



The diagnostic and prognostic significance of soluble urokinase plasminogen activator receptor in Crimean-Congo hemorrhagic fever

Gurdal Yilmaz^{a,*}, Ahmet Mentese^b, Selcuk Kaya^a, Aysegul Uzun^b, S. Caner Karahan^b, Iftihar Koksali^a

^a Department of Infectious Diseases and Clinical Microbiology, Faculty of Medicine, Karadeniz Technical University, KTU Tip Fakültesi, Enfeksiyon Hastahkları, Trabzon, Turkey

^b Department of Biochemistry, Karadeniz Technical University, School of Medicine, Trabzon, Turkey

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ABSTRACT

Background: Crimean-Congo hemorrhagic fever (CCHF) is a potentially fatal disease caused by a tick-borne virus from the Bunyaviridae family. It has recently been reported that soluble urokinase-type plasminogen activator receptor (suPAR), secreted from endothelial cells and the mononuclear phagocyte system, one of the main targets of the CCHF virus, is a potential biomarker for several bacterial and viral infection diseases.

Objectives: This study was intended to determine the diagnostic and prognostic significance of suPAR levels in CCHF.

Study design: This retrospective study was conducted between June 2006 and August 2009 using plasma from patients monitored with a diagnosis of CCHF and from healthy blood donors. Levels of plasma suPAR were determined using an enzyme-linked immunosorbent assay (ELISA) kit according to the manufacturer's instructions.

Results: One hundred CCHF patients were enrolled in the study. The control group was made up of 53 healthy blood donors. suPAR values of 6.2 ± 4.2 were determined in the CCHF patients and of 2.3 ± 0.6 in the control group ($p < 0.0001$). A suPAR level optimum diagnostic cut-off point of 3.06 ng/mL was determined, with an area underneath the ROC (AUROC) curve of 0.94 (95% CI: 0.89–0.97), sensitivity of 87% (95% CI: 79–93%), specificity of 92% (95% CI: 82–98%), PPV of 95% and NPV of 79%. Five of the patients died. suPAR was 18.4 ± 9.1 in the patients that died and 5.6 ± 2.6 in the survivors ($p = 0.034$). In terms of mortality, suPAR level had an optimum diagnostic cut-off point of 10.6 ng/mL, AUROC of 0.97 (95% CI: 0.94–0.99), sensitivity of 100% (95% CI: 48–100%), specificity of 96% (95% CI: 90–99%), PPV of 50% and NPV of 100%.

Conclusions: Plasma suPAR level, a new biomarker, is a test that can be used in the differential diagnosis and monitoring of CCHF in patients admitted to hospital with suspected infection. The test is at the same time important in being a possible predictor of mortality.

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1. Background

Crimean-Congo hemorrhagic fever (CCHF), which is caused by a tick-borne virus belonging to the Bunyaviridae family, may present with a mild clinical course or else exhibit a severe profile with potentially fatal hemorrhaging.^{1,2} Patients mostly present with complaints such as fever, fatigue, generalized pain, myalgia, nausea or vomiting, while severe cases exhibit epistaxis, hematemesis, melena, hematuria, gingival bleeding, vaginal bleeding, petechiae or ecchymosis.^{1–3} The main target of the CCHF virus is

the mononuclear phagocyte system and endothelial cells.¹ Soluble urokinase-type plasminogen activator receptor (suPAR), secreted from these cells (neutrophils, lymphocytes, macrophages, endothelial cells) has recently been reported to be a potential biomarker for several bacterial and viral infection diseases.^{4,5}

2. Objectives

This study was intended to determine the diagnostic and prognostic significance of suPAR levels in CCHF.

3. Study design

This retrospective study was conducted on patients with CCHF hospitalized between June 2006 and August 2009 at the Department of Infectious Diseases and Clinical Microbiology of the

* Corresponding author. Tel.: +90 4623775698; fax: +90 4623775344.

E-mail addresses: gurdalyilmaz53@hotmail.com (G. Yilmaz),

amentese28@gmail.com (A. Mentese), eselkaya@yahoo.com (S. Kaya),

uzunaysegul@hotmail.com (A. Uzun), scaner61@yahoo.com (S.C. Karahan),

iftihar@yahoo.com (I. Koksali).

Karadeniz Technical University Medical Faculty in Turkey. The study was approved by Local Ethical Committee. Patients whose CCHF diagnoses were confirmed through detection of IgM using ELISA and/or the genomic segment of the virus using RT-PCR in the Virology Laboratory of the Refik Saydam National Hygiene Center of the Turkish Ministry of Health were enrolled. The presence of CCHF-specific serum IgM antibodies was determined by in-house ELISA method. The antigens used in ELISA tests were obtained from the Centers for Disease Control and Prevention (CDC, Atlanta, GA, USA). Demographic characteristics, occupation, tick exposure history, incubation time, symptoms, duration between onset of symptoms and admission, clinical findings, and laboratory tests (white blood cell count [WBC], platelet count [PLT], hemoglobin [Hb], aspartate aminotransferase [AST], alanine aminotransferase [ALT], lactate dehydrogenase [LDH], creatine phosphokinase [CK], myoglobin [Mb], blood-urea-nitrogen [BUN], plasma creatinine [Cr], C-reactive protein [CRP], prothrombin time [PT], activated partial thromboplastin time [aPTT] and international normalized ratio [INR]) of all patients were recorded onto forms on admission. A control group made up healthy blood donors was also established.

Blood samples were obtained on admission from the patient and control groups from the antecubital vein in tubes containing K3EDTA and serum separator. Plasma and serum supernatants were obtained after the centrifugation of blood samples at 3000 rpm for 10 min. The remaining serum and plasma samples were kept at -80°C until suPAR analysis. Levels of plasma suPAR were determined using an enzyme-linked immunosorbent assay (ELISA) kit (ViroGates A/S, Denmark, Product No. 203EK1-1) according to the manufacturer's instructions. The absorbance of samples was measured at 450 nm using a VERSA max tunable microplate reader (designed by Molecular Devices, California, USA).

3.1. Statistical analysis

Descriptive statistical analysis was performed for all the studied variables. The data obtained in measurements of normal distribution were analyzed using the Kolmogorov–Smirnov test. Data in conformity with normal distribution were analyzed using Student's *t*-test, and those not conforming to normal distribution using the Mann–Whitney–*U* test. Data obtained by measurements are given as mean \pm standard deviation. Data obtained by counting are given as numbers (%); analyses were performed using the chi-square test. The area beneath the receiver operating characteristics (ROC) curve was used to calculate the discriminative ability of the suPAR to determine patients with CCHF. Sensitivity, specificity, negative predictive values (NPV) and positive predictive values (PPV) were calculated according to ROC curves for suPAR. $p < 0.05$ was regarded as significant.

4. Results

A hundred consecutive patients with CCHF, 55 females and 45 males, with a mean age of 48.8 ± 16.1 , were included in the study. The control group consisted of 53 individuals (25 females and 28 males), with a mean age of 34.1 ± 8.6 . The patients were from various towns in the southern parts of the Black Sea region of Turkey. Eighty-six patients worked in farming or animal husbandry. A history of tick bite was established in 66 patients. Exhaustion (84%), fever (82%), headache (74%), nausea (72%), myalgia (69%), vomiting (34%), bleeding (29%), hepatomegaly (12%) and splenomegaly (8%) were observed in patients. No patients were given ribavirin. suPAR value of 6.2 ± 4.2 was determined in the CCHF patients and 2.3 ± 0.6 in the control group ($p < 0.0001$). While suPAR value was negatively correlated with PLT count, it was positively correlated with WBC, AST, ALT, LDH, Mb, BUN, Cr, CRP, PT, aPTT and INR

Table 1

Correlation analysis between suPAR and other laboratory markers.

Biochemical markers	Mean \pm SD	Correlation analysis (suPAR)
suPAR (ng/mL)	6.2 \pm 4.2	$r = 1$
WBC (μL^{-1})	2700 \pm 2100	$r = 0.547$; $p = 0.000$
Hb (g/dL)	13.8 \pm 1.4	$r = -0.094$; $p = 0.352$
PLT (μL^{-1})	93000 \pm 45000	$r = -0.314$; $p = 0.001$
AST (U/L)	270 \pm 570	$r = 0.313$; $p = 0.002$
ALT (U/L)	133 \pm 170	$r = 0.391$; $p = 0.000$
LDH (U/L)	700 \pm 800	$r = 0.580$; $p = 0.000$
CK (U/L)	680 \pm 890	$r = 0.089$; $p = 0.376$
Mb (g/dL)	130 \pm 193	$r = 0.654$; $p = 0.000$
BUN (mg/dL)	19.5 \pm 15.5	$r = 0.303$; $p = 0.002$
Cr (mg/dL)	0.9 \pm 0.7	$r = 0.206$; $p = 0.040$
CRP (mg/dL)		$r = 0.519$; $p = 0.000$
PT (s)	13.6 \pm 2.1	$r = 0.471$; $p = 0.000$
aPTT (s)	32.9 \pm 6.0	$r = 0.327$; $p = 0.001$
INR	1.1 \pm 0.4	$r = 0.653$; $p = 0.000$

values (Table 1). When the suPAR results were analyzed using the ROC curve method, the optimum diagnostic cut-off point was 3.06 ng/mL, the area underneath the ROC curve (AUROC) was 0.94 (95% CI: 0.89–0.97), sensitivity 87% (95% CI: 79–93%), specificity 92% (95% CI: 82–98%), PPV 95% and NPV 79% (Fig. 1). Five of the patients died. A suPAR value of 18.4 ± 9.1 was determined in the patients that died and of 5.6 ± 2.6 in the survivors ($p = 0.034$) (Fig. 2). In terms of mortality, when the suPAR results were analyzed using the ROC curve method, the optimum diagnostic cut-off point was 10.6 ng/mL, AUROC 0.97 (95% CI: 0.94–0.99), sensitivity 100% (95% CI: 48–100%), specificity 96% (95% CI: 90–99%), PPV 50% and NPV 100%.

5. Discussion

suPAR is a new biomarker, and there have so far been few studies on the subject. Studies have reported that plasma suPAR levels rise in patients with bacterial and viral infection, stating that suPAR emission increases during acute inflammation.^{5–7} Concentrations of suPAR increase in conditions that involve immune activation, and studies have shown that high concentrations of suPAR portend a poor clinical outcome in such diverse infections as tuberculosis, malaria, HIV infection and pneumococcal bacteremia.^{5,6,8} suPAR levels have also been reported to rise in patients with urosepsis and bacterial meningitis.^{9,10} In our study, suPAR was significantly higher in patients with infection compared to those

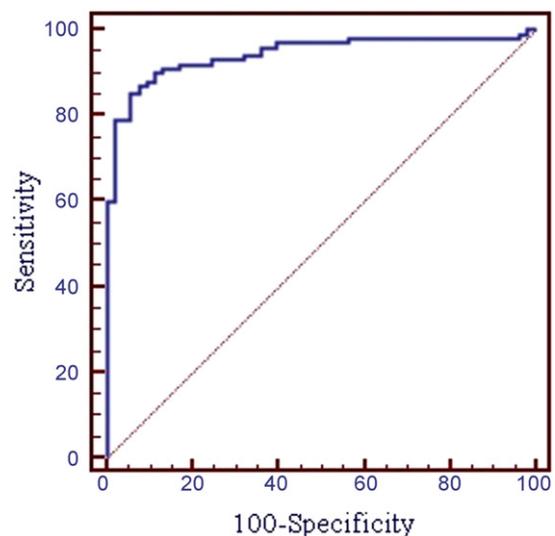


Fig. 1. Receiving operating characteristics of suPAR.

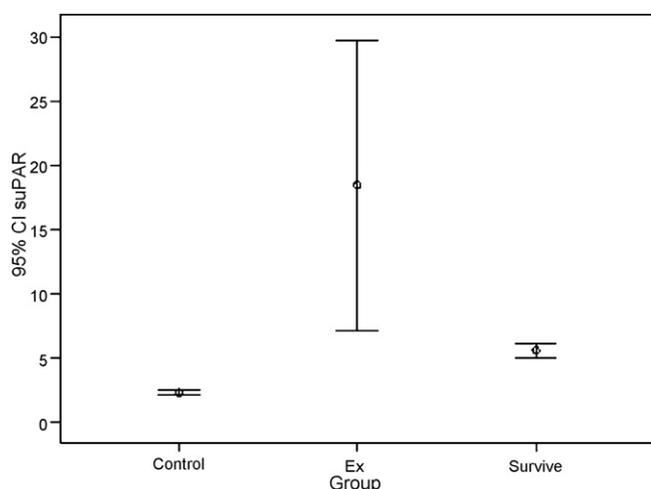


Fig. 2. suPAR values for the control group, surviving and fatal CCHF patients.

without ($p < 0.0001$). A suPAR cutoff value of 3.06 ng/mL was associated with an NPV of 79% and PPV of 95%, with 87% sensitivity and 92% specificity.

In a previous study of ours, low platelet count and Hb; prolonged PT and aPTT, high INR, AST, ALT, AST/ALT, LDH, CK and CRP were determined to be predictors for severity. Similar results have been obtained by Ergonul et al., Cevik et al. and Swanepoel et al. There was a negative correlation between suPAR values and PLT count in our study, and a positive correlation with WBC, AST, ALT, LDH, Mb, BUN, Cr, CRP, PT, aPTT and INR values. This result suggests that suPAR values can be an indicator of severity in CCHF patients. The age difference between the CCHF patients and the control group in our study is noteworthy. This stems from the control group consisting of healthy blood donors. But because of the lack of accompanying disease, apart from hypertension in five CCHF patients and diabetes mellitus in two, this suggests that suPAR values rose because of CCHF rather than due to age and accompanying diseases.

Patients with CCHF may present with a clinical profile spectrum varying from mild to fatal.^{1,11} Those patients should be monitored closely in terms of vital parameters and hemodynamic values; adequate support should be provided, and they should be transferred to the intensive care unit if necessary.¹² Parameters that might serve as a guide to the clinician in the early determination of patients with CCHF that may follow a severe course, will contribute to the planning, implementation and management the planning of intensive care units with support therapy and blood and blood products replacement when necessary.¹² In addition, prior knowledge of these parameters will also assist the clinician in terms of the proper use of available means and manpower.¹² In that context, in our study in which we investigated the prognostic value of plasma suPAR levels, these were notably high in patients with CCHF following a mortal course compared to those who survived ($p = 0.034$). This also shows that suPAR levels are of prognostic importance in CCHF. Elevated suPAR value in patients with CCHF may predict mortality with 100% sensitivity and 96% specificity. In agreement

with our study, other research has also shown elevated suPAR levels to be of prognostic value for patients with tuberculosis, UTI, HIV infection, bacterial meningitis and streptococcal bacteremia.⁸ Although the prognostic value of elevated suPAR was established in our study, this result was studied in only five patients. Large-scale studies determining more accurate prognostic criteria are therefore required.

In conclusion, the measurement of plasma suPAR levels, a new biomarker, is a test that can be used in the differential diagnosis and monitoring of CCHF in patients admitted to hospital with suspected infection. The test is at the same time important in being a possible predictor of mortality.

Conflicts of interest

None of the authors had any financial or personal relationships with other individuals or organizations that might inappropriately influence their work during the submission process.

Ethical approval

The study had been approved by judgement with 2005-33 reference number of Local Ethical Committee.

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