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Plasma soluble urokinase-type plasminogen activator receptor level is independently associated with coronary microvascular function in patients with non-obstructive coronary artery disease

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ABSTRACT

Background: Soluble urokinase-type plasminogen activator receptor (suPAR) is a novel biomarker released from leukocytes and endothelial cells that has been associated with atherosclerotic cardiovascular disease. We hypothesized that plasma suPAR level is an independent predictor of coronary microvascular function. **Methods**: Coronary blood flow velocity and plasma suPAR levels were evaluated in patients with non-obstructive coronary artery disease. Coronary flow reserve (CFR) was calculated as the ratio of hyperemic to basal average peak blood flow velocity and coronary microvascular dysfunction was defined as CFR $\leq$ 2.0 in the setting of a fractional flow reserve value of $\geq$0.75. Plasma suPAR levels were measured using ELISA technique. The association between suPAR and CFR was investigated using univariate and multivariate regression analyses. **Results**: In 66 patients, 47% were men, 26% had diabetes, 68% had hypertension and 76% had dyslipidemia. Mean age was 55 ± 12 years and median suPAR level 2.82 (2.08–3.40) ng/mL. Plasma suPAR levels correlated with age ($r = 0.31$, $p = 0.01$), body mass index ($r = 0.25$, $p = 0.04$) and high-sensitivity C-reactive protein (hs-CRP) ($r = 0.33$, $p = 0.009$). While median suPAR level was not significantly different in patients with different cardiovascular risk factors, patients on statin therapy had significantly higher suPAR level ($p = 0.03$). SuPAR correlated negatively with CFR and, after multivariate adjustment for established cardiovascular risk factors, medications profiles and hs-CRP, suPAR remained an independent predictor of CFR ($B = -0.30$, $p = 0.04$), indicating an independent association between suPAR level and coronary microvascular function. **Conclusions**: In this cross-sectional study, plasma suPAR level was an independent predictor of coronary microvascular function. Larger prospective clinical trials are warranted to investigate the prognostic value of this novel biomarker and the role of immune dysregulation in coronary microvascular disease.

1. Introduction

Cardiovascular risk factors and inflammation play pivotal roles in the pathogenesis of both epicardial and microvascular coronary artery disease (CAD) [1,2]. While clinical research in coronary atherosclerosis has focused on the epicardial disease, microvascular disease is an under-recognized entity and has been associated with increased oxidative stress, inflammation and adverse clinical outcomes [3–7].

Soluble urokinase-type plasminogen activator receptor (suPAR)

Abbreviations: CAD, coronary artery disease; CFR, coronary flow reserve; FFR, fractional flow reserve; hs-CRP, high sensitivity C-reactive protein; suPAR, soluble urokinase-type plasminogen activator receptor; uPA, urokinase-type plasminogen activator, also known as urokinase; uPAR, urokinase-type plasminogen activator receptor.

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is a novel proinflammatory biomarker and chemotactic agent [8] that is released by cleavage of the membrane-bound urokinase-type plasminogen activator receptor (uPAR). SuPAR is ubiquitous in body fluids including the plasma, urine and cerebrospinal fluid, is involved in the inflammatory processes and promotes the body's immune response. Up-regulation of uPAR is manifested by elevated blood suPAR levels and has been observed in rheumatologic conditions, malignancies, infections, sepsis and cardiovascular diseases [9–19].

Unlike c-reactive protein (CRP), suPAR is highly stable and is not subject to circadian variation [8]. Although suPAR modestly correlates with CRP, it has been shown to predict cardiovascular outcomes independent of CRP [18,20–23] and the Framingham Risk Score [24]. While CRP may reflect metabolic changes of the various pathways involving adhesion molecule expression, nitric oxide induction, complement function alteration or fibrinolysis [25], suPAR is thought to be involved in other aspects of the atherosclerotic process such as endothelial dysfunction, macrophage-mediated inflammation and immune dysregulation [26].

The association between coronary microvascular dysfunction and inflammatory markers has been previously reported [3,27,28]. Elevated suPAR signifies activation of the inflammatory and immune pathways and its association with coronary microvascular disease is unknown. Coronary microvascular function can be evaluated in the cardiac catheterization laboratory using coronary flow reserve (CFR), which is defined as the ratio of hyperemic to basal average peak velocity. A normal value is around 4–5 and a value of $\leq$2.0 is indicative of significant microvascular dysfunction. We aimed to investigate the relationship between suPAR and coronary microvascular function with the hypothesis that, after adjusting for cardiovascular risk factors and hs-CRP, plasma suPAR is an independent predictor of coronary microvascular function.

2. Methodology

2.1. Study population

For this cross-sectional study, between August 2007 and February 2012 we recruited 66 patients with either an abnormal non-invasive stress test, stable angina or stabilized acute coronary syndrome (unstable angina or non-ST segment elevation myocardial infarction) who presented to our institution for clinically indicated cardiac catheterization and had non-obstructive CAD by coronary angiography and physiologic assessment (fractional flow reserve (FFR) $\geq$0.75). Patients with previous ST segment elevation myocardial infarction, hemodynamic instability, history of coronary artery bypass grafting, or severe valvular heart disease were excluded.

All study patients provided informed written consent prior to enrollment and the study was approved by the Institutional Review Board of Emory University. Clinical and demographic data including age, sex, race, diabetes mellitus (hemoglobin A1c > 6.5, fasting blood glucose $\geq$ 126 mg/dL or treatment with oral anti-hyperglycemic medications or insulin), hypertension (systolic blood pressure >140 mmHg, diastolic blood pressure >90 mmHg or treatment with antihypertensive medications), dyslipidemia (low-density lipoprotein >130 mg/dL, total cholesterol $\geq$200 mg/dL, high-density lipoprotein <40 mg/dL or treatment with cholesterol-lowering medications), smoking, body-mass index (BMI, defined as weight in kilograms/height in meters squared), obesity (BMI $\geq$ 30), fasting lipid profiles and high-sensitivity C-reactive protein (hs-CRP) levels were recorded.

2.2. Coronary angiography and assessment of coronary physiology

A 6F coronary guide catheter was used to perform coronary angiography. Following intravenous administration of heparin, a 0.014-in. combined Doppler flow and pressure wire (ComboWire® XT Guide Wire, Volcano Corporation, San Diego, CA) was advanced through the guide catheter to continuously record Doppler flow velocity and pressure signals. Adenosine was infused intravenously at a rate of 140 mcg/kg/min for 3 min to induce maximal hyperemia. Reproducibility of velocity measurements from our laboratory has been previously reported, where in 60 measurements we found a concordance correlation coefficient of 0.979 (95% CI 0.966–0.988) [29].

Fractional flow reserve (FFR) was calculated as a ratio of distal to proximal (aortic) pressure and an FFR value of $\geq$0.75 in the study vessel was considered non-flow limiting [30]. Coronary flow velocity reserve was calculated as a ratio of hyperemic to basal average peak blood flow velocity. Coronary microvascular dysfunction was defined as CFR $\leq$ 2.0 in the setting of non-flow limiting epicardial lesions (FFR $\geq$ 0.75) [30]. Fig. 1 illustrates the acquisition of coronary pressure and flow measurements using the ComboMap® Pressure and Flow System (Volcano Corporation, San Diego, CA).

2.3. Measurement of suPAR and hs-CRP

At the time of cardiac catheterization, blood samples were collected in ethylenediaminetetraacetic acid-anticoagulated tubes from the femoral arterial sheath and were centrifuged at 3000 rpm for 10 min. Plasma was extracted and stored at −80 °C. Plasma suPAR levels were measured using commercially available ELISA assays (suPARnostic® assay, ViroGates, Copenhagen, Denmark), which has intra-assay coefficients of variations of 2.3–6.0% over suPAR sample levels of 2.3–7.2 ng/mL. Blood was also collected for hs-CRP measurement at the time of angiography and was measured using the Dade-Behring Nephelometry system (Deerfield, IL) [44]. Patient samples were run with a set of controls with known hs-CRP concentrations and the deviation between the expected value and obtained calculated value was 4.04%.

2.4. Statistical analyses

Continuous variables are described as mean ± standard deviation (SD) and categorical variables as proportions. Student's t-test and chi-square test were used for continuous and categorical variables, respectively. Non-normally distributed variables were compared using Mann–Whitney test and were log transformed as required. Both suPAR and CFR were not normally distributed and therefore were log transformed for univariate analysis using Pearson Correlation. The association between suPAR and log CFR was investigated using multivariate linear regression analyses after adjustment for traditional cardiovascular risk factors, including age, sex, race, diabetes mellitus, hypertension, dyslipidemia and smoking. A two-sided p-value of <0.05 was considered statistically significant. The statistical analyses were performed using SPSS 21 (SPSS Inc., Chicago, IL) and SAS 9.3 (SAS Institute, Cary, NC).

3. Results

3.1. Patient characteristics

In 66 patients with non-flow limiting coronary lesions (FFR $\geq$0.75), plasma suPAR assessment and CFR measurement were studied. Demographic and clinical characteristics of the patients are presented in Table 1. The majority of patients (74%) had stable

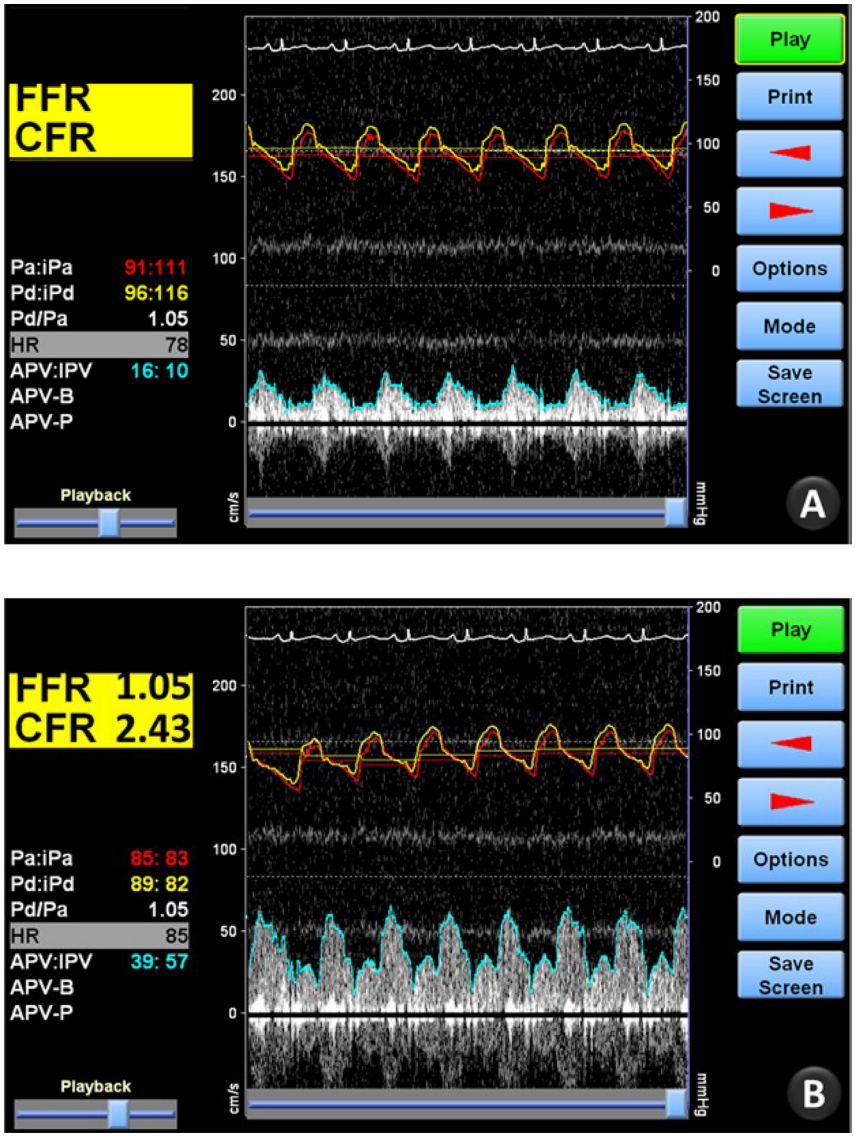

Fig. 1. Illustration of the acquisition of coronary physiologic measurements (FFR and CFR) at baseline (panel A) and following hyperemia with IV adenosine (panel B).

angina and the remaining patients presented with non-ST segment elevation myocardial infarction. When patients were classified based on CFR (CFR $\leq$ 2.0 or >2.0), patients with abnormal CFR (CFR $\leq$ 2.0) had greater $P2Y_{12}$-inhibitor ($p = 0.047$), beta blocker ($p = 0.03$) and statin ($p = 0.003$) usage, and lower cholesterol levels compared with the normal CFR group.

3.2. Relationship between cardiovascular risk factors, medication profile and biomarkers

In univariate analysis, there was a modest positive correlation between log suPAR and log hs-CRP ($r = 0.33$, $p = 0.009$), age ($r = 0.31$, $p = 0.01$) and body mass index ($r = 0.25$, $p = 0.04$). Dichotomous comparison of median suPAR level was not significantly different for the following variables: age (cutoff 55), race (white vs. black), sex, hypertension, diabetes mellitus, dyslipidemia, obesity and smoking status. While median suPAR level was significantly higher in patients on statin therapy [3.1 (2.3–3.8) vs. 2.4 (2.0–3.0), $p = 0.03$], we did not observe any significant difference in patients with beta blocker, aspirin, $P2Y_{12}$ inhibitor, angiotensin converting enzyme inhibitor (ACEI), calcium channel blocker or nitrates usage. Median CRP levels were significantly higher in patients with hypertension [2.38 (1.35–4.92) vs. 0.90 (0.40–4.10), $p = 0.03$], obesity [3.80 (1.43–8.00) vs. 1.45 (0.70–2.50), $p = 0.008$] and ACEI usage [3.4 (1.90–6.00) vs. 1.40 (0.50–3.88), $p = 0.003$].

3.3. Relationship between cardiovascular risk factors, medication profile and CFR

There was no significant correlation between log CFR and age or BMI. Median CFR value was significantly lower in diabetics [1.83 (1.47–2.33) vs. 2.31 (1.89–2.69), $p = 0.03$], as well as in patients taking statins [1.9 (1.60–2.45) vs. 2.37 (1.98–2.88), $p = 0.009$], beta blockers [1.91 (1.73–2.42) vs. 2.34 (1.93–2.91), $p = 0.03$] and $P2Y_{12}$ inhibitors [1.90 (1.39–2.33) vs. 2.30 (1.79–2.70), $p = 0.045$].

3.4. Relationship between biomarkers and CFR

In univariate analysis, log suPAR level correlated significantly

Table 1
Demographic and clinical characteristics for study participants based on CFR responses.

Characteristics	n (%)	CFR ≤ 2.0 (n = 30)	CFR >2.0 (n = 36)	p-value
Demographics				
Age (yrs)[a]	55 ± 12	55 ± 13	54 ± 11	0.80
Female sex (n, %)	35 (53)	17 (57)	18 (50)	0.59
Race				
Caucasian	42 (64)	16 (53)	26 (72)	0.27
African American	21 (32)	12 (40)	9 (25)	
Other	3 (4)	2 (7)	1 (3)	
Cardiovascular Risk Factors				
BMI (kg/m^2)[a]	31 ± 7	32 ± 7	30 ± 7	0.13
Diabetes	17 (26)	11 (37)	6 (17)	0.06
Dyslipidemia	50 (76)	24 (80)	26 (72)	0.46
Hypertension	45 (68)	23 (77)	22 (61)	0.18
Smoking	24 (36)	12 (40)	12 (33)	0.58
Medications				
Aspirin	44 (67)	22 (73)	22 (61)	0.29
$P2Y_{12}$-Inhibitor	11 (17)	8 (27)	3 (8)	0.047
Beta blocker	25 (38)	14 (47)	11 (31)	0.03
ACEi/ARB	28 (42)	16 (53)	12 (33)	0.10
Calcium channel blocker	18 (27)	11 (37)	7 (19)	0.12
Nitrate	16 (24)	4 (13)	12 (33)	0.06
Statin	33 (50)	21 (70)	12 (33)	0.003
Lipids (mg/dl)[a]				
Total Cholesterol	163 ± 35	151 ± 36	172 ± 32	0.02
LDL	96 ± 34	85 ± 35	105 ± 30	0.02
HDL	43 ± 12	41 ± 9	46 ± 16	0.09
Triglyceride	118 ± 79	131 ± 93	107 ± 61	0.23
Biomarkers[a]				
Hs-CRP (mg/L)	2.17 (0.90–4.57)	2.13 (1.00–4.05)	2.17 (0.70–5.50)	0.77
SuPAR (ng/dL)	2.82 (2.08–3.40)	3.04 (2.33–3.70)	2.53 (2.00–3.25)	0.12

Abbreviations: ACEI: angiotensin-converting enzyme inhibitor; ARB: angiotensin receptor blocker; BMI: body mass index; CAD: coronary artery disease; CFR: coronary flow reserve; HDL: high-density lipoprotein; hs-CRP: highly-sensitive C-reactive protein; LDL: Low-density lipoprotein; suPAR: soluble urokinase-type plasminogen activator receptor.
[a] Data reported as median (Interquartile Range).

with log CFR ($r = -0.32$, $p = 0.008$). In a multivariate regression analysis, after adjusting for age, sex, race, diabetes mellitus, hypertension, dyslipidemia, smoking, statin therapy, beta blocker therapy, $P2Y_{12}$ therapy and hs-CRP level, suPAR remained an independent predictor of CFR (Table 2). Moreover, a doubling in plasma suPAR level was associated with a 30% decrease in CFR value.

4. Discussion

In this cross-sectional study we demonstrate, for the first time, that plasma suPAR level is an independent predictor of coronary microvascular function in patients with non-obstructive CAD, even after adjustment for demographic variables, clinical risk factors, medication profiles and hs-CRP. Doubling of plasma suPAR is associated with about one-third decrease in CFR. This finding depicts the role of inflammation and immune dysregulation on coronary microvascular function.

Activated macrophages and impaired endothelial function play prominent roles in atherosclerosis initiation and development. Urokinase-type plasminogen activator receptor (uPAR) is expressed on a variety of cells including hematopoietic, endothelial and smooth muscle cells, and interactions between uPAR and β2 integrin may stimulate leukocyte chemotaxis and facilitate adhesion of macrophages and neutrophils to endothelial cells [8,31]. As a result, uPAR can be detected within the same areas of activated macrophages and lipids in immunohistological cross-sections of human carotid plaques [32].

Table 2
Multivariate linear regression analysis with biomarkers, cardiovascular risk factors and medications profiles.

Variable	log CFR		
	Beta	SE	p-value
Age	0.14	0.001	0.31
Race (Caucasian vs. Black)	−0.18	0.03	0.16
Diabetes Mellitus	−0.14	0.04	0.36
Dyslipidemia	−0.04	0.05	0.79
Hypertension	−0.04	0.04	0.76
Smoking	−0.002	0.04	0.99
Statin Therapy	−0.19	0.04	0.22
Beta Blocker Therapy	−0.11	0.03	0.42
$P2Y_{12}$ Therapy	−0.06	0.05	0.70
CRP biomarker	0.19	0.003	0.16
suPAR biomarker	−0.30	0.02	0.04

Higher expression of uPAR was detected in ruptured plaque segments compared to non-ruptured segments, suggesting that the uPAR system may be associated with plaque vulnerability [33]. Several longitudinal population-based studies have linked plasma suPAR levels with increased risk of cardiovascular disease and mortality [17,18,24]. Indeed, suPAR has been associated with the presence of carotid plaques, higher rates of stroke, myocardial ischemia and cardiovascular death, independent of traditional risk factors and hs-CRP [19,21,24].

The activation of both the innate and adaptive immunity (including cellular and humoral responses) has been linked to epicardial CAD [34–38]; however, to the best of our knowledge, this study is the first to demonstrate an independent relationship between suPAR, a surrogate of systemic inflammation and immune function, and coronary microvascular function. Even in the absence of significant epicardial stenoses, coronary microvascular dysfunction has been implicated as the cause of anginal symptoms and worse outcomes [5–7,39].

Therefore, accurate diagnosis and prognostication of coronary microvascular dysfunction remains crucial to the management of this clinical population. Although contemporary techniques for coronary microvascular function assessment include non-invasive imaging and invasive physiologic measurements [40–43], these methods can be impractical for routine surveillance. Serum biomarkers have been shown to have additive prognostic value over and above traditional risk factors and the Framingham Risk Score, and may become a convenient tool for the practicing clinician [44]. Our results suggest that suPAR may also be a valid biomarker for coronary microvascular function as the association between suPAR levels and log CFR remained significant even after adjustment of cardiovascular risk factors and hs-CRP. As a result, suPAR may prove useful in the surveillance of patients with non-obstructive CAD.

5. Limitations

First, this is a relatively small cross-sectional study and this precludes any inference about the causality of the association observed. Second, each patient had only one measurement of baseline suPAR level at time of catheterization which may not be fully reflective of an individual's baseline level given that it was collected under ‘stressful’ conditions. Third, more patients with abnormal CFR were receiving statin therapy, thus leading to lower serum LDL levels. More patients with normal CFR were receiving clopidogrel, which may improve rheological parameters in patients with subclinical atherosclerosis [45]. Additionally, more patients in the group with abnormal CFR were on beta blockers likely due to

more ischemic symptoms necessitating initiation of these agents for their antianginal effects [46,47]. However, significant association between suPAR and CFR remained even after adjustment for these statistically significant covariates, established cardiovascular risk factors and hs-CRP.

6. Conclusions

In patients with non-obstructive CAD, after adjusting for traditional cardiovascular risk factors and hs-CRP, plasma suPAR level remains independently associated with coronary microvascular function. Larger prospective clinical trials are warranted to investigate the role of immune dysregulation and the prognostic value of suPAR in microvascular dysfunction.

Conflict of interest/financial disclosure

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