Clinical Kidney Journal

Clinical Kidney Journal, 2023, vol. 16, no. 8, 1355–1358

https://doi.org/10.1093/ckj/sfad072
Advance Access Publication Date: 5 April 2023
Letter to the Editor

LETTER TO THE EDITOR

CD44 staining in parietal epithelial cells and early steroid response in patients with minimal change disease

Antoine Morel [1,2], Philippe Remy[1,2], Hamza Sakhi[1,2], Khalil El Karoui[1,2], Dil Sahali[1,2], Vincent Audard[1,2] and Anissa Moktefi [2,3]

[1]Assistance Publique des Hôpitaux de Paris (AP-HP), Hôpitaux Universitaires Henri Mondor, Service de Néphrologie et Transplantation, Centre de Référence Maladie Rare «Syndrome Néphrotique Idiopathique», Fédération Hospitalo-Universitaire "Innovative Therapy for Immune Disorders", Créteil, France, [2]Univ Paris Est Créteil, Institut National de la Santé et de la Recherche Médicale (INSERM) U955, Institut Mondor de Recherche Biomédicale (IMRB), Equipe 21, Créteil, France and [3]AP-HP, Hôpitaux Universitaires Henri Mondor, Département de Pathologie, Créteil, France

Correspondence to: Anissa Moktefi; E-mail: anissa.moktefi@aphp.fr

We read with great interest the article by Roca *et al.* [1] suggesting that positive staining for CD44 in parietal epithelial cells (PECs) may be associated with a higher prevalence of steroid resistance and a poorer renal outcome in both adults and children with minimal change disease (MCD). These results add to the growing evidence from experimental [2] and clinical studies [3, 4] that PEC activation, identified on the basis of *de novo* CD44 expression, can be considered an early sign of focal and segmental glomerulosclerosis (FSGS).

Our group led a randomized controlled open-label trial comparing low-dose corticosteroid plus enteric-coated mycophenolate sodium with standard corticosteroid treatment for adult patients with biopsy-proven MCD (MSN Study, NCT01197040) [5]. We showed that treatment with low-dose prednisone plus enteric-coated mycophenolate sodium was not superior to a standard high-dose prednisone regimen for inducing complete remission (CR) from MCD. As part of the MSN trial, we conducted a retrospective ancillary study [5] in which we compared PEC CD44 staining between patients with steroid-sensitive (steroid-S) MCD and patients without remission after 8 weeks of treatment.

The patients were considered to have steroid-S MCD if CR had been achieved 8 weeks after randomization. CR was defined as the return of urinary protein concentration (UPC) to values within the normal range (UPC ratio <30 mg/mmol or trace or negative results on repeat urine albumin dipstick tests) associated with an albumin concentration >30 g/L. It should be noted that the French criteria used to define CR in the MSN trial are slightly different from the KDIGO 2021 GN guidelines [6]. The absence of remission was defined as persistent proteinuria (>300 mg/mmol) and a persistent decrease in albumin concentration (to <30 g/L) after 8 weeks of treatment.

Nine of the 109 patients included in the MSN trial did not achieve remission after 8 weeks of treatment. Biopsy samples were unavailable or inadequate for three of these patients. We therefore compared PEC CD44 (Abcam, Cambridge, UK) staining between 15 patients with steroid-S disease and 6 patients without remission after 8 weeks of treatment.

Like Roca *et al.*, we scored CD44 staining as positive if at least one cell at the site of the PECs or at the anatomic site of the visceral epithelial cells over the glomerular tuft was positive. Unlike experimental murine models, in which the PECs on Bowman's capsule are CD44 positive, the activated PECs in human renal biopsy specimens are mostly found at visceral sites [2, 3]. As CD44 is also expressed by macrophages and leukocytes,

Received: 28.12.2022; Editorial decision: 3.4.2023

Table 1: Baseline characteristics of the 21 adult patients with minimal change nephrotic syndrome included in the ancillary study of the MSN trial.

Characteristics	Whole cohort, N = 21	Steroid-sensitive at Week 8, N = 15	No remission at Week 8, N = 6	P-value
Age, years	44 (36–56)	44 (37–56)	40 (31–56)	.39
Male	13 (62)	9 (60)	4 (67)	1.00
Standard corticosteroid treatment[a]	13 (61.9)	9 (60)	4 (67)	1.00
Medical history of atopic disease	8 (40)	7 (46.7)	1 (20)	.60
Medical history of diabetes mellitus	2 (10)	2 (13.3)	0 (0)	1.00
Basal weight (kg)	73 (65–87)	73 (65–87)	77 (65–90)	.94
Weight minus basal weight (kg)	3 (2–6)	3 (0–5)	5.5 (3–9)	.09
Systolic blood pressure (mmHg)	130 (120–143)	130 (120–143)	132 (129–145)	.41
Diastolic blood pressure (mmHg)	80 (71–87)	75 (70–87)	82 (80–90)	.35
Hemoglobin concentration (g/dL)	14.2 (13.5–15.1)	14.2 (13.6–15.1)	13.9 (13.5–15.9)	.94
Platelet count (10^9/L)	303 (256–342)	294 (244–336)	331 (280–368)	.21
Neutrophil count (10^9/L)	4.74 (2.94–5.57)	4.95 (2.97–6.1)	3.87 (2.59–4.68)	.23
Serum albumin concentration (g/L)	15.9 (14–22.3)	15.4 (14–23.7)	19.6 (9–22)	.88
Serum creatinine concentration (µmol/L)	87 (68.1–110)	78 (59–101)	105 (72–123)	.16
eGFR (mL/min/1.73 m^2)	80 (63.9–112.3)	86.6 (63.9–125.3)	67.3 (52.5–76.9)	.14
Hemoglobin A1c level (%)	5.45 (5.1–5.5)	5.5 (5–5.6)	5.3 (5.3–5.5)	.86
C-reactive protein concentration (mg/L)	2.6 (0.8–5)	4 (0.8–5)	1.3 (1–11.9)	.86
Hematuria (/mL)	10 000 (2000–12 000)	10 000 (7000–18 000)	5200 (10–10 000)	.19
Leukocyturia (/mL)	10 000 (6000–18 000)	10 000 (8000–18 000)	5500 (198–58 000)	.41
Urine protein/creatinine ratio (mg/mmol)	934 (491–1175)	951 (595–1175)	712 (483–1516)	1.00
Total cholesterol concentration (g/L)	3.77 (3.4–4.94)	3.52 (3.19–5.41)	3.88 (3.87–4.0)	.52
Triglyceride concentration (mmol/L)	2.72 (1.58–3.52)	2.50 (1.46–3.4)	3.28 (2–3.52)	.73
HDL (g/L)	0.62 (0.51–0.87)	0.62 (0.51–0.81)	0.61 (0.48–0.87)	.73
LDL (g/L)	2.67 (1.46–3.5)	2.51 (1.45–3.63)	3.07 (2.69–3.08)	.59
ACE inhibitors or ARB use	7 (33)	4 (27)	3 (50)	.60
PEC CD44-positive staining	4 (19)	3 (20)	1 (17)	1.00
Ectopic PEC PAX8-positive staining	4 (19)	3 (20)	1 (17)	1.00
Ectopic PEC CK19-positive staining	1 (5)	1 (7)	0 (0)	1.00

Categorical variables are expressed as N (%). Continuous data are presented as medians (interquartile range, Q1–Q3).
Glomerular filtration rate was estimated with the abbreviated Modification of Diet in Renal Disease formula.
Quantitative and qualitative variables were analyzed in Mann–Whitney and Fisher's tests, respectively.
[a]Standard corticosteroid treatment (1 mg/kg/day) relative to low-dose corticosteroid (0.5 mg/kg/day) plus enteric-coated mycophenolate sodium for adult MCD patients included in a published randomized controlled open-label trial (the MSN Study) [5].
ACE inhibitors, angiotensin converting enzyme inhibitors; ARB, angiotensin receptor blockers; eGFR, estimated glomerular filtration rate; HDL, high-density lipoprotein cholesterol; LDL, low-density lipoprotein cholesterol.

we considered only the cells overlying the capillary tuft on the outside of the glomerular basement membrane [4]. We also performed immunohistochemistry with two constitutive PEC markers [PAX8 (Zytomed, Berlin, Germany) and CK19 (Leica)] to detect ectopic PECs over the glomerular tuft [7]. Staining for PAX8 and CK19 was considered positive if at least one of the cells on the tuft was positive.

The characteristics of the patients at baseline are reported in Table 1. Most of the patients in the total cohort were male (62%), and the median age was 44 (36–56) years. Median serum albumin concentration was 15.9 (14–22.3) g/L, median UPC was 934 (491–1175) mg/mmol and estimated glomerular filtration rate was 80 (63.9–112.3) mL/min/1.73 m^2. The baseline characteristics of the patients and the proportion of patients on standard corticosteroid treatment were similar in the two groups (60% vs 67%, respectively, $P = 1.00$).

In our cohort, CD44-positive PECs were observed in 19% of MCD biopsy specimens and similarly in patients without remission [$n = 1$ (17%)] and in patients with steroid-S disease [$n = 3$ (20%)] (Fig. 1). The proportion of patients with PAX8- and CK19-positive PECs over the glomerular tuft was similar in the two groups ($P = 1.00$ for both markers).

The overall prevalence of CD44-positive PECs was lower than reported by Roca *et al.* (35.2%) [1]. Smeets *et al.* reported that immunohistochemical staining for three markers (CD44, Claudin1 and LKIV69) could detect early sclerotic lesions in 25% of the biopsy specimens originally diagnosed as MCD [3]. However, CD44, which was expressed in only 60% of cases, was the least sensitive marker for detecting early sclerotic lesions, which were small and often located close to the glomerular tip. They may, therefore, have been glomerular tip lesions [3], a histologic FSGS variant known to be associated with a response to steroid treatment similar to that of MCD [8, 9].

In our cohort of prospectively followed adult patients with a first episode of biopsy-proven MCD, PEC CD44 staining was not associated with early steroid-response status after 8 weeks of treatment. The primary endpoint of the MSN study was the CR rate after 4 weeks of treatment. Patients not achieving CR by Week 8 were eligible for a second-line regimen in accordance with the usual practices of the investigating center (maintenance dose of steroids ± cyclosporine). By contrast, Roca *et al.* evaluated steroid response after a minimum of 16 weeks of exposure to corticosteroids. For these reasons, our data obtained after 8 weeks of treatment are not directly comparable

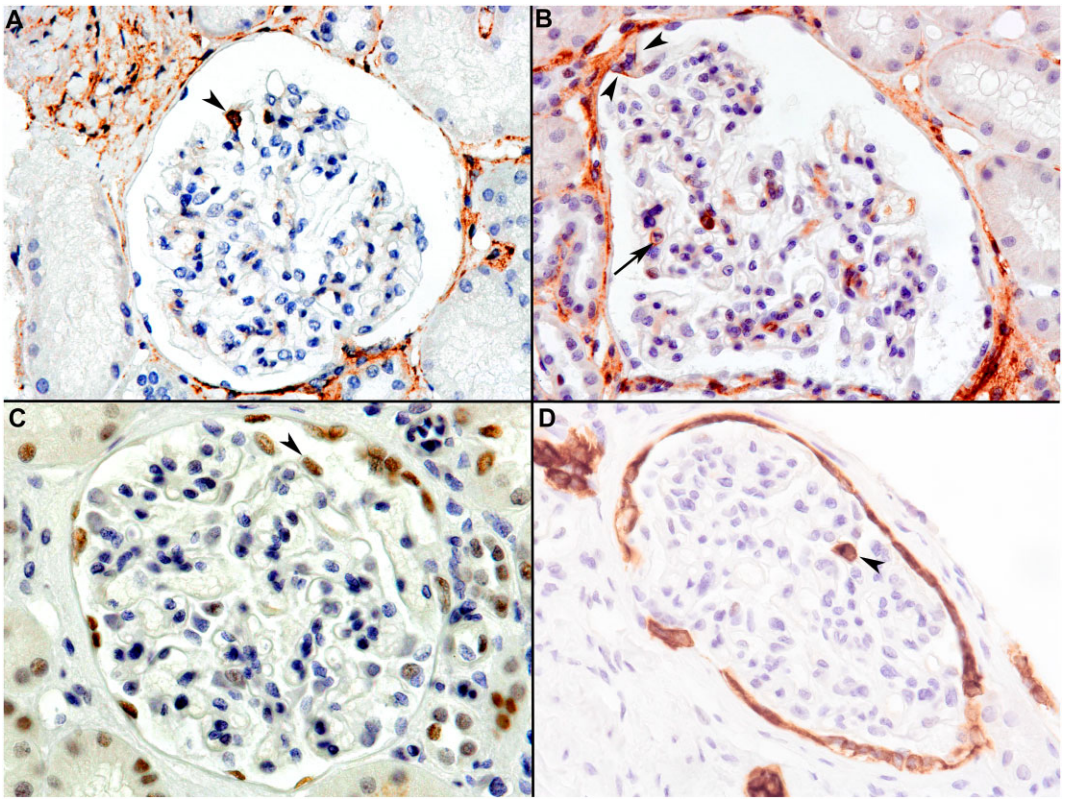

Figure 1: (**A**) CD44-positive activated PEC over the glomerular tuft, on the outside of the glomerular basement membrane (arrowhead) (immunohistochemistry for CD44 ×400). (**B**) CD44-positive staining at the anatomic site of the PECs on Bowman's capsule (arrowhead). CD44 expression in intracapillary leukocytes (black arrow) (immunohistochemistry for CD44 ×400). (**C**) PAX8-positive PEC over the glomerular tuft (arrowhead) (immunohistochemistry for PAX8 ×400). (**D**) CK19-positive PEC over the glomerular tuft (arrowhead) (immunohistochemistry for CK19 ×400).

to those obtained by Roca after 16 weeks of steroid therapy. The small sample size of our cohort also limits the conclusions that can be drawn concerning the relevance of CD44 expression in PECs as a biomarker for differentiating between steroid-S MCD and patients without remission. Further studies are required to determine whether histological markers are predictive of steroid response in adult MCD patients.

ACKNOWLEDGEMENTS

We thank Prof. David Buob (Pathology Department, Tenon Hospital, Paris), Prof. Jean-Paul Duong Van Huyen (Pathology Department, Necker Hospital, Paris), Dr Elodie Miquelestorena Standley (Pathology Department, Tours University Hospital, Tours) and Dr Carole Cordonnier (Pathology Department, Amiens University Medical Center, Amiens) for providing with renal biopsy samples from the patients included in this study.

We thank Julie Sappa for her editorial assistance.

FUNDING

This work was funded by ORKiD (Orphan Kidney Diseases) network.

PATIENT CONSENT

The study was carried out in accordance with the Declaration of Helsinki and was approved by the institutional review board (Comité de Protection des Personnes Ile- de-France II # 2009-04-02). Written informed consent was obtained from all patients.

CONFLICT OF INTEREST STATEMENT

V.A. reports payment or honoraria for lectures, presentations, speakers bureaus, manuscript writing or educational events from Travere outside the submitted work, support for attending meetings and/or travel from Sanofi Genzyme outside the submitted work, and membership of a Data Safety Monitoring Board or Advisory Board for Alnylam, Addmedica and Travere outside the submitted work. A.M., P.R., H.S., K.E.K., D.S. and A.M. declare no conflicts of interest. The results presented in this paper have not been published previously, in whole or in part, except in abstract format.

REFERENCES

1. Roca N, Jatem E, Abo A *et al*. CD44-negative parietal–epithelial cell staining in minimal change disease: association with clinical features, response to corticosteroids and kidney outcome. *Clin Kidney J* 2021;**15**:545–52. https://doi.org/10.1093/ckj/sfab215.
2. Smeets B, Kuppe C, Sicking EM *et al*. Parietal epithelial cells participate in the formation of sclerotic lesions in focal segmental glomerulosclerosis. *J Am Soc Nephrol* 2011;**22**:1262–74. https://doi.org/10.1681/ASN.2010090970.

3. Smeets B, Stucker F, Wetzels J *et al*. Detection of activated parietal epithelial cells on the glomerular tuft distinguishes early focal segmental glomerulosclerosis from minimal change disease. *Am J Pathol* 2014;**184**:3239–48. https://doi.org/10.1016/j.ajpath.2014.08.007.
4. Fatima H, Moeller MJ, Smeets B *et al*. Parietal epithelial cell activation marker in early recurrence of FSGS in the transplant. *Clin J Am Soc Nephrol* 2012;**7**:1852–8. https://doi.org/10.2215/CJN.10571011.
5. Rémy P, Audard V, Natella PA *et al*. An open-label randomized controlled trial of low-dose corticosteroid plus enteric-coated mycophenolate sodium versus standard corticosteroid treatment for minimal change nephrotic syndrome in adults (MSN Study). *Kidney Int* 2018;**94**:1217–26. https://doi.org/10.1016/j.kint.2018.07.021.
6. 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**:S1–276.
7. D'Agati VD, Shankland SJ. Recognizing diversity in parietal epithelial cells. *Kidney Int* 2019;**96**:16–9. https://doi.org/10.1016/j.kint.2019.02.036.
8. Stokes MB, Markowitz GS, Lin J *et al*. Glomerular tip lesion: a distinct entity within the minimal change disease/focal segmental glomerulosclerosis spectrum. *Kidney Int* 2004;**65**:1690–702. https://doi.org/10.1111/j.1523-1755.2004.00563.x.
9. D'Agati VD, Alster JM, Jennette JC *et al*. Association of histologic variants in FSGS clinical trial with presenting features and outcomes. *Clin J Am Soc Nephrol* 2013;**8**:399–406. https://doi.org/10.2215/CJN.06100612.