Title

Analytical and Biological Variability of a Commercial Modified Aptamer Assay in Plasma Samples of Patients with Chronic Kidney Disease.

Journal

The Journal of Applied Laboratory Medicine, 8(3)

ISSN

2576-9456

Authors

Dubin, Ruth F
Deo, Rajat
Ren, Yue
et al.

Publication Date

2023-05-04

DOI

10.1093/jalm/jfac145

Peer reviewed

Published in final edited form as:

J Appl Lab Med. 2023 May 04; 8(3): 491–503. doi:10.1093/jalm/jfac145.

Analytical and Biological Variability of a Commercial Modified Aptamer Assay in Plasma Samples of Patients with Chronic Kidney Disease

Ruth F. Dubin[a,*], **Rajat Deo**[b], **Yue Ren**[c], **Hongzhe Lee**[c], **Haochang Shou**[c], **Harold Feldman**[c], **Paul Kimmel**[d], **Sushrut S. Waikar**[e], **Eugene P. Rhee**[f], **Adrienne Tin**[g,h], **Jingsha Chen**[i], **Joseph Coresh**[h,j], **Alan S. Go**[k], **Tanika Kelly**[l], **Paduranga S. Rao**[m], **Teresa K. Chen**[n], **Mark R. Segal**[o], **Peter Ganz**[p]

[a]Division of Nephrology, University of Texas Southwestern Medical Center, San Francisco, CA, USA

[b]Division of Cardiology, University of Pennsylvania Perelman School of Medicine, Philadelphia, PA, USA

[c]Department of Biostatistics, Epidemiology, and Informatics, Perelman School of Medicine at the University of Pennsylvania, Philadelphia, PA, USA

[*]Address correspondence to this author at: 5323 Harry Hines Blvd. H5.122E, Dallas, TX 75390. ruth.dubin@utsouthwestern.edu.

Author Contributions: The corresponding author takes full responsibility that all authors on this publication have met the following required criteria of eligibility for authorship: (a) significant contributions to the conception and design, acquisition of data, or analysis and interpretation of data; (b) drafting or revising the article for intellectual content; (c) final approval of the published article; and (d) agreement to be accountable for all aspects of the article thus ensuring that questions related to the accuracy or integrity of any part of the article are appropriately investigated and resolved. Nobody who qualifies for authorship has been omitted from the list.

Authors' Disclosures or Potential Conflicts of Interest: *Upon manuscript submission, all authors completed the author disclosure form. Disclosures and/or potential conflicts of interest:* **Employment or Leadership:** P.L. Kimmel is the co-editor of *Chronic Renal Disease* 2nd Edition, and *Psychosocial Aspects of Chronic Kidney Disease*. **Consultant or Advisory Role:** J. Coresh received consulting fee from Healthy.io. and Somalogic. H. Feldman received a consulting fee from Kyowa-Hakko Kirin, Inc consultancy, InMed Physicians Coaching, and is a member of the NKF Scientific Advisory Board. P. Ganz is a member of SomaLogic Medical Advisory Board (unpaid). P.L. Kimmel serves on the Board of Directors Academy of Medicine, Washington, DC (unpaid). H. Lee received a consulting fee from Eli Lilly. **Stock Ownership:** J. Coresh owns stock of Healthy.io. H. Lee owns stock in Biomarin, Crispr, Editas, Gilead, Intercept. **Honoraria:** H. Feldman received honoraria from UCLA (invited speaker) and Rogosin Institute (invited speaker). H. Feldman received funding for travel from the International Society of Nephrology and KDIGO. T.K. Chen, American Society of Nephrology. **Research Funding:** R. Deo received funding from NIH 5U01DK108809. P. Rao received funding from NIH-DHHS-US U01 DK061028–22. H. Shou received funding from NIH U01-DK103225. T.K. Chen received funding from NIH/NIDDK (K08DK117068) and Yale University via NIH/NIDDK P30DK079310. R.F. Dubin received funding from NIH NIDDK Biomarker Consortium- U01DK108809. CRIC is funded by NIH NIDDK U01DK060990, U01DK060984, U01DK061022, U01DK061021, U01DK061028, U01DK060980, U01DK060963, U01DK060902. H. Feldman received funding and is also the Steering Committee Chair of NIH-NIDDK. P. Ganz received funding from NIH NIDDK 5U01DK108809. A. Go received funding from NIDDK, NHLBI, NIA, Novartis, CSL Behring, Bristol Meyers-Squibb and Pfizer Alliance, Amarin Pharmaceuticals, Janssen Research and Development. J. Coresh, National Institutes of Health. **Expert Testimony:** H. Feldman received payment from Essure Litigation (DLA Piper LLP). **Patents:** None declared. **Other Remuneration:** T.K. Chen received funding from Yale University (O'Brien Center Pilot and Feasibility Grant) via NIH/NIDDK. P.L. Kimmel received payment from the New York Academy of Medicine, January 2020, for travel, lodging, and meals to NIDDK for attending meeting on Social Determinants of Kidney Health. H. Feldman, support for attending meetings and/or travel from International Society of Nephrology and KDIGO.

Disclaimer: The opinions expressed in this paper do not necessarily reflect those of the National Institute of Diabetes Digestive and Kidney Disease, the National Institutes of Health, the Department of Health and Human Services, or the government of the United States of America.

SUPPLEMENTAL MATERIAL

Supplemental material is available at *The Journal of Applied Laboratory Medicine* online.

[d]Division of Kidney, Urologic, and Hematologic Diseases, National Institute of Diabetes and Digestive and Kidney Diseases, Washington, DC, USA

[e]Division of Nephrology, Boston University School of Medicine, Boston, MA, USA

[f]Division of Nephrology, Massachusetts General Hospital/Harvard Medical School, Boston, MA, USA

[g]Department of Medicine, University of Mississippi Medical Center, Jackson, MS, USA

[h]Department of Epidemiology, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, USA

[i]Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, USA

[j]Welch Center for Prevention, Epidemiology, and Clinical Research, Johns Hopkins University, Baltimore, MD, USA

[k]Division of Research, Kaiser Permanente Northern California Division of Research, Oakland, CA, USA

[l]Department of Epidemiology, Tulane University, New Orleans, LA, USA

[m]Department of Medicine, University of Michigan Ann Arbor, Ann Arbor, MI, USA

[n]Division of Nephrology, Johns Hopkins School of Medicine, Baltimore, MD, USA

[o]Department of Epidemiology and Biostatistics, University of California, San Francisco, San Francisco, CA, USA

[p]Division of Cardiology, University of California, San Francisco, San Francisco, CA, USA.

Abstract

Background: We carried out a study of the aptamer proteomic assay, SomaScan V4, to evaluate the analytical and biological variability of the assay in plasma samples of patients with moderate to severe chronic kidney disease (CKD).

Methods: Plasma samples were selected from 2 sources: (*a*) 24 participants from the Chronic Renal Insufficiency Cohort (CRIC) and (*b*) 49 patients from the Brigham and Women's Hospital–Kidney/Renal Clinic. We calculated intra-assay variability from both sources and examined short-term biological variability in samples from the Brigham clinic. We also measured correlations of aptamer measurements with traditional biomarker assays.

Results: A total of 4656 unique proteins (4849 total aptamer measures) were analyzed in all samples. Median (interquartile range [IQR] intra-assay CV) was 3.7% (2.8–5.3) in CRIC and 5.0% (3.8–7.0) in Brigham samples. Median (IQR) biological CV among Brigham samples drawn from one individual on 2 occasions separated by median (IQR) 7 (4–14) days was 8.7% (6.2–14). CVs were independent of CKD stage, diabetes, or albuminuria but were higher in patients with systemic lupus erythematosus. *Rho* correlations between aptamer and traditional assays for biomarkers of interest were cystatin C=0.942, kidney injury model-1 = 0.905, fibroblast growth factor-23 = 0.541, tumor necrosis factor receptors 1 = 0.781 and 2 = 0.843, $P < 10^{-100}$ for all.

Conclusions: Intra-assay and within-subject variability for SomaScan in the CKD setting was low and similar to assay variability reported from individuals without CKD. Intra-assay precision was excellent whether samples were collected in an optimal research protocol, as were CRIC samples, or in the clinical setting, as were the Brigham samples.

INTRODUCTION

The Slow Off-rate Modified Aptamer proteomic assay (SomaScan V4) is a high throughput microarray platform designed to measure nearly 5000 unique proteins in 55 μl of plasma with high sensitivity and specificity. In preparation for using the SomaScan V4 in a study of cardiovascular and renal outcomes in the Chronic Renal Insufficiency Cohort (CRIC), we conducted a pilot study to establish the intra-assay analytical variability and short-term biological variability of SomaScan V4 in plasma samples of patients with moderate to severe chronic kidney disease (CKD) not on dialysis. Prior studies of SomaScanV4 in plasma from individuals without CKD have shown low analytical and biological variability. Somalogic's in-house tests of SomaScan V4 among healthy individuals indicate that triplicate samples have median (interquartile range [IQR]) intra-assay CVs of 3.2% (2.5–5.1). Tin et al. performed a pilot study of the assay's biological variability in plasma samples from participants of the ARIC cohort. Among 22 subjects with estimated glomerular filtration rate (eGFR) >60 mL/min/1.73 m^2, each of whom had 2 plasma samples at median (IQR) 36 (30–38) days apart; median (IQR) within-subject CVs were 6.7% (5.1–9.7) (1).

Since plasma samples from patients with CKD typically have higher analyte concentrations and might introduce interference from nonanalyte substances, we designed a pilot study to evaluate the assay's technical and biological variability among patients with CKD. We hypothesized that intra-assay and short-term biological variability of SomaScan V4 in samples of patients with CKD would be similar to that in samples from individuals without CKD. We designed the study to include samples collected both in research and clinical settings. We further examined whether intra-assay variability varied by clinical factors such as stage of CKD, albuminuria, and diabetes, and we obtained preliminary information on the correlations of aptamer measurements with select traditional biomarker assays.

MATERIALS AND METHODS

Complete study design is found in Supplemental Methods.

Study Design

Plasma EDTA samples were selected from 2 sources: research samples from CRIC and clinical samples from the Brigham and Women's Hospital–Kidney/Renal Clinic. A subset of CRIC samples was used to test analytical variability, and the larger CRIC cohort was utilized for orthogonal tests. For analytical variability, 24 participants were selected from CRIC who had single samples drawn at a study visit 3 years after baseline, spanning the years 2006 to 2011. Among the 24 CRIC samples, we included 8 participants with each CKD stage G3A (eGFR 45–59 mL/min/1.73 m^2), G3B (eGFR 30–44), and G4 (eGFR 15–29) based on the CRIC eGFR equation (2). Larger numbers of CRIC samples were used for the orthogonal tests. We analyzed correlations between aptamer and nonaptamer measures in

the following numbers of CRIC participants: cystatin-C (n = 3309 participants), fibroblast growth factor (FGF)-23 (n = 1672), kidney injury model (KIM)-1 (n = 312), tumor necrosis factor (TNF)-R1 (n = 312), and TNF-R2 (n = 312). The second source of CKD plasma was 49 patients from the Brigham and Women's Hospital–Kidney/Renal Clinic who had samples drawn at each of 2 clinic visits (spanning 2011–2014); we assessed both technical variability and short-term biological variability in these samples. All 49 patients returned for a second visit at interval ranging from 1 to 135 days (median, IQR: 7, 4–14 days). Out of 49 patients, 9 had a renal biopsy concurrent with the first plasma sample. Sample processing methods at originating labs are described in Supplemental II. All study participants gave informed consent, and the study was approved by the Institutional Review Board. All procedures were in accordance with the Declaration of Helsinki (3).

SomaScan V4 Assay

SomaScan V4 is an assay based on modified aptamers, which are chemically modified single strands of DNA approximately 40 nucleotides long, as binding reagents for target proteins (4–9). Modified aptamers bind to proteins with high affinity similar to antibodies (lower limit of detection 10^{-15} moles per liter) (4, 6, 7). "Pull-down" studies, in which the aptamer-protein complexes were isolated and the identities of the bound proteins were verified by targeted mass spectrometry and SDS gel electrophoresis, have been performed for 920 proteins among 1305 proteins in a previous version of the assay (8). These studies showed that >95% of aptamers correctly targeted the intended proteins (for those proteins in concentrations sufficient to be detected by mass spectrometry). These findings are corroborated by cis protein quantitative trait loci and biologically plausible trans protein quantitative trait loci. In rare instances (<5%), the aptamer was selected for an impurity rather than for the intended protein. These "wrong" aptamers have been identified by SomaLogic and associated with the correct protein (8). The samples on the SomaScan assay are run at 3 different dilutions to assay each analyte within its linear range of concentrations. The assay results are quantified on a hybridization microarray and reported in relative fluorescent units (RFU). SomaLogic has procedures for data calibration, standardization, and internal controls, typical of microarray technologies (Supplemental I, reproduced with permission from SomaLogic). After running the assay, SomaLogic standardizes the entire protein dataset using adaptive normalization by maximum likelihood (ANML) to remove bias in the assay. This is an iterative procedure that adjusts values for analytes that fall outside expected measurements from a reference distribution. Efficacy of standardization is demonstrated, reducing variation in replicate samples.

Nonaptamer Biomarkers

Additional laboratory tests were performed on the CRIC samples from year 1 visit: cystatin C (Dade Behring BNII); FGF-23 C-terminal assay (Immunotopics); KIM-1, TNF receptor 1, TNF receptor 2 (Meso Scale Discovery platform). eGFR for CRIC samples was calculated using the CRIC equation that utilizes creatinine, cystatin, age, gender, and race (2). eGFR for Brigham samples was calculated by the abbreviated Modification of Diet in Renal Disease equation: $\text{GFR} = 186 \times \text{Serum Cr}^{(-1.154)} \times \text{age}^{(-0.203)} \times 1.212$ (if patient is Black) × 0.742 (if female) (10).

Statistical Analyses

First, prior to unblinding, we performed hierarchical clustering based on Euclidean distances to determine if we could correctly identify the duplicate pairs based on their protein levels. After pairs were unblinded, the CV for each protein in each pair was calculated using absolute RFUs. In each of the separate CRIC and Brigham analyses we looked for differences in average CV by CKD stage, albuminuria, and diabetic status. For Brigham within-subject variability, we also checked the CV in 3 separate subgroups of potential concern: (*a*) samples from patients with lupus (as circulating DNA autoantibodies potentially interfere with the aptamer assay), (*b*) samples from patients who had a biopsy concurrent with the first clinic visit, and (*c*) plasma samples flagged by SomaLogic for technical concerns. We examined whether longitudinal CV was associated with the length of the interval between clinic visits or change in eGFR between visits. Additionally, we evaluated CVs using non-ANML formatted (raw RFU) data. We examined change in individual proteins by calculating the fold-change in non-log-transformed RFU from visit 1 to visit 2. We also compared RFU at visit 1 and 2 using the Student t-test and addressed multiple comparisons using a false discovery rate (FDR) of 10%. As a pilot orthogonal test, we calculated Spearman correlations between aptamer and nonaptamer measures of cystatin C and several other renal biomarkers in CRIC samples from Visit 1 (n = 3209 participants) that have been assayed with SomaScan V4.

RESULTS

Baseline Characteristics of the Study Participants

Baseline characteristics of the CRIC and Brigham cohort participants who contributed samples to this pilot study are summarized in Table 1. Age, gender, systolic blood pressure, body mass index, albuminuria, and eGFR were similar between CRIC and Brigham participants. Etiology of CKD in the CRIC patients was limited to diabetes, hypertension, glomerulonephritis (none had lupus), or congenital disease. Etiology of CKD in Brigham patients included nephrectomy, drug toxicity, lupus, and polycystic kidney disease. In Brigham within-subject variability samples, 9 of 49 patients had a renal biopsy at visit 1; median (IQR) change in eGFR in all Brigham patients was −1.28 (−4.28, 0.5), while in patients who underwent biopsy it was −6.37 (−7.59, −1.37) mL/min/1.73 m^2.

Quality Control Results for Samples and Aptamer Reagents

Among 48 CRIC samples from 24 participants, one sample was flagged by a technician at the SomaLogic laboratory as having a technical problem during the assay. We included this sample in our initial blinded analysis to determine whether it and its pair could be matched based on protein levels. The flagged sample could not be paired correctly to any other sample, and it was excluded from subsequent analyses.

From the Brigham cohort, 113 samples from 49 patients were analyzed by SomaLogic. Six samples (4 patients) were flagged for quality control by SomaLogic staff. Three of these 4 patients with flagged samples had systemic lupus erythematosus. Prior quality control studies performed by SomaLogic had shown lower precision in samples from patients

with lupus, due to apparent interaction between the DNA aptamers and circulating DNA autoantibodies.

Out of 4776 unique human proteins measured in the assay, 189 were matched to more than one aptamer (including 3 proteins matched to >2 aptamers), for a total of 4979 aptamer measures of proteins. All multiple aptamer measurements for one protein were included in our analyses. After removing 130 proteins whose aptamer pairs are still investigational, there remained 4656 unique proteins (4849, including those paired to multiple aptamers) included in the analyses. Additional proteins were flagged by SomaLogic technicians for various reasons in the CRIC assays (91 proteins) and the Brigham assays (208 proteins), but we retained them in these analyses.

Analytic Variability: Intra-Assay Results in Optimal Research Conditions (CRIC Samples)

Among CRIC duplicate samples, median (IQR) intra-assay CV was 3.7% (2.8–5.3) and Spearman correlation was 0.95 (0.90–0.98) (Table 2 and Fig. 1). Among 4849 aptamers in CRIC samples, 95% had CV ≤ 10 and 99% had a CV ≤ 20%. CVs averaged over all proteins did not differ by CKD stage, diabetic status, or degree of albuminuria ($P > 0.1$ for all). Among the 23 intra-assay duplicates from CRIC participants, no individual protein's CVs differed significantly by CKD stage (using Kruskal–Wallis method and FDR $q < 0.1$ as the statistical threshold).

Analytic Variability: Intra-Assay Results in Clinical Conditions (Brigham Clinic Samples)

Among Brigham clinic duplicates, median (IQR) intra-assay CV was 5.0% (3.8–7.0), and Spearman correlation was 0.88 (0.75–0.95) (Table 2 and Fig. 1) and CVs did not differ by CKD stage, diabetic status, or degree of albuminuria ($P > 0.1$ for all). Intra-assay CV did not differ according to lupus status (median intra-assay CV, n = 2 participants, 4.1%), or according to whether patient underwent biopsy (median intra-assay CV, n = 4 participants, 4.2%). We examined CVs of samples by their plate location and patient characteristics. Among 13 duplicate pairs in the Brigham cohort, 6 were run on the same plate, with median CV= 3.7%. Seven were run on different plates, on the same day; these had a median CV= 5.2%. These intra- and inter-plate CVs were not statistically different ($P = 0.44$).

We also checked proteins flagged by SomaLogic staff as having potential technical issues in CRIC and Brigham duplicate samples. Flagged proteins had slightly higher CVs: CRIC median CVs were 5.3% vs 3.7%; Brigham median CVs were 6.0% vs 5.0%. Among all intra-assay duplicates there were 56 proteins with CV > 25% and only 8 with CV >50%. Proteins with median intra-assay CVs > 25% among CRIC or Brigham samples are listed in Supplemental II, Table 1. Additionally, we ran CV analyses on raw RFU data that had not been ANML formatted. As shown in Supplemental II, Table 2, CVs are somewhat higher when calculated using raw data.

Biological Variability: Short-Term Within-Subject Variability (Brigham Clinic Samples)

In the 44 Brigham within-subject paired samples, median (IQR) CV was 8.7% (6.2–14), and Spearman correlation was 0.77 (0.63, 0.87) (Table 2 and Fig. 2). Limiting the sample to the 39 without lupus and who did not undergo kidney biopsy, median (IQR) CV between

samples drawn median (IQR) 6 (3.5–7.5) days apart was median 7.2% (6.0–9.1). CVs did not differ between groups stratified by time interval between visits or by change in eGFR between visits ($P > 0.2$ for both). Samples labeled as having a technical assay problem did not have higher CVs. Average CVs for paired samples of 9 patients undergoing kidney biopsy were higher than the other pairs (19% vs 8.1%, $P = 0.003$). We examined changes in individual protein levels between 2 visits for each of 44 patients with serial samples. Using a *t*-test and FDR threshold of 10%, no protein had significant changes in plasma levels. The median (IQR) absolute fold change in proteins was 1.009 (1.00, 1.02), with 90% having less than 1.05 absolute fold change. In Supplemental II, Table 3, we list the 20 proteins with largest absolute fold change.

To explore the influence of analytical variability on biological variability, we compared within-subject short-term variability CVs and intra-assay duplicate CVs among 13 Brigham participants (Fig. 3). We found that, overall, most proteins (98%) had intra-assay CV ≤ 20%, and, among these, the within-subject CVs ranged from 1.2% to 54.9%, with a median of 7.2%. Overall, the median (IQR) ratio of intra-assay CV/within-subject CV was 0.76 (0.06–2.85). There were 759 proteins for which the longitudinal CVs exceeded split duplicate sample CVs (using the paired Wilcoxon rank sum test at FDR adjusted q value <0.1). The 20 proteins with the largest difference between longitudinal and split duplicate CVs are shown in Supplemental II, Table 4. For 22 proteins with intra-assay CVs >30%, within-subject CVs ranged from 21.7% to 61.5%, suggesting that for these individual proteins with high analytical variability, the within-subject CV largely reflected this higher analytical variability. The entire SomaScan V4 menu of proteins, along with CVs observed in our study, may be found in Supplemental III.

Correlations of Aptamer and Nonaptamer Biomarker Assays

Using available measurements from all samples from CRIC Visit 1, we found robust correlations between aptamer and nonaptamer measures for cystatin (n = 3309, $\rho = 0.942$) and KIM-1 (n = 312, $\rho = 0.905$). Correlations were also good for TNF receptor 1 (n = 312, $\rho = 0.781$) and TNF receptor 2 (n = 312, $\rho = 0.843$). Correlation was weakest for FGF-23 (n=1672, $\rho = 0.541$). All these Spearman correlations were highly significant ($P < 10^{-100}$). Scatterplots for these correlations are shown in Supplemental II, Fig. 1. Notably, agreement between antibody-based FGF-23 assays varies depending on whether antibodies bind intact FGF-23, C-terminal FGF-23, or both (11). The immunoassay used in CRIC measured C-terminal FGF-23, which may differ from the aptamer assay.

DISCUSSION

We have shown that the SomaScan V4 has low intra-assay CVs when applied to plasma samples from patients with moderate to severe CKD, whether samples were drawn under optimal research conditions (CRIC samples) or in a clinical setting (Brigham samples). Short-term within-subject variability was low in patients with stable CKD. These important features suggest the assay has the necessary precision to be an appropriate tool for large-scale proteomic investigations among patients with CKD.

Intra-assay CVs in our study were similar to metrics obtained in previous studies. Candia and colleagues recently published a study of technical variability for the current version of the SomaScan platform that measures 7000 proteins (12). The authors conducted tests of technical variability using duplicates of plasma samples from 102 human subjects and found the median CV among all the aptamers was 4.5% using normalized data, as opposed to 15% for the raw data. We observed a similar but smaller difference in CVs calculated from different data formats (CRIC technical variability was 3.7% for ANML normalized data vs 6.7% for raw data). In Candia and coworkers' study, normalization was performed for samples using the study set as a reference. In our study and in the most recent SomaScan applications, ANML normalization is performed using an external relatively healthy reference population; the authors stated that the custom normalization they used had similar results to ANML normalized data. In Supplemental II, Table 1, we list aptamers in our study that had technical CV >25%, and for comparison we list the CV data on the same aptamers studied by Candia et al. While the CVs are calculated from data subject to different normalization schemes, it seems that the higher CVs for these aptamers are consistent in both studies. Only 12 aptamers had technical CV > 25% in the prior study and in both cohorts in our study, so while inspection of individual markers of interest is important, only a small percentage of aptamers in the SomaScan platform have consistently high CVs.

Our observation that within-subject short-term variability is low is also consistent with prior studies (13). Authors of a reproducibility study in participants of the ARIC study, using the same version of the SomaScan platform as we did, calculated CVs among 22 subjects with eGFR >60 mL/min/1.73 m^2, each of whom had 2 plasma samples at median (IQR) 36 (30–38) days apart, and median (IQR) within-subject CVs were 6.7% (5.1–9.7) (1). A recent study of 9 selected aptamers in serum samples of participants with CKD showed the certain aptamers, such as those for interleukin-6, are not highly correlated with traditional immunoassays (14). While technical variability of the assay in serum has been shown to be slightly higher than variability in plasma (15), we know from personal data that interleukin-6 aptamers have very low correlation with immunoassays in plasma samples as well. Prior studies comparing SomaScan and O-link, a multiplexed antibody assay, have shown acceptable correlations for inflammatory markers but low correlation for FGF-23 (rho 0.47) (16), while a separate study comparing the SomaScan FGF-23 to an ELISA showed a correlation rho = 0.61 (17). Given that antibody-based FGF-23 assay results vary depending on whether antibodies bind intact FGF-23, C-terminal FGF-23, or both (11), it is plausible that differences in binding sites account for the lower correlation we observed for aptamer vs ELISA quantification of FGF-23. Overall, we observed a relatively low correlation for FGF-23 (0.541), but for the other selected markers correlations were 0.781 to 0.940.

For any samples assayed by SomaScan v4, there is an extensive set of quality controls for samples and performance of aptamers performed by SomaLogic (see Supplemental I). Our study provides an opportunity to apply similar criteria for studies of patients with CKD, an important population where the performance of the assay has not been previously investigated. Among all samples we examined, samples from patients with lupus undergoing biopsy had higher CVs. One may assume these were patients with active lupus who had higher levels of DNA autoantibodies, which may interfere with the DNA aptamer binding reagents, as per communication from SomaLogic. SomaLogic has developed alternate

protocols specific for lupus to mitigate this effect. CVs among all duplicate samples were low, indicating that technical variability of the SomaScan assay is similar in CKD as compared to plasma samples from the general population, considering the 5% intra-assay CV in CRIC samples compared to SomaLogic's internal studies showing intra-assay CV of 3.2%. Short-term within-subject variability was also low: median (IQR) CV between samples drawn median (IQR) 6 (3.5–7.5) days apart in our study, among patients with stable kidney function not undergoing biopsy, was 7.2% (6.0–9.1), compared to samples from ARIC participants without CKD, drawn median (IQR) 36 (30–38) days apart, in whom median (IQR) within-subject CVs were 6.7% (5.1–9.7) (1).

A small proportion (2%–12%) of samples in the CRIC and Brigham cohort were flagged for various quality control concerns by SomaLogic staff. Flagged aptamers had marginally higher CVs than those that were not flagged, although the median CV was still low for flagged proteins (≤6%). Overall, our results suggest that SomaLogic's criteria for flagging samples or aptamers may be more stringent than what the individual investigator may use to determine whether the sample or aptamer should be excluded from any particular study. In upcoming clinical applications of SomaScan in patients with CKD, one might exclude proteins whose CV is too large based on predetermined criteria (e.g., CVs > 50%). Conversely, one might argue that that the magnitude of any CVs should be interpreted in the context of the size of the measured "signal." Thus, even a large CV may not hinder detection of a large signal.

We were able to utilize a small group (n = 13) of participants from the Brigham clinic whose samples were used to measure both analytical variability and short-term within-subject biological variability. Historically, authors such as Fraser et al. have proposed that optimally for any given analyte, the (analytical CV) ≤0.5 (within-subject CV) (18). We note that, for 25% of the proteins, the analytical CV is less than ½ of the observed within-subject variability, and, for 78%, the analytical CV < within-subject CV. While these data are based on a small number of samples, these estimates make us optimistic that individual proteins discovered with this platform could become viable biomarkers.

Limitations

The excellent intra-assay reproducibility of the SomaScan assay in plasma from patients with CKD argues against substantial interference from substances retained due to reduced eGFR. Mixing studies could additionally be performed in future studies to further exclude interference from renally retained solutes for individual aptamers and their proteins of interest. We studied intra-assay but not inter-assay CVs. Although prior SomaLogic studies show inter-assay CVs to be similarly low to intra-assay CVs, we do recommend running blinded calibrators to evaluate assay drift within a large study where samples might be assayed over extended periods of time. Circulating titers of double-stranded DNA in lupus patients were not available, so we could not determine if there was a relationship between DNA autoantibody levels and higher CVs in lupus patients undergoing kidney biopsy for clinical indications. Additionally, orthogonal tests were limited in number of biomarkers and utilized alternate biochemical or binding assays, rather than mass spectrometry.

CONCLUSIONS

In this study, we show that SomaScan V4 intra-assay precision is excellent in plasma samples from patients with moderate to severe CKD, whether these samples are collected in an optimal research protocol, as were CRIC samples, or in the clinical setting, as were the Brigham samples. In patients with stable CKD, short-term within-subject variability is low for the majority of proteins measured, even in the setting of small interval change in eGFR. Based on these findings, we anticipate that the assay will perform well in the upcoming large-scale proteomic study of cardiovascular disease and CKD progression in patients with CKD in CRIC and other future investigations of CKD patients using this proteomic platform.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments:

We thank the CRIC study investigators for their assistance: Lawrence J. Appel, Jing Chen, James P. Lash, Robert G. Nelson, Mahboob Rahman, Vallabh O Shah, Raymond R. Townsend, Mark L. Unruh. We thank SomaLogic for performing the proteomic assays for this study. We thank SomaLogic for granting permission to use SomaLogic assay technical documents, located in Supplemental I.

Role of Sponsor:

The funding organizations played no role in the design of study, choice of enrolled patients, review and interpretation of data, preparation of manuscript, or final approval of manuscript.

DATA AVAILABILITY

To request access to data from Brigham and Women's Renal Clinic, contact Dr. Sushrut Waikar swaikar@bwh.harvard.edu. Because the CRIC data for the current study were obtained under a Data Use Agreement, they are not currently publicly available. The CRIC data are available from the CRIC study group upon request and with a Data Use Agreement. Data requests can made be by contacting the CRIC Scientific and Data Coordinating Center at cri-projmgmt@lists.upenn.edu.

Nonstandard Abbreviations:

CRIC	Chronic Renal Insufficiency Cohort
CKD	chronic kidney disease
IQR	interquartile range
eGFR	estimated glomerular filtration rate
FGF	fibroblast growth factor
KIM	kidney injury model
TNF	tumor necrosis factor

RFU	relative fluorescent units
ANML	adaptive normalization by maximum likelihood
FDR	false discovery rate

REFERENCES

1. Tin A, Yu B, Ma J, Masushita K, Daya N, Hoogeveen RC, et al. Reproducibility and variability of protein analytes measured using a multiplexed modified aptamer assay. J Appl Lab Med 2019;4:30–9. [PubMed: 31639705]
2. Anderson AH, Yang W, Hsu CY, Joffe MM, Leonard MB, Xie D, et al. Estimating GFR among participants in the Chronic Renal Insufficiency Cohort (CRIC) study. Am J Kidney Dis 2012;60:250–61. [PubMed: 22658574]
3. World Medical Association Declaration of Helsinki: ethical principles for medical research involving human subjects. JAMA 2000;284:3043–5. [PubMed: 11122593]
4. Brody EN, Gold L, Lawn RM, Walker JJ, Zichi D. High-content affinity-based proteomics: unlocking protein biomarker discovery. Expert Rev Mol Diagn 2010;10:1013–22. [PubMed: 21080818]
5. Ganz P, Heidecker B, Hveem K, Jonasson C, Kato S, Segal MR, et al. Development and validation of a protein-based risk score for cardiovascular outcomes among patients with stable coronary heart disease. JAMA 2016;315:2532–41. [PubMed: 27327800]
6. Gold L, Ayers D, Bertino J, Bock C, Bock A, Brody EN, et al. Aptamer-based multiplexed proteomic technology for biomarker discovery. PLoS One 2010;5:e15004. [PubMed: 21165148]
7. Rohloff JC, Gelinas AD, Jarvis TC, Ochsner UA, Schneider DJ, Gold L, et al. Nucleic acid ligands with protein-like side chains: modified aptamers and their use as diagnostic and therapeutic agents. Mol Ther Nucleic Acids 2014;3:e201. [PubMed: 25291143]
8. Sun BB, Maranville JC, Peters JE, Stacey D, Staley JR, Blackshaw J, et al. Genomic atlas of the human plasma proteome. Nature 2018;558:73–9. [PubMed: 29875488]
9. Williams SA, Kivimaki M, Langenberg C, Hingorani AD, Casas JP, Bouchard C, et al. Plasma protein patterns as comprehensive indicators of health. Nat Med 2019;25:1851–7. [PubMed: 31792462]
10. Levey AS, Bosch JP, Lewis JB, Greene T, Rogers N, Roth D. A more accurate method to estimate glomerular filtration rate from serum creatinine: a new prediction equation. Modification of Diet in Renal Disease Study Group. Ann Intern Med 1999;130:461–70. [PubMed: 10075613]
11. Smith ER. The use of fibroblast growth factor 23 testing in patients with kidney disease. Clin J Am Soc Nephrol 2014;9:1283–303. [PubMed: 24578336]
12. Candia J, Daya GN, Tanaka T, Ferrucci L, Walker KA. Assessment of variability in the plasma 7k SomaScan proteomics assay. Sci Rep 2022;12:17147. [PubMed: 36229504]
13. Kim CH, Tworoger SS, Stampfer MJ, Dillon ST, Gu X, Sawyer SJ, et al. Stability and reproducibility of proteomic profiles measured with an aptamer-based platform. Sci Rep 2018;8:8382. [PubMed: 29849057]
14. Lopez-Silva C, Surapaneni A, Coresh J, Reiser J, Parikh CR, Obeid W, et al. Comparison of aptamer-based and antibody-based assays for protein quantification in chronic kidney disease. Clin J Am Soc Nephrol 2022;17:350–60. [PubMed: 35197258]
15. Candia J, Cheung F, Kotliarov Y, Fantoni G, Sellers B, Griesman T, et al. Assessment of variability in the SOMAscan assay. Sci Rep 2017;7:14248. [PubMed: 29079756]
16. Daniels JR, Ma JZ, Cao Z, Beger RD, Sun J, Schnackenberg L, et al. Discovery of novel proteomic biomarkers for the prediction of kidney recovery from dialysis-dependent AKI patients. Kidney360 2021;2:1716–27. [PubMed: 34913041]
17. Yu LR, Sun J, Daniels JR, Cao Z, Schnackenberg L, Choudhury D, et al. Aptamer-based proteomics identifies mortality-associated serum biomarkers in dialysis-dependent AKI patients. Kidney Int Rep 2018;3: 1202–13. [PubMed: 30197987]

18. Fraser CG, Harris EK. Generation and application of data on biological variation in clinical chemistry. Crit Rev Clin Lab Sci 1989;27:409–37. [PubMed: 2679660]

IMPACT STATEMENT

Large-scale proteomics is now feasible as a powerful method of finding novel biomarkers in epidemiological cohorts. We present a pilot study of SomaScanV4, an assay for nearly 5000 proteins, in plasma from patients with chronic kidney disease, collected in both research and clinical settings. The assay has excellent reproducibility, a finding that supports the use of SomaScan in ongoing studies aimed at finding novel biomarkers for patients with chronic kidney disease.

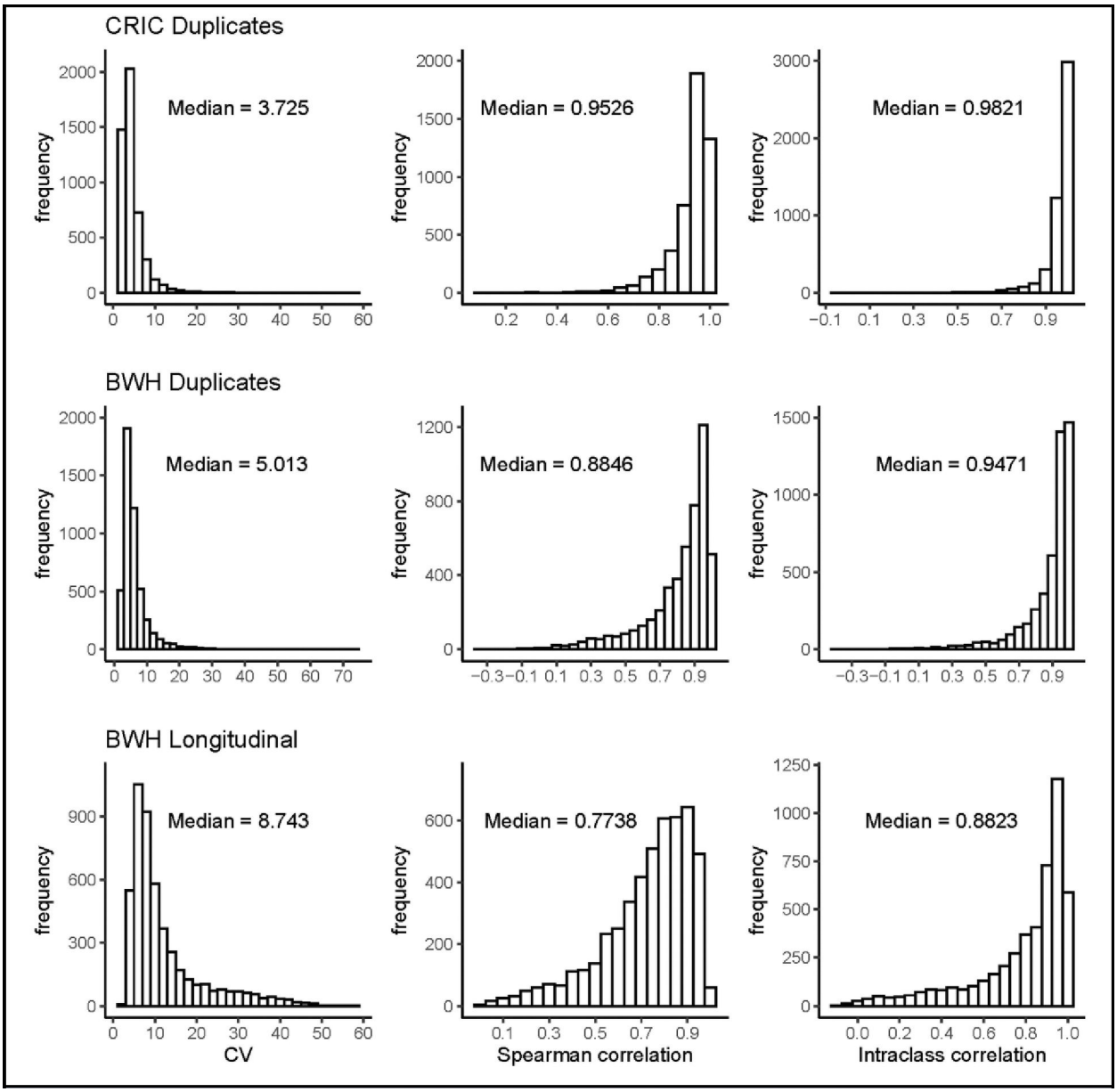

Fig. 1.

Histograms representing summary reproducibility metrics. Histograms show distribution of CVs, Spearman correlations, and intraclass correlations among 4849 proteins in each set of samples pairs. X-axes for histograms from left to right are CV, Spearman correlation, and intraclass correlation. Each of these metrics quantifies variation for each individual protein between pairs of samples. Abbreviations: BWH, Brigham and Women's Hospital–Kidney/Renal Clinic.

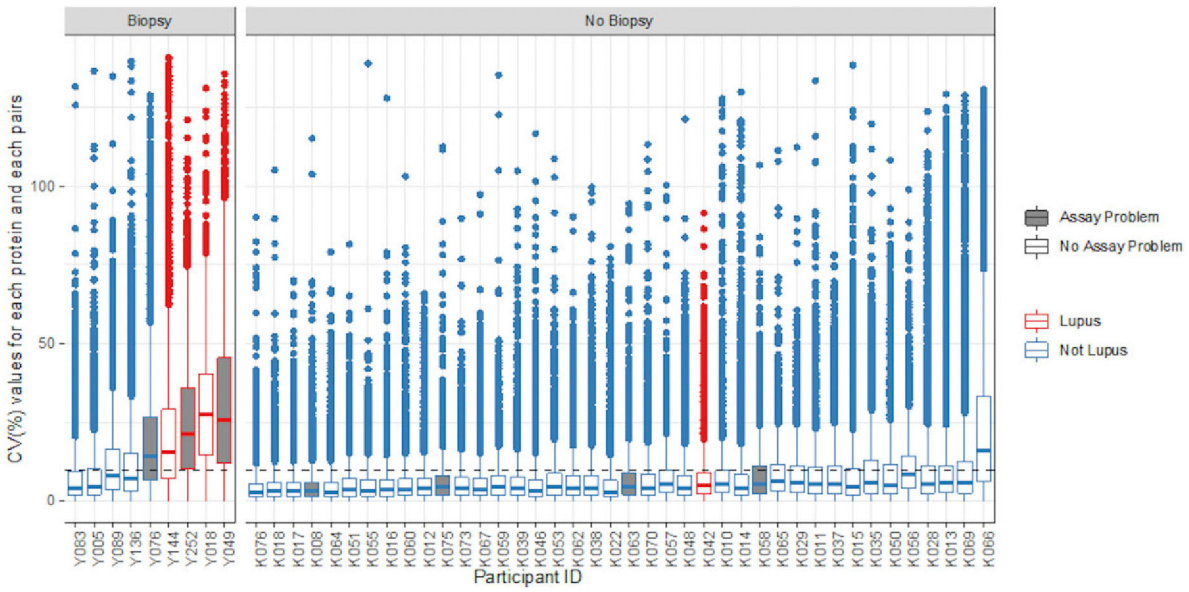

Fig. 2.

Brigham samples: longitudinal CVs in 49 patients. Longitudinal coefficients of variation (CV) were calculated in 49 participants who had 2 samples separated by median (IQR) 7 (4, 14) days. Each box represents median, (IQR) CV for all proteins in each participant. The dashed line marks 10% CV.

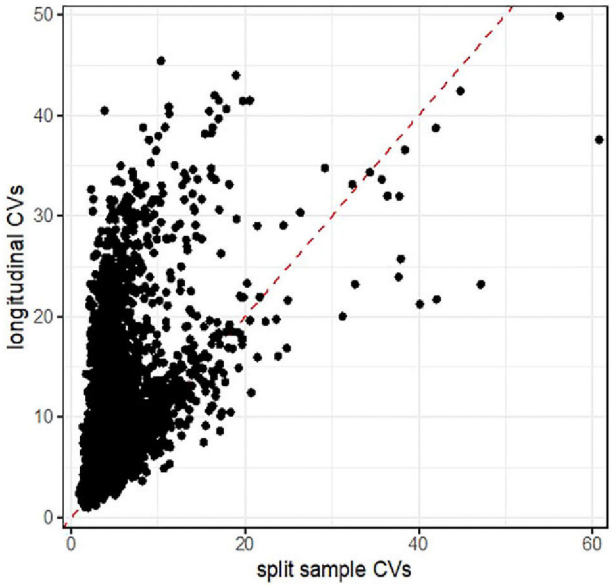

Fig. 3.

Scatterplot for longitudinal vs duplicate split sample CVs. Scatterplot of longitudinal CVs (vertical axis) and split duplicate sample CVs (horizontal axis) in 13 patients from the Brigham cohort.

Table 1.

Baseline characteristics of participants included in the quality control study.

	Brigham (n = 44 participants) n (%) mean (SD) or median (IQR)	Brigham (n = 13 participants in split duplicate CV analysis)	CRIC (n = 23 participants) n (%) mean (SD) or median (IQR)
N participants contributing split duplicate samples	13	13	23
Time interval between longitudinal samples (days)	7 (4, 14)	—	—
Age, years	59 (17)	56 (13)	59 (13)
Male	26 (59)	7 (54)	14 (61)
Race			
White	32 (73)	9 (69)	8 (35)
Black or African American	11 (25)	3 (23)	8 (35)
Unknown or other race	1 (2)	1 (8)	7 (30)
Diabetes	12 (27)	3 (23)	12 (52)
CKD stage			
1 (eGFR 90 + mL/min/1.73 m^2)	3 (6.8)	0	0
2 (eGFR 60–89 mL/min/1.73 m^2)	6 (13.6)	3 (23.1)	0
3A (eGFR 45–59 mL/min/1.73 m^2)	10 (22.7)	3 (23.1)	8 (34.8)
3B (eGFR 30–44 mL/min/1.73 m^2)	13 (29.5)	3 (23.1)	7 (30.4)
4 (eGFR 15–29 mL/min/1.73 m^2)	10 (22.7)	3 (23.1)	8 (34.8)
5 (eGFR <15 mL/min/1.73 m^2)	2 (4.55)	1 (7.7)	0
CKD etiology			
Diabetes	11 (25)	3 (23.1)	11 (48)
Hypertension	9 (21)	1 (7.7)	5 (22)
Glomerulonephritis	8 (18)	4 (30.8)	2 (9)
Nephrectomy	6 (14)	1 (7.7)	0
Drug toxicity	3 (7)	0	0
Polycystic kidney disease	1 (2)	1 (7.7)	0
Hereditary or congenital	1 (2)	0	1 (4)
Other	5 (11)	3 (23.1)	1 (4)
ACE/ARB	31 (71)	8 (62)	11 (48)
Diuretic	17 (39)	8 (62)	15 (65)
SBP, mmHg	134 (19)	134 (11)	124 (21)

	Brigham (n = 44 participants) n (%) mean (SD) or median (IQR)	Brigham (n = 13 participants in split duplicate CV analysis)	CRIC (n = 23 participants) n (%) mean (SD) or median (IQR)
DBP, mmHg	78 (15)	80 (12)	67 (10)
BMI	30 (8)	32 (7)	29 (7)
eGFR, mL/min/1.73 m^2	46 (23)	40 (18)	38 (14)
Interval change in eGFR, mL/min/1.73 m^2	−1.275 (4.65)	—	—
Creatinine, mg/dL	2.00 (1.23)	2.16 (1.09)	2.02 (0.75)
ACR, mg/g	645 (1084)	772 (1432)	514 (1186)

Five participants of Brigham Renal Clinic with lupus are excluded from this table.

ACE/ARB, angiotensin converting enzyme inhibitors or angiotensin receptor blockers; SBP, systolic blood pressure; DBP, diastolic blood pressure; BMI, body mass index; ACR, albumin to creatinine ratio.

Table 2.

Summary metrics of intra-assay and short-term biological variability for the SomaScan assay.

Sample subgroup	N pairs	CV (%) (Median, IQR)	Spearman correlation (median, IQR)	Intraclass correlation (median, IQR)
CRIC split duplicates	23	3.73 (2.8–5.3)	0.95 (0.90–0.98)	0.98 (0.96–0.99)
Brigham split duplicates	13	5.01 (3.8–7.0)	0.88 (0.75–0.95)	0.95 (0.86, 0.98)
Brigham longitudinal pairs	44	8.7 (6.2–14)	0.77 (0.63–0.87)	0.88 (0.70–0.95)

Reproducibility for each of 4849 proteins were measured in split duplicate and longitudinal paired samples, using ANML formatted proteomic data. Five participants of Brigham Renal Clinic with lupus are excluded from this table.