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The Exostosin Immunohistochemical Status Differentiates Lupus Membranous Nephropathy Subsets With Different Outcomes



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KEYWORDS: exostosin; immunohistochemistry; membranous nephropathy; renal biopsy; systemic lupus erythematosus

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Exostosin (EXT) 1 and EXT2 proteins are recently discovered major components of the subepithelial deposits in a subset of patients with membranous nephropathy (MN).^{1,2} Although the detection of EXT1/2 seems to be a distinctive feature of MN related to systemic lupus erythematosus (SLE),¹ the exact frequency of EXT-positive, SLE-associated MN (SLE-MN) and its potential clinical significance are not well determined.

Here, we retrospectively evaluated 86 consecutive patients with biopsy-proven pure class 5 SLE-MN diagnosed between January 2010 and December 2018 in 2 nephropathology centers (La Pitié Salpêtrière and Tenon hospitals) in Paris, France; mixed classes (3 + 5 and 4 + 5 of the 2003 ISN/Renal Pathology Society classification)³ were excluded. Immunohistochemistry with anti-EXT1 antibody revealed that 38.4% of the patients (33 of 86) had EXT-positive SLE-MN. The clinical and laboratory features comparing patients with SLE-MN according to the EXT status are described in Table 1. At the time of diagnosis, the EXT-positive patients were younger (33.3 ± 12.9 years vs. 39.9 ± 13.4 years, $P = 0.0267$), with higher estimated

glomerular filtration rate (eGFR) (122.6 ± 52.4 ml/min per 1.73 m^2 vs. 95.5 ± 48.0 , $P = 0.0197$), and higher amount of urinary protein (3.0 g/d [1.4–4.6] vs. 1.7 [0.9–3.3], $P = 0.0334$) than EXT-negative patients. Kidney biopsy specimens of EXT-positive patients revealed less glomerular scars (9.1% [3 of 33] with $\geq 10\%$ sclerosed glomeruli vs. 34.0% [18 of 53] in the EXT-negative group, $P = 0.009$). MN stages according to the Ehrenreich and Churg classification were evaluated at light microscopy examination and did not differ between the 2 groups. Interestingly, EXT-positive patients were less likely to undergo a repeat biopsy during follow-up and, in such event, had less proliferative lupus nephritis compared with the EXT-negative group (7.7% [2 of 26] vs. 35.7% [15 of 42], $P = 0.0189$). Data regarding treatment were available in most patients (97.0% [32 of 33] in the EXT-positive group and 94.3% [50 of 53] in the EXT-negative group). In both groups, most patients received hydroxychloroquine (81.3% in the EXT-positive and 82.0% in the EXT-negative patients) and steroids (84.4% in the EXT-positive and 86.0% in the EXT-negative patients). The number of additional

Table 1. Clinicopathologic characteristics, follow-up, and outcomes of EXT-positive and EXT-negative SLE-MN

| Variables | EXT+ n = 33 | EXT- n = 53 | P value |
|---|--------------------------|--------------------------|---------------------|
| At presentation | | | |
| Age, mean \pm SD, yr | 33.3 \pm 12.9 | 39.9 \pm 13.4 | 0.0267 ^a |
| Female, n (%) | 27 (81.8) | 45 (84.9) | 0.7061 ^b |
| SCr, median (IQR), mg/dl | 0.61 (0.50–0.87), n = 31 | 0.75 (0.60–1.00), n = 49 | 0.0546 ^c |
| eGFR, mean \pm SD, ml/min per 1.73 m ² | 122.6 \pm 52.4, n = 31 | 95.5 \pm 48.0, n = 49 | 0.0197 ^d |
| Proteinuria, median (IQR), g/24 h | 3.0 (1.4–4.6), n = 32 | 1.7 (0.9–3.3), n = 49 | 0.0334 ^c |
| Proteinuria >3.0 g/24 h, n (%) | 16 (50.0), n = 32 | 15 (30.6), n = 49 | 0.0793 ^b |
| Hematuria, n (%) | 8 (40.0), n = 20 | 18 (51.4), n = 35 | 0.4141 ^b |
| Sclerosed glomeruli | | | 0.0090 ^b |
| <10% | 30 (90.9) | 35 (66.0) | |
| \geq 10% | 3 (9.1) | 18 (34.0) | |
| Interstitial fibrosis | | | 0.0803 ^b |
| Grade 0 | 28 (84.8) | 36 (67.9) | |
| Grade \geq 1 | 5 (15.2) | 17 (32.1) | |
| Follow-up | | | |
| Repeat biopsy | n = 26 | n = 42 | 0.0189 ^d |
| Class 3 or 4 | 2 (7.7) | 15 (35.7) | |
| Other | 3 (11.5) | 2 (4.8) | |
| No renal biopsy | 21 (80.8) | 25 (59.5) | |
| At end of follow-up | | | |
| SCr, median (IQR), mg/dl | 0.60 (0.55–0.72), n = 27 | 0.81 (0.68–0.93), n = 38 | 0.0017 ^c |
| eGFR, mean \pm SD, ml/min per 1.73 m ² | 114.6 \pm 34.5, n = 27 | 84.6 \pm 32.3, n = 38 | 0.0006 ^d |
| Proteinuria, median (IQR), g/24 h | 0.30 (0.11–0.60), n = 27 | 0.39 (0.20–1.36), n = 38 | 0.2413 ^c |
| Proteinuria >3.0 g/24 h, n (%) | 2 (7.4), n = 27 | 3 (7.9), n = 38 | 1.0000 ^d |
| Clinical remission | n = 27 | n = 38 | 0.0056 ^b |
| Complete | 19 (70.4) | 21 (55.3) | |
| Partial | 7 (25.9) | 5 (13.2) | |
| No remission | 1 (3.7) | 12 (31.6) | |
| Time of follow-up, median (IQR), mo | 39.0 (22.0–79.0), n = 27 | 57.0 (27.0–75.0), n = 41 | 0.4620 ^c |

eGFR: estimated glomerular filtration rate; EXT, exostosin; IQR, interquartile range; SCr, serum creatinine; SLE-MN, systemic lupus erythematosus-associated membranous nephropathy.

^aStudent's *t* test.

^bPearson's chi-square test.

^cWilcoxon rank sum test.

^dFisher's exact test.

Interstitial fibrosis grading is based on Banff classification.

immunosuppressive drugs (mainly mycophenolate mofetil, rituximab, cyclophosphamide, and azathioprine) was not different between the 2 groups ($P = 0.3278$) (Supplementary Table S1). At the end of the follow-up period (50.0 [23.5–75.0] months), EXT-positive patients had better renal function with lower serum creatinine (0.60 [0.55–0.72] mg/dl vs. 0.81 [0.68–0.93] mg/dl, $P = 0.0017$) and higher estimated glomerular filtration rate (114.6 \pm 34.5 vs. 84.6 \pm 32.3, $P = 0.0006$). Clinical remission, either complete (defined as proteinuria \leq 0.5 g/d, albuminemia > 30 g/l, and normal serum creatinine) or partial (defined as a decrease in proteinuria by at least 50% with a final value between 0.5 and 3.5 g/d, an increase in albuminemia, and stable serum creatinine), was more frequent in the EXT-positive group (96.3% [26 of 27] vs 68.4% [26 of 38], $P = 0.0056$). In multivariate logistic regression model after adjustment on the follow-up period and MN stage, EXT staining at baseline was associated with complete or partial remission at last follow-up (odds ratio [95% confidence interval] 17.68 [1.78–175.58]) (Table 2).

In a very recent publication, Ravindran *et al.*⁴ reported the clinicopathologic characteristics of a large series of 374 SLE-MN (263 pure class 5 SLE-MN and 111 mixed 3 + 5 and 4 + 5 classes of the 2003 ISN/Renal Pathology Society classification) dichotomized according to the presence or absence of EXT in membranous deposits. Overall, immunohistochemistry revealed that 32.6% (122 of 374) and 35.0% (92 of 263) of the whole cohort and the pure class 5 cases, respectively, were EXT-positive. Kidney biopsy specimens from patients with EXT-positive SLE-MN revealed less chronicity features (glomerulosclerosis, interstitial fibrosis, and tubular atrophy) compared with those from EXT-negative patients. Furthermore, clinical follow-up data, available in 160 patients (129 pure class 5 and 31 mixed 3 + 5/4 + 5 classes), revealed that EXT-negative patients were less likely to reach end-stage kidney disease than EXT-positive patients.

Our results are strikingly similar to those of Ravindran *et al.*⁴ First, the ratios of EXT-positive among pure class 5 SLE-MN were very similar in Mayo Clinic's

Table 2. Multivariable logistic regression model of the risk of remission at last follow-up (n = 47 of 60)

| Variables | Univariable model | | Multivariable model | |
|--|---------------------|--------------------------|---------------------|--------------------------|
| | OR (95% CI) | P value of type III test | OR (95% CI) | P value of type III test |
| Follow-up period (mo) ^a | 1.01 (0.99–1.03) | 0.4609 | 1.01 (0.98–1.03) | 0.6110 |
| MN stages ^a | | 0.1082 | | 0.1416 |
| I | 1 | | 1 | |
| II | 0.52 (0.09–2.91) | | 0.72 (0.11–4.62) | |
| III–IV | 0.15 (0.02–0.99) | | 0.12 (0.01–1.18) | |
| EXT status ^a | | 0.0194 | | 0.0142 |
| Negative | 1 | | 1 | |
| Positive | 12.52 (1.50–104.12) | | 17.68 (1.78–175.58) | |
| eGFR <90 ml/min per 1.73 m ² | | 0.1581 | | |
| No | 1 | | | |
| Yes | 0.40 (0.11–1.43) | | | |
| Interstitial fibrosis = 0 | | 0.0433 | | |
| No | 1 | | | |
| Yes | 4.27 (1.04–17.46) | | | |
| ≥2 Immunosuppressive drugs (except hydroxychloroquine and steroids) | | 0.1416 | | |
| No | 1 | | | |
| Yes | 0.39 (0.11–1.37) | | | |

CI, confidence interval; eGFR, estimated glomerular filtration rate; EXT, exostosin; MN, membranous nephropathy; OR, odds ratio.

^aVariable forced in the multivariable model.

cohort and the current study, being 35.0% and 38.4%, respectively. Second, both studies remarkably revealed that, at baseline, EXT-positive patients were younger, with better renal function, greater daily proteinuria, and less renal parenchyma scars on biopsy specimens. Third, after a similar follow-up period, both studies found that renal prognosis was better in the EXT-positive cases, in terms of both renal function and proteinuria. Thus, our series can be considered as an independent validation cohort of the study by Ravindran *et al.*⁴

Our work brings additional original findings. First, owing to a closer histologic follow-up in our series (patients are mostly sedentary), we found that EXT-negative patients had significantly more repeat biopsies with proliferative class 3 or 4 lupus nephritis since the diagnosis of SLE-MN. The possibility for patients with SLE-MN to evolve toward proliferative glomerulonephritis is a well-known pejorative and relatively frequent event challenging the management of patients with lupus.^{5,6} Although other studies are needed to confirm this result, EXT immunohistochemical typing may help in stratifying patients with SLE-MN according to their risk of evolution toward proliferative lupus nephritis and therefore dictate personalized clinical monitoring. The presence of EXT in the immune deposits seems to be associated with a better prognosis also in the patients with mixed (3 + 5 and 4 + 5) classes as they did not develop end-stage kidney disease despite proliferative lesions as compared with those that were EXT negative in the Mayo Clinic study.⁴ Second,

multivariable analysis suggested that the EXT status independently predicted clinical remission at the end of follow-up. Nevertheless, this analysis should be considered as exploratory given the relatively small number of patients and the large confidence intervals of odds ratio, and the results should be confirmed by studies focusing on time to clinical response postbiopsy.

The mechanisms leading to the subepithelial accumulation of EXT1/2 and its pathophysiological consequences remain to be elucidated. It is not known whether EXT1/2 is an antigen or a biomarker as antibodies have not been identified yet. The heterodimer formed by EXT1 and EXT2 seems to play a key role in the making of the glomerular basement membrane by adding glycosyl residues to the protein core of proteoglycans.⁷ One may hypothesize that diversion of this enzyme to immune deposits might alter the glomerular basement membrane biosynthesis and account for the higher proteinuria at the time of kidney biopsy, but the discrepancy between the better prognosis of EXT-positive SLE-MN and the higher proteinuria at baseline remains intriguing and unexplained.

In summary, our findings echo to those of the Mayo Clinic⁴ and suggest that patients with EXT-positive SLE-MN have better renal outcome compared with patients with EXT-negative SLE-MN despite greater proteinuria at diagnosis. In light of these results, we recommend that immunohistochemical phenotyping of SLE-MN with anti-EXT antibody should be systematically performed by pathologists. Further studies are

needed to determine whether therapeutic approach should be different according to the EXT status of patients with SLE-MN.

DISCLOSURE

All the authors declared no competing interests.

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SUPPLEMENTARY MATERIAL

[Supplementary File \(PDF\)](#)

Methods.

Table S1: Treatment of SLE-MN.

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