Soluble urokinase-type plasminogen activator receptor as a biomarker for focal segmental glomerulosclerosis; a retrospective analysis

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Abstract

Background: Focal segmental glomerulosclerosis (FSGS) is a clinicopathological syndrome that presents with proteinuria, usually in the nephrotic range and evidence of histologic lesions of focal and segmental glomerular sclerosis with diffuse foot-process effacement. Recently, suPAR (soluble urokinase-type plasminogen activator receptor) was proposed as the potential circulating causative factor for primary FSGS.

Objectives: We performed a cross-sectional study with the aim to determine whether there is a relationship between suPAR serum levels and primary FSGS. The secondary aim was to associate serum suPAR levels with kidney dysfunction.

Patients and Methods: We enrolled a total of 90 patients with both suPAR serum levels and proteinuria. From these, 61 patients performed a renal biopsy.

Results: The mean age was 49.8 ± 17.2 years, 37 were females (60.7%) and 54 were Caucasian race (91.5%). FSGS was diagnosed in 30 patients (49%). suPAR levels were positive in 34 patients (55.7%) and negative in 27 (44.3%). Concerning the positive results, 17 patients had the histologic diagnosis of FSGS, which gives the test a sensibility of 28%. Concerning the negative results, 14 patients had a different histologic diagnosis other than FSGS, which gives the test a specificity of 23%. The predicted positive value was 50% and the predicted negative value was 52%. suPAR serum levels were not correlated with 24 hours proteinuria ($P=0.5$), but we found a positive correlation with C-reactive protein ($P=0.037$) and an inverse correlation with estimated glomerular filtration rate (eGFR) ($P<0.001$).

Conclusions: We found that a positive suPAR test is not a marker of FSGS, but it can be a marker of podocyte and glomerular lesion, as it is inversely correlated with renal function in a cohort of proteinuric patients. Further studies are needed to further validate suPAR as a specific biomarker of glomerular damage.

Implication for health policy/practice/research/medical education:
Some research groups proposed soluble urokinase-type plasminogen activator receptor (suPAR) as a potential circulating causative factor for primary focal segmental glomerulosclerosis (FSGS). In this study we obtained very low sensibility (28%) and specificity (23%) of SuPAR for FSGS, and very low predicted positive value (50%) and predicted negative value (52%) was seen too. We found an inverse correlation with eGFR ($P<0.001$). Our results show that suPAR test is not a marker of FSGS, but it can be a marker of podocyte and glomerular lesions in a cohort of proteinuric patients.


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1. Background
Focal segmental glomerulosclerosis (FSGS) is a clinicopathological syndrome that was first described in an autopsy series (1). It presents with proteinuria, usually in the nephrotic range and evidence of histologic lesions of focal and segmental glomerular sclerosis with diffuse foot-process effacement (2,3). FSGS is one of the leading causes of end-stage renal disease (ESRD) in children and adults (4), it affects both native and transplanted kidneys (5-7), with the highest rate of recurrence (30%) after transplantation comparing with other glomerular diseases, manifested by proteinuria and accelerated renal allograft dysfunction and loss (8,9).

The lesions of FSGS have various etiologies other than primary FSGS, comprising genetic, as well as secondary forms, being primary FSGS the largest group (2), and accounting for approximately 40% of the causes of idiopathic nephrotic syndrome (10). The pathogenesis of primary FSGS remains unknown. However, it was postulated that circulating factors may be the potential trigger of renal injury (11). This was suggested due to high rate of recurrence in kidney transplants, sometimes hours after surgery, with some patients improving with plasmapheresis (12,13). Additionally a case report explained a potential transmission of a permeability factor from a pregnant woman with primary FSGS to her newborn infant, who presented transient proteinuria (14); and observation that infusion of plasma from FSGS patients causes proteinuria in rats (15-17).

A soluble form of uPAR (soluble urokinase-type plasminogen activator receptor) was proposed as being a potential causative circulating factor for primary FSGS. The urokinase-type plasminogen activator receptor (uPAR) is a cell membrane glycosylphosphatidylinositol (GPI)-anchored three domain protein (DI, DII and DIII) (18) expressed in various cell types, such as endothelial cells (19), immune cells (20-22), tumor cells (23), tubular epithelial cells (24) and podocytes (25). The soluble urokinase plasminogen activator receptor (suPAR) is released through cleavage of uPAR from its GPI-anchor (18). suPAR can be further cleaved between the DI and DII/DIII domains, thus depending on the degree of glycosylation and the size of cleaved proteins, suPAR’s size ranges from 25 to 50 kDa (18,26).

Low levels of suPAR are present in the serum of healthy individuals, regulating neutrophil trafficking and stem cell mobilization (18). An increase in suPAR levels may be seen in inflammatory diseases and infections, including HIV, indicating a role as an acute phase reactant (27-29). Similarly elevated levels may also be observed in malignant neoplasms (30).

Recent observations found that serum suPAR levels are elevated in patients with primary FSGS, with the highest concentrations observed in patients with recurrent FSGS (31,32). Additionally, it was proposed that suPAR levels could differentiate between primary and secondary forms of FSGS (33), and that suPAR levels could predict steroid-responsiveness of FSGS, as patients who presented higher levels had a better response to steroid treatment (34).

Subsequent reports, however, indicated that suPAR might not be a specific marker for primary FSGS (35), with several studies describing an inverse correlation of suPAR levels with the estimated glomerular filtration rate (eGFR) (36-39).

2. Objectives
The primary aim of our study was to determine, in a population of proteinuric patients, whether there is a relationship between suPAR serum levels and primary FSGS. The secondary aim was to associate serum suPAR levels with kidney dysfunction.

3. Patients and Methods
3.1. Study population
We performed a cross-sectional study in a cohort of adult (>18 years old) proteinuric patients (proteinuria superior to 300 mg in 24 hours) with at least one determination of suPAR serum levels, admitted to nephrology ward from January 2015 to December 2016.

3.2. Predictor variable
The predicted variable was suPAR levels. These were determined by enzyme-linked immunosorbent assays (ELISA). The positive and negative values depend on the gender; for males, a negative value is less than 5 ng/mL. For a female, a negative value is less than 5.5 ng/mL.

3.3. Outcome variable
For the primary aim of the study, the outcome variable was the histologic diagnosis of renal disease, determined by a renal biopsy. Kidney biopsies were stained with hematoxylin-eosin, Masson trichrome and silver, and Congo red birefringence detected serum amyloid deposits. Immunofluorescence (IF) was performed on frozen sections using labeled human immunoglobulin (IgA, IgG, IgM, C3, C4, C1q and fibrinogen). When no frozen fragment was available, we made indirect immunoperoxidase using formalin-fixed paraffin embedded section. FSGS was diagnosed by glomerular scarring and fusion/effacement of foot processes, without immune deposits, or with nonspecific binding of...
IgM and C3.
For the secondary aim of the study, our outcome variable was the estimated glomerular filtration rate by EPI formula, as well as proteinuria levels.

3.4. Ethical issues
The present study followed the tenets of the Declaration of Helsinki on medical protocol and ethics and was approved by the Institutional Review Board.

3.5. Covariates
Age, gender, race, etiology of chronic kidney disease, C-reactive protein, 24 hours proteinuria, and eGFR at the time of suPAR levels determination were also analyzed.

3.6. Statistical analysis
We presented continuous variables as mean ± standard deviation (SD) and categorical variables as frequencies. Primary aim: for this proposes we performed a cross-sectional analysis, including all patients of our study who had both suPAR serum levels and a renal biopsy. We calculated the sensibility and specificity of a positive/negative suPAR test, and the positive and negative value of the test. Secondary aim: for this proposes we performed a cross-sectional analysis, including all patients who had suPAR serum levels, eGFR and 24 hours proteinuria determined. A univariate analysis between suPAR serum levels and age, eGFR and 24 hours proteinuria (by Spearman’s correlation) and between suPAR and gender, race, and diabetes (t test) was performed. After dividing suPAR serum levels into quartiles, correlations between quartiles and eGFR and proteinuria were made using one-way analysis of variance (ANOVA).

All data were analyzed using STATA package version 13.1. A two-sided P<0.05 was considered significant for the whole analysis.

4. Results
We enrolled a total of 90 patients admitted to the nephrology department with both suPAR serum levels and proteinuria from January 2015 to December 2016.

4.1. SuPAR serum levels didn’t predict the existence of FSGS lesions
Of all patients, 61 patients performed a renal biopsy. The mean age was 49.8 ± 17.2 years, 37 females (60.7%), 54 Caucasian race (91.5%).
FSGS was diagnosed in 30 patients (49%). Table 1 illustrates all the histologic diagnosis that were found. The median suPAR levels were 5.4 (3.5–7.4) ng/mL, 5.3 (2.9–7) ng/mL in those with FSGS lesions and 5.4 (3.6–7.9) in those without FSGS lesions. In 34 patients, the suPAR levels were positive (55.7%) and in 27 patients, the suPAR levels were negative (44.3%). Concerning the positive results (n = 34), 17 patients had the histologic diagnosis of FSGS, which gives the test a sensibility of 28%. Concerning the negative results (n = 27), 14 patients had a different histologic diagnosis other than FSGS, which gives the test a specificity of 23%. The predicted positive value was 50% and the predicted negative value was 52%.

4.2. SuPAR serum levels are correlated with eGFR
We studied 90 proteinuric patients with at least one serum suPAR evaluation, 48 females (53.3%) and 42 males, 78 Caucasian (90.7%), with mean age of 54 ± 17 years.
Medical history was positive for diabetes in 20 patients (22.2%) and hypertension in 71 (78.9%).
Median suPAR levels were 5.7 (3.8–7.5) ng/mL, median eGFR 39.5 (23.2–86.7) mL/min, median 24 hours proteinuria 2120 (960–4581) mg/d, median C-reactive protein was 2.7 (1.2–8.3) mg/dL.
SuPAR serum levels were not correlated with age (P=0.15), gender (P=0.08), but were correlated
with race \((P=0.01)\) with higher values for Caucasian race. SuPAR serum level was not correlated with 24h proteinuria \((P=0.5)\), but we found a positive correlation with C-reactive protein \((r=0.3, P=0.037)\); and an inverse correlation with eGFR \((r=-0.5, P<0.001)\).

After dividing suPAR serum levels into quartiles \(\text{(see Table 2)}\), we found that from the first to the fourth quartile of suPAR, estimated GFR decreased \((P<0.001)\), but age increased \((P=0.002)\). No other correlations were found. Adjusting for age, and comparing with the first quartile of suPAR, we confirmed a decreasing in eGFR \((\text{second quartile } P=0.6; \text{third quartile } [P=0.003]; \text{fourth quartile } [P<0.001])\).

5. Discussion

Our study showed a poor sensibility and specific of suPAR as a serum marker of FSGS, but showed that suPAR is a good marker of renal dysfunction \(\text{(because it is inversely correlated with eGFR in proteinuric patients, adjusting for age)}\), being an eventual marker of podocyte lesion, other than FSGS.

Nevertheless, we have to acknowledge some limitation to this study. It is a retrospective, cross-sectional study, with a small number of patients.

The hypothesis of a circulating factor being implied in the pathogenesis of FSGS has been first proposed in 1972, when a case series of patients with recurrent FSGS after kidney transplant has been described by Hoyer et al \(\text{(40)}\).

Wei et al, first reported that induced uPAR expression in podocytes could cause foot process effacement and proteinuria \(\text{(25)}\). Subsequently, they identified significantly elevated levels of suPAR in approximately 70% of patients with primary and recurrent FSGS when compared to other forms of primary glomerulopathies. Additionally, suPAR levels remain elevated after kidney transplant in subjects who developed recurrent FSGS, being suPAR-mediated activation of \(\alpha_\beta_3\)-integrin on podocyte foot processes the mechanism of injury induced by elevated suPAR concentrations. The authors also proposed a cut-off level of suPAR of 3000 pg/mL \(\text{(31)}\). Subsequently it was showed that suPAR decreases nephrin expression in human podocytes through suppression of Wilms tumor-1 transcription factor. This phenomenon would be time and dose-dependent and would only be mediated by full-length suPAR \(\text{(41)}\).

In addition, co-injection of suPAR with anti-CD40 autoantibody, a potentially pathogenic antibody identified in the serum of patients with recurrent FSGS after kidney transplantation, elicited greater proteinuria in mice, suggesting that suPAR can also cooperate with other molecules to produce renal injury \(\text{(42)}\).

Despite the limitations we acknowledge, our results are in accordance with the subsequent studies that failed to validate suPAR as a biomarker of FSGS, however we found a correlation with renal function.

Maas et al, was the first to refute the hypothesis, as no difference was found in serum suPAR concentrations among a cohort of patients with primary FSGS, secondary FSGS and minimal change disease \(\text{(MCD)}\) \(\text{(35,43)}\). A subsequent report by the same group compared serum suPAR levels in 54 patients with biopsy-proven idiopathic FSGS and 476 non-FSGS patients; no difference in suPAR levels was noted in primary FSGS and control patients. However, multivariate analysis revealed an inverse association of suPAR with estimated glomerular filtration rate and serum albumin, while a positive association with age and C-reactive protein was found \(\text{(37)}\).

The relationship between suPAR levels and eGFR was confirmed in a Japanese cohort with primary glomerular diseases, including FSGS. In this study, suPAR levels were also significantly higher in the patients with ANCA-associated glomerulonephritis, which is in accordance with previous observations that inflammation might affect suPAR concentration \(\text{(39)}\). Musetti et al also established an association between elevated serum suPAR levels with reduced eGFR and presence of proteinuria in both primary and secondary glomerulonephritis \(\text{(GN)}\), through a cross-sectional analysis of suPAR levels on 42 patients with primary non-FSGS and 140 patients with secondary GN with known autoimmune disease \(\text{(44)}\). It was also proposed that an elevated serum suPAR levels are independently associated with incident chronic kidney disease, with greater levels associated with accelerated decline in eGFR \(\text{(45)}\).

6. Conclusions

In conclusion, we found that a positive suPAR test is not a marker of FSGS, but it can be a marker of podocyte and glomerular lesion, as it is inversely correlated with renal function in a cohort of proteinuric patients. Further studies are needed to further validate suPAR as a specific biomarker of glomerular damage.

Limitations of the study

We acknowledge the fact that it is a retrospective, cross-sectional study, with a small number of patients.

Authors’ contribution

MV collected the data; prepared the primary draft of the manuscript; ACF designed the study, analyzed
and interpreted the data, prepared the primary draft of the manuscript; MCS, MG, HV and FC contributed to the acquisition and interpretation of the data; FR and FN performed critical revision of the article and interpretation of the data. All authors approved the final version of the manuscript.

Conflicts of interest
The authors declare that they have no conflict of interest.

Ethical considerations
Ethical issues (including plagiarism, data fabrication, double publication) have been completely observed by the authors.

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