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ORIGINAL ARTICLE

Relationship between soluble urokinase-type plasminogen activator receptor and serum biomarkers of endothelial activation in patients with idiopathic nephrotic syndrome

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ABSTRACT

Background. Serum levels of soluble urokinase-type plasminogen activator receptor (suPAR) are high in some patients with idiopathic nephrotic syndrome (INS). Given that suPAR constitutes a predictor of vascular disease and has been associated with endothelial dysfunction, we hypothesized that suPAR levels are related to endothelial activation or dysfunction in INS patients. The aims of this study were to evaluate the relationship between serum concentrations of endothelial biomarkers and suPAR in patients with different histological patterns of INS and healthy controls, and to determine the demographic, clinical and biochemical characteristics of INS patients that influence suPAR serum levels.

Methods. This observational, cross-sectional study included patients with INS, diagnosed with minimal change disease (MCD), focal segmental glomerulosclerosis (FSGS) or membranous nephropathy (MN) by renal biopsy. Patient demographic, clinical and biochemical characteristics were recorded and blood samples were obtained at the time of diagnosis. Measurements of suPAR and endothelial molecules via serum levels were performed using Enzyme-Linked ImmunoSorbent Assay kits.

Results. Patients with nephrotic syndrome ($n = 152$) caused by FSGS, MCD or MN had increased circulating levels of endothelial markers. suPAR levels positively correlated with age and the serum levels of almost all endothelial markers. Generally, endothelial cell molecules positively correlated with each other. suPAR levels were not associated with the histopathological pattern of INS.

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Conclusions. In patients with INS secondary to FSGS, MCD and NM, circulating levels of suPAR are independent of the primary renal disease, and significantly associated with age, glomerular filtration rate and the levels of various endothelial markers.

Keywords: biomarkers, endothelial activation, nephrotic syndrome, suPAR

INTRODUCTION

Idiopathic nephrotic syndrome (INS) is caused by podocyte and endothelial damage due to three main glomerular diseases: minimal change disease (MCD), focal segmental glomerulosclerosis (FSGS) and membranous nephropathy (MN). These glomerular diseases are similar in presentation, and given the inability of commonly used clinical and biochemical criteria to differentiate them, a kidney biopsy is required to confirm the diagnosis [1]. In this context, this research focuses on identifying novel biomarkers able to differentiate between the three causes of INS.

In recent years, various authors have consistently described very high levels of serum-soluble urokinase-type plasminogen activator receptor (suPAR) in certain FSGS patients, pointing to suPAR as one of the potential factors responsible for podocyte injury in FSGS [2–4]. However, subsequent studies have shown that suPAR levels are heterogeneously distributed among patients with FSGS and strongly influenced by many factors, including inflammation, ethnics, age and glomerular filtration rate (GFR) [3, 5–7]. These studies have also shown that suPAR levels overlap between patients with different glomerular diseases and even between INS patients and healthy subjects. Consequently, suPAR levels are unable to distinguish FSGS from other causes of nephrotic syndrome [3, 5–7].

suPAR is released to body fluids during inflammatory stimulation and has been associated with endothelial dysfunction and atherosclerosis [8–11]; likewise, its levels correlate with proinflammatory markers such as the tumour necrosis factor and traditional biomarkers of cardiovascular disease [12]. In fact, suPAR might outperform C-reactive protein as a prognostic marker and emerge as a potential new biomarker of cardiovascular risk [9]. Furthermore, suPAR is associated with certain conventional cardiovascular risk factors, including smoking and physical inactivity, and with subclinical vascular disease [10, 13]. In this regard, patients with nephrotic syndrome show signs of endothelial dysfunction, evidenced by reduced flow-mediated vasodilation and by increased circulating levels of various molecules associated with endothelial cell injury or activation, including von Willebrand Factor (VWF), vascular cell adhesion molecule-1 (VCAM-1), E-selectin and syndecan-1 (sCD138) [13, 14].

Despite findings regarding increased suPAR levels in certain patients with nephrotic syndrome and the emerging role of suPAR as a biomarker of subclinical vascular disease, the relationship between suPAR and endothelial dysfunction in nephrotic syndrome has not been investigated. We hypothesized that increased suPAR levels observed in certain patients with nephrotic syndrome are related to the extent of endothelial cell injury, activation or dysfunction. Therefore, the aims of this study were: (i) to evaluate the relationship between serum levels of endothelial biomarkers and of suPAR in a large cohort of patients with different histological patterns of INS and a healthy control group and (ii) to determine the demographic, clinical and biochemical characteristics of INS patients that influence serum suPAR levels.

MATERIALS AND METHODS

Study design and population

This was an observational, cross-sectional study including patients with INS and diagnosed with MCD, FSGS or MN by renal biopsy between 2012 and 2018 in two tertiary referral hospitals. The diagnosis of MCD, FSGS and MN was performed based on the following clinical criteria: patients were diagnosed with MCD if they showed no glomerular changes assessed by morphological and optical analysis and presented negative immunofluorescence results and evidence of diffuse podocyte effacement determined by electron microscopy. Patients were diagnosed with FSGS if they met all the following criteria: clinical nephrotic syndrome at the time of diagnosis, evidence of FSGS lesions assessed by light microscopy with diffuse podocyte effacement assessed by electron microscopy, no family history of chronic kidney disease or renal replacement therapy and exclusion of secondary aetiologies, including reduction of renal mass, morbid obesity, human immunodeficiency virus-associated nephropathy, heroin or cocaine use, use of analgesics, vesicoureteral reflux and obstructive sleep apnoea. Finally, MN was diagnosed in patients with characteristic morphological features and subepithelial immunoglobulin G (IgG) and C3 deposits assessed by immunofluorescence; in addition, the presence of anti-phospholipase A2 receptor antibodies was confirmed in all MN patients, and potential secondary causes were also excluded. Patients receiving treatment with corticosteroids, immunosuppressants, angiotensin II receptor antagonists, aldosterone receptor antagonists or statins at the time of blood sample collection (diagnosis) were excluded from the study. The control group included serum samples from age- and sex-matched healthy subjects selected from an electronic database containing demographical data from 450 healthy blood donors. All study participants signed the corresponding informed consent before their inclusion. The study was performed in accordance with the parameters established by the Helsinki Declaration and the local personal data protection law (LOPD 15/1999). The study protocol was approved by the independent bioethics committee of the participating centres.

Variables and assessments

Patient demographic, clinical and biochemical characteristics were recorded, and blood samples were obtained at the time of diagnosis. Clinical characteristics included estimated GFR (eGFR), calculated using the Schwartz formula in children and the Chronic Kidney Disease Epidemiology Collaboration equation in adults, and 24-h urinary protein excretion (proteinuria), which was considered to be in the nephrotic range when its values were >3.5 g/day and >40 mg/m^2/h in adults and children, respectively. Nephrotic syndrome was defined as the presence of proteinuria in the nephrotic range associated with hypoalbuminaemia (<3.5 g/dL) and oedema. Kidney biopsies, obtained at the time of INS diagnosis, were stained with haematoxylin and eosin, periodic acid–Schiff–methenamine and Masson's trichrome

Table 1. Baseline characteristics of study patients

	FSGS ($n=49$)	MCD ($n=57$)	MN ($n=46$)	Control ($n=50$)	P1	P2	P3	P4	P5	P6
Demographic characteristics										
Age [mean (SD)], years	50.3 (18.5)	48.4 (26.6)	45.5 (17.7)	46.4 (20.7)	0.600	0.300	0.500	0.400	0.600	0.800
Gender: male, *n* (%)	38 (22.4)	54 (94.7)	16 (34.8)	27 (54)		0.879			0.691	
Clinical characteristics										
Venous thrombosis, *n* (%)	6 (12.2)	4 (7.0)	6 (13.0)	0 (0.0)	0.070	0.070	0.070	–	–	–
GFR [mean (SD)], mL/min/1.73 m^2	90.5 (24.7)	102.5 (16.4)	94.4 (15.5)	94.1 (16.7)	**0.049**	1.000	0.590	1.000	0.320	1.000
Biochemical characteristics										
Proteinuria [mean (SD)], g/dL	7.4 (3.2)	6.4 (3.0)	8.0 (3.3)	0.0 (0.0)	**0.000**	**0.005**	**0.002**	**0.000**	0.376	**0.000**
Albumin [mean (SD)], g/dL	2.3 (0.4)	2.6 (0.6)	2.2 (0.5)	4.4 (0.2)	**0.002**	0.317	**0.000**	**0.000**	**0.000**	**0.000**
Cholesterol [mean (SD)], mg/dL	339.2 (140.4)	305.0 (52.6)	336.3 (22.6)	159 (38.8)	**0.025**	0.853	**0.043**	**0.000**	**0.000**	**0.000**
suPAR [mean (SD)], ng/mL	4347.5 (1734.0)	3905.3 (1547.3)	3726.7 (1467.2)	3160.1 (1234.0)	0.130	**0.046**	0.500	**0.000**	**0.011**	0.067
VWF [mean (SD)], ng/mL	175.4 (79.3)	180.4 (45.7)	185.8 (43.3)	50.8 (31.6)	0.800	0.270	1.000	**0.001**	0.069	0.404
VCAM-1 [mean (SD)], ng/mL	173.5 (135.4)	118.4 (98.3)	159.0 (133.9)	36.0 (16.4)	**0.009**	0.509	0.560	**0.000**	**0.000**	**0.000**
E-selectin [mean (SD)], ng/mL	47.5 (13.0)	33.8 (15.4)	33.2 (15.4)	16.6 (11.7)	**0.000**	**0.000**	0.835	**0.000**	**0.000**	**0.000**
Syndecan-1 [mean (SD)], ng/mL	198 (21.5)	197.8 (34.1)	176.3 (25.2)	38.9 (15.7)	0.960	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**

P1: FSGS versus MCD; P2: FSGS versus MN; P3: MCD versus MN; P4: FSGS versus control; P5: MCD versus control; P6: MN versus control. Bold values are the ones with statistical significance.

for morphological analysis. Immunofluorescence was performed using antibodies against IgA, IgG, IgM, C3, fibrinogen and light chains, and stained samples were subsequently processed for electron microscopy analysis. FSGS lesions were classified following the criteria of D'Agati *et al.* [15].

Regarding biochemical variables, serum creatinine was measured by the isotope dilution-mass spectrometry-traceable compensated method (Hitachi Modular P-800 Roche Diagnostics, Germany), and serum suPAR levels and endothelial markers were measured using commercial Enzyme-Linked ImmunoSorbent Assay (ELISA) kits, including Human uPARQuantikine® ELISA kit (R&D Systems, Minneapolis, MN, USA), syndecan-1 (Abcam, Cambridge, UK), VWF (antibodies-online GmbH, Aachen, Germany), VCAM-1 (R&D Systems, Minneapolis, MN, USA) and E-selectin (Abcam, Cambridge, UK). The reproducibility of suPAR measurements was assessed by analysing the coefficients of variation of suPAR in three or more samples obtained during the nephrotic phase before starting treatment in 11 patients. Standard laboratory procedures were used to determine serum levels of albumin and total cholesterol. Regarding complications (i.e. vein thrombosis), deep vein thrombosis of the lower extremities was diagnosed based on clinical criteria and confirmed by Doppler, whereas renal vein thrombosis was diagnosed by echo-Doppler and confirmed by angio-computed tomography of renal veins, both indicated upon clinical suspicion.

Statistical analysis

Quantitative variables were presented as the mean and standard deviation (SD), whereas qualitative variables were presented as frequencies and percentages. The former were compared using Student's *t*-test for independent data or an analysis of variance, whereas the latter were compared using the Chi-squared test or Fisher's exact test. Correlations between quantitative variables were analysed using the Pearson correlation test. The relationship between the circulating levels of endothelial adhesion molecules and suPAR and the demographic and clinical variables (transformed into a logarithmic scale) was assessed by multiple regression analyses, which included all factors yielding $P \leq 0.05$ in their respective single regression analyses. The significance threshold was set at a bilateral alpha value of 0.05. All analyses were performed using the SPSS 20.0 software.

RESULTS

Demographic, clinical and biochemical characteristics of the study population

During the recruitment period, 164 patients with INS were referred to the participating sites. Of them, 14 patients were excluded from the record for receiving treatment with corticosteroids, immunosuppressants, angiotensin II receptor antagonists, aldosterone receptor antagonists or statins at the time of blood sample collection (diagnosis). The final study cohort consisted of 152 patients diagnosed with INS, caused by either FSGS ($n=49$; 32%), MCD ($n=57$; 38%) or MN ($n=46$; 30%). Table 1 summarizes the demographic, clinical (when applicable) and biochemical characteristics of study patients and 50 selected controls. No significant differences were observed in age and gender distribution between patients and controls or between patient groups. No significant differences were found in pairwise comparisons regarding GFR, except for MCD patients, who showed higher values than FSGS patients. A total of 16 (10.5%) patients had developed venous thrombosis, irrespective of the diagnosis. With regard to biochemical characteristics, patients with MCD showed higher levels of serum albumin than those with MN and FSGS, whereas patients with MN presented significantly higher levels of proteinuria than those with FSGS and MCD.

Compared with controls, FSGS patients showed higher serum levels of VWF, those with FSGS and MCD showed higher serum levels of suPAR and all groups of patients showed significantly higher serum levels of VCAM-1, E-selectin and syndecan-1. Levels of suPAR and endothelial markers were highly variable within each group; nevertheless, significant differences between groups were found for all of them, except VWF. Particularly, VCAM-1 levels were significantly higher in patients with FSGS compared with patients with MCD, and E-selectin levels were higher in patients with FSGS compared

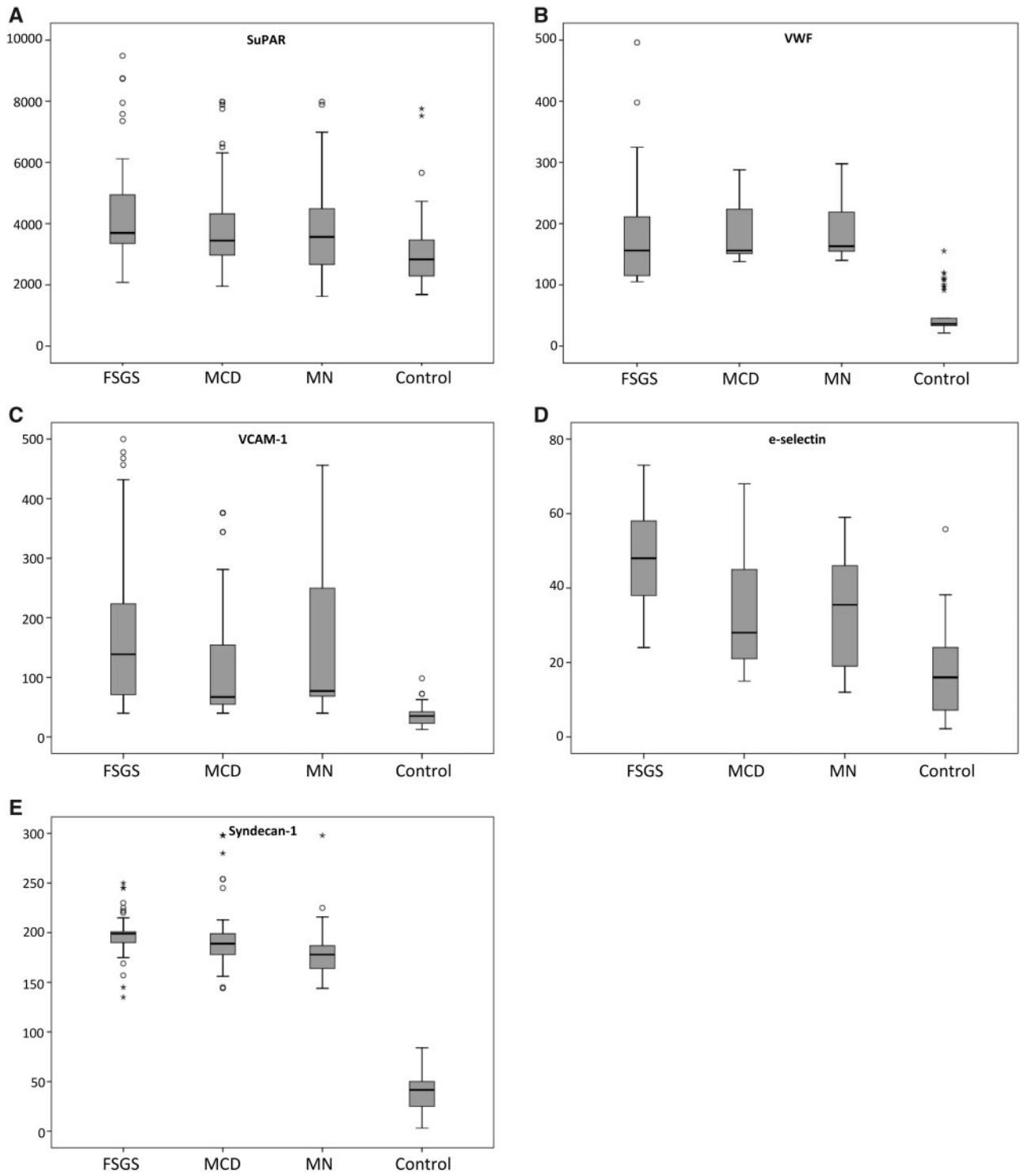

FIGURE 1: Boxplot diagrams of the serum levels of suPar and biomarkers of endothelial cell injury/activation in the four subgroups of analysis: FSGS, MCS, MN and control. (**A**) suPAR. (**B**) VWF. (**C**) VCAM-1. (**D**) E-selectin. (**E**) Syndecan-1. Serum levels are presented in nanograms per millilitre. Outliers and extreme outliers are represented with small circles and asterisks, respectively.

with the other two patient groups. Levels of syndecan-1 were significantly lower in patients with MN than in patients with FSGS and MCD, and suPAR levels were significantly lower in patients with MN compared with patients with FSGS (Table 1). Figure 1 shows the boxplot diagrams of the serum levels of suPAR and biomarkers of endothelial cell injury or activation. Coefficients of variation for the serum concentrations of suPAR used to assess the reproducibility of the measurements were <10.0%.

When comparing the characteristics of patients with and without deep venous thrombosis, significant differences between groups were found in albumin, VWF and VCAM-1 serum

Table 2. Baseline characteristics of patients with and without venous thrombosis, concomitant to INS

	Venous thrombosis ($n = 16$)	No venous thrombosis ($n = 136$)	P-value
Demographic characteristics			
Age [mean (SD)], years	49.1 (21.7)	48.1 (21.7)	0.864
Sex, *n* (%)			
Male	7 (43.7)	101 (74.3)	0.314
Female	9 (56.3)	35 (25.7)	
Clinical characteristics			
GFR [mean (SD)], mL/min/1.73 m^2	87.2 (21.4)	94.7 (24.7)	0.248
Biochemical characteristics			
Proteinuria [mean (SD)], g/dL	8.1 (2.5)	7.1 (3.3)	0.236
Albumin [mean (SD)], g/dL	2.0 (0.3)	2.4 (0.6)	**0.003**
Cholesterol [mean (SD)], mg/dL	332.3 (64.7)	324.6 (89.7)	0.740
suPAR [mean (SD)], ng/mL	4192.7 (1672.2)	3970.4 (1592.8)	0.600
VWF [mean (SD)], ng/mL	238.9 (84.8)	173.5 (50.0)	**0.000**
VCAM-1 [mean (SD)], ng/mL	206.0 (142.6)	141.7 (120.3)	**0.049**
E-selectin [mean (SD)], ng/mL	42.5 (15.1)	37.5 (16.0)	0.235
Syndecan-1 [mean (SD)], ng/mL	202.9 (37.2)	190 (28.3)	0.098

Table 3. Correlation matrix among variables of patients with INS

	Age	suPAR	VWF	VCAM-1	E-selectin	Syndecan-1	Cholesterol	Albumin	GFR
suPAR	0.31**								
VWF	0.05	0.39**							
VCAM-1	0.26**	0.43**	0.38**						
E-selectin	0.12	0.03	0.05	0.24**					
Syndecan-1	0.08	0.29**	0.29**	0.33**	0.33**				
Cholesterol	−0.11	−0.23	0.103	0.11	0.18*	0.12			
Albumin	0.12	0.04	−0.23**	−0.25**	−0.39**	−0.09	−0.34**		
GFR	−0.17*	−0.39**	−0.11	−0.13	−0.08	−0.07	0.18	−0.16	
Proteinuria	−0.02	0.08	−0.12	0.001	0.11	−0.11	0.23**	−0.41	−0.13

*$P < 0.05$; **$P < 0.01$.

levels: patients with venous thrombosis had lower albumin and higher VWF and VCAM-1 serum levels than patients without venous thrombosis (Table 2).

Relationships between demographic, clinical and biochemical variables

Table 3 summarizes all correlations between age, GFR and the levels of all biochemical variables investigated. The scatter plots corresponding to these correlations are displayed in Supplementary data, Figure S1. suPAR levels positively correlated with age and the serum levels of all endothelial markers except E-selectin and negatively correlated with GFR. Endothelial cell molecules showed positive correlations with each other—except E-selectin and VWF—and negative correlations with serum albumin.

Table 4 summarizes the results of the multivariate analyses regarding the demographic, clinical and biochemical factors that can potentially predict the serum levels of all endothelial markers and suPAR, showing only the variables with a significant contribution to each model. All models succeeded in finding significant associations between the serum levels of all analysed molecules and at least two factors, including between syndecan-1 levels and E-selectin, proteinuria and suPAR levels; between E-selectin and VCAM-1 levels and age, serum albumin and FSGS; and between VWF levels and age, serum albumin and GFR. The multiple regression model also showed an association between suPAR levels and GFR, age and VWF, syndecan-1 and VCAM-1 serum levels, explaining 37.6% of the variability in circulating suPAR levels.

Table 4. Multivariate regression models for predicting the levels of suPAR and biomarkers

Dependant variables	Independent predictors	β	t	Sig.	R^2
Log suPAR	GFR	−0.005	−5.2	0.000	0.376
	Age	0.003	2.37	0.019	
	VWF	0.001	2.372	0.019	
	Syndecan-1	0.002	2.106	0.037	
	VCAM-1	0.001	3.212	0.002	
Log VWF	GFR	−0.24	−3.04	0.003	0.100
	Albumin	−0.29	−3.65	0.001	
	Age	0.19	1.99	0.048	
Log VCAM-1	FSGS	0.18	2.16	0.032	0.100
	Albumin	−0.15	−1.98	0.049	
	Age	0.24	2.9	0.005	
Log E-selectin	FSGS	0.39	5.8	0.000	0.190
	Albumin	−0.43	−5.6	0.000	
	Age	0.16	2.3	0.027	
Log syndecan-1	E-selectin	0.329	4.48	0.000	0.210
	Proteinuria	−0.149	−1.99	0.048	
	suPAR	0.213	2.67	0.008	

DISCUSSION

In this cross-sectional study, we found that patients with nephrotic syndrome caused by FSGS, MCD or MN had increased circulating levels of endothelial markers such as VWF, VCAM-1, syndecan-1 and E-selectin. Furthermore, circulating levels of suPAR were significantly and independently associated with the levels of almost all these endothelial molecules. However, although all three diagnoses were associated with serum suPAR concentrations in univariate analyses, the multivariate analysis showed that suPAR levels were not associated with the histopathological pattern of the nephrotic syndrome.

In line with previous studies, we observed an increase in mean circulating levels of endothelial markers in patients with nephrotic syndrome [16, 17], which is possibly due to a state of endothelial activation and/or dysfunction associated with the nephrotic syndrome itself. However, in our study, the serum levels of the different endothelial molecules showed high interindividual variability within the three groups of patients analysed, ranging from very low values, comparable to those of healthy controls, to extremely high values. This variability suggests that only a subset of patients with nephrotic syndrome have high levels of endothelial cell molecules. The multivariate analysis of the variables potentially associated with the levels of endothelial cell molecules revealed associations between each of the endothelial cell molecules and demographic, clinical and/or biochemical characteristics. However, these explained only a small part of the variability, suggesting that, for the most part, it was associated with variables not included in our analysis.

The data from our study indicate that all three types of glomerular disease studied showed an increase in the levels of endothelial molecules, with the exception of VWF. However, although in the descriptive analysis both VCAM-1 and E-selectin levels were significantly higher in patients with FSGS, in the multivariate analyses, after adjusting for age, albumin level and GFR, the histopathological pattern did not significantly contribute to explaining the circulating levels of these two molecules. Increased levels of endothelial markers in nephrotic syndrome have been associated with the presence of endothelial dysfunction, assessed by a reduced arterial flow-mediated vasodilation and with an increased risk of venous thrombosis events [16]. Consistently with these observations, our results showed that patients with clinical venous thrombosis at the time of diagnosis had lower albumin levels and higher concentrations of VWF and VCAM-1 than those patients without venous thrombosis. However, as occurred in previous studies [18, 19], the measurement of endothelial molecule levels was performed concomitantly with the diagnosis of the thrombotic event and, therefore, the clinical significance of the different concentrations of biomarkers in patients with and without venous thrombosis must be evaluated cautiously. In this regard, the available data do not allow determination of whether an increase in the concentration of endothelial markers should be interpreted as a risk factor or as a mere consequence of the thrombotic complication. Furthermore, in the absence of a specifically directed systematic study, the presence of patients with subclinical venous thrombosis in the non-thrombosis group cannot be ruled out.

Since the identification of circulating levels of suPAR as potential biomarkers of FSGS, multiple studies have been carried out to analyse its clinical and/or pathogenic significance in nephrotic syndrome [2, 3, 5, 20]. The distribution of suPAR levels described in most studies performed so far indicates the existence of high interindividual variability that has been mainly associated with age, GFR and certain inflammatory biomarkers [2, 3], which is similar to our study. Our multivariate model to predict suPAR levels showed that VWF, syndecan-1 and VCAM-1 were independently associated with suPAR levels. This finding suggests concomitant high levels of suPAR and endothelial molecules, regardless of the aetiology of nephrotic syndrome. Although the clinical and/or pathogenic significance of the association between suPAR and endothelial marker levels is currently unknown, this relationship mirrors that observed in subjects without nephrotic syndrome, in which suPAR has been identified as a key molecule of endothelial dysfunction with predictive capacity for cardiovascular events [10].

The main limitation of our study was the cross-sectional design, which precluded the determination of endothelial molecules and suPAR before the diagnosis of venous thrombosis. Thus, we could not determine whether an increase in serum levels of endothelial molecules was a risk factor or a result of venous thrombosis. Furthermore, although our analysis revealed significant differences in the levels of suPAR and most endothelial markers between aetiological groups, the high interindividual variability of some of these markers—particularly suPAR—suggests that these results are likely to be influenced by the patient's profile.

In conclusion, the data from our study indicate that in patients with nephrotic syndrome secondary to FSGS, MCD and NM, the circulating levels of suPAR are independent of the primary renal disease and are significantly associated with age, GFR and levels of various endothelial markers (i.e. VWF, syndecan-1 and VCAM-1). The levels of these endothelial molecules, with the exception of syndecan-1, are associated with age and albumin levels, although the variability explained by demographic and biochemical characteristics are small. Thus, further studies are needed to identify the molecular mechanisms underlying an increase in suPAR and endothelial molecules in some patients with nephrotic syndrome. Finally, given the associations between suPAR and endothelial molecules and the risk of vascular events reported by other authors, we believe that future prospective studies must investigate the potential role of these molecules as biomarkers of vascular disease in patients with chronic-evolving nephrotic syndrome.

SUPPLEMENTARY DATA

Supplementary data are available at ckj online.

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CONFLICT OF INTEREST STATEMENT

None declared.

REFERENCES

1. Eddy AA, Symons JM. Nephrotic syndrome in childhood. *Lancet* 2003; 362: 629–639
2. Wei C, El Hindi S, Li J *et al*. Circulating urokinase receptor as a cause of focal segmental glomerulosclerosis. *Nat Med* 2011; 17: 952–960
3. Kronbichler A, Saleem MA, Meijers B *et al*. Soluble urokinase receptors in focal segmental glomerulosclerosis: a review on the scientific point of view. *J Immunol Res* 2016; 2016: 1
4. Bertelli R, Bonanni A, Di Donato A *et al*. Regulatory T cells and minimal change nephropathy: in the midst of a complex network. *Clin Exp Immunol* 2016; 183: 166–174
5. Meijers B, Maas RJH, Sprangers B *et al*. The soluble urokinase receptor is not a clinical marker for focal segmental glomerulosclerosis. *Kidney Int* 2014; 85: 636–640
6. Spinale JM, Mariani LH, Kapoor S *et al*. A reassessment of soluble urokinase-type plasminogen activator receptor in glomerular disease. *Kidney Int* 2015; 87: 564–574
7. Maas RJH, Wetzels JFM, Deegens J. Serum suPAR concentrations in patients with focal segmental glomerulosclerosis with end-stage renal disease. *Kidney Int* 2014; 85: 711
8. Edsfeldt A, Nitulescu M, Grufman H *et al*. Soluble urokinase plasminogen activator receptor is associated with inflammation in the vulnerable human atherosclerotic plaque. *Stroke* 2012; 43: 3305–3312
9. Cyrille NB, Villablanca PA, Ramakrishna H. Soluble urokinase plasminogen activation receptor–An emerging new biomarker of cardiovascular disease and critical illness. *Ann Card Anaesth* 2016; 19: 214–216
10. Sehestedt T, Lyngbæk S, Eugen-Olsen J *et al*. Soluble urokinase plasminogen activator receptor is associated with subclinical organ damage and cardiovascular events. *Atherosclerosis* 2011; 216: 237–243
11. Mekonnen G, Corban MT, Hung OY *et al*. Plasma soluble urokinase-type plasminogen activator receptor level is independently associated with coronary microvascular function in patients with non-obstructive coronary artery disease. *Atherosclerosis* 2015; 239: 55–60
12. Lyngbæk S, Sehestedt T, Marott JL *et al*. CRP and suPAR are differently related to anthropometry and subclinical organ damage. *Int J Cardiol* 2013; 167: 781–785
13. Zhang Q, Zeng C, Fu Y *et al*. Biomarkers of endothelial dysfunction in patients with primary focal segmental glomerulosclerosis. *Nephrology (Carlton)* 2012; 17: 338–345
14. Sharma B, Saha A, Dubey NK *et al*. Endothelial dysfuntion in children with idiopathic nephrotic syndrome. *Atherosclerosis* 2014; 233: 704–706
15. D'Agati VD, Fogo AB, Bruijn JA *et al*. Pathologic classification of focal segmental glomerulosclerosis: a working proposal. *Am J Kidney Dis* 2004; 43: 368–382
16. Tkaczyk M, Czupryniak A, Owczarek D *et al*. Markers of endothelial dysfunction in children with idiopathic nephrotic syndrome. *Am J Nephrol* 2008; 28: 197–202
17. Malyszko J, Malyszko JS, Mysliwiec M. Markers of endothelial cell injury and thrombin activatable fibrinolysis inhibitor in nephrotic syndrome. *Blood Coagul Fibrinolysis* 2002; 13: 615–621
18. Smith NL, Rice KM, Bovill EG *et al*. Genetic variation associated with plasma von Willebrand factor levels and the risk of incident venous thrombosis. *Blood* 2011; 117: 6007–6011
19. Koster T, Blann AD, Briët E *et al*. Role of clotting factor VIII in effect of von Willebrand factor on occurrence of deep-vein thrombosis. *Lancet* 1995; 345: 152–155
20. Segarra A, Jatem E, Quiles MT *et al*. Value of soluble urokinase receptor serum levels in the differential diagnosis between idiopathic and secondary focal segmental glomerulosclerosis. *Nefrologia* 2014; 34: 53–61