

	usC5b-9 ≤ LOD (n=68)	usC5b-9 > LOD (n=56)	Overall (n=124)
Patients characteristics			
Female	36 (52.9%)	22 (39.3%)	58 (46.8%)
Age (years)	55.5 [39.0; 71.0]	59.5 [45.5; 72.5]	59.0 [41.5; 72.0]
Obesity (BMI ≥ 30 kg/m ²)	16 (23.5%)	8 (14.3%)	24 (19.4%)
Diabetes Mellitus	9 (13.2%)	9 (16.1%)	18 (14.5%)
Serum creatinine (□mol/L)	146 [93; 195]	145 [101; 227]	145 [96; 209]
Estimated glomerular filtration rate	42.5 [30.0; 71.8]	43.5 [25.0; 59.0]	43.0 [28.0; 66.5]
Urinary albumin to creatinine ratio	30.0 [5.8; 108.0]	205.0 [131.0; 649.0]	106.0 [15.3; 314.0]
Serum sC5b-9	168 [124; 242]	210 [164; 316]	181 [142; 266]
Serum Bb	1.12 [0.87; 1.52]	1.18 [0.91; 1.77]	1.15 [0.88; 1.60]
Renal pathology features			
Type of nephropathy			
Glomerular	32 (47.1%)	38 (67.9%)	70 (56.5%)
Tubulo-interstitial	23 (33.8%)	6 (10.7%)	29 (23.4%)
Vascular	13 (19.1%)	12 (21.4%)	25 (20.2%)
Glomerular immune deposits			
	19 (27.9%)	21 (37.5%)	40 (32.3%)*
Activity score (0-12)*			
0	29 (42.6%)	30 (53.6%)	59 (47.6%)
1	18 (26.5%)	7 (12.5%)	25 (20.2%)
2	10 (14.7%)	6 (10.7%)	16 (12.9%)
3	8 (11.8%)	4 (7.1%)	12 (9.7%)
4	0 (0%)	4 (7.1%)	4 (3.2%)
5	2 (2.9%)	2 (3.6%)	4 (3.2%)
6	1 (1.5%)	1 (1.8%)	2 (1.6%)
7	0 (0%)	1 (1.8%)	1 (0.8%)
8	0 (0%)	1 (1.8%)	1 (0.8%)
Activity score components			
Crescent score			
0	61 (89.7%)	45 (80.4%)	106 (85.5%)
1	2 (2.9%)	3 (5.4%)	5 (4.0%)
2	3 (4.4%)	3 (5.4%)	6 (4.8%)
3	2 (2.9%)	5 (8.9%)	7 (5.6%)
Endocapillary hypercellularity score			
0	63 (92.6%)	46 (82.1%)	109 (87.9%)
1	5 (7.4%)	6 (10.7%)	11 (8.9%)
2	0 (0%)	1 (1.8%)	1 (0.8%)
3	0 (0%)	3 (5.4%)	3 (2.4%)
Mesangial hypercellularity score			
0	56 (82.4%)	48 (85.7%)	104 (83.9%)
1	10 (14.7%)	3 (5.4%)	13 (10.5%)
2	2 (2.9%)	4 (7.1%)	6 (4.8%)
3	0 (0%)	1 (1.8%)	1 (0.8%)

Interstitial inflammatory score			
0	40 (58.8%)	38 (67.9%)	78 (62.9%)
1	16 (23.5%)	14 (25.0%)	30 (24.2%)
2	7 (10.3%)	3 (5.4%)	10 (8.1%)
3	5 (7.4%)	1 (1.8%)	6 (4.8%)
Chronicity score (0-7)			
0	8 (11.8%)	8 (14.3%)	16 (12.9%)
1	11 (16.2%)	14 (25.0%)	25 (20.2%)
2	13 (19.1%)	6 (10.7%)	19 (15.3%)
3	11 (16.2%)	4 (7.1%)	15 (12.1%)
4	16 (23.5%)	5 (8.9%)	21 (16.9%)
5	6 (8.8%)	9 (16.1%)	15 (12.1%)
6	3 (4.4%)	6 (10.7%)	9 (7.3%)
7	0 (0%)	4 (7.1%)	4 (3.2%)
Chronicity score components			
Glomerulosclerosis score			
0	22 (32.4%)	18 (32.1%)	40 (32.3%)
1	23 (33.8%)	19 (33.9%)	42 (33.9%)
2	12 (17.6%)	5 (8.9%)	17 (13.7%)
3	11 (16.2%)	14 (25.0%)	25 (20.2%)
Interstitial fibrosis score			
0	13 (19.1%)	16 (28.6%)	29 (23.4%)
1	25 (36.8%)	13 (23.2%)	38 (30.6%)
2	23 (33.8%)	20 (35.7%)	43 (34.7%)
3	7 (10.3%)	7 (12.5%)	14 (11.3%)
Vascular score			
0	58 (85.3%)	34 (60.7%)	92 (74.2%)
1	10 (14.7%)	22 (39.3%)	32 (25.8%)

Supplemental Table S1: Characteristics of included patients with detectable or undetectable urinary sC5b-9.

Abbreviations: LOD, limit of detection (3.7 ng/mL for sC5b-9).

* Activity score empty classes 9 to 12 are not reported

	usC5b-9 / uCr		uBb -9 / uCr	
	τ or median by group	p-value	τ or median by group	p-value
Sex	0.66 - 0	0.165	0 - 0	0.150
Age	0.07	0.291	0.14	0.038
Obesity	0 - 0	0.168	0 - 0	0.925
Diabetes mellitus	0 - 0.28	0.517	0 - 0	0.190
Serum creatinine	0.04	0.548	0.10	0.166
eGFR	-0.05	0.478	-0.11	0.103
Urinary ACR	0.48	<0.001	0.44	<0.001
Serum sC5b-9	0.17	0.011	0.15	0.036
Serum Bb	0.03	0.712	0.13	0.073
Type of nephropathy	1.06 - 0 - 0	0.015	0 - 0 - 0	0.098
IgG deposits	0.13	0.097	0.03	0.749
IgA deposits	-0.07	0.370	-0.08	0.357
IgM deposits	0.06	0.464	-0.05	0.548
c3 deposits	0.03	0.714	0.05	0.572
c1q deposits	-0.09	0.252	-0.04	0.628
Any glomerular immune deposit	0.05	0.503	0.04	0.613
Activity score	-0.08	0.259	-0.11	0.169
Crescents score	0.01	0.892	-0.14	0.086
Interstitial inflammation score	-0.12	0.120	-0.07	0.416
Endocapillary hypercellularity score	0.12	0.145	0.07	0.433
Mesangial hypercellularity score	-0.06	0.468	-0.09	0.288
Chronicity score	0.06	0.427	0.15	0.047
Glomerulosclerosis score	0.04	0.598	0.08	0.325
Interstitial fibrosis score	0.02	0.831	0.18	0.018
Vascular score	0.25	0.002	0.24	0.004

Supplemental Table S2: Kendall correlation (τ) and associated p-value for ordered variables. For categorical non-ordered variables, median by group are presented as well as the p-value of exact Wilcoxon test or Kruskal-Wallis. No correction for multiplicity of test is applied.

Kidney pathology scores

Histopathology score was adapted from the C3 glomerulopathy histopathology index devised by Bomback et al⁵⁶. The index includes both activity and chronicity scoring categories.

Activity score (0-12).

Crescent score, Crescent formation score corresponding to % of glomeruli with cellular and/or fibrocellular crescents (0% = 0; 1-10% = 1; 11-25% = 2; > 25% = 3).

Endocapillary hypercellularity score, Endocapillary hypercellularity/proliferation corresponding to % of glomeruli with increased number of cells within glomerular capillary lumina causing luminal narrowing (none = 0; 1-25% = 1; 26-50% = 2; > 50% = 3).

Mesangial hypercellularity score, Mesangial hypercellularity corresponding to % of glomeruli with > 3 mesangial cells per mesangial area (none = 0; 1-25% = 1; 26-50% = 2; > 50% = 3).

Interstitial inflammatory score, Interstitial inflammation score corresponding to % of cortical tubulointerstitial area of inflammation in non-fibrotic cortex (< 10% = 0; 10-25% = 1; 26-50% = 2; > 50% = 3).

Chronicity score (0-7).

Glomerulosclerosis score, Glomerulosclerosis corresponding to % of glomeruli with global sclerosis (< 10% = 0; 10-25% = 1; 26-50% = 2; > 50% = 3).

Interstitial fibrosis score, corresponding to % of cortical tubulointerstitial area involved with interstitial fibrosis (< 10% = 0; 10-25% = 1; 26-50% = 2; > 50% = 3).

Vascular score, grading the degree of arterial and arteriolar lesions (0 or 1).

Methods

Between the 1st of January 2021 and the 31st of January 2023, 124 consecutive patients undergoing a kidney biopsy (KB) in the department of Nephrology and Hypertension at CHUV, Lausanne, were prospectively included in the study. For all patients, urine samples were collected at the time of KB (two hours before the procedure). Samples were stored on ice, immediately transferred to the immunology laboratory with a measurement done in routine within 2 hours, without any storage, freezing and thawing processes that may induce complement activation ex vivo. uBb and usC5b-9 levels were measured using commercially available kits (Quidel, San Diego, CA, USA) and expressed as a ratio to urine creatinine (uCr): uBb/uCr ratio and usC5b-9/uCr ratio in $\mu\text{g}/\text{mmol}$ and ng/mmol , respectively. All patients gave informed consent for inclusion in the study that was approved by the local ethics committee

(CER-VD; number 2023-00410). Relevant clinical data were extracted from patients' medical records. KB were blindly reviewed, and inflammatory and fibrotic changes were scored using a semi-quantitative scale (see Kidney pathology score in Supplemental Data).

Categorical data were described by count and frequencies, continuous data were described by 1st quartile, median and 3rd quartile. uBb/uCr and usC5b9/uCr ratio cannot be computed for values below LOD. Values below LOD were imputed to 0 (the choice of this value is not important as statistical analysis relies only on non-parametric approach). To assess the correlation uBb/uCr and usC5b9/uCr ratio and other variables, Kendall correlation (τ) and associated p-value for ordered variable are reported. For categorical non-ordered values, the median of uBb/uCr and usC5b9/uCr by group are presented as well as the p-value of exact Wilcoxon test or Kruskal-Wallis. No correction for multiplicity of tests was applied, however, in order to avoid too many false positive a threshold of 0.01 was chosen.

Additionally, data were split in two groups: the one with a detectable level of usC5b-9 and the one without. Characteristics of these groups were described by count and frequencies, continuous data were described by 1st quartile, median and 3rd quartile.

.Analysis was performed with R version 4.3.0 (2023-04-21 ucrt)^{S7}. R packages Kendall^{S8}, pRoC^{S9} and coin^{S10} were used.

Supplemental References

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