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ORIGINAL RESEARCH

The Prognostic Value of suPAR Compared to Other Inflammatory Markers in Patients with Severe Sepsis

Anna Gustafsson[1], Lennart Ljunggren[1], Mikael Bodelsson[2] and Ingrid Berkestedt[2]

[1]Department of Biomedical Science, Malmö University, Malmö, Sweden. [2]Department of Anaesthesiology and Intensive Care, Skåne University Hospital and Lund University, Lund, Sweden. Corresponding author email: anna.gustafsson@mah.se

Abstract: It has been suggested that soluble urokinase plasminogen activator (suPAR) can be used as a marker of disease severity and risk of mortality in sepsis. The aim with the present study was to compare plasma levels of suPAR in patients with severe sepsis to control subjects and correlate it with the level of inflammatory activation, severity and mortality. Samples were collected from 27 sepsis patients at the intensive care unit (ICU), Lund, Sweden; 90-day mortalities were registered. The suPAR level was significantly elevated in sepsis patients compared to controls, but not significantly higher in nonsurvivors than survivors. Plasma levels of suPAR did correlate weakly with the SOFA score and myeloperoxidase (MPO) but not with CRP, PCT, IL-6 or IL-10 in patients with severe sepsis. The weak correlation between suPAR and other inflammatory markers might suggest that suPAR reflects general activation of the immune system rather than exerting inflammatory actions.

Keywords: inflammatory markers, sepsis, SIRS, soluble urokinase plasminogen activator receptor, suPAR

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Introduction

Sepsis is a clinical syndrome defined as a systemic inflammatory response to infection; severe sepsis, which is characterised by failing vital functions is a major cause of mortality in the intensive care unit (ICU).[1] The diagnosis of sepsis and evaluation of its severity is complicated. A standardised assessment tool to identify patients who are at higher risk of a poor, or fatal even, outcome would be of high value to ensure the optimal use of health care resources.[2,3]

Biomarkers, biological molecules that are characteristics of normal or pathogenic processes can be useful indicators to clinicians. An ideal biomarker for identifying patients that need more intense monitoring and treatment should be both accurate and readily obtainable bedside. The two biomarkers that have been most widely studied and used in patients with severe sepsis are the C-reactive protein (CRP) and procalcitonin (PCT).[4–6] The urokinase plasminogen activator receptor (uPAR) is expressed on most leucocytes including neutrophils, lymphocytes, monocytes and macrophages, which are crucially important in the pathogenesis of sepsis. The interaction of uPAR with its ligand, the urokinase plasminogen activator (uPA), results in numerous immunologic events including cell migration, adhesion, proliferation and fibrinolysis.[7] Through inflammatory stimulation, uPAR is cleaved from the cell surface to the soluble form of the receptor, suPAR.[7] Recently, suPAR has been suggested as a novel prognostic marker to identify high-risk patients.[8] In healthy individuals, suPAR levels are low and quite stable while the concentration increases in conditions that involve immune activation.[9] Several studies indicate that an elevated suPAR level in plasma is associated with a negative outcome in critically ill patients with systemic inflammatory response syndrome (SIRS), bacteriemia, sepsis, and septic shock and predict mortality in these patients.[8,10–12] However, studies have also showed that suPAR does not appear to be superior to other biomarkers like CRP and PCT, in diagnosing sepsis.[4,13]

The aim with the present study was to investigate the relationship between suPAR and the more commonly used clinical biomarkers CRP and PCT as well as the inflammatory markers IL-6, IL-10, and myeloperoxidase (MPO) in patients with severe sepsis. In addition, we investigated if plasma levels of suPAR can predict mortality in patients with severe sepsis and if suPAR levels correlate with total Sequential Organ Failure Assessment (SOFA) scores, a sepsis mortality risk algorithm including multiple laboratory and clinical measures.

Materials and Methods

Patients

The patient material of the present study has been used in another already published study.[14] The local research ethics committee approved the project and informed consent was obtained from patients or next of kin of the unconscious patients. Sepsis patients admitted to the ICU of Lund University Hospital, Sweden, between 23 March 2004 and 7 December 2007 were included. All patients fulfilled at least 2 out of 4 criteria for SIRS, had a suspected or verified underlying infection and respiratory and/or circulatory dysfunctions requiring intensive care. Therefore, they fulfilled the criteria for severe sepsis or septic shock. Severity of organ dysfunction was defined with the SOFA score on the basis of measurements during the first 24 h of admission. The mortality rate within 90 days after admission was registered. The median age of the septic patients was 65 years (range, 28–87 years; n = 27); 10 males and 17 females. Survival of the septic patients in 90 days post submission to the ICU was 56% (15/27 patients). Controls consisted of patients scheduled for neurosurgery. Informed consent was obtained. None of the control patients had ongoing steroid treatment. The median age of the controls was 61.5 years (range, 22–85 years; n = 22); 11 males and 11 females.

Sample collection

Within 24 h after admission to the ICU, blood samples were drawn from an already existing arterial catheter and were collected in EDTA-treated vacuettes. In six patients, additional blood samples were obtained 4 days later. Arterial blood samples from the control group were similarly drawn before induction of anesthesia. The samples were immediately centrifuged for 10 minutes at 800 *g* at room temperature. The plasma supernatants were removed and stored at −70 °C until analysis.

Measurements of PCT and CRP

PCT was measured in the plasma samples using an immunoluminometric assay from BRAHMS

(Henningsdorf, Germany) according to the manufacturer's instructions. CRP concentration in venous blood was measured by routine latex-enhanced immunoturbidimetry (Roche Diagnostics).

ELISA assays

Plasma suPAR concentrations were analysed using a commercially available enzyme immunoassay (suPARnostic™, Virogates, Copenhagen, Denmark) according to the manufacturer's instructions. The assay is a double monoclonal antibody sandwich assay that measures all circulating suPAR, including full-length and cleaved forms of the receptor.

IL-6 and IL-10 levels were quantified using R&D Systems High Sensitivity ELISA according to the manufacturer's instructions. MPO levels were quantified using an ELISA from Diagnostics Development, Uppsala, Sweden.

Statistical analysis

The differences in the plasma levels of suPAR between patients and controls, men and women, and between survivors and nonsurvivors were assessed using the Wilcoxon rank sum test. The difference between levels at admission compared to the four-day samples was assessed with the Wilcoxon signed rank test. Correlations between suPAR and the other inflammatory markers or age were calculated with linear regression on log-transformed data. Differences and correlations were considered as statistically significant when $P < 0.05$.

Results

Patient characteristics

A total of 27 patients fulfilling at least two out of four SIRS criteria were included in this study. Patient characteristics are summarized in Table 1. Medians of IL-6, IL-10, MPO and PCT were higher in patients with severe sepsis compared to controls ($P < 0.001$). In the sepsis patients, the levels of these inflammatory markers had decreased at day 4 compared with the levels at admission ($P < 0.01$) (data not shown). Blood culture was positive for *E. coli* in four patients, *Streptococcus pneumoniae* species in four patients, *Enterococcus* species in three patients, coagulase-negative *staphylococci* in two patients, *pseudomonas* species in one patient and *enterobacter* species in one patient. One of the patients had positive blood culture for both *enterococcus* and *enterobacter* species. In twelve of the patients, the blood culture was negative.

Table 1. Patient characteristics.

	Sepsis	Control
Patients	27	22
Age, years	65 (28–87)	61.5 (22–85)
Male/female	10/17	11/11
Survival rate, %	56	
CRP, mg/L	219 (54–542, n = 22)	
PCT, ng/mL	47.2 (0.5–445)	0.013 (0.003–0.11)
IL-6, pg/mL	2 348 (159–494 575)	0.5 (0.2–0.5)
IL-10, pg/mL	122 (5–857)	3.6 (0.9–17.0)
MPO, ng/mL	255 (54–2 000)	53 (35–184)
suPAR, ng/mL	12.2 (5.9–36.1)	2.5 (1.5–6.2)
Renal SOFA score	2 (0–4, n = 20)	
Total SOFA score	14 (5–18, n = 24)	

Note: Data are medians, with range in parenthesis.

Plasma suPAR

As depicted in Figure 1A, the median plasma suPAR level of patients with severe sepsis was significantly higher ($P < 0.001$) than that of the controls. The plasma level of suPAR did not significantly correlate with mortality (Fig. 1B) although the median suPAR values were higher (n.s.) in non-survivors (15.4 ng/mL, range

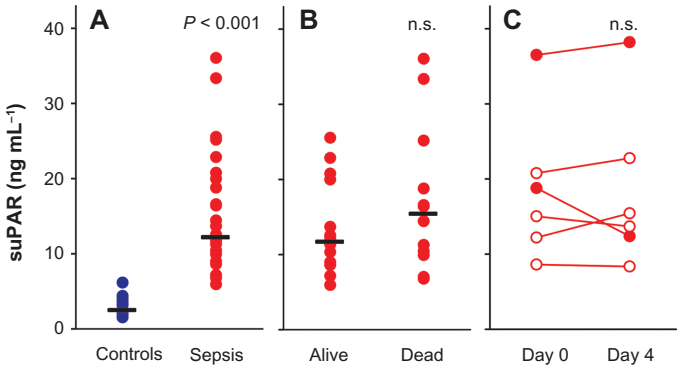

Figure 1. Plasma levels of suPAR in patients with severe sepsis, and controls.
Notes: *P*-values refer to statistically significant differences in median levels of sepsis patients compared to controls, between deceased and surviving sepsis patients at 90 days after admission or of sepsis patients at day 4 compared to the day of admission. The circles represent individual patients and the median is indicated with a horizontal line. In (**C**), open circles represent surviving patients.
Abbreviation: n.s., not significant.

6.8–36.1, n = 12) compared to survivors (11.7 ng/mL, range 5.9–25.5, n = 15). The suPAR level of patients with severe sepsis four days after submission had not changed significantly compared with the level at admission (16.9 ng/mL, range 8.6–36.5 vs. 14.6, range 8.4–38.2, n = 6) (Fig. 1C). Plasma suPAR levels did not correlate with age or gender, neither in sepsis patients, nor in controls (data not shown).

Correlation between levels of suPAR, mediators of inflammation and SOFA scores

No significant correlation was found between plasma levels of suPAR and the levels of CRP, PCT, IL-6 or IL-10 (Fig. 2A–D). The plasma suPAR levels of patients with severe sepsis correlated weakly with admission SOFA scores ($P = 0.05$) (Fig. 3A) but not with renal SOFA scores. SOFA scores also correlated with levels of PCT ($P < 0.05$) but not with CRP or mortality. suPAR levels correlated with the levels of the neutrophil granule protein MPO ($P = 0.05$) (Fig. 3B).

Discussion

Despite the relatively small number of patients included in this study, we found significantly higher levels of plasma suPAR in patients with severe sepsis compared to non-sepsis patients upon admission to the ICU. The result suggests that suPAR is a powerful marker of inflammation in patients with sepsis, concordant with previous studies.[8,10–12] We measured suPAR plasma concentrations upon admission to the ICU, at the start of intensive care treatment, and in six patients after four days. In contrast to CRP, PCT, MPO, IL-6, and IL-10, plasma suPAR concentrations did not significantly differ within the first four days of ICU treatment. This suggests that the clearance of suPAR is low and/or that the production and release persists over a longer time compared to the other inflammatory markers. It has been demonstrated that suPAR plasma concentrations are correlated to renal function, suggesting that failing renal clearance might additionally contribute to elevated circulatory suPAR.[13] In the present study no correlation between renal SOFA and suPAR levels was found. Our study

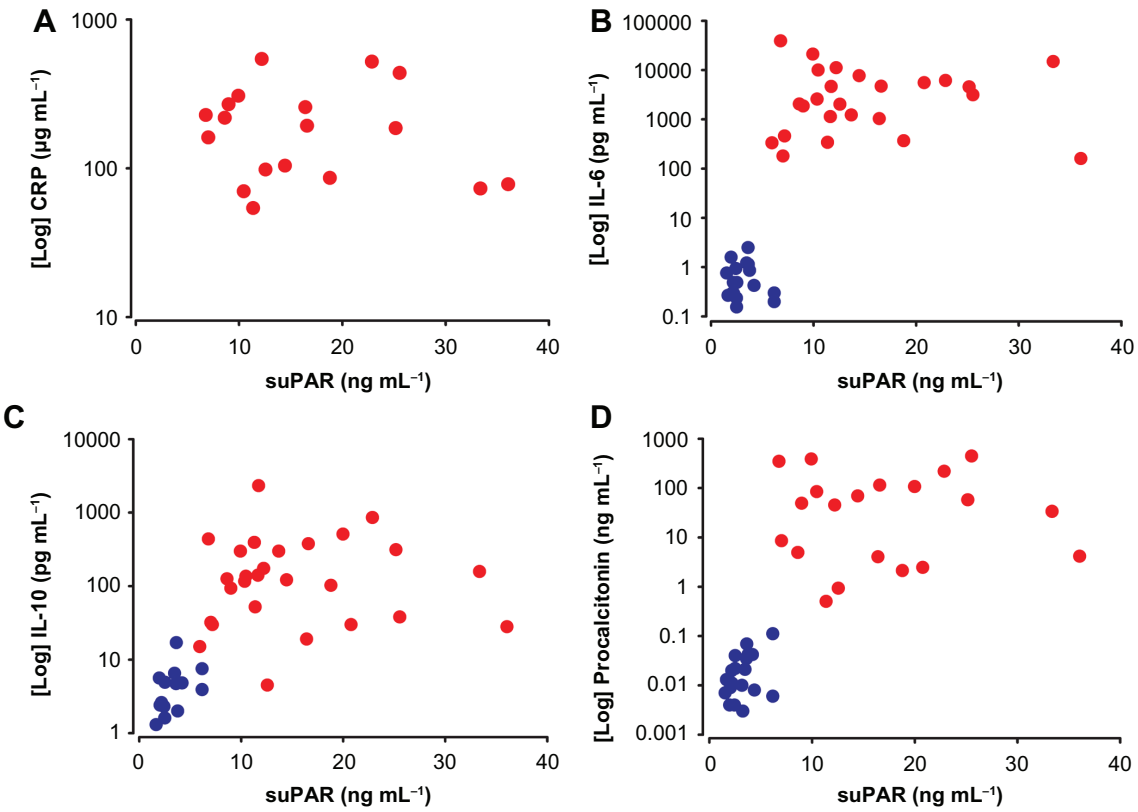

Figure 2. Correlation between suPAR and CRP (**A**, $P = 0.53$, r = 0.15), IL-6 (**B**, $P = 0.84$, r = 0.040), IL-10 (**C**, $P = 0.93$, r = 0.019), and PCT (**D**, $P = 0.98$, r = 0.0062), respectively.
Notes: The red circles represent sepsis patients and the blue circles controls. CRP was not measured in the controls.

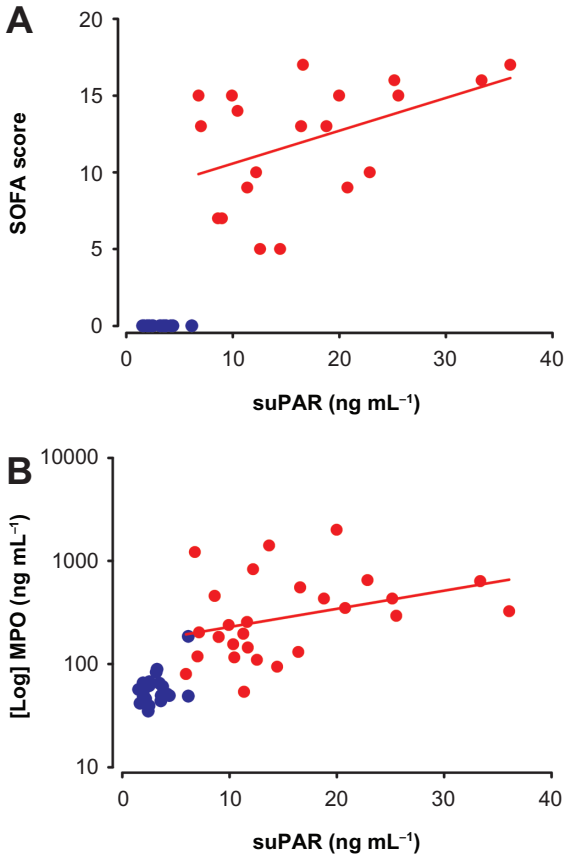

Figure 3. Correlation between suPAR and total SOFA score (**A**, $P = 0.05$, r = 0.43) and MPO (**B**, $P = 0.05$, r = 0.39), respectively.

did not include follow-up measurement after full recovery but it has been demonstrated in clinical trials that effective treatment of infectious diseases resulted in a decrease in suPAR levels after full recovery.[15,16]

Supporting previous findings[3,10,13] suPAR levels were correlated to SOFA scores in patients with severe sepsis ($P = 0.05$, r = 0.43). The SOFA score is a sepsis mortality risk algorithm including multiple laboratory and clinical measures and is based on six different scores, one each for the respiratory, cardiovascular, hepatic, coagulation, renal and neurological systems. It was surprising that suPAR levels did not correlate directly with mortality, only with severity. A probable explanation is the low number of patients in the present study (n = 27, ICU mortality = 44%), since several large studies have shown good correlation between high levels of suPAR and mortality in sepsis patients.[11–13]

At present, it is unclear whether plasma suPAR actually exerts proinflammatory actions or if it just reflects general inflammation. Further studies are needed for a satisfying understanding of the regulatory mechanisms of suPAR in order to evaluate whether suPAR could be a potential novel therapeutic target in critically ill patients. In the present study suPAR levels were not correlated with the non-specific inflammatory markers CRP and PCT in sepsis patients, which is consistent with another study.[8] Neither did suPAR correlate with the proinflammatory cytokine IL-6 nor the anti-inflammatory cytokine IL-10. The correlation between suPAR and MPO suggests that suPAR reflects activation of the cellular immune system rather than exerting proinflammatory or anti-inflammatory actions.

In conclusion, plasma levels of suPAR are increased in sepsis patients upon admission to the ICU, likely reflecting the activation state of the immune system, and remain elevated during the first four days of treatment. The suPAR level appears to have almost equal prognostic value as admission SOFA scores. The fact that suPAR did not significantly correlate with mortality may be explained by the relatively low number of patients included in this study (n = 27, ICU mortality = 44%).

Author Contributions

Conceived and designed the experiments: MB, IB. Analysed the data: AG, MB, IB. Wrote the first draft of the manuscript: AG. Contributed to the writing of the manuscript: AG, LL, MB, IB. Agree with manuscript results and conclusions: AG, LL, MB, IB. Jointly developed the structure and arguments for the paper: AG, LL, MB, IB. Made critical revisions and approved final version: AG, LL, MB, IB. All authors reviewed and approved of the final manuscript.

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Disclosures and Ethics

As a requirement of publication author(s) have provided to the publisher signed confirmation of compliance with legal and ethical obligations including but not limited to the following: authorship and contributorship, conflicts of interest, privacy and confidentiality and (where applicable) protection of

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