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Title: Soluble urokinase plasminogen activator receptor levels reflect organ damage in systemic lupus erythematosus

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ABSTRACT

Assessments of disease activity and organ damage in systemic lupus erythematosus (SLE) remain challenging due to lack of reliable biomarkers and to disease heterogeneity. However, ongoing inflammation can be difficult to distinguish from permanent organ damage caused by previous flares or medication side effects. Circulating soluble urokinase plasminogen activator receptor (suPAR) has emerged as a potential marker of inflammation and disease severity, and an outcome predictor in several disparate conditions. This study was done to evaluate suPAR as a marker of disease activity and organ damage in SLE.

Sera from 100 healthy donors and 198 SLE patients fulfilling the 1982 American College of Rheumatology (ACR) classification criteria and/or the 'Fries criteria' were analyzed for suPAR by enzyme immunoassay. 18 patients with varying degree of disease activity were followed longitudinally. Disease activity was assessed by SLE disease activity index-2K and the physician's global assessment. Organ damage was evaluated by the Systemic Lupus International Collaborating Clinics/ACR damage index (SDI).

Compared to healthy controls, serum suPAR levels were significantly elevated in SLE patients. No association was recorded regarding suPAR levels and SLE disease activity in cross-sectional or consecutive samples. However, a strong association was observed between suPAR and SDI ($p < 0.0005$). Considering distinct SDI domains, renal, neuropsychiatric, ocular, skin and peripheral vascular damage had significant impact on suPAR levels.

This study is the first to demonstrate an association between serum suPAR and irreversible organ damage in SLE. Further studies are warranted to evaluate suPAR and other biomarkers as predictors of evolving organ damage.

Keywords: systemic lupus erythematosus; disease activity; organ damage; soluble urokinase plasminogen activator receptor

Abbreviations: ACR = American College of Rheumatology; ANA = antinuclear antibody; C = complement protein; CRP = C-reactive protein; D_I = domain I; D_{II} = domain II; D_{III} = domain III; dsDNA = double-stranded DNA; ELISA = enzyme-linked immunosorbent assay; ESR = erythrocyte sedimentation rate; Fas = apoptosis stimulating fragment; HEp-2 = Human Epithelial cell line type-2; IFN = interferon; Ig = immunoglobulin; IL = interleukin; PGA = physician's global assessment; SDI = Systemic Lupus International Collaborating Clinics/ACR damage index; sFas = soluble Fas; SLE = systemic lupus erythematosus; SLEDAI-2K = SLE disease activity 2000; suPAR = soluble urokinase plasminogen activator receptor; TNF = tumor necrosis factor; uPAR = urokinase plasminogen activator receptor

INTRODUCTION

Systemic lupus erythematosus (SLE) is a rheumatic disease characterized by multi-organ involvement with episodes of disease flares and remission over time. The pathogenesis is believed to relate to abnormal apoptosis and deficient elimination of apoptotic material, such as nuclear proteins and nucleic acids, eventually leading to autoantibody production and formation of circulating or tissue-bound immune complexes.¹ Autoantibody-binding to tissue-exposed autoantigens and/or insufficient receptor-mediated clearance of circulating immune complexes via the reticuloendothelial system are explanations to extrahepatic immune complex formation/deposition.²⁻⁴

Although autoantibodies, complement proteins, blood cell counts and erythrocyte sedimentation rate (ESR) can be helpful markers of diagnosis, prognosis, and/or degree of ongoing inflammation, distinction of disease activity from irreversible organ damage remains a challenge.⁵ C-reactive protein (CRP) is usually a reliable marker of systemic inflammation, but this is not the case in SLE^{6, 7} or viral infections⁸ probably due to interferon alpha (IFN α) dependent inhibition of hepatic CRP production.⁹ Other biomarkers may reflect specific organ involvements, most notably lupus nephritis which is often mirrored by raised levels of autoantibodies against double-stranded (ds) DNA, nucleosomes and/or complement protein (C) 1q.^{2, 5, 10}

The Systemic Lupus International Collaborating Clinics/American College of Rheumatology damage index (SDI)¹¹ covers 12 organ systems and measures accumulated organ damage that has occurred since the onset of SLE. SDI is scored regardless of whether the damage can be attributed to SLE or to other causes. A limited number of cross-sectional studies have demonstrated associations between certain biomarkers (*e.g.* apoptosis stimulating fragment

(Fas/CD95), both membrane bound and soluble (sFas), CRP and osteopontin), and organ damage.¹²⁻¹⁵ However, only plasma levels of osteopontin have been shown predict organ damage, and this was shown for a relatively small study group of SLE patients.¹⁵

Soluble urokinase plasminogen activator receptor (suPAR) is part of the plasminogen activation system and is involved in inflammation, tissue remodeling and cancer metastasis.¹⁶ Many cell types express uPAR (CD87), including vascular smooth muscle cells,¹⁷ endothelial cells,^{18, 19} megakaryocytes,²⁰ monocytes, neutrophils,^{21, 22} and activated T-cells.²³ Cell-surface uPAR expression is up-regulated upon stimulation with growth factors and cytokines such as interleukin (IL-)1 β and tumor necrosis factor (TNF),^{24, 25} the latter possibly involved in the pathogenesis of SLE.²⁶ The full length suPAR shed from the cell surface contains three domains (D_{I-III}), but suPAR may also occur in different cleaved forms consisting of only D_I or D_{II-III}, with different biological functions.^{16, 27} In the first studies on circulating suPAR, levels were found to be elevated and to predict disease outcome in various forms of cancer and infectious diseases.^{16, 28} It has also been suggested to be a biomarker of value in rheumatoid arthritis,²⁹ and to reflect organ damage in liver and kidney disease.³⁰⁻³² The aims of the present study were to investigate if circulating suPAR reflects inflammatory activity and/or organ damage in lupus.

METHODS

Patients and controls

198 SLE patients (22 men, 176 women; mean age 50.6 years; range 18-88) were recruited to the study. All patients took part in a prospective structured follow-up programme at the rheumatology clinic of Linköping university hospital, Sweden. 160 (81 %) patients met the 1982 American College of Rheumatology (ACR-82) classification criteria,³³ whereas 38 (19%) had a clinical diagnosis of SLE based on a history of abnormal antinuclear antibody (ANA) titer, and at least two typical organ manifestations at the time of diagnosis (referred to as the 'Fries criteria').³⁴ Presence of anti-cardiolipin antibodies of IgG- and or IgM class detected by ELISA and/or positive lupus anticoagulant test (not classified as an immunological criterion according to ACR-82) was found in 26 of the 38 individuals in the Fries group. Patients were recruited consecutively; most were prevalent cases (91%), but a few (9%) had recent-onset disease at the time of sampling. The physician's global assessment of disease activity (PGA 0-4) and the 'SLE disease activity 2000' (SLEDAI-2K)³⁵ was recorded at each visit. Disease severity/organ damage was estimated by the SDI.¹¹ 184 (93%) of the patients were Caucasians. 79 (40 %) of the patients were prescribed antimalarials (AM) alone, 56 (28%) other disease-modifying anti-rheumatic drugs $\pm$ AM and 130 (66%) oral prednisolone. Further characteristics of the patients are summarized in Table 1.

Table 1. Baseline characteristics of SLE patients with SLE and differences between patients fulfilling the 1982 ACR criteria and patients meeting the Fries criteria only

<i>Characteristics</i>	<i>All patients (n=198) Mean (range)</i>	<i>ACR-82 (n=160) Mean (range)</i>	<i>Fries (n=38) mean (range)</i>	<i>Fries vs. ACR-82 p-value*</i>
Number of fulfilled ACR criteria	4.6 (3-9)	5.0 (4-9)	3.0 (3-3)	<0.0005
SLICC/ACR damage index	1.1 (0-8)	1.2 (0-8)	1.2 (0-8)	ns
SLEDAI	2.2 (0-16)	2.5 (0-16)	0.9 (0-7)	<0.0005
Physician's global assessment (PGA)	0.4 (0-4)	0.4 (0-4)	0.2 (0-1)	ns
Disease duration (years)	11.4 (0-45)	11.6 (0-45)	11.0 (0-36)	ns
Age (years)	50.6 (18-88)	49.5 (18-88)	55.0 (28-80)	ns
<i>ACR criteria</i>	<i>Frequency (%)</i>			<i>p-value[†]</i>
Malar rash	43.9	49.4	21.1	0.002
Discoid rash	16.2	19.4	2.6	0.012
Photosensitivity	53.5	58.8	31.6	0.003
Oral ulcers	8.6	10.6	0.0	0.047
Arthritis	77.3	76.9	78.9	ns
Serositis	38.9	40.0	34.2	ns
Renal disorder	21.2	26.3	0.0	<0.0005
Neurologic disorder	5.1	6.3	0.0	ns
Hematological disorder	53.5	62.5	18.4	<0.0005
Immunological disorder	46.0	53.8	13.2	<0.0005
Antinuclear antibody (IF)	98.5	98.1	100	ns
<i>SLICC/ACR damage index ≥ 1</i>	<i>Frequency (%)</i>			<i>p-value[†]</i>
Total score	47.0	50.0	34.2	ns
Ocular	7.6	8.1	5.3	ns
Neuropsychiatric	19.2	20.0	15.8	ns
Renal	4.5	5.0	2.6	ns
Pulmonary	3.0	2.5	5.3	ns
Cardiovascular	13.1	14.4	7.9	ns
Peripheral vascular	8.1	9.4	2.6	ns
Gastrointestinal	3.0	2.5	5.3	ns
Musculoskeletal	13.1	14.4	7.9	ns
Skin	4.0	3.8	5.3	ns
Premature gonadal failure	0.0	0.0	0.0	ns
Diabetes	5.1	5.6	2.6	ns
Malignancy	3.0	2.5	5.3	ns

Abbreviations: SLE, systemic lupus erythematosus; ACR-82, 1982 American College of Rheumatology classification criteria; SLICC/ACR, Systemic Lupus International Collaborating Clinics/American College of Rheumatology; NS, not significant; SLEDAI, SLE disease activity index; ACR, American College of Rheumatology; IF, immunofluorescence

*Mann-Whitney U test

[†]Fisher's exact test

Peripheral venous blood was drawn from each individual at baseline. Serum was prepared and stored at -70°C until analyzed. In addition, serial serum samples were drawn from 18 of the 198 recruited patients (2-13 visits per patient), presenting with signs of raised disease activity defined as a SLEDAI-2K peak score of at least 4 (mean 8.4; range 4-19) over time. The selection was also made to represent different disease manifestations. All 18 patients met the 1982 ACR classification criteria. For each of the 18 patients, two visits were chosen to represent the lowest and highest disease activity, respectively (based on PGA and SLEDAI-2K). In 13 of the 18 patients, the disease activity was evaluated by the same physician at all visits.

100 healthy controls (50 men, 50 women; mean age 45.8 years; range 22-70) without ongoing medication served as controls.

Routine laboratory analyses

At all visits, laboratory analyses included blood cell count (erythrocytes, leukocytes and platelets), urine albumin, urine erythrocytes, ESR and circulating levels of CRP, creatinine, creatine kinase, ANA, C3 and C4 as well as classical hemolytic complement function. High sensitivity CRP (detection limit of 0.12 mg/L) was analyzed by turbidimetry at the clinical chemistry, Linköping university hospital. Complement analyses were performed at Uppsala Akademiska hospital, Sweden. IgG-class ANA was analyzed by indirect immunofluorescence microscopy using multispot slides with fixed Human Epithelial cell line type-2 (HEp-2) cells (ImmunoConcepts, Sacramento, CA, USA) as substrate. Gamma-chain specific fluorochrome-labeled polyclonal anti-human IgG was used as secondary antibody. Positive ANA was defined as nuclear immunofluorescence staining at a serum dilution of 1:200, corresponding to $>95^{\text{th}}$ percentile based on 150 healthy female blood donors. Microscope slides with fixed *Crithidia luciliae* (ImmunoConcepts) were used to analyze IgG class anti-

dsDNA antibodies by immunofluorescence (cut-off titer 1:10, corresponding to >99th percentile among healthy female blood donors). Anti-dsDNA antibody levels were end-point titration in 2-fold dilution steps.

suPAR and cytokine analyses

For suPAR analyses sera were diluted 1:100 and assayed in duplicates by enzyme-linked immunosorbent assay (ELISA) (ViroGates, Copenhagen, Denmark; kindly provided by Electra-Box AB, Tyresö, Sweden). Serum samples from SLE patients and healthy controls were interdispersed on the multi-well plates to avoid biased results due to inter-assay variation. IL-1 receptor antagonist (IL-1ra) was analyzed by a commercial ELISA from R&D Systems (Abingdon, UK). IFN α was measured by a dissociation-enhanced lanthanide fluorescent immunoassay (DELFI) at Uppsala university, Sweden.³⁶ In sera from 155 of the patients fulfilling the 1982 ACR classification criteria, IL-10, IL-6, IL-1 β and TNF were analyzed with a high sensitivity multiplex magnetic bead assay (Milliplex, Millipore, Solna, Sweden).

Statistics

Relations between disease activity or organ damage with suPAR or CRP were assessed using multiple linear regression models with suPAR or log-transformed CRP as the response variables. Because of known or potential age- and sex-dependent variations of suPAR³⁷ and CRP³⁸ levels, age and sex were included as independent variables for all multiple linear regression analyses with suPAR or logCRP as the dependent variable. Due to significant age and sex differences between the healthy controls and patients, univariate analysis of variance with adjustment for age and sex was used to assess differences between these groups.

Since 19% of the patients only met the Fries criteria, all statistical analyses were run for (1) all patients, and (2) only the patients fulfilling ACR-82.

Fisher's exact test or the Mann whitney U test was used to determine differences in disease characteristics between patients fulfilling ACR-82 and patients only meeting the Fries criteria. Wilcoxon matched-paired sign rank test was used to compare individual differences in suPAR level at lowest and highest disease activity.

A two-tailed p -value of <0.05 was considered significant. All statistical analyses were performed with the SPSS Statistics 19-20 (IBM, Armonk, NY, USA) or GraphPad Prism version 5.03 (GraphPad Software, San Diego, CA, USA) softwares.

Ethics

Informed consent was obtained from all subjects. The study protocol was approved by the regional ethics committee in Linköping (M75-08/2008).

RESULTS

Associations of suPAR with cytokines and routine laboratory measures

As shown in Table 2, suPAR levels were positively associated with all cytokines measured apart from IL-6. suPAR was positively associated with creatinine, CRP, ESR, leukocyte count, platelet count, C4 and urine albumin, whereas it was inversely associated with erythrocyte count. In addition, among patients fulfilling ACR-82, C3 and urine erythrocytes were also positively associated with suPAR.

Table 2. Association of cytokines and laboratory measures with suPAR levels

Variable *	All patients			ACR-82 patients		
	<i>n</i>	$\beta^{\dagger}$	<i>p</i> -value	<i>n</i>	$\beta^{\dagger}$	<i>p</i> -value
IL-1 β		NT	NT	155	0.37	<0.0005
TNF		NT	NT	155	0.34	<0.0005
IL-10		NT	NT	155	0.31	<0.0005
IL-1ra		NT	NT	155	0.24	0.002
IL-6		NT	NT	155		NS
IFN α	198	0.19	0.005	160	0.20	0.006
CRP	198	0.39	<0.0005	160	0.36	<0.0005
ESR	198	0.20	0.003	160	0.24	0.002
Leukocyte count	198	0.24	<0.0005	160	0.23	0.003
Platelet count	198	0.18	0.008	160	0.20	0.009
C4	197	0.15	0.027	159	0.19	0.017
C3	198		NS	160	0.17	0.035
Classical complement function	189		NS	153		NS
Urine albumin	198	0.19	0.005	160	0.20	0.008
Urine erythrocytes	198		ns	160	0.17	0.026
Anti-dsDNA titer	198		NS	160		NS
Creatinine	197	0.41	<0.0005	159	0.41	<0.0005
Hemoglobin	198	-0.14	0.041	160	-0.18	0.021
Creatine kinase	198		NS	160		NS

Abbreviations: suPAR, soluble urokinase plasminogen activator receptor; ACR-82 American College of Rheumatology classification criteria; IL, interleukin; IL-1ra, interleukin 1 receptor antagonist; NT, not tested; TNF, tumor necrosis factor; IFN, interferon; NS, not significant; CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; dsDNA, double-stranded DNA.

*All analyses are adjusted for sex and age

$\dagger$ Standardized beta coefficient (SD increase)

$\ddagger$ not significant ($p < 0.05$)

suPAR versus SLE disease activity

The difference between healthy controls ($n=100$) and SLE patients at baseline ($n=198$) regarding suPAR levels was only borderline significant ($p=0.050$) (Figure 1). However, when excluding patients with leukocytopenia ($<3.5 \times 10^9/L$) at sampling the difference was statistically significant ($p=0.034$). Because of a relatively low average SLE disease activity at baseline, we also compared suPAR levels in patients with active disease at baseline ($PGA \geq 2$, $n=16$) with healthy controls, and then found a significant difference ($p=0.004$).

Figure 1. Serum soluble urokinase plasminogen activator receptor (suPAR) levels demonstrated in healthy controls (n=100) and at baseline in SLE patients: all included patients (n=198); patients fulfilling the 1982 American College of Rheumatology (ACR) classification criteria (n=160); patients fulfilling only the Fries criteria (n=38); patients with leukocyte count (LC) $\geq 3.5 \times 10^9/L$ (n=184); patients with raised disease activity (PGA ≥ 2) (n=16); and patients with significant organ damage (SDI) (n=22). Lines represent mean values. *P*-values refer to comparisons between healthy controls and patient subgroups in a univariate analysis of variance adjusting for age and sex.

In the regression analysis, there was no significant association between suPAR levels and disease activity, neither defined as SLEDAI-2K (all patients: $p=0.84$; ACR-82 patients: $p=0.71$, respectively) nor as PGA (all patients: $p=0.20$; ACR-82 patients: $p=0.34$, respectively). uPAR is expressed and shed from immune cells, and since SLE patients often present with cytopenia, we also included leukocyte count and platelet count as independent variables in the regression analysis, but still without significant association of suPAR with disease activity. Finally, we also included prednisolone dose into the regression analysis, but without receiving a significant association (not shown).

Association of CRP and suPAR with organ damage

The mean SDI for all SLE patients was 1.1 ± 1.6 and the median value was 0 (range 0-8). The organs affected are presented in Table 1. The relation between SDI and suPAR is shown in Figure 2 and the statistical association assessed by multiple linear regression is shown in Table 3. A highly significant positive association was found between suPAR and organ damage ($p < 0.0005$) as well as a borderline significant association between logCRP and SDI ($p = 0.05$, $\beta = 0.14$), the latter not significant when the study population was limited to ACR-82 only. Dissecting SDI into organ systems in a multiple regression analysis, we found renal ($p < 0.0005$), ocular ($p < 0.0005$) neuropsychiatric ($p < 0.0005$), skin ($p = 0.001$) and peripheral vascular ($p = 0.019$) organ damage to have a significant positive impact on suPAR levels, whereas no isolated organ affection had any significant impact on CRP levels. Adjustments for cell counts and prednisolone dose did not reveal any important changes in the association between SDI and suPAR (Table 3), whereas adjustment for prednisolone dose and/or cell counts eliminated the significant association between CRP and total SDI found for all patients ($n = 198$) (not shown).

Figure 2. Correlation between soluble urokinase plasminogen activator receptor (suPAR) levels and the Systemic Lupus erythematosus International Collaborating Clinics/American College of Rheumatology damage index (SDI) in the 198 SLE patients. Correlation coefficient and *p*-values not shown since the association is not adjusted for age and sex.

Table 3. The impact of organ damage (SLICC/ACR DI) on suPAR levels

Variable	All patients				ACR-82 patients			
	Model 1*		Model 2 [†]		Model 1*		Model 2 [†]	
	$\beta^{\ddagger}$	<i>p</i> -value	$\beta^{\ddagger}$	<i>p</i> -value	$\beta^{\ddagger}$	<i>p</i> -value	$\beta^{\ddagger}$	<i>p</i> -value
Global SLICC/ACR DI	0.50	<0.0005	0.48	<0.0005	0.45	<0.0005	0.43	<0.0005
Organ systems[§]								
Renal	0.34	<0.0005	0.31	<0.0005	0.34	<0.0005	0.31	<0.0005
Ocular	0.23	<0.0005	0.23	<0.0005	0.21	0.003	0.20	0.005
Neuropsychiatric	0.21	<0.0005	0.22	<0.0005	0.19	0.004	0.21	0.002
Skin	0.19	0.001	0.19	0.001	0.20	0.002	0.20	0.003
Peripheral vascular	0.14	0.019	0.13	0.023	0.15	0.020	0.13	0.026

Abbreviations: SLICC/ACR, Systemic Lupus International Collaborating Clinics/American College of Rheumatology; suPAR, soluble urokinase plasminogen activator receptor; ACR-82, 1982 American College of Rheumatology classification criteria; DI, damage index.

*Adjusted for age and sex

[†]Adjusted for age, sex, leukocyte count, platelet count and prednisolone dose

[‡]Standardized beta coefficient (SD increase)

[§]Only organ systems that were retained in the model after a stepwise analysis are shown

Individual suPAR level variations in consecutive samples

For the 18 patients where consecutive serum samples were analyzed, the suPAR level at the lowest and highest recorded disease activity was compared (Figure 3). No significant difference was seen ($p=0.542$).

Figure 3. Soluble urokinase plasminogen activator receptor (suPAR) concentrations at lowest and highest disease activity in the 18 patients selected for consecutive analysis. The dashed line represents the mean value of healthy controls. See Materials and methods section for details about disease activity measures.

Few extreme drops in suPAR level over time in consecutive samples

The maximum drop in suPAR level during the study period was calculated for each of the 18 patients that were monitored consecutively. Maximum drop was defined as the greatest decline in suPAR over time. The median value of maximum drop was 0.6 ng/mL (range 0-20.7 ng/mL). Two patients had extreme drops in suPAR levels (9.4 ng/mL and 20.7 ng/mL, respectively) compared to the others (≤ 2.2 ng/mL). The patient representing the second highest suPAR drop had a viral infection and a minor myocardial infarction at the time point

of the high suPAR level, whereas the patient with the highest drop had no known infection or other signs of active disease at the time of high serum suPAR level.

DISCUSSION

SLE is a profoundly heterogeneous disease entity. It therefore appears unlikely that one single biomarker could cover all lupus phenotypes and serve as a general disease activity or severity marker. Nevertheless, suPAR has emerged as a biomarker reflecting inflammatory activity and predicting outcome in several infectious and malignant diseases. We found it worthwhile to evaluate the potentials of suPAR as a biomarker in SLE since earlier observations have been contradictory.^{39, 40} Based on the results of this study, we conclude that circulating suPAR is an unreliable marker of SLE disease activity. However, we found that the level appears to reflect irreversible organ damage, especially in the renal, ocular and neuropsychiatric domains of SDI.

Lupus patients commonly present with cytopenia and we found that suPAR levels strongly associated with leukocyte count in line with previous observations.⁴¹ Thus, it is likely that the absence of a general increase in suPAR levels among SLE patients could be a reflection of decreased leukocyte count (Figure 1). Many cytokines and routine laboratory measures were associated with suPAR (Table 2). Convincing correlations between suPAR and the pro-inflammatory cytokines IL-1 β and TNF as well as CRP and creatinine have been shown also in other conditions,^{29, 41-43} and could be expected since uPAR is up-regulated and shed from immune cells during inflammation. Interestingly, suPAR levels were not inversely associated with complement proteins or complement function, which further demonstrates a lack of apparent association between suPAR and disease activity in lupus.

To our knowledge, no biomarkers apart from lymphocyte Fas expression,¹⁴ sFas,¹² CRP¹³, and osteopontin¹⁵ have been shown to associate with organ damage as defined by SDI.

Importantly, age has been found to correlate with levels of sFas, but the associations with organ damage in the Fas and sFas studies were not adjusted for age. Lee and collaborators found CRP to be associated with pulmonary, musculoskeletal and global SDI in a cross-sectional study involving 610 SLE patients.¹³ In line with their results, we identified a weak association of CRP with global SDI, but in contrast to the study by Lee *et al.*, we found no significant impact of isolated organ systems on CRP levels. This discrepancy can possibly be due to differences in study size and ethnicity, but may also be explained by differences in statistical adjustments made in the regression analyses.

Due to its reflection of permanent organ damage, one would expect suPAR levels to be stable over time, apart from further raised levels upon additional organ damage. In fact, suPAR levels fluctuated moderately over time in patients followed longitudinally. Although the median value regarding maximal drop in suPAR was very low, two patients showed a substantial decline in suPAR over time. A plausible explanation was found only for one of these patients who had an ongoing infection at the time of high suPAR level. Age and lifestyle factors such as smoking and physical activity have impact on baseline suPAR levels in a healthy population.⁴³ These factors, however, appear unlikely as explanations to such great variations, in particular since this patient was not unique regarding disease manifestations or medication.

Some affected organ systems were associated with suPAR levels when the score was divided into specific domains. However, the lack of association with other domains is most likely due to lack of power in the statistical analysis and not necessarily to an absence of association with suPAR. Of all organ systems considered, renal damage had the most pronounced impact on suPAR levels. Interestingly, SDI of the renal domain has previously shown to predict

mortality in SLE patients.^{44, 45} In addition, several studies have shown a convincing correlation between SDI and severe outcome of the disease, particularly if damage occurs early.^{44, 46-48} Two other domains also significantly associated with suPAR levels in the regression analyses were neuropsychiatry and skin. Interestingly, expression of uPAR has been reported to be increased in the frontal cortex of patients with epilepsy.⁴⁹ It is also possible that urokinase-type plasminogen activator and uPAR synergetically contributes to extensive alopecia, epidermal thickening and subepidermal blisters.⁵⁰ One study reported that raised suPAR levels predict mortality, not only in patients with severe diseases, but also in apparently healthy subjects.³⁷ Hypothetically, permanently raised levels of circulating suPAR in SLE may thus be a subtle sign of deteriorated health and outcome regardless of current disease activity.

Further research is needed to understand the biological roles of suPAR, its turnover in health and different diseases, as well as to pinpoint potential pitfalls in the use of suPAR as a biomarker. Besides its role in the plasminogen activation system, where urokinase-type plasminogen activator is one of the serine proteases generating plasmin that degrades fibrin, suPAR/uPAR seems to be involved in a number of immune regulation mechanisms, including cell migration and adhesion.¹⁶ Regarding organ damage in the present as well as in previous studies,^{30, 31} it is not known whether suPAR exerts a direct harmful effect or if it just reflects damage. Since suPAR levels correlate strongly to leukocyte counts in inflammation, it is interesting to note that neutrophils may be stimulated *in vitro* to release the chemotactic suPAR form D_{II-III}, that is capable of causing a formyl peptide receptor-like 1-dependent migration of *e.g.* transfected kidney cells.⁵¹ Synovial fluid neutrophils from rheumatoid arthritis patients also release more D_{II-III} than peripheral neutrophils,⁵² further supporting a role for suPAR in recruiting formyl-peptide receptor expressing cells at inflammatory sites.

suPAR also interacts with integrins, and kidney damage in focal segmental glomerulosclerosis was recently suggested to be due to a direct effect on podocyte behaviour via integrin $\alpha_3\beta_v$.³² This interesting finding is currently under debate.^{53, 54} Another observation making uPAR particularly interesting in relation to SLE is that uPAR stimulates efferocytosis, *i.e.* phagocytic uptake of apoptotic cells,⁵⁵ including apoptotic neutrophils.⁵⁶ Briassouli *et al.* also recently suggested that there is an interaction between the SLE-associated autoantigen Ro60 and uPAR, and that autoantibodies against Ro60 may promote enhanced uPAR expression and interfere with efferocytosis of apoptotic fetal cardiocytes.⁵⁷ The same authors also recently suggested that an autoantibody-triggered uPAR-dependent increase in plasmin activity may activate transforming growth factor- β , which in turn could promote fibrosis.⁵⁸ Speculatively, altered expression/shedding of uPAR may be affected by autoantibodies and reflect, or even contribute to, a deficient waste disposal process.

In conclusion, circulating suPAR reflects disease severity/organ damage in SLE and is thus a promising biomarker candidate. However, further prospective studies are warranted to (i) answer the question of whether suPAR not only reflects prevalent tissue damage, but also predicts risk of future organ damage; as well as to (ii) understand the biological relevance of raised suPAR levels in SLE patients with severe disease.

COMPETING INTERESTS

The authors declare that they have no competing interests.

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AUTHORS' CONTRIBUTIONS

HE contributed to laboratory work, interpretation and analysis of data, intellectual discussion and manuscript writing. **JW** contributed to the original idea and study design, interpretation of data, intellectual discussion and manuscript writing. **TS** contributed to the original idea and study design, interpretation of data, intellectual discussion and manuscript writing. **CS** contributed to the original idea and study design, patient characterization, interpretation of data, intellectual discussion and manuscript writing. All authors approved the final version of the manuscript.

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