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RESEARCH LETTER

Assay-related differences in SuPAR levels: implications for measurement and data interpretation

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Soluble urokinase plasminogen activator receptor (suPAR) is an immune-derived glycoprotein implicated in kidney disease and associated with various clinical outcomes [1]. The rising interest in exploring suPAR's role in kidney and cardiovascular disease has led to its measurement in various cohorts [1]. Different assays have been used to measure suPAR levels including enzyme-linked immunosorbent assays (ELISA) such as the Human uPAR Quantikine ELISA (R&D Systems, Minneapolis, MN) and the suPARnostic ELISA (Virogates, Copenhagen, Denmark); and proteomics platforms such as the aptamer-based assay SomaLogic SOMAscan (SomaLogic, Boulder, CO) and the proximity extension assay Olink Explore (Olink, Uppsala, Sweden). SuPAR values and associations with clinical outcomes have differed greatly according to the assay used [2–5], warranting an exploration of assay-related differences in suPAR levels. To that end, we leveraged cohorts in which suPAR was measured using two different approaches, and examined the correlation between assays, the association between levels and clinical characteristics, and their risk discrimination ability for relevant outcomes.

Data on plasma suPAR measurement, clinical characteristics and outcomes were obtained from the following cohorts: a subset ($n=4637$) of the Malmö Diet and Cancer Study (MDCS)which is a Swedish population-based cohort in which suPAR was measured using the suPARnostic ELISA and Olink CVD-I panel; a subset ($n=1492$) of the Jackson Heart Study (JHS) which recruited African American participants from Jackson, Mississippi in whom suPAR was measured using the Human uPAR Quantikine ELISA and SOMAscan; and lastly, a subset ($n=487$) of the Emory Cardiovascular Biobank (EmCAB) which enrolled patients undergoing coronary catheterization, in whom suPAR was measured with the suPARnostic and Human uPAR Quantikine ELISAs [1].

Data transformation and normalization procedures were performed for the SOMAscan and Olink assays according to standard protocols developed by the manufacturers as part of the data processing in the original assays. The procedures are necessary to account for intra- and inter-assay variation and for the interpretation of raw data. In JHS, SOMAscan values were natural log-transformed, standardized to a mean of 0 and an SD of 1 within each of three batches, and then inverse-normalized across batches. In MDCS, Olink's normalized protein expression units were log-based 2 transformed. We used Spearman-Rank to report the pair-wise correlation between assays. We report standardized estimates for the association between clinical characteristics and suPAR measures derived using linear regression, with the suPAR level as the dependent variable and clinical characteristics as independent covariates. Lastly, we computed Harrell's C-statistic as a measure of risk discrimination for

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each suPAR assay for the following outcomes: all-cause mortality, cardiovascular mortality, and incident chronic kidney disease (defined as a decrease in creatinine-derived eGFR to below 60 ml/min/1.73 m^2). Analyses were performed using SPSS 24 (IBM, NY, USA) and R (R Core Team, 2014).

We found extensive variation in the correlation between suPAR assays (Table 1). The correlation between the proteomics platforms and ELISAs were modest at best, with a correlation of 0.285 between the Human uPAR Quantikine and SOMAScan, and 0.586 between the suPARnostic and Olink. The correlation between immunoassays (suPARnostic and Human uPAR Quantikine) was relatively better at 0.753, with values obtained using the suPARnostic assay on average 50% higher than the Quantikine-derived values, albeit with significant variability given a standard deviation of 50%. Associations with relevant clinical characteristics and their directionality were mostly consistent across measures, except for the lack of an association between SOMAScan-derived suPAR levels and diabetes mellitus (Table 1). Risk discrimination as quantified using Harrell's C-statistic for all-cause death, cardiovascular death and incident chronic kidney disease differed between suPAR assays, with the most notable differences between the SOMAScan and Quantikine measures (Table 1).

This study highlights major discrepancies between suPAR measurements across commonly used assays, with modest correlations and differences in associations with outcomes. These findings warrant caution in deriving conclusions related to suPAR measures, notably when comparing results from different assays. Proteomics platforms, while useful for discovery, have limitations including cross-reactivity, lack of specificity, protein complexes and single nucleotide polymorphisms altering aptamer/antibody affinities, amongst others which may be more relevant for some assayed proteins such as suPAR [3]. The correlation in suPAR levels measured with SOMAScan and Olink has been previously reported to be modest ($r = 0.48$) [2]. Most importantly, proteomics-derived suPAR measures have not been previously cross-validated with ELISAs. We also found

Table 1 Correlation, associations with clinical characteristics, and C-statistics stratified by cohort and assay

	MDCS ($N = 4637$)		JHS ($n = 1905$)		EmCAB $n(=487)$	
	SuPARnostic	Olink	Quantikine	SOMAScan	SuPARnostic	Quantikine
Spearman's rank correlation						
Overall cohort	0.566		0.285		0.753	
Predictors of suPAR levels						
Standardized β, P-value						
Age, per 1 year	0.09, $P < 0.001$	0.09, $P < 0.001$	0.07, $P = 0.035$	0.08, $P = 0.010$	− 0.12, $P = 0.002$	− 0.09, $P = 0.024$
Male gender	− 0.08, $P < 0.001$	− 0.06, $P < 0.001$	− 0.47, $P < 0.001$	− 0.27, $P < 0.001$	− 0.16, $P < 0.001$	− 0.18, $P < 0.001$
Body-mass index, per 1 kg/m^2	0.04, $P = 0.005$	0.02, $P = 0.13$	0.07, $P = 0.002$	− 0.06, $P = 0.022$	0.01, $P = 0.80$	0.01, $P = 0.90$
Current smoker	0.30, $P < 0.001$	0.35, $P < 0.001$	0.44, $P < 0.001$	0.34, $P < 0.001$	0.00, $P = 0.92$	0.03, $P = 0.41$
Hypertension	0.01, $P = 0.50$	0.02, $P = 0.22$	0.02, $P = 0.55$	− 0.01, $P = 0.90$	0.03, $P = 0.39$	0.00, $P = 0.94$
Diabetes mellitus	0.05, $P = 0.001$	0.05, $P < 0.001$	0.29, $P < 0.001$	0.06, $P = 0.26$	0.18, $P < 0.001$	0.18, $P < 0.001$
High-density lipoprotein level, per 1 mg/dl	− 0.12, $P < 0.001$	− 0.09, $P < 0.001$	− 0.03, $P = 0.20$	− 0.03, $P = 0.29$	− 0.04, $P = 0.35$	− 0.03, $P = 0.42$
Creatinine-derived eGFR, per 1 ml/min	− 0.15, $P < 0.001$	− 0.18, $P < 0.001$	− 0.38, $P < 0.001$	− 0.17, $P < 0.001$	− 0.57, $P < 0.001$	− 0.49, $P < 0.001$
Harrell's C-statistic						
All-cause mortality	$n = 1799$		$n = 446$		$n = 107$	
	0.628	0.603	0.678	0.588	0.685	0.655
Cardiovascular mortality	$n = 530$		$n = 67$		$n = 49$	
	0.619	0.582	0.742	0.566	0.678	0.628
Incident chronic kidney disease	$n = 977$		$n = 128$		$n = 75$	
	0.620	0.616	0.614	0.537	0.529	0.502

Chronic kidney disease is defined as a decrease in eGFR to below 60 ml/min/1.73 m^2 at follow-up. Harrell's *C*-statistic is derived from time to event models which include only suPAR levels

MDCS Malmö Diet and Cancer Study, *JHS* Jackson Heart Study, *EmCAB* Emory Cardiovascular Biobank, *eGFR* estimated glomerular filtration rate

differences between the ELISA measurements, consistent with a prior report by Winnicki et al. in which suPAR levels using the suPARnostic assay were higher and outperformed the Human Quantikine uPAR ELISA in differentiating between patients with and without focal segmental glomerulosclerosis [4]. SuPAR exists in circulation in different forms originating from splice variants and proteolytic processing with varying levels of glycosylation and distinct biological activity. The suPARnostic ELISA consists of two monoclonal capture antibodies: one targeting the D^{III} subunit, and the other the D^{II} subunit, thus capturing full-length suPAR ($D^{I}D^{II}D^{II}$) and the $D^{II}D^{III}$ fragment, but not D^{I}. The Human uPAR Quantikine assay uses a monoclonal capture antibody and polyclonal detection antibodies. The specific suPAR forms detected by the various proteomics platforms are unknown. The discrepancy between assays may relate to their differing ability in detecting the various suPAR forms, with the suPARnostic assay's ability to detect both full length and cleaved suPAR forms explaining the higher levels reported and overall better risk discrimination. As suPAR is increasingly measured in clinical settings, understanding the differences in the methods of suPAR measurements is crucial for interpreting findings.

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Declarations

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Conflict of interest SSH and JR are scientific advisory board members of Walden Biosciences Inc.

Authors' contributions Research idea and study design: Laura M. Raffield, Gunnar Engstrom, Jochen Reiser, and Salim S. Hayek; data acquisition: Salim S. Hayek, Gunnar Engstrom, Yan Gao, Arshed A. Quyyumi, and Jochen Reiser; data analysis and interpretation: Alexi Vasbinder, Yan Gao, Arshed A. Quyyumi, Alexander P. Reiner, and Salim S. Hayek. All authors reviewed the initial draft and provided critical revisions. All authors reviewed, edited, and approved the final version of the manuscript.

Study approval The IRBs of Jackson State University, Tougaloo College, and University of Mississippi Medical Center approved the Jackson Heart Study (JHS) protocol. The IRB of Emory University, Atlanta, GA approved the Emory Cardiovascular Biobank (EmCAB) protocol. The IRB of Lund University, Sweden approved the Malmo Diet and Cancer Study (MDCS) procotol.

Informed consent Informed consent was obtained from all individual participants included in the study.

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