Atherosclerosis xxx (2017) 1–7

Contents lists available at ScienceDirect

Atherosclerosis

journal homepage: www.elsevier.com/locate/atherosclerosis

Circulating soluble urokinase plasminogen activator receptor levels and peripheral arterial disease outcomes

Ayman Samman Tahhan [a], Salim S. Hayek [a], Pratik Sandesara [a], Jamal Hajjari [a], Muhammad Hammadah [a], Wesley T. O'Neal [a], Heval M. Kelli [a], Ayman Alkhoder [a], Nima Ghasemzadeh [a], Yi-An Ko [b], Hiroshi Aida [a], Mohamad Mazen Gafeer [a], Naser Abdelhadi [a], Kareem Hosny Mohammed [a], Keyur Patel [a], Shipra Arya [c], Jochen Reiser [d], Viola Vaccarino [a, e], Laurence Sperling [a], Arshed Quyyumi [a, *]

[a] Emory Clinical Cardiovascular Research Institute, Division of Cardiology, Emory University School of Medicine, Atlanta, GA, United States
[b] Department of Biostatistics and Bioinformatics, Emory University, United States
[c] Department of Surgery, Emory University School of Medicine, United States
[d] Department of Medicine, Rush University Medical Center, United States
[e] Department of Epidemiology, Rollins School of Public Health, Emory University, Atlanta, GA, United States

ARTICLE INFO

Article history:
Received 25 March 2017
Received in revised form
26 May 2017
Accepted 7 June 2017
Available online xxx

Keywords:
SuPAR
PAD
CAD
Atherosclerosis
Cardiovascular outcomes
PAD-Related outcomes

ABSTRACT

Background and aims: Circulating soluble urokinase plasminogen activator receptor (suPAR) is a marker of immune activation associated with atherosclerosis. Whether suPAR levels are associated with prevalent peripheral arterial disease (PAD) and its adverse outcomes remains unknown and is the aim of the study.

Methods: SuPAR levels were measured in 5810 patients (mean age 63 years, 63% male, 77% with obstructive coronary artery disease [CAD]) undergoing cardiac catheterization. The presence of PAD (n = 967, 17%) was classified as carotid (36%), lower/upper extremities (30%), aortic (15%) and multisite disease (19%). Multivariable logistic and Cox regression models were used to determine independent predictors of prevalent PAD and outcomes including all-cause death, cardiovascular death and PAD-related events after adjustment for age, gender, race, body mass index, smoking, diabetes, hypertension, hyperlipidemia, renal function, heart failure history, and obstructive CAD.

Results: Plasma suPAR levels were 22.5% ($p < 0.001$) higher in patients with PAD compared to those without PAD. Plasma suPAR was higher in patients with more extensive PAD (≥2 compared to single site) $p < 0.001$. After multivariable adjustment, suPAR was associated with prevalent PAD; odds ratio (OR) for highest compared to lowest tertile of 2.0, 95% CI (1.6–2.5) $p < 0.001$. In Cox survival analyses adjusted for clinical characteristics and medication regimen, suPAR (in the highest *vs.* lowest tertile) remained an independent predictor of all-cause death [HR 3.1, 95% CI (1.9–5.3)], cardiovascular death [HR 3.5, 95% CI (1.8–7.0)] and PAD-related events [HR = 1.8, 95% CI (1.3–2.6) $p < 0.001$ for all].

Conclusions: Plasma suPAR level is predictive of prevalent PAD and of incident cardiovascular and PAD-related events. Whether SuPAR measurement can help screen, risk stratify, or monitor therapeutic responses in PAD requires further investigation.

1. Introduction

Atherosclerosis is a systemic disease that can involve coronary, cerebrovascular, aortic, renal, upper and lower extremity arteries [1,2] Although widely prevalent, peripheral artery disease (PAD) remains underdiagnosed and is associated with excessive morbidity and mortality [3]. Despite sharing common risk factors with coronary artery disease (CAD) including hypertension,

* Corresponding author. Division of Cardiology, Department of Medicine, Emory University School of Medicine, 1462 Clifton Rd. NE, Suite 507, Atlanta, GA 30322, United States.
E-mail address: aquyyum@emory.edu (A. Quyyumi).

http://dx.doi.org/10.1016/j.atherosclerosis.2017.06.019
0021-9150/

advanced age, diabetes, hyperlipidemia and smoking, only 20–30% of patients with CAD develop concomitant significant PAD. In addition, endothelial dysfunction, reduced regenerative capacity, inflammation and immune dysregulation also play a fundamental role in the development and progression of PAD [1,2,4–6]. However, none of the markers reflecting these processes have been translated into clinical practice either for screening, risk stratification, or to monitor therapeutic response in patients with PAD.

Recently, we and others have shown that higher levels of soluble urokinase plasminogen activator receptor (suPAR), a circulating marker of inflammation, thromobogenesis, and immune regulation, are associated with hypertension, diabetes, CAD, stroke, and chronic kidney disease [7–10]. SuPAR is produced by cleavage of membrane-bound urokinase-type plasminogen activator receptor, which is a membrane linked G-protein expressed on numerous inflammatory cells including monocytes, activated T-lymphocytes and macrophages as well as fibroblasts and endothelial cells. Both the circulating and membrane-bound forms are directly involved in the regulation of cell adhesion and migration through binding of integrins. The soluble form has direct chemotactic properties that may facilitate recruitment of inflammatory cells such as neutrophils and monocytes and the mobilization of hematopoietic stem cells [11–13]. Finally, SuPAR is associated with increased inflammatory activity in atherosclerotic plaques [10].

The relationship between circulating suPAR levels and PAD and its related outcomes has not been systematically studied to date. Whether suPAR levels predict presence of PAD and whether its' levels are a measure of long-term incident risk remains unknown. The aim of this study is to examine the association between plasma suPAR levels and the presence of PAD and adverse cardiovascular and PAD-related events.

2. Materials and methods

2.1. Study design and population

We measured suPAR levels in 5810 adults ≥18 years from the Emory Cardiovascular Biobank, a prospective cohort of patients undergoing left heart catheterization for suspected or confirmed CAD at Emory Healthcare sites in Atlanta, GA, between 2003 and 2015. Subjects with congenital heart disease, heart transplantation, severe anemia, active inflammatory diseases, and cancer were excluded. Participants were interviewed to collect demographic characteristics, medical history, medication use, and behavioral habits as previously described [14]. Medical records and ICD-9 diagnostic codes were reviewed to confirm self-reported medical history. Angiograms performed at enrollment were reviewed, and obstructive CAD was defined as the presence of ≥50% stenosis in at least one coronary artery. The study was approved by the institutional review board at Emory University (Atlanta, GA). All subjects provided written informed consent at the time of enrollment.

2.2. Defining peripheral arterial disease

We extensively reviewed patients' self-reported and physician-documented medical history and imaging reports to identify the presence of PAD as previously described [6]. PAD was defined as a history of symptomatic or asymptomatic non-coronary atherosclerotic disease in at least one of the following arteries: carotid, thoracic or abdominal aorta, subclavian, brachial, iliac, femoral, or popliteal arteries. No routine testing was performed to screen for asymptomatic PAD. PAD of the lower extremities was diagnosed when at least one of the following were present: documented ankle-brachial index <0.90; lower limb revascularization; atherosclerotic plaques or stenosis on imaging ≥50% (computed tomography, ultrasound, or fluoroscopy) in the iliac, femoral, or popliteal arteries; and history of amputation for critical limb ischemia. All subjects with extremities PAD were symptomatic. PAD of the carotid artery was diagnosed if there was ≥20% stenosis in any carotid artery. Subgroup analysis was done to compare those with mild *vs.* severe carotid disease using higher cutoffs of 25% and 50%. Aortic disease was diagnosed when there was a history abdominal (greater than 4 cm) or thoracic (ascending greater than 4 cm or descending diameter larger than 3 cm) aneurysms after (excluding subjects with aortic root aneurysm associated with bicuspid aortic valves) or evidence of moderate-to-severe (frequent, large plaques) atherosclerotic plaques of the aorta or renal arteries on computed tomography imaging.

2.3. Sample collection and measurement of suPAR

Fasting arterial blood samples were collected at the time of catheterization and stored at −80 °C. Plasma levels of suPAR were measured (suPARnostic kit; ViroGates, Copenhagen, Denmark) with a lower detection limit of 100 pg/mL and intra- and inter-assay variation of 2.75% and 9.17%, respectively [8]. Serum high-sensitivity C-reactive protein (CRP) levels were determined in 3452 patients (59.4%) using a particle-enhanced immunoturbidimetry assay (FirstMark, a division of GenWay Biotech) that has a lower limit of detection of 0.03 mg per liter [15].

2.4. Follow-up and outcomes

We conducted follow-up as previously described to identify prespecified incident adverse cardiovascular outcomes including death, cardiovascular death and PAD-related events. In brief, follow-up and adjudication were conducted by personnel blinded to the SuPAR data by phone, electronic medical record review, as well as and social security death index and state records. PAD-related events such as peripheral revascularization, amputation and stroke were identified using standard current procedural terminology codes for vascular procedures, chart review and follow-up questionnaire [6].

2.5. Statistical analysis

We reported subject characteristics as descriptive statistics with means, standard deviations, frequency counts, percentages, medians, and interquartile ranges. Differences between groups were assessed using the *t*-test for continuous variables, and chi-square for categorical variables. Two-tailed P-value ≤0.05 were considered statistically significant. For non-normally distributed variables such as suPAR and CRP levels, the Mann-Whitney *U* test was used to compare groups in unadjusted analyses. For multivariable analyses, suPAR levels were examined both as a categorical variable stratified by tertiles, and as a continuous variable after log-transforming, and reported as “per 25% increase in suPAR”. Covariates incorporated in multivariable analyses included age, gender, race, body mass index (BMI), smoking history, hypertension, diabetes, estimated glomerular filtration rate (eGFR calculated using the CKD-EPI equation), presence of obstructive CAD, statin use, antiplatelet therapy, angiotensin pathway antagonist use, beta-blocker therapy, suPAR and hs-CRP levels. Logistic regression was performed to investigate independent predictors of prevalent PAD. Regression coefficients are presented as point estimates with 95% confidence intervals. We examined the collinearity among all the continuous variables using Pearson and Spearman correlation when appropriate. We found that the highest two correlations observed were 0.4 and 0.5. We further examined the tolerance values and none of them were <0.1. We also did not find a large standard errors for any of the estimates.

Please cite this article in press as: A. Samman Tahhan, et al., Circulating soluble urokinase plasminogen activator receptor levels and peripheral arterial disease outcomes, Atherosclerosis (2017), http://dx.doi.org/10.1016/j.atherosclerosis.2017.06.019