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Original article

Plasma level of soluble urokinase plasminogen activator receptor (suPAR) predicts long-term mortality after first acute alcohol-induced pancreatitis

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ABSTRACT

Background: Soluble urokinase plasminogen activator receptor (suPAR) is a biomarker associated with inflammatory and certain malignancies. Earlier we have shown that plasma suPAR (P-suPAR) predicts severity of acute alcohol-induced pancreatitis (AAP) on admission. Our aim was to investigate whether P-suPAR levels predict AAP recurrences or mortality during long-term follow-up after first AAP.

Methods: Eighty-three patients (median age 47.5, range 25–71 years) suffering their first AAP during 2001–2005 were recruited and followed prospectively for 9 years with a median follow-up time of 7.0 (range 0.3–9.8) years. P-suPAR was measured by enzyme-linked immunosorbent assay (ELISA) from the samples taken at follow-up visits. Survival was registered in November 2014.

Results: P-suPAR level on admission or after recovery of the first AAP did not predict the recurrence of AAP. However, higher P-suPAR measured after recovery of first AAP (3.6 vs. 2.9 ng/mL) predicted mortality during follow-up period (hazard ratio 1.48, $p = .008$). Cut-off value for P-suPAR indicating a higher risk for 10-year mortality resulted a value of ≥3.4 ng/mL. When adjusted for other covariates, P-suPAR above cut-off level retained its statistical significance as an independent factor.

Conclusions: P-suPAR level on admission or after recovery of the first AAP does not predict the recurrence of AAP during long-term follow-up. However, P-suPAR ≥3.4 mg/mL measured after recovery from first AAP is associated with an increased risk of 10-year mortality as an independent factor. This can be used to detect patients with highest risk after AAP, in order to focus the preventive healthcare actions.

1. Introduction

Acute pancreatitis (AP) has an increasing incidence in Finland, and it has serious effects on the public health and health care costs. The major cause of AP is alcohol, which is responsible for 70% of the cases [1,2]. The risk to develop a recurrent episode of acute pancreatitis (RAP) is high among these patients: after the first alcohol-induced AP (AAP) about half of the patients will develop a RAP. Recurrence pattern and risk factors concerning the recurrence after first AAP have been reported previously, and among patients developing RAP, 80% of patients had a recurrence during the first 4 years, and 90% of patients during the first 6 years [3]. The known risk factors for RAP include continuing alcohol consumption and usage of sedative medication prior to first AAP episode [4]. In addition, younger age, smoking, obesity, pancreatic malfunction and milder first AAP are suggested to be risk factors for RAP, but the evidence is not distinct [3,5]. Finding of individuals with the highest risk for RAP and mortality would help to focus the limited resources on these patients.

Soluble urokinase plasminogen activator receptor (suPAR) is a biomarker associated with inflammatory and certain malignant diseases. Circulating blood suPAR level reflects the activation level of overall human immune system [6] and serves as a marker for disease severity and increased risk for mortality [7]. Our group has recently reported that plasma suPAR (P-suPAR) levels are elevated in first AAP, and high P-suPAR at admission of AAP predicts a more severe disease [8].

Based on the promising results of P-suPAR predicting the severity of AAP at admission, we wanted to investigate whether P-suPAR also

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predicts the AAP recurrence or prognosis when measured on admission, after recovery and during the long-term follow-up after the first AAP.

2. Methods

Eighty-three patients [median age 48 years (range 25–71 years); 90% male] with their first AAP treated in Tampere University Hospital during 2001–2005 formed the study population. Exclusion criterias were previous history of pancreatitis of other etiology of pancreatitis. Follow-up was based on voluntarism and required sufficient co-operation. The severity of the first acute AAP was classified according the revised Atlanta criteria [9], and the moderately severe and severe grades were combined for the analysis as a non-mild group.

The patients were prospectively followed up for 9 years, with a median follow-up time of 7.0 (range 0.3–9.8) years. BMI, smoking (cigarettes per day), alcohol consumption (grams per week) and co-morbidities such as diabetes and cardiovascular diseases (e.g. coronary disease, hypertension, hypercholesterolemia) were registered on admission, at 24 months (median, range 5–32 months) after recovery (after recovery sample, months from index admission), and during the long-term follow-up at 5, 7 and 9 years. Medical reports were reviewed to complete any missing data. P-suPAR values were retrospectively measured from plasma samples collected on control visits [3], and survival was registered in November 2014. Fully informed written consent was obtained from each patient, and the Ethics Committee of Tampere University Hospital, Finland approved the study protocol (decision number R00126). Demographic data is presented in Table 1. Drop out percentage was 33% in 5-year, 47% in 7-year, and 55% in 9-year control point. Survival data was available from all patients.

P-suPAR was measured by enzyme-linked immunosorbent assay (ELISA) from blood samples stored at −70 °C using enzyme-linked immunosorbent assay (ELISA) according to manufacturer's instructions (suPARnostic®, ViroGates A/S, Birkerød, Denmark).

Statistical analysis was run with IBM SPSS Statistics for Windows (Version 22 and 23). Univariate analysis of P-suPAR values was performed by using Mann-Whitney *U* test as the data was not normally distributed. *P*-values ≤.05 were considered significant. 10-year mortality was estimated using ROC curve analysis, and cut-off value was calculated using Youden's J statistic. Survival curves were established with Kaplan-Meier method and differences were estimated with log rank test. Hazard ratio and multivariate analysis were established with COX regression analysis.

Table 1
Demographic and clinical characteristics of patients (n = 83) recruited on admission of their first alcohol-induced pancreatitis (AAP).

Characteristics	
Gender (male/female)	75/8 (90%/10%)
Age on admission, years, median (range)	48 (25–71)
Follow-up time, months, median (range)	84 (18–117)
Survival follow-up time, median, months (range)	138 (38–166)
Death during follow-up time	19 (23%)
Severity of first AAP (mild/non-mild)	55/28 (66%/34%)
Recurrent pancreatitis during follow-up	32 (39%)
Chronic pancreatitis	12 (14%)
Diabetes mellitus	35 (42%)
BMI (kg/m^{-2}), median on admission (range)	28 (19–38)
Smoking (cigarettes/day)	10 (0–50)
Alcohol abuse (g/2 months)[a]	3048 (288–15,456)
Cardiovascular disease (HBP/CHD)[b]	16 (19%)
Malignancy	3 (4%)

[a] Data missing on 1 patient.
[b] HBP = high blood pressure; CHD = coronary heart disease.

3. Results

Among the 83 patients with their first AAP, pancreatitis was classified as mild in 66% (n = 55), moderately severe in 28% (n = 23) and severe in 6% (n = 5). During the 9-year prospective follow-up, 39% (n = 32) of the patients had at least one RAP, 14% (n = 12) developed chronic pancreatitis and 23% (n = 19) died. Among the 19 patients who died, the median survival after the first AAP was 8 years (range 3.2–13.3 years) and median age in deceased patients was 60.6 years (range 44.1–76.0 years). The cause of death was registered in 14/19 patients, and was accidental in 7 (falling in 3, suffocation in 2, drowning in 1 and burning in 1 cases), malignancy in 3, intoxication in 2, gastrointestinal bleeding in 1, acute pancreatitis in 1 and cardiac arrhythmia in 1 patient.

P-suPAR level remained higher for 12 months after recovery in patients with a non-mild AAP compared to the patients with a mild AAP [median 3.7 (IQR 3.3–3.9) vs. 3.1 (2.6–3.6) ng/mL, p = .047]. However, two years after the first AAP no difference was seen between the non-mild and mild AAP patients [2.7 (2.3–3.3) vs. 2.9 (2.5–3.6) ng/mL; NS]. P-suPAR levels of the patients with 2 or more RAPs did not differ from the patients with 1 or no RAP at any time point. P-suPAR values on admission [4.5 (3.6–5.3) vs. 4.1 (3.1–6.2) ng/mL; NS] or after recovery of the first AAP [3.0 (2.6–3.8) vs. 3.1 (2.5–3.3) ng/mL; NS] did not differ between the patients with or without RAPs, respectively.

P-suPAR level measured on first control visit after the first AAP was associated with 10-year mortality, whereas P-suPAR level measured on admission or later during the follow-up were not. In those patients who died during the follow-up, P-suPAR after recovery of the first AAP was significantly higher compared to the patients who survived [3.6 (2.7–4.1) vs. 2.9 (2.5–3.3) ng/mL; hazard ratio 1.48, p = .008]. The cut-off value for P-suPAR indicating a higher risk for 10-year mortality was estimated using ROC curve analysis shown in Fig. 1.

Area under the curve (AUC) was 0.75 (CI 0.61–0.89); p = .002. Cut-off P-suPAR value calculated using Youden's J statistic was 3.4 ng/mL with the sensitivity and specificity predicting long-term mortality of 58% and 82%, respectively. Patients were further divided into high or low level of P-suPAR after recovery of the first AAP (P-suPAR < 3.4 ng/mL or ≥ 3.4 ng/mL), and Kaplan-Meier survival curves were established to indicate mortality (Fig. 2). The risks for 10-year mortality for patients with P-suPAR < 3.4 ng/mL or ≥ 3.4 ng/mL were 13% and 49%, respectively.

A multivariate analysis was performed to study other factors with a possible influence. Factors studied were smoking (tobaccos per day), BMI, existence of diabetes and cardiovascular diseases (coronary syndrome, high blood pressure and/or high blood cholesterol), age, gender

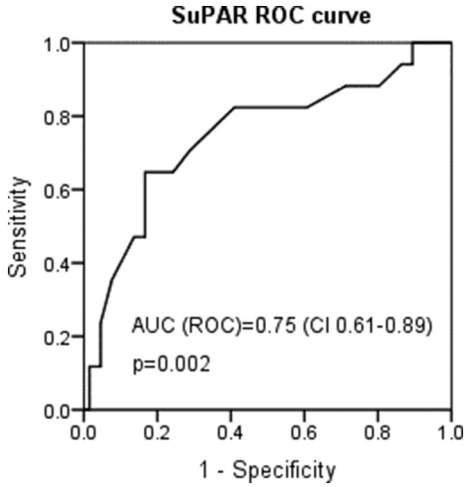

Fig. 1. ROC (receiver operating characteristics) curve for P-suPAR measured after recovery from the first acute AAP to evaluate mortality risk in 10 years.

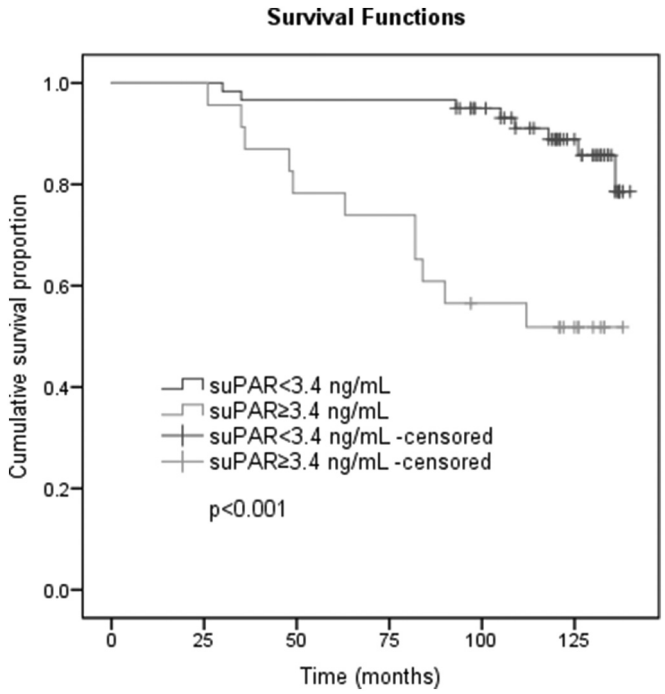

Fig. 2. Cumulative survival in patients with P-suPAR after recovery ≥3.4 ng/mL and < 3.4 ng/mL. Out of the 19 patients who died during the follow-up, 11 (58%) had the P-suPAR value above the cut-off level 3.4 ng/mL.

Table 2
Risk factors for mortality after recovery from first alcohol-induced pancreatitis (AAP) using multivariate Cox regression analysis (n = 82).

Covariate	HR (95% CI)	95% CI	p-Value
P-suPAR ≥3.4 mg/mL	4.14	1.31–13.0	0.015
BMI (kg/m^{-2})	0.90	0.75–1.07	0.210
Smoking	0.77	0.16–3.70	0.740
Age	1.05	0.99–1.12	0.111
Cardiovascular disease	1.56	0.42–5.72	0.515
Diabetes mellitus (DM)			
No DM	1.00		
DM before 1. AAP	0.65	0.07–5.86	0.702
DM after AAP	0.87	0.25–2.99	0.818
Alcohol consumption[a] (kg/2 months)	1.12	0.97–1.30	0.116
Recurrent AAP	2.44	0.76–7.79	0.133
Chronic pancreatitis	3.19	0.77–13.2	0.108
Severity of 1. AAP			
Mild	1.00		
Moderate	1.14	0.33–3.95	0.837
Severe	0.51	0.04–6.85	0.613

[a] Data available on 82/83 patients

and alcohol amount used (grams per week). Covariates and their hazard ratios are shown in Table 2. When adjusted for all other covariates, P-suPAR above cut-off level retained its statistical significance. P-suPAR ≥3.4 mg/mL measured after recovery from first AAP was associated with increased risk of mortality (hazard ratio 4.14, p = .015) as an independent factor.

4. Discussion

Earlier studies have suggested that elevated suPAR level may be associated with poorer prognosis in gastrointestinal disorders [10–14]. Recently we have shown that elevated P-suPAR on admission predicts the severity of AAP [8]. In this study we went further to investigate whether P-suPAR measured on the first AAP admission, on its recovery or during follow-up could be used to identify those patients who are in a high risk of developing RAP episodes or have a poorer long-term prognosis. To our knowledge no previously published data on suPAR about the long-term follow-up after AAP exists. We found that P-suPAR did not predict the RAP episodes but was able to detect the patients with a high risk for 10-year mortality. A cut-off value of 3,4 ng/mL after recovery of first AAP was an independent risk factor for long-term mortality.

Urokinase plasminogen activator receptor (uPAR) is expressed by various immunologically active and inflammatory cells, such as monocytes, activated T-lymphocytes, macrophages and certain cancer cells. uPAR seems to be up-regulated in cells that are in motion, whereas cells that are lacking stimulus do not express uPAR [15]. In certain inflammatory conditions - after uPAR interaction with its urokinase plasminogen activator (uPA) ligand - uPAR is proteolytically cleaved by various kind of proteases from the cell surface to its soluble form suPAR, which can then be measured from blood, cerebrospinal fluid and urine [6]. About half of patients with continuous alcohol abuse after AAP develop a RAP. Our hypothesis was that the recurrent inflammatory episodes could be detected as permanently elevated P-suPAR levels. However, we found no significant difference between the patients with and without RAPs in short or long-term follow-up. Since smoking, morbid obesity > 40 kg/m^2, alcohol consumption, age, sex, history of cardiovascular diseases and many other factors are shown to have effect on P-suPAR levels [7], the possible differences caused by elevated pancreatic inflammation state in this population may not be detected.

Elevated P-suPAR levels are associated with short-term (30–90 –day) mortality in many different inflammatory diseases, for example sepsis [16] as well as in heterogeneous medical patient population [17]. P-suPAR correlation with long-term mortality in general population has also been shown [18], and this data correlates with our findings. In our study the mortality rate is very high comparing to median age, showing that these patients have high risk to die young. Reasons of death were diverse, including intoxication, malignancies and trauma. It has been shown that suicidal and depressive patients have elevated P-suPAR levels, referring to an inflammatory background of depressive behavior [19]. People with alcohol abuse commonly persist depressive and high-risk behavior [20]. The accidental deaths, intoxications and pancreatitis-associated death were regarded as related to alcohol abuse and thus could potentially be prevented.

Additional studies and larger study populations are needed to examine if P-suPAR levels could explain long-term mortality in other disease conditions as well, and if development of chronic pancreatitis could be predicted with P-suPAR level measurements.

The severity of pancreatitis ranges from mild abdominal pain to life-threatening multi-organ failure (MOF) with mortality of 2–16%. The amount of alcohol used prior to first AAP is suggested to correlate with severity of AAP [21]. P-suPAR of patients with non-mild first AAP remains significantly higher than in patients with mild first AAP. This may be due to the longer healing process from a more severe disease. In two years after AAP, these levels become and stays even. The study cohort is estimated to represent average patients after the first AAP.

P-suPAR levels of these patients in follow-up samples are higher than in normal population measured in earlier studies [7,18]. We do not know the P-suPAR levels of these patients before their first AAP. This study awakens the question, if P-suPAR levels after recovery from first AAP are a result from pancreatitis itself or reflect the overall inflammatory state of human body. Despite the initial cause of elevated P-suPAR, it is useful in identifying the crucial patients who are at high risk for mortality, needing an intervention and intensive follow-up after their first AAP.

Limitations of this study include the lack of possibility to compare P-suPAR levels to general population, small patient number and the number of drop-outs over time. The association between suPAR-level and high accidental mortality rate is also difficult to interpret. There were a lot of drop-outs between 100 and 120 months in patients with

low suPAR values. Whether the patients who are asymptomatic are less motivated to control visits than the ones with symptoms is not known. What we do know is that it is challenging to motivate patients with alcohol-related diseases for control visits. True amount of RAP episodes may differ from the reported, because of disease can be so mild that patients do not admit to hospital, or they may have been treated elsewhere. This patient data is, however, prospective, though P-suPAR -analyses are retrospective, and patients were interviewed for possible recurrent attacks so the amount of recurrences is regarded reliable. Previous use of sedative medication is shown to have effect on AAP recurrence [4]. Medication data of these patients was not reported, so this possible influence has not been evaluated. The strength of our study lies on prospective and long-time follow-up.

We conclude that P-suPAR level measured on admission or after recovery of the first AAP does not predict the RAP during the long-term follow-up. However, a high P-suPAR ≥ 3.4 mg/mL measured after recovery from the first AAP is associated with an increased risk of 10-year mortality as an independent factor. This may help to detect the patients with the highest risk after AAP, in order to focus the preventive healthcare actions to the ones in the greatest need, as well as to inform the patients about their prognosis and motivate them for a lifestyle change.

Conflict of interest statement

Anu Aronen, Janne Aittoniemi, Reetta Huttunen, Anssi Nikkola, Jussi Nikkola, Olli Limnell, Isto Nordback, Juhani Sand and Johanna Laukkarinen have no conflicts of interest to declare.

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