Supplementary Material

Supplementary Methods

Study design and patients

All patients, who underwent native kidney biopsy and were diagnosed with primary or secondary membranous nephropathy (MN) from 2005 to 2018 at Jikei University Hospital (Tokyo, Japan), Jikei Kashiwa Hospital (Chiba, Japan), and Jikei Daisan Hospital (Tokyo, Japan), were included in this study.

Patients with rheumatoid arthritis (RA) were identified by reviewing the medical records of all enrolled MN patients. RA diagnosis was made according to the 2010 American College of Rheumatology (ACR)/ European Alliance of Associations for Rheumatology (EULAR) classification criteria. No patients fulfilled the following classification criteria for systemic lupus erythematosus (SLE): the 1997 ACR criteria, the 2012 Systemic Lupus International Collaborating Clinics (SLICC) classification criteria, or the 2019 EULAR/ACR criteria.

This study was approved by the ethics review board of Jikei University School of Medicine (33-386 [11010]) and was conducted in accordance with the Declaration of Helsinki. Since this was a retrospective study, information about the research plan was

posted, patients were offered the opportunity to refuse participation, and individual

informed consent was not required.

Clinical measurements

Clinical characteristics, including age, sex, body mass index (BMI), medical

history, medications, clinical details of RA (diagnosis date, diagnosis basis, involved

joints, treatments, interval to nephropathy onset, drug therapy duration), MN treatment,

and MN outcome were obtained from the medical records of patients. Laboratory data,

including serum albumin level, serum creatinine (Cr) level, 24-hour urine protein

quantitation at kidney biopsy, rheumatoid factor, C-reactive protein (CRP) level,

erythrocyte sedimentation rate (ESR), anti-citrullinated protein/peptide antibody

(ACPA) level, matrix metalloproteinases 3 (MMP-3) level, and anti-nuclear antibody

(ANA) level, were also collected. Estimated glomerular filtration rate (eGFR) was

defined by the following formula for Japanese subjects: eGFR (mL/min per 1.73 m^2) =

$194 \times Cr^{-1.094} \times age^{-0.287}$ ($\times 0.739$ for women). BMI was calculated as the body weight

divided by the square of height.

Histopathological findings from kidney biopsy

Paraffin-embedded kidney biopsy specimens from all enrolled MN patients were subjected to immunostaining for NELL1. Briefly, 3-μm thick sections were stained with rabbit polyclonal anti-NELL1 primary antibody (1:100 dilution; HPA012657, Sigma-Aldrich) after heat-induced antigen retrieval. NELL1 positivity was confirmed by frozen tissue staining if available. Tissue samples from all enrolled MN cases were stained for IgG, IgA, IgM, C3, and C1q. All NELL1-positive cases and NELL1-negative cases with RA were stained for phospholipase A2 receptor (PLA2R) (1:100 dilution; HPA051535, Sigma-Aldrich). Electron microscopy findings were evaluated for the presence of electron-dense deposits. MN stage was determined according to the Ehrenreich-Churg classification. Specimens containing five or more glomeruli were eligible for NELL1-staining evaluation. MN was classified as segmental if subepithelial deposits were present in at least 25% but no more than 75% of the total glomerular capillaries by light microscopy, immunofluorescence, or electron microscopy.

Supplementary References

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Supplementary Table S1. Comparison of clinical characteristics at the time of diagnostic kidney biopsy and histopathological findings between NELL1-positive and NELL1-negative cases suspected with bucillamine-induced MN.

| NELL1 | Case | Clinical characteristics at the time of kidney biopsy | | | | | | | | Kidney biopsy findings | | | | | | |
		Age (years)	Sex	BMI (kg/m²)	Duration of RA (years)	eGFR (ml/min/ 1.73 m²)	UPCR (g/gCr)	Treatment for RA	Interval from BUC initiation to proteinuria (months)	IgG	IgA	IgM	C3	C1q	Distribution of deposits	Ehrenreich-Churg Stage
Positive	1	61	F	18	4	40	1.4	PSL, BUC	3	(+)	(-)	(+)	(-)	(+)	Seg	III
	2	54	M	23	1	66	3.4	BUC	6	(+)	(-)	(+)	(-)	(+)	Diff	I
	4	81	M	22	0.4	47	4.6	BUC, mPSL	3	(+)	(-)	(-)	(-)	(+)	Seg	I
Negative	5	58	F	19	10	95	3.7	BUC	57	(+)	(-)	(+)	(-)	(+)	Diff	I
	6	53	F	17	33	86	0.6	BUC	1	(+)	(-)	(+)	(-)	(+)	Diff	I
	7	58	F	22	18	16	3.8	BUC	16	(+)	(+)	(-)	(-)	(+)	Diff	I

Abbreviations: BMI, body mass index; BUC, bucillamine; Cr, creatinine; Diff, diffuse; eGFR, estimated glomerular filtration rate; MN, membranous nephropathy; mPSL, methylprednisolone; PSL, prednisolone; RA, rheumatoid arthritis; Seg, segmental; UPCR, urine protein/creatinine ratio.