suPAR as a biomarker for risk of readmission and mortality in the acute medical setting

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ABSTRACT

INTRODUCTION: The urokinase-type plasminogen activator receptor (uPAR) and its ligand (suPAR) are involved in numerous physiological and pathological pathways. Previous studies have shown that an elevated plasma suPAR level is associated with disease severity and mortality. The aim of this prospective observational study was to determine if the suPAR level was associated with readmission and mortality in the acute medical setting.

METHODS: Plasma suPAR levels were measured in 1,036 patients at admission. Follow-up ranged 3-10 months. Cox proportional hazards model was used to assess the relative contribution of different risk factors to mortality and readmission. The ANOVA test and Pearson's chi-squared test were used to compare suPAR tertile level with various variables.

RESULTS: The highest suPAR tertile level was significantly associated with mortality within 30 days after discharge, with a 6.66 hazard ratio (HR). Similar associations were found with readmission within the maximum observation period (HR = 2.26) and within 30 days (HR = 2.08), although the latter became insignificant when covariates were included.

CONCLUSIONS: This study confirms previous findings of increased mortality and adds the finding that increased long-term readmission rates are associated with elevated suPAR levels. The present data do, however, not indicate that suPAR may serve as an independent biomarker for increased risk of short-term readmission in the acute medical setting.

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In the health sector, one important strategy towards disease prevention is to identify high-risk patients who need prevention; and the use of biomarkers for this purpose, including immunological and inflammatory mediators, has been discussed over the past several decades [1].

The plasma level of the inflammatory marker soluble urokinase plasminogen activator receptor (suPAR) has been positively correlated with disease severity and survival rates [2-4]. Through interactions with proteins present in the extracellular matrix, the urokinase-type plasminogen activator receptor (uPAR) and its ligand (suPAR) are involved in numerous physiological and pathological pathways, which include the plasminogen activating pathway, regulation of pericellular proteolysis, and modulation of cell adhesion, migration and proliferation. uPAR is anchored to the external plasma membrane through a glycolphosphatidylinositol (GPI)-anchor in a variety of body cells including neutrophils, lymphocytes, macrophages, endothelial and malignant cells. By binding urokinase-type plasminogen activator (uPA) to the receptor, a variety of extracellular signalling is initiated. Furthermore, the formation of the uPA-uPAR complex results in cleavage of the GPI anchor, meaning that uPAR will be released into the extra cellular matrix in a soluble form. The soluble receptor, suPAR, is measurable in human body fluids including plasma, serum, urine, sputum, saliva and cerebrospinal fluid [5, 6]. The plasma level of suPAR reflects immune activation and is increased in several infectious diseases, such as HIV-1-infection [7], malaria [8], tuberculosis [9], *Streptococcus pneumoniae* bacteraemia [10], sepsis [11], bacterial and viral central nervous system infection [12], cancer, cardiovascular diseases, and type 2 diabetes mellitus [13]. Furthermore, high suPAR levels are associated with increased inflammation, disease progression and fatal outcome [2-4]. Measuring suPAR levels may thus serve as a feasible marker to monitor disease progression and treatment.

Three previous studies have focused specifically on suPAR as a risk marker for readmission or mortality in the general acute medical setting. Haupt et al [3] found that suPAR was significantly associated with 90-day mortality, whereas no association between baseline suPAR and readmission within 30 days was observed among 543 patients with various diseases from a Danish acute medical unit. Uusitalo-Seppälä et al [14] found that suPAR was significantly associated with 28-day mortality in 539 patients with suspected infection seen in the emergency department. Finally, Huttunen et al [4] found that suPAR was significantly associated with 30-day mortality among 132 patients admitted with bacteraemia.

In this prospective observational study, we aimed to further investigate the association between suPAR level and risk of readmission and mortality in the acute medical setting.

ORIGINAL ARTICLE

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METHODS

Study population

This study included patients admitted to the Acute Care Ward, the Department of Internal Medicine at Bispebjerg & Frederiksberg Hospital, Denmark, from March to October 2010. At the time of hospitalisation, patients were invited to participate in the study. If informed consent was obtained, a blood sample for measurement of the plasma suPAR level was taken together with routine admission samples. Knowledge of the suPAR level was not available for either the staff or the patients, and no further specific intervention was done during admission based on the suPAR measurement. Data on primary diagnosis, specific co-morbidities (i.e. diabetes, chronic obstructive pulmonary disease (COPD), ischaemic heart disease (IHD), or a previous diagnosis of cancer), vital signs (Early Warning Score, EWS), C-reactive protein (CRP) level, length of stay, age, gender and any information about readmission and or death until 31 December 2010 were obtained from the hospital record and the Danish civil registration register (CPR).

suPAR assay

The sample was taken from peripheral venous blood, drawn in a standard 4 ml ethylenediaminetetraacetic acid (EDTA) tube. SuPAR was measured using a commercially available sandwich enzyme-linked immunosorbent assay (ELISA)-kit (ViroGates A/S, Birkerød, Denmark) according to the manufacturer's instructions. Briefly, plasma samples and standards with known suPAR concentrations were added to anti-suPAR-coated microtiter plates and incubated in dilution buffer containing a horseradish peroxidase-conjugated secondary antibody for one hour. After washing to remove any unbound secondary antibody, 3,3',5,5'-tetramethylbenzidine substrate was added, and a colour reaction developed for 20 minutes. The colour reaction was terminated by the addition of a sulphuric acid stop-solution. The plate was read at 450 nm with wavelength correction at 650 nm. The optical densities of the standards with known concentrations were used to calculate the concentrations of the plasma samples by creating a standard curve. The ELISA-kit manufacturer provided the quantification software. All samples were measured in duplicate, and the mean suPAR concentration of the two measurements was used for analysis.

Statistics

All statistical analyses were performed using SPSS Statistics (version 19.0.0.1). Age, length of stay, suPAR level, CRP level, vital signs (EWS) and total follow-up period were modelled as continuous variables. Sex, COPD, cancer, diabetes, and IHD were fitted as categorical variables.

An ANOVA test was conducted to compare the suPAR tertile level with individual factors for continuous

TABLE 1

Descriptive statistics of study population according to tertile range of plasma soluble urokinase plasminogen activator receptor concentration.

		Tertile				
	Total population (N_{tot} = 1,036)	1st: 0.0-4.0 ng/ml (N = 345)	2nd: 4.1-6.0 ng/ml (N = 344)	3rd: 6.1-57.6 ng/ml (N = 347)	p-value ANOVA	χ^2-test
Continuous variables, median (range)						
Age, yrs	69 (85)	57 (77)	73 (82)			
76 (85)		0.000	–			
EWS	0 (7)	0 (6)	0 (7)	1 (7)	0.000	–
CRP, mg/l	10 (435)	3 (329)	11 (388)	23 (435)	0.000	–
Length of stay, days	4 (194)	3 (146)	4 (84)	6 (194)	0.000	–
Categorical variables, n (%)						
Male	425 (41.0)	137 (39.7)	133 (38.7)	155 (44.7)	–	0.229
Female	611 (59.0)	208 (60.3)	211 (61.3)	192 (55.3)	–	–
COPD	145 (14.0)	30 (8.7)	60 (17.4)	55 (15.9)	–	0.002
Cancer	125 (12.1)	32 (9.3)	47 (13.7)	46 (13.3)	–	0.148
IHD	140 (13.5)	25 (7.2)	48 (14.0)	67 (19.3)	–	0.000
DM	120 (11.6)	20 (5.8)	31 (9.0)	69 (19.9)	–	0.000
Death < 30 days	42 (4.1)	4 (1.2)	12 (3.5)	26 (7.5)	–	0.000
Death < 90 days	77 (7.4)	9 (2.6)	16 (4.7)	52 (15.0)	–	0.000
Readmission < 30 days	93 (9.0)	21 (6.1)	32 (9.3)	40 (11.5)	–	0.042
Readmission maximum period	216 (20.8)	49 (14.2)	72 (20.9)	95 (27.4)	–	0.000

COPD = chronic obstructive pulmonary disease; CRP = C-reactive protein concentration; DM = diabetes mellitus; EWS = Early Warning Score; IHD = ischaemic heart disease.

variables. Pearson's chi-squared test was used to compare the suPAR tertile level with other independent categorical variables. A two-tailed p-value < 0.05 was considered statistically significant.

The Cox proportional hazards model was used to assess the relative contribution of different risk factors to mortality and readmission within three months. Final models resulted from conditional forward and backward analyses. The results are presented as hazard ratios (HR) with 95% confidence intervals (CI). Kaplan-Meier plots were used to check for proportionality.

Trial registration: No: H-B-2009-075

RESULTS

A total of 3,564 patients were admitted during the sampling period (male/female ratio 1,508/2,056, median age 69 years). Informed consent for participation was obtained in 1,070 primary admissions. A total of 32 suPAR samples were lost during processing, and two patients were lost to follow-up, leaving 1,036 cases available for analysis.

The median suPAR level was 5.9 ng/ml (range: 0.0-57.6 ng/ml) with tertile ranges as follows: Q1 1.0-4.0 ng/ml; Q2 4.1-6.0 ng/ml; Q3 > 6.0 ng/ml. **Table 1** displays the distribution of demographic characteristics, co-morbidity, vital signs, CRP level, length of stay and incidence of readmission and death as present within the suPAR tertile groups. In total, 216 readmissions and 99 deaths were recorded within the observation period (from 1 March 2010 to 31 December 2010), whereas 93 patients were readmitted and 42 had died within 30 days following discharge. In general, the indicators of acute disease severity as well as age and the prevalence of co-morbidity progressed with increasing suPAR tertile level (Table 1).

In Cox proportional hazard analyses, increasing suPAR level significantly increased the risk of death (30 and 90 days after discharge) and the risk of readmission (within the maximum observation period (median 152 days, maximum 302 days). Furthermore, significant covariates in the final models for death were age, EWS and cancer, and for readmission age, IHD and COPD (**Table 2** and **Table 3**). When the CRP level was included as a covariate in the final models, the significance of the suPAR level was lost (p = 0.15) in the 30-day mortality analyses, but otherwise it was preserved. The suPAR tertile level was significant for readmission within 30 days in the crude analysis, but not when adjusted for age (Table 3).

Receiver operating characteristic (ROC) areas under the curve with suPAR level as test variable were 0.73, 0.74, 0.58 and 0.59 for death (30 and 90 days) and readmission (30 and maximum observation period), respectively.

TABLE 2

Incidence of mortality events in relation to plasma soluble urokinase plasminogen activator receptor concentration in 1,036 patients.

	Tertile		
	1st: 0.0-4.0 ng/ml	**2nd: 4.1-6.0 ng/ml**	**3rd: 6.1-57.6 ng/ml**
Death < 30 days			
Events, n/N (%)	4/345 (1.2)	12/344 (3.5)	26/347 (7.5)
Model, HR (95% CI):			
1[a]	1.0	3.03 (1.0-9.4)	6.66 (2.3-19.1)
2[b]	1.0	2.21 (0.7-7.0)	4.41 (1.5-13.1)
3[b, c]	1.0	1.91 (0.6-6.0)	3.22 (1.1-9.6)
4[b, c, d]	1.0	1.92 (0.6-6.0)	3.28 (1.1-9.8)
Death < 90 days			
Events, n/N (%)	9/345 (2.6)	16/344 (4.7)	52/347 (15.0)
Model, HR (95% CI):			
1[a]	1.0	1.81(0.8-4.1)	6.14(3.0-12.5)
2[b]	1.0	1.22 (0.5-2.8)	3.69(1.8-7.7)
3[b, c]	1.0	1.06 (0.5-2.4)	3.07 (1.5-6.4)
4[b, c, d]	1.0	1.04 (0.5-2.4)	3.21 (1.5-6.7)

CI = confidence interval; HR = hazard ratio.
a) Unadjusted.
b) Adjusted for age.
c) Adjusted for Early Warning Score.
d) Adjusted for cancer.

TABLE 3

Incidence of readmission rate events in relation to plasma soluble urokinase plasminogen activator receptor concentration in 1,036 patients.

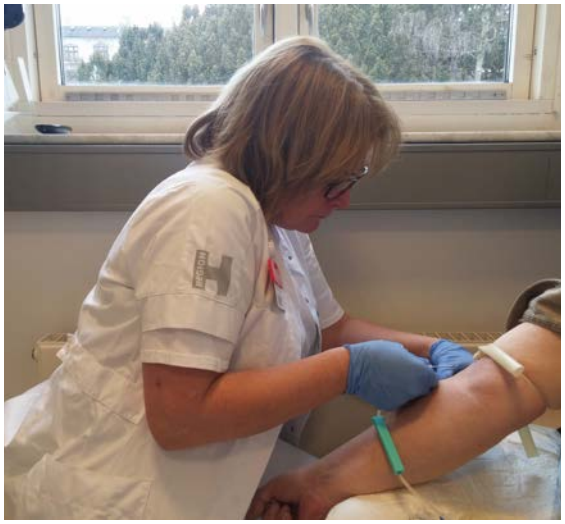
	Tertile		
	1st: 0.0-4.0 ng/ml	**2nd: 4.1-6.0 ng/ml**	**3rd: 6.1-57.6 ng/ml**
Readmission maximum period			
Events, n/N (%)	49/345(14.2)	72/344(20.9)	95/347(27.4)
Model, HR (95% CI):			
1[a]	1.0	1.54 (1.1-2.2)	2.26 (1.6-3.2)
2[b]	1.0	1.23 (0.9-1.8)	1.69 (1.2-2.4)
3[b, c]	1.0	1.23 (0.8-1.8)	1.65 (1.1-2.4)
4[b, c, d]	1.0	1.17 (0.8-1.7)	1.60 (1.1-2.3)
Readmission < 30 days			
Events, n/N (%)	21/345 (6.1)	32/344 (9.3)	40/347 (11.5)
Model, HR (95% CI):			
1[a]	1.0	1.59 (0.9-2.8)	2.08 (1.2-3.5)
2[b]	1.0	1.27 (0.7-2.2)	1.56 (0.9-2.7)

CI = confidence interval; HR = hazard ratio.
a) Unadjusted.
b) Adjusted for age.
c) Adjusted for ischaemic heart disease.
d) Adjusted for chronic obstructive pulmonary disease.

DISCUSSION

This study adds to existing findings of suPAR as a biomarker for readmission and death in the acute medical setting [3]. It basically confirms previous findings of increased mortality and adds the finding of an increased long-term readmission rate with elevated suPAR levels.

The absence of an effect on short-term readmission



Blood sample for measurement of plasma soluble urokinase plasminogen activator receptor level was taken together with routine admission samples.

(within 30 days following discharge) is in accordance with previous findings [3]. As our 30-day readmission rate was considerably lower than the rate reported by Haupt et al [3] (10% versus 25%), it could be argued that our cohorts may differ with respect to disease severity. Also, 30-day mortality differed between the two cohorts (4% versus 5%, 90 days mortality 7% versus 9%). The difference in disease severity may be due to selection bias towards not requesting consent from the most ill patients. Our sampling rate was, indeed, lower than in the study by Haupt et al [3] (30% versus 50%). Nevertheless, we included twice the number of patients compared with any of the previous studies [3, 4, 14] and found the same associations between elevated suPAR levels and indicators of disease severity.

We were unable to calculate the full Charlson Comorbidity Index from our data. Instead, we included each of four major co-morbidity diagnoses (diabetes, ischaemic heart disease, chronic obstructive pulmonary disease and cancer (previous or known active)). In the Charlson Comorbidity Index, malignancy contributes significantly more than diabetes, ischaemic heart disease and COPD (six points versus one each). Our finding that a specific association exists between mortality and cancer, and the absence of an association with the other co-morbidities, is in accordance with the Charlson weighting. With regard to readmission risk, which is not the scope of the Charlson Index, it appears from our findings that specific co-morbidity covariates to be included in future algorithms should be analysed separately, and possibly weighting, e.g., COPD and IHD higher than cancer rather than aggregating them according to the Charlson Index.

At the end of the day, the value of suPAR as a marker of risk must be demonstrated through intervention, i.e. showing that access to suPAR level knowledge leads to improved treatment results. However, in accordance with previous studies, our study does not suggest that a higher plasma suPAR level as a biomarker can be used in the acute medical setting for predicting increased risk of short-term readmission (within 30 days following discharge).

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