biopsy in LN.

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Beyond ISN/RPS lupus nephritis classification: adding chronicity index to clinical variables predicts kidney survival

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KEY POINTS

- Older age and delay between clinical onset of LN and kidney biopsy were significantly correlated with baseline chronicity index.
- Chronicity index and its components, but not activity index, were significantly associated with long-term impairment of kidney function.
- Baseline serum creatinine, arterial hypertension, chronic glomerular lesions, delay in kidney biopsy predicted Kidney Function Impairment.

ABSTRACT

Background. A renewed interest for activity and chronicity indices as predictors of lupus nephritis (LN) outcome has emerged. Revised National Institutes of Health (NIH) activity and chronicity indices have been proposed to classify LN lesions but should be validated by future studies. Aims of this study: i) to detect the histological features associated with the development of Kidney Function Impairment (KFI); ii) to identify the best clinical-histological model to predict KFI at time of kidney biopsy.

Methods. LN patients with kidney biopsy containing >10 glomeruli per specimen were admitted to the study. Univariate and multivariate logistic regression and Cox proportional hazards model were used to investigate whether activity and chronicity indices could predict KFI development.

Results. Among 203 LN participants followed for 14 years, correlations were found between activity index and its components and clinical-laboratory signs of active LN at baseline. Chronicity index was correlated with serum creatinine. Thus, serum creatinine was significantly and directly correlated with both activity and chronicity indexes. At multivariate analysis glomerular sclerosis (OR:3.0478, CI:1.173-7.91, P=0.022) and fibrous crescents (OR:6.8352, CI:3.218-14.519, P<0.001) associated with either moderate/severe tubular atrophy (OR:3.1697, CI:1.042-9.643, P=0.0421), or with interstitial fibrosis (OR:2.361, CI:1.047-5.322, P=0.0383) predicted KFI. Considering both clinical and histological features, serum creatinine (OR:1.677; 1.311-2.145; P<0.001), arterial hypertension (OR:4.641, CI: 1.902-11.324, P<0.001), glomerular sclerosis (OR:2.123, CI:1.001-4.503, P=0.049), and fibrous crescents (OR:5.182, CI: 2.433-11.037, P<0.001) independently predicted KFI. Older age

(P<0.001) and longer delay between clinical onset of LN and kidney biopsy (P<0.001) were significantly correlated with baseline chronicity index.

Conclusions. Chronicity index and its components, but not activity index, were significantly associated with an impairment of kidney function. The Cox model showed that serum creatinine, arterial hypertension, chronic glomerular lesions and delay in kidney biopsy predicted KFI. These data reinforce the importance of timely kidney biopsy in LN.

INTRODUCTION

Lupus Nephritis (LN), a common complication of Systemic Lupus Erythematosus (SLE) is associated with a high risk of morbidity and mortality(1). Kidney biopsy is the gold standard for diagnosis and management of LN(2). In 2003 the International Society of Nephrology and Renal Pathology Society (ISN/RPS) updated the histological classification of LN(3). This classification assessed only the glomerular pathology, but there is growing evidence that tubulointerstitial and vascular lesions are critical to define kidney prognosis. Over the last decades, progresses in the therapeutic approach improved the outcome of LN and mitigated the prognostic differences among histological classes. At the same time, a renewed interest has arisen in the activity and chronicity indices, proposed years ago by Austin et al.(4-6). Several studies pointed out the importance of assessing activity and chronicity changes in all kidney compartments(7-10) and the Working Group for LN proposed to add the activity and chronicity indices to all classes of the ISN/RPS classification to improve the prognostic value of kidney biopsy(11). Among other changes the Working Group suggested to separate fibrinoid necrosis from karyorrhexis and to associate karyorrhexis with neutrophils infiltration(11). Purposes of this retrospective long-term study are: i) To define the association between the clinical features at presentation and the components of the activity and chronicity indices at basal kidney biopsy. ii) To detect the histological features associated with long-term Kidney Function Impairment (KFI) and to identify the best model to predict KFI considering both clinical and histological features at time of kidney biopsy.

METHODS

Inclusion criteria were: i) patients older than 16 years with SLE classified according to the American College of Rheumatology (ACR) criteria(12); ii) patients who had a kidney biopsy-proven LN, performed between January 1984 and December 2019 and with a follow-up longer than 1 year; iii) patients with kidney biopsy including >10 glomeruli for light microscopy and immunofluorescence.

Exclusion criteria comprised patients needing kidney replacement therapy at presentation or patients with inadequate kidney biopsy.

The study was approved by the Ethics Committee of Ospedale Maggiore Policlinico di Milano (protocol number 505_2019bis). Before biopsy, all participants signed an informed consent for the scientific use of their anonymized records. This study adheres to the tenets of the Declaration of Helsinki.

We considered as baseline of the study the histologic diagnosis of LN classified according to the ISN/RPS criteria(3). Histological diagnosis was assessed by 2 experienced nephrologists (G.B.,G.M.), based on light microscopy, immunofluorescence(13), and on electron microscopy when necessary; disagreements were adjudicated by consensus. As already reported(7), renal biopsies performed before 2002 were reclassified according to the last ISN/RPS classification(3). The activity and chronicity indices were estimated by a semiquantitative scoring system according to Austin et al.(4-6).

For the aims of the study, Kidney Function Impairment (KFI) was defined by decrease in creatinine clearance (14) of ≥ 30% over the baseline.

Table1 reports the categorization of the histological variables and the definition of kidney outcomes.

We re-evaluated the terms “neutrophils infiltration” and “fibrinoid necrosis/karyorrhexis” according to the new proposal for histological LN classification(11). Accordingly, fibrinoid necrosis was considered alone whereas karyorrhexis had to be associated with neutrophils infiltration. We retrospectively evaluated the data of immunofluorescence and classified as present or absent the extraglomerular immune deposits. We also specified if the deposits were localized in tubular basement membrane, interstitial capillary wall, and/or small arteries.

The demographics, clinical, histological, laboratory and therapeutic variables, collected from the time of kidney biopsy to the last follow-up, were reported in a database (Table 2).

After kidney biopsy, therapy was based on histological and clinical data. Patients were followed by a dedicated team in our Unit. They were evaluated one month after the diagnosis, then every 2-3 months at one year, and every 3-6 months thereafter. At each follow-up visit clinical, laboratory and therapeutic data were regularly recorded until the last check-up in December 2020.

Statistical analysis

Descriptive statistics were calculated as median and interquartile ranges, since the distribution of most variables was not normal (according to the Shapiro normality test). For the same reason, the difference of continuous variables between groups was tested with t-test or non-parametric Wilcoxon test for independent samples. Chi-square test was used to test associations between qualitative or dichotomized variables. Fisher's Exact test was used instead of Chi-square when expected cell counts were <=5. Kaplan-Meier estimate was used for survival. Linear multivariate regression was used to find the predictors of activity and chronicity index at baseline kidney biopsy among the demographic characteristics. The Cox proportional hazards model, both univariate and multivariate, was used to find the predictors of KFI development over time. The ISN/RPS histological classes, activity and chronicity indices and all their components, and clinical features at LN biopsy have been tested as predictors of KFI development. Correction for possible confounders, namely the calendar year of biopsy, age and sex, and duration of lupus nephritis before renal biopsy, was considered for all the models.

Different dichotomizations of ordinal variables ranging 0-3 were tested (e.g., 0 vs 1-3, 0-1 vs 2-3) and the best one according to its p-value in the statistical models was retained. Activity and chronicity indexes were dichotomized according to their median values. The R statistical package has been used for all the analyses(15).

RESULTS

This study included 203 patients. Their median age was 32.4 years (Interquartile ranges (IQR) 24.15-42.52); 177 (87.2%) participants were females and 184 Caucasians (90.6%). The clinical characteristics of the whole group at kidney biopsy are reported in **Table 2.** The median serum creatinine was 0.9 mg/dL (0.7-1.4), creatinine clearance was 80.45 ml/min (IQR 51.61-110.1). Creatinine clearance was >60 ml/min in 138 (68%) participants, while it was <60 ml/min in 65 (32%). Proteinuria >3.5 g/day and arterial hypertension were respectively seen in 101 and 102 patients (50%). Most participants had class III (45 or 22%), class IV (108 or 53%), or class V (48 or 24%).

The median number of glomeruli was 20 (IQR 13-25). The median activity index was 6 (IQR 2-9) and the median chronicity index was 2 (IQR 1-3).

After kidney biopsy, all patients received an induction therapy consisting of methylprednisolone pulses in 84.1% of patients while the remaining patients were given oral prednisone. Cyclophosphamide or mycophenolate were added in 87% of patients. Maintenance therapy consisted of low dose prednisone associated with azathioprine or mycophenolate or cyclosporine in 62.1%.

Since the treatment regimens used in LN have evolved during the follow up of the study, in **Supplementary Table 1** we reported changes in immunosuppressive therapy that occurred over the two established time spans (P1: years 1984-2001, P2: years 2002-2019). The rate of KFI was significantly higher in the first period (27 out of 94 patients: 28.7%) compared to the second period (12 out of 109 patients: 11%; P=0.001).

Twenty five of the 203 patients (12.3%) were lost to follow-up. The others 178 were followed up regularly and continued to adhere to prescriptions. (The comparison between the clinical data at one year of the these groups is reported in **Supplementary Table 2**).

During a median follow-up of 14.03 years (IQR 4.94,20.67) kidney function impaired in 39 (19%) patients in median 6.6 years after kidney biopsy (IQR 1.43-19.49) and 13

(33.3%) of them reached end stage renal disease (ESRD). The KFI-free survival was 85.2% at 10 years and 77.4% at 20 years **(Figure 1a)**.

Thirteen patients (6.4%) who did not develop KFI, died after a median of 11.43 years (IQR 3.91-20.87). Infections (3 patients), cardiovascular accidents (5) and neoplasia (5) were the causes of death. The KFI-free survival and death-free survival were 81.6% and 72% respectively at 10 and at 20 years **(Figure 1b**).

Among the demographic variables, only the calendar year of biopsy($p=0.05$) was weakly correlated with activity index >6. Based on logistic regression analysis, older age ($P<0.001$) and longer delay between clinical onset of LN and kidney biopsy ($P=0.001$) were directly correlated with higher baseline chronicity index. In patients aged ≥30years, chronicity index was 2.9±2.4, in comparison to 1.6±2.2 in those aged <30 years ($P<0.001$). When kidney biopsy was performed ≥3 or <3 months after the clinical LN onset, chronicity indexes were respectively 2.9±2.8 and 1.8±2 ($P=0.001$).

Correlations between clinical features at kidney biopsy and activity and chronicity indices (Table 3).

As for activity index >6 was significantly more frequent in class IV than in class III or V. Patients with activity index >6 had significantly higher serum creatinine and number of urine erythrocytes, significantly lower levels of serum complement and hematocrit compared to patients with activity index <6. Similar correlations were observed for endocapillary hypercellularity, hyaline deposits, cellular crescents and interstitial infiltration. In addition, hyaline deposits were also significantly associated with higher proteinuria and lower serum albumin levels. In comparison to the old variable “neutrophils infiltration”, including karyorrhexis(11), continued not to be associated with serum creatinine, but had significant correlation with proteinuria. On the other hand, “fibrinoid necrosis” alone correlated with serum creatinine, and with C3**(Supplementary Table 3).**

As demonstrated for activity index >6, also for chronicity index >2 and all its components, there was a significative direct correlation with serum creatinine. In addition serum C3 and C4 levels were directly correlated with chronicity index.
The presence of extraglomerular deposits correlated with higher serum creatinine, high number of urinary erythrocytes, low C3 and hematocrit.

Histological predictors of Kidney Function Impairment in the long-term (Table 2 and 4*).*

Among ISN/RPS histological classes, only class IV showed a weak correlation with KFI at univariate analysis (P=0.048).
The presence of interstitial inflammation (P=0.018) was the only component of activity index associated with development of KFI at univariate analysis. However, when we evaluated separately interstitial infiltration in patients with or without interstitial chronic lesions, the correlations with KFI were maintained only in presence with tubular atrophy/interstitial fibrosis (P<0.001), while it was lost in presence of normal cortex (P=0.677).
Chronicity index >2 (P<0.001) and moderate/severe degree of glomerular sclerosis (P<0.001), fibrous crescents (P<0.001), tubular atrophy (P<0.001), interstitial fibrosis (P<0.001), presence of extraglomerular deposits (P=0.0376) and vascular immune deposits (P=0.00738) were significantly associated with KFI at univariate analysis.
At multivariate analysis, two models with the same power were associated with the development of KFI (likelihood ratio p=5e-10 in both). Both models included moderate severe glomerular sclerosis (model 1:OR:3.0478, CI:1.173-7.917, P=0.022; model 2: OR:3.937, CI:1.840-8.426, P<0.001) and fibrous crescents (model 1: OR 6.8352, CI:3.218-14.519, P<0.0010; model 2: OR:5,769, CI:2.572-12.938, P<0.001). In the first model, glomerular sclerosis and fibrous crescents included moderate/severe tubular atrophy (OR:3.1697, CI:1.042-9.643, P=0.0421), while in the second model glomerular sclerosis and fibrous crescents were associated with interstitial fibrosis (OR:2.361 CI:1.047-5.322, P=0.0383). The quasi-equivalence of

the two models can be explained by the strong correlation between tubular atrophy and interstitial fibrosis (r=0.6583, P<0.001).

The histological predictors of KFI in the subgroup of patients with class IV LN at univariate and at multivariate analysis are reported in **Supplementary Table 4.** Similarly, to what observed in the whole group, at multivariate analysis glomerular sclerosis (OR:4.092, CI:1.553-10.780,P=0.005), fibrous crescent (OR:4.273,CI:1.518-12.027, P=0.006), and interstitial fibrosis (OR:3.227,CI:1.151-9.049,P=0.027) were the independent predictor of KFI.

Clinical and histological predictors of Kidney Function Impairment (Table 5)

Among the clinical variables, serum creatinine (OR:1.793 for any mg/dl increase in serum creatinine (CI:1.450-2.216,P<0.001), arterial hypertension (OR:6.154,CI:2.598-14.579, P<0.001) and among the possible confounders, months from clinical LN onset to kidney biopsy (OR:1.006 for each month delay in performing renal biopsy (CI:1.001-1.012,P=0.019) were independent predictors of KFI at multivariate analysis.

Adding the histological characteristic to the clinical data, at multivariate analysis, serum creatinine (OR:1.677 for any mg/dl increase in serum creatinine (CI:1.311-2.145,P<0.001), arterial hypertension (OR:4,641, CI:1.902-11.324,P<0.001), glomerular sclerosis (OR:2,123,CI:1.001-4.503,P=0.049), fibrous crescents (OR:5.182,CI:2.433-11.037,P<0.001), and months from LN onset to kidney biopsy (OR:1.007,CI:1.001-1.013,P=0.034), were found to be independent predictors of KFI.

DISCUSSION

In this study we evaluated if the demographic characteristics of the population had an impact on the severity of activity and chronicity indices at baseline kidney biopsy. No significant association was found between demographic parameters and activity index. Instead, we found that older age at the biopsy and longer time between onset of clinical renal signs and kidney biopsy were significantly correlated with chronicity

index. Previous studies reported that delay in starting treatment after clinical onset of LN significantly increases the probability of ESRD(16,17). We confirmed the deleterious impact of delayed biopsy on kidney function and found a significant association between delay in performing biopsy and increase in baseline chronicity index. This reinforces the importance of performing biopsy as soon as any sign of kidney involvement appears. This point was also stressed by recent guidelines for LN(2).

One purpose of this study was to evaluate if the clinical renal manifestations were able to predict the type and the severity of baseline histological lesions. At univariate analysis, there was a strong correlation between laboratory signs of LN and activity index and most of its components (**Table 3**). Hyaline deposits had the best correlation with clinical activity. This is not surprising if one considers that LN is probably initiated by glomerular deposit of immune complexes containing nucleic acids(18).

However, both activity and chronicity indexes were significantly and directly correlated with serum creatinine. This suggests that in presence of high serum creatinine, without kidney biopsy, it is difficult to establish if the renal impairment is due to active or chronic lesions. In addition proteinuria is not of help as this parameter did not correlate neither with activity nor with chronicity indexes.

Our results, in line with recent guidelines(2), confirm that kidney biopsy remains crucial for its diagnostic and prognostic value. The correlation between serum creatinine and chronicity index was also confirmed by Leatherwood et al.(19) and Broder et al.(20).

The correlation between serum creatinine and extraglomerular deposits was present at univariate analysis but the significance was lost at multivariate analysis. However, patients with extraglomerular deposits seemed to have a more active LN (higher urinary erythrocyte number, lower C3 and hematocrit), in agreement with Wang et al.(21), and in contrast to Hill et al.(22).

An important aim of this study was to define the value of baseline histological features in predicting the development of KFI in the long-term. Several studies

evaluated separately or together the impact of interstitial inflammation(20,23-26), chronic tubulo-interstitial lesions(19,20,23,24), or extraglomerular deposits(21,22,25,27) in predicting kidney outcome but with conflicting results. In this study, class IV showed a weak association with KFI at univariate analysis, but it was lost at multivariate analysis (**Supplementary Table 4).** Recent studies also reported the poor prognostic value of ISN/RPS classification (7-10). Interstitial inflammation was the only element of activity index associated with KFI at univariate analysis, but not at multivariate analysis. Our results are consistent with two previous studies(20,26). In three other studies, interstitial inflammation was found to be an independent predictor of poor kidney outcome(23,24,28). Different criteria in the evaluation of interstitial infiltration may explain the discrepant results. As observed in kidney transplantation, interstitial inflammation in normal tubulointerstitial areas, may be considered as an expression of active rejection potentially responsive to therapy(26,29). Conversely, interstitial inflammation in areas with interstitial fibrosis/tubular atrophy is hardly reversible and is associated with poor kidney survival(30). In our study, interstitial infiltration lost the correlation with KFI if tubulointerstitial area was normal, while correlation with KFI was maintained in case of tubular atrophy/interstitial fibrosis. Thus, to better assess the prognostic value of interstitial inflammation, it is important to define the setting and the type of inflammatory components(11,23).

The correlation between interstitial inflammation and immune complexes in tubulo-interstitium is controversial(25,31-33).

No significant correlation was found between interstitial inflammation and extraglomerular immune deposits (data not shown), disproving the pathogenetic interdependence between the two lesions. The presence of extraglomerular immune complexes was associated with KFI at univariate analysis but not at multivariate analysis(21). Thus, as already suggested by others(22,27) tubulointerstitial immune deposits play only a minor role in the progression of LN.

In this large cohort of LN patients followed for a median of 14 years, all the glomerular and tubulo-interstitial components of chronicity index were significantly associated with poor kidney survival at multivariate analysis.

The impact of tubulointerstitial chronic lesions on CKD was already outlined by many reports(19,20,23-25,28,34). However, only few studies evaluated simultaneously the impact of chronic glomerular and tubulointerstitial with conflicting results. Hsiech et al.(23) found that tubulointerstitial inflammation and fibrosis but not glomerular injury identified patients at risk of ESRD. In a multicenter Chinese study interstitial inflammation, tubular atrophy and interstitial fibrosis were the only independent risk factors of kidney outcome(24). In another study, the proportion of globally sclerotic glomeruli predicted kidney survival at univariate analysis but not at multivariate analysis(28).

Instead, in 105 patients followed for 9.9 years, fibrinoid necrosis, fibrous crescents, interstitial fibrosis/tubular atrophy, together with kidney function and non-white race predicted ESRD(35). The recent revision of the ISN/RPS classification was used to evaluate the outcome of 101 LN Chinese patients followed for about ten years. At multivariate analysis, fibrous crescents, tubular atrophy/interstitial fibrosis and NIH Chronicity Index were independent risk factors for a composite renal outcome that includes eGFR reduction≥30%, ESDR and death(36).

These data and our results, obtained in a larger cohort and with a longer follow-up, can support the thesis that glomerulosclerosis and interstitial fibrosis are linked pathogenetically. Insufficient blood supply from damaged glomeruli causes chronic hypoxia, which releases interleukins 1 and 6, angiotensin II, and transforming growth factor Beta, resulting in accumulation of extracellular matrix (ECM) and fibrosis. The excessive deposition of ECM can replace functional parenchyma, release inflammatory mediators and reactive oxygen species, and induce epithelial-mesenchymal transition, eventually leading to interstitial fibrosis and chronic kidney failure(37-40). However, tubulointerstitial damage may lead to KFI through different mechanisms. An alternative mechanism for the development could rest on the “in

loco" production of autoantibodies from lymphoid-like structures presents in the interstitium(23). The divergent isotypes of antibodies deposited in the glomeruli and in the interstitium may validate this hypothesis(31). Regardless of the relationship between glomerular and tubulointerstitial damage, our data point out that chronic glomerular and tubulointerstitial damage are both the determinants of kidney prognosis.

However, we found that the best model for predicting KFI resulted from the combination of histological and clinical characteristics at presentation. Moreover, adding activity and chronicity index to the ISN/RPS LN classification, improves the prognostic value of kidney biopsy.

Our study has limitations due to its retrospective nature and extended follow-up period, over which the treatment regimens used in LN have evolved. Most participants were Caucasians, and these results cannot be extended to other ethnicities. Data were from a real-world LN cohort, treatment and duration of follow-up were not standardized.

Except for the re-evaluation of "Fibrinoid/karyorrhexis necrosis", and "neutrophil infiltrations" we have not performed an evaluation of other changes of activity and chronicity indices proposed by the recent revision of the ISN/RPS classification(11). Larger prospective studies are needed to confirm these results.

Disclosures

G. Moroni reports the following: Consultancy Agreements: Glaxo. L. Sacchi reports the following: Scientific Advisor or Membership: Journals editorial board: BMC Medical Informatics and Decision Making; Artificial Intelligence in Medicine; Journal of Biomedical Informatics; PLOS ONE (Academic editor); Scientific Societies Board; SIBIM (Italian Society of Biomedical Informatics – Chair. The remaining authors have nothing to disclose.

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Author Contributions

Gabriella Moroni: Conceptualization; Data curation; Formal analysis; Investigation; Methodology; Resources; Supervision; Validation; Writing - original draft; Writing - review and editing. Giulia Porata: Data curation; Formal analysis; Investigation; Methodology; Resources; Validation; Visualization; Writing - original draft; Writing - review and editing. Francesca Raffiotta: Data curation; Formal analysis; Investigation. Silvana Quaglini: Data curation; Formal analysis; Validation. Giulia Frontini: Data curation; Formal analysis; Investigation. Lucia Sacchi: Data curation; Formal analysis; Validation. Valentina Binda: Data curation; Formal analysis; Investigation. Marta Calatroni: Visualization. Francesco Reggiani: Visualization. Giovanni Banfi: Supervision; Validation; Visualization; Writing - review and editing. Claudio Ponticelli: Conceptualization; Supervision; Validation; Visualization; Writing - original draft; Writing - review and editing.

Supplemental material

Supplementary Table 1: Change in immunosuppressive therapy in the different periods of the study.

Supplementary Table 2: Clinical findings at one year in the 25 patients lost to follow-up and in the 178 who were followed until the end of the study.

Supplementary Table 3: Comparison of correlations with clinical features at renal biopsy of old definitions of “neutrophil infiltration” and “fibrinoid necrosis karyorrhexis”, and the new definitions suggested by the recent revision of histological classification of LN “neutrophil infiltration/karyorrhexis” and “fibrinoid necrosis”.

Supplementary Table 4: Histological predictors of KFI among histological features at renal biopsy in 108 patients with class IV Lupus Nephritis. Univariate and multivariate analysis.

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Table 1: Definition of kidney variables and histological assessment

Definition of kidney variables				
Kidney function impairment (KFI)	Decrease in creatinine clearance ≥ 30 % confirmed by at least three determinations for at least 3 months			
Nephrotic syndrome	Proteinuria> 3.5 g/24 h, with hypoalbuminemia and hypercholesterolemia.			
Arterial hypertension*:*	Systolic blood pressure >140 mm/Hg and/or diastolic blood pressure >90 mm/Hg in sitting position (mean of three consecutive measurements).			
Histological assessment				
Light microscopy	Specimen fixed in 5% formalin Stains used: hematoxylin and eosin, periodic acid-Schiff, silver methenamine and Masson's trichrome/AFOG (Acid Fuchsin Orange G).			
	0	**1+**	**2+**	**3+**
Neutrophil infiltration Endocapillary hypercellularity Hyaline deposits Cellular/fibrocellular crescents Fibrous crescents Fibrinoid necrosis Glomerular sclerosis	absent	Mild (< 25% of glomeruli)	Moderate (25-50% of glomeruli)	Severe (> 50% of glomeruli)
Interstitial inflammation Interstitial fibrosis Tubular atrophy	absent	Mild (< 25 % of the cortex)	Moderate (25-50% of the cortex)	Severe (> 50% of the cortex)
Immunofluorescence staining	formalin-fixed paraffin-embedded kidney sections incubated with pronase[15].			
IgG, IgA, IgM, C1q, C3, fibrinogen	absent	present	present	present

Kidney biopsy specimens were fixed in 5% formalin. For immunofluorescence staining was performed in formalin-fixed paraffin-embedded kidney sections incubated with pronase(13). The intensity was scored on a 0-3+ scale; deposits were considered present when the intensity was >1.
Creatinine clearance was assessed by Cockcroft and Gault Formula(14).

Table 2: Clinical and histological characteristics of 203 patients with lupus nephritis at kidney biopsy.

P values are evaluated with t-test or non-parametric Wilcoxon test for independent samples and with Chi-square test between qualitative or dichotomized variables. If no differently specified data are expressed as median and interquartile ranges.

	All patients 203	Patients who developed KFI: 39	Patients who did not develop KFI: 164	P
Months from SLE to lupus nephritis	8.06 (0-61.6)	3.9 (0-49.4)	8.5 (0-70)	0.0389
Months from renal manifestations to kidney biopsy	3.12 (0.92-13.01)	7.9 (1.6-32.6)	2.6 (0.7-10.7)	**0.040**
Female/male, n° of pts	177/26	33/6	144/20	0.592
Caucasians/others, n of pts	184/19	36/3	148/16	0.691
Age at renal biopsy (years)	32.4 (24.15-42.52)	31.3 (24.1-39)	33.9/24.5-47.6)	0.351
Serum Creatinine (mg/dl)	0.9 (0.7-1.4)	1.5 (1.1-2.4)	0.8 (0.7-1.2)	**<0.001**
Creatinine clearance (ml/min)	80.45 (51.6-110.1)	47 (30.15-74.9)	88.5 (68.1-112.4)	**<0.001**
Proteinuria g/die	3 (1.9-5)	4.2 (3-5.6)	2.9 (1.7-4.9)	**0.009**
Arterial hypertension, number of pts (%)	102 (50.2)	32 (82)	70 (42.7)	**<0.001**
ACE inhibitors N° (%)	160 (78.8)	34 (87.2)	126 (76.8)	0.155
Urinary erythrocytes number/HPF	11 (3-40)	15 (4.3-40)	10 (3-40)	0.505
Serum C3 mg/dL (normal values > 90 mg/dL)	59 (46-79)	56 (47.5-81)	60 (46-78)	0.967
Serum C4 mg/dL (normal values 10-40 mg/dl)	10 (6-15)	11 (5-18.3)	10 (6-15)	0.654
Hematocrit (%)	33.6 (29.2-37.7)	30 (27.5-37)	34 (30-37.7)	0.098
Serum Albumin (g/dL)	2.9 (2.4-3.5)	2.6 (2.3-3.4)	3 (2.5-3.5)	0.096
White blood cells (mmc)	5700(3900-7540)	6225 (5000-8450)	5600 (3850-7500)	0.498
Platelets (µL)	239 (109-304)	219500 (175250-303750)	246000 (183250-299750)	0.313
Ab anti-dsDNA positivity, number of pts (%)	172 (87.7)	32 (86.5)	140 (88)	0.605
Ab anti-phospholipid, number of pts (%)	44 (23.3)	7 (18.9)	37 (24.3)	0.529
ENA, positivity % pf pts				
Anti-SM	23.3	19.2	23.9	0.786
Anti-SSA	35.5	32	36.1	0.892
Anti-SSB	15.3	16	15.3	0.971
Anti-RNP	28.4	24	29.2	0.632
Clinical manifestations % of pts				
Skin	61.7	63.1	61.34:	0.918
Arthralgias	74.2	74.3	74.2	0.988
Serositis	24.7	33.3	22.7	0.351
Cerebritis	5.4)	10.2	4.3	0.244
Fever	52.5	56.4	51.5	0.826
Lymphadenopathy	13.4	12.8	13.5	0.895
Therapy				
Methylprednisolone pulses, number of pts (%)	169 (84.1)	31 (81.6)	138 (84.7)	0.484
IST Induction, n° (%) No IST/CY/MMF/AZA/ CsA/others; n°	174 (87.4) 25/99/43/19/4/9	32 (84.2) 6/18/3/6/0/5	142 (88.2) 19/81/40/13/4/4	0.445
Maintenance, n° (%) No IST/MMF/ AZA/CsA/others; n°	120 (60.9) 77/71/38/10/1	15 (39.5) 23/9/5/1/0	105 (66) 54/62/33/9/1	**0.002**
Histological characteristics				
Histological classes II/III/IV/V n° pts (%)	2(0.9)/45 (22.2)/108 (53.2)/48(23.6)	0/5 (12.8)/26 (66.7)/8 (20.5)	2(1.2)/40 (24.4)/82 (50)/40(24.3)	0.531/ 0.118/ 0.061/ 0.608
Activity index	6 (2-9)	6 (2-9)	6 (2-9)	0.797
Activity index >6, pts (%)	88 (43.3)	17 (43.6)	71 (43.3)	0.993
Endocapillary hypercellularity* >0, pts (%)	133 (65.5)	26 (66.6)	107 (65.2)	0.937
Neutrophil infiltration* >0, pts (%)	153 (75.4)	29 (74.35)	124 (75.6)	0.891
Hyaline deposits/wire loops* >0, pts (%)	105 (51.7)	25 (64.1)	80 (49.1)	0.085
Cellular/fibrocellular crescents** >1, pts (%)	40 (19.7)	9 (23.1)	31 (18.9)	0.556
Fibrinoid necrosis/ karyorrhexis ** >1, pts (%)	71 (34.97)	8 (20.5)	63 (38.4)	**0.035**
Interstitial inflammation* >0, pts (%)	101 (49.75)	25 (64.1)	76 (46.3)	**0.046**

Chronicity index	2(1-3)	3 (1-7)	1 (1-3)	**<0.001**
Chronicity index >2, pts (%)	70 (34.5)	23 (58.97)	47 (28.65)	**<0.001**
Glomerular sclerosis** >1, pts (%)	28 (13.8%)	14 (35.9)	14 (8.5)	**<0.001**
Fibrous crescents** >1, pts (%)	27 (13.3)	13 (33.3)	14 (8.5)	**<0.001**
Tubular atrophy** >1, pts (%)	14 (6.9)	9 (23.1)	5 (3.04)	**<0.001**
Interstitial fibrosis** >1, pts (%)	20 (9.85)	13 (33.3)	7 (4.3)	**<0.001**
Neutrophil infiltration/ karyorrhexis * >0, pts (%)	152 (74.9)	29 (74.35)	123 (75)	0.972
Fibrinoid necrosis ** >1, pts (%)	45 (22.2)	4 (10.25)	41 (25)	**0.046**
Extraglomerular immunedeposits * positive pts (%)**	95 (46.8)	25 (64.1)	70 (15.2)	**0.016**
Tubular basement Dep ***, pts (%)	71 (34.97)	16 (41)	55 (33.5)	0.378
Interstitial capillary wall Dep ***, pts (%)	18 (8.9)	5 (12.8)	13 (7.9)	0.334
Vascular Dep *** pts (%)	45 (22.2)	15 (38.5)	30 (18.3)	**0.006**

Legend: Pts: patients, Ab: antibodies; HPF: high power field; IST: immunosuppressive therapy; CY: cyclophosphamide, MMF: mycophenolate Mofetil, AZA: azathioprine, CsA: cyclosporine; (iQR): interquartile ranges; N°: number; pts: patients; Dep: deposits.
§: data are not available in 34 patients; *All these variables were categorized as 0 vs1+2+3; ** all these variables were categorized as 0+1 vs 2+3 being 0= absent, 1: in less than 25% of glomeruli, or of interstitial or of tubular cortex, 2: from 25 to 50% of glomeruli, or of interstitial or of tubular cortex, 3: more 50% of glomeruli, or of interstitial or of tubular cortex.
*** patients with extraglomerular immune deposits (%)

Table 3: Value of activity index (3a) and of chronicity index and extraglomerular deposits (3b) in predicting clinical features at time of renal biopsy.

White boxes identify significant differences between the two compared groups ($p<0.05$); grey boxes identify non-significant differences between the two compared groups.

3a	Activity Index		Endoc. Hyper		Neutr. Infiltration		Hyaline deposits		Cellular crescents		Fibrinoid necrosis		Interst. infiltration	
	≤6	>6 (43.3%)	0	>0 (65.5%)	0	>0 (75.4%)	0	>0 (52.2%)	≤1	>1 (19.7%)	≤1	>1 (35%)	0	>0 (49.8%)
Serum creatinine, mg/dl	1.1 (±1)	1.4 (±1)	1.2 (±1)	1.3 (±1)	1 (±1.1)	1.3 (±1)	1.1 (±1)	1.4 (±1.1)	1.2 (±0.9)	1.7 (±1.3)	1.2 (±1)	1.3 (±1)	1 (±1)	1.5 (±1)
Proteinuria, g/24h	3.7 (±2.9)	4.4 (±3.8)	3.7 (±3)	4.2 (±3.5)	3.3 (±2.5)	4.2 (±3.5)	3.6 (±2.8)	4.4 (±3.6)	3.9 (±3.5)	4.2 (±2.4)	4.3 (±3.7)	3.5 (±2.3)	3.9 (±3.6)	4.1 (±2.9)
Urinary eryth. number/HPF	17 (±25)	32.6 (±32)	13.1 (±25)	29.8 (±31)	10.6 (±25)	28.4 (±30)	17.2 (±28)	30.6 (±30)	22.2 (±29)	32.3 (±31)	20.2 (±28)	31 (±32)	20.4 (±29)	28.3 (±30)
Serum albumin, mg/dl	3 (±0.8)	2.8 (±0.6)	2.9 (±0.8)	2.9 (±0.7)	3.1 (±0.8)	2.9 (±0.7)	3 (±0.8)	2.8 (±0.7)	2.9 (±0.7)	2.8 (±0.6)	2.9 (±0.8)	3 (±0.7)	3 (±0.8)	2.8 (±0.7)
C3, mg/dl*	69.2 (±26)	52.3 (±23)	74.6 (±26)	54.9 (±23)	77.4 (±27)	56.3 (±23)	70.9 (±26)	53.2 (±23)	62.8 (±27)	56.5 (±22)	65.3 (±28)	54.9 (±21)	61.5 (±26)	61.6 (±26)
C4, mg/dl*	14.3 (±11)	9.9 (±8.5)	15.5 (±11)	10.7 (±9)	16.9 (±12)	10.8 (±8.8)	14.1 (±10)	10.7 (±9.5)	12.3 (±9.6)	12.6 (±12)	14 (±11)	9.4 (±8)	12.4 (±9.5)	12.2 (±11)
Hematocrit, %	35.1 (±6.6)	31.5 (±4.5)	35.3 (±6.7)	32.5 (±5.4)	36.7 (±5.8)	32.4 (±5.7)	35.1 (±6.7)	32 (±4.9)	33.7 (±6.1)	32.4 (±5.2)	34.3 (±6.2)	32.1 (±5.3)	34.1 (±6)	32.8 (±5.9)
Class III, n° (%)	35 (30.4)	10 (11.4)	16 (22.9)	29 (21.8)	4 (8)	41 (26.8)	29 (29.9)	16 (15.1)	39 (23.9)	6 (15)	27 (20.4)	18 (25.4)	24 (23.5)	21 (20.8)
Class IV, n° (%)	31 (26.9)	77 (87.5)	13 (18.6)	95 (71.4)	6 (12)	102 (66.7)	20 (20.6)	88 (83)	74 (45.4)	34 (85)	56 (42.4)	52 (73.2)	46 (45.1)	62 (61.4)
Class V, n° (%)	47 (40.9)	1 ** (1.1)	39 (55.7)	9 (6.8)	38 (76)	10 (6.5)	46 (47.4)	2 (1.9)	48 (29.4)	0	47 (35.6)	1 (1.4)	30 (29.4)	18 (17.8)

3b	Chronicity Index		Glom sclerosis		Fibrous crescents		Tubular atrophy		Interstitial fibrosis			Extraglomerular deposits		
	≤2	>2 (34.5%)	≤1	>1 (13.8%)	≤1	>1 (13.3%)	≤1	>1 (6.9%)	≤1	>1 (9.8%)		No	Yes (46.3%)	
Serum creatinine, mg/dl	1.1 (±1)	1.5 (±1.2)	1.2 (±0.9)	1.8 (±1.5)	1.2 (±1)	1.7 (±1.3)	1.2 (±0.9)	2.5 (±1.8)	1.2 (±0.9)	2.1 (±1.6)		1.1 (±0.8)	1.5 (±1.3)	
Proteinuria, g/24h	4.1 (±3.6)	3.8 (±2.8)	3.9 (±3.3)	4.5 (±3.2)	4.1 (±3.4)	3.7 (±2.6)	4.1 (±3.4)	3.7 (±2.2)	4.1 (±3.5)	3.7 (±1.9)		4.3 (±3.9)	3.7 (±2.5)	
Urinary eryth number/HPF	24.9 (±32)	23.2 (±26)	24.7 (±31)	21.8 (±18)	24.6 (±30)	22.2 (±26)	24.5 (±30)	21.5 (±21)	25.1 (±31)	19.1 (±19)		19.8 (±29)	29.4 (±31)	
Serum albumin, mg/dl	2.8 (±0.7)	3.1 (±0.8)	2.9 (±0.7)	2.9 (±0.8)	2.9 (±0.7)	3.1 (±0.8)	2.9 (±0.7)	3.1 (±0.8)	2.9 (±0.7)	3 (±0.8)		2.9 (±0.7)	2.9 (±0.8)	
C3, mg/dl*	59 (±26)	63.5 (±25)	60.3 (±25)	68.8 (±29)	61.1 (±26)	64.5 (±26)	61.4 (±26)	64.2 (±31)	60.7 (±25)	66.6 (±32)		69.2 (±25)	53.1 (±25)	
C4, mg/dl*	12.2 (±11)	12.5 (±8.1)	11.7 (±10)	15.7 (±10)	12.2 (±10)	12.8 (±9.4)	12.3 (±9.9)	12.8 (±12)	12 (±10)	14.2 (±10)		12.7 (±9.5)	11.9 (±11)	
Hematocrit, %	33.6 (±5.9)	33.3 (±6.2)	33.5 (±5.9)	33.2 (±6.4)	33.5 (±5.9)	33.4 (±6.3)	33.6 (±5.9)	31.9 (±7.1)	33.5 (±5.9)	33.3 (±6.5)		34.7 (±6.5)	32.1 (±5.1)	
Class III, n° (%)	25 (18.8)	20 (28.6)	39 (22.3)	6 (21.4)	41 (23.3)	4 (14.8)	43 (22.7)	2 (14.3)	41 (22.4)	4 (20)		26 (23.8)	19 (20.2)	
Class IV, n° (%)	69 (51.9)	39 (55.7)	90 (51.4)	18 (64.3)	87 (49.4)	21 (77.8)	98 (51.8)	10 (71.4)	96 (52.5)	12 (60)		48 (44)	60 (63.8)	
Class V, n° (%)	37 (27.8)	11 (15.7)	44 (25.1)	4 (14.3)	46 (26.1)	2 (7.4)	46 (24.3)	2 (14.3)	44 (24)	4 (20)		33 (30.3)	15 (16)	

All the histological variables were evaluated as 0 if absent; 1+ if mild (in less than 25% of glomeruli and/or in tubulointerstitial area); 2+ if moderate (in between 25% and less than 50% of glomeruli and/or in tubulointerstitial area), and 3+ if severe (in more than 50% of glomeruli and/or in tubulointerstitial area).

* Normal value of C3 (normal values 90-180 mg/dl) and of C 4 (normal values 10-40 mg/dl)

Legend: Endoc. Hyper: Endocapillary hypercellularity; Neutr: Neutrophils; Interst: interstitial; Glom: glomerular; Eryth: erythrocytes; HPF: High power field.

** This patient had overimposed thrombotic microangiopathy

Table 4: Histological predictors of KFI. Univariate and multivariate analysis.

	Univariate analysis			Multivariate analysis 1			Multivariate analysis 2		
	OR	CI	P	OR	CI	P	OR	CI	P
Histological classes III+IV vs II+V	1.679	0.77-3.68	0.198						
Histological class IV vs all the other classes	1.952	1.008-3.78	**0.048**						
Activity index ≤6 vs >6	1.081	0.574-2.037	0.810						
Endocapillary hypercellularity*	1.1362	0.581-2.221	0.709						
Neutrophil infiltration*	1.1278	0.5475-2.323	0.744						
Hyaline deposits/wire loops*	0.6195	0.322-1.193	0.152						
Cellular/fibrocellular crescent* *	1.7279	0.813-3.67	0.155						
Fibrinoid necrosis/ karyorrhexis**	0.6177	0.282-1.353	0.229						
Interstitial inflammation*	2.2120	1.146-4.269	**0.018**						
Interst. Inflamm pure >0	0.742	0.326-1.688	0.677						
Intest. Inflamm in presence of chronic T-I lesions >0	3.149	1.647-6.018	**0.000052**						
Neutrophil infiltration/ karyorrhexis*	1.153	0.559-2.373	0.385						
Fibrinoid necrosis* *	0.4779	0.169-1.349	0.163						
Chronicity index ≤2 vs >2	3.9763	2.075-7.62	**0.0000319**						
Glomerular sclerosis**	4.8758	2.492-9.54	**0.0000037**	3.0478	1.173-7.917	0.022	3.937	1.840-8.426	0.000415
Fibrous crescents**	6.9968	3.433-14.26	**0.000000085**	6.8352	3.218-14.519	0.0000006	5,769	2.572 - 12.938	0.000002
Tubular atrophy**	9.2968	4.301-20.1	**0.000000014**	3.1697	1.042-9.643	0.0421			
Interstitial fibrosis**	7.2684	3.680-14.369	**0.000000011**				2.361	1.047-5.322	0.0383
Extraglomerular deposits* **	2.0039	1.041-3.859	**0.0376**						
Tubular deposits***	1.1370	0.5997-2.156	0.694						
Interstitial capillary wall deposits***	1.6939	0.6603-4.345	0.273						
Vascular deposits ***	2.4393	1.27-4.684	**0.00738**						

Likelihood ratio test = Multivariate analysis 1: 46.25 on 3 df, p=5e-10; Multivariate analysis 2: 46.17 on 3 df, p=5e-10

Legend: OR: Odd ratio, CI: confidential interval Inflamm: inflammation, T-I: tubule-interstitial

*These variables were categorized as: 0 vs 1+2+3, **These variables were categorized as: 0+1 vs 2+3 being: 0 if absent; 1+ if mild (in less than 25% of glomeruli and/or in tubulointerstitial cortex); 2+ if moderate (in between 25% and less than 50% of glomeruli and/or in tubulointerstitial cortex), and , 3+ if severe (in more than 50% of glomeruli and/or in tubulointerstitial cortex).

*** these variables were categorized as present absent.

Table 5: Clinical and histological predictors of KFI . Univariate and multivariate analysis.

Clinical predictors of KFI						
	Univariate			Multivariate analysis		
	OR	CI	P	OR	CI	P
Serum creatinine, mg/dl	1.908	1.564-2.327	<0.001	1.793	1.450-2.216	<0.001
Creat. Clearance, ml/min	0.981	0.971-0.991	<0.001			
Arterial hypertension	8.624	3.670-20.26	<0.001	6.154	2.598-14.579	<0.001
Hematocrit, %	0.926	0.879-0.978	0.005			
Serositis	2.420	1.229-4.766	0.011			
Months from LN onset to kidney biopsy				1.006	1.001-1.012	0.019

Clinical and histological predictors of KFI			
	Multivariate analysis		
	OR	CI	P
Serum creatinine, mg/dl	1.677	1.311-2.145	<0.001
Arterial hypertension	4.641	1.902-11.324	<0.001
Glomerular sclerosis**	2.123	1.001-4.503	0.049
Fibrous crescents**	5.182	2.433-11.037	<0.001
Months from LN onset to kidney biopsy	1.007	1.001-1.013	0.034

Legend: OR: Odd ratio, CI: confidential interval, LN: lupus nephritis

**These variables were categorized as: 0+1 vs 2+3 being: 0 if absent; 1+ if mild (in less than 25% of glomeruli and/or in tubulointerstitial cortex); 2+ if moderate (in between 25%
and less than 50% of glomeruli and/or in tubulointerstitial cortex), and 3+ if severe (in more than 50% of glomeruli and/or in tubulointerstitial cortex).

The histological variables were evaluated as 0 if absent; 1+ if mild (in less than 25% of glomeruli); 2+ if moderate (in between 25% and less than 50% of glomeruli), and 3+ if severe (in more than 50% of glomeruli). Legend: Eryth: erythrocytes; HPF: High power field

Legend of figures

Figure 1a: Kidney Function Impairment-free survival curve of the study population during the entire follow-up period (85.2% at 10 years and 77.4% at 20 years).

Figure 1b: Kidney Function Impairment and death-free survival curve (81.6% at 10 years and 72% at 20 years).

Figure 1

Figure 1a: Kidney Function Impairment-free survival curve of the study population during the entire follow-up period (85.2% at 10 years and 77.4% at 20 years).
Figure 1b: Kidney Function Impairment and death-free survival curve (81.6% at 10 years and 72% at 20 years).