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suPAR and WT1 modify the adhesion of podocytes and are related to proteinuria in class IV lupus nephritis

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ABSTRACT

Introduction: Lupus nephritis (LN) affects up to 60 % of the patients with Systemic Lupus Erythematosus (SLE) and renal damage progression is associated with proteinuria, caused in part by the integrity of the glomerular basement membrane (GBM) and by podocyte injury. The soluble urokinase plasminogen activator receptor (suPAR) and Wilms Tumor 1 (WT1) have been related to podocyte effacement and consequently with proteinuria which raises questions about its pathogenic role in LN.

Objective: Define whether suPAR levels and WT1 expression influence in podocyte anchorage destabilization in LN class IV.

Materials and methods: This is a cross-sectional study of cases and controls. We studied patients with SLE without renal involvement (n = 12), SLE and LN class IV with proteinuria ≤0.5 g/24 h (n = 12), LN class IV with proteinuria ≥0.5 g/24 h (n = 12) and compared them with renal tissue control (CR) (n = 12) and control sera (CS) (n = 12). The CR was integrated by cadaveric samples without SLE or renal involvement and the CS was integrated by healthy participants. The expression and cellular localization of WT1, urokinase-type plasminogen activator receptor (uPAR), ac-α-tubulin, vimentin, and β3-integrin was assessed by immunohistochemistry (IHC). The concentration of suPAR in serum was analyzed by enzyme-linked immunosorbent assay (ELISA).

Results: In patients with LN, the activation of anchoring proteins was increased, such as podocyte β3-integrin, as well as the acetylation of alpha-acetyl-tubulin and uPAR, in contrast to the decrease in vimentin; interestingly, the cellular localization of WT1 was cytoplasmic and the number of podocytes per glomerulus decreased. The concentrations of suPAR was increased in patients with LN.

Conclusion: The destabilization of podocyte anchorage modulated by β3-integrin activation, and tubulin acetylation, associated with decreased WT1 cytoplasmic expression, and increased suPAR levels could be involved in kidney damage in patients with LN class IV.

1. Introduction

SLE is an autoimmune multisystem disease that affects different clinical domains including the kidney; many clinical and serologic data are reflected in the American College of Rheumatology (ACR) criteria for disease classification [1–3]. The hallmark of SLE is the presence of

Abbreviations: ACR, american college of rheumatology; AMC, adhesive molecular complex; ANA, antinuclear antibodies; Anti-dsDNA, anti-double-stranded DNA; anti-Sm, anti–Smith; AUC, area under the curve; C, control; CR, control renal tissue; CS, control sera; C3, Complement 3; C4, Complement 4; DAB, 3,3'-diaminobenzidine; DAPI, 4',6-phenylindole Diamidine-2'dihydrochloride; ELISA, enzyme-linked immunosorbent assay; EULAR, European League Against Rheumatism; FITC, fluorescein isothiocyanate; FSGS, focal segmental glomerulosclerosis; GBM, glomerular basement membrane; H&E, hematoxylin and eosin; H_2O_2, hydrogen peroxide; IFA, indirect immunofluorescence; IHC, immunohistochemistry; ISN/RPS, international society of nephrology/renal pathology society; LN, Lupus nephritis; mAbs, monoclonal antibodies; PAS, periodic acid-Schiff; PBS, phosphate buffered saline; R, Overlap coefficient; r_s, Spearman correlation coefficient; sFas, soluble Fas; SLE, systemic lupus erythematosus; SLEDAI, SLE disease activity index; SPSS, statistical software package; suPAR, soluble urokinase-type plasminogen activator receptor; uPAR, urokinase-type plasminogen activator receptor; WT1, Wilms tumour 1.

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autoantibodies including the antinuclear antibodies (ANA), anti-double-stranded DNA (anti-dsDNA), and anti–Smith (anti-Sm) autoantibodies, also the modification of the C3 and C4 complement fractions levels is observed especially during disease activity [4,5]. Kidney involvement is present in 25–60 % of adult patients depending on the population studied, and renal damage is classified according to histological findings, in which morphology and extension of the lesions serve as the real indicator of the degree of injury, activity, and chronicity [6,7].

The presence of proteinuria is a clinical indicator of glomerular involvement; therefore this **sign** depends in part on the integrity of the glomerular basement membrane (GBM), its normal intraglomerular pressure, and podocyte function. Podocytes are identified by expression of WT1, a transcription factor used as a biomarker to evaluate podocyte injury in kidney disease. Decreased levels of WT1 negatively regulate the transcription of PODXL (podocalyxin) and NPHS1 (nephrin). The podocytes embrace the glomerular capillaries and integrate ultrafiltration diaphragm, that is composed of proteins of the nephrin family, they are linked to adhesive molecular complex (AMC) (including the integrin alpha 3 beta, collagen IV, uPAR, vitronectin, nidogen, laminin, paxillin, and talin among others), the AMC is anchored to the cytoskeleton and intermediate filaments, and allows the interaction of the GBM with the capillary wall, in consequence, the integrity of the glomerular filtration barrier is important for homeostasis since it allows the selective filtration of plasma, water, and solutes and prevents the escape of macromolecules such as albumin, damage to this barrier results in proteinuria [8–10].

In addition of the immune complexes deposition, the existence of a circulating factor involved in GBM permeabilization, arise an interesting hypothesis since nearly 40 % of transplanted patients develop early proteinuria, among the possible inducer factors is the urokinase-type plasminogen activator receptor (uPAR), which has been related with the podocyte effacement and consequently with proteinuria, the uPAR plays an important role in motility and other podocitary functions [11–16], the extracellular domain of uPAR can be cleaved by proteolysis in suPAR fragments with molecular weight ranging between 30 and 50 kDa, they display different biological activities that activate some components of AMC, and induce proteinuria in focal segmental glomerulosclerosis (FSGS) and LN [17], this truncated fragment is also related with atherosclerosis, sepsis, tumoral process, and liver disease [18,19]. Taking into account the aforementioned, we infer that suPAR is not a specific marker of renal disease however, undoubtedly is involved in the pathophysiology of LN.

So, our hypothesis is that WT1 expression levels and suPAR concentrations destabilize the molecular adherent complex of podocytes and cause proteinuria in LN class IV. To demonstrate our proposal, we studied kidney biopsies of patients with LN and controls without kidney disease. Additionally, suPAR concentrations and serum autoantibody profile were evaluated.

2. Materials and methods

2.1. Specimen collection and handling

This is a cross-sectional study of cases and controls. Samples were collected for convenience. The study population was formed with patients who attended a public sector Nephrology Service in Mexico. In the control group, sera were collected from healthy participants and cadaveric kidney biopsies who died from craniocerebral injury. This investigation was performed according to the principles of the Declaration of Helsinki and was approved by the bioethics committees of our institutions. All patients, controls and/or relatives signed informed consent.

Serum samples: Serum samples were collected from three subgroups: The first subgroup was patients with SLE with no evidence of kidney damage with a n = 12 (10 women and 2 men) with a median age of 27.5 (16.3–44). The second subgroup was patients with LN and proteinuria ≤0.5 g/h with a n = 12 (9 women and 3 men) and a median age of 28 (18.25–33.25). The third subgroup was patients with LN and proteinuria ≥0.5 g/h with a n = 12 (10 women and 2 men) and a median age of 26 (15–35). All patients meet the American College of Rheumatology/European League Against Rheumatism (EULAR/ACR) 2019 criteria for the classification of SLE [20]. The control (C) consisted of 12 healthy participants (10 women and 2 men) with a median age of 29.25 (24.25–34) years. All C were negative for antibodies against ANA and anti-dsDNA. The detection of anti-ANA antibodies was performed by indirect immunofluorescence (IFA) using HEp-2 cells and *Crithidia luciliae* (EUROIMMUN US Inc.) for anti-dsDNA antibodies. The autoantibodies anti-Ro-60 kDa (EA 1595–9601 G), anti-La-48 (EA 1957–9601 G), anti-Sm (EA 1593–9601 G), and anti-GBM (EA 1251–9601 G) were determined by ELISA according to the protocols of (EUROIMMUN US Inc).

Kidney biopsies: The adherent component of podocytes in kidney biopsies was studied in a group of patients with LN and CR who were initially hospitalized for a head injury due to a car accident, and then died within 48 h as a consequence of this trauma, and then were subjected to a legal medical autopsy. All renal biopsies were analyzed by light microscopy with conventional H&E, silver, and PAS staining. The samples met the criteria of the ISN/RPS [6]. One segment of each biopsy was stained by IFA and IHC.

2.2. The expression of WT1, vimentin, and β3-integrin was determined by double-IFA

Samples of 4 μm thick kidney tissues from LN patients and CR mounted on microscope slides were maintained for 5 min in 1X phosphate buffered saline (PBS), and permeabilized with Triton X-100 (0.1 % Triton with 0.1 % sodium citrate in PBS 1X). After washing, the samples were immersed for 20 min in 0.1 M HCl-Tris pH 7.5, 3 % bovine serum albumin, and 20 % fetal bovine serum to block nonspecific labeling, then washed and incubated for 4 h with anti-β3-integrin (Integrin β3[N-20]: sc-6627, INC mAbs-IgG goat), anti-vimentin conjugate (Vimentin [D21H3] XP® mAbs-IgG rabbit, Alexa Fluor® 647 Conjugate Cell Signaling TECHNOLOGY) diluted 1:100 in PBS. After washing, the samples were incubated for 2 h with secondary antibodies anti-IgG (goat) conjugated with fluorescein isothiocyanate (FITC) diluted 1:100 in PBS, followed by three washes were incubated for 4 h with monoclonal antibodies (mAbs) anti-WT1 (WT-1 [F-6]: sc-7585, mAbs-IgG$_1$ mouse), and after washing, they were incubated with secondary anti-IgG antibody mouse conjugated with Texas Red for 1 h. After this, the samples were washed and counterstained with DAPI (Roche) for 30 min, then washed with methanol-PBS and mounted in PBS/glycerol to be observed and photographed at 10X and 40X in a microscope (Olympus BX-40). The WT1 and vimentin were identified by emitting fluorescence in red, and the β3-integrin in green; 4′,6-phenylindole Diamidine-2′dihydrochloride (DAPI) (blue fluorescence) was used to determine the cellular localization of WT1. The podocytes display the WT1 protein as a marker of their phenotype, therefore all glomerular cells that express WT1 correspond to podocytes. With the nuclei contrasted with DAPI, co-localization between WT1/DAPI was analyzed. The analysis was conducted independently by 2 researchers.

2.3. The expression of uPAR, α-tubulin, and acetyl-α-tubulin was detected by IHC

The specimens of 4 μm were dewaxed, permeabilized with 0.01 % Triton X-100/PBS, and then washed with PBS, the endogenous peroxidase was blocked for 10 min with 3 % hydrogen peroxide (H_2O_2) dissolved in methanol, after an additional wash, the tissues were incubated for 18 h with a monoclonal anti-uPAR (E−3-HRP-sc-376494-HRP), anti-α-tubulin (B-7, sc-5286) and anti-ac-α-tubulin (Lys40, D20G3 #5335S) diluted 1: 100 in PBS, the tissues were then washed with PBS, and the

bound antibodies were identified with detection system Histostain–sp kits (Invitrogen #959943). The color reaction was induced by the 3,3′-diaminobenzidine (DAB) chromogen kit (901-BDB2004-082,417). The slides were examined under a light microscope. The assays were performed in triplicate and evaluated by two pathologists in a blinded fashion. The intensity of the IFA, the cellular co-localization of WT1, and the color reaction obtained by IFA e IHC were analyzed in the software Image-Pro Plus Version 7.0 (Media Cybernetics, USA).

2.4. Determination of suPAR by ELISA

We used a solid-phase ELISA to determine the soluble fraction of suPAR in the sera of patients and CS. For this purpose, a Quantikine® ELISA Human uPAR kit (Immunoassay R&D Systems, Minneapolis, MN, USA) the level was expressed in pg/mL of suPAR.

2.4.1. Statistical analysis

The differences between the groups were analyzed using median values and interquartile ranges (25th – 75th percentile) for non-normally distributed variables by Mann-Whitney *U* Test using GraphPad Prism 9 program with a confidence interval of 95 %, also was used the Spearman rank correlation (r_s) between the clinical parameters and the concentration of suPAR. The ROC curves and area under the curve (AUC) were analyzed using a statistical software package (SPSS) with a confidence interval of 95 %.

3. Results

Clinic pathologic features. All patients meet the EULAR/ACR criteria for the classification of SLE and LN, all patients with LN were receiving Cyclophosphamide. None of the CS had positive lupus autoantibodies. The clinical characteristics of the patients are shown in (Table 1).

suPAR levels positively correlated with proteinuria in the group of 24 patients with LN. We observed a positive correlation between anti-Ro 60 kDa/Anti-Sm in the group of patients with LN (greater or lower than 0.5 g/24 h) (Table 2).

Kidney histology. In 8 biopsies of LN with proteinuria ≥0.5 g/24 h exhibited glomerular changes greater than 50 % of the glomeruli, this tissues showed diffuse endocapillary and extracapillary hypercellularity and mesangial proliferation, with an increase of fibrous matrix and fibrinoid necrosis of the basement membrane, and also hyaline deposits. Biopsies of LN with proteinuria ≤0.5 g/h showed a median activity of 5 (4–8.5) and a chronicity index of 4 (1.75–5.5) in contrast to LN with proteinuria ≥0.5 g/h that showed a median activity of 8 (6–8) and a chronicity index of 2 (0–5.) classifying as LN class IV nephritis. CR showed normal characteristics in the glomeruli, mesangium, urinary spaces, and Bowman's capsules.

Adherent molecular complex. WT1 was considered the podocyte marker and was detected in all biopsies. However, the number of podocytes was significantly reduced in LN 8.34 (4.37–10.36) when compared with the CR 22.27 (14.80–47.63) ($p = 0.01$). The cell colocalization was determined by the overlap coefficient (R) and in WT1 we detected an R = 0.616 in the CR, in contrast to LN which had R = 0.439 ($p = 0.0028$), interestingly the cytoplasmic expression of WT1 and vimentin decreases 5.75 (3.18–9.58) and 13.24 (7.23–23.77) in podocytes from patients with LN, whereas in CR increased 33.42 (13.79–53.61) and 49.91 (20.72–82.96) ($p = 0.001$). On the other hand, the β3-integrin was overexpressed in LN 45.47 (22.49–67.09) in contrast to the CR (6.81 (3.36–10.35) ($p = 0.001$) (Figs. 1 and 2).

The expression of uPAR and ac-α-Tubulin increases in LN. The ac-α-tubulin was negatively correlated with Tubulin in the group of patients with LN (r_s.= -0.485 p = 0.015). The expression of uPAR 0.07 (0.06–0.08) and ac-α-Tubulin 0.07 (0.06–0.08) in the CR decreases significantly in contrast to patients with LN 0.46 (0.29–0.59) and 0.58 (0.33–0.87) ($p = 0.001$) (Figs. 3 and 4).

4. Discussion

The proteinuria in the LN is a sign of the progression of glomerular damage, which is clinically translated as generalized edema by podocyte damage.

We previously found alternate biomarkers in serum and urine that are associated with kidney damage, such as podocyturia and decreased WT1 expression in kidney biopsies [21]. Another biomarker linked to apoptosis is soluble Fas (sFas), an increase in sFas levels in patients with LN is also associated with proteinuria and with high titers of anti-dsDNA antibodies [22]. In any case, is evident that podocyte dysfunction is involved in the pathophysiology of LN, therefore the podocytopathy in SLE could be related to the destabilization of its AMC. For this reason, in this study, in addition to analyzing the expression and cellular localization of WT1, uPAR, vimentin, acetyl-α-tubulin, and β3-integrin in kidney samples from patients with LN, we determined the association between clinical parameters and the concentration of suPAR..

We observed a decrease in the expression cytoplasmatic of WT1, and vimentin, as well as an increase in the expression of uPAR, acetyl-α-tubulin, and β3-integrin in the kidney biopsies.

The increase of β3-integrin expression is related to the conformational change of proteins. Zhang B. et al. induced dysfunction of the podocytes and proteinuria in a murine model using LPS, and in diabetic nephropathy, it was observed that the signaling pathway of uPAR in podocytes leads to the effacement of the pedicels and that this mechanism includes the increased the activation of αvβ3-integrin [23,24]. Furthermore, it has been demonstrated that the plasma of patients with steroid-resistant FSGS activates the β3-integrin of podocytes *in vitro* [25–27].

These findings suggest a physiological role of suPAR in the signaling regulation that affects the permeability of the kidney. However, experimental studies should continue to find the possible effects of blocking the union of suPAR or β3-integrin and their potential application in humans.

Table 1
Clinical characteristics of the groups studied.

	Control	SLE	LN ≤ 0.5 g/h	LN ≥ 0.5 g/h
Male n (%)	2 (16)10	2 (16)	3 (25)	2 (16)
Female n (%)	(84)	10 (84)	9 (75)	10 (84)
Disease duration (years), median (25th-75th percentile)	ND	3 (2.5–4.5)	4 (3.5–7.25)	5 (2–8)
SLEDAI median (25th-75th percentile)	ND	2 (1.5–4)	6.5 (4.75–8)	6 (5–8)
Anti-Ro-60 kDa (UR/mL), median (25th-75th percentile)	0.53 (0.10–0.98)	0.56 (0.48–1.10)	0.73 (0.54–1.49)	0.82 (0.50–5.57)
Anti-La-48 (UR/mL), median (25th-75th percentile)	0.46 (0.40–0.60)	0.48 (0.36–0.92)	0.66 (0.38–0.81)	0.52 (0.39–1.85)
Anti-Sm (UR/mL), median (25th-75th percentile)	0.63 (0.43–0.93)	0.57 (0.48–3.59)	0.83 (0.52–1.45)	0.89 (0.59–2.65)
Anti-GBM (UR/mL), median (25th-75th percentile)	0.69 (0.61–0.80)	0.69 (0.39–1.56)	0.75 (0.50–0.82)	0.72 (0.59–0.82)
Proteinuria (g/24 h) median (25th-75th percentile)	ND	0.08 (0.06–0.10)	0.12 (0.10–0.14)	1.02 (0.64–2.7)
ANA (Screening dilution), median (25th-75th percentile)	0 (0–0)	320 (80–2560)	160 (160–1280)	960 (160–1280)
suPAR (pg/mL), median (25th-75th percentile)	763.8 (560.8–994.8)	758.8 (558.8–1908.8)	972.8 (746.8–1666.8)	1381.8 (796.8–2152.8)

ND, undetermined.

Table 2
Relationship between autoantibodies and clinic pathologic characteristics in SLE and LN.

	SLE (r_s)	*p*	LN ≤ 0.5 g/h (r_s)	*p*	LN ≥ 0.5 g/h (r_s)	*p*	NL g/h (r_s)	*p*
suPAR/Proteinuria							0.386	0.05
Anti-Ro-60 kDa/Anti-La-48 kDa	0–712	0.009						
Anti-Ro-60 kDa/Anti-Sm	0.779	0.002	0.861	0.005	0.833	0.008	0.885	0.001
Anti-La-48 kDa/Anti-Sm	0.624	0.022						
suPAR/Anti-GBM			0.838	0.007				
Proteinuria/Anti-Sm					0.667	0.042		

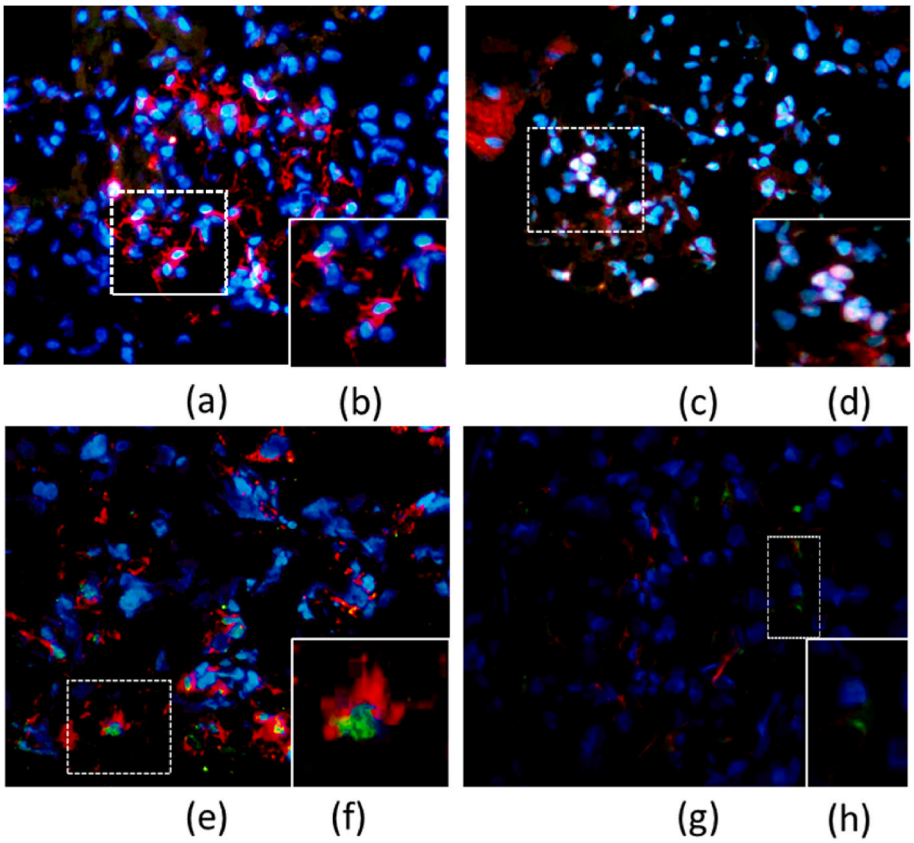

Fig. 1. Double-IFA shows the cellular co-localization of vimentin, β3-integrin, and WT1 in renal tissue of 12 patients with LN and 12 CR. The upper panels (a) and (c) show the CR tissue, and the lower panels (e) and (g) show tissue-representative LN. Panels (b) and (f) show the expression of vimentin red and WT1 green. Panel (d) and (h) depict the expression of β3-integrin green and WT1 red. Nuclei were counterstained with DAPI in blue. Squares of the crop show representative podocytes. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

Vimentin is a fibrous protein that forms the intermediate filaments of the cytoskeleton and is involved in cell motility. Presently a the decreased expression of vimentin and the cytoplasmic localization of WT1 was observed in the podocytes of patients with LN, and we believe that WT1 is not functioning enough as a transcription factor for essential proteins of the podocyte, thus altering the shape of the podocytes. This is consistent with Macconi et al., who used vimentin and WT1 as a marker of podocytes in a rat model without renal involvement and found that this filament is in the nuclei [27].

Tiantian et al. [28] demonstrated that in renal samples from patients with diabetic nephropathy, the enzyme HDAC6 induces the depolymerization of microtubules through α-tubulin deacetylation, decreasing autophagy and increases podocyte mobility, what would cause the movement of the podocytes and the loss of adherence to the GBM. On the other hand, Regne et al. [29] in mice prone to SLE, observed that acetylation increases NF-κB activation. Our data suggest that increased tubulin acetylation in class IV LN biopsies is a mechanism associated to podocyte damage since NF-κB transcribes WT1, which is a transcription factor for essential podocyte proteins such as nephrin, podocin, and podocalyxin. Some mechanisms in the progression of LN have been identified, showing that podocyte detachment is the main cause of proteinuria, which makes it a potential pharmacological target for LN.

Recently, the discovery of suPAR as a biomarker of FSGS led to clinical studies to determine its relationship with other glomerular diseases [30].

We found that suPAR concentration is increased in patients with LN and was associated with proteinuria. These results are consistent with previous reports [23,29,30], which shows that high levels of suPAR are associated with a decrease in the glomerular filtration rate and proteinuria greater than 0.5 g/24 h in primary and secondary glomerulonephritis.

This suggests that high suPAR concentration has a pathogenic effect on the podocytes, causing proteinuria, which is a clinical feature of renal damage in patients with LN.

To determine whether suPAR can be used as a biomarker of LN, we used ROC curves that provide the accuracy of the test. Our results indicate a high sensitivity and specificity for LN.

Even though the sample was small, we believe that with these data,

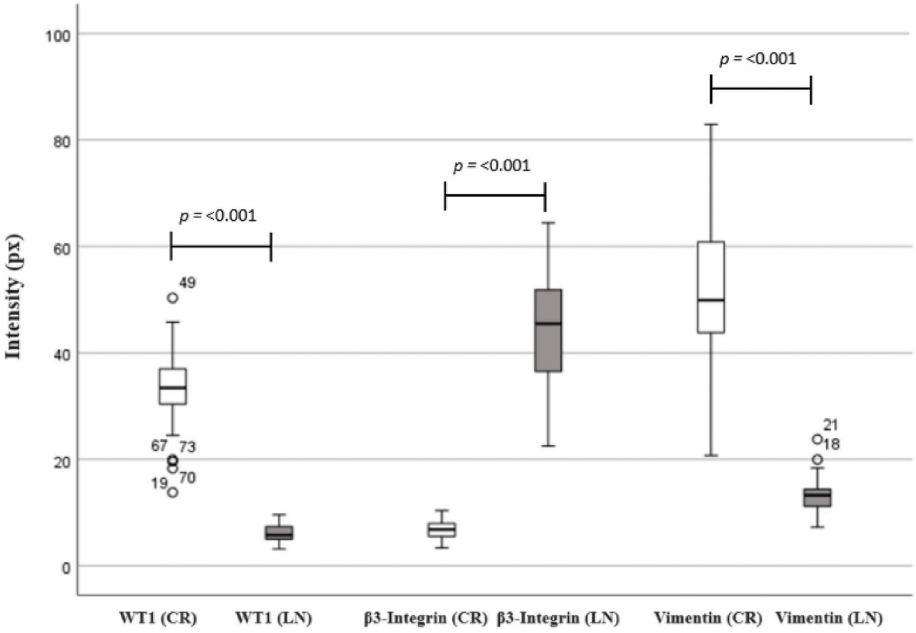

Fig. 2. Boxplots representing the expression of vimentin, β3-integrin, and WT1 in kidney tissue from 12 patients with LN and 12 CR. The box represents the interquartile range with the line inside the box indicating the median value.

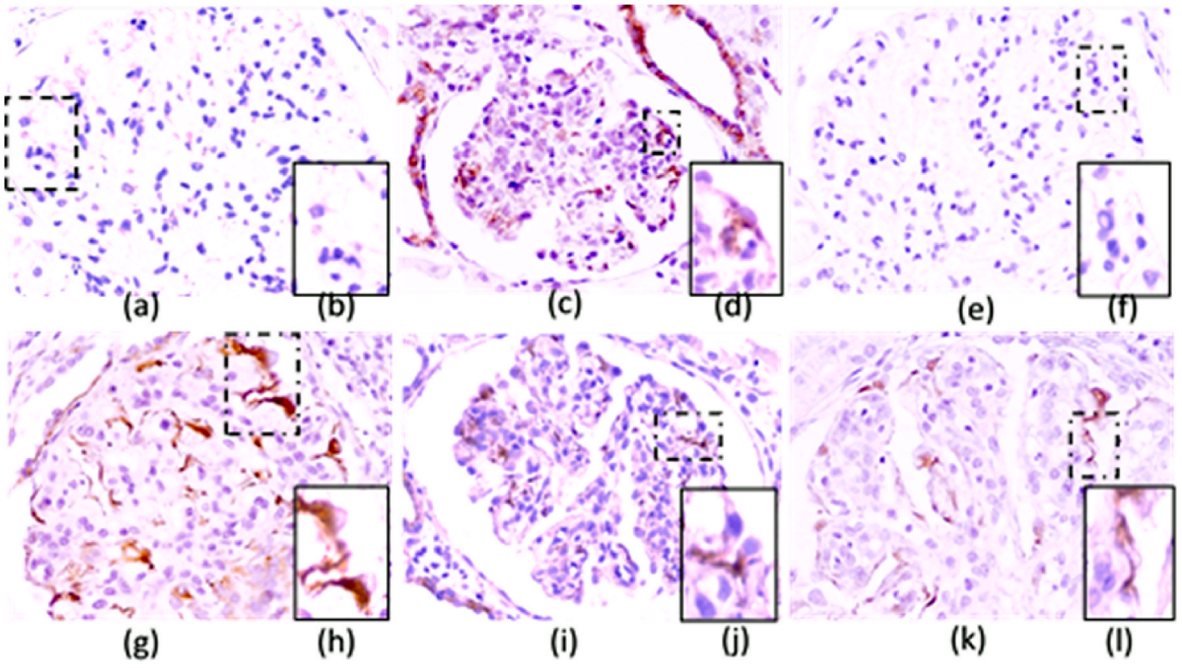

Fig. 3. Expression of uPAR, ac-α-Tubulin, and Tubulin in 12 CR and 12 patients with LN by IHC. The assay shows the expression of uPAR, ac-α-Tubulin, and Tubulin in podocytes of BCG and patients with LN. The upper panels (a, c, and e) show the CR and the lower panels (g, I, and k show tissue representative LN. Panel (b) and (h) show the expression of ac-α-Tubulin. Panels (d) and (j) depict the expression of tubulin. Panels (f) and (l) show uPAR expression. Squares of the crop show representative podocytes.

more investigation can be done in this field to evaluate if it is of benefit to evaluate the concentration of suPAR in patients with SLE with or LN to delay renal involvement in these patients.

5. Conclusions

Apparently the destabilization of the podocytes is related to the elevations of suPAR, it affects the dynamics of the anchoring proteins, and this is related to the cytoplasmic expression of WT1, with the acetylation of tubulin and the activation of B3 integrin, all together it induces proteinuria. We consider that these initial results will help in clinical practice to better understand the complex pathophysiology of lupus nephritis.

Credit author statement

Juan-José Bollain-y-Goytia: Conceptualization, Validation, Investigation, Writing - original draft, Supervision. Felipe-de-Jesús Torres-Del-

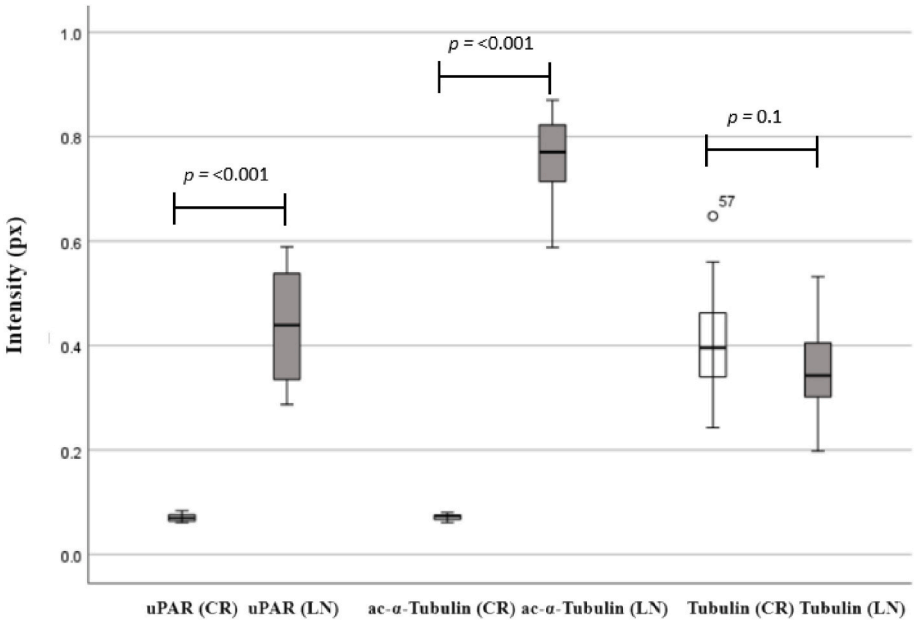

Fig. 4. Boxplots representing the expression of uPAR, ac-α-Tubulin, and Tubulin in 12 CR and 12 patients with LN. The box represents the interquartile range with the line inside the box indicating the median value. suPAR increases in lupus nephritis. In CS we detected a median concentration of 763.80 (560.80–994.80) pg/mL of suPAR, in contrast with patients of SLE 758.80 (558.80–1908.80). After grouping patients with LN according to their proteinuria levels (greater or lower than 0.5 g/24 h), we observed that the concentrations of suPAR were increased in patients with LN and proteinuria ≥0.5 g/24 h 1381.80 (796.80–2152.80) in contrast to patients with SLE 758.8 (558.8–1908.8) ($p = 0.004$) and CG 763.8 (560.8–994.8) ($p = 0.002$) (Fig. 5).

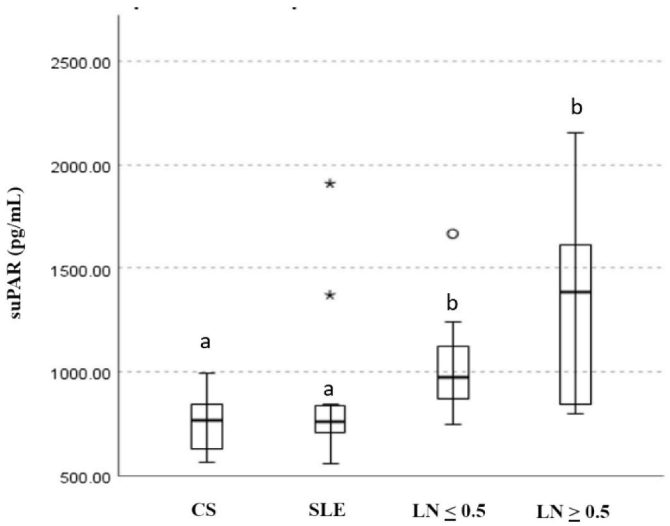

Fig. 5. Boxplots representing suPAR concentration in 12 CS, 12 of SLE, and 12 of LN samples stratified by proteinuria. The box represents the interquartile range with the line inside the box indicating the median value. Finding the following significant differences SGC vs LN ≤ 0.5 ($p = 0.021$), SLE vs LN ≤ 0.5 ($p = 0.034$). suPAR concentration differentiates patients with LN. Using a ROC curve we analyzed suPAR as a biomarker of kidney damage in patients with LN. A statistically significant difference was observed compared to a control group in the AUC = 0.875 ($p \leq 0.001$, 95 % CI: 0.741, 1.009); taking the concentration of 750 ρg/mL as the cut-off point with a sensitivity of 93 % and a specificity of 60 %.

muro: Methodology. Sara-Paola Hernández-Martínez: Formal analysis. Esperanza Avalos-Díaz: Methodology, Recourses. Rafael Herrera-Esparza: Writing: review and editing, Conceptualization.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

The data that has been used is confidential.

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References

[1] A. Fernández-Nebro, S. Marsal, W. Chatham, A. Rahman, Systemic lupus erythematosus: genomics, mechanisms, and therapies, Clin. Dev. Immunol. (2012), 926931, https://doi.org/10.1155/2012/926931.
[2] E.M. Hay, M.L. Snaith, ABC of rheumatology. Systemic lupus erythematosus and lupus-like syndromes, Bmj 13 (1995) 1257–1261.
[3] J. Robinson, The patient's journey: systemic lupus erythematosus, BMJ 10 (2006) 1374–1376, https://doi.org/10.1136/bmj.332.7554.1374.
[4] S.T. Faezi, M. M H Almodarresi, M. Akbarian, F. Gharibdoost, M. Akhlaghi, A. Jamshidi, et al., Clinical and immunological pattern of systemic lupus erythematosus in men in a cohort of 2355 patients, IJRD 17 (2014) 394–399, https://doi.org/10.1111/1756-185x.12268.
[5] R. Saxena, T. Mahajan, C. Mohan, Lupus nephritis: current update, Arthritis Res. Ther. 13 (2011) 240, https://doi.org/10.1186/ar3378.
[6] H.J. Anders, R. Saxena, M.h Zhao, et al., Lupus nephritis. Nat Rev Dis Primers 6 (2020) 7, https://doi.org/10.1038/s41572-019-0141-9.
[7] I.M. Bajema, S. Wihemus, C.E. Alprs, et al., Revision of the International Society of Nephrology/Renal Pathology Society classification for lupus nephritis: clarification of definitions, and modified National Institutes of Health activity and chronicity indices, Kidney Int. 93 (2018) 789–796, https://doi.org/10.1016/j.kint.2017.11.023.

[8] I. Correia, D. Chu, Y.H. Chou, R.P. Goldman, et al., Integrating the actin and vimentin cytoskeletons adhesion-dependent formation of fimbrin-vimentin complexes in macrophages, J. Cell Biol. 23 (1999) 831–842, https://doi.org/10.1083/jcb.146.4.831.

[9] X. Ge, et al., LIM-nebulette reinforces podocyte structural integrity by linking actin and vimentin filaments, J. Am. Soc. Nephrol. 31 (2020) 2372–2391, https://doi.org/10.1681/ASN.2019121261.

[10] R. Lennon R, M.J. Randles, M.J. Humphries, The importance of podocyte adhesion for a healthy glomerulus, Front. Endocrinol. 14 (2014) 160, https://doi.org/10.3389/fendo.2014.00160.

[11] A. Kronbichler, M.A. Saleem, B. Meijers, J.I. Shin, Soluble urokinase receptors in focal segmental glomerulosclerosis: a review on the scientific point of view, Journal of Immunology Research (2016), 2068691, https://doi.org/10.1155/2016/2068691.

[12] S.S. Hall, Omen in the blood, Science 20 (2018) 254–258, https://doi.org/10.1126/science.360.6386.254.

[13] M. Praga, suPAR and focal segmental glomerulosclerosis, Nefrologia 34 (2014), https://doi.org/10.3265/nefrologia.pre2014.jan.12452.

[14] C. Wei, S. Hindi, J. Li, A. Fornoni, N. Goes, J. Sageshima, et al., Circulating urokinase receptor as a cause of focal segmental glomerulosclerosis, Nat. Med. 17 (2011) 952–960, https://doi.org/10.1038/nm.2411.

[15] C. Wei, C.C. Möller, M.M. Altintas, J. Li, K. Schwarz, S. Zacchigna S, et al., Modification of kidney barrier function by the urokinase receptor, Nat Med 14 (2008) 55–63, https://doi.org/10.1038/nm1696.

[16] J. Ma, B. Zhang, S. Liu, S. Xie, Y. Yang, J. Ma, et al., 1,25-Dihydroxyvitamin D(3) inhibits podocyte uPAR expression and reduces proteinuria, PLoS One 85 (2013), e64912, https://doi.org/10.1371/journal.pone.0064912.

[17] H. Enocsson, C. Sjöwall, J. Wetterö, Soluble urokinase plasminogen activator receptor A valuable biomarker in systemic lupus erythematosus? Clin. Chim. Acta 444 (2015) 234–241, https://doi.org/10.1016/j.cca.2015.02.031.

[18] J.M. Spinale, L.H. Mariani, S. Kapoor, J. Zhang, R. Weyant, P.X. Song, et al., A reassessment of soluble urokinase-type plasminogen activator receptor in glomerular disease, Kidney Int. 87 (2015) 564–574, https://doi.org/10.1038/ki.2014.346.

[19] A. Segarra, E. Jatem, M.T. Quiles, M.A. Arbós, E. Ostos, H. Ostos, et al., Diagnostic value of soluble urokinase-type plasminogen activator receptor serum levels in adults with idiopathic nephrotic syndrome, Nefrologia 34 (2014) 46–52, https://doi.org/10.3265/Nefrologia.pre2013.Oct.12256. https://www.revistanefrologia.com/en-diagnostic-value-soluble-urokinase-type-plasminogen-articulo-X2013251414053654.

[20] M. Aringer, K. Costenbader, D. Daikh, R. Brinks, et al., European League against rheumatism/American College of rheumatology classification criteria for systemic lupus erythematosus, Arthritis Rheumatol. 71 (2019) 1400–1412, https://doi.org/10.1002/art.40930, 2019.

[21] J.J. Bollain-y-G, M. Gonzalez-Castaneda, F. Torres-Del-Muro, L. Daza-Benitez, P. Zapata-Benavides, C. Rodriguez-Padilla, et al., Increased excretion of urinary podocytes in lupus nephritis, Indian J. Nephrol. 21 (2011) 166–171, https://doi.org/10.4103/0971-4065.83029.

[22] J.J. Bollain-y-G, M. Arellano-Rodriguez, F.de J. Torres-Del-Muro, L. Daza-Benitez, J.F. Munoz-Valle, E. Avalos-Diaz, et al., Soluble fas and the -670 polymorphism of fas in lupus nephritis, Int J Nephrol 2014 (2014), 780406, https://doi.org/10.1155/2014/780406.

[23] B. Zhang B, W. Shi, J. Ma, A. Sloan, C. Faul, C. Wei, et al., The calcineurin–NFAT pathway allows for urokinase receptor-mediated beta3 integrin signaling to cause podocyte injury, J Mol Med 90 (12) (2012) 1407–1420, https://doi.org/10.1007/s00109-012-0960-6.

[24] R.R. Dande, V. Peev, M.M. Altintas, J. Reiser, Soluble urokinase receptor and the kidney response in diabetes mellitus, J. Diabetes Res. (2017), 20173232848, https://doi.org/10.1155/2017/3232848.

[25] M. Bitzan, S. Babayeva, A. Vasudevan, P. Goodyer, E. Torban, TNFα pathway blockade ameliorates toxic effects of FSGS plasma on podocyte cytoskeleton and β3 integrin activation, Pediatr. Nephrol. 27 (12) (2012) 2217–2226, https://doi.org/10.1007/s00467-012-2163-3.

[26] O. Staeck, T. Slowinski, I. Lieker, K. Wu, B. Rudolph, D. Schmidt, et al., Recurrent primary focal segmental glomerulosclerosis managed with intensified plasma exchange and concomitant monitoring of soluble urokinase-type plasminogen activator receptor–mediated podocyte β3-integrin activation, Transplantation 99 (12) (2015) 2593–2597, https://doi.org/10.1097/tp.0000000000000914.

[27] D. Macconi, M. Bonomelli, A. Benigni, T. Plati, F. Sangalli, L. Longaretti, et al., Pathophysiologic implications of reduced podocyte number in a rat model of progressive glomerular injury, Am. J. Pathol. 168 (2006) 42–54, https://doi.org/10.2353/ajpath.2006.050398.

[28] T. Liang, C. Qi, Y. Lai, et al., HDAC6-mediated α-tubulin deacetylation suppresses autophagy and enhances motility of podocytes in diabetic nephropathy, J. Cell Mol. Med. 24 (2020) 11558–11572, https://doi.org/10.1111/jcmm.15772.

[29] N.L. Regna, M.D. Vieson, A.M. Gojmerac, X.M. Luo, D.L. Caudell, C.M. Reilly, HDAC expression and activity is upregulated in diseased lupus-prone mice, J.int imp. 29 (2015) 494–503, https://doi.org/10.1016/j.intimp.2015.10.006.

[30] M. Alfano, P. Cinque, G. Giusti, S. Proietti, M. Nebuloni, S. Danese, et al., Full-length soluble urokinase plasminogen activator receptor down-modulates nephrin expression in podocytes, Sci. Rep. 5 (1) (2015), 13647, https://doi.org/10.1038/srep13647.