KI REPORTS
KIReports.org
CLINICAL RESEARCH

Characteristics and Outcomes of NELL1 Membranous Nephropathy in Lipoic Acid Users and Nonusers

Rupali S. Avasare[1], Samantha Clark[2], Rebecca I. Spain[3,4], Raghav Wusirika[1], Robert Rope[1], Susan Gurley[1], Madison Stanaway[1], Miroslav Sekulic[5], Dominick Santoriello[5], Andrew S. Bomback[6], Pietro Canetta[6], Sitalakshmi J. Iyer[7], Vanderlene Kung[8], Vivek Charu[9], Megan L. Troxell[9,10], Satoru Kudose[5,10] and Nicole K. Andeen[8,10]

[1]Division of Nephrology and Hypertension, Department of Medicine, Oregon Health and Science University, Portland, Oregon, USA; [2]School of Medicine, Oregon Health and Science University, Portland, Oregon, USA; [3]Department of Neurology, Oregon Health and Science University, Portland, Oregon, USA; [4]Veterans Administration Portland Healthcare System, Portland, Oregon, USA; [5]Department of Pathology and Cell Biology, Columbia University Irving Medical Center, New York, New York, USA; [6]Division of Nephrology, Department of Medicine, Columbia University Irving Medical Center, New York, New York, USA; [7]Kidney Care Physicians, LLC, Salem, Oregon, USA; [8]Department of Pathology and Laboratory Medicine, Oregon Health and Science University, Portland, Oregon, USA; and [9]Department of Pathology, Stanford University, Palo Alto, California, USA

Introduction: Neural epidermal growth factor like 1 membranous nephropathy (NELL1 MN) is associated with various secondary etiologies. However, previous studies on the frequency of these associations and their impact on outcomes are limited. We report a large multiinstitutional series of patients with NELL1 MN with a focus on secondary associations, pathology findings, and their impact on outcome.

Methods: We retrospectively reviewed clinicopathologic features of NELL1 MN from 3 institutions and analyzed clinical and histologic associations with outcome.

Results: Of 70 patients, 53% were male with a median age of 66 years; median proteinuria was 5.9 g/d. NELL1 MN was associated with lipoic acid (36%), heavy nonsteroidal antiinflammatory drug (NSAID) use (27%), autoimmune disease (23%), malignancy (10% recent, 23% any), mercury exposure (1%), and 11% had no known secondary association. At median follow-up of 11 months, 72% achieved complete or partial remission. Remission rate was 91% in patients with lipoic acid-associated NELL1 MN and ≥6 months of follow-up. On multivariable analyses, patients with primary NELL1 MN (adjusted odds ratio [OR]: 19.7, $P = 0.01$) and increasing degree of tubular atrophy and interstitial fibrosis (IFTA) (adjusted OR 1.1, $P = 0.01$) were less likely to achieve any remission, whereas complete remission (CR) was associated with lipoic acid use (adjusted OR: 10.9, $P = 0.04$, 95% confidence interval [CI]: 1.2–100) and lesser degrees of IFTA (adjusted OR: 0.79, $P = 0.16$, 95% CI: 0.66–0.96).

Conclusion: Our findings strengthen the association between lipoic acid and NELL1 MN. Furthermore, our findings suggest that discontinuation of lipoic acid without immunosuppression should be considered as the first-line treatment.

Kidney Int Rep (2024) **9,** 1379–1386; https://doi.org/10.1016/j.ekir.2024.02.1401
KEYWORDS: drug-induced kidney disease; glomerular disease; lipoic acid; membranous nephropathy; NELL1; NSAID

Membranous nephropathy (MN) is an autoimmune disease caused by circulating antibodies directed against podocyte antigens.[1-3] In 2019, 2 groups identified the NELL1 podocyte antigen in a subset of MN with distinct histologic and immunologic features, namely segmental or incomplete glomerular capillary loop involvement with predominant IgG1 staining.[4,5] NELL1 MN has also been identified in association with thiol-containing medications, namely lipoic acid,[6,7] bucillamine,[8] and tiopronin[9]; traditional indigenous medications, including those with high mercury content[10]; and with underlying conditions, including malignancy,[5] infections (including HIV[11]), sarcoidosis,[12] hematopoietic stem cell transplant and graft-versus-host disease,[13] and rheumatoid arthritis.[8]

Previous series of patients with NELL1 MN either lack outcome data[4] or predate the discovery of these

Correspondence: Rupali S. Avasare, Division of Nephrology and Hypertension, Oregon Health and Science University Medicine, 3181 SW Sam Jackson Park Road, SJH6, Portland, Oregon 97239, USA. E-mail: avasare@ohsu.edu

[10]MLT, SK, and NKA contributed equally.

Received 15 September 2023; revised 15 February 2024; accepted 19 February 2024; published online 24 February 2024

secondary associations.[5,10,14] Therefore, the frequency of possible secondary associations in patients with NELL1 MN may not be representative and their outcomes likely do not reflect the potentially excellent prognosis in a subset of these patients, as seen with lipoic acid use.[6]

In this study, we present clinicopathologic features of a large, multiinstitutional series of patients with NELL1 MN focusing on the frequency of secondary associations and their outcomes.

METHODS

This study was approved by the institutional review boards of Oregon Health and Science University, Columbia University Irving Medical Center, and Stanford University; and adheres to the Declaration of Helsinki. Renal pathology biopsy databases were searched for all available NELL1 MN cases in patients over the age of 18. Dates searched varied between institutions; however, as a rule, cases before 2020 were selected for segmental, phospholipase A2 receptor-negative MN, yielding 68 biopsy-proven NELL1 MN cases. Immunohistochemistry was performed on formalin-fixed paraffin embedded tissue using anti-NELL1 antibodies (Sigma HPA051535). Biopsies had multiple levels cut and stained with Jones methenamine silver, periodic acid Schiff, hematoxylin and eosin and trichrome. For immunofluorescence, frozen tissue was stained with antibodies against IgG, IgA, IgM, C3, C1q, fibrin or fibrinogen, kappa light chain, lambda light chain, and albumin. IgG subclass staining was performed in 29 cases and electron microscopy was available for 64 cases. Paraffin immunofluorescence was not performed. Two additional cases were included: 1 from a clinical trial of lipoic acid for multiple sclerosis who developed nephrotic syndrome after lipoic acid use and had resolution of disease before planned kidney biopsy (reported in our earlier series of nephropathy after lipoic acid exposure),[6] and 1 case of segmental phospholipase A2 receptor-antibody negative MN in a lipoic acid user.

Clinical history was obtained through chart review and discussion with nephrologists. Charts were reviewed by the study team for clinical and histologic information, specifically demographics, comorbidities, prescribed and documented over-the-counter supplements, laboratory values at diagnosis and at last follow-up. Race and ethnicity were obtained from medical charts where it is usually self-reported or provided by the treating providers. The designation of "heavy" (NSAID) use was self-reported and defined as approximately daily NSAID use for more than 1 week and within 1 month of diagnosis. Nonmelanoma skin cancers were not included in assessment of underlying malignancy. Clinical characteristics were not mutually exclusive, reflecting the daily clinical environment and retrospective design; no variable hierarchy was used.

For proteinuria, spot urine protein-to-creatinine ratio or 24-hour urine protein was analyzed interchangeably.[15] Outcomes were based on Kidney Disease Improving Global Outcome guidelines and were classified as follows: CR (urine protein-to-creatinine ratio $<$500 mg/g, and stable glomerular filtration rate [GFR], within 25% of GFR from time of diagnosis), partial remission (urine protein-to-creatinine ratio $<$3.5 g/g and decrease by at least 50% from time of diagnosis and GFR $<$25% reduction), or failure to achieve any remission.[16] Estimated GFR (eGFR) was calculated using the 2021 Chronic Kidney Disease-Epidemiology Collaboration Creatinine equation.[17] Statistical analyses were performed in Graphpad Prism 8 (San Diego, CA, and College Station, TX) for univariate analyses. Descriptive statistics are summarized as median and range for continuous variables, and continuous variables were compared between groups using Mann-Whitney U-tests. Categorical variables were compared between groups using Fisher exact tests. Outcome and multivariable analyses were performed in Stata 13 (College Station, TX). Because not all patients had precise time to remission available, regression analysis for the overall cohort was done using available outcome data ($n = 64$). Survival analysis with Cox proportional hazard models was performed for those in whom relatively precise time-to-remission, or follow-up time without remission, was recorded ($n = 51$), because time to follow-up is not interchangeable with time to remission for NELL1 MN. Due to uncertain time-to-remission, this subgroup for survival analysis functionally excluded 13 patients with $\geq$12 months follow-up, all of whom were in remission (all but 1 in CR) at last follow-up. Given the bias this introduced, both logistic regression and Cox proportional hazard analyses were performed and are provided for significant variables. Univariable analyses were used to evaluate the association of potentially relevant covariates with the primary outcome. Having $P < 0.2$ for individual covariates was the threshold for subsequent inclusion in a multivariable model. Associations are provided as univariate analysis, except where they are indicated to be multivariable analyses. Goodness-of-fit of the final multivariable model was assessed using chi-square likelihood ratio.

RESULTS

Baseline Characteristics

In the overall cohort ($n = 70$, Table 1), median age at diagnosis was 66 years (range, 29–86), with 33 (47%)

Table 1. Clinical features and outcomes of NELL1 MN

Variables	Overall cohort ($N = 70$)	Lipoic acid ($n = 25$, 36%)	Nonlipoic acid ($n = 41$, 59%)[a]
Age, yr	66 (29–86)	66 (42–83)	63 (29–86)
Female	47%	76%[b,c]	27%
Race	58% White/30% NA/7% Hispanic/6% Black/4% Asian	64% White/20% NA/0% Hispanic/8% Black/8% Asian	46% White/39% NA/12% Hispanic/5% Black/2% Asian
Hypertension	51%	52%	52%
Diabetes	27%	36%	24%
Malignancy	23% total/10% within 1 yr	28% total/12% within 1 yr	22% total/10% within 1 yr
Autoimmune disease	23%	36%	17%
Lipoic acid use	36%	100%	0%
Heavy NSAID-use	27%	12%	37%
Proteinuria at biopsy, g	5.9 (0.1–35)	6 (0.1–19)	6 (1.6–35)
Initial proteinuria ≥3.5 g	80%	80%	83%
Immunosuppression for MN	29%	4%[b,c]	41%
Follow-up time, mo	11 (1–96)	10 (1–84)	11 (2–96)
Remission	67% any/47% CR/20% PR	88% any[b,c]/72% CR[b,c]/12% PR	62% any/38% CR/24% PR

CR, complete remission; MN, membranous nephropathy; NA, not available; NELL1, neural epidermal growth factor-like; NSAID, nonsteroidal antiinflammatory; PR, partial remission.
[a]Four patients for whom lipoic acid use was not described but could not be entirely excluded are not included.
[b]Statistically significant when compared between listed subgroup *vs.* overall cohort ($P < 0.05$).
[c]Statistically significant when compared between lipoic acid use *vs.* no lipoic acid use ($P < 0.05$).
Values provided as median and range.

female and 37 (53%) male patients. At the time of biopsy, median proteinuria was 5.9 (range 0.11–35 g), with 56 (80%) having ≥3.5 g; 21 patients (30%) had an eGFR <60 ml/min per 1.73 m^2. Four patients (6%) had deep venous thrombosis attributed to nephrotic syndrome. Thirty-six patients (51%) had hypertension, 19 (27%) had diabetes, and 16 (23%) had a preexisting autoimmune disease. A wide spectrum of autoimmune diseases was observed, including multiple sclerosis ($n = 4$), psoriasis ($n = 2$), Hashimoto's thyroiditis (2), and 1 each of rheumatoid arthritis, Sjögren, Celiac disease, polymyositis, CREST syndrome, autoimmune hepatitis, Evan's syndrome, Guillan-Barre, and sarcoidosis (Supplementary Data).

Sixteen patients (23%) had a history of malignancy, which was recent (within 1 year of kidney biopsy) in 7 (10%). The most common malignancy was lung cancer ($n = 5$), followed by prostate cancer ($n = 3$), gastrointestinal carcinomas ($n = 2$), and breast cancer ($n = 1$). Two patients had myeloid neoplasms treated with hematopoietic stem cell transplantation, which was complicated by graft-versus-host disease. Cancer remission status varied among patients, with some developing NELL1 MN after undergoing cancer treatment and during clinical and radiologic remission (Supplementary Data).

Twenty-five patients (36%) used lipoic acid (Supplementary Table S1). They had median age of 66 (range, 42–83) years, and included 19 females (76%). Twenty patients (80%) had available race and ethnicity data, and 16 were White (64%), 2 were Black (8%), and 2 were Asian (8%). At the time of biopsy, lipoic acid users had median proteinuria of 6 g/g (range, 0.1–19), including 80% with nephrotic range proteinuria. The average dosage of lipoic acid was 840 mg/d. Neuropathy or other nerve-related diseases (48%) were the most common indications. Patients who used lipoic acid included a notably higher proportion of females ($n = 19$, comprising 76% of lipoic acid users vs. 47% in the overall cohort, $P < 0.01$); however, there was no other statistically significant differences with the overall cohort for baseline variables including proportion with diabetes.

Nineteen patients (27%) reported heavy NSAID-use before diagnosis. One patient had occupational mercury exposure and elevated blood mercury levels. Only 8 patients (11%) had no known secondary associations[12] (including autoimmune disease, lipoic acid use, NSAID use, or malignancy).

Among tested baseline variables, initial eGFR <60 ml/min per 1.73 m^2 was associated with hypertension ($P = 0.04$), male sex ($P = 0.04$), and remote or recent malignancy ($P = 0.04$) with a trend for association with age ($P = 0.08$), but not with diabetes, race or ethnicity, NSAID-use or lipoic acid use. Initial proteinuria ≥3.5 g was associated with hypertension ($P = 0.04$), but not age, sex, diabetes, race or ethnicity, malignancy, NSAID-use nor lipoic acid use.

Pathology

Pathologic findings are summarized in Table 2 ($n = 68$) and a representative image is shown in Figure 1. Overall, the biopsy showed mild chronicity, including 57 (84%) with global sclerosis <25%, and 55 (81%) with mild tubulointerstitial scarring. Subepithelial

Table 2. Kidney biopsy findings in NELL1 MN ($n = 68$)

Variables	Overall cohort	Lipoic acid user	Nonlipoic acid user
Number of glomeruli	22 (1–70)	16 (1–69)	23 (1–70)
Global glomerulosclerosis	6% (0%–70%); 57 (84%) with <25%	6% (0%–70%); 90% with <25%	6% (0%–42%); 90% with <25%
Segmental glomerulosclerosis	12 (18%)	3 (15%)	9 (22%)
Segmental/incomplete MN pattern	36 (55%)	12 (60%)	21 (51%)
Extraglomerular deposits of IgG by immunofluorescence[a]	8 (12%): 7 with TBM deposits, 1 with PTC + arteriolar deposits	2 (10%)	6 (15%)
IgG1 (co)dominant deposits[b]	86%	91%	81%
Dual PLA2R staining [c]	1 (1%)	0%	1 (3%)
Oligoclonal immune deposits[d]	1 (1%)	0%	1 (3%)
MN stage by electron microscopy	Stage 1–2: 50 (82%) / Stage 3: 10(16%) /Stage 4: 1(2%)	Stage 1–2: 13 (72%) / Stage 3: 5 (28%) /Stage 4: 0	Stage 1–2: 33 (85%) / Stage 3: 5 (13%) /Stage 4: 1 (2%)
Mesangial deposits	10 (16%)	2 (12%)	8 (21%)
Subendothelial deposits	4 (6%)	1 (6%)	3 (8%)
Tubuloreticular inclusions[e]	3 (5%)	0 (0%)	3 (8%)
IFTA	5% (0%–70%); 55 (81%) with <25%	10% (0–65%); 18 (90%) with <25%	10% (0–70%); 34 (83%) with <25%
Additional disease on biopsy	5 (8%): 2 diabetic nephropathy, 1 smoking associated nodular glomerulopathy, 1 interstitial nephritis associated with NSAIDs, 1 chronic ischemic tubulointerstitial nephropathy	1 (5%): 1 smoking associated nodular glomerulopathy	4 (10%): 2 diabetic nephropathy, 1 interstitial nephritis associated with NSAIDs, 1 chronic ischemic tubulointerstitial nephropathy

IFTA, tubular atrophy and interstitial fibrosis; MN, membranous nephropathy; NSAID, nonsteroidal antiinflammatory drug; PLA2R, phospholipase A2 receptor; PTC, peritubular capillary; TBM, tubular basement membrane.
[a]One case had corresponding granular NELL1 staining along TBMs.
[b]Of 29 cases in which IgG subclasses were performed, IgG1 was dominant in 20 (69%), and codominant with IgG3 (in 3, 10%) or IgG4 (in 2, 7%). IgG3 and IgG4 were dominant in 2 cases (7%) each.
[c]PLA2R was negative in all tested cases except one equivocal and one positive, which showed diffuse subepithelial without subendothelial or mesangial deposits; serum PLA2R results not available.
[d]All cases had staining for IgG, kappa and lambda light chains except one case with IgG1, IgG4, and kappa light chain staining without lambda light chain; serum protein electrophoresis demonstrated a faint monoclonal lambda band, discordant with light chain restriction of glomerular deposits.
[e]Three patients had endothelial tubuloreticular inclusions, two of whom had a history of metastatic carcinoma and one had CREST syndrome.
Values provided as median and range.

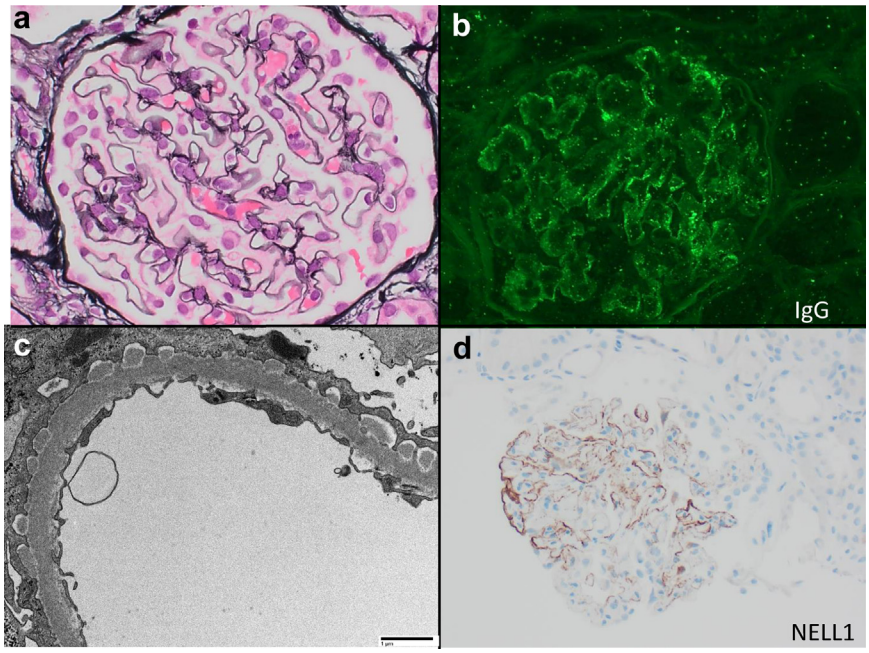

Figure 1. (a) Subtle membranous nephropathy, with "stiff" appearing capillary loops and faint irregularities of the glomerular basement membrane (Jones methenamine silver stain, original magnification ×400). (b) Segmental granular capillary wall staining for IgG (original magnification ×200). (c) Ultrastructural studies show subepithelial electron dense immune deposits with associated podocyte foot process effacement (transmission electron microscopy, direct magnification ×4800). (d) Corresponding segmental granular capillary wall staining for neural epidermal growth factor-like 1 (NELL1) by immunohistochemistry (original magnification ×200). NELL1, neural epidermal growth factor-like.

deposits were segmental or incomplete in 36 (55%) biopsies and 50 biopsies (82%) were stage 1 to 2.

Among tested baseline characteristics, initial eGFR <60 ml/min per 1.73 m^2 was associated with pathology findings of ≥25% global glomerulosclerosis ($P = 0.003$) and ≥25% IFTA ($P = 0.001$). At a cut-off of ≥3.5 g, initial proteinuria was not significantly associated with MN stage, segmental versus global distribution of deposits, nor focal segmental glomerulosclerosis (FSGS). There were no statistically significant pathologic differences among various associations of NELL1 MN. Among 8 cases of NELL1 MN without known secondary association, 2 had mesangial deposits, 3 others had a segmental MN (1 of which had IgG1 and IgG4 kappa restricted deposits), and a sixth was dual positive for phospholipase A2 receptor and NELL1.

Outcomes

At a median observation time of 11 months (range, 1 month–8 years), follow-up information was available in all but 6 patients (i.e., in 91%). A total of 46 patients (72%) achieved remission, including 33 (52%) CR and 13 (20%) partial remission. Median proteinuria at last-follow-up was 0.33 (range 0–11.3) g. Of those with a follow-up eGFR <60 ml/min per 1.73 m^2 ($n = 17$, 27%), most (15, 88%) had had an initial eGFR <60 ml/min per 1.73 m^2 at time of biopsy. Twenty (29%) patients received 1 or more immunosuppressive agents for MN, including rituximab ($n = 13$), prednisone monotherapy ($n = 2$), cyclophosphamide ($n = 2$), tacrolimus ($n = 3$, 2 of 3 whom also received rituximab), cyclosporine ($n = 1$), and/or azathioprine ($n = 1$).

On logistic regression analysis, failure to achieve any remission was significantly associated with NELL1 MN without identified secondary association (OR: 19.2, $P = 0.01$, 95% CI: 2.0–179.9), as well as Black race or Hispanic ethnicity (OR: 12.4, $P \leq 0.001$, 95%: CI 2.3–66.3). The latter numbers were small and included 2 of 4 Black patients and 4 of 5 Hispanic patients not achieving remission; of these 9 patients, 3 also had NELL1 MN without secondary association. Patients receiving immunosuppression were less likely to achieve remission (OR: 5.8, $P = 0.006$, 95% CI: 6–18.6). It is possible that those who were treated with immunosuppression may have had more aggressive disease as determined by the treating provider. Other clinical variables, including sex, diabetes, hypertension, autoimmune disease, malignancy, NSAID, lipoic acid, initial proteinuria ≥3.5 g/g, and initial GFR <60 ml/min per 1.73 m^2, were not significantly associated with overall remission. Of the tested pathology variables (including global glomerulosclerosis, FSGS, segmental vs. global deposit distribution, MN stage, and IFTA), failure to achieve any remission was associated only with increasing degrees of IFTA (OR: 0.95, $P = 0.03$, 95% CI: 0.91–0.99) with a trend for association with FSGS ($P = 0.08$). On multivariable analysis, primary NELL1 MN (adjusted OR: 19.7, $P = 0.01$, 95% CI: 1.9–209) and IFTA (adjusted OR: 1.1, $P = 0.01$, 95% CI: 1.01–1.1) but not lipoic acid use were associated with failure to achieve any remission.

CR was associated with lipoic acid use (OR: 4.2, $P = 0.01$, 95% CI: 1.4–12.6), underlying autoimmune disease (OR: 3.9, $P = 0.04$, 95% CI: 1–13.7) and absence of Black race or Hispanic ethnicity (OR: 0.18, $P = 0.05$, 95% CI: 0.3–1.00), but not other aforementioned clinical variables. Biopsy variables associated with CR were less IFTA (OR: 0.89, $P = 0.008$, 95% CI: 0.82–0.97) and lack of FSGS (OR: 0.16, $P = 0.03$, 95% CI: 0.032–0.82). On multivariable analyses for CR, lipoic acid use (adjusted OR 10.9, $P = 0.04$, 95% CI: 1.2–100) and IFTA (adjusted OR: 0.79, $P = 0.16$, 95% CI: 0.66–0.96) but not race or ethnicity nor FSGS were significantly associated with CR.

Considering that lipoic acid users comprised 36% ($n = 25$, all with available follow-up data) of our NELL1 MN cohort, we further examined the details of remission in these patients. At a median follow-up time of 10 months (range, 1 month–7 years), patients with NELL1 MN associated with lipoic acid use had significantly higher rates of remission (88% vs. 62%; $P = 0.04$) and CR (72% vs. 38%; $P = 0.01$) compared with confirmed nonlipoic acid users ($n = 41$); there was no significant difference in follow-up rates for lipoic acid users versus nonusers. Patients with lipoic acid-associated NELL1 MN also had a significantly lower rate of immunosuppressive therapy (4% vs. 41%; $P < 0.001$) than those without lipoic acid exposure (Table 1). Most (23/25, 92%) had confirmed cessation of lipoic acid use after kidney biopsy. Among patients in the lipoic acid group with ≥6 months of follow-up (22 of 25 patients), all but 2 (i.e., 20 of 22, 91%) achieved remission, which was complete in 16 (73%). The 2 patients not achieving remission had >50% reduction in proteinuria with follow-up proteinuria of 0.09 g and 2.2g but had had an initial eGFR <60 ml/min per 1.73 m^2, and were considered no remission based on eGFR criteria of >25% reduction in eGFR at follow-up. Twenty-three of 25 patients had confirmed cessation of lipoic acid use after kidney biopsy, although CR was also achieved in 1 patient who continued taking lipoic acid inconsistently and in another patient who continued lipoic acid use and was treated for breast cancer with cyclophosphamide-based regimen.

For 34 patients for whom time-to-remission was recorded in addition to overall follow-up time, the overall median time to remission for NELL1 MN was 6

months (range, 1.5–14 months), which was similar for the lipoic acid subgroup ($n = 15$).

Cox proportional hazard analyses was performed in a subgroup ($n = 51$) where the precise time-to-remission or no remission data were available, using the same clinical and histologic variables above. This subgroup for survival analysis functionally excluded 13 patients with ≥12 months follow-up, all of whom were in remission (all but 1 in CR) at last follow-up, but with uncertain time-to-remission. This analysis yielded overall similar results to the logistic regression analysis. Specifically, failure to achieve any remission remained significantly associated with primary NELL1 (hazard ratio [HR]: 8.0, $P = 0.001$, 95% CI: 2.32–27.77), Black race or Hispanic ethnicity (HR: 3.75, $P = 0.047$, 95% CI: 1.02–13.88), as well as global rather than segmental distribution of deposits on biopsy (HR: 4.7, $P = 0.01$, 95% CI: 1.4–15.5), but not any other clinical or pathology variable. Of these, only primary NELL1 MN was statistically associated with failure to achieve any remission (adjusted HR: 5.4, $P = 0.026$, 95% CI: 1.2–24.3) on multivariable analysis. CR was significantly associated only with lipoic acid use (HR: 2.72, $P = 0.032$, 95% CI: 1.09–6.77), and less IFTA (HR: 0.93, $P = 0.02$, 95% CI: 0.87–0.99). Both lipoic acid use (adjusted HR: 4.2, $P = 0.007$, 95% CI: 1.5–11.7) and IFTA (adjusted HR: 0.91, $P = 0.007$, 95% CI: 0.84–0.97) remained significant in the multivariable model for CR.

DISCUSSION

In this multiinstitutional study of NELL1 MN, we identified significant differences in outcome based on underlying associations, and specifically demonstrated high remission rates for lipoic acid-associated NELL1 MN (without immunosuppressive therapy) compared with NELL1 MN with no identifiable underlying association. Race or ethnicity, and pathology variables of IFTA, FSGS, and segmental deposit distribution were also associated with outcomes in NELL1 MN. Our findings strengthen the etiologic association between NELL1 MN and lipoic acid use,[6,7] and underscore the practical importance of both reviewing supplements and classifying MN by its association when known, in addition to its antigen.

Lipoic acid is an over-the-counter dietary supplement used for general wellness and neuropathy with an estimated market value in the hundreds of millions of US Dollars. Lipoic acid is also being investigated as a therapeutic because of its antioxidant properties (clinicaltrials.gov). Side effects of lipoic acid include gastrointestinal upset, rashes, and rarely, insulin autoimmune syndrome in genetically susceptible individuals.[18,19] Several different brands of lipoic acid were used by patients in this series, thus the adverse event of NELL1 MN does not appear specific to a particular brand. The lipoic acid used in the multiple sclerosis clinical trials previously reported,[6] tested negative for other contaminants, thus implicating lipoic acid itself as the culprit compound (R. Spain, personal communication). The underlying mechanism of lipoic acid-associated NELL1 MN remains unknown, but increasing reports of thiol-containing medications associated with NELL1 MN raise the possibility that the sulfhydryl group may be involved in disease development.[6-9]

Underlying associations in NELL1 vary in global reports.[4,5,10,14] The reason for the substantially higher rate of lipoic acid use in our series compared with others (36% vs. 13%)[7] may be at least partially due to growing recognition in the nephrology community about the link between supplements and NELL1 MN.[6,7] Although NELL1 MN had an overall similar sex distribution, there was a significantly greater proportion of females than males in the lipoic acid cohort; a statistically significant female predilection was also seen in traditional indigenous medication-associated MN.[6] This may reflect the greater use of supplements by females than males[20,21]; however, whether sex may be an important biological risk factor needs further investigation.

The majority of lipoic acid users in our series discontinued lipoic acid use and experienced remission without immunosuppressive therapy, strikingly similar to a series of patients with MN who had a high rate of NELL1 MN remission after discontinuation of traditional medications.[10] These data suggest that certain patients with lipoic acid-associated NELL1 MN may be carefully monitored after supplement discontinuation, without immunosuppression. Notably, however, there were 2 patients in our series who continued using lipoic acid and achieved remission. Whether a subset of patients may safely continue, perhaps at a lower dose, or restart using lipoic acid after remission, requires further investigation.

The rate of malignancies in our patients with NELL1 MN (23% overall, including 10% within 1 year of biopsy) was lower than previously reported by Caza *et al.*[5] (33%) and had no specific association with outcomes. Because malignancy is more common in an older population, we believe that some occurrences may be coincidental although multiple series have shown a higher prevalence of malignancy in patients with NELL1 MN[4] than with phospholipase A2 receptor MN (~4%).[5,22,23] In the absence of mechanistic data, the role of malignancy in development of NELL1 MN remains unclear.

In addition to malignancy, we identified enrichment for patients with NSAID-use in our series, particularly

"heavy" use.[24] However, unlike lipoic acid, the strength of this possible etiologic association appears weaker; NSAID-associated NELL1 MN did not have specific associations with remission, and NSAID-associated MN appears to be associated with a different antigen, namely proprotein convertase subtilisin/kexin type 6.[25] Furthermore, determining what constitutes heavy use is particularly fraught because NSAID-use cannot be confirmed through medication lists and is often noted in the chart using qualitative descriptors.

Limitations of our study include those inherent with retrospective studies, specifically nonstandardized reporting of supplemental medications, that are often not included in the medication section of the electronic medical record, and wide variability in timing and availability of serial laboratory tests. For a limited number of cases, staining for NELL1 was requested by the treating provider, which may introduce bias in the frequency of different disease-associations. We demonstrate a high rate of remission for lipoic acid-associated NELL1 MN without immunosuppressive use; however, we cannot draw further conclusions about the utility of immunosuppression for all-comers with NELL1 MN, particularly those not associated with thiol-containing medications. The decision to treat with immunosuppression was also not standardized; those treated may have more severe disease. Our study does not compare outcomes across different antigen-driven MNs. Finally, we used the Kidney Disease Improving Global Outcome definition of remission, which includes both proteinuria and kidney function, although in NELL1 MN, the former may be a more accurate marker of immunologic disease whereas kidney function incorporates multifactorial parenchymal injury. Specifically, though limited by sample size, our study suggests a link between chronic injury on biopsy, initial eGFR, race, and hypertension. Preexisting chronic injury may have confounded our outcome data without precisely reflecting the immunologic behavior of NELL1 MN, thereby highlighting the importance of developing commercially available serum testing for anti-NELL1 antibodies.

In summary, approximately one-third of patients in the U.S. with NELL1 MN may have exposure to lipoic acid. The overall good prognosis of NELL1 MN is part of an emerging pattern described in NELL1 MN associated with traditional indigenous medications containing mercury[10] as well as certain thiol-containing medications, such as lipoic acid.[9] Although exceptions exist, our data support drug discontinuation and careful monitoring without immunosuppression as the first-line treatment for NELL1 MN associated with lipoic acid use in otherwise low-risk patients.

DISCLOSURE

RSA and SG receive research support from the Medical Research Foundation of Oregon. RA is on the Data and Safety Monitoring Board for Lipoic acid in Progressive Multiple Sclerosis clinical trial (LAPMS; NCT03161028); RIS is the principal investigator of the LAPMS trial and receives research support from Department of Veterans Affairs (I01 RX002682); SG receives research support from grant NIH DK098382; SK receives research support from the Young Investigator Grant of the National Kidney Foundation. All the other authors declared no competing interests.

SUPPLEMENTARY MATERIAL

Supplementary File (PDF)

Supplementary Data.

Table S1. Details on lipoic acid dose and indication for use.

Strobe Statement.

REFERENCES

1. Debiec H, Ronco P, Ronco P. PLA2R autoantibodies and PLA2R glomerular deposits in membranous nephropathy. *N Engl J Med*. 2011;364:689–690. https://doi.org/10.1056/NEJMc1011678
2. Beck LH Jr, Bonegio RG, Lambeau G, et al. M-type phospholipase A2 receptor as target antigen in idiopathic membranous nephropathy. *N Engl J Med*. 2009;361:11–21. https://doi.org/10.1056/NEJMoa0810457
3. Sethi S. New 'antigens' in membranous nephropathy. *J Am Soc Nephrol*. 2021;32:268–278. https://doi.org/10.1681/ASN.2020071082
4. Sethi S, Debiec H, Madden B, et al. Neural epidermal growth factor-like 1 protein (NELL-1) associated membranous nephropathy. *Kidney Int*. 2020;97:163–174. https://doi.org/10.1016/j.kint.2019.09.014
5. Caza TN, Hassen SI, Dvanajscak Z, et al. NELL1 is a target antigen in malignancy-associated membranous nephropathy. *Kidney Int*. 2021;99:967–976. https://doi.org/10.1016/j.kint.2020.07.039
6. Spain RI, Andeen NK, Gibson PC, et al. Lipoic acid supplementation associated with neural epidermal growth factor-like 1 (NELL1)-associated membranous nephropathy. *Kidney Int*. 2021;100:1208–1213. https://doi.org/10.1016/j.kint.2021.10.010
7. Caza TN, Larsen CP. Lipoic acid in neural epidermal growth factor-like 1-associated membranous nephropathy: more than a coincidence? *Kidney Int*. 2022;101:418–419. https://doi.org/10.1016/j.kint.2021.12.001
8. Miyazaki R, Ueda H, Hayashi A, et al. Neural epidermal growth factor-like 1-positive membranous nephropathy with rheumatoid arthritis. *Kidney Int Rep*. 2023;8:921–924. https://doi.org/10.1016/j.ekir.2022.12.026
9. Dominick Santoriello RR, Kudose S, Markowitz GS, Markowitz GS. Segmental NELL-1 membranous nephropathy complicating tiopronin therapy. *Kidney Int Rep*. 2023;8:1683–1686. https://doi.org/10.1016/j.ekir.2023.05.023

10. Kurien AA, Ks P, Walker PD, Caza TNJ, walker PD, Caza TN. Traditional indigenous medicines are an etiologic consideration for NELL1-positive membranous nephropathy. *Kidney Int*. 2022;102:1424–1426. https://doi.org/10.1016/j.kint.2022.09.001
11. Dinesh KP, Charu V, Troxell ML, Andeen NKCV, Troxell ML, Andeen NK. NELL1-positive HIV-associated lupus-like membranous nephropathy with spontaneous remission. *Glomerular Dis*. 2022;2:184–188. https://doi.org/10.1159/000525541
12. Sethi S. The many faces of NELL1 MN. *Clin Kidney J*. 2022;16:442–446. https://doi.org/10.1093/ckj/sfac237
13. Kudose S, Sekulic M, Mehring CJ, et al. NELL1-associated membranous glomerulopathy after hematopoietic stem cell transplantation. *Kidney Int Rep*. 2021;6:1992–1995. https://doi.org/10.1016/j.ekir.2021.04.033
14. Wang G, Sun L, Dong H, et al. Neural epidermal growth factor-like 1 protein-positive membranous nephropathy in Chinese patients. *Clin J Am Soc Nephrol*. 2021;16:727–735. https://doi.org/10.2215/CJN.11860720
15. Teo BW, Loh PT, Wong WK, et al. Spot urine estimations are equivalent to 24-hour urine assessments of urine protein excretion for predicting clinical outcomes. *Int J Nephrol*. 2015;2015:156484. https://doi.org/10.1155/2015/156484
16. Kidney Disease: Improving Global Outcomes (KDIGO) Glomerular Diseases Work Group. KDIGO 2021 Clinical Practice Guideline for the Management of Glomerular Diseases. *Kidney Int*. 2021;100:S1–S276. https://doi.org/10.1016/j.kint.2021.05.021
17. Inker LA, Eneanya ND, Coresh J, et al. New creatinine- and cystatin C-based equations to estimate GFR without race. *N Engl J Med*. 2021;385:1737–1749. https://doi.org/10.1056/NEJMoa2102953
18. Ishida Y, Ohara T, Okuno Y, et al. Alpha-lipoic acid and insulin autoimmune syndrome. *Diabetes Care*. 2007;30:2240–2241. https://doi.org/10.2337/dc07-0689
19. Yamada T, Imai J, Ishigaki Y, Hinokio Y, Oka Y, Katagiri H. Possible relevance of HLA-DRB1*0403 haplotype in insulin autoimmune syndrome induced by alpha-lipoic acid, used as a dietary supplement. *Diabetes Care*. 2007;30:e131. https://doi.org/10.2337/dc07-1636
20. Tan ECK, Eshetie TC, Gray SL, Marcum ZA. Dietary supplement use in middle-aged and older adults. *J Nutr Health Aging*. 2022;26:133–138. https://doi.org/10.1007/s12603-022-1732-9
21. Kantor ED, Rehm CD, Du M, White E, Giovannucci EL. Trends in dietary supplement use among US adults from 1999-2012. *JAMA*. 2016;316:1464–1474. https://doi.org/10.1001/jama.2016.14403
22. Ronco P, Beck L, Debiec H, et al. Membranous nephropathy. *Nat Rev Dis Primers*. 2021;7:69. https://doi.org/10.1038/s41572-021-00303-z
23. Bobart SA, Tehranian S, Sethi S, et al. A target antigen-based approach to the classification of membranous nephropathy. *Mayo Clin Proc*. 2021;96:577–591. https://doi.org/10.1016/j.mayocp.2020.11.028
24. Iwakura T, Ema C, Isobe S, et al. Prevalence of neural epidermal growth factor-like 1- and exostosin 1/exostosin 2-associated membranous nephropathy: a single-center retrospective study in Japan. *Sci Rep*. 2022;12:2967. https://doi.org/10.1038/s41598-022-07037-2
25. Sethi S, Casal Moura M, Madden B, et al. Proprotein convertase subtilisin/kexin type 6 (PCSK6) is a likely antigenic target in membranous nephropathy and nonsteroidal anti-inflammatory drug use. *Kidney Int*. 2023;104:343–352. https://doi.org/10.1016/j.kint.2023.04.006