## RESEARCH LETTER

### Plasma Kidney Injury Molecule-1 in Systemic Lupus Erythematosus: Discordance Between ELISA and Proximity Extension Assay

*To the Editor:*

Oligonucleotide-labeled antibodies (Olink, Olink Proteomics) and aptamers (SomaScan, SomaLogic) are increasingly used in biomarker discovery and research. However, few studies have compared the protein levels measured on proteomics platforms with those measured using conventional immunoaffinity assays.[1-5] This may be especially relevant in the setting of autoimmune diseases, such as systemic lupus erythematosus (SLE), where anti–double-stranded DNA (anti-dsDNA) antibodies could interfere with immunoassay technologies that use oligonucleotide-based antibodies (Olink) or aptamers (SomaScan). In this study, we compared measurements of plasma kidney injury molecule-1 (KIM-1), a sensitive marker of tubular injury with prognostic value,[6,7] across 2 different immunoassay technologies in patients with and without SLE.

We measured plasma KIM-1 levels in 446 individuals enrolled in the Boston Kidney Biopsy Cohort, a prospective, observational cohort study of patients with chronic kidney disease (Item S1), using the microbead-based sandwich enzyme-linked immunosorbent assay (ELISA) and a proximity extension assay (Olink). The proximity extension assay uses oligonucleotide-labeled antibodies that bind to the target protein. The measurements and assay performance of both ELISA and the Olink platform in the Boston Kidney Biopsy Cohort were described previously in detail.[7,8] Using Spearman correlation coefficients, we investigated differences in plasma KIM-1 measurements between the 2 assays in individuals with and without SLE and in individuals with negative versus positive anti-dsDNA levels (assay reference range, ≤25 vs >25 IU/mL). The corresponding 95% confidence intervals (CIs) were reported as bias-corrected and accelerated bootstrap estimates.[9]

Sixty-eight Boston Kidney Biopsy Cohort participants had SLE and were compared with 378 individuals with diseases other than SLE (Table S1). The mean ages were 39 ± 15 and 55 ± 16 years, the mean estimated glomerular filtration rates were 85 ± 37 and 52 ± 33 mL/min/1.73 $m^2$, and the median proteinurias (interquartile range) were 1.5 (0.7-3.2) and 1.7 (0.4-4.2) g/g creatinine in individuals with and without SLE, respectively. Eighty-seven percent of individuals with SLE were women and 46% of those without SLE were women. Among those with SLE, 62% had proliferative and 27% had non-proliferative histopathologic lesions in the kidneys. Spearman's ρ between plasma KIM-1 measurements from both assays in the entire cohort was 0.89 (95% CI, 0.85-0.92; $P < 0.001$). Spearman's ρ was 0.69 (95% CI, 0.47-0.82; $P < 0.001$) in individuals with SLE, and Spearman's ρ was 0.90 (95% CI, 0.85-0.93; $P < 0.001$) in individuals with diseases other than SLE (Fig 1). Forty-three individuals with SLE had available anti-dsDNA levels measured using the chemiluminescent QUANTA Flash dsDNA assay (Werfen). The correlation between the 2 assays was lower in 34 individuals with anti-dsDNA

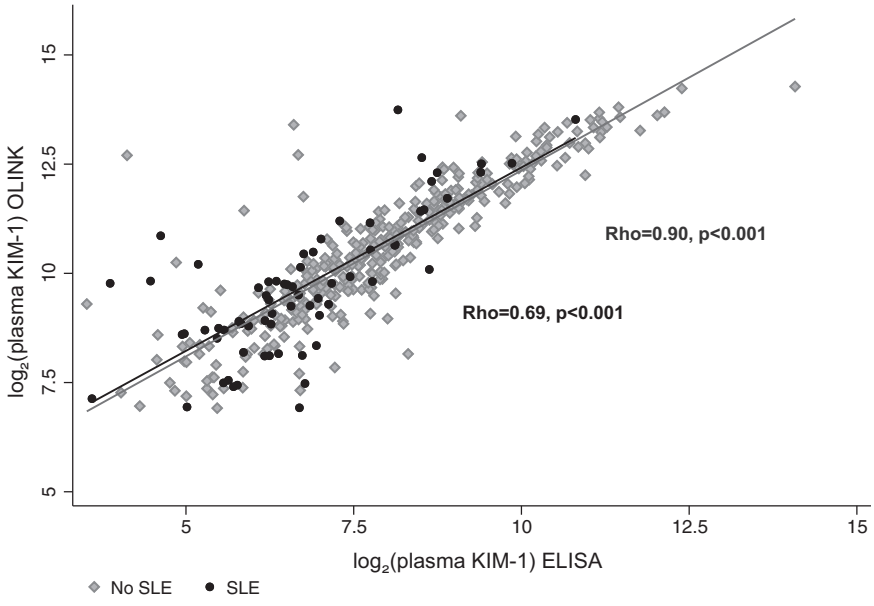


**Figure 1.** Correlation between plasma kidney injury molecule-1 (KIM-1) measurements using enzyme-linked immunosorbent assay (ELISA) and a proximity extension assay (Olink) according to systemic lupus erythematosus (SLE) status. Plasma KIM-1 (Olink) values are expressed as normalized protein expression values on a $\log_2$ scale. Plasma KIM-1 (ELISA) values were measured in pg/mL and $\log_2$-transformed.

antibody levels >25 IU/mL ($\rho = 0.70$; 95% CI, 0.37-0.88; $P < 0.001$) than in 9 individuals with anti-dsDNA levels ≤25 IU/mL ($\rho = 0.92$; 95% CI, 0.68-1.0; $P = 0.001$).

In this study, we found differences in the concordance of plasma KIM-1 measurements by proximity extension assay versus ELISA according to SLE status. Few studies have compared assay specificity and correlation between different assay technologies.[1-5] In a prior study, correlations between ELISA and SomaScan aptamer–based assays for multiple proteins ranged from high for some proteins (eg, C-reactive protein) to none (eg, platelet endothelial cell adhesion molecule-1).2 In a recent study that compared soluble urokinase plasminogen activator receptor levels measured by conventional ELISA with the Olink and SomaScan platforms in 3 different cohorts, assay correlations varied widely across the cohorts (Spearman's $\rho = 0.3$-$0.8$). Soluble urokinase plasminogen activator receptor had a weaker magnitude of association with adverse clinical outcomes when measured on the proteomics platforms compared with ELISA.[1] Although cross-reactivity, epitope availability, and negative cooperative binding could all influence the concordance between assay technologies,[2] it is possible that autoantibodies (eg, anti-dsDNA antibodies, which can bind to diverse DNA structures and nucleoprotein complexes, and may have DNA-related catalytic activity)[10] interfere with measurements dependent on proximity-based oligonucleotide amplicons used in the Olink platform.

Although our sample size of individuals with available anti-dsDNA levels was small, our results showing lower correlation with higher anti-dsDNA levels highlight a potential limitation to the use of oligonucleotide-based assay technologies in patients with diseases characterized by the presence of autoantibodies. More broadly, our results emphasize the possibility of disease-specific interference phenomena for biomarker assays and the importance of cross-platform validation studies.

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## SUPPLEMENTARY MATERIAL

**Supplementary File (PDF)**

**Item S1:** Supplemental Methods.

**Table S1:** Baseline Characteristics of the Boston Kidney Biopsy Cohort.

## ARTICLE INFORMATION

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## REFERENCES

1. Hayek SS, Raffield LM, Gao Y, et al. Assay-related differences in SuPAR levels: implications for measurement and data

interpretation. *medRxiv*. Preprint posted online June 28, 2021. https://doi.org/10.1101/2021.06.23.21259148
2. Raffield LM, Dang H, Pratte KA, et al. Comparison of proteomic assessment methods in multiple cohort studies. *Proteomics*. 2020;20(12):e1900278.
3. Katz DH. Abstract 9892: proteomic profiling of cardiometabolic phenotypes: a comparison of aptamer-based and antibody-based affinity platforms. *Circulation*. 2021;144(Suppl 1):A9892. Accessed March 10, 2022. https://www.ahajournals.org/doi/abs/10.1161/circ.144.suppl_1.9892
4. Liu RX, Thiessen-Philbrook HR, Vasan RS, et al. Comparison of proteomic methods in evaluating biomarker-AKI associations in cardiac surgery patients. *Transl Res*. 2021;238:49-62.
5. Tin A, Yu B, Ma J, et al. Reproducibility and variability of protein analytes measured using a multiplexed modified aptamer assay. *J Appl Lab Med*. 2019;4(1):30-39.
6. Bonventre JV. Kidney injury molecule-1 (KIM-1): a specific and sensitive biomarker of kidney injury. *Scand J Clin Lab Invest Suppl*. 2008;241:78-83.
7. Schmidt IM, Srivastava A, Sabbisetti V, et al. Plasma kidney injury molecule 1 in CKD: findings from the Boston Kidney Biopsy Cohort and CRIC studies. *Am J Kidney Dis*. 2022;79(2):231-243.e1. doi:10.1053/j.ajkd.2021.05.013
8. Schmidt IM, Mothi SS, Wilson PC, et al. Circulating plasma biomarkers in biopsy-confirmed kidney disease. *Clin J Am Soc Nephrol*. 2022;17(1):27-37.
9. Chan W, Chan DW. Bootstrap standard error and confidence intervals for the correlation corrected for range restriction: a simulation study. *Psychol Methods*. 2004;9(3):369-385.
10. Wang X, Xia Y. Anti-double stranded DNA antibodies: origin, pathogenicity, and targeted therapies. *Front Immunol*. 2019;10:1667.