

Serum Albumin at Partial Remission Predicts Outcomes in Membranous Nephropathy



Taewoo Lee^{1,2}, Yunro Chung^{3,4}, Caroline J. Poulton¹, Vimal K. Derebail¹, Susan L. Hogan¹, Heather N. Reich⁵, Ronald J. Falk¹ and Patrick H. Nachman^{1,6}

¹UNC Kidney Center, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina, USA; ²Department of Medicine, Division of Nephrology, St. Louis University, St. Louis, Missouri, USA; ³College of Health Solutions, Arizona State University, Phoenix, Arizona, USA; ⁴Virginia G. Piper Center for Personalized Diagnostics, Biodesign Institute, Arizona State University, Tempe, Arizona, USA; ⁵Department of Medicine, Division of Nephrology, University Health Network, University of Toronto, Toronto, Ontario, Canada; and ⁶Division of Renal Diseases and Hypertension, University of Minnesota, Minneapolis, Minnesota, USA

Background: In primary membranous nephropathy (MN), partial remission (PR) ($\geq 50\%$ reduction of proteinuria to < 3.5 g/d) is associated with a greater risk of relapse and end-stage kidney disease (ESKD) compared with complete remission (CR). We aimed to determine factors associated with relapse or renal failure in patients who attain the standard definition of PR.

Methods: We captured PR, CR, relapse, and the composite of doubling of serum creatinine or ESKD in a cohort of 267 patients with MN, nephrotic syndrome, and > 12 months of follow-up. Characteristics at the time of PR associated with the composite outcome or relapse were evaluated using a time-to-event analysis.

Results: A total of 192 patients attained PR and 86 attained CR. Serum albumin at PR (hazard ratio [HR]: 1.58 per 0.5 g/dl decrease from 4.0 g/dl; 95% confidence interval [CI]: 1.03–2.43) and duration of nephrotic proteinuria (HR: 1.01 per month increase; 95% CI: 1.00–1.03) were independent risk factors for the composite endpoint. Serum albumin at PR was associated with an increased risk of relapse (HR: 1.58 per 0.5 g/dl decrease below 4.0 g/dl; 95% CI: 1.24–2.01). A cutoff for serum albumin ≤ 3.5 g/dl at PR performed best in predicting relapse and composite outcome.

Conclusions: Patients with serum albumin > 3.5 g/dl at PR have decreased risk of composite outcome or relapse compared with PR with low albumin. A definition of PR that includes normalization of serum albumin may be a more robust surrogate endpoint in MN than the traditional definition of PR.

Kidney Int Rep (2020) 5, 706–717; <https://doi.org/10.1016/j.ekir.2020.02.1030>

KEYWORDS: albuminemia; membranous nephropathy; nephrotic syndrome; proteinuria; remission; surrogate endpoint

© 2020 International Society of Nephrology. Published by Elsevier Inc. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

Patients with primary MN typically present with nephrotic syndrome and preserved renal function.^{1,2} The severity and duration of proteinuria are associated with increased risk of loss of renal function.^{1,3–5} The reduction of proteinuria has been considered a reasonable therapeutic goal and has been used as a primary outcome measure in clinical trials.^{6–11} CR, defined as attaining a near-normal protein excretion (< 0.3 g/d) with preservation of glomerular

filtration rate (GFR), reflects a clinical absence of disease activity and is associated with a low risk of relapse and excellent long-term patient and renal survival. As such, CR is considered suitable for use as a surrogate endpoint in clinical trials for drug licensing.¹² PR, defined as attaining protein excretion < 3.5 g/d and relative reduction $\geq 50\%$ with preserved GFR, is also associated with a reduction in the risk of progressive loss of GFR or ESKD compared with patients with no remission (NR).¹³ However, it is difficult to determine when the residual proteinuria characteristic of PR is due to persistent disease activity or is a result of kidney scarring. PR is also associated with a significantly higher rate of relapse than CR. For these reasons, PR is considered a reasonably likely surrogate endpoint for use in clinical trials for drug licensing.¹² In this study,

Correspondence: Patrick H. Nachman, Division of Nephrology and Hypertension, University of Minnesota, 717 Delaware Street SE, Minneapolis, Minnesota 55414, USA. E-mail: pnachman@umn.edu

Received 14 September 2019; revised 11 February 2020; accepted 24 February 2020; published online 4 March 2020

we investigated the clinical course and outcome in patients who achieved PR. Based on the retrospective analysis of a large inception cohort of patients with primary MN from the Glomerular Disease Collaborative Network of the University of North Carolina,² we evaluated which clinical parameters at the time of PR are associated with (i) progressive renal dysfunction and (ii) relapse. We also evaluated whether incorporating serum albumin measures can improve the value of PR in predicting renal outcomes.

METHODS

Data Collection and Study Cohort Development

Data were collected from the previously described primary MN inception cohort of the Glomerular Disease Collaborative Network of the University of North Carolina, which involves more than 300 participating clinics and academic sites from predominantly the southeastern United States.² We identified 466 patients with primary MN who underwent a renal biopsy between 1977 and 2012 and were followed until March of 2014. Clinical and laboratory test result data were prospectively accrued from patients' records of each clinic visit. For inclusion in this study, patients must have had primary MN with nephrotic range proteinuria at presentation (>3.5 g/d); at least 12 months of follow-up; and sufficient information on treatments, laboratory values, and clinical course to assess remission, relapse, and long-term renal and patient outcomes.

Study Design

We retrospectively investigated the clinical course of patients from the time of kidney biopsy to the last follow-up date or to ESKD or death. We captured the time to PR, CR, relapse, and ESKD. We also evaluated time to a composite renal outcome, defined as doubling of serum creatinine from baseline, an estimated GFR (eGFR) less than 15 ml/min per 1.73 m², or initiation of renal replacement therapy. Relapse was defined as the reemergence of nephrotic range proteinuria after CR or PR. Patients who attained at least a PR were categorized in the *Remission* group. Patients who attained a CR (after a PR) were categorized in the CR group. Patients who remained in PR until the last follow-up were categorized in the PR group. (The Remission group encompassed the PR + CR groups.) We used the definitions of CR (attaining proteinuria <0.3 g/d with a stable eGFR), PR ($\geq 50\%$ reduction in proteinuria to <3.5 g/d with a stable eGFR), and relapse (increase in proteinuria to >3.5 g/d after reaching CR or PR) described in the 2012 Kidney Disease: Improving Global Outcomes guidelines.^{12,14} A stable eGFR was defined as <25 ml/min per 1.73 m² decline from

baseline. Two consecutive measurements of proteinuria and serum creatinine were required for each event determination, and the first date meeting the defining criteria was used as the time point of the event. Chronic kidney disease (CKD) stage was categorized according to the National Kidney Foundation Kidney Disease Outcomes Quality Initiative guidelines.¹⁵

For the analysis simulating a clinical trial using normal albumin PR (NAPR) at 18 months (NAPR₁₈) as the endpoint, patients were categorized as NAPR₁₈ group if they were in PR with serum albumin >3.5 g/dl at that time point after kidney biopsy, whereas those in PR but with serum albumin ≤ 3.5 g/dl at 18 months were categorized in the low albumin PR (LAPR) at 18 months (LAPR₁₈) group. The LAPR₁₈ and NAPR₁₈ groups are distinct from the LAPR and NAPR groups, respectively, because serum albumin could have improved from the time of first PR to the 18-month time point.

We evaluated time to first event for the composite renal endpoint and ESKD with start date defined as the first kidney biopsy date. Time to relapse was calculated from the time a patient first attained PR. Models for each outcome controlled for influences of clinical characteristics at the time of biopsy, at the time of PR, and treatment during follow-up. Immunosuppressive therapy used was categorized in 3 groups: no treatment, treatment with glucocorticoids only, and dual immunosuppression with either cyclophosphamide or a calcineurin inhibitor in addition to glucocorticoids. Treatment categories were used as an intention to treat in all analyses.

Statistical Analysis

Continuous variables were described as mean \pm SD for normal distributions or median with interquartile range (IQR) for skewed distributions. Categorical variables were described as percentages. Baseline characteristics for relapse and no-relapse groups were compared using Student's *t* test for normally distributed variables, Wilcoxon-rank sum test for variables with skewed distributions, and χ^2 test for categorical variables. Cumulative incidence rates of the composite renal endpoint and relapse were calculated and plotted using Kaplan-Meier analysis. The comparison of incidence rates of outcomes between PR and other remission groups were performed with a log-rank test.

Cox proportional hazard models were used to assess risk factors of outcomes. Age at biopsy was converted to an ordinal categorical variable divided by quartiles, and race was evaluated as a binary variable (white vs. nonwhite). For renal disease parameters, we used CKD stages as ordinal categories, gram increase of proteinuria, and 0.5 g/dl decrease of serum albumin level from

4.0 g/dl. HRs reaching a statistically predictive value at the $P < 0.1$ level of significance in univariate analyses were used in multivariable modeling for each outcome. Multivariable models also included clinically relevant covariates.¹⁴ Backward elimination was conducted for the final risk prediction model with variable selection threshold as an α -level of 0.05. Stata version 14.2 (Stata Corp, College Station, TX) was used for statistical analyses and graphing. The proportionality assumption of our models was evaluated by examination of Schoenfeld residuals. A receiver operating characteristic (ROC) analysis was used to determine the best cutoff value of serum albumin level for the prediction of relapse of nephrotic proteinuria or the combined outcome,^{16,17} using R package survival ROC (<https://CRAN.R-project.org/package=survivalROC>). Our ROC analysis was supplemented by examination of Net Classification Improvement at years 2, 3, and 4.

RESULTS

We identified 267 patients with primary MN, nephrotic range proteinuria at presentation (>3.5 g/d), and at least 12 months of follow-up who met inclusion criteria (Figure 1). Table 1 summarizes baseline characteristics, treatment, and outcomes for the cohort. At presentation, the median proteinuria was 8 g/d (IQR, 5–12 g/d)

Table 1. Baseline characteristics of entire study cohort ($N = 267$) of patients with MN

Variables	Median [IQR]
Demographics	
Age at biopsy, yr	52 [42–63]
Sex, male, %	61
Race, white/black/other, %	75/17/8
Laboratory findings	
Serum creatinine, mg/dl	1.1 [0.9–1.4]
Estimated GFR, ml/min per 1.73 m ²	72 [53–86]
Serum albumin, g/dl	2.4 [1.9–2.9]
Proteinuria, g/d	8 [5–12]
Immunosuppressant use,^a %	
None	20
Monotherapy	42
Combination therapy	38
ACEi/ARB	77
Follow-up, mo	34 [16–66]

ACEi, angiotensin-converting enzyme inhibitor; ARB, angiotensin receptor blocker; GFR, glomerular filtration rate; IQR, interquartile range; MN, membranous nephropathy.
^aMonotherapy: treatment with single immunosuppressive agent (including corticosteroids alone). Combination therapy includes combined corticosteroids in addition to cytotoxic agent or other immunosuppressant.

and the median serum albumin was 2.4 g/dl (IQR, 1.9–2.9 g/dl). Eighty percent of patients received immunosuppressive treatment. The combination of cyclophosphamide and corticosteroids was prescribed to 38% of patients (median duration of treatment 7.7

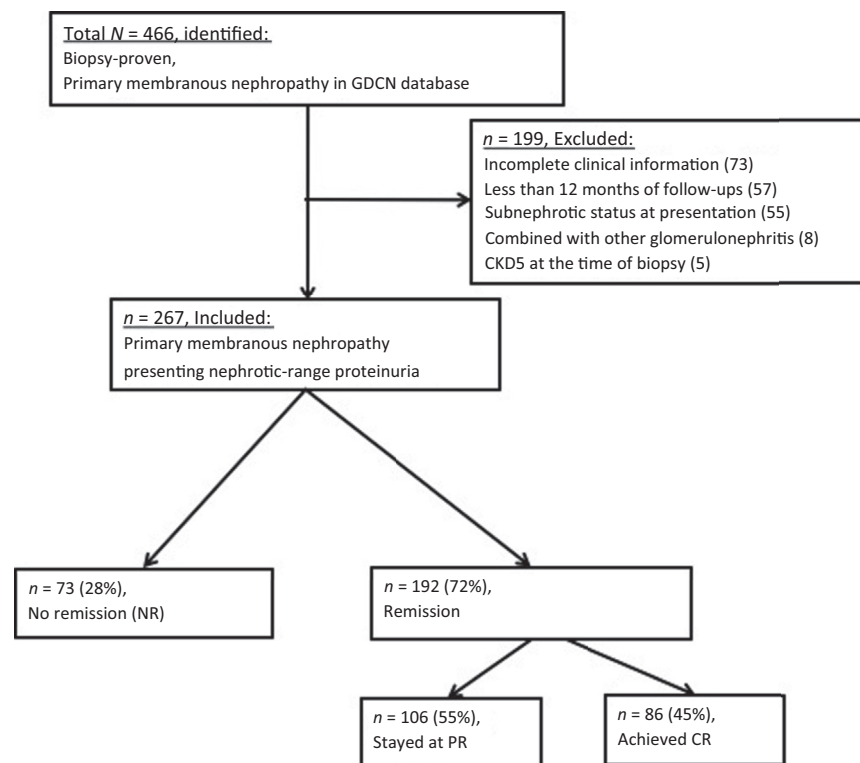


Figure 1. Study flow diagram of patient selection and outcomes observed. Of the 267 patients with primary membranous nephropathy who met inclusion criteria, 72% attained a remission. Of these, 45% subsequently attained a complete remission (CR) in a median of 10 months (interquartile range, 4–17 months), whereas the remaining 55% remained in partial remission (PR) until the last follow-up or had a relapse. CKD, chronic kidney disease; GDCN, Glomerular Disease Collaborative Network.

months [IQR, 5.2–12.2 months]). Calcineurin inhibitors were prescribed to 19% of patients (median duration of treatment 9.9 months [IQR, 4.9–19.4 months]). Renin-angiotensin system blockers were used by 77% of patients. Among patients treated with immunosuppressive therapy, 78% initiated treatment within 3 months of biopsy, 6% between 3 and 6 months after biopsy, and 16% after 6 months.

During a median observation period of 34 months (IQR, 19–66 months), 28% of the entire cohort (75 of 267) either had no remission or progressed to the composite

renal endpoint. Seventy-two percent of patients (192 of 267) achieved PR or CR (*Remission* group). Twenty percent of patients who entered into remission did so without immunosuppressive therapy. The median time from kidney biopsy to PR was 7 months (IQR, 3–13 months). Among those who achieved a remission, 45% (86 of 192) subsequently attained a CR in a median of 10 months (IQR, 4–17 months), whereas the remaining 55% remained in PR until the last follow-up or had a relapse. Only 2 patients who attained a remission had a doubling of serum creatinine without a relapse.

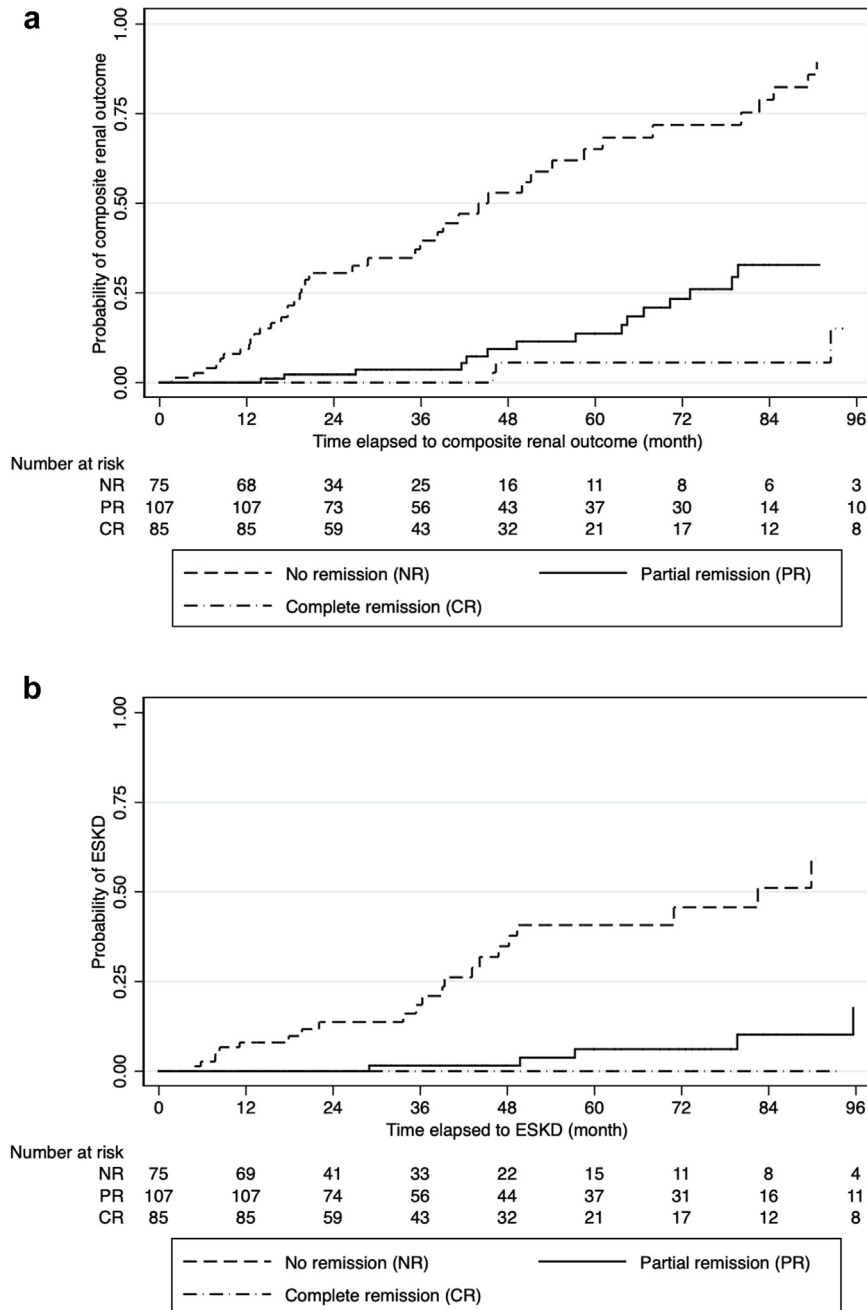


Figure 2. Kaplan-Meier curves of the composite renal endpoint (a) and end-stage kidney disease (ESKD) (b) of the 3 remission groups (no remission [NR], partial remission [PR] and complete remission [CR]). There was statistically significant difference in the cumulative probability of reaching the composite renal endpoint among the 3 groups (log-rank test, $P < 0.05$).

Risk of Composite Renal Endpoint

Sixty-two patients (23% of the entire cohort) reached the composite renal endpoint (doubling of serum creatinine, eGFR <15 ml/min per 1.73 m², or initiation of renal replacement therapy), 27 (10%) of whom progressed to ESKD. Figure 2 illustrates Kaplan-Meier curves for the composite endpoint (Figure 2a) and ESKD (Figure 2b) for each remission group. Over 5 years of follow-up, the cumulative probability of reaching the composite renal endpoint was 65% for NR, 12% for PR, and 5.5% for the CR groups, respectively (log-rank test $P < .05$).

Among the 192 patients who achieved a remission, characteristics at baseline and at the time of PR were evaluated for their association with renal outcomes (Tables 2 and 3¹⁴). In the univariable model, greater proteinuria at baseline, time exposed to nephrotic range proteinuria, and lower serum albumin level at PR were significantly associated with reaching the composite renal endpoint. In a multivariable model, serum albumin at PR (HR, 1.58 per 0.5 g/dl decrease in serum albumin from 4.0 g/dl; 95% CI, 1.03–2.43) and duration of nephrotic range proteinuria (HR, 1.01 per month increase; 95% CI, 1.00–1.03) were independent risk factors of reaching the composite renal endpoint. Examination of the Schoenfeld residuals of this model

Table 2. Comparison of clinical characteristics by the composite renal endpoint among patients with MN who achieved remission

Variables ^a	Total	Reached composite renal endpoint		P value
		No	Yes	
<i>n</i>	192	170	22	
Age at biopsy, yr	52 [42–63]	53 [42–63]	50 [39–59]	0.31
Sex, male, %	59	59	59	0.98
Race, white/black/others, %	78/14/8	80/11/9	60/39/1	0.02
eGFR, ml/min per 1.73 m ²	71.6 [53.1–86.2]	73.5 [56–88]	76 [50–99]	0.26
CKD stage ^b 4/3b/3a/2/1, %	5/8/18/46/23	6/8/17/47/22	0/9/18/41/32	0.67
Proteinuria, g/d	8 [5–12]	7.4 [4.8–11.1]	6.2 [4–10.6]	0.46
Serum albumin, g/dl	2.54 ± 0.66	2.53 ± 0.66	2.64 ± 0.65	0.57
Serum albumin at PR, g/dl	3.36 ± 0.54	3.39 ± 0.52	3.18 ± 0.67	0.07
Time in no remission, mo	9.3 [4.2–20.2]	7.8 [3.8–16.2]	40.9 [17.2–50.4]	<0.01
ACEi/ARB, %	79	79	84	0.44
Immunosuppression ^c , %				0.08
No immunosuppression	24	25	9	
Monotherapy	39	41	32	
Combination therapy	37	35	59	

ACEi, angiotensin-converting enzyme inhibitor; ARB, angiotensin receptor blocker; CKD, chronic kidney disease; eGFR, estimated glomerular filtration rate; MN, membranous nephropathy; PR, partial remission.

^aContinuous variables present with median value [interquartile range].

^bCKD stage is based on 2012 Kidney Disease: Improving Global Outcomes guideline.¹⁴

^cMonotherapy: treatment with single immunosuppressive agent (including corticosteroids alone). Combination therapy includes combined corticosteroids in addition to cytotoxic agents or other immunosuppressant.

Laboratory test results are presented at baseline (time of biopsy) unless otherwise indicated.

Table 3. Risk factors of the composite renal outcome among patients with MN who achieved remission

Variables (reference)	P value	Hazard ratio (95% CI)
Univariable model ^a		
Older age, per quartile increase (age <42 yr)	0.89	0.97 (0.67–1.41)
Sex, male (female)	0.42	0.71 (0.30–1.66)
Race, nonwhite (white)	0.17	1.57 (0.82–3.00)
Baseline proteinuria, per gram increase	0.04	1.04 (1.01–1.09)
Proteinuria at remission, per gram increase	0.96	0.99 (0.66–1.49)
CKD stage ^b , per higher stage (CKD stage 1)	0.12	0.76 (0.50–1.18)
Baseline serum albumin, per 0.5 mg/dl decrease (>4.0)	0.99	1.00 (0.69–1.44)
Serum albumin at PR, per 0.5 mg/dl decrease (>4.0)	0.04	1.52 (1.00–1.03)
Time in no-remission, per mo increase	0.03	1.01 (1.01–1.03)
Combination therapy ^c (none or monotherapy)	0.07	1.41 (0.98–2.01)
Multivariable model ^a		
Serum albumin at PR, per 0.5 mg/dl decrease (>4.0)	0.038	1.58 (1.03–2.43)
Time in no-remission, per mo increase	0.028	1.01 (1.00–1.03)

CI, confidence interval; CKD, chronic kidney disease; MN, membranous nephropathy; PR, partial remission.

^aCox proportional hazard model: variables with $P < 0.1$ in univariable models (baseline proteinuria, serum albumin at PR, time in no-remission, combination immunosuppressants) and clinically relevant (race and CKD stage) were adjusted in the multivariable model. Backward elimination was used to develop the final model, with variables retained if $\alpha \leq 0.05$.

^bCKD stage is based on 2012 Kidney Disease: Improving Global Outcomes guideline.¹⁴

^cMonotherapy: treatment with single immunosuppressive agent (including corticosteroids alone). Combination therapy includes combined corticosteroids in addition to cytotoxic agents or other immunosuppressant.

Composite renal outcome is defined as doubling of serum creatinine from baseline, an estimated glomerular filtration rate less than 15 ml/min per 1.73 m², or initiation of renal replacement therapy.

upheld the proportionality assumption (Supplementary Figure S1).

Risk of Relapse

Sixty of 192 patients (31%) who reached a remission experienced a relapse, with a median time to relapse of 41 months (IQR, 10–110 months). In the 5 years after PR, the cumulative probability of relapse was 65% for patients who remained in PR, but only 25% for patients in CR (Figure 3). Compared with patients who did not relapse, those who did were significantly younger (median age, 46 years [IQR, 40–58 years] vs. 56 years [IQR, 43–64 years], $P < 0.01$), had a lower serum albumin level at the time of PR (median, 3.2 g/dl [IQR, 3.0–3.7 g/dl] vs. 3.5 g/dl [IQR, 3.2–3.8 g/dl], $P < 0.01$), and were more likely to be on dual immunosuppressive therapy (50% vs. 32%, respectively) (Table 4). Table 5¹⁴ summarizes the result of time-to-relapse analysis using Cox proportional hazard models. Attaining CR was associated with lower risk of relapse compared with PR (HR, 0.44; 95% CI, 0.24–0.80; $P = 0.007$). A low serum albumin level at the time of PR was associated with an increased risk of relapse (HR, 1.58; 95% CI, 1.24–2.01; $P = 0.002$) for each 0.5 g/dl decrease in serum albumin level below 4.0 g/dl). Examination of the Schoenfeld residuals of this model upheld the proportionality assumption (Supplementary Figure S2).

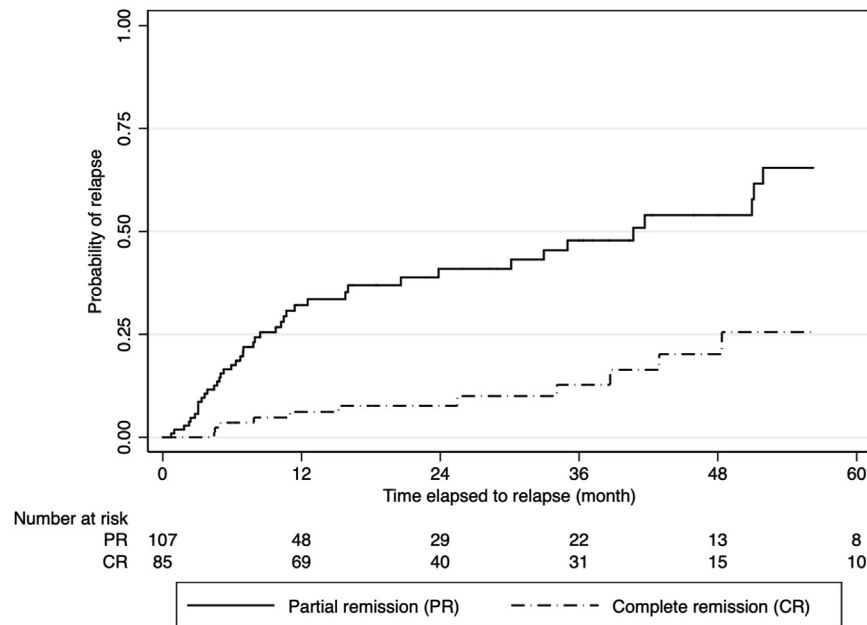


Figure 3. Kaplan-Meier curves depicting the cumulative probability of relapse of nephrotic range proteinuria for patients attaining a partial remission (PR) ($n = 107$) compared with complete remission (CR) ($n = 85$).

Risk of Relapse or Composite Renal Endpoint Based on Serum Albumin Level at the Time of PR

We sought to identify a cutoff serum albumin level at the time of PR that could be used clinically to assess the risk of relapse. In ROC analysis, we compared models with a binary categorical variable for serum albumin

levels at PR and ranged the cut point from 3.3 to 3.7 g/dl. A cutoff serum albumin concentration of ≤ 3.5 g/dl best delineated the predication of relapse (Figure 4, Supplementary Table S1). Using this cutoff level, we categorized patients in PR into 2 groups: NAPR, with serum albumin level >3.5 g/dl, and LAPR, with

Table 4. Comparison of clinical characteristics between patients who did and those who did not relapse after partial remission

Variables ^a	Total	Relapse	No relapse	P value
<i>n</i>	192	60	132	
Age at biopsy, yr	52 [42–63]	46 [40–58]	56 [43–64]	<0.01
Sex, male, %	59	68	55	0.08
Race, white/black/others, %	78/14/8	75/17/8	79/13/8	0.73
eGFR, ml/min per 1.73 m ²	71.6 [53.1–86.2]	71.6 [53.1–86.2]	78.6 [57.7–94.5]	0.11
CKD stage ^b 4/3b/3a/2/1, %	5/8/18/46/23	3/5/20/44/28	6/10/16/48/20	0.52
Proteinuria, g/d	8 [5–12]	7.8 [5.9–11.6]	7.1 [4.5–10.9]	0.28
Serum albumin, g/dl	2.4 [1.9–2.9]	2.5 [2.0–2.8]	2.6 [2.1–3.0]	0.28
Serum albumin at PR, g/dl	3.4 [3.0–3.7]	3.2 [3.0–3.7]	3.5 [3.2–3.8]	<0.01
Time from baseline to PR, mo	6.9 [3.2–13.4]	7.7 [2.7–11.4]	6.7 [3.6–14.0]	0.94
ACEi/ARB, %	79	83	77	0.15
Immunosuppression, ^c %				0.03
No immunosuppression	24	13	27	
Monotherapy	39	37	41	
Combination therapy	37	50	32	

ACEi, angiotensin-converting enzyme inhibitor; ARB, angiotensin receptor blocker; CKD, chronic kidney disease; eGFR, estimated glomerular filtration rate; PR, partial remission.
^aContinuous variables present with median value [interquartile range].
^bCKD stage is based on 2012 Kidney Disease: Improving Global Outcomes guideline.¹⁴
^cMonotherapy: treatment with single immunosuppressive agent (including corticosteroids alone). Combination therapy includes combined corticosteroids in addition to cytotoxic agents or other immunosuppressant. Laboratory test results are presented at baseline (time of biopsy) unless otherwise indicated.

Table 5. Risk factors of relapse of nephrotic range proteinuria after partial remission

Variables (reference)	P value	Hazard ratio (95% CI)
Univariable model ^a		
Older age, per quartile increase (age <42 yr)	0.08	0.81 (0.64–1.03)
Sex, male (female)	0.21	1.43 (0.82–2.47)
Race, nonwhite (white)	0.35	1.25 (0.83–1.87)
Baseline proteinuria, per gram increase	0.42	1.02 (0.97–1.08)
Proteinuria at remission, per gram increase	0.21	1.18 (0.91–1.53)
CKD stage, ^b per higher stage (CKD stage 1)	0.06	0.77 (0.59–1.01)
Baseline serum albumin, per 0.5 mg/dl decrease (>4.0)	0.11	1.20 (0.96–1.51)
Serum albumin at PR, per 0.5 mg/dl decrease (>4.0)	<0.01	1.58 (1.26–1.98)
Complete remission before relapse	<0.01	0.32 (0.18–0.57)
Combination therapy ^c (none or monotherapy)	0.11	1.34 (0.94–1.92)
Multivariable model ^a		
Serum albumin at PR, per 0.5 mg/dl decrease (>4.0)	0.002	1.58 (1.24–2.01)
Complete remission before relapse	0.007	0.44 (0.24–0.80)

CI, confidence interval; CKD, chronic kidney disease; PR, partial remission.
^aCox proportional hazard model: variables with $P < 0.1$ from univariable models (age, CKD stage, and serum albumin at PR, and complete remission before relapse) and clinically relevant. (Baseline serum albumin and combination immunosuppressive therapy) were adjusted in the multivariable model. Backward elimination was employed to develop the final model, with variables retained if $\alpha \leq 0.05$.
^bCKD stage is based on 2012 Kidney Disease: Improving Global Outcomes guideline.¹⁴
^cMonotherapy: treatment with single immunosuppressive agent (including corticosteroids alone). Combination therapy includes combined corticosteroids in addition to cytotoxic agents or other immunosuppressant.

albumin ≤ 3.5 g/dl. Figure 5 compares the cumulative probability of the composite renal endpoint (Figure 5a) and relapse (Figure 5b) of the 3 remission groups (LAPR, NAPR, and CR). The risk of reaching the composite renal endpoint was statistically significantly higher among patients with LAPR compared with NAPR (log-rank test, $P = 0.049$), but was not significantly different between patients with NAPR and CR (log-rank test, $P = 0.128$). The risk of relapse was statistically significantly higher in patients with LAPR than NAPR, and in patients with NAPR than CR (log-rank test, $P < 0.001$; trend $P < 0.001$). Examination of the Net Classification Improvement at years 2, 3, and 4 was highest at a serum albumin cutoff of 3.5 g/dl, consistent with the findings of our ROC analysis (Supplementary Table S2, Supplementary Figure S3).

Simulation: Use of NAPR as the Endpoint in a Clinical Trial

We explored the feasibility of a clinical trial using NAPR at 18 months (NAPR₁₈) as the primary endpoint. We observed the relapse of nephrotic range proteinuria as an outcome in patients who achieved and remained in PR by the 18-month time point after kidney biopsy. Of the original cohort, 83 patients who achieved PR at 18 months and extended follow-up >24 months were included in the analysis (Table 6). The median follow-

up was 52.6 months (IQR, 37.7–79.9 months) from the time of biopsy. At the 18-month time point, 70 patients (84%) attained a serum albumin level >3.5 g/dl (NAPR₁₈); and 13 patients (16%) had a low serum albumin concentration ≤ 3.5 g/dl at 18 months (LAPR₁₈). The time to relapse of nephrotic range proteinuria between the NAPR₁₈ and LAPR₁₈ groups was significantly different (log-rank test, $P < 0.001$; Figure 6). In the LAPR₁₈ group, the median time to relapse was 25 months (IQR, 6–34 months) with an incidence of 4.3 relapse/100 patient-months. In contrast, among patients with NAPR₁₈, the median time to relapse was 68 months (IQR, 44–101 months) with an incidence of 0.6 relapse/100 patient-months.

DISCUSSION

Although associated with significant morbidity and mortality,^{2,18,19} the course of progressive loss of renal function in primary MN typically occurs over many years. Among patients who do not attain reduction of proteinuria to a subnephrotic range, the risk of ESKD is approximately 25% over 8 years and approximately 50% by 10 to 15 years.²⁰ This relatively slow rate of progression makes it necessary to use a surrogate endpoint to complete clinical trials for drug licensing within a realistic time frame. CR has previously been deemed suitable for use as a surrogate endpoint.¹² Unfortunately, with current therapies, only 20% to 30% of patients attain a CR within the relatively short time frame of most clinical trials (2 years).^{5–7,11,21,22}

The benefits of PR on patient and renal outcomes have been studied and well recognized.¹³ Nevertheless, as defined in the 2012 Kidney Disease: Improving Global Outcomes guidelines and elsewhere,^{6,8,13,14} PR is considered only a “reasonably likely surrogate endpoint,” in part because PR does not reflect the full disappearance of signs of disease.¹² In addition, although associated with improved outcome compared with NR, PR is associated with a relatively high relapse rate and a significantly greater risk of adverse renal and patient outcomes compared with CR.^{13,23} In the United States, a reasonably likely surrogate endpoint may be used as a surrogate endpoint under the accelerated pathway for drug licensing. However, such a trial may need to be followed by a postmarketing clinical trial to confirm the benefit of the treatment studied on hard endpoints.²⁴

There is no uniformly used definition of PR. The most common definition of PR rests on a 50% reduction of proteinuria and attaining a level of protein excretion <3.5 g/d per 1.73 m² body surface area.^{12,14} Other studies, including recent randomized and controlled trials, have used a definition that includes the improvement of serum albumin level.^{9,11,22}

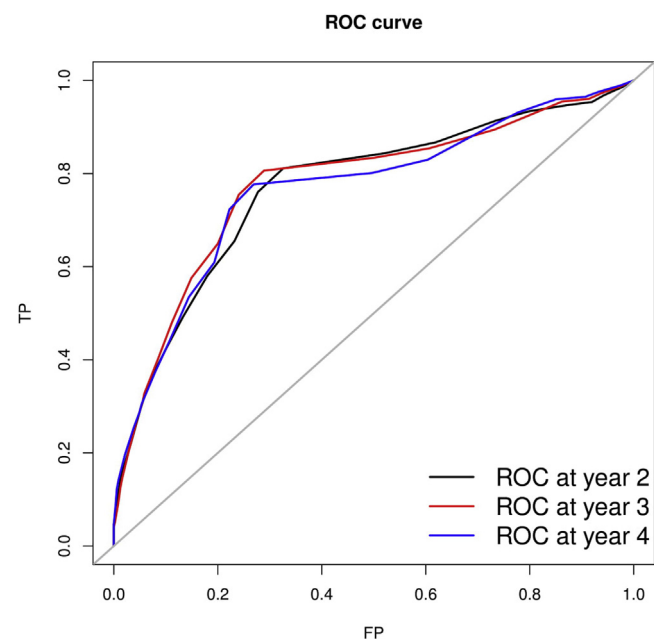


Figure 4. Receiver operating characteristic (ROC) curves were generated to determine the level of serum albumin at the time of partial remission (PR) that best predicted relapse of nephrotic proteinuria at 2 (black line), 3 (red line), and 4 years (blue line) after PR. The areas under the curve for the 2-, 3-, and 4-year curves were 0.77, 0.78, and 0.77, respectively. For each analysis, the optimal cut of level of serum albumin was 3.6 g/dl (Youden's indices tabulated in Supplementary Figure S1). The 3 curves show that the association of serum albumin level at PR and relapse is stable when time-to-event is varied from 2 to 4 years. FP, false positive; TP, true positive.

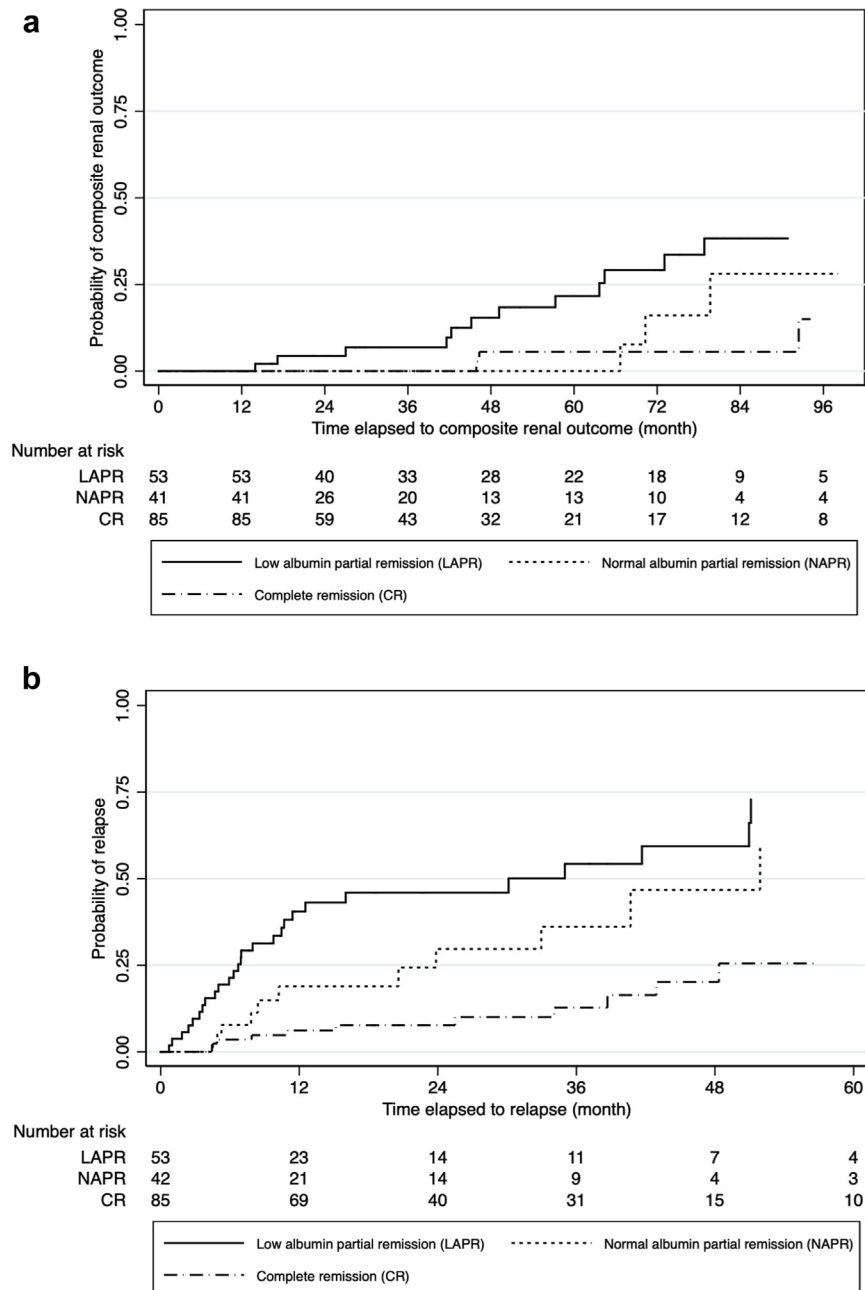


Figure 5. Kaplan-Meier curves of the composite renal endpoint (a) and relapse (b) of the 3 remission groups (low albumin partial remission [LAPR], normal albumin partial remission [NAPR], and complete remission [CR]). There was a statistically significant difference between LAPR (≤ 3.5 g/dl) and NAPR (> 3.5 g/dl) in attaining the composite renal endpoint (log-rank test, $P = 0.049$) but not between normal albumin complete remission and CR (log-rank test, $P = 0.128$). The risk of relapse was statistically significantly higher in patients with LAPR than NAPR, and in patients with NAPR than CR (log-rank, $P < 0.001$; trend $P < 0.001$).

We aimed to assess the outcomes of patients with MN who attain a PR and evaluate the risk factors of renal endpoints and of disease relapse focusing specifically on patients with PR. We specifically evaluated whether incorporating a measure of serum albumin concentration at the time of PR can improve its predictive value with respect to relapse or long-term renal outcome. If so, this modified definition of PR could be a more robust surrogate endpoint for use in clinical trials in primary MN.

Our results confirm the association of PR with improved renal outcomes and decreased risk of relapse compared with NR, as well as the association of a shorter duration of time in NR with improved renal prognosis, as previously reported.^{13,25} The latter finding raises the important question as to the optimal timing for initiating immunosuppressive therapy in patients with nephrotic range proteinuria. The 2012 Kidney Disease: Improving Global Outcomes Guidelines for Glomerulonephritis recommend

Table 6. Comparison of clinical characteristics between patients who attained a PR by 18 months after biopsy with persistently low serum albumin (LAPR₁₈) and those with normal serum albumin (NAPR₁₈) level

Variables ^a	Total	LAPR ₁₈ ^b	NAPR ₁₈
<i>n</i> (%)	83 (100)	13 (17)	70 (83)
Demographics			
Age at biopsy, yr	50.4 ± 14.2	49.3 ± 14.5	50.6 ± 14.2
Sex, male, %	62	69	61
Race, white/black/others, %	77/15/8	54/31/15	82/11/7
Follow-up, mo from biopsy			
Median [IQR]	52.6 [37.7–79.9]	44.7 [23.8–72.3]	58.9 [30.5–79.9]
At biopsy			
eGFR, ml/min per 1.73 m ²	74 [56–86]	76 [64–97]	73 [54–85]
Proteinuria, g/d	7.1 [4.2–11.7]	7.1 [6.0–11.0]	7.1 [4.0–11.7]
Serum albumin, g/dl	2.55 ± 0.69	2.33 ± 0.63	2.59 ± 0.71
At 18 mo			
Serum albumin, g/dl	3.79 ± 0.50	3.05 ± 0.46	3.93 ± 0.38
Immunosuppressant used by 18 mo, %			
No immunosuppression	17	8	22
Corticosteroids	52	46	50
Corticosteroids + cytotoxic agents	31	46	28

eGFR, estimated glomerular filtration rate; IQR, interquartile range; PR, partial remission.
^aData presented with mean ± SD for continuous variable with normal distribution or median [IQR] for continuous variable with skewed distribution or percentage for categorical.

^bLow-albumin group has serum albumin level ≤ 3.5 mg/dl at 18 mo from biopsy; hence, normal albumin group >3.5 mg/dl. All variables except serum albumin at 18 mo have no statistical difference between 2 albumin level groups.

initiating treatment with non-immunosuppressive anti-hypertensive and anti-proteinuric agents, and reserving immunotherapy for patients who fail to demonstrate significant improvement in proteinuria during an observation period of at least 6 months.¹⁴ If

the benefit-to-risk ratio of new immunosuppressive therapies¹¹ improves, the decision to delay such treatment will warrant reevaluation, especially considering that some serious complications of MN tend to occur early in the course of disease during periods of active nephrosis.^{2,18,26}

By multivariable analysis, we found that CR after PR, or higher serum albumin level at the time of PR, was independently associated with more favorable renal outcomes and a lower risk of relapse. By ROC analysis, we also found that attaining PR with a normal serum albumin concentration (>3.5 g/dl) is associated with a significantly lower risk of relapse and of reaching a doubling of serum creatinine or ESKD. Although the most optimal cutoff value of serum albumin should be confirmed in separate, prospective studies, our results suggest that incorporating a measure of serum albumin normalization into the definition of PR would significantly improve its predictive value for traditional clinically meaningful (*hard*) endpoints, such as doubling of serum creatinine and ESKD. This finding is supported by the results of the randomized controlled trial of rituximab or non-immunosuppressive anti-proteinuric therapy for severe MN, in which between-group differences in serum albumin preceded those in proteinuria.¹¹

A key question we explored was the feasibility of using of NAPR as a surrogate endpoint in a clinical trial. We chose 18 months as the time point to assess patient outcome as a reasonable duration of patient engagement in a trial, and to detect differences in

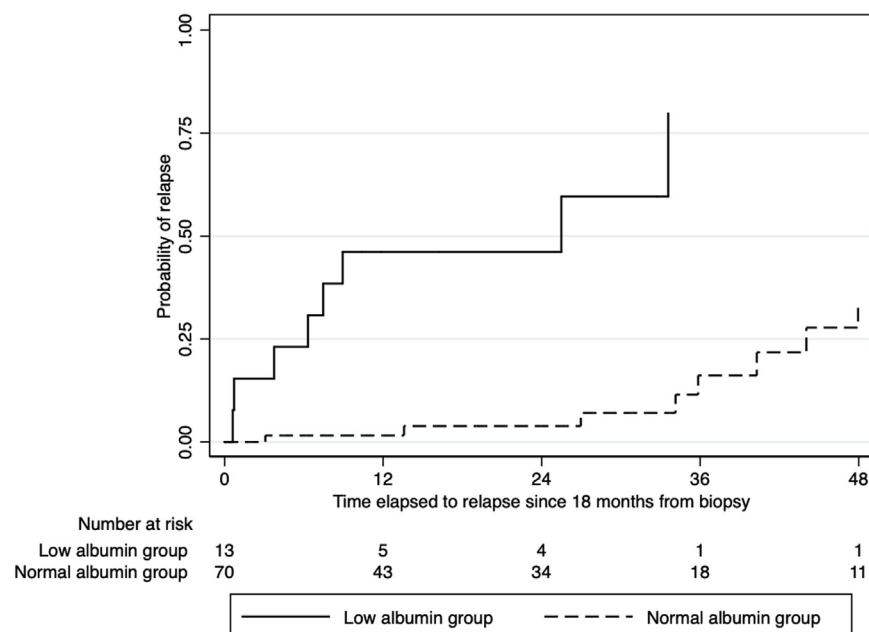


Figure 6. Kaplan-Meier curves of relapse of nephrotic proteinuria among patients who attained a normal albumin partial remission compared with low albumin partial remission at 18 months post biopsy (log-rank test, $P = 0.019$).

outcomes between treatment groups based on the results of previous clinical trials.^{7,8,11} Our results suggest that using NAPR as a surrogate endpoint at the 18-month time point would be associated with a significantly improved relapse-free renal survival and long-term composite renal endpoint compared with LAPR. Whether this would be sufficient to establish NAPR as a surrogate endpoint in a clinical trial for drug licensing would require further analysis. Ideally, such an analysis could be done in the setting of a prospective treatment protocol to demonstrate that a treatment effect on NAPR is predictive of the treatment effect on long-term *hard* endpoints, such as doubling of serum creatinine, ESKD, or death.

Our study has a number of limitations. Our results are based on a retrospective cohort study spanning 4 decades with incomplete and heterogeneously obtained data, as well as suspected heterogeneity in measuring serum albumin concentration. In common clinical practice, measuring serum albumin concentration can be performed using several methods and procedures that are associated with differences in results. Unfortunately, it was not possible to ascertain the methods used in our cohort of patients who were followed by multiple clinical practices over many years.²⁷ We therefore acknowledge that the presumed heterogeneity in testing methods may affect the accuracy of the optimal cutoff value for serum albumin based on the ROC curves, but not the association between a normalization of serum albumin concentration and the improved outcomes in MN. Our results warrant validation in a prospective study with uniformly measured serum albumin levels. Our results also reflect treatment patterns that have evolved over the past few decades, most notably the older use of corticosteroids alone and the more recent use of rituximab. Nevertheless, we suspect our results on the value of serum albumin as an additional marker of remission (when incorporated into the definition of PR) are relevant regardless of the treatments used considering the results of the multivariate analysis. Perhaps the most important limitation of our study is the lack of data on histologic detection of PLA2R or measurement of serum anti-PLA2R antibodies. Emerging data suggest prognostic value to anti-PLA2R in the follow-up and management of patients with MN. Studies have demonstrated that decrease in anti-PLA2R levels precedes clinical remission,^{28,29} and that most patients who attain remission of proteinuria also have marked reduction in anti-PLA2R levels.^{22,30,31} Limited data also suggest that reappearance of anti-PLA2R may precede a relapse and that persistence of anti-PLA2R before transplantation is associated with an increased risk of subsequent relapse.³² Nevertheless, the value of anti-PLA2R as a possible surrogate

endpoint in clinical trials has not been formally evaluated, and awaits future prospective studies. In addition, the prognostic value of anti-PLA2R levels do not apply to patients with anti-PLA2R-negative MN.

Whether the results of our analysis of serum albumin at the time of remission are generalizable to other proteinuric diseases, such as focal segmental glomerulosclerosis, warrants investigation. This is particularly pertinent in light of the recent analysis suggesting that a different definition of proteinuria reduction may be a better predictor of outcome in focal segmental glomerulosclerosis.³³

Our results indicate an added prognostic value of including serum albumin achieved in the definition of PR in MN. Whether NAPR is robust enough to be used as a surrogate endpoint in clinical trials of MN warrants formal prospective study. Future studies should consider other potential markers of active disease in MN, especially anti-PLA2R levels and anti-PLA2R target domains.^{28,31,33}

DISCLOSURE

In addition to their respective institutions, the authors disclose the following sources of funding:

National Institutes of Health (NIH): VKD, SLH, CJP, RJF, and PHN; NIH, NephCure Kidney International, and University of Michigan via the Nephrotic Syndrome Study Network Consortium (NEPTUNE): VKD, PHN, and HNR; Food and Drug Administration: VKD and PHN; Kidney Foundation of Canada and Canadian Institutes of Health Research Canadians Seeking Solutions and Innovations to Overcome Chronic Kidney Disease (Can-SOLVE) Network: HNR; RTI International: VKD; and honoraria from the American Society of Nephrology: PHN and HNR. HNR holds the University of Toronto Gabor Zellerman Chair in Nephrology Research. RJF is the Nan and Hugh Cullman Eminent Professor of Medicine.

Although none of the following are related to the work presented in this article, VKD is, or has been, a site primary investigator in clinical trials for Mallinckrodt, Gilead, Bristol-Myers Squibb, InflaRx, Chemocentryx, Otsuka, and Retrophin. PHN is or has been a site primary investigator in clinical trials for Aurinia, Chemocentryx, InflaRx, Omeros, and Otsuka. All the other authors declared no competing interests.

ACKNOWLEDGMENTS

This work was supported by the University of North Carolina Kidney Center.

AUTHOR CONTRIBUTIONS

TL, VKD, HNR, SLH, RJF, and PHN development the concept and reviewed and analyzed data. TL, YC, and VKD performed statistical analysis. CJP and TL carried out

database management, cohort identification, and primary data review. All authors prepared and reviewed the manuscript.

SUPPLEMENTARY MATERIAL

Supplementary File (Word)

Table S1. Summary of Youden's index derived from the ROC curves for the association of serum albumin levels at PR with relapse of nephrotic range proteinuria at years 2, 3, and 4 after PR (Figure 4).

Table S2. Net Reclassification Index for the ROC curves for the association of serum albumin levels at PR with relapse of nephrotic range proteinuria at years 2, 3, and 4 after PR (Figure 4).

Figure S1. Test for proportional hazards assumption analysis for Cox proportional hazard analysis of risk factors of the composite renal outcome among patients with MN who achieved remission (Table 3).

Figure S2. Test for proportional hazards assumption analysis for Cox proportional hazard analysis of risk factors of relapse of nephrotic range proteinuria after partial remission (Table 5).

REFERENCES

1. Pei Y, Cattran D, Greenwood C. Predicting chronic renal insufficiency in idiopathic membranous glomerulonephritis. *Kidney Int.* 1992;42:960–966.
2. Lionaki S, Derebail VK, Hogan SL, et al. Venous thromboembolism in patients with membranous nephropathy. *Clin J Am Soc Nephrol.* 2012;7:43–51.
3. Cattran DC, Pei Y, Greenwood CM, et al. Validation of a predictive model of idiopathic membranous nephropathy: its clinical and research implications. *Kidney Int.* 1997;51:901–907.
4. Hladunewich MA, Troyanov S, Calafati J, et al. The natural history of the non-nephrotic membranous nephropathy patient. *Clin J Am Soc Nephrol.* 2009;4:1417–1422.
5. Ponticelli C, Zucchelli P, Passerini P, et al. A 10-year follow-up of a randomized study with methylprednisolone and chlorambucil in membranous nephropathy. *Kidney Int.* 1995;48:1600–1604.
6. Ponticelli C, Passerini P, Salvadori M, et al. A randomized pilot trial comparing methylprednisolone plus a cytotoxic agent versus synthetic adrenocorticotropic hormone in idiopathic membranous nephropathy. *Am J Kidney Dis.* 2006;47:233–240.
7. Jha V, Ganguli A, Saha TK, et al. A randomized, controlled trial of steroids and cyclophosphamide in adults with nephrotic syndrome caused by idiopathic membranous nephropathy. *J Am Soc Nephrol.* 2007;18:1899–1904.
8. Praga M, Barrio V, Juárez GF, et al. Tacrolimus monotherapy in membranous nephropathy: a randomized controlled trial. *Kidney Int.* 2007;71:924–930.
9. Chan TM, Lin AW, Tang SC, et al. Prospective controlled study on mycophenolate mofetil and prednisolone in the treatment of membranous nephropathy with nephrotic syndrome. *Nephrol.* 2007;12:576–581.
10. Ramachandran R, Hn HK, Kumar V, et al. Tacrolimus combined with corticosteroids versus Modified Ponticelli regimen in treatment of idiopathic membranous nephropathy: a randomized control trial. *Nephrol.* 2016;21:139–146.
11. Dahan K, Debiec H, Plaisier E, et al. Rituximab for severe membranous nephropathy: a 6-month trial with extended follow-up. *J Am Soc Nephrol.* 2017;28:348–358.
12. Thompson A, Cattran DC, Blank M, et al. Complete and partial remission as surrogate end points in membranous nephropathy. *J Am Soc Nephrol.* 2015;26:2930–2937.
13. Troyanov S, Wall CA, Miller JA, et al. Idiopathic membranous nephropathy: definition and relevance of a partial remission. *Kidney Int.* 2004;66:1199–1205.
14. Kidney Disease: Improving Global Outcomes (KDIGO) Glomerulonephritis Work Group. KDIGO clinical practice guideline for glomerulonephritis. *Kidney Int Suppl.* 2012;2:139–274.
15. National Kidney Foundation. K/DOQI clinical practice guidelines for chronic kidney disease: evaluation, classification, and stratification. *Am J Kidney Dis.* 2002;39(suppl 2):S1–S266.
16. Heagerty PJ, Lumley T, Pepe MS. Time-dependent ROC curves for censored survival data and a diagnostic marker. *Biometrics.* 2000;56:337–344.
17. Saha-Chaudhuri P, Heagerty PJ. Non-parametric estimation of a time-dependent predictive accuracy curve. *Biostat.* 2013;14:42–59.
18. Lee T, Derebail VK, Kshirsagar AV, et al. Patients with primary membranous nephropathy are at high risk of cardiovascular events. *Kidney Int.* 2016;89:1111–1118.
19. Barbour SJ, Greenwald A, Djurdjev O, et al. Disease-specific risk of venous thromboembolic events is increased in idiopathic glomerulonephritis. *Kidney Int.* 2012;81:190–195.
20. Polanco N, Gutiérrez E, Covarsí A, et al. Spontaneous remission of nephrotic syndrome in idiopathic membranous nephropathy. *J Am Soc Nephrol.* 2010;21:697–704.
21. Cattran DC, Appel GB, Hebert LA, et al. Cyclosporine in patients with steroid-resistant membranous nephropathy: a randomized trial. *Kidney Int.* 2001;59:1484–1490.
22. Fervenza FC, Appel GB, Barbour SJ, et al. Rituximab or cyclosporine in the treatment of membranous nephropathy. *N Engl J Med.* 2019;381:36–46.
23. Caro J, Gutiérrez-Solís E, Rojas-Rivera J, et al. Predictors of response and relapse in patients with idiopathic membranous nephropathy treated with tacrolimus. *Nephrol Dial Transplant.* 2015;30:467–474.
24. Food and Drug Administration, Center for Drug Evaluation and Research (CDER), Center for Biologics Evaluation and Research (CBER). Guidance for Industry: Expedited Programs for Serious Conditions – Drugs and Biologics. 2014. Available at: <https://www.federalregister.gov/documents/2014/05/30/2014-12534/guidance-for-industry-on-expedited-programs-for-serious-conditions-drugs-and-biologics-availability>. Accessed April 12, 2020.
25. Cattran DC, Kim ED, Reich H, et al. Membranous nephropathy: quantifying remission duration on outcome. *J Am Soc Nephrol.* 2017;28:995–1003.
26. Lee T, Biddle AK, Lionaki S, et al. Personalized prophylactic anticoagulation decision analysis in patients with membranous nephropathy. *Kidney Int.* 2014;85:1412–1420.

27. Bachmann LM, Yu M, Boyd JC, et al. State of harmonization of 24 serum albumin measurement procedures and implications for medical decisions. *Clin Chem.* 2017;63:770–779.
28. Hoxha E, Thiele I, Zahner G, et al. Phospholipase A2 receptor autoantibodies and clinical outcome in patients with primary membranous nephropathy. *J Am Soc Nephrol.* 2014;25:1357–1366.
29. Ramachandran R, Yadav AK, Kumar V, et al. Temporal association between PLA2R antibodies and clinical outcomes in primary membranous nephropathy. *Kidney Int Rep.* 2018;3:142–147.
30. Ruggerenti P, Debiec H, Ruggiero B, et al. Anti-phospholipase A2 receptor antibody titer predicts post-rituximab outcome of membranous nephropathy. *J Am Soc Nephrol.* 2015;26:2545–2558.
31. Seitz-Polski B, Dahan K, Debiec H, et al. High-dose rituximab and early remission in PLA2R1-related membranous nephropathy. *Clin J Am Soc Nephrol.* 2019;14:1173–1182.
32. Gupta G, Fattah H, Ayalon R, et al. Pre-transplant phospholipase A2 receptor autoantibody concentration is associated with clinically significant recurrence of membranous nephropathy post-kidney transplantation. *Clin Transplant.* 2016;30:461–469.
33. Seitz-Polski B, Dolla G, Payré C, et al. Epitope spreading of autoantibody response to PLA2R associates with poor prognosis in membranous nephropathy. *J Am Soc Nephrol.* 2016;27:1517–1533.