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Comparison of symmetric dimethylarginine with creatinine, cystatin C and their eGFR equations as markers of kidney function☆

Joe M. El-Khoury [a], Dustin R. Bunch [b], Bo Hu [c], Drew Payto [b], Edmunds Z. Reineks [b], Sihe Wang [b,*]

[a] Department of Laboratory Medicine, Yale University, New Haven, CT 06510, United States
[b] Department of Laboratory Medicine, Cleveland Clinic, Cleveland, OH 44195, United States
[c] Department of Quantitative Health Sciences, Cleveland Clinic, Cleveland, OH 44195, United States

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ABSTRACT

Objectives: Symmetric dimethylarginine (SDMA) is a catabolic product of arginine-methylated proteins and is an emerging biomarker for kidney function. A limited number of studies in selected populations have shown good correlation between SDMA and a few known markers of glomerular filtration rate (GFR). However, a comprehensive comparison of SDMA with all existing serum endogenous markers in a population with varied kidney function and against measured GFR is lacking. The objective of this study was to compare the correlations of SDMA, creatinine, cystatin C and their eGFR equations against GFR measured by iothalamate clearance in an adult population with varied kidney function.

Design & methods: Left-over serum and plasma specimens were collected from 40 adults with normal and reduced kidney function. GFR was measured using a radioactive iothalamate procedure. Creatinine and cystatin C were measured on Roche Cobas 8000. SDMA was measured by a published liquid chromatography-tandem mass spectrometry method.

Results: SDMA correlated highly with measured GFR ($r = -0.84$), which was better than creatinine ($r = -0.70$) but equivalent to cystatin C ($r = -0.86$) and the eGFR equations [MDRD and CKD-EPI (separate and combined)].

Conclusions: SDMA is a strong marker of kidney function and further studies are needed to establish an eGFR formula that includes it for widespread clinical use.

1. Introduction

Chronic kidney disease (CKD) impacts 13.1% of the U.S. population, with an estimated 80,000 new cases introduced yearly [1,2]. Glomerular filtration rate (GFR) is the best overall index of kidney function, and is essential for the diagnosis, classification, management, and monitoring of kidney disease [3]. Serum creatinine and cystatin C (cysC) are the most widely used markers for estimating GFR (eGFR). However, both markers are impacted by several factors unrelated to kidney function, which limits their sensitivity and specificity for estimating GFR [4]. While equations using these markers have been developed to account for variations in age, sex and race, other factors remain unaccounted for [5]. These factors include diet, medications that inhibit tubular secretion and extremes of muscle mass for creatinine, and thyroid disease, obesity, inflammation and atherosclerosis for cysC [4,6]. As a result, endogenous markers with higher specificity for kidney function are highly desirable.

Symmetric dimethylarginine (SDMA) is the catabolic product of post-translationally methylated arginine-containing proteins and is primarily eliminated by the kidneys [7]. The plasma level of SDMA has been shown to increase in patients with kidney disease and to correlate with GFR in patients with CKD [8]. In addition, SDMA has the advantage of not being influenced by non-renal factors that are proven to influence creatinine and/or cystatin C, such as muscle mass, diet, inflammation, diabetes, and estrogen therapy [9]. Furthermore, SDMA is minimally influenced by obesity, gender, age, and polycystic ovary syndrome [9]. Most impressively, SDMA was shown to be consistent among species (cats and dogs) and is used for assessing kidney function in veterinary medicine [9]. However, an across the board study comparing the performance of SDMA with cysC, creatinine and their eGFR equations against a direct measure of GFR in an adult population including both normal and

Abbreviations: SDMA, symmetric dimethylarginine; GFR, glomerular filtration rate; cysC, cystatin C; CKD, chronic kidney disease; eGFR, estimated GFR; mGFR, measured GFR; MDRD, Modification of diet in renal disease; CKD-EPI$_{cr}$, Chronic kidney disease epidemiology collaboration creatinine-based equation; CKD-EPI$_{cys}$, CKD-EPI cystatin c-based equation; CKD-EPI$_{cr + cys}$, CKD-EPI combined creatinine and cystatin c-based equation.

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* Corresponding author at: Department of Laboratory Medicine, Robert J. Tomsich Pathology and Laboratory Medicine Institute, Cleveland Clinic, 9500 Euclid Ave, LL3-140, United States.
E-mail address: wangs2@ccf.org (S. Wang).

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reduced kidney function is lacking in the literature. This comparison is essential to evaluate plasma SDMA as a marker of kidney function in humans.

The aim of this study was to investigate the correlation between SDMA and measured GFR (mGFR), as determined by a radioactive iothalamate method, across a wide range of GFR values, and to compare it to that of creatinine, cysC and their estimated GFR (eGFR) equations.

2. Materials and methods

2.1. Study population

Participants selected for this study by chart review included all patients for whom there was an order for accurate GFR measurement by the radioactive method and who had sufficient left-over serum and plasma specimens for SDMA, creatinine, and cysC analyses over a 1-year period beginning September 2011. Patients referred for GFR measurement were being evaluated as kidney donors, for CKD staging or for drug dosing with nephrotoxic medication. Other important characteristics are summarized in Table 1. The Cleveland Clinic's Institutional Review Board approved this study.

2.2. Laboratory analysis

GFR was measured as the renal clearance of an exogenous molecule ^{125}I – sodium iothalamate (Glofil®, Isotex Diagnostics, Friendswood, TX). Briefly, this procedure involved administering 15 ± 5 μCi of Glofil® subcutaneously in one arm of the patient followed by bracketed collection of urine and blood samples. Whole blood samples were collected in serum separator tubes, centrifuged for 10 min at 3000 rpm (1000*g*), and resulting serum was aliquoted into 0.5 mL gamma counting tube with 0.25 mL analyzed by the Wallac Wizard® 1470 gamma counter (Perkin Elmer, Waltham, MA). Urine was collected in plain collection devices and was aliquoted and analyzed without centrifugation. Left-over EDTA plasma used for SDMA measurement was collected on the same day as the mGFR procedure was performed. Left-over serum and plasma were frozen at −70 °C up to two years until analysis. Long term stability studies (>1 year) for SDMA in frozen samples are lacking, but anecdotal evidence supports that it is stable for at least 5 years at −20 °C or −80 °C [9]. Quantification of SDMA was performed using our published liquid chromatography-tandem mass spectrometry (LC–MS/MS) assay [10]. In brief, the SDMA assay requires 50 μL of EDTA plasma, is linear from 0.06 μM to 5.15 μM, with 101–118% recovery, <7% total imprecision and a reference interval of 0.32 μM to 0.65 μM. Left-over serum in serum separator tubes, collected at the same time as EDTA plasma, was used for the analysis of creatinine and cysC. Creatinine and cysC were analyzed on the Cobas 8000® (Roche Diagnostics, Indianapolis, IN) with manufacturer reported interassay CV of <1.5% and <2.7%, respectively.

2.3. Statistical analysis

Subject characteristics were summarized as medians and ranges or frequencies and percentages as appropriate. Spearman correlations were computed between SDMA, creatinine, cysC, MDRD, CKD-EPI$_{cr}$, CKD-EPI$_{cys}$, CKD-EPI$_{cr\ +\ cys}$ and mGFR, respectively (Table 2). The *t*-test was used to compare the SDMA concentrations between those with GFR <60 and those with GFR >90. Statistical analysis was conducted with the use of Excel 2010 (Microsoft, Redmond, WA). Two-tailed *P* values of <0.05 were considered statistical significance.

3. Results

3.1. Study participant characteristics

We analyzed samples in the Cleveland Clinic Renal Laboratory from 40 randomly selected patients who had clinical indication for measuring GFR and sufficient left-over serum and plasma samples for SDMA, creatinine, and cysC analysis. Participants included 22 males and 18 females with ages ranging from 21 to 76 years and the vast majority being white (n = 35/40). Their GFR levels, as measured by the radioactive iothalamate method, ranged from 13 to 143 mL/min/1.73 m^2 with 16 and 13 participants having GFR values <60 and >90 mL/min/1.73 m^2, respectively. Other important characteristics are summarized in Table 1.

3.2. SDMA concentrations

Plasma concentrations of SDMA were significantly lower in adults with GFR > 90 mL/min/1.73 m^2 versus adults with GFR <60 mL/min/1.73 m^2 [mean (SD); 0.45 (0.1) vs 1.09 (0.8) μM, P = 0.006]. Using the previously established upper-end of the reference interval of 0.65 μM for SDMA as a cut-off for CKD yielded the following clinical performance: sensitivity = 81.3%, specificity = 100%, positive predictive value = 100%, negative predictive value = 88.9%.

3.2.1. Correlation of SDMA, CysC, creatinine, and eGFRs with mGFR

The associations between mGFR and other markers of kidney function: creatinine, SDMA and cysC were negatively associated with mGFR (r = −0.70, −0.84 and −0.86, respectively) (Fig. 1), while MDRD, CKD-EPI$_{cr}$, CKD-EPI$_{cys}$, CKD-EPI$_{cr\ +\ cys}$ were positively associated with mGFR (r = 0.84, 0.86, 0.88, and 0.90 respectively) (data not shown).

Table 1
Characteristics of study participants.

Characteristic	Overall (n = 40)
Age, years, mean (range)	52 (21–76)
Male sex, n (%)	22 (55)
White race, n (%)	35 (87.5)
mGFR$_{iothalamate}$, mL/min/1.73 m^2, median (range)	66 (13–151)
Creatinine, μM, median (range)	81 (42–151)
Cystatin C, mg/L, median (range)	0.96 (0.53–3.53)
SDMA, μM, median (range)	0.57 (0.33–3.88)
MDRD, mL/min/1.73 m^2, median (range)	75 (22–140)
2009 CKD-EPI$_{cr}$, mL/min/1.73 m^2, median (range)	80 (22–135)
2012 CKD-EPI$_{cys}$, mL/min/1.73 m^2, median (range)	79 (14–137)
2012 CKD-EPI$_{cr\ +\ cys}$, mL/min/1.73 m^2, median (range)	84 (20–139)

MDRD, Modification of diet in renal disease; CKD-EPI$_{cr}$, Chronic kidney disease epidemiology collaboration creatinine-based equation; CKD-EPI$_{cys}$, Chronic kidney disease epidemiology collaboration cystatin c-based equation; CKD-EPI$_{cr\ +\ cys}$, Chronic kidney disease epidemiology collaboration combined creatinine and cystatin c-based equation; SDMA, symmetric dimethylarginine.

Table 2
Correlations of SDMA, creatinine, cystatin C and their eGFR equations with mGFR as determined by iothalamate clearance.

Marker	Spearman correlation[a]
Creatinine	−0.70
Cystatin C	−0.86
MDRD	0.84
2009 CKD-EPI$_{cr}$	0.86
2012 CKD-EPI$_{cys}$	0.88
2012 CKD-EPI$_{cr\ +\ cys}$	0.90
SDMA	−0.84

MDRD, Modification of diet in renal disease; CKD-EPI$_{cr}$, Chronic kidney disease epidemiology collaboration creatinine-based equation; CKD-EPI$_{cys}$, Chronic kidney disease epidemiology collaboration cystatin c-based equation; CKD-EPI$_{cr\ +\ cys}$, Chronic kidney disease epidemiology collaboration combined creatinine and cystatin c-based equation; SDMA, symmetric dimethylarginine.

[a] All *p*-values are <0.001.

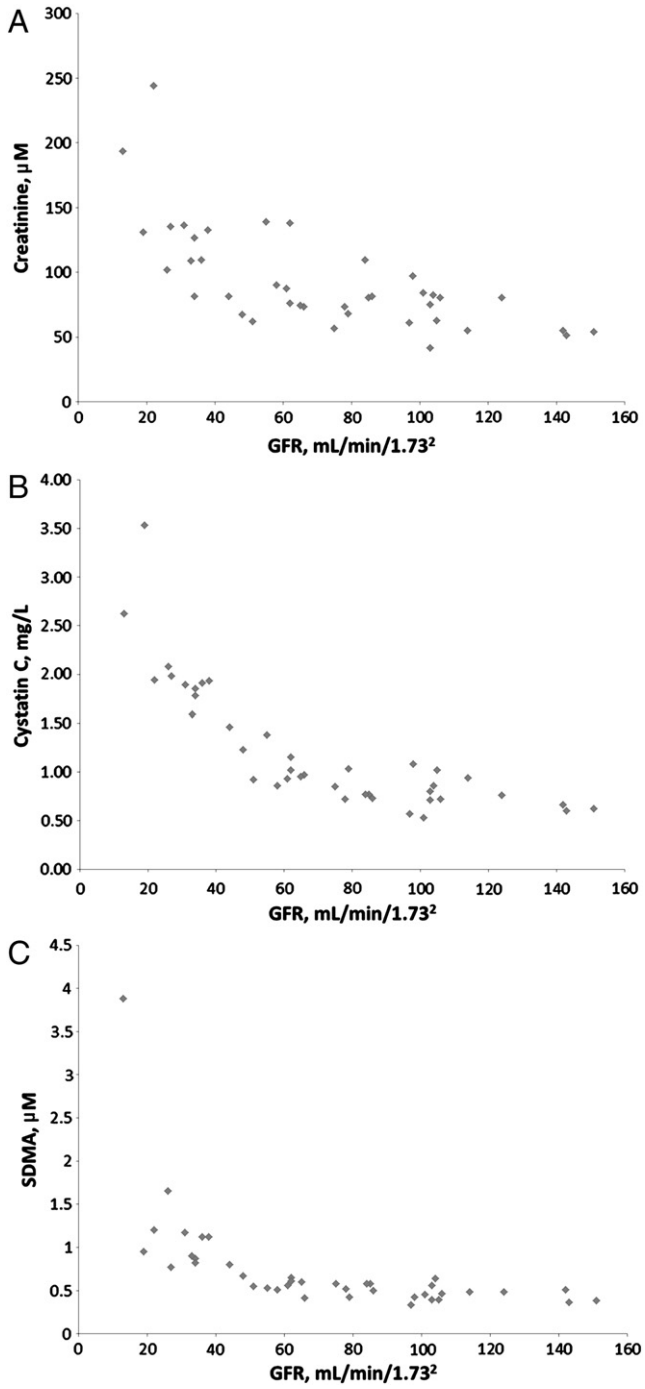

Fig. 1. Correlations of creatinine (A), CysC (B), and SDMA (C) with mGFR. Solid line represents a power trendline of the data.

4. Discussion

In this study, we investigated the relationship between SDMA and mGFR in a randomly selected sample ($n = 40$) with a wide range of GFR values, and compared its correlation to those of creatinine, cysC and their eGFR equations with mGFR. We observed that SDMA is significantly lower in adults with GFR >90 mL/min/1.73 m^2 when compared with adults with GFR <60 mL/min/1.73 m^2 ($P = 0.006$) and that it strongly correlates with GFR ($r = -0.84$, $P < 0.001$). These results are in-line with published studies that compared the correlation of SDMA with mGFR in patients with CKD or type 1 diabetes and reported r values of 0.78–0.90 in populations of 24 to 394 subjects [11–13]. Our study establishes this strong correlation across the entire range of GFR values. In addition, comparison with existing markers for kidney function revealed that SDMA outperforms creatinine, but is not statistically different from cysC or the eGFR equations. This is the first study to report these findings against a direct measure of GFR. A previous study examined the performance of SDMA versus creatinine-based equations using a cysC-based equation as the standard, and SDMA also outperformed creatinine [14].

The limitations of this study are its small sample size and that the majority of participants are white. Further validation using a larger and more ethnically diverse set of participants is needed. Nevertheless, the present study demonstrates that SDMA is a strong marker for kidney function that deserves further validation. Further studies investigating its potential confounding non-GFR factors are needed to assist in developing an SDMA specific eGFR equation. In that regard, it has already been established that SDMA increases with age and that it is slightly higher in men (95% reference interval: 0.30–0.67 μM) versus women (95% reference interval: 0.27–0.63 μM) [15]. Furthermore, no racial differences have been observed for SDMA in a study examining levels in Caucasian versus African men, which represents another advantage over creatinine [16]. Therefore, an eGFR equation accounting for variations in age and gender is highly desirable.

Other advantages of SDMA as a marker include low intra-individual biological variability (5.8%) when compared with cysC (8.6%) [17,18]. SDMA has also been shown to be an early marker of change in GFR after living-related kidney donation, along with creatinine and cysC within 6 h post-transplantation [19]. However, there are currently no automated immunoassays available for the measurement of SDMA and thus measurement of SDMA values for now will be available only to clinical laboratories with LC–MS/MS capabilities.

5. Conclusions

This study demonstrates that SDMA is a strong marker of kidney function that correlates better with mGFR than creatinine but is similar to cystatin C. Commercial availability of a standardized immunoassay along with the development of a SDMA-specific eGFR equation will significantly bolster research on SDMA and improve its utilization in the clinical field.

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