SuPAR, an emerging biomarker in kidney and inflammatory diseases

Lamiaa Hamie,1 Georges Daoud,1 Georges Nemer,2 Tarek Nammour,3 Alissar El Chediak,3 Imad W Uthman,3 Abdul Ghani Kibbi,4 Assaad Eid,1 Mazen Kurban2,4

ABSTRACT
Soluble urokinase plasminogen activator receptor (suPAR) is a circulating form of a physiological and pathophysiological important cell surface receptor implicated in inflammation. Recent studies showed that suPAR is a promising biomarker, useful for diagnosis, assessment and prognosis of several diseases. This review summarises the majority of preliminary studies and analyses the significance and the clinical application of suPAR in various clinical conditions. SuPAR seems to have a significant value in the diagnosis as well as prognosis of many diseases; nonetheless, it merits large-scale studies to set cut-off values that help physicians in following up their patients and accordingly tailor their treatment plans.

INTRODUCTION
Biomarkers have shown great importance in the diagnosis, prognosis, treatment choices, response to treatment as well as follow-up markers for several medical conditions.1 Biomarkers are grossly divided into two categories: biomarkers of exposure and biomarkers of disease. Biomarkers of exposure are used to predict patients at risk of developing specific diseases, whereas biomarkers of disease help in ‘screening, diagnosis and monitoring of disease progression’.2 In the era of personalised medicine, different biomarkers hold promising value for serving as disease surrogate endpoints. Recently, soluble urokinase plasminogen activator receptor (suPAR) has been closely studied to determine its accuracy and reliability as a novel biomarker in a large number of diseases.1 2 In this work, we will be reviewing and analysing the significance of suPAR as a novel biomarker in various clinical entities which are summarised in tables 1 and 2.

PLASMIN-PLASMINOGEN SYSTEM
The plasmin-plasminogen system controls the degradation of fibrin-containing clots; nonetheless, over the past 40 years, it has been found to facilitate different pathways based on the type of cell it is bound to. Its protease activity mediates several cellular pathways including cell migration, activation, adhesion, angiogenesis and wound healing. The system depends on the conversion of plasminogen to plasmin (a serine protease) through either tissue-type plasminogen activator or urokinase type plasminogen activator (uPA) (figure 1).3 SuPAR is the soluble form of the urokinase-type plasminogen activator receptor (uPAR); a glycosyl-phosphatidylinositol linked membrane protein. In its membrane-bound form, suPAR is present on various cells including monocytes, activated T-lymphocytes, endothelial cells, keratinocytes, macrophages, smooth muscle cells, fibroblasts and megakaryocytes, whereas in its soluble form, it is found in blood, serum, urine and cerebrospinal fluid.4 SuPAR is one of the few mammalian members of the three-finger-like proteins. On crystallography, suPAR was found to have three domains, each forming finger folds arranged in a manner that creates an internal cavity and a large surface area. SuPAR’s three-dimensional structure explains its ability to interact with extracellular matrix proteins such as vitronectin, integrins and other receptors. These interactions mediate the influence of suPAR on proteolysis, cell migration and adhesion.5 SuPAR was first described in the 1990s as a marker of cancer progression as well as a marker of several infectious diseases. Since then, suPAR has been closely studied for its clinical, diagnostic, prognostic and surveillance relevance.6 7

NEPHROLOGY
In the field of nephrology, prognostic markers of renal diseases are limited, yet several markers linked to podocyte injury may be of great significance in predicting the disease course. SuPAR, a marker of podocyte injury, has been implicated in the pathogenesis of various kidney diseases. It activates αvβ3 integrins on podocytes, stimulating small GTPase Rac-1 proteins8 which in turn affects podocyte foot process motility and effacement. Hence, suPAR affects the αvβ3 integrin pathway, resulting in the inability of podocytes to adapt to physiological events.9 This highlights a proposed mechanism behind which suPAR is involved in the development of different pathologies including chronic kidney diseases (CKD), focal segmental glomerulosclerosis (FSGS) and diabetic nephropathy (DN).10 Two major studies with several thousands of individuals showed that plasma suPAR is associated with new onset kidney disease.10 Higher suPAR level at baseline was associated with a greater decline in the estimated glomerular filtration rate (eGFR) during subsequent visits and was associated with incident CKD.10 These studies also suggested that suPAR was an adequate predictive biomarker for CKD even in healthy individuals, as an increase in suPAR levels was associated with CKD even before eGFR decline.
The relationship between suPAR and other kidney diseases has been the centre of several studies. The level of suPAR was compared in glomerular diseases. A study examined whether suPAR levels were useful in predicting kidney diseases secondary to FSGS, minimal change disease or idiopathic membra-
nephropathy. The conclusion was that the elevated suPAR levels alone did not distinguish between the three disease enti-
ties. Another study from Taiwan explored the expression of suPAR and found that it was most elevated in minimal change
disease and lowest in chronic interstitial nephritis; however, it was elevated in all examined kidney diseases.

Focal segmental glomerulosclerosis (FSGS)

The effacement of the podocyte foot processes is a landmark characteristic of FSGS. As such, and based on the mechanism of podocyte injury caused by activation of the αvβ3 integrins pathway, it was hypothesised that suPAR might be a circulating
Table 2 SuPAR cut-off values for clinical studies

<table>
<thead>
<tr>
<th>Disease entity</th>
<th>SuPAR cut-off (ng/mL)</th>
<th>Specificity (%)</th>
<th>Sensitivity (%)</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Severe AP vs mild/moderate AP</td>
<td>4.75</td>
<td>93</td>
<td>93</td>
<td>35</td>
</tr>
<tr>
<td>Predict 28-day mortality in patients with ALF</td>
<td>14.4</td>
<td>71</td>
<td>71</td>
<td>39</td>
</tr>
<tr>
<td>Screen for tuberculosis</td>
<td>≥5</td>
<td>62</td>
<td>82</td>
<td>32</td>
</tr>
<tr>
<td>Screen for HCC</td>
<td>9.56</td>
<td>90</td>
<td>76</td>
<td>70</td>
</tr>
</tbody>
</table>

ALF, acute liver failure; AP, acute pancreatitis; HCC, hepatocellular carcinoma; suPAR, soluble urokinase plasminogen activator receptor.

factor in FSGS. We learnt that a lower eGFR at baseline, such as that found in FSGS, was independently associated with elevated suPAR levels. This is not surprising since other molecules such as fibroblast growth factor 23 having a similar molecular size to suPAR are correlated with a reduced renal clearance.

Moreover, circulating factors have been implicated in the aetiology of FSGS based on significant observations. FSGS has been found to recur after renal transplantation in about 30% of patients. Second, plasma infusion from patients with FSGS can induce proteinuria in rats. In addition, a case of transient proteinuria in a newborn was suggested to occur by the transmission of a permeability factor from a mother suffering from FSGS.

Henceforth, circulating suPAR levels were found to be elevated in two large cohorts of patients with FSGS. Consequently, lowering serum suPAR concentrations, through plasmapheresis, resulted in less podocyte β3-integrin activation and stabilisation of recurrent FSGS disease. Moreover, elevated pretransplant suPAR levels indicated an increased risk for recurrence of FSGS after transplantation. Hence, suPAR is an important circulating factor in FSGS.

IgA nephropathy

High suPAR levels were associated with decreased eGFR as well as podocyte damage in patients with IgA nephropathy. There was also a positive correlation between suPAR levels and IgA severity reflected by the degree of effacement of podocytes, thus possibly explaining the development of proteinuria in patients with IgA nephropathy. Another study found that plasma suPAR levels were significantly associated with increased crescent formation in patients with IgA nephropathy. This suggests that suPAR can be a potential indicator for FSGS lesions in patients with IgA nephropathy.

Other glomerular diseases

Serum suPAR levels were associated with proteinuria in primary non-FSGS glomerulonephritis and secondary autoimmune related glomerulonephritis. Thus serum suPAR may represent a key biomarker in diseases of glomerulonephritis. Interestingly, in a large cohort of patients with lupus nephritis, it was shown that suPAR levels were elevated in patients with systemic lupus erythematosus (SLE) with renal involvement as compared with...
patients with SLE without renal involvement. Significantly elevated suPAR levels were also found in patients with active lupus nephritis. Nonetheless, using suPAR as a biomarker in patients with lupus nephritis merits additional confirmatory data.

CARDIOVASCULAR SYSTEM
Coronary artery disease (CAD) is a very common disease worldwide. It has established guidelines that focuses on screening scores and imaging techniques, all aiming at early detection and disease control. Although CAD has been associated with significant morbidity and mortality, no ideal screening marker is available for targeted therapy and/or prevention.

SuPAR has been hypothesised to play an important role in the pathogenesis of CAD, since it plays a major role in inflammation. Interestingly, it was found that low levels of suPAR (3.1±1.6 ng/mL) were associated with normal coronary artery angiography as compared with patients with significant coronary atherosclerosis who had suPAR levels of 3.5±1.9 ng/mL. Additionally, the 1-year risk of death and myocardial infarction (MI) were found to increase from 2.8% to 8.3% when patients had a suPAR level of ≥3.5 ng/mL. Likewise, elevated suPAR levels were associated with higher risk of ischaemic stroke, especially when combined with carotid plaques on ultrasound examination. High suPAR levels were associated with worse prognosis in patients presenting with chest pain suspected to have non-ST-elevation acute coronary syndrome. These patients were more likely to be readmitted with heart failure symptoms as well as fatal or non-fatal MI. In line with the association of suPAR with the inflammatory state in atherosclerosis, its levels correlated with the presence of peripheral arterial disease (PAD). Patients with PAD were found to have higher suPAR levels when compared with patients with only CAD. In addition, suPAR levels were highest when patients were afflicted with both PAD and CAD. Conforming to the prognostic value of suPAR, elevated suPAR levels were also associated with higher mortality rates.

SEPSIS AND INFECTIOUS DISEASES
Different scoring systems and biomarkers have been developed to assist physicians in triaging patients with systemic inflammatory response syndrome. Physicians mainly rely on their intuition and experience, yet as important as they are, they are prone to fail when standardised or attempted to be replicated. Hence, elaborate predictive models have been established such as Sequential Organ Failure Assessment Score (SOFA), Simplified Acute Physiology Score (SAPS) II and Acute Physiology and Chronic Health Evaluation II (APACHE II). These scoring systems rely on physiological markers measured within 24–48 hours after admission. Such systems are important in assessing patient morbidity and mortality. Since scoring systems are time-consuming and impractical in the emergency department setting, suPAR was compared with SAPS and SOFA. suPAR predicted the 30-day and 180-day mortality especially when combined with age and other predictive markers. Specifically, when suPAR levels were >9 ng/mL, the rate of mortality increased by 13-fold and 3-fold in patients younger and older than 70 years of age, respectively.

Due to the high burden of disease, several severity scoring systems have been implemented, many of which are time consuming in the acute setting and depend on several markers. For this reason, suPAR has been compared with previously known prognostic tools such as BISAP (Basic Index of Severity in Acute Pancreatitis), suPAR showed better sensitivity and specificity compared with the BISAP. In addition, suPAR was superior to the BISAP in predicting mortality rates. This superiority can be attributed to suPAR’s physiological characteristics. suPAR is a marker of inflammation and is also elevated in states of ischaemia and hypoxia, which are clinically associated with worse prognosis.

GASTROENTEROLOGY
suPAR has also been investigated in hepato-pancreato-biliary conditions. Acute pancreatitis (AP) is an inflammatory process with a wide spectrum of disease severities. AP is one of the most common gastrointestinal diseases requiring hospitalisation. Severe pancreatitis can develop in 15%–20% of patients with AP in association with serious complications including multiple organ failure and death. Thus, early identification of pancreatitis and the ability to determine its severity are essential for early medical intervention.

As in sepsis, multiple factors have been previously studied for their value to predict the outcomes of AP. These include CRP, procalcitonin, interleukins and others. However, such markers were non-specific and can lag behind the onset of inflammation. suPAR has been studied as a marker of AP severity. A suPAR cut-off value of 4.75 ng/mL was confidently found to differentiate between severe AP and mild/moderate AP with an equal sensitivity and specificity of 93%.

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Serum suPAR levels were also studied in acute liver failure (ALF) and were shown to be elevated, irrespective of the cause (toxin-induced, viral hepatitis or autoimmune). SuPAR levels correlated with markers of liver injury (serum aspartate and alanine aminotransferase activities) as well as with markers of liver function depicted by an increase in international normalised
ratio. In a subgroup analysis, a tendency for suPAR elevation in patients with worse prognosis was found. However, such differences did not reach statistical significance. SuPAR as a single biomarker correlated closely with the model of end stage liver disease (MELD), an important prognostic score for ALF.46

SuPAR can also play a role beyond acute states of inflammation such as seen in liver cirrhosis. Liver fibrosis is a scarring process resulting from the liver’s reaction to a variety of injurious stimuli that would eventually lead to a chronic inflammatory response. Percutaneous liver biopsy remains the ‘gold standard’ to classify the stages of liver fibrosis. Attempts were made to discover novel reliable non-invasive biomarkers of liver fibrosis.47 Interestingly, high suPAR levels were correlated with fibrosis severity in two groups of patients with chronic liver disease: non-alcoholic fatty liver disease and chronic hepatitis C virus.48 Similar findings were more recently replicated in chronic hepatitis B.49

 Decompensated liver cirrhosis is the deterioration of liver function in a cirrhotic patient with a 1-year mortality rate reaching 50%.50 Spontaneous bacterial peritonitis (SBP), bacteraemia and pneumonia can occur in one-third of decompensated patients and contribute to the high mortality through sepsis and multi-organ failure. SuPAR's predictive ability of short-term mortality was assessed in patients with decompensated liver cirrhosis and was found to be significantly elevated in decompensated patients compared with compensated controls, correlating with MELD and Child-Pugh scores. Patients who died at day 28 were found to have higher suPAR level on presentation as compared with survivors and to patients who underwent liver transplantation. A serum suPAR concentration of 14.4 ng/mL can serve as cut-off for 28 day mortality prediction with a sensitivity and specificity of 71%, identical to that of MELD score and superior to CRP.51

Elevated suPAR levels were also found to predict survival even at 90 days of follow-up. Another pertinent finding was the ascitic fluid suPAR levels which were indicative of SBP, independent of blood suPAR levels, suggesting a role of suPAR in the detection of local infections.52

Additionally, suPAR was also evaluated in inflammatory bowel disease.40 Yet its value remains to be determined.

PULMONARY

Although asthma and chronic obstructive pulmonary disease (COPD) are common worldwide healthcare diseases, there are no reliable inflammatory biomarkers to evaluate disease severity and to predict patients prone to recurrent exacerbations.41 COPD is characterised by airway inflammation and tissue remodelling resulting in decreased efficiency of the respiratory system to achieve gas exchange. On the basis that sputum suPAR levels were elevated in patients with COPD, serum suPAR’s ability to correlate with the COPD severity was tested. It was found that the plasmin-plasminogen system is implicated in local inflammation but the disease status was not reflected by the serum suPAR in this study.41

On the other hand, a study performed on patients with acute COPD exacerbation, where serum suPAR levels were measured on admission and after 7 days showed that suPAR levels correlated with the acute exacerbation. SuPAR levels subsequently decreased with management after 7 days but were still higher than healthy individuals. Interestingly, suPAR was a superior marker when compared with fibrinogen and CRP in acute COPD exacerbation.42

Interestingly, when suPAR’s diagnostic ability was tested in pregnant patients with asthma, it was found that the serum levels were lower in pregnant patients with asthma as compared with non-pregnant asthmatics. These findings were explained by the immune tolerant state of pregnancy.43

Finally, with respect to therapeutic targeting of suPAR in pulmonary diseases, a study by Schuliga et al proposed uPA inhibition in patients with idiopathic pulmonary fibrosis (IPF). These patients suffer from a declining lung function that can lead to death within 5 years of diagnosis. Interestingly, uPAR was found to promote IL-6 production by fibroblasts, a key player in IPF pathogenesis. uPA inhibition significantly inhibited IL-6 production; nonetheless, these observations are yet to be validated.44

DERMATOLOGY AND RHEUMATOLOGY

The serine protease activity of plasmin has been found to be involved in inflammatory reactions, tissue remodelling and degradation of matrix proteins in several immune-mediated dermatological and rheumatological diseases.45 Such conditions are characterised by multifactorial pathways leading to systemic inflammation with end-organ damage. In addition, patients are often at a higher baseline inflammatory states, putting them at risk of developing inflammation-associated comorbidities such as cardiovascular diseases and diabetes. Hence, inflammation control is vital to prevent these complications.46 Currently, there is an ongoing search for an ideal biomarker that could reflect the inflammatory states of such conditions.

Psoriasis is a common immune-mediated dermatological condition that affects about 3% of the population in the USA and carries a significant burden on affected patients. SuPAR was highly expressed in the dermis of patients with psoriasis, yet its serum levels failed to correlate with disease severity. Nonethe- less, suPAR’s value needs to be better studied in patients with various stages of psoriasis to confirm these findings.47 SLE is another dermatological autoinflammatory disease, characterised by systemic inflammation that involves nearly all the vital organs. Although erythrocyte sedimentation rate (ESR) is nowadays used as an SLE-monitoring biomarker, it is a non-specific marker elevated in a number of different conditions.48 In order to determine whether suPAR could be used as a biomarker in SLE, 89 patients with SLE were categorised according to their Systemic Lupus Erythematosus Activity Index (SLEDAI) of 0, 1–8 and more than 8 into mild, moderate and severe, respectively.49 It was found that levels of SuPAR, in contrary to CRP and ESR, were different between different SLEDAI scores. SuPAR levels were higher in patients with higher disease activity, hence, this might imply its future use in disease monitoring.48 Furthermore, it has been found that suPAR can be used as follow-up marker for irreversible organ damage in patients with SLE, such as the renal, neuropsychiatric and ocular systems.48

Similar studies were done on patients with rheumatoid arthritis (RA), systemic sclerosis (Ssc) and ankylosing spondylitis. suPAR was found to correlate well with the clinical symptoms given by patients even when a unified RA scoring system, DAS28, reflected disease remission. suPAR levels also positively correlated with complications resulting from the fibrotic activity in Ssc. This was mirrored by decreased diffusing lung capacity for carbon monoxide and forced vital capacity, the presence of pulmonary artery hypertension and microvascular changes in 83 patients with Ssc.47

HAEMATOLOGY AND ONCOLOGY

SuPAR levels were also investigated in various liquid and solid tumours, due to its role in cell migration and angiogenesis. A study analysed the suPAR levels of 30 patients with acute
myeloid leukaemia (AML) and found that the mortality risk approximated doubled once the suPAR level was higher than 6.71 ng/mL. It was also noticed that patients with low suPAR were more likely to have complete remission as compared with patients with higher levels.49 Similar prognostic uses have been emphasised in multiple myeloma, 50 lymphomas, 51 ovarian, breast, stomach, prostate, endometrial, colon, non-small cell lung and hepatocellular cancers.49 52

In addition, high suPAR levels were associated with inadequate response of patients with leukaemia following more chemotherapy cycles, highlighting the importance of suPAR in leukaemia characterisation and prediction of chemosensitivity.53 Finally, a 7-year prospective cohort followed patients with pre-existing liver disorders and determined that suPAR is superior to alpha-fetoprotein (AFP) in monitoring the frequency and time to develop hepatocellular carcinoma (HCC).34

DIABETES
Diabetes mellitus type 2 (DM2) is another worldwide prevalent disease contributing to high morbidity and mortality rates and helpful biomarkers can aid in the early detection of patients at risk. A 13.8-year follow-up Danish study on 2353 individuals, showed that individuals with higher suPAR levels were at a greater risk of developing DM type 2.55 Overall, by following up on seemingly healthy individuals for a significantly long duration, this study has shown that suPAR can be used to predict patients who are at risk of developing DM2, well ahead of developing insulin resistance. Furthermore, it has been suggested that suPAR may play a role in the early immune and inflammatory reactions that eventually lead to beta cell defects, insulin resistance and DM2.55

One of the prominent examples of diabetic end-organ damage is DN. DN is a major health problem and a common cause of end-stage renal disease. In common practice, urinary microalbuminuria is used to screen for impending DN. Interestingly, suPAR correlated with the development of microalbuminuria in patients at a higher risk for type 2 diabetes, even after adjustment of baseline eGFR.56 Moreover, serum suPAR levels increased with the severity of DN.12

SuPAR plays a major role in podocyte function and its production appears to be inhibited by the blockade of the renin angiotensin system (RAS).57 38 The RAS is usually targeted in the prevention or delay of DN. After the administration of a RAS inhibitor, irbesartan 300 mg daily, urine suPAR levels were reduced significantly, although the serum levels were not affected.57 Thus, altogether, suPAR can represent a biomarker for disease screening, progression or improvement.57

CONCLUSION
SuPAR is emerging as a novel major player in inflammation as we have reviewed some of its physiological and pathophysiological contributions to the inflammatory processes (figure 2, tables 1 and 2)
Main messages

► Biomarkers are important for screening, establishing diagnosis and prognosis.
► Soluble urokinase plasminogen activator receptor (suPAR) seems to be a promising biomarker in different disease entities.
► SuPAR seems to be more reliable than ESR and C reactive protein in inflammatory conditions.

Current research questions

► Should suPAR be used to screen for malignancies?
► Should suPAR be used to assess the clinical condition of patients with sepsis presenting to the emergency department?
► Should suPAR be used to screen for an insulin-resistant state?
► Should suPAR be used to monitor the response of various inflammatory conditions to treatment?

Key references


Self assessment questions

1. ESR, CRP and suPAR values are all affected by food intake.
2. SuPAR can be used to predict postmyocardial infarction mortality.
3. SuPAR is currently being used to triage patients in the emergency departments of some centres.
4. SuPAR can predict the course of kidney transplant in patients affected with focal segmental glomerulosclerosis.
5. SuPAR can predict the future development of Diabetes type II in previously healthy patients.

and 2). Recent data in multiple inflammatory based diseases have shown that suPAR, in the correct settings, may serve as both a diagnostic and prognostic marker. Thus, suPAR holds significant potential to be used as a biomarker in different fields of medicine.

Interestingly, SuPAR, when compared with ESR and CRP showed a better circadian stability; suPAR remained stable when measured in short increments of time. Furthermore, suPAR was neither affected by food intake nor by fasting state of patients. All in all, suPAR has shown its superiority over CRP and ESR in multiple conditions. Nonetheless, when combined with these makers, more insight can be provided for the diagnosis as well as the prognosis of different diseases.

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REFERENCES


**Answers**

1. **False**
2. **True**
3. **True**
4. **True**
5. **True**