Exploring Biomarker Profiles in the Screening of Sickle Cell Nephropathy

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Background: Sickle cell nephropathy is a major complication of sickle cell disease (SCD), leading to health and economic burdens. Early detection is important to prevent irreversible damage, however, conventional biomarkers often lack sensitivity for detecting early-stage nephropathy. This study aims to identify biomarker profiles across the most common SCD phenotypes (hemolysis-dominant and vaso-occlusive-dominant) to enhance early diagnosis.

Materials and methods: This cross-sectional study included SCD patients from the Radboud Iron Biobank, enrolled during routine follow-up visits. Patients were categorized by predominant phenotype for HbSS and HbSβ^0 genotypes: hemolysis-dominant or vaso-occlusive dominant, and HbSC/HbSD genotypes. Urine and blood biomarkers were assessed during routine visits, with no signs of inflammation or crisis.
Glomerular markers included urinary albumin, total protein, and cystatin C; tubular markers included α1-microglobulin (α1MG), kidney injury molecule-1 (KIM-1), neutrophil gelatinase-associated lipocalin (NGAL), and urine osmolality. Vascular markers included soluble fms-like tyrosine kinase-1 (sFlt-1) and endothelin-1 (ET-1). Clinical data were extracted from electronic records, and informed consent was obtained from all participants.

Results: Thirty-one patients (median age 20.4 years, range 3.1-58.3) were included: 12 in the vaso-occlusive dominant group, 13 in the hemolysis-dominant group, and 6 in the HbSC/HbSD-group. Most patients (n=21) were receiving hydroxyurea. Seven adults (23%) had microalbuminuria (range 3.7-12.0 mg/mmol) (**Table 1**). Serum creatinine and α1-microglobulin-to-creatinine ratio (α1MGCR) were significantly higher in albuminuric patients.
Serum cystatin C levels were highest in the HbSC/HbSD-group and did not vary with age or albuminuria status (**Figure 1**). Albumin-to-creatinine ratio (ACR) and serum creatinine were similar across subphenotypes (**Table 2**). Hydroxyurea treatment was associated with lower cystatin C in the HbSC/HbSD and hemolysis-dominant groups but not in the vaso-occlusive dominant group (**Figure 1**).
The tubular markers α1MG and KIM-1 showed no significant differences by subphenotype or hydroxyurea use, although there was a trend toward lower levels with hydroxyurea. Results for NGAL and KIM-1 will follow.
Among vascular markers, sFlt-1 was highest in the hemolysis-dominant group and correlated with hemolytic parameters, with no differences between hydroxyurea-treated and untreated patients (**Figure 1**).

Conclusion:
This exploratory study revealed that cystatin C-levels were highest in the HbSC/HbSD-group, with significantly lower levels in hydroxyurea-treated patients in both the HbSC/HbSD- and hemolysis-dominant groups, suggesting a potentially shared inflammatory pathway. Higher sFlt-1 levels in the hemolytic-dominant group indicated hemolysis-associated endothelial damage. The novel biomarkers showed no correlation with ACR, except for α1MGCR.
Further research with larger sample sizes is needed to validate these findings and explore the underlying biological processes, potentially leading to more targeted treatment strategies.

Table 1. Demographic characteristics of patients with sickle cell disease

	Total population n = 31	Vaso-occlusive dominant n = 12	Hemolysis-dominant n = 13	HbSC/HbSD n = 6
Clinical findings				
Age (years), median (range)	19.9 (3.1-58.3)	19.8 (3.1-40.8)	19.9 (6.2-45.7)	16.7 (8.6-58.3)
Gender				
- Male	15	7	6	2
- Female	16	5	7	4
Genotype				
HbSS	24	11 (92%)	13 (100%)	-
HbSβ^0	1	1 (8%)	-	-
HbSC	5	-	-	5 (83%)
HbSD	1	-	-	1 (17%)
Alpha thalassemia	9 (31%)	2 (17%)	6 (46%)	1 (17%)
Treatment				
None	8	2	4	2
Hydroxyurea	19	8	8	3
Red cell transfusions (RCT)	2	1	-	1
Hydroxyurea and RCT	1	1	-	-
Hydroxyurea and ESA	1	0	1	-
Normoalbuminuria	24	10	9	5
Microalbuminuria	7	2	4	1
Acute chest syndrome in history	10 (31%)	2 (17%)	5 (36%)	3 (50%)

Table 2. Biomarkers associated with sickle cell nephropathy

	Vaso-occlusive dominant		Hemolysis-dominant		HbSC/HbSD		p-value Kruskal Wallis
Tubular markers	Median	Range	Median	Range	Median	Range	
α1MG/creatinine (mg/mmol)	1.31	(0.97-1.54)	1.23	(0.55-3.76)	1.62	(0.69-4.33)	0.850
KIM-1/creatinine (ng/mmol)	437	(63-702)	431	(168-503)	438	(259-527)	0.377
Urine osmolality (mOsm/kg)	0.41	(0.09-0.76)	0.83	(0.26-1.87)	0.37	(0.11-1.03)	0.805
Glomerular markers							
Albumin/creatinine (mg/mmol)	1.13	(0.41-8.75)	1.53	(0.50-8.75)	0.82	(0.27-12.00)	0.561
Total protein/creatinine (mg/mmol)	0.01	(0.00-0.02)	0.01	(0.00-0.02)	0.01	(0.00-0.02)	0.891
Serum cystatin C (mg/L)	0.66	(0.59-0.93)	0.64	(0.43-0.99)	0.81	(0.72-1.10)	0.014*
Serum creatinine (mmol/L)	54	(22-68)	52	(26-79)	54	(22-68)	0.453
Endothelial markers							
Serum sFlt-1 (ng/L)	81.6	(66.1-112.0)	111.0	(84.9-127.0)	97.1	(74.3-114.0)	0.006*

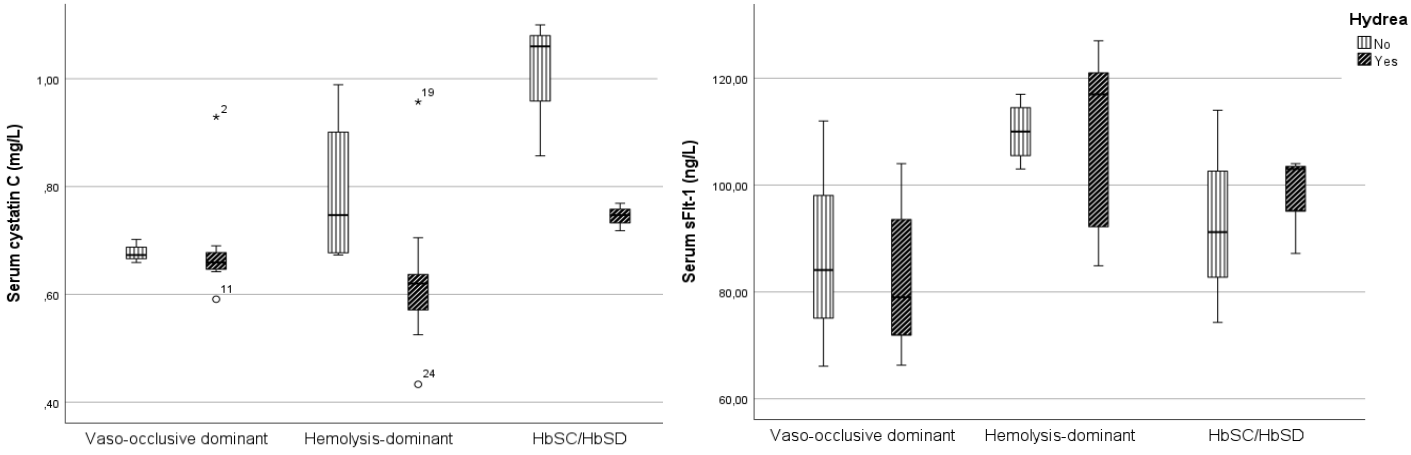

Figure 1. Boxplots represent markers per subgroup, grouped by treatment with or without hydroxyurea.
Left: Serum cystatin C levels were significantly higher in the HbSC/HbSD-group, while hydroxyurea-treated patients showed significantly lower cystatin C levels in both the hemolysis-dominant and HbSC/HbSD-group compared to patients without hydroxyurea.
Right: Serum sFlt-1 levels were highest in the hemolysis-dominant group, with no significant differences between patients with or without hydroxyurea treatment.