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Persistent Isolated C3 Hypocomplementemia as a Strong Predictor of End-Stage Kidney Disease in Lupus Nephritis

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Introduction: Proliferative lupus nephritis (LN) progresses to end-stage kidney disease (ESKD) in roughly 10% of the cases despite treatment. Other than achieving <0.8 g/24h proteinuria at 12 months after treatment, early biomarkers predicting ESKD or death are lacking. Recent studies encompassing not only LN have highlighted the central role of the alternative complement pathway (ACP), with or without histological evidence of thrombotic microangiopathy (TMA), as a key promotor of renal death.

Methods: We assessed whether persistent isolated C3 hypocomplementemia (PI-LowC3), that is not accompanied by C4 hypocomplementemia, 6 months after kidney biopsy, is associated with an increased risk of death or ESKD in proliferative LN.

Results: We retrospectively followed-up 197 patients with proliferative LN (51 with PI-LowC3) for a median of 4.5 years (interquartile-range: 1.9–9.0), 11 of whom died and 22 reached ESKD. After adjusting for age, gender, ethnicity, hypertension, mycophenolate, or cyclophosphamide use, PI-LowC3 was associated with a hazard ratio [HR] of the composite outcome ESKD or death of 2.46 (95% confidence interval [CI]: 1.22–4.99, $P = 0.012$). These results were confirmed even after controlling for time-varying estimated glomerular filtration rate (eGFR) measurements in joint longitudinal-survival multiple regression models. After accounting for the competing risk of death, PI-LowC3 patients showed a strikingly increased risk of ESKD (adjusted HR 3.41, 95% CI: 1.31–8.88, $P = 0.012$).

Conclusion: Our findings support the use of PI-LowC3 as a low-cost readily available biomarker, allowing clinicians to modify treatment strategies early in the course of disease and offering a rationale for complement blockade trials in this particularly at-risk subgroup of LN patients.

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LN arguably represents the most fearsome complication of systemic lupus erythematosus (SLE). The prognosis of LN has radically changed with the introduction of cyclophosphamide and mycophenolate mofetil as the mainstay of remission-induction immunosuppressive regimens. Despite LN being common in SLE, developing in 38.3% of patients,[1] with the most common class being proliferative LN (roughly 70% in most series), only a fraction currently reaches ESKD, ranging from 1.3% to 16% in large retrospective cohorts,[2–5] with an estimated incidence rate of ESKD of 3.26 cases per million patient-years.[5] Patients developing ESKD are those at the highest risk of dying.[6,7] Early identification of patients with proliferative LN

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who will progress to ESKD and possibly death despite best practice is therefore crucial, informing prognosis and treatment, and holds the potential of advancing the understanding of LN pathophysiology.

Treatment failure may depend on a plethora of causes, many of which are not well defined. Considering the diverse nature of tissue injury in SLE, and the wide variety of histological lesions observed in proliferative LN, which can coexist or occur alone, it is reasonable to hypothesize that nonresponders are not a homogenous population, but instead include several subgroups with different disease mechanisms at play, which may or may not overlap. Low eGFR or an elevated National Institutes of Health (NIH) chronicity index at baseline predict bad long-term renal outcome but they reflect an already-accrued and nonspecific renal damage rather than identifying a specific phenotype of a high-risk subgroup. On the other hand, the only early predictor of (good) long-term renal outcome in modern cohorts is represented by low proteinuria at 12 months.[8]

Recently, dysregulation of the complement system, and particularly of the ACP, emerged as a key pathogenetic mechanism in several glomerulonephritis. PI-LowC3, that is not accompanied by C4 hypocomplementemia, has been described as an indirect marker of ACP overactivation in postinfectious glomerulonephritis,[9,10] C3 glomerulopathy, and immune complex-mediated membranoproliferative glomerulonephritis.[11] In the prototypical condition of ACP overactivation, atypical hemolytic-uremic syndrome, PI-LowC3 occurs in up to 50% of all genetic cases, reaching 70% to 100% of those with C3 or *CFB* mutations.[12] In sera of patients affected by C3 glomerulopathy and immune complex-mediated membranoproliferative glomerulonephritis, circulating autoantibodies acting as C3 nephritic factors were identified, particularly anti-Factor B and anti-C3b antibodies. Interestingly, circulating anti-C3b antibodies leading to chronic ACP overactivation were well characterized also in some cases of LN.[13] TMA, the histological hallmark of ACP dysregulation in atypical hemolytic-uremic syndrome, occurs in up to 24% of LN cases, and is associated with worse renal prognosis; the pathophysiology of TMA in LN is a matter of debate, and thought to potentially arise either from dysregulation of the classical complement pathway or the ACP, or both.[14,15] Whether ACP overactivation defines a subset of proliferative LN patients who more often progress to ESKD, and how large a part they represent of the relatively small portion of LN patients who eventually progress to ESKD, is an unresolved issue. The answer to this question holds promise as a key prognostic tool. One that would finally allow the clinician to identify high-risk LN patients early in the course of disease.

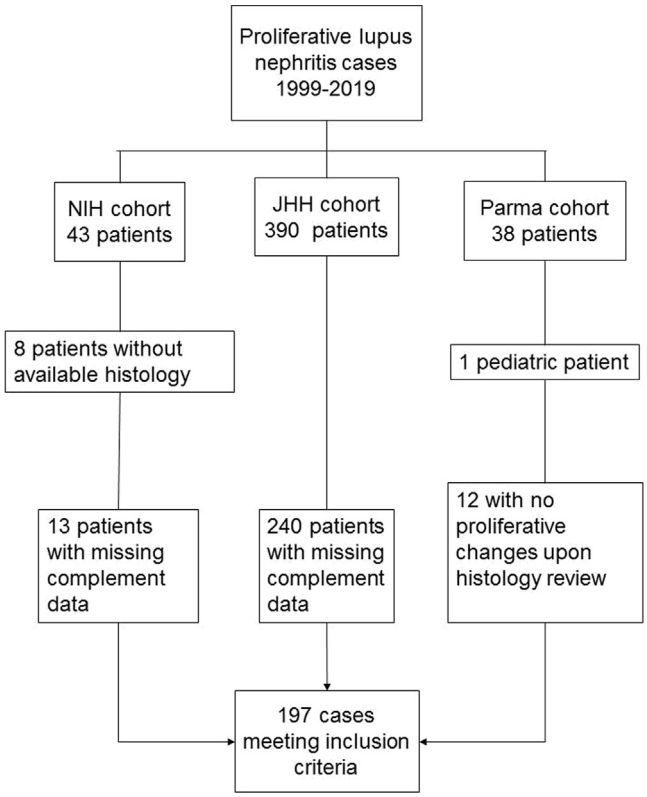

Figure 1. Flow-chart of cohort study. JHH, John Hopkins Hospital; NIH, National Institutes of Health.

In this multicenter, retrospective study, we explored the association between PI-LowC3 6 months after kidney biopsy and ESKD or death in a large, multiethnic cohort of proliferative LN patients.

METHODS

We included adult patients (>18 years old) with the following criteria: (i) received a new histological diagnosis of proliferative LN (i.e., not on repeat biopsies) between December 1999 and January 2020 at Parma University Hospital (Parma, Italy), the NIH (Bethesda, Maryland, USA), and Johns Hopkins Hospital (Baltimore, Maryland, USA), (ii) had at least 6 months of follow-up, and (iii) had available serum C3 and C4 concentration at the time of kidney biopsy and at 6 months of follow-up (Figure 1).

Patients were classified as having SLE if they fulfilled the latest American College of Rheumatology classification criteria.[16] Demographic, clinical, and laboratory data were collected from electronic medical records. These data included the following: age, sex, ethnicity, body mass index, relevant comorbidities (obesity, diabetes, hypertension, heart failure, and cancer), antinuclear antigen antibodies, antiextractable nuclear antigens, including anti-dsDNA,

antiphospholipid antibodies, serum C3 and C4 concentration, serum creatinine, 24-hour proteinuria or urinary protein-to-creatinine ratio, complete blood count, peripheral blood smear, serum lactate dehydrogenase, haptoglobin, as well as remission-induction and maintenance treatment regimens. eGFR was calculated with the Chronic Kidney Disease Epidemiology Collaboration equation.[17]

Histology slides were retrieved for all cases and reevaluated by 3 experienced renal pathologists. All biopsies were reclassified according to the International Society of Nephrology and The Renal Pathology Society classification of LN.[18] Because biopsies, per our inclusion criteria, showed proliferative LN (i.e., classes III, IV, and either III or IV plus V), they were also scored with the NIH activity index and chronicity index. In additiony, histological signs of TMA were assessed, including the following: glomerular fibrin thrombi, double contours of glomerular capillary loops, arteriolar fibrin thrombi, mucoid edema of the intima in arterioles with or without parietal fragmented red blood cells, and hyperplastic arteriolosclerosis (''onion skinning'' of arterioles). Any of these features, alone or presenting together, qualified for TMA, except for double contours of glomerular capillary loops, which were not considered sufficient when occurring alone. Fibrin thrombi were deemed as such when positive for fibrinogen antiserum on immunofluorescence microscopy and/or when directly observed on electron microscopy. Strictly thrombotic features (platelet-rich and/or fibrin thrombi) were not considered mandatory if microangiopathy (mucoid edema of the intima and/or ''onion skinning'') was present, reflecting recent recommendations.[19] TMA was then scored as either present or absent.

Further histological lesions were assessed using a semiquantitative score derived from the Banff classification of renal allograft pathology,[20] namely arteriolar hyalinosis, arteriosclerosis, and a composite score of and interstitial fibrosis and tubular atrophy excluding subcapsular cortical parenchyma. The percentage of globally sclerosed glomeruli was also extracted.

PI-LowC3 was defined as C3 hypocomplementemia without concomitant C4 hypocomplementemia 6 months ($\pm$ 4 weeks) after kidney biopsy. A 6-month interval was deliberately chosen to reflect the standard duration of current remission-induction treatment regimens, in order to identify those patients in which ACP overactivation was still ongoing despite treatment. Circulating immune complexes leading to the activation of the classical complement pathway and therefore a concurrent decrease in serum C3 and C4 levels, are expected to wane upon effective treatment, leading to a normalization of C4 levels.[21] In keeping with this view, we hypothesized that the persistence of isolated C3 hypocomplementemia despite treatment (and therefore the switching off of the classical complement pathway) would allow us to identify those patients with ongoing selective ACP overactivation, in analogy with primary disorders of ACP regulation such as atypical hemolytic-uremic syndrome.[12] This patient subgroup would therefore include those who present either with low C3 and normal C4 both at the time of biopsy and after treatment or with low C3 and low C4 at the time of biopsy and low C3 and normal C4 after treatment (Figure 2).

The study was approved by the Institutional Review Board of the 3 Institutions involved (Parma University Hospital–protocol number 6069 2019-02-22, NIH, and Johns Hopkins Hospital), and undertaken in accordance with the principles of the Declaration of Helsinki.

Data Analysis

All the analyses were performed using the Stata Statistical Software Release 17.0. (StataCorp, 2021, College Station, TX, USA) and R version 4.1.3 (2022, https://www.R-project.org).

A 2-tailed P value of less than 0.05 was regarded as statistically significant. Differences between groups in continuous variables were examined by Kruskal–Wallis and by Mann–Whitney 2-sample test, and in categorical variables by Fisher's exact test. Time at risk started from 6-month since biopsy (the time at which patients were classified according to the PI-LowC3 exposure category) and continued until the composite clinical outcome of death or ESKD, whichever came first. We used Kaplan-Meier plots to compare survival curves between PI-LowC3 and the rest of the patients, and Cox proportional hazards regression model (and Fine-Gray models for ESKD risk) to adjust the comparison for potential confounders. All multivariable-adjusted regression models included the following variables, which we selected based on background knowledge[4,6]: age (continuous), and indicator variables for gender, Black race, hypertension, use of mycophenolate mofetil, and use of cyclophosphamide. Models that were additionally adjusted for antiphospholipid syndrome, activity index, chronicity index, interstitial fibrosis and tubular atrophy provided similar results. Nevertheless, to avoid having to report several regression models that included more variables than the data could support, we did not report them in the final results. Rather, we included longitudinal eGFR measurements in the Cox proportional hazard regression model; eGFR may not be regarded as a confounder on the relation between PI-LowC3 and death/ESKD, because it is an intermediate

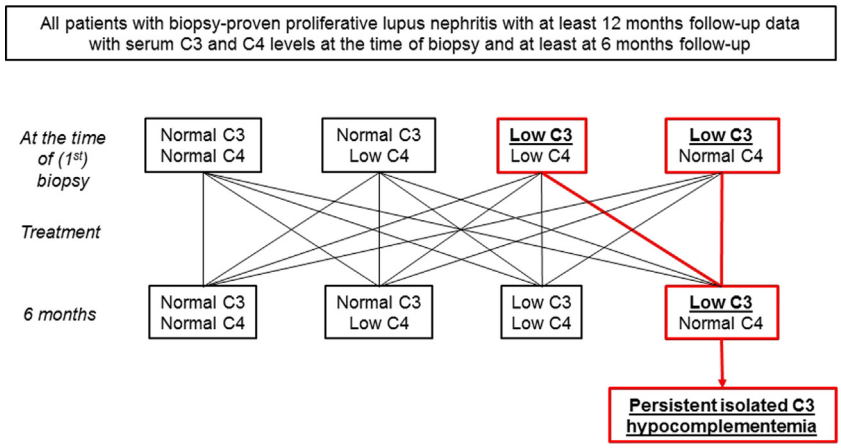

Figure 2. Definition criteria of persistent isolated low C3.

variable that lies in the causal pathway between the exposure (PI-LowC3) and the composite outcome (death/ESKD). Accordingly, time-varying eGFR-adjusted HRs may be regarded as estimates of the (possibly causal) relation between PI-LowC3 and outcome that is independent of any change in eGFR. To include eGFR among the covariates, we fitted a multivariable-adjusted random-coefficient joint longitudinal survival model,[22] in which time-varying eGFR and survival time were analyzed jointly under the assumption that the longitudinal and survival processes are underpinned by shared latent patient random effects. The joint longitudinal survival model consists of 2 submodels, namely a longitudinal submodel (i.e., a linear mixed effects model for eGFR), and a time-to-event submodel (i.e., a Cox proportional hazards model for ESKD/death), which are linked using an association parameter, which is the HR of ESKD/death associated with reduced eGFR. Because the 2 events ESKD/death and eGFR might be highly correlated, joint analysis can reduce bias of estimated parameters. In addition, major results available from joint analyses, as opposed to standard Cox regression analysis with time-varying eGFR, are that they naturally deal with eGFR measurement error, interval missing data, lack of consistency among subjects in timing of eGFR assessment; they provide an optimal "adjustment" for pre-ESKD longitudinal eGFR. We fitted separate joint models in which time-varying eGFR was included as the current value (i.e., last outpatient visit), slope eGFR until the current value, and area under the curve until the current value. Joint analyses were performed using R package JMbayes2.[23] Because of Bayesian inference, results are reported as 95% credible intervals and P values representing 2 times the probability PI-LowC3 having better outcome compared to the rest of the cohort. Further details on the joint longitudinal survival analysis are reported in the Supplementary Material. We also reported the findings from standard Cox proportional hazard regression models with eGFR included as a time-varying covariate for the sake of comparison with results from the more traditional analyses. The Stata and R code for all analyses is freely available at https://github.com/UMaggiore/LN-LowC3.

RESULTS

The study population included 197 patients, 51 of which with PI-LowC3. Population characteristics are reported in Table 1, which shows that demographics, clinical characteristics, and immunosuppressive treatment did not differ between PI-LowC3 and the rest of the cohort. Apart from the obvious difference in serum C3 levels at 6 months reflecting inclusion criteria, the only remaining differences between groups concerned histological findings. The main difference concerned the higher prevalence of TMA in the PI-LowC3 group (31.4% vs. 13.7%, $P = 0.010$). In addition, a slightly higher prevalence of chronic changes ($P = 0.044$), along with a borderline-statistically significant higher prevalence of acute inflammatory lesions ($P = 0.078$) and a higher degree of interstitial fibrosis and tubular atrophy ($P = 0.038$), was observed in the PI-LowC3 group (Table 1). Nevertheless, 6 months after biopsy (the starting point of the follow-up period), eGFR was similar between PI-LowC3 and the rest of the cohort (78.7 [SD 43.9] vs. 83.9 [37.2] ml/min per 1.73 m^2; $P = 0.387$) (Table 1).

Association Between PI-LowC3 and Clinical Outcomes

After a total follow-up of 1038 person-years, and a median follow-up of 4.5 years (interquartile range: 1.9–9.0), 11 patients died and 22 reached ESKD. Half of the cases of ESKD occurred within 5.0 years of follow-

Table 1. Patients' characteristics at baseline

	All patients		PI-LowC3		Rest of the cohort		*P*-value
Number of patients	197		51		146		
Age, yrs (mean±SD)	197	36.3±12.1	51	35.1±10.9	146	36.8±12.4	0.536
Male (%)	46	23.4	14	27.5	32	21.9	0.445
Ethnicity (n,%)							
White	58	29.4	12	23.5	46	31.5	0.647
Black	88	44.7	23	45.1	65	44.5	
Hispanic	13	6.6	5	9.8	8	5.5	
Asian	22	11.2	7	13.7	15	10.3	
Unspecified	16	8.1	4	7.8	12	8.2	
Diabetes (n,%)	5	2.5	3	5.8	2	1.3	0,110
Hypertension (n,%)	69	35	19	37.3	50	34.2	0.735
Obesity (n,%)	25	12.7	5	9.8	20	13.7	0.627
eGFR, ml/min/1.73 m^2	197	82.6±39.0	51	78.7±43.9	146	83.9±37.2	0.387
Proteinuria, g/d	160	2.2±2.7	40	2.5±2.9	120	2.1±2.7	0.182
C3, mg/dl(n,%)	197	64.7±29.0	51	52.2±21.5	146	69.1±30.0	0.001
C4, mg/dl	197	12.5±8.5	51	11.2±6.4	146	12.9±9.1	0.585
LDH, IU/l	44	303.4±185.8	14	340.0±207.5	30	286.3±175.9	0.436
Haptoglobin, mg/dl	23	176.8±146.6	7	191.3±161.8	16	170.5±144.6	0.535
Platelets (×1000/mm^3)	56	205.6±91.3	16	182.9±62.6	40	214.6±99.8	0.189
APL(n,%)	52	26.0	11	21.6	41	28.1	0.461
Histology							
TMA(n,%)	36	18.3	16	31.4	20	13.7	0.010
ISN/RPS Class (n,%)							
Class III or III+V	100	50.8	25	49.0	75	51.4	0.871
Class IV or IV +V	97	49.2	26	51.0	71	48.6	
GS score	191	13.8±17.5	50	16.4±19.4	141	12.9±16.8	0.123
AI score	188	6.2±3.9	47	7.2±4.2	141	5.9±3.8	0.078
CI score	189	3.1±2.4	48	3.7±2.6	141	2.9±2.3	0.044
IFTA score	190	1.1±0.9	50	1.3±1.0	140	1.0±0.9	0.038
AH score	180	0.4±0.7	46	0.3±0.7	134	0.4±0.7	0.756
Treatment							
Hydroxychloroquine (n,%)	145	73.6	33	64.7	112	76.7	0.100
Cyclophosphamide (n,%)	42	21.3	15	29.4	27	18.5	0.114
Mycophenolate (n,%)	127	64.5	29	56.9	98	67.1	0.234
Rituximab (n,%)	5	2.5	2	3.9	3	2.1	0.606

AH, arteriolar hyalinosis; AI, NIH activity index; APL, antiphospholipid antibodies; CI, NIH chronicity index; eGFR, estimated glomerular filtration rate; GS, glomerulosclerosis; ISN/RPS, International Society of Nephrology and the Renal Pathology Society; IFTA, interstitial fibrosis and tubular atrophy; PI-LowC3, persistent isolated low C3; sCr, serum creatinine; TMA, thrombotic microangiopathy.
Baseline laboratory characteristics refer to 6 month after kidney biopsy. Continuous variables are reported as nonmissing data, mean (SD), categorical variables as number (%).

up. The difference in crude composite event-free survival (i.e., free of death or ESKD) between the 2 groups are reported in Figure 3. The crude 5-year event-free survival was 70.1% (95% CI: 52.1−82.4) in PI-LowC3 and 92.4% (95% CI: 85.3−96.2) in the rest of the cohort ($P = 0.004$). After adjustment for potential confounders at baseline (i.e., 6 months after kidney biopsy), the composite outcome rate was more than 2 times higher in the PI-LowC3 (adjusted HR: 2.46 [95% CI: 1.22−4.99; $P = 0.012$]). Despite TMA being more prevalent in PI-LowC3 (Table 1), when patients were classified according to the presence of TMA, event-free survival did not significantly differ between the groups (Figure 4), with a crude 5-year event-free survival of 78.6% (95% CI: 55.4−90.7) in patients with TMA, and 88.1% (95% CI: 80.6−92.8) in patients without TMA ($P = 0.498$). After adjustment for potential confounders at baseline, the adjusted HR associated with TMA was 1.23 (95% CI: 0.51−1.92; $P = 0.651$).

With the aim of additionally adjusting for eGFR at baseline and over the course of follow-up, we fitted joint longitudinal survival regression models. Overall, there were 4656 eGFR measurements (median 13, mean 23.8, range 1−194, interquartile range 4−34 per patient) collected at uneven time points between subjects. The longitudinal multivariable linear mixed model showed that there was no difference between the groups in eGFR slope during the course of the study (slope −0.04 ml/min per 1.73 m^2 per year [95% CI: −0.05 to 0.12; $P = 0.396$] comparing PI-LowC3 with others. The joint model showed that patients with lower current eGFR levels (i.e., eGFR at the last outpatient visit) were at an increased risk of the composite outcome ESKD or death (adjusted HR: 1.16 per 10 ml/min per 1.73 m^2 eGFR decrease [95% CI:

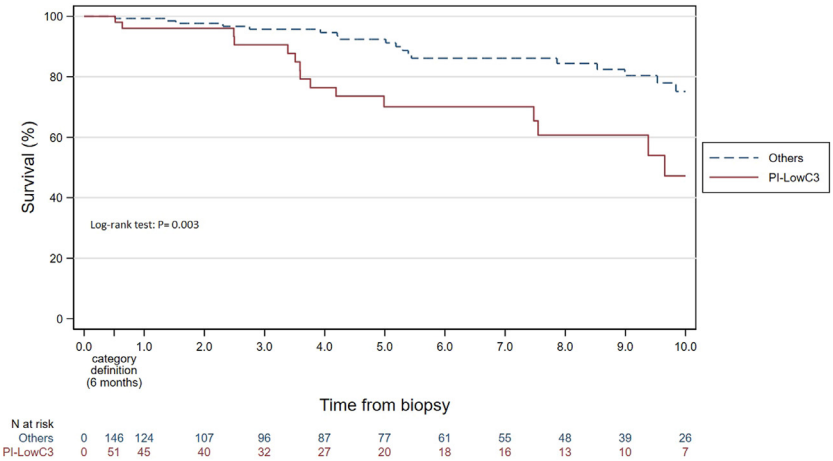

Figure 3. Event-free survival in PI-LowC3 as compared to the rest of the cohort. Time at risk was measured from 6 months postbiopsy (time of classification on patients between the PI-LowC3 and Others category) until the composite outcome ESKD or death (whichever came first). CI, confidence interval; HR, hazard ratio; PI-LowC3, persistent isolated low C3.

1.06−1.29; $P = 0.008$]). In contrast to current eGFR levels (i.e., last follow-up visit), the eGFR slope until the current visit was not significantly associated with the composite outcome (see Supplementary Materials for further details). Moreover, the joint models showed that current eGFR levels had similar association with ESKD compared to cumulative average of eGFR over follow-up (see Supplementary Materials). Altogether, the findings from joint analyses suggested that patients at risk of ESKD were mainly those who started with low eGFR and maintained a low eGFR over the follow-up. In other words, it was the current eGFR over the study rather than the eGFR slope that heralded the onset of ESKD. Moreover, after controlling for current eGFR, the composite outcome rate was still more than 2-fold higher in PI-LowC3 patients (HR: 2.78 [95% CI: 1.03−7.32; $P = 0.0447$]): adjustment for eGFR did not nullify the relation between PI-LowC3 and ESKD, as one might have expected. Altogether, these findings might suggest that the available data from outpatient could not capture the latest eGFR drop heralding the onset of ESKD.

Finally, we sought to estimate the effect of PI-LowC3 and the incidence of ESKD, after accounting for the competing risk of death. As shown in Figure 5, in which the crude cumulative incidence of death and ESKD are reported, in PI-LowC3 patients there was a striking increase in the cumulative incidence of ESKD compared to the rest of the cohort, whereas death rate was not affected. After adjusting for confounding factors, the rate of ESKD progression was more than 3-fold higher in PI-LowC3 (adjusted HR of ESKD associated with PI-LowC3: 3.41 [95% CI: 1.31−8.88; $P = 0.012$]), whereas the death rate did not differ between groups (adjusted HR 1.14 [95% CI: 0.32−4.03; $P = 0.839$]). As shown in Table 2, PI-LowC3 was associated with more than 3-fold increased risk of ESKD irrespective of the model used to adjust for potential confounders.

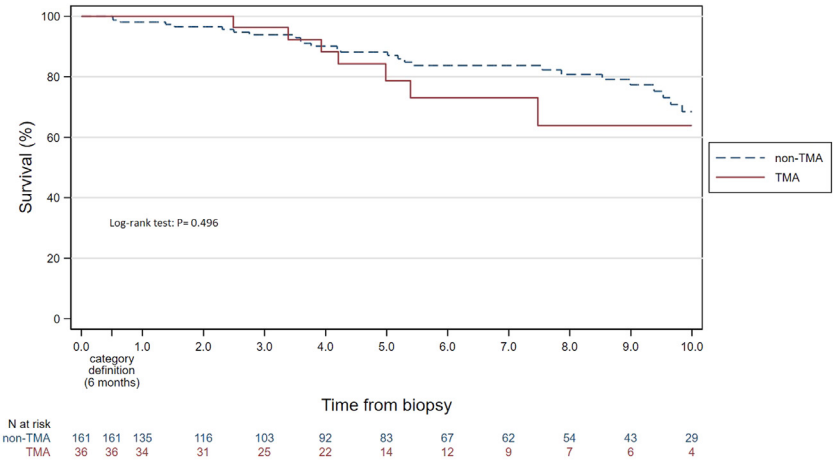

Figure 4. Event-free survival in TMA and non-TMA categories. Time at risk was measured from 6 months after kidney biopsy (the time at which patients were classified as having persistent isolated low C3 or not) until the composite outcome of ESKD or death (whichever came first). CI, confidence interval; HR, hazard ratio;TMA, thrombotic microangiopathy.

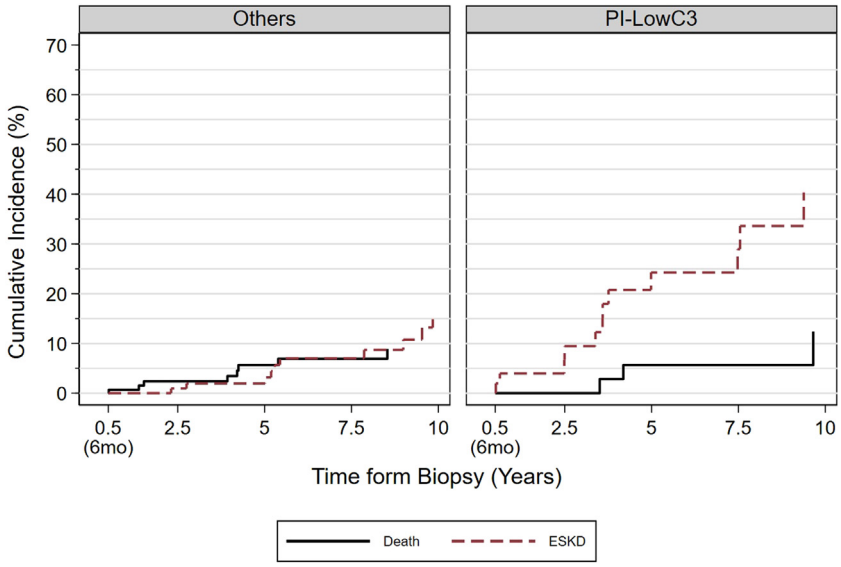

Figure 5. Cumulative incidence of ESKD and death in PI-LowC3 and the rest of the cohort, estimated by nonparametric competitive risk analysis. Time at risk was measured from 6 months after kidney biopsy (the time at which patients were classified as having persistent isolated low C3 or not) until ESKD or death. The difference in the incidence of ESKD was significantly different between PI-LowC3 and the rest of the cohort even after adjusting for potential confounding factors (age at biopsy, sex, black race, hypertension, use of mycophenolate mofetil, and use of cyclophosphamide) via competing-risk multiple regression ($P = 0.012$), whereas the difference in the incidence of death did not differ between groups ($P = 0.839$). ESKD, end-stage kidney disease; PI-LowC3, persistent isolated low C3.

DISCUSSION

Our retrospective long-term follow-up study, carried out in patients with a new histological diagnosis of proliferative LN, provides evidence that PI-LowC3, a low-cost and readily available biomarker, is strongly associated with an increased risk of ESKD.

The association of low serum C3 with LN is well known,[24] as well as the complex relationship between renal flares and transient decreases in serum C3 and/or C4 levels.[25,26] The association of the normalization of complement activity induced by immunosuppressive therapy with a favorable renal outcome has long been established,[27] and likewise that of low serum C3 any time in the course of disease with ESKD.[28] These findings represent important clinical observations, but they do not help to single out the individual patient at high risk of developing ESKD. Moreover, low serum C3 in these studies was considered independently of fluctuations in serum C4 levels, thus grouping together patients who had isolated C3 hypocomplementemia with patients who had concurrent C3 and C4 hypocomplementemia, thereby, including all nonresponders/treatment failures, and strongly limiting the clinical effect of such findings. Curiously, our study is the first to assess the prevalence of PI-LowC3 among proliferative LN patients.

We showed that early in the course of disease, PI-LowC3 identifies a specific group carrying the highest risk of progression to ESKD despite best practice. PI-LowC3 accounts for as much as 54% of all patients who progressed to ESKD in this large, multiethnic retrospective cohort. The immediate effect of this observation on clinical practice is that early in the course of LN, upon completion of a standard remission induction treatment regimen, the clinician can identify a large part of those patients who will fare worse, and thus benefit from more aggressive or different treatment, more intense follow up, and timely ESKD care.

In our analyses, the following observations were made: (i) eGFR current value (i.e., eGFR at the last outpatient visit), as opposed to eGFR slope, was associated with clinical outcomes, (ii) PI-LowC3 was not associated with a decrease in eGFR slope in the long term, but still (iii) PI-LowC3 was associated with an increased risk of ESKD (after accounting for the competing risk of death). Taken together, our findings provide evidence that in patients treated for proliferative LN, ESKD might develop as the result of a sudden nephron loss during renal flares which might be associated with or related to to ACP overactivation or dysregulation (as in PI-LowC3 patients) rather than chronic kidney disease progression.

Currently, clinicians caring for lupus patients can rely on very few predictors of unfavorable long-term renal outcome, namely ethnicity, with Blacks and Hispanics faring worse than others; sex, with males faring worse than females; age at onset or SLE duration, with younger age and/or longer disease duration as risk factors; and baseline or persistent hypertension.[29,30] Other

Table 2. Sensitivity analysis on confounders included in the model

	1	2	3	4	5	6	7
PI-LowC3	4.01[b]	3.81[b]	3.81[b]	3.72[b]	3.41[a]	3.46[b]	3.91[b]
	[1.74, 9.22]	[1.61, 9.03]	[1.61, 9.03]	[1.56, 8.88]	[1.31, 8.88]	[1.36, 8.81]	[1.47, 10.37]
Age, yrs		0.98	0.98	0.98	0.98	0.98	0.99
		[0.94, 1.02]	[0.94, 1.02]	[0.94, 1.02]	[0.94, 1.02]	[0.94, 1.02]	[0.95, 1.04]
Male sex		1.09	1.09	1.11	1.13	1.07	1.20
		[0.45, 2.65]	[0.45, 2.65]	[0.46, 2.73]	[0.47, 2.72]	[0.43, 2.68]	[0.46, 3.12]
Black ethnicity		1.10	1.10	1.03	1.13	1.16	0.92
		[0.43, 2.85]	[0.43, 2.85]	[0.37, 2.87]	[0.41, 3.15]	[0.41, 3.30]	[0.25, 3.43]
Hypertension				1.46	1.52	1.50	2.21
				[0.60, 3.59]	[0.59, 3.93]	[0.57, 3.91]	[0.71, 6.93]
Cyclophosphamide					0.76	0.77	0.74
					[0.22, 2.58]	[0.22, 2.67]	[0.19, 2.80]
Mycophenolate					0.52	0.50	0.54
					[0.18, 1.50]	[0.16, 1.55]	[0.15, 1.93]
TMA						0.78	0.66
						[0.22, 2.70]	[0.15, 2.96]
AI score							0.98
							[0.83, 1.17]
CI score							1.27[a]
							[1.06, 1.52]
Number of observations	197	197	197	197	197	197	188

AI, NIH activity index; CI, NIH chronicity index; PI-LowC3, persistent isolated low C3; TMA, thrombotic microangiopathy.
[a]$P < 0.05$.
[b]$P < 0.01$.
The table reports hazard ratio of ESKD and [95% confidence intervals] from 7 different competing risk multiple regression models. Each column represents a different multiple regression model (identified by a number from 1 to 7).

parameters, including histological indices such as the NIH activity index, or laboratory data such as thrombocytopenia or anemia, have not proven consistent in terms of predictive value across different studies.[30,31] Reducing proteinuria to <0.7−0.8 g/24h at 12 months has been considered an early predictor of good long-term renal outcome in LN in several pharmacological clinical trials. However, this finding is counterbalanced by the observation that persistent proteinuria may not always indicate ongoing SLE disease activity but can result from irreversible kidney scarring despite resolution of inflammation, thus limiting the value of low-level proteinuria in clinical practice.[32] One might also question the "earliness" of a 12-month biomarker, considering that most patients who progress to ESKD do so in the first 5 years after renal disease onset,[2] and that initial undertreatment is one of the key mistakes to avoid in caring for LN patients.[33]

On the other hand, baseline eGFR and NIH chronicity index (which mostly reflects interstitial fibrosis and tubular atrophy, and therefore tubulointerstitial scarring) at baseline biopsies do consistently predict worse long-term renal outcome, but this is somewhat expected, because they both reflect an already-accrued damage, similarly to prior SLE duration,[34] another predictor of unfavorable outcome. They are important signals, which, however, merely tell the clinician that disease processes have been working for some time and a nonreversible damage has already established.

PI-LowC3, rather than signaling an already accrued damage or a nonmodifiable condition, identifies patients at the highest risk of renal death. The observation that an indirect marker of selective ACP overactivation (PI-LowC3) is associated with worse renal outcome and that this holds true upon adjusting for several confounders, the most important being treatment choices, raises the question of whether this group represents a LN subphenotype with distinct pathophysiology. One postulated mechanism is that dysregulated innate immunity, initially triggered by immune complexes, relentlessly elicits injury, leading to irreversible inflammatory damage despite adequate immunosuppression and the waning of initial activation signals.[35] We hypothesized that PI-LowC3 in LN is the expression of chronic ACP overactivation. The mechanisms leading to ACP overactivation in LN are still unclear, but several studies have offered intriguing clues. Recently, a few studies assessed the relative role of anti-complement antibodies in LN: Birmingham *et al*.[36] showed that C3 levels predicted renal flares in those patients who carried anti-C3b antibodies, the presence of which has been documented in 30% to 36% of LN patients; Vasilev *et al*.[13] elegantly showed how these antibodies might work, that is, by inhibiting the interactions between factor H and complement receptor 1 with C3b, thus leading to unrestricted ACP activation, in a similar fashion to the so-called C3 nephritic factor. A similar mechanism has been well elucidated in post-infectious glomerulonephritis, where more than 90% of

patients carry anti-factor B antibodies leading to an unrestricted ACP activity via increased C3 convertase activity.[37]

Our findings are partly in contrast with previous studies that identified an association between worse renal prognosis and TMA in LN,[14,38,39] because we did not find that TMA significantly predicted ESKD by itself, despite the independent correlation between PI-LowC3 and TMA.

This study has limitations. Due to its retrospective fashion, as for study protocol we excluded a significant number of patients because they did not have 2 complement detections in the first 6 months after renal biopsy. Moreover, ESKD due to renal flares resulting in hospitalization could not be captured by our dataset that is based on outpatient visits. Furthermore, the time interval we chose to define PI-LowC3 was arbitrary, although it reflected the standard duration of induction-remission regimens, and we could not collect relevant data such as follow-up 24-hour proteinuria. Future prospective studies might be designed to verify whether a definition of PI-LowC3 relying on a shorter time interval (e.g., 3 months) is more appropriate or, even better, to use more unbiased, but less readily available in clinical practice, biomarkers of selective ACP overactivation, such as complement fraction Bb.[35] The sooner in their disease history these patients are identified, the sooner the therapeutic adjustments.

Despite these limitations, we identified an affordable, reliable, and easily reproducible time-dependent marker which predicts ESKD early in the course of proliferative LN. This is arguably the first reliable early predictor of ESKD in LN identified in a large multiethnic cohort. The association between PI-LowC3 and TMA strongly suggests a causal link between ACP overactivation and renal damage, warranting on the one hand the exploration of the peculiar pathophysiology at play in this high-risk subgroup and on the other hand, an immediate solid rationale for future trials involving complement blockade.

DISCLOSURE

GMR has received funding from the Gary Hill award. He has no conflict of interest to declare. LM serves on a GSK and a Vifor advisory board. All the other Authors have no funding or conflict of interest to declare.

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SUPPLEMENTARY MATERIAL

Supplementary File (PDF)

Supplementary methods and output of the full main joint model with the covariate coefficients.

Figure S1. Scatter plot of eGFR values in patients with PI-LowC3 (red circles) and in Others patients (blue circles).

STROBE Statement.

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