RESEARCH LETTER

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TO THE EDITOR:

Use of multiple urinary biomarkers for the early detection of chronic kidney disease in sickle cell anemia

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Sickle cell anemia (SCA) affects ~100 000 people in the United States, primarily African Americans.[1] The life expectancy for individuals with SCA is lower than that of African Americans without SCA (54 years vs 76 years, respectively).[1] Around the third decade of their lives, patients develop long-term complications, which result in a lower quality, adjusted life expectancy.[1] Chronic kidney disease (CKD) is one of the most common complications, affecting ~28% to 68% of patients and is associated with ~16% to 18% early mortality.[2,3]

The mechanism of CKD is multifactorial and includes hemolysis, inflammation, and iron overload leading to glomerular, tubular, and endothelial injury. Identification of the early stages of CKD in SCA can lead to the initiation of early treatment. High glomerular filtration rates (GFRs) and reduced urine concentrating ability are common in SCA and significantly impact CKD detection. Thus, novel biomarkers of early-stage CKD are highly desired. Recently, positive correlations between kidney injury molecule 1 (KIM-1) and N-acetyl-b-D-glucosaminidase (NAG) with persistent albuminuria was demonstrated in a longitudinal study of 303 patients with SCA.[4,5]

We identified urinary biomarkers of CKD, which reflect basic pathophysiological mechanisms of SCA, including iron homeostasis (ceruloplasmin [CP])[6] and inflammation (orosomucoid [ORM]).[7] However, the accuracy of each biomarker was not enough to be used individually.

In this study, we tested the hypothesis that the combination of biomarkers for basic pathological mechanisms of SCA improves the accuracy of early detection of CKD.

We re-evaluated results for 45 patients with SCA from the University of Illinois Chicago. The protocol was approved by the institutional review board, and individuals provided written informed consent.

General and renal function characteristics of patients are shown in Table 1. An estimated GFR (eGFR) was calculated using the creatinine (Cr)–based equation without race adjustment.[8] CKD was defined in accordance with the National Kidney Foundation Kidney Disease Outcomes Quality Initiatives guidelines: stage 0 (without CKD if eGFR >90 mL/min per 1.73 m^2 and albuminuria <30 mg/g Cr) and stage 1 (eGFR >90 mL/min per 1.73 m^2 and albuminuria ≥30 mg/g Cr).

Random urine samples were collected during a clinic visit when patients were in a steady state. Urine levels of CP,[6] hemoglobin (Hb),[4] and ORM[7] were measured using an enzyme-linked immunosorbent assay and normalization of urinary Cr levels.

Differences in the accuracy of biomarker combinations were compared with the simple model (Hb) and the complete model, with 3 biomarkers. We used Hb as a reference single biomarker model because it was strongly associated with CKD in a longitudinal study of 356 patients.[9] We used patients with stage 1 CKD as the at-risk group, and without CKD (stage 0) as the reference group. A 2-sided $P < .05$ was

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Table 1. General and renal function characteristics of patients with SCA

Variable	Without CKD	With CKD	*P*
No. of patients	23	22	
Female/male	9/14	13/9	.238
Mean age (SD), y	33.7 (2.3)	37.6 (2.0)	.137
Mean eGFR (SD), mL/min per 1.73 m^2	118.7 (4.0)	119.7 (2.7)	.617
Mean urinary albumin/Cr (SD), mg/g	14.2 (1.6)	271.0 (68.2)	<.001
No. of patients with hyperfiltration	4	3	1.000

Hyperfiltration was defined as eGFR >130 mL/min per 1.73 m^2 for women and >140 mL/min per 1.73 m^2 for men.

Fisher exact test and Kruskal-Wallis rank test were used to determine statistical significance for categorical and continuous variables, respectively.

SD, standard deviation.

considered significant. Statistical analysis was done using Stata version 15 (StataCorp, College Station, TX).

Our first analysis of receiver operating characteristic curve evaluated cutoffs that differentiate patients with stage 1 of CKD from those without CKD for the individual biomarker (Hb, CP, and ORM). All biomarkers showed low Youden indexes (YIs, ≤75%) that limited their usage as single biomarkers (Table 2; models A, 1, and 2). The combination of 3 biomarkers significantly improved the YI (Table 2) from 69.6% (model A) to 78.1% (model B) and increased the area under the curve (AUC) from 0.858 to 0.948 ($P = .018$). It also increased the positive predictive value (PPV) from 69.5% to 79.2% but slightly reduced the NPV from 100% to 96.3%. Then, the combination of 2 biomarkers were compared for models A and B. The combinations of Hb + CP (model 3) and CP + ORM (model 5) did not improve the YI but demonstrated trends for the improvement of AUC (0.923 [model 3] and 0.927 [model 5]) compared with model A (0.858). In contrast, the Hb + ORM combination (model 4) improved YI (77.9%) and AUC (0.919). Thus, all combinations of the 2 biomarkers slightly improved the accuracy of the stage 1 detection. The lack of statistical significance for the 2-biomarker models might be because of the small sample size.

The main limitation in the determination of early stages of CKD in patients with SCA is the sole use of microalbuminuria to differentiate between stage 0 and stage 1 because most patients have an eGFR >90 mL/min per 1.73 m^2 for both stages (Table 1). Transient albuminuria is common in patients with SCA and does not always lead to CKD.[5,10] The calculation of eGFR based on the CKD Epidemiology Collaboration Cr-cystatin equation for the cohort from the University of Illinois Chicago resulted in an estimate of 60.9% of patients without renal disease and 63.2% with stage 1 CKD and hyperfiltration.[11] Recalculation of the eGFR without adjusting for race significantly reduced the number of patients with hyperfiltration (17.4% of patients without CKD and 13.6% with stage 1). In our study, patients without renal disease and stage 1 CKD had similar eGFRs (118.7 ± 4.0 mL/min per 1.73 m^2 and 119.7 ± 2.7 mL/min per 1.73 m^2, respectively; $P = .617$) (Table 1). Thus, eGFR calculation did not affect our analysis.

In a multicenter study of 356 patients with SCA, hemoglobinuria levels were strongly associated with the reduction of eGFR and progression of albuminuria.[4,5,9] The advantage of Hb detection is the availability of clinical dipstick urinalysis tests for rapid screening in communities or low-resource settings. In this article, the measurement of hemoglobinuria demonstrates low specificity (69.6%) and low PPV (69.5) for the detection of stage 1 CKD.

Glomerular filtration barrier provides an electrostatic barrier to the filtration of negatively charged proteins, and the loss of negative charge selection is observed before the increase of albumin secretion.[12,13] CP and ORM are more negatively charged proteins than albumin and might be secreted at early stages of CKD, before the onset of albuminuria. Urinary CP has been used as a biomarker of CKD in patients with diabetes.[14] In this article, the CP-based test (Table 2, model 1) has a specificity of 91.3% and a sensitivity of 77.3% (Table 2), similar to the sensitivity (90% to 91%) and specificity (61% to 66%) in diagnosing diabetic kidney disease.[15]

High levels of urinary ORM have been detected in diabetic nephropathy and systemic lupus erythematosus–associated renal diseases.[16,17] Urinary ORM test has high diagnostic efficiency for the early screening of renal disease in type 2 diabetes with the cutoff value of 3.69 mg/g (83.3% sensitivity and 90.3% specificity).[17] In our evaluation, the cutoff value and test sensitivity are higher (Table 2; model 2: 5.75 mg/g cutoff and 90.9% sensitivity) but specificity is lower (82.6%). The combination of any 2 biomarkers slightly improves detection of stage 1 CKD, but only the combination of 3 biomarkers significantly improve YI, AUC, specificity, and PPV.

Table 2. Accuracy of using different combinations vs the single biomarker of hemoglobinuria for the detection of CKD in patients with SCA

Model (biomarker combination)	Sensitivity (n = 22), % (n)	Specificity (n = 23), % (n)	PPV, %	NPV, %	YI, %	AUC		
						AUC value	*P*	*P*
A (Hb)	100.0 (22)	69.6 (16)	69.5	100.0	69.6	0.858	**Reference**	0.018
B (Hb + CP + ORM)	95.5 (21)	82.6 (19)	79.2	96.3	78.1	0.948	0.018	**Reference**
1 (CP)	77.3 (17)	91.3 (21)	86.1	85.3	68.6	0.843	0.938	0.011
2 (ORM)	90.9 (20)	82.6 (19)	78.4	92.9	73.5	0.868	0.729	0.039
3 (Hb + CP)	77.3 (17/22)	91.3 (21/23)	86.1	85.3	68.3	0.923	0.059	0.245
4 (Hb + ORM)	90.9 (20/22)	86.9 (20/23)	82.9	93.2	77.9	0.919	0.069	0.272
5 (CP + ORM)	95.5 (21/22)	78.3 (18/23)	75.3	96.1	73.8	0.927	0.177	0.214

Criteria or cutoffs for each model was model A, ≥0.529 ng/mg; model B, ≥2 markers; model 1, ≥1.747 mg/g; model 2, ≥5.750 mg/g; and model 3 to model 5, ≥2 markers.

A test for the equality of the AUC compared with the simple and complete models was performed. Receiving operating characteristic curves were constructed to determine appropriate cutoffs for each biomarker based on the highest values of YI.

PPV and NPV were calculated in the scenario that the prevalence of patients with stage 1 CKD is 48.9% (95% confidence interval, 33.7%-64.2%).

Bold letters were used to indicate the reference model.

NPV, negative predictive value.

The limitations of this study are the small sample size and the limited number of biomarkers. In addition, the enzyme-linked immunosorbent assays used for the determination of levels of CP and ORM in urine have been validated only in a small cohort. The development of a simple panel test may facilitate the usage of multiple biomarkers as a routine test in clinical settings. Markers of renal glomerular and tubular endothelial injury such as neutrophil gelatinase–associated lipocalin, nephrin, NAG, and KIM-1 can be included in larger studies. Future studies will also include direct measurements of GFR because of the limitations of Cr-based equations for the calculation of eGFR in patients with SCD.[18] Random urine samples may increase the interperson variability of each biomarker, and the results may need to be confirmed using a 24-hour urine collection or first morning void urine samples.[19]

The application of multiple biomarkers is valuable in the prediction of death in cases of heart failure, diabetes mellitus, and atrial fibrillation.[20-22] CKD in patients with SCA is a multifactorial condition and is likely to be the result of different pathological mechanisms. The use of multiple biomarkers could facilitate the development of new treatment strategies and risk stratification.

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