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The clinical value of soluble urokinase plasminogen activator receptor (suPAR) levels in autoimmune connective tissue disorders

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ABSTRACT

The assessment of the general inflammatory condition of patients with autoimmune connective tissue disorders (ACTD) is a major challenge. The use of traditional inflammatory markers including CRP-levels and erythrocyte sedimentation rate (ESR) is limited by several preanalytical factors and their low specificities. Soluble urokinase plasminogen activator receptor (suPAR) is one of the novel candidate markers that is increasingly used in immune mediated disorders. In our studies we compared suPAR levels of patients with rheumatoid arthritis (RA), systemic lupus erythematosus (SLE), systemic sclerosis (SSc) and ankylosing spondylitis with those of healthy controls.

suPAR provided valuable clinical information on disease activity in RA, SLE and SSc. We identified a subgroup of remitted RA patients, who presented still clinical symptoms of inflammatory activity which correlated to high plasma suPAR (while ESR and CRP were normal). In SLE we established specific suPAR cut-off values that support the discrimination between patients with high and those with moderate SLE activity. In patients with SSc suPAR correlated with objective measures of lung and other complications.

In the majority of ACTDs including SLE, SSc or RA, suPAR is seemingly a good biomarker that would provide valuable clinical information. However, before the introduction of this novel parameter in laboratory repertoire important issues should be elucidated. These include the establishment of appropriate and disease specific cutoff values, clarification of interfering preanalytical values and underlying conditions and declaration of age- and gender-specific reference ranges.

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INTRODUCTION

Inflammation is a characteristic hallmark of relapsed autoimmune connective tissue disorders (ACTD). The treating physician's challenge is to determine the extension of inflammation and to decide whether the patient requires an intervention or therapy should be modified. Inflammatory markers, therefore, are generally used to assess ACTD patients' general condition. CRP-levels and erythrocyte sedimentation rate are among the most frequently ordered lab tests. However, the information provided by these tests is limited by their low sensitivity and the number of interfering preanalytical factors such as diurnal cycle, way of sampling or even physical exercise. Therefore, novel biomarkers that indicate the presence of severe inflammation in ACTD are highly warranted. suPAR is one of the promising candidates that we investigated extensively in patients with different ACTDs.

Urokinase-type plasminogen activator receptor (uPAR) is expressed on various cell types, including immune, smooth muscle and endothelial cells (1,2). When this receptor dissociates from the cell surface, suPAR, the soluble form of uPAR is created. suPAR is detectable with standard ELISA tests in low concentrations in non-diseased people. Its benefits over traditional acute phase proteins are that its levels do not depend on diurnal variation and fasting state (3). suPAR is readily resistant to preanalytical conditions such as freezing and thawing (4). Due to its stability it may be a candidate as an assessable biomarker for inflammation. According to the data available, inflammatory response leads to elevated plasma suPAR levels in many inflammatory diseases (5) which is predictive to a worse prognosis. The clinical value of suPAR was investigated most extensively in systemic inflammatory response syndrome (SIRS) and in patients with septic conditions. Current evidence unanimously indicates that levels of suPAR are increased in SIRS and may be used for risk stratification of patients with SIRS (6,7). Findings indicate that suPAR predicts better adverse outcome following sepsis than traditional markers including CRP levels (8,9,10).

During the last five years our team made an extensive work to assess the clinical utility of suPAR levels in ACTD. In our studies we compared plasma suPAR levels of different and well-characterized patient ACTD subgroups such as those with rheumatoid arthritis (RA) (11), systemic lupus (SLE) (12), systemic sclerosis (SSc) (13), and ankylosing spondylitis (AS) (14) with 29 healthy control subjects. Healthy controls had a negative history of rheumatic symptoms and negative status upon detailed physical and laboratory examination. Written informed consent was obtained from all participants, and our study was reviewed and approved by the Ethics Committee of the institution. The studies were adhered to the tenets of the most recent revision of the Declaration of Helsinki.

For the purpose of suPAR determination we collected EDTA anticoagulated fasting blood samples from patients and controls, separated plasma and stored at −80°C until measurement. Plasma suPAR concentrations were measured with the suPARnostic Flex ELISA assay (ViroGates A/S, Birkerod, Denmark) and were related to ESR, CRP and clinical status.

Table Summary of suPAR, C-reactive protein (CRP) and erythrocyte sedimentation rate (ESR) values in autoimmune connective tissue disorders (ACTD) including rheumatoid arhtritis (RA), ankylosing spondylitis (AS), systemic lupus (SLE) and systemic sclerosis (SSc)

Demographic characteristics and inflammatory markers	Healthy controls n = 29	RA (n=120)	AS (n=33)	SLE (n= 89)	SSc (n = 83)
Age, years	55 [46-69]	61 (48 – 72)	41* [35–45]	44 [34–59]	51.5 [44-60]
Gender, male/female	10/19	46/74	24/9	10/79	16/67
suPAR, ng/mL	2.80 [2.06-3.42]	4.24 (3.19 – 5.40)	2.97 [2.57–3.80]	4.58* [3.72–6.30]	4.02* [3.19-5.53]
CRP, mg/L	2.70 [BLD-4.15]	4.00 (BLD – 9.83)	10.00* [2.75–25.80]	3.90 [BLD-9.55]	3.50 [1.80-8.40]
ESR, mm/h	10 [7-14]	21 (12 – 36)	17* [10–33]	28* [17–50]	18* [8-28]

*BLD=below the level of detection; * p<0.05 compared to the control*

Based on data published in refs 11–14.

suPAR IN RHEUMATOID ARTHRITIS (11)

Rheumatoid arthritis (RA) is a chronic inflammatory disease leading to the erosion of the cartilage and bone, and invasive growth of synovial pannus tissue. The Disease Activity Score (DAS28) reflecting the severity of RA is based on clinical signs and symptoms along with CRP and ESR (15). RA is regarded as active above a DAS28 score of 2.6, however, patients in remission (DAS28 score ≤2.6) might also be affected by inflammatory activity.

In our RA study we enrolled 120 RA patients at various stages of disease duration and activity and related their clinical parameters and DAS28 score to suPAR levels. The median DAS28 score (calculated at the time of sampling) was 2.8, corresponding to a median low disease activity. All the 120 RA patients received a variety of disease modifying anti-rheumatic drugs (DMARDs); 34 and 60 RA patients received add-on anti-tumor necrosis factor (TNF) therapy and glucocorticoid treatment, respectively.

suPAR, CRP and ESR values were higher in RA patients compared to healthy individuals (see Table). We identified correlation between suPAR and DAS28 in RA patients (p=0.02, r=0.26), suPAR values and ESR values in RA patients (p=0.05, r=0.30) and suPAR values and CRP values in healthy individuals (p=0.02, r=0.32). CRP and ESR values were also analyzed according to DAS28 scores. Irrespectively of anti-TNF and glucocorticoid therapy, CRP and ESR values were higher with a DAS28 score >2.6 than in RA patients in remission (DAS28 score ≤2.6) or in healthy individuals.

The evaluation of RA patients' condition is based on laboratory markers and clinical symptoms.

Appropriate determination of disease activity has a significant impact on therapeutic decision making process. The elevated suPAR, CRP and ESR values are in agreement with earlier results indicating higher plasma suPAR levels in RA. Furthermore, Slot et al's study indicated a positive correlation between suPAR and CRP and ESR in RA (16). We also found a correlation between ESR and DAS28 but not CRP values and suPAR levels when all RA patients were analyzed. A reason for this apparent controversy might be that patients enrolled in our study were more heterogeneous in terms of disease severity, including patients with milder RA. In contrast with the findings in RA, CRP values were correlated to suPAR levels in healthy individuals in our study either.

When RA patients were grouped according to anti-TNF and glucocorticoid therapy, or CRP and ESR values, no differences were detected between the corresponding therapeutic subgroups. However, when we compared RA subgroups according to DAS28 scores, a difference between remitted RA patients (DAS28 ≤2.6) and patients with different stages of active disease (DAS28 >2.6) was detected.

Of note, while CRP and ESR values were comparable with healthy individuals in remitted patients, suPAR values were still elevated (but were lower than in patients with DAS28 >2.6). In addition, the number of affected joints was strongly correlated to elevated plasma suPAR levels, indicating that suPAR levels represent well ongoing inflammatory activity in remission. While CRP and ESR values were similar in all subgroups of RA patients in remission to the levels seen in healthy individuals, suPAR values were elevated indicating the inflammatory activity in patients with 2–3 or four affected joints. Highest suPAR values were observed in patients with the highest number of affected joints. This subgroup represented almost 10% of the whole RA group of our study and over 20% of remitted RA patients, indicating that in remitted RA regular monitoring of plasma suPAR values would support the early detection of inflammatory activity. This is of particular importance as recent data indicate that patients in remission according to DAS28 scores could have slowly progressive structural damage without relevant clinical symptoms and with normal CRP and ESR (17). In such cases only ultrasound investigation of the joints supports the presence of synovitis. However, the use of ultrasound has limited as its availability is restricted, it is time-consuming, and investigator-dependent. Our analysis indicated measuring suPAR with a 4.8 ng/mL cut-off value would support the identification of patients under risk.

These results suggest that suPAR is a sensitive marker of inflammatory activity even in remitted RA patients. We identified a subgroup of RA patients in remission according to DAS28 scores, who present still with clinical symptoms of inflammatory activity (tender and/or swollen joints) which correlate to elevated plasma suPAR levels. Importantly, ESR and CRP values showed no alteration in these patients compared to healthy controls. Hence, suPAR levels might help the follow-up of remitted RA patients with mild clinical signs. Our finding might also have important therapeutic consequences, since this subgroup identified by elevated suPAR levels may benefit from earlier anti-RA treatment.

suPAR IN BECHTEREW'S DISEASE (14)

Ankylosing spondylitis (AS) is an immune-mediated rheumatic disease characterized by chronic inflammation. The autoimmune reaction principally affects the axial and sacroiliac joints in AS eventually leading to spondylitis, extra bone formation and vertebral fusion (ankylosis). In later stages of the disease, systemic autoimmune reactions are hallmarked by the

inflammatory involvement extraskeletal organs (eye, gastrointestinal tract or heart). Therefore, early and reliable detection and monitoring of inflammation and the initiation of targeted therapy are of utmost importance in AS.

In order to determine whether suPAR is a marker of inflammation in AS, we enrolled 33 AS patients. AS patients were classified according to the modified New York criteria (18). The median of Bath ankylosing spondylitis disease activity index (BASDAI) was 5.49, indicating an active disease. Ten of 33 patients received sulfasalazine treatment, while 15 of 33 AS patients received anti- TNF therapy.

In AS CRP and ESR values were higher than normal, while suPAR values were comparable to the control (see Table). When suPAR levels were analyzed according to different subgroups of AS patients, AS patients with an ESR value greater than 20mm/h exhibited higher suPAR levels than those with an ESR value ≤20mm/h and healthy controls. suPAR correlated with CRP and ESR values in AS patients. Of note, while BASDAI scores correlated with CRP and ESR, they did not interact with suPAR.

These observations indicated that suPAR failed to detect the ongoing inflammation in AS. This pilot study does not support the usefulness of suPAR in the assessment of AS.

suPAR AND SYSTEMIC LUPUS ERYTHEMATOSUS (12)

Systemic lupus erythematosus (SLE) is a systemic autoimmune disease that affects almost all vital organs and tissues and is characterized by a wide spectrum of clinical signs and symptoms.

Nowadays, C-reactive protein (CRP) is regarded as the gold-standard for the assessment of systemic inflammation. However, SLE is an important exception, as CRP levels are not necessarily elevated and do not reflect inflammation in SLE (19). In clinical practice, a significantly elevated erythrocyte sedimentation rate (ESR) with a normal CRP is a strong indicator of SLE. ESR is, however, a rather unspecific marker of inflammation.

In 89 SLE patients with various stages of disease duration and activity we aimed to assess plasma suPAR levels and to determine if suPAR could serve as an inflammatory biomarker in SLE. SLE patients were diagnosed and classified according to the updated American College of Rheumatology (ACR97) criteria (20). The median of SLE duration was 8 years, and the median of Systemic Lupus Erythematosus Disease Activity Index (SLEDAI) score was 2, corresponding to moderate disease activity (21). Patients with a SLEDAI score of 0 were considered to be in remission, a SLEDAI score between 1 and 8 was regarded as moderate disease activity, and a SLEDAI score above 8 was regarded as high disease activity.

suPAR and ESR values were higher in SLE patients than in controls, while CRP levels were comparable (see Table). We performed further analyses of suPAR levels based on several subgroups created according to SLE complications. Of note, suPAR levels of patients with vasculitis in their history was higher than that of patients with no vasculitis (5.84 [4.12–7.01] vs. 4.21 [3.57–5.47] ng/mL, $p = 0.04$). CRP and ESR values did not differ between subgroups of SLE patients with different disease activity. suPAR levels behaved in a different manner; patients with high disease activity exhibited higher suPAR levels than those with moderate disease activity or in remission.

ROC analysis to discriminate healthy individuals and SLE patients based on suPAR yielded an AUC of 0.85 (ESR performed in a comparable manner with an AUC of 0.87). The cut-off value of suPAR was 3.54 ng/mL (sensitivity%: 82.02, specificity%: 79.31). ROC analysis of suPAR values in SLE

patients according to SLEDAI scores yielded an AUC of 0.68; the cut-off value of suPAR to discriminate between patients with high and with moderate disease activity was 5.70 ng/mL (sensitivity%: 61.54, specificity%: 78.72).

These findings indicate that suPAR levels are elevated in SLE. The use of CRP in SLE is limited its insensitivity to inflammation in this condition. This is due to different factors including decreased responsiveness of monocytes producing CRP-inducing cytokines in SLE, the common presence of CRP gene variations associated with lower CRP levels and an increased risk of SLE and the presence of the autoantibodies against CRP in SLE (12)). In contrast with CRP suPAR levels are increased in SLE patients, particularly in those with vasculitis. One might hypothesize that inflammatory cell adhesion to endothelial cells leading to extravasation into the intima is promoted by higher suPAR levels in SLE. (Indeed, experimental evidence suggests that suPAR might promote cell adhesion by binding to very late antigen-4 (VLA-4) on inflammatory cells as a ligand, promoting their extravasation via the activation of other molecules regulating cellular adhesion and migration (22)).

Importantly, these results indicate that suPAR is a novel marker that may help to discriminate between patients with high disease activity and those with moderate disease activity or in remission.

suPAR IN SYSTEMIC SCLEROSIS (13)

Systemic sclerosis (SSc) is a chronic connective tissue disorder characterized by microvascular injury, fibrosis and autoimmunity that affects the skin and internal organs (23). There are two major subtypes of SSc; dcSSc (dominantly affecting the skin) and lcSSc (involving the lung). To date, there is still no systemic marker that supports the clinical follow-up of organ specific disease activity. Currently, erythrocyte ESR and CRP levels are routinely used to assess SSc disease activity and severity (24).

We measured suPAR in 83 SSc patients who fulfilled the criteria proposed by the American College of Rheumatology (25). While CRP levels were comparable, suPAR and ESR values were higher than normal in SSc patients (see Table). suPAR values were higher in lcSSc than in dcSSc and correlated with the presence of anti-Scl70. Interstitial lung disease assessed by diffusing capacity for carbon monoxide (DLCO) and forced vital capacity (FVC) was more severe in patients with high suPAR; these parameters correlated inversely with suPAR levels. SSc patients with pulmonary fibrosis and pulmonary arterial hypertension also exhibited higher suPAR levels than those without these complications. Microvascular changes including the presence of digital ulcers, Raynaud phenomenon and NC abnormalities and arthritis were also more prevalent with high suPAR values.

These findings support the notion that suPAR may provide additional information to traditional biomarkers that help the objective assessment of complicated SSc.

CONCLUSIONS

These studies indicate that suPAR may be a useful biomarker of inflammation in several types of ACTD characterized by low-grade or transient inflammatory periods. However, the clinical use of suPAR in these conditions requires the clarification of several issues.

What suPAR levels should be used for decision making?

In several conditions the decision should be based on well defined cutoff values; suPAR above the limit may indicate an increased risk of inflammation and/or complications. However, based on the currently available data it is still uncertain which cut-off values are to be used. (The similar

uncertainty exists for sepsis, a much more extensively investigated condition with different cut-off values suggested [6,8].)

This uncertainty is partly due to methodological issues. It is still unclear whether any change in lot numbers / manufacturers would affect the results. In addition, the lack of external quality control programs for this parameter still prevents the routine use of suPAR.

What is the physiological basis of suPAR alteration?

suPAR is cleaved from the cell membranes by the action of proteases including cathepsin-G, PI-PLC, plasmin, chymotripsin, matrix metalloproteases (MMPs) etc. Inflammatory and endocrine milieu clearly influences the activity of these enzymes, and, probably, suPAR levels. The over-activation of the adrenal gland is a common feature in the diseased patients. Of note, adrenal hormones clearly influence MMP activity and, therefore, may contribute to suPAR production (26). Therefore, one may assume that any increase of suPAR levels may be a surrogate marker of increased adrenal activities. The increase in adrenal hormone levels including glucocorticoids may be due to the progression of disease, but is also inherent with therapeutic interventions routinely applied in autoimmune disorders. However, still there are no data to test this possibility.

What clinical factors additional to systemic inflammation influence suPAR levels?

The factors and conditions interacting with suPAR levels are less clarified. Patients with autoimmune disorders often suffer from a polymorbid state and from complications partly associated with their background condition. Some data indeed demonstrated an inverse association between suPAR and renal function and a positive association between suPAR and age (6). It is unknown, however, how other factors such as hepatic failure, or different therapeutic regimes influence suPAR levels.

From these pilot data suPAR is seemingly a good biomarker to obtain an impression whether patients with SLE, SSc or RA are subjected to an increased inflammatory status. However, the introduction of such a novel parameter in everyday practice requires more extensive clinical observations collected during prospective studies. The results will serve to decide whether suPAR is suitable to be used as a clinical biomarker in patients with autoimmune connective tissue disorders.

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