Diagnostic accuracy of soluble urokinase plasminogen activator receptor (suPAR) for prediction of bacteremia in patients with systemic inflammatory response syndrome

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Abstract

Objectives: Soluble urokinase plasminogen activator receptor (suPAR) serum concentrations have recently been described to reflect the severity status of systemic inflammation. In this study, the diagnostic accuracy of suPAR, C-reactive protein (CRP), procalcitonin (PCT), and interleukin-6 (IL-6) to predict bacteremia in patients with systemic inflammatory response syndrome (SIRS) was compared.

Methods: A total of 132 patients with SIRS were included. In 55 patients blood cultures had resulted positive (study group 1, Gram positive bacteria: Staphylococcus aureus and Streptococcus spp., n = 15; study group 2, Gram-negative bacteria, n = 40) and 77 patients had negative blood culture results (control group, n = 77). Simultaneously with blood cultures suPAR, CRP, PCT, IL-6 and white blood count (WBC) were determined.

Results: SuPAR values were significantly higher in study group 1 (median 8.11; IQR 5.78–15.53; p = 0.006) and study group 2 (median 9.62; IQR 6.52–11.74; p < 0.0001) when compared with the control group (median 5.65; IQR 4.30–7.83). ROC curve analysis revealed an AUC of 0.726 for suPAR in differentiating SIRS patients with bacteremia from those without. The biomarkers PCT and IL-6 showed comparable results. Regarding combinations of biomarkers multiplying suPAR, PCT and IL-6 was most promising and resulted in an AUC value of 0.804. Initial suPAR serum concentrations were significantly higher (p = 0.028) in patients who died within 28 days than in those who survived. No significant difference was seen for PCT, IL-6 and CRP.

Conclusion: In conclusion, suPAR, IL-6 and PCT may contribute to predicting bacteremia in SIRS patients.

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Introduction

Sepsis is defined as systemic inflammatory response syndrome (SIRS) in response to a confirmed infectious process (e.g., bacteremia) and is a major cause of morbidity and mortality [1]. Accurate and timely diagnosis of infection remains, in some cases, challenging to both clinicians and laboratories, with the use of highly sensitive clinical and laboratory markers being limited by poor specificity. Consequently, the need for a reliable marker arose, but only C-reactive protein (CRP), procalcitonin (PCT) and interleukin 6 (IL-6) are currently used broadly in clinical routine [2]. While the search for a single marker covering all the needs might ultimately be fruitless, a combination of established and novel biomarkers may improve diagnosis, prognosis, treatment efficacy and thereby survival [3].

The urokinase-type plasminogen activator system consists of a proteinase (uPA), a receptor (uPAR) and inhibitors. suPAR is the soluble form of uPAR. The system is involved in pericellular proteolysis, cell migration, and tissue remodeling by multiple modes of action: proteolysis, signal transduction, and chemokine-like activities. Under physiological conditions, uPA and uPAR are predominantly expressed by blood cells, including neutrophils, monocytes, macrophages and activated T-cells, for which they are believed to play important roles in cell activation, adhesion, migration, and extravasation [4]. It was shown that uPAR expression is up-regulated on cytokine-activated inflammatory cells, including activated neutrophils, and also that circulating serum concentrations of suPAR are increased in inflammatory and infectious processes, including bacteremia with endotoxemia, HIV infection, viral infections, malaria and rheumatoid arthritis [5–9]. suPAR serum concentrations in healthy individuals are known to be stable throughout the day [10]. Circadian changes in plasma concentration of suPAR were shown to be very limited, even repeated freeze-thaw

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procedures of plasma samples do not affect the suPAR concentrations [11]. Thus, suPAR measurements based on a biological fluid derived from a subject will be valid, independent of whether the subject is fasting or not, and largely independent of the sampling schedule.

Several studies have described that suPAR serum concentrations are elevated during S. aureus and Streptococcus pneumoniae bacteremia [12–14]. In those studies, however, suPAR values were determined from samples taken after the blood cultures already had turned positive and empirical antiinfective treatment had been administered. While most studies concentrated on the role of suPAR as prognostic marker in infectious diseases, studies elucidating a possible role of suPAR in prediction/diagnosis of infection are rare. Kofod and colleagues have evaluated 151 patients with SIRS of which 91 had bacterial infection and found an AUC of 0.500 for suPAR for prediction of the infection [3]. Yilmaz and colleagues found significantly higher serum concentrations in SIRS patients with infection (n = 76) than in SIRS patients without (n = 9, p = 0.001). Among patients with SIRS and infection they also found serum concentrations to be highest in patients with bacteremia. Results, however, were not significant due to low subgroup numbers and causative pathogens were not reported [15].

In this clinical study we compared the early diagnostic accuracy of suPAR, PCT and IL-6 in differentiating between SIRS patients with and without bacteremia. Evaluation of prognostic potential was a secondary objective.

Methods

This prospective explorative study was conducted January to April 2011 at the Medical University Hospital Graz, Graz, Austria. Within the study period all adult patients presenting with SIRS at the emergency department of our hospital were screened for study inclusion. Study flow is displayed in Supplementary material, Fig. 1a. Eight patients with positive blood cultures due to coagulase negative staphylococci and Corynebacterium spp. were excluded due to likelihood of contamination and false positive results.

One-hundred thirty-two patients fulfilling SIRS criteria as described previously [16,17] were included. Inclusion criteria were (i) age above 18 years, (ii) presentation at or admitted to the University Hospital of Graz, Austria, (iii) clinical suspicion of bacteremia/septicaemia by the attending physician and consecutive order of blood cultures, (iv) fulfillment of SIRS criteria, (v) absence of antibacterial therapy during the last 5 days before blood cultures were taken and (vi) informed consent. Exclusion criteria were (i) age below 18 years, (ii) no order of blood cultures at the emergency department, (iii) no fulfillment of SIRS criteria, (iv) antibacterial therapy during the last 5 days before blood cultures were taken and (v) patient refused to give informed consent.

Enrollment started with obtaining the first blood cultures in the emergency department. Three pairs of blood cultures per patient were collected simultaneously, placed in the BACTEC blood culture systems (Becton Dickinson, Cockeysville, MD) and incubated for a maximum of seven days. Blood cultures were processed at the Microbiological Laboratory, Department of Internal Medicine, and the Bacteriology Laboratory, Institute of Hygiene, Microbiology and Environmental Medicine, Medical University of Graz. In the emergency department (and before initiation of antibacterial therapy) also serum and whole blood samples for determination of suPAR and the routine laboratory infection markers (CRP, PCT, IL-6 and white blood count) were collected. The study population consisted of patients with SIRS and bacteremia (study groups 1 and 2) and patients with SIRS in which blood cultures resulted negative (control group 0). Study population only included patients who had not received antibacterial therapy 5 days prior to collection of the blood cultures. CRP, PCT, IL-6 (from serum samples) as well as neutrophil count and white blood count (from whole blood) were determined directly after sample collection. SuPAR was determined retrospectively from serum samples. In all study patients another sample was collected 24 h after the initial sample.

Samples for suPAR testing were immediately aliquoted, frozen and stored at −80 °C until tested. In samples taken after 24 h suPAR values were measured only in patients with positive blood cultures (study groups 1 and 2), otherwise (in control group 0) samples were discarded. Prior to testing, specimens were thawed and analyzed within 1 h; left-over specimens from this aliquot were discarded. Plasma suPAR serum concentrations were determined using the suPARnostic™ ELISA kit (ViroGates, Copenhagen, Denmark) according to the manufacturers instructions with a detection limit of 0.1 ng/mL and CVs for intra- and inter assay imprecision being within 1.7–3.5% and 2.3–6.0% respectively [4]. One kit serves to process 40 samples in duplicate within less than 2 h. Measurements were performed at the Clinical Institute of Medical and Chemical Laboratory Diagnostics, Medical University Graz on a ELISA platform reader (Flex Station 3, Molecular Devices, Munich, Germany). CRP, PCT, and IL-6 were determined on a fully automated analyzer (Cobas 8000 system, Roche Diagnostics, Rotkreuz, Switzerland). Glomerular filtration rate (GFR) was calculated using the GFR- Modification of Diet in Renal Disease (MDRD) formula [18].

Statistical analysis was performed using SPSS, version 19 (SPSS Inc., Chicago, IL, USA). Continuous data (e.g. suPAR values) are presented as medians (inter-quartile ranges [IQR]) or means (± standard deviation) and categorical data as proportions. All three patient groups were compared using Kruskal Wallance test. Each two patient groups were compared using Mann–Whitney U test. The p-values of the Mann–Whitney U tests were not corrected for multiple comparisons and are therefore only descriptive. Receiver operating characteristics (ROC) curve analysis was performed for biomarkers and combinations. Area under the curve (AUC) values were displayed including 95% confidence interval (CI) and compared according to Hanley and Mc Neil’s method. Cut-off values were determined using Youdends Index. Univariate and multivariate logistic regression analysis was performed for biomarkers and odds ratios (OR) were displayed. A p-value of less than 0.05 was considered statistically significant.

The study was conducted in accordance with the Declaration of Helsinki, 1996, Good Clinical Practice, and applicable local regulatory requirements and law. The study protocol has been approved by the local ethics committee, Medical University Graz, Austria (EC-number 21–469 ex 09/10).

Results

One hundred thirty-two patients fulfilling SIRS criteria (all patients were febrile > 38 °C) were included in the study. In 15 of 55 patients with positive blood cultures Staphylococcus aureus (n = 10) or Streptococcus spp. (n = 5) were isolated (group 1). In 40 of 55 patients Escherichia coli (n = 27) or other Gram-negative bacteria (total n = 13; Klebsiella spp. n = 9, Pseudomonas spp. n = 4) were isolated (group 2). Control group 0 comprised 77 patients with SIRS in which blood cultures remained negative. Demographic data are displayed in Table 1. Overall suPAR (p < 0.001), PCT (p < 0.001), IL-6 (p < 0.001) and to a lesser extend CRP (p = 0.028) were significantly higher in patients with bacteremia than those without, while WBC and absolute neutrophil count were not (data not shown).

SuPAR (p < 0.001), PCT (p < 0.001), IL-6 (p = 0.001) and also CRP (p = 0.03) differed significantly between the three patient groups (negative blood culture vs. gram-positive vs. gram-negative bacteremia). Initial suPAR values were significantly higher in group 1 (15 patients, median 8.11 ng/mL; IQR 5.78–15.53; p = 0.006) and group 2 (40 patients, median 9.62 ng/mL; IQR 6.52–11.74; p < 0.001) than in control group 0 in which blood cultures resulted negative (77 patients, median 5.65 ng/mL; IQR 4.30–7.83).

PCT (group 1, p = 0.001; group 2, p = 0.002) and IL-6 (group 1, p < 0.001; group 2, p = 0.017) were also significantly higher in patients with bacteremia than those without. In contrast CRP was not
significantly higher in group 2 (patients with Gram-negative bacteremia) versus the control group \((p = 0.441)\) while for group 1 it was \((p = 0.007)\). Medians and IQRs for suPAR, PCT, IL-6 and CRP are displayed in Table 1.

Overall, a median of 6.95 ng/mL \((IQR 4.75–10.05)\) was found in the study population \((n = 132)\) for suPAR values determined from samples taken simultaneously with initial blood cultures. Sensitivity, specificity, PPV and NPV for predicting bacteremia for a suPAR cut-off of 7.9 ng/mL were 62%, 77%, 65%, and 74%, respectively.

Receiver operating characteristics (ROC) curve analysis revealed an area under the curve (AUC) value of 0.726 for suPAR \((95\% CI 0.638–0.814)\) in differentiating patients with bacteremia from patients without \((p < 0.001)\). AUC values for comparators were as follows: PCT 0.744 (95% CI 0.650–0.838), IL-6 0.735 (95% CI 0.632–0.838), CRP 0.601 (95% CI 0.494–0.708; Fig. 1), and WBC 0.569 (95% CI 0.458–0.679). According to Hanley and McNeill’s method only PCT exhibited a significantly higher AUC value when compared to CRP \((p = 0.015)\), suPAR \((p = 0.063)\) and IL-6 \((p = 0.053)\) did not. Regarding combinations of biomarkers multiplying suPAR, PCT and IL-6 as the optimal cut-off point; multiplying PCT and IL-6 gave an AUC of 0.791 (95% CI 0.703–0.880), while multiplying IL-6 with suPAR gave an AUC of 0.780 (95% CI 0.684–0.875) in ROC curve analysis.

In the univariate analysis CRP was not considered to be statistically significant. Strong potential univariate predictors of bacteremia and their optimal cut-off points were suPAR 7.9 ng/mL \((p < 0.001); \text{OR} \, 4.94, 95\% \text{CI} \, 2.33–10.48\), PCT 0.78 ng/mL \((p = 0.001); \text{OR} \, 4.0, 95\% \text{CI} \, 1.71–9.33\) and IL-6 493 pg/mL \((p = 0.007); \text{OR} \, 3.4, 95\% \text{CI} \, 1.39–8.32\). Multivariate logistic regression analysis was performed for investigated biomarkers. In the final stepwise logistic regression, the significant predictors of bacteremia were suPAR >7.9 ng/mL \((p < 0.001); \text{OR} \, 5.82, 95\% \text{CI} \, 2.29–14.81\) and PCT >0.78 ng/mL \((p = 0.02); \text{OR} \, 3.11, 95\% \text{CI} \, 1.19–8.15\).

Patients with impaired renal function \((\text{GFR} \leq 60 \text{mL/min})\) had significantly higher suPAR values than patients with normal function \((p = 0.017)\). When analyzing groups with and without bacteremia separately concerning impact of renal function on suPAR serum concentrations, however, no difference was found. Neutropenia \(\left(\text{WBC} < 2 \times 10^9/\mu L\right)\) and underlying diseases did not influence suPAR values significantly (data not shown).

Initial suPAR serum concentrations were significantly higher \((p = 0.028)\) in patients who died within 28 days \((n = 11)\; \text{median} \, 10.72, \text{IQR} \, 7.34–19.16\) than in those who survived \((n = 121)\; \text{median} \, 6.80, IQR \, 4.72–9.61\). In contrast no significant difference was seen for PCT \((p = 0.211)\), IL-6 \((p = 0.618)\) and CRP \((p = 0.437)\). Bacteremic patients with suPAR serum concentrations increasing after 24 h had a significantly higher mortality than patients with decreasing serum concentrations \((\text{mortality rate} \, 8/22 \text{with increasing values versus} \, 3/27 \text{with decreasing values}; \, p = 0.046)\). In contrast to outcome suPAR values were not associated with length of hospital stay in our patients \((\text{data not shown})\).

**Discussion**

In this study, the potential of suPAR in predicting bacteremia in patients with SIRS was investigated. Two main findings are evident: First, suPAR serum concentrations were significantly higher among SIRS patients with bacteremia than among those without. Second, suPAR values in the study population were found to be high when compared to values published previously for healthy controls. With regard to the first finding the potential of suPAR was greater in predicting Gram-negative bacteremia, whereas potential of PCT and IL-6 seemed to be greater in Gram-positive bacteremia. According to

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Demographic data of study population as well as results for suPAR, PCT, IL-6 and CRP (median and IQR or mean and standard deviation (SD) displayed).</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>SIRS + gram-positive bacteremia ((n = 15))</td>
</tr>
<tr>
<td>Sex (male/female)</td>
<td>7.19 ± 8.1</td>
</tr>
<tr>
<td>Impaired renal function ((\text{GFR} &lt; 60 \text{mL/min})) ((%)</td>
<td>11 (73%)</td>
</tr>
<tr>
<td>Neutropenia (\left(\text{WBC} &lt; 2 \times 10^9/\mu L\right)) ((%)</td>
<td>2 (13%)</td>
</tr>
<tr>
<td>Malignancies ((%)</td>
<td>7 (47%)</td>
</tr>
<tr>
<td>Cardiovascular disease ((%)</td>
<td>12 (8%)</td>
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<tr>
<td>Hematological disease ((%)</td>
<td>8 (33%)</td>
</tr>
<tr>
<td>suPAR ((\text{ng/mL})) (\text{IQR})</td>
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</tr>
<tr>
<td>PCT ((\text{ng/mL})) (\text{IQR})</td>
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<td>IL-6 ((\text{pg/mL})) (\text{IQR})</td>
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<td>CRP ((\text{mg/L})) (\text{IQR})</td>
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<tr>
<td>Neutrophil count ((\text{g/L})) (\text{IQR})</td>
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<tr>
<td>WBC ((\text{g/L})) (\text{IQR})</td>
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</tbody>
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Fig. 1. ROC curve analysis: suPAR, PCT, IL-6 and CRP for differentiation between positive and negative blood cultures in SIRS patients.

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results obtained, combining suPAR and/or PCT with IL-6 may contribute to better prediction of bacteremia in SIRS patients.

Previously, several studies have shown that suPAR serum concentrations are elevated during bacteremia [19]. Wittenhagen and colleagues found that serum concentrations of suPAR were increased significantly (median 5.5; range 2.4–21.0 ng/mL) in 141 patients with pneumococcal bacteremia compared to 31 healthy controls (median 2.6, range 1.5–4.0 ng/mL, p = 0.001). Furthermore, suPAR serum concentrations were elevated significantly in patients who died from the infection compared to survivors (p < 0.001). In multivariate analysis, only suPAR remained a significant predictor of death (mortality rate of 13 for suPAR serum concentrations of > 10 ng/mL; 95% CI: 1.1–158) [12]. Other studies confirmed the potential of suPAR to predict mortality also in S. aureus bacteremia. Suggested cut-offs were 11.0 [13] and 9.25 ng/mL [14], respectively.

While these studies concentrated on the role of suPAR as prognostic marker in infectious diseases, data elucidating a possible role of suPAR in prediction/diagnosis of infection are insufficient. Koch and colleagues revealed that critically ill patients had higher serum suPAR concentrations at admission than healthy controls [20,21]. Another ongoing study had found that a suPAR cutoff value of 5.5 ng/mL had a sensitivity of 75% and specificity of 72% for diagnosing sepsis [22]. The major drawback of these studies is the lack of records regarding underlying infectious disease and/or causative pathogen. The rate of septic patients with proven bloodstream infection can therefore not be assessed and may be small to marginal in those studies.

Kofoed reported an AUC of 0.500 for suPAR in predicting bacterial infection in SIRS patients [3]. This paper was followed up by another Kofoed publication showing that the reverse conclusion could be made with regard to prognosis; in other words, those markers good in diagnosing were poor in prognosis and vice versa [23]. Reasons for the differing AUC found in this study for suPAR could be that all patients included had bacteremia, while only 15% of patients with bacterial infection had bacteremia in Kofoed's study. In addition, not excluding patients having received prior antibiotic therapy from the control group demonstrated a relevant confounder in that study. In a recent study from Turkey suPAR values at admission were found to be significantly higher in 85 SIRS patients (44 had bacteremia, 20 urinary tract infections, 12 pneumonia and 9 had no infection) compared to 53 individuals not fulfilling SIRS criteria. Among patients with SIRS and infection suPAR serum concentrations were highest in bacteremia, followed by urinary tract infection and pneumonia; results, however, were not statistically significant [15].

Our second main finding was that suPAR serum concentrations were high in our SIRS cohort. Median of suPAR serum concentrations in over 6000 healthy controls in Denmark has been reported as 3.38 (IQR 2.75–4.30 ng/mL) [24,25]. Haugaard and colleagues published suPAR values of 4.21 ± 1.35 ng/mL for over 2000 healthy controls between 40 and 70 years of age [26]. The suPAR cut-offs provided by suPARnostic™ for patients above 70 years of age are 4.3 for males and 4.5 for females [27]. SuPAR values obtained in our collective of patients presenting with SIRS (median 6.95; IQR 4.75–10.05) were therefore significantly higher compared to above mentioned previously published values for healthy controls. In contrast to healthy controls to date no suPAR cut-offs exist for critically ill patients with SIRS or sepsis. In our opinion it is vital having more and bigger studies being performed to define cut-off values those patients, since intensive care units may benefit from introducing suPAR testing.

In contrast to PCT, CRP and IL-6 suPAR serum concentrations were found significantly higher in patients who died within 28 days than in those who survived. This is in concordance with previous study results [23]. Interestingly bacteremic patients with suPAR concentrations increasing within a 24 h interval had a significantly higher mortality than patients showing a decrease of suPAR levels (p = 0.046).

The suPARnostic assay showed meaningful precision and linearity and is feasible for batch/large scale testing. The application in its present standard form based on 96well ELISA format, however, limits the introduction of this test performed in a central routine laboratory allowing 24 h/7 day testing. To enable clinical use in patients with SIRS single sample testing and/or a quick test (e.g. lateral flow immunoassay) is desirable. The latter will soon be introduced with cut-offs for healthy controls. However, cut-offs derived from healthy controls will have to be evaluated and probably adapted for differentiation in critically ill patients where finally a test with higher cut-offs may be needed.

In conclusion, results suggest that suPAR may help predicting bacteremia in clinical routine and analyzing suPAR may be of additional benefit in patients presenting with SIRS to an emergency department. More data are needed to find clinically relevant implications for suPAR testing.

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.clinbiochem.2012.11.004.

Conflicts of interest

The authors declare no conflicts of interest.

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