Supplemental Material

Table of Contents

Supplemental Materials and Methods

Clinical and laboratory evaluation

Estimated glomerular filtration rate (eGFR) was calculated using the Chronic Kidney Disease Epidemiology Collaboration equation[1]. Hypocomplementemia of C3 and C4 were considered with cutoffs of 60 mg/dl and 12 mg/dl. The abnormal serum free light chain ratio was identified beyond the range of 0.26–1.65 for patients with eGFR≥60 ml/min/1.73 m^2 and beyond the range of 0.37–3.10 for patients with eGFR> 60 ml/min/1.73 m^2 [2]. Complete remission was defined as remission of proteinuria to <500 mg/d with normal kidney function; partial remission was defined as a reduction in proteinuria by at least 50% and to <2 g/d with stable kidney function (no more than a 20% increase in serum creatinine compared with the baseline); and persistent kidney dysfunction was defined as failure to meet the criteria for either complete remission or partial remission but without kidney failure and included patients with unremitting proteinuria or progressive chronic kidney disease.

Kidney pathology

All kidney biopsies were examined by standard light microscopy, immunofluorescence, and electron microscopy. Paraffin tissues were stained with hematoxylin and eosin, periodic acid-Schiff, Masson trichrome, and periodic acid–silver methenamine. Immunofluorescence staining on 3-μm frozen tissues included IgG, IgM, IgA, C3, C1q, fibrinogen, albumin, κ, λ, and IgG1-4. Two kidney pathologists made histopathologic diagnoses independently. Differences in diagnosis between them were resolved by rereviewing the biopsy slides to reach a consensus.

Proteomic analysis of glomeruli by laser microdissection and mass spectrometry (LMD/MS)

Briefly, 10-μm-thick sections of formalin-fixed paraffin-embedded tissues were stained with

hematoxylin. Nonsclerotic glomeruli were laser microdissected using a Leica dissector (Leica LMD7). Peptides extracted from the microdissected tissue were subjected to liquid chromatography-tandem mass spectrometry. Raw data files of mass spectrometry were queried using the Sequest algorithm. The results were combined and assigned peptide and protein probability scores in Proteome Discoverer 2.4. The proteins for which the expression value accounted for ≥50% of any group of samples were retained. The proteins with a missing value of ≤50% were filled with the mean value of the same group samples, and credible proteins were obtained by median normalization and log2 conversion.

Detection of complement components deposited in kidneys by immunohistochemistry and immunofluorescence

The primary antibodies used included fluorescein isothiocyanate (FITC)-conjugated rabbit anti-human antibodies against C3c and C1q (Dako, Glostrup, Denmark), murine anti-human factor Bb (Quidel, San Diego, CA, USA), mouse anti-human mannose-binding lectin (Hycult Biotech, Uden, the Netherlands), rabbit anti-human C4d antibody (Biomedica, Vienna, Austria) and mouse anti-human C5b-9 antibody (Abcam, Cambridge, UK). The primary antibodies were all diluted in 0.01 mmol/L phosphate buffered saline in 1:50. Sections of kidney tissue from patients with lupus nephritis and minimal change disease were used as positive and negative controls, respectively.

Statistical analysis

In order to adjust for the influence of urinary protein excretion and increased levels of plasma complements on urinary complements concentration, an analysis of covariance model was used for adjustment: the model regarded the urinary level of complement components as a dependent variable, group as a factor and urinary protein excretion as a covariate. The nonnormally distributed data were

converted to normal distribution representation by logarithmic transformation, and values of zero were substituted by the minimum value.

Supplemental Table 1. Identification of immunoglobulins deposited in glomeruli

Case No.	LMD/MS	Positive immunoglobulins on immunofluorescence
P1	IgG3	IgG (3+), IgG3 (1+), κ (2-3+), λ Trace
P2	IgG3λ	IgG (4+), IgG3 (3+), κ Trace, λ (4+)
P3	IgG3κ	IgG (3+), IgG3 (2+), κ (3+),λ Trace
P4	IgG3λ	IgG (3+), IgG3 (2+), κ Trace, λ (3+)
P5	IgG3κ	IgG (2+), IgG3 (3+), κ (2-3+), λ Trace
P6	IgG1κ	IgG (1+), IgG1 (1-2+), κ (2-3+), λ Trace
P7	IgG1	IgG (3+), IgG1 (3+), κ (3+), λ (-)
P8	IgG1	IgG (2-3+), IgG1 (2+), κ (2+), λ Trace
P9	IgG1	IgG (3+), IgG1 (2+), κ (2+), λ (-)
P10	IgG3κ	IgG (2+), IgG3 (2+), κ (2-3+), λ Trace

Supplemental Table 2. Proteomic analysis of glomeruli by laser microdissection and mass spectrometry associated with IgG subtype and light chain isotype

Description	Accession	Abundance									
		P1	P2	P3	P4	P5	P6	P7	P8	P9	P10
Immunoglobulin heavy constant gamma 1	P01857	383.4	166.7	434.7	671.9	857.3	3330.9	3190.0	4324.3	8609.9	319.0
Immunoglobulin heavy constant gamma 2	P01859	218.4	106.0	90.0	156.5	194.7	85.7	113.4	129.0	259.5	46.3
Immunoglobulin heavy constant gamma 3	P01860	2852.6	6031.5	4855.2	6682.9	5592.9	329.6	6.7	23.7	54.1	2635.0
Immunoglobulin heavy constant gamma 4	P01861	6.8	7.9	5.5	106.1	24.2	26.5	60.4	3.5	39.6	10.8
Immunoglobulin kappa constant	P01834	771.7	137.2	458.2	472.4	758.1	1278.2	932.2	255.6	564.1	390.7
Immunoglobulin kappa joining 1	A0A0A0MT89				40.8						
Immunoglobulin kappa light chain	P0DOX7	19.6	253.6	771.7	477.1	1272.9	15.1	13.6	604.3	1105.4	499.0
Immunoglobulin kappa variable 1-17	P01599						6.6	5.5	5.1	17.1	
Immunoglobulin kappa variable 1-27	A0A075B6S5	14.3	3.8	1.2	3.4	13.4	6.0	46.1	9.4	49.8	
Immunoglobulin kappa variable 1-6	A0A0C4DH72	175.5	83.2	8.1	83.2	83.2	522.0		26.2	84.3	16.0
Immunoglobulin kappa variable 1D-33	P01593				3.7						4.3
Immunoglobulin kappa variable 2-29	A2NJV5			117.4							
Immunoglobulin kappa variable 2-40	A0A087WW87	68.0	56.8		45.6	468.8	73.0		95.2	198.6	47.9
Immunoglobulin kappa variable 3-15	P01624	53.0		1349.3	73.2	66.0	67.7	54.5	97.8	143.9	59.8
Immunoglobulin kappa variable 3-20	P01619	198.8	40.8	69.2	185.8			97.7	174.9	114.2	114.0
Immunoglobulin kappa variable 3D-11	A0A0A0MRZ8		33.8	71.3	34.7	45.2		38.3		94.7	37.4

Supplemental Table 2. Proteomic analysis of glomeruli by laser microdissection and mass spectrometry associated with IgG subtype and light chain isotype

Description	Accession	Abundance									
		P1	**P2**	**P3**	**P4**	**P5**	**P6**	**P7**	**P8**	**P9**	**P10**
Immunoglobulin kappa variable 3D-15	A0A087WSY6			7.8						25.7	
Immunoglobulin kappa variable 3D-20	A0A0C4DH25	6.4		89.5		114.8	59.9			136.8	
Immunoglobulin kappa variable 4-1	P06312	10.6		8.2	20.2	7.6	13.0			17.8	6.0
Immunoglobulin lambda constant 2	P0DOY2	378.2	2439.0	409.7	577.1	544.0	292.6	384.8	439.9	537.1	94.9
Immunoglobulin lambda constant 7	A0M8Q6				1525.9					48.8	
Immunoglobulin lambda variable 10-54	A0A075B6I4									7.6	
Immunoglobulin lambda variable 1-44	P01699									6.3	
Immunoglobulin lambda variable 1-47	P01700	22.5		35.9	24.3	38.7	30.9	27.7	65.5		4.2
Immunoglobulin lambda variable 1-51	P01701		453.4			55.2		53.7		177.0	
Immunoglobulin lambda variable 2-11	P01706						4.7				
Immunoglobulin lambda variable 3-1	P01715	63.6								95.3	
Immunoglobulin lambda variable 3-19	P01714		36.6			9.1					
Immunoglobulin lambda variable 3-21	P80748	13.4	15.2	8.8	38.9	28.3	9.3	21.8	32.1	42.3	16.7
Immunoglobulin lambda variable 3-27	P01718	7.5		7.0	6.5	9.9		6.4		22.0	11.3
Immunoglobulin lambda variable 4-69	A0A075B6H9	9.8	6.2			12.8	8.4	10.1	9.6	13.0	
Immunoglobulin lambda variable 7-46	A0A075B6I9	7.9		11.5	13.7	27.8	11.5	13.7		33.4	8.5

Supplemental Table 2. Proteomic analysis of glomeruli by laser microdissection and mass spectrometry associated with IgG subtype and light chain isotype

Description	Accession	Abundance									
		P1	**P2**	**P3**	**P4**	**P5**	**P6**	**P7**	**P8**	**P9**	**P10**
Immunoglobulin lambda variable 9-49	A0A0B4J1Y8	19.9		2.2		7.6	5.3	3.8			2.7
Immunoglobulin lambda-1 light chain	P0DOX8	310.3									214.9
Immunoglobulin lambda-like polypeptide 1	P15814				2.5						
Immunoglobulin lambda-like polypeptide 5	B9A064		72.4	147.7	105.6	301.5	196.5	80.1	127.8	296.6	

Supplemental Table 3. Correlation of urinary complements with clinicopathological parameters

Parameter	Serum creatinine at biopsy (mg/dl)		Urinary protein excretion (g/d)		Percentage of crescents (%)		Percentage of global glomerulosclerosis (%)	
	r	***P***	***r***	***P***	***r***	***P***	***r***	***P***
Urinary C3a/creatinine (ng/mg)	0.54	<0.001	0.55	<0.001	0.46	0.002	0.54	<0.001
Urinary C5a/creatinine (ng/mg)	0.58	<0.001	0.58	<0.001	0.46	0.002	0.43	0.004
Urinary sC5b-9/creatinine (ng/mg)[a]	0.47	0.001	0.54	<0.001	0.36	0.02	0.53	<0.001
Urinary C4d/creatinine (μg/mg)	0.48	0.001	0.59	<0.001	0.46	0.002	0.54	<0.001
Urinary C1q/creatinine (ng/mg)	0.26	0.09	0.32	0.04	0.40	0.01	0.63	<0.001
Urinary MBL/creatinine (ng/mg)[b]	0.56	<0.001	0.51	0.001	0.44	0.003	0.43	0.004
Urinary Bb/creatinine (μg/mg)	0.46	0.002	0.49	0.001	0.36	0.02	0.48	0.001

[a] sC5b-9, soluble C5b-9;

[b] MBL, mannose-binding lectin.

References

1. Levey AS, Stevens LA, Schmid CH, Zhang YL, Castro AF, Feldman HI, Kusek JW, Eggers P, Van Lente F, Greene T, Coresh J: A new equation to estimate glomerular filtration rate. *Ann Intern Med*, 150**:** 604-612, 2009
2. Hutchison CA, Harding S, Hewins P, Mead GP, Townsend J, Bradwell AR, Cockwell P: Quantitative assessment of serum and urinary polyclonal free light chains in patients with chronic kidney disease. *Clinical journal of the American Society of Nephrology : CJASN*, 3**:** 1684-1690, 2008 10.2215/cjn.02290508