


ORIGINAL ARTICLE

CD44-negative parietal–epithelial cell staining in minimal change disease: association with clinical features, response to corticosteroids and kidney outcome

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ABSTRACT

Background. Activation of parietal–epithelial cells (PECs) with neo-expression of CD44 has been found to play a relevant role in the development of focal and segmental glomerulosclerosis (FSGS). The aim of this study was to analyse whether the expression of CD44 by PECs in biopsies of minimal change disease (MCD) is associated with the response to corticosteroids, with kidney outcomes and/or can be considered an early sign of FSGS.

Methods. This multicentric, retrospective study included paediatric and adult patients with MCD. Demographic, clinical and biochemical data were recorded, and biopsies were stained with anti-CD44 antibodies. The association between PECs, CD44 expression and the response to corticosteroids, and kidney outcomes were analysed using logistic, Kaplan–Meier and Cox regression analyses.

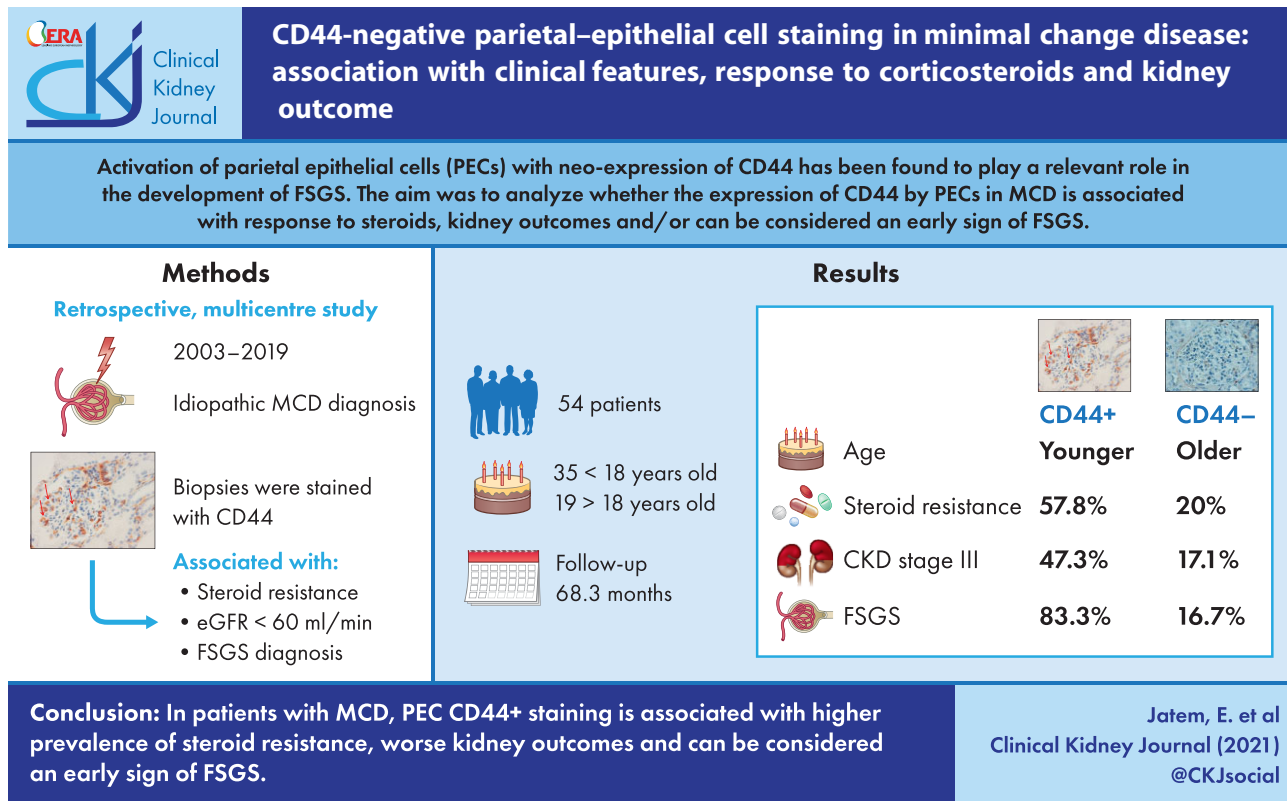
Results. A total of 54 patients were included: 35 (65%) <18 years and 19 (35%) adults. Mean follow-up was 68.3 ± 37.9 months. A total of 19/54 patients (35.2%) showed CD44-positive staining. CD44-positive patients were younger (14.5 ± 5 versus 21.5 ± 13, $P = 0.006$), and showed a higher incidence of steroid-resistance [11/19 (57.8%) versus 7/35 (20%), $P = 0.021$; odds ratio: 5.5 (95% confidence interval 1.6–18), $P = 0.007$] and chronic kidney disease [9/19 (47.3%) versus 6/35 (17.1%), $P = 0.021$; relative risk: 3.01 (95% confidence interval 1.07–8.5), $P = 0.037$]. Follow-up re-biopsies of native kidneys ($n = 18$), identified FSGS lesions in 10/12 (83.3%) of first-biopsy CD44-positive patients versus 1/6 (16.7%) of first-biopsy CD44-negative patients ($P = 0.026$).

Conclusions. In patients with a light microscopy pattern of MCD, CD44-positive staining of PECs is associated with a higher prevalence of steroid resistance and worse kidney outcomes, and can be considered an early sign of FSGS.

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GRAPHICAL ABSTRACT



Keywords: CD44 staining, focal and segmental glomerulosclerosis, idiopathic nephrotic syndrome, minimal change disease, parietal-epithelial cells

INTRODUCTION

Minimal change disease (MCD) and focal and segmental glomerulosclerosis (FSGS) are two of the main causes of nephrotic syndrome in both children and adults. In both entities, idiopathic or secondary forms are distinguished depending on whether or not an aetiology responsible for them is identified [1, 2]. In the idiopathic forms of both diseases, the response to corticosteroid treatment has been identified as the main long-term prognostic variable, even independently of the histopathological substrate [3]. The evidence of morphological lesions of FSGS is associated with a higher prevalence of corticosteroid resistance [3]. However, approximately 10–15% of children and up to 25% of adults with unequivocal histological lesions of MCD suffer corticosteroid resistance, either at the first cycle of treatment or later, after one or more relapses, during the course of the disease [2, 3]. Both diseases have a very similar clinical presentation and, to differentiate them, it is necessary to perform a kidney biopsy. However, the distinction between MCD versus early FSGS may be difficult, particularly when biopsy samples contain only a few glomeruli or when glomerular injury is at an early stage [4–6]. Recent evidence coming from experimental and clinical studies indicates that focal activation of parietal-epithelial cells (PECs), and the formation of cellular adhesions to the capillary tuft is one of the earliest stages of FSGS [7–12]. When the irreversible loss of podocytes exceeds a certain threshold,

compensatory hyperplasia of the remnant podocytes is not enough to avoid the appearance of denuded capillary areas that dilate and come into contact with the PECs, forming synechia. As a consequence of this interaction, the parietal cells become activated and change their phenotype, acquiring proliferation and migration properties [10, 13]. One of these phenotypic changes is the neo-expression of CD44 [6, 8, 11]. Recent studies have shown that CD44 expression in PECs is useful for differentiating between MCD and FSGS [14] and is one of the earliest signs seen in FSGS lesion recurrence after kidney transplantation [7]. Furthermore, in paediatric patients with FSGS, the expression of CD44 in parietal cells has been associated with a lower survival of renal function [8].

In the present study, we analysed whether the expression of CD44 by PECs in kidney biopsies tagged as MCD by light microscopy can be considered an early sign of FSGS, is associated with the response to the treatment to corticosteroids and/or with long-term kidney outcomes.

MATERIALS AND METHODS

This retrospective multicentre study was performed at four university hospitals. Paediatric (age ≤18 years) and adult (age >18 years) patients diagnosed with idiopathic MCD by renal biopsy between 2003 and 2019 were identified from the biopsy databases of each centre. Idiopathic MCD was diagnosed in the

absence of glomerular changes in light microscopy analysis, with evidence of diffuse podocyte effacement in electron microscope and in the absence of any associated aetiology, including lymphoma, drugs or identified genetic mutations. The demographic and biochemical variables at the time of diagnosis were recorded retrospectively from clinical cards. Hypertension was defined in paediatric patients as measured systolic blood pressure (BP) and/or diastolic BP \geq 95th percentile (on the basis of age, sex and height percentiles) of new normative BP tables [15] and in adults as a systolic pressure \geq 140 mmHg or diastolic pressure \geq 90 mmHg [16]. Other variables included were serum creatinine, estimated glomerular filtration rate (eGFR), serum albumin, serum cholesterol and urinary protein excretion. In paediatric patients, urinary protein excretion was recorded as protein-to-creatinine ratio (uPCR) and expressed in mg/mg. In adult patients, proteinuria was recorded in g/24 h. All the initial biochemical variables were recorded before starting any treatment.

We also recorded the indications for the practice of renal biopsy, the first-line treatment prescribed, including the dose and the time of exposure to corticosteroids, the response to the treatment with corticosteroids, the total follow-up period after diagnosis and the evolution of renal function throughout the follow-up period.

The files of the histopathological departments or the biobanks of each centre were also reviewed to determine the availability of paraffin blocks of kidney biopsies for immunohistochemical studies.

From the initial cohort of patients with idiopathic MCD identified in the four databases, those who met at least one of the following criteria were excluded: (i) unavailability or inadequate (<6 glomeruli) paraffin blocks for immunohistochemical studies, (ii) first-line treatment other than corticosteroids, (iii) non-evaluable or lower exposure to corticosteroid treatment, in terms of dose and duration, than that recommended in the Kidney disease improving global outcomes (KDIGO) guidelines to assess steroid-resistance [17], (iv) follow-up lower than 8 weeks (paediatric patients) or 16 weeks (adult patients) after diagnosis and (v) refusal or unavailability to obtain an informed consent.

A genetic study to rule out pathogenic genetic mutations was performed on patients who showed corticosteroid resistance and were <35 years old. Genetic studies were performed using a kidney panel that has previously been described and validated [18].

Operative definitions

Nephrotic syndrome was defined according to KDIGO guidelines of glomerular diseases [19].

Paediatric patients. Complete remission: uPCR <0.2 mg/mg. Partial remission: proteinuria reduction of 50% or greater from the presenting value and absolute uPCR between 0.2 and 2 mg/mg. No remission: failure to reduce urine protein excretion by 50% from baseline or persistent excretion uPCR >2 mg/mg.

Adult patients. Complete remission: reduction of proteinuria <0.3 g/day, stable serum creatinine and serum albumin >3.5 g/dL. Partial remission: proteinuria decrease of 50% or greater from the presenting value and absolute proteinuria reduction to 0.3–3.5 g/day with normoalbuminemia. No remission: failure to reduce urine protein excretion by 50% from baseline or persistent excretion 3.5 g/day with hypoalbuminemia despite prednisone for >16 weeks.

Outcome variables

Steroid resistance. In paediatric patients, steroid resistance was defined as lack of complete remission after 8 weeks of therapy with prednisone or prednisolone at standard dose (60 mg/m²/day). In adult patients, steroid resistance was defined as no remission after a minimum exposure of 16 weeks of prednisone at a daily single dose of 1 mg/kg (maximum 80 mg) or alternate-day single dose of 2 mg/kg (maximum 120 mg).

Kidney outcome. Kidney outcome was defined as the development of moderate or severe chronic kidney disease (CKD) stage 3 or lower (GFR categories G3a–G5), with an eGFR <60 mL/min/1.73 m² for >3 months, along the follow-up period, according to the KDIGO guidelines [17].

FSGS. The pathologic diagnosis of FSGS was established by the finding of at least one glomerulus with a segmental lesion and some of the remaining glomeruli were relatively normal.

Methods

Serum creatinine was measured by a traceable IDMS compensated method (Hitachi Modular P-800 Roche Diagnostics, Germany). The eGFR was calculated using the CKD Epidemiology Collaboration formula in adults and modified Schwartz equation in children.

Pathological analysis of renal biopsies. All kidney biopsies were centralized at the same centre (HUAV) and were analysed by the same pathologists. Kidney biopsies were stained with haematoxylin and eosin, periodic acid-Schiff-methenamine and Masson's trichrome for morphological analysis, and immunofluorescence studies were carried out with antibodies against IgA, IgG, IgM, C3, fibrinogen and light chains, and were processed for an electron microscope study.

Formalin-fixed paraffin-embedded tissue blocks were sectioned at a thickness of 3 μ m, dried for 1 h at 65°C before pre-treatment procedure of deparaffinization, rehydration and epitope retrieval in the Pre-Treatment Module, PT-LINK (Agilent Technologies-DAKO, Santa Clara, CA, USA) at 95°C for 20 min in 50 \times Tris/EDTA buffer, pH 9. Before staining the sections, endogenous peroxidase was blocked. The antibody used was against CD44 (1:100 dilution, clone 156-3C11, ABCAM, Cambridge, UK). After incubation with primary antibody, the reaction was visualized with the EnVision™ FLEX Detection Kit (Agilent Technologies-DAKO) using diaminobenzidine chromogen as a substrate. Sections were counterstained with haematoxylin. Appropriate negative controls including no primary antibody were also tested.

In each biopsy, all glomeruli, except for those with global sclerosis, were evaluated individually for the number of CD44-positive epithelial cells in an anatomical PEC location and over the glomerular tuft in an anatomical visceral epithelial cell (VEC) location. CD44 immune staining in PECs or in VECs was scored positive when at least one cell was positive for CD44.

Statistical analysis. Quantitative variables were expressed as the mean \pm 1 SD, and qualitative variables were expressed as proportions. Differences between two means were analysed by the Student's *t*-test for independent data. Comparisons between proportions were done using the Chi-squared test or the Fisher exact test. A logistic regression analysis was performed to analyse the association between CD44 PECs staining and the

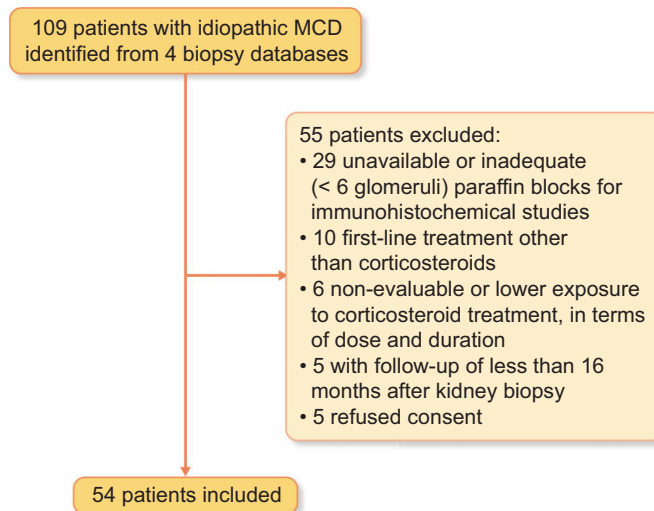


FIGURE 1: Flowchart of patient selection.

Table 1. Clinical and biochemical characteristics and outcomes of patients classified according to the presence of CD44 parietal cell staining

| | All, n = 54 | CD44 positive, n = 19 | CD44 negative, n = 35 | Sig. |
|---------------------------------------------------|-------------|-----------------------|-----------------------|--------------|
| Age (years), mean (SD) | 21.9 (17) | 14.5 (5) | 21.5 (13) | 0.006 |
| Gender (male), n (%) | 22 (40.7) | 19 (47.4) | 13 (37.1) | 0.33 |
| Creatinine (mg/dL), mean (SD) | 0.63 (0.48) | 0.64 (0.5) | 0.65 (0.4) | 0.98 |
| eGFR (mL/min/1.73 m ²), mean (SD) | 141 (27.8) | 135 (26) | 144 (27.8) | 0.27 |
| Albumin (g/dL), mean (SD) | 2.09 (0.52) | 2.1 (0.56) | 2.08 (0.50) | 0.89 |
| Cholesterol (mg/dL), mean (SD) | 373 (141) | 401 (130) | 358 (146) | 0.26 |
| uPCR paediatric (mg/mg), median (IQR), n = 35 | 12 (8.4–19) | 11.5 (9–17.5) | 12 (8–25) | 0.91 |
| Proteinuria adults (g/24 h), median (IQR), n = 19 | 11 (9–15) | 13 (9–16) | 10.5 (8.2–12) | 0.54 |
| Hypertension, n (%) | 1 (1.9) | 1 (5.3) | 0 | 0.35 |
| Microhematuria, n (%) | 2 (3.7) | 1 (5.3) | 1 (2.9) | 0.58 |
| Follow-up (months), mean (SD) | 68.3 (38) | 79.1 (39) | 62.5 (29.3) | 0.12 |
| Re-biopsy, n (%) | 18 (33.3) | 12 (63.1) | 6 (17.1) | 0.000 |
| Steroid resistance, n (%) | 18 (33.3) | 11 (57.8) | 7 (20) | 0.006 |
| CKD, n (%) | 15 (27.8) | 9 (47.3) | 6 (17.1) | 0.021 |

The bold values are the parameters with statistical significance.

response to corticosteroid treatment. Kaplan–Meier and Cox proportional hazards analysis were performed to evaluate the association between C44 staining and kidney outcome.

A P-value <0.05 was considered to be statistically significant. Statistical calculations were carried out using the SPSS 20.0 software.

This study adhered to the parameters established by the declaration of Helsinki. All patients gave their written informed consent. The bioethics committees of each centre approved the study.

RESULTS

Patient selection and clinical, biochemical and histological characteristics

A total of 109 patients with idiopathic MCD were initially screened. Of these, 55 patients were excluded for the following reasons: 29 patients (53%) had unavailability or inadequate (<6 glomeruli) paraffin blocks for immunohistochemical studies, 10 (18%) received as first-line other treatment than corticosteroids,

6 (11%) had either a non-evaluable or an inadequate exposure to corticosteroid treatment in terms of dose and duration, 5 (9%) had a follow-up shorter than 8 (paediatric) or 16 (adults) months after kidney biopsy, and 5 (9%) refused to give their written consent (Figure 1).

The final study group included 54 patients; the mean follow-up after diagnosis was 68.3 ± 37.9 months (max: 144 months, min: 24 months). At the time of diagnosis, 35 patients (65%) were aged <18 years, and 19 patients (35%) were adults (≥ 18 years). In paediatric patients, kidney biopsy was indicated because of corticosteroid resistance in 12 patients and because of steroid-dependence or frequent-relapses with poor response to treatment in 23 patients. In adult patients, kidney biopsy was indicated as a routine diagnostic procedure, after the onset of nephrotic syndrome.

Table 1 summarizes the main clinical and biochemical characteristics and the kidney outcome of the whole group after stratifying it according to the presence or absence of CD44-positive staining of PECs in the kidney biopsies. A total of 19/54 patients (35.2%) showed positive staining of CD44 in PECs. Of those, 17 patients (89.5%) also showed evidence of CD44 cells

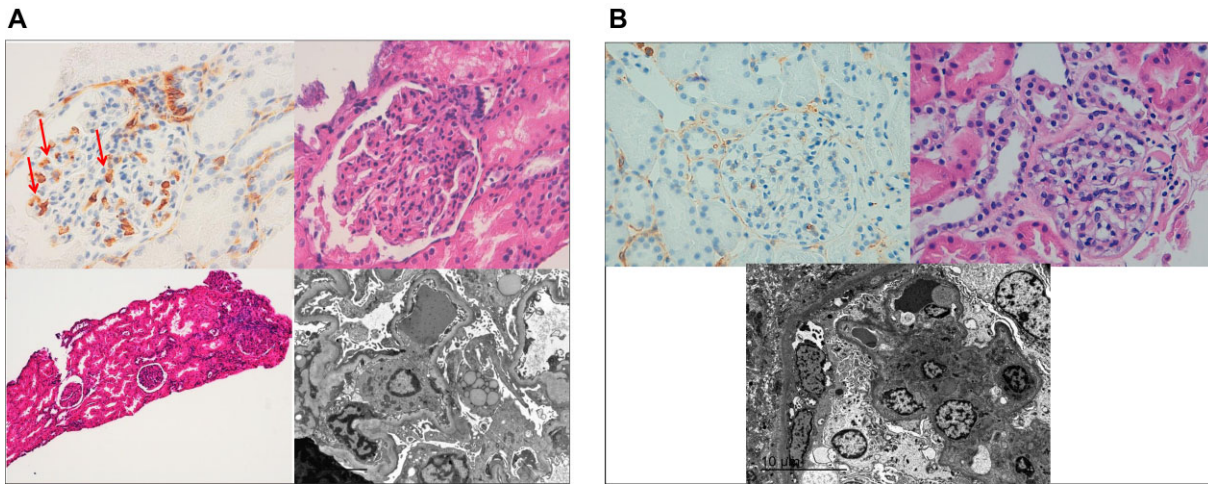


FIGURE 2: Examples of PEC CD44 staining in renal tissue of MCD patients. Panel (A) shows CD44-positive staining in Bowman's capsule and migration into glomerular tuft. The images show the glomerulus stained with CD44 ($\times 40$), the same glomerulus in haematoxylin and eosin (H&E) staining ($\times 40$), the H&E low magnification picture ($\times 10$) and the images in electron microscopy. Panel (B) shows CD44-negative staining in PECs. The images show the glomerulus stained with CD44 ($\times 40$), the same glomerulus in H&E staining ($\times 40$) and the images in electron microscopy.

Table 2. Patient characteristics according to the response to treatment with corticosteroids

| | Corticosteroid-sensitive, n = 36 | Corticosteroid-resistant, n = 18 | P |
|---------------------------------------------------|----------------------------------|----------------------------------|--------------|
| Clinical characteristics | | | |
| Age (years), mean (SD) | 23.3 (14.7) | 17.3 (9.4) | 0.057 |
| Serum creatinine (mg/dL), mean (SD) | 0.62 (0.5) | 0.60 (0.5) | 0.827 |
| eGFR (mL/min/1.73 m ²), mean (SD) | 138.1 (27.6) | 146.2 (26.8) | 0.863 |
| Albumin (g/dL), mean (SD) | 2.08 (0.5) | 2.2 (0.5) | 0.520 |
| uPCR paediatric (mg/mg), median (IQR) | 11.5 (8.6–26.5) | 13 (8–19) | 0.96 |
| Proteinuria adults (g/24 h) (mg/mg), median (IQR) | 9.5 (8–13.5) | 10.6 (9–12.2) | 0.34 |
| Cholesterol (mg/dL), mean (SD) | 384.6 (147.4) | 339.4 (120.6) | 0.352 |
| Histopathological characteristics | | | |
| CD44-positive PECs, n (%) | 8 (22.2) | 11 (61.1) | 0.006 |
| CD44-positive PECs migration, n (%) | 8 (22.2) | 9 (50) | 0.040 |

The bold values are the parameters with statistical significance.

inside the glomerular tuft. Figure 2 shows representative images of biopsies with positive (Figure 2A) and negative (Figure 2B) PECs CD44 staining. Different patterns of segmental CD44 staining in PECs coinciding with areas of synechiae between glomerular tuft and parietal epithelium are shown in Supplementary Data, Figure S1, and some examples of CD44-negative staining in healthy controls are shown in Supplementary Data, Figure S2.

When compared with CD44-negative patients, those with CD44-positive staining of PECs showed lower age, higher prevalence of steroid resistance, higher frequency of re-biopsy and higher incidence of CKD, but no significant differences in other clinical or biochemical variables at diagnosis.

Variables associated with the response to corticosteroid treatment

Overall, 18/54 patients (33.3%) showed steroid resistance. Table 2 summarizes the baseline characteristics of patients, classified according to the response to corticosteroid treatment. There were no differences in age, renal function, albumin, cholesterol or proteinuria between steroid-sensitive and steroid-resistant patients. Steroid-resistant patients showed a signifi-

cantly higher prevalence of CD44 immunostaining in comparison with steroid-sensitive patients [11/18 (61.1%) versus 8/36 (22.2%), $P = 0.006$]. Steroid-resistant patients also showed a higher prevalence of CD44-positive cells inside the capillary tuft than steroid-sensitive patients [9/18 (50%) versus 8/36 (22.2%), $P = 0.040$]. The odds ratio for steroid resistance associated with CD44-positive staining, was 5.5 [95% confidence interval (CI) 1.6–18, $P = 0.007$] with an area under the curve (AUC) of 0.69 [95% CI 0.54–0.85, $P = 0.021$].

Variables associated with CKD

During follow-up, 15 patients (27.8%) developed moderate or severe CKD with GFR categories G3a–G5. Table 3 summarizes the baseline clinical, biochemical and histological characteristics of patients according their kidney outcome. There were no differences in age, albumin, cholesterol or proteinuria between CKD and non-CKD groups. CKD patients showed higher initial serum levels of creatinine than non-CKD patients. According to the histological findings, the CKD group showed a higher prevalence of CD44-positive PECs (66.7% versus 23%, $P = 0.001$) and PECs migration (66.7% versus 17.9%, $P = 0.001$). In Kaplan–Meier

Table 3. Patient characteristics according to development of moderate or severe CKD with GFR categories G3a–G5

| | CKD, n = 15 | Non-CKD, n = 39 | P |
|-------------------------------------------------------|---------------|-----------------|--------------|
| Clinical characteristics | | | |
| Age (years), mean (SD) | 19.6 (9.1) | 22.1 (14.9) | 0.054 |
| Serum creatinine (mg/dL), mean (SD) | 0.75 (0.5) | 0.56 (0.5) | 0.003 |
| Initial eGFR (mL/min/1.73 m ²), mean (SD) | 144.4 (18.5) | 139.4 (30.8) | 0.052 |
| Albumin (g/dL), mean (SD) | 2.3 (0.4) | 2.1 (0.5) | 0.907 |
| uPCR paediatric (mg/mg), median (IQR) | 10.5 (8.1–18) | 13 (8.5–20) | 0.51 |
| Proteinuria adults (g/24 h) (mg/mg), median (IQR) | 11 (9.5–13.5) | 10.5 (8–12.5) | 0.48 |
| Cholesterol (mg/dL), mean (SD) | 340 (124) | 378 (143) | 0.822 |
| Corticosteroid-resistance, n (%) | 10 (66.7) | 8 (20.5) | 0.002 |
| Histopathological characteristics | | | |
| CD44 positive PECs, n (%) | 10 (66.7) | 9 (23) | 0.001 |
| CD44 positive PECs migration, n (%) | 10 (66.7) | 7 (17.9) | 0.001 |

The bold values are the parameters with statistical significance.

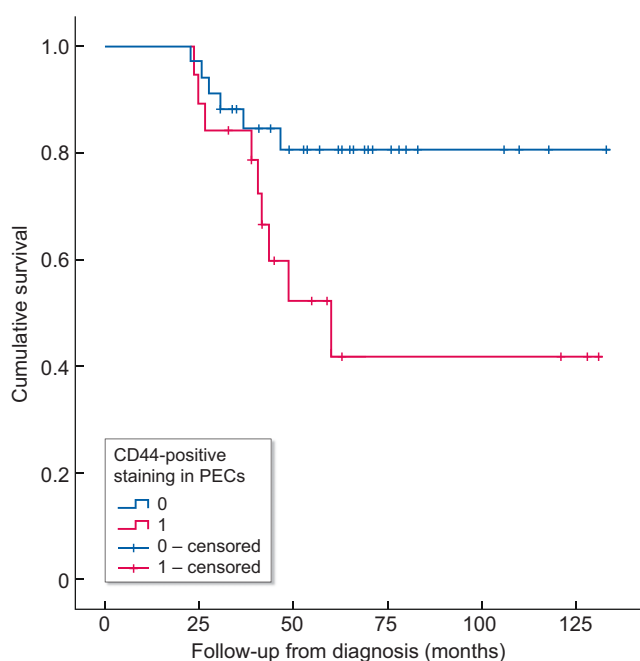


FIGURE 3: Kaplan-Meier analysis of the mean survival of kidney function according to CD44-positive staining in PECs.

analysis, the mean survival of kidney function was significantly higher in CD44-negative patients than in CD44-positive patients [mean survival 113.5 (95% CI 99.3–127) months versus 78.7 (95% CI 55.5–102), $P = 0.02$] (Figure 3). In Cox regression analysis, the relative risk of CKD was significantly higher in patients with CD44-positive staining [relative risk: 3.01 (95% CI 1.07–8.5), $P = 0.037$].

Re-biopsy studies

Follow-up biopsies of native kidneys were performed in 18/54 patients (33.3%) because of resistance to treatment and/or declining renal function after a median time of 31 months (P_{25} – P_{75} 12–43) from the first biopsy. A total of 12 out of 18 patients (66.6%) showed CD44-positive staining and 6 patients (33.3%) showed CD44-negative staining in their respective first kidney biopsies. In follow-up biopsies FSGS lesions were observed in 10/12 (83.3%) of CD44-positive patients versus 1/6 (16.7%) of CD44-negative pa-

tients ($P = 0.026$). A total of 8 out of the 15 patients who developed CKD received a kidney transplant (53.3%). Two out of them developed proteinuria within the first month after kidney transplantation, and were re-biopsied. In both cases, the kidney biopsy findings showed absence of morphological lesions in light microscopy but CD44-positive staining of PECs. Figure 4 shows a representative image of an early post-transplant biopsy of one of these patients. Both patients were treated with plasmapheresis, intravenous cyclosporine and corticosteroid pulses. Follow-up kidney biopsies performed 3 months later showed FSGS lesions in both cases.

DISCUSSION

This study shows that in idiopathic nephrotic syndrome patients with a histopathological pattern of MCD, CD44-positive staining of PECs is associated with higher risk of corticosteroid-resistance and worse clinical outcomes, and can be considered an early sign of an underlying FSGS.

CD44 is a cell surface adhesion molecule involved in cell-to-cell and cell-to-matrix interactions. Hyaluronic acid is one of the main ligands of CD44 as well as other components of the extracellular matrix [12]. Activated PECs show *de novo* expression of CD44, as has been described in both experimental models and human biopsies of FSGS and crescentic glomerulonephritis [7, 13, 14].

The reasons why parietal cells are activated are not well-known and may be different in each type of kidney disease. In FSGS, it has been proposed that the activation of parietal cells could occur as a consequence of their direct contact with products of the filtered plasma in the regions of the glomerulus where, secondary to the loss of podocytes, the capillary walls become denuded, lose their filtration selectivity, expand and come into contact with parietal cells, forming synechiae. After their activation, the parietal cells acquire a migratory phenotype with the capacity to synthesize extracellular matrix. Through the synechiae, they can migrate into the capillary tuft, where they produce extracellular matrix, which contributes to the formation of segmental sclerosis lesions, probably in an attempt to repair the glomerular damage [7]. This mechanism could explain the presence of CD44-positive cells inside the capillary tuft [13]. According to this hypothesis, the expression of CD44 by the PECs could be an indirect sign of the reduction in the number of podocytes below a critical threshold and, consequently, could be an early sign of an underlying FSGS. Supporting this, CD44-positive staining of PECs has been shown to be a

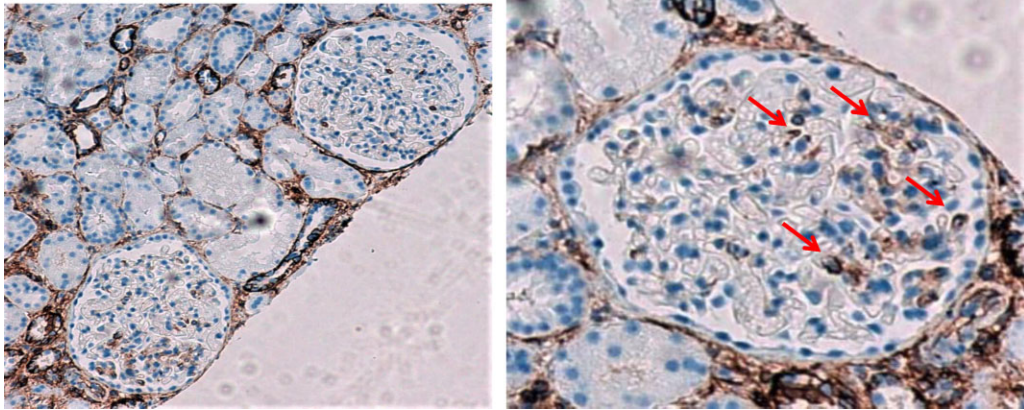


FIGURE 4: Early post-transplant biopsy of a patient with CD44-positive staining ($\times 40$).

potential marker to distinguish MCD from early FSGS as described in a recent study, in which 25% of biopsies originally tagged as MCD showed the presence of small sclerosing lesions that were undetectable with standard staining procedures, after immunostaining with PEC markers (CD44, claudin-1 and LKIV69) [13]. These data are in agreement with those we observed in our group of patients who were re-biopsied, as most of them showed CD44-positive staining in their first biopsy and developed a morphological pattern of FSGS during follow-up. The data observed in the biopsies of the transplant-recipient patients who suffered recurrence of proteinuria shortly after transplantation are in agreement; in both groups, the glomeruli showed no morphological lesions in light microscopy, but, as described in previous studies [14], PECs showed a CD44-positive staining, and, in follow-up biopsies performed 3 months after starting treatment, evident lesions of FSGS were observed. As steroid-resistance is more prevalent in FSGS than in MCD [1, 2] overall, the above considerations can account for the relationship between the presence of CD44-positive PECs and steroid resistance that we have found in our study. However, the AUC to predict steroid-resistance associated with CD44 expression is weak and this relationship, being purely morphological, only indicates that steroid-resistance is associated with greater podocyte loss, which is the final consequence of the injury. Moreover, it must be taken into account that even selecting biopsies with a minimum of six glomeruli, since early FSGS lesions are typically focal, some of the patients classified as CD44 negative could be false negatives and show CD44 expression in other renal areas not observed in the biopsy, especially in samples with a low glomerular number.

Besides its potential role to differentiate between MCD and early FSGS, a recent study has shown a significant association between CD44-positive staining of PECs and kidney outcome in paediatric patients with FSGS [13]. Our data indicate that the same relationship can be observed in patients initially tagged as having MCD (according to the histopathological data observed in light microscopy), since those who progressed to CKD also had a higher prevalence of CD44-negative PECs-positive cells, and CD44-negative PECs-positive cell migration into the glomerular capillary tuft.

The study of the variables associated with corticosteroid resistance in MCD patients in retrospective multicentre studies has to cope with two major difficulties, the low prevalence of corticosteroid resistance (which in general terms stands at

percentages close to 10% in paediatric patients and 20% in adult patients) and the differences in the definition of steroid resistance among centres, which can be based on different doses and exposure times. One of the main strengths of our study is the inclusion of a cohort of patients with MCD, using explicit and homogeneous criteria to define steroid resistance. These criteria limited the sample size, since they forced us to discard patients for whom no reliable information was available to assess steroid resistance adequately. Even though the sample size is limited, steroid-resistant patients are widely represented due to the fact that the study group includes a large number of paediatric patients in whom renal biopsy was not indicated as an initial diagnostic procedure, but only in the case of poor response to initial treatment in terms of corticosteroid resistance or corticosteroid dependence or frequent relapses with poor response to usual immunosuppressive treatment. This also explains why the prevalence of corticosteroid resistance in paediatric patients was higher than that observed in adult patients, in whom biopsy was indicated as the initial diagnostic procedure, and the percentage of steroid-resistant patients was very similar to that described in most studies [2]. It must be noted, however, that the prevalence of corticosteroid resistance observed in our cohort of patients should not be considered representative of that expected in cohorts of patients with MCD [1, 2].

In conclusion, our data indicate that in patients with a histopathological pattern of MCD in light microscopy, PEC activation, defined by CD44-positive staining, is associated with a significantly higher prevalence of corticosteroid resistance and with an increased risk of renal function deterioration, and can be considered an early sign of FSGS. Further research needs to be done to explain its implication in MCD and FSGS pathogenesis and to define its potential role in the clinical practice.

SUPPLEMENTARY DATA

Supplementary data are available at [ckj](#) online.

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CONFLICT OF INTEREST STATEMENT

None declared.

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