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Appendix: study group members

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To the editor:

Severe anemia early in life as a risk factor for sickle-cell kidney disease

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Patients with sickle-cell disease (SCD) are at high risk for morbidity and mortality from SCD nephropathy.[1,2] During adolescence, 20% to 35% of patients develop microalbuminuria (MiA; 30-300 mg/g Cr) classified as stage A2 chronic kidney disease (CKD) according to Kidney Disease Improving Global Outcomes (KDIGO) definition.[3-8] As adults, 60% of SCD patients progress to either stage A2 or A3 (>300 mg/g Cr) CKD.[9,10] The concern with progression to nephropathy is that SCD patients (1) develop renal failure at an early age and (2) have a higher mortality rate than non-SCD black patients after developing end-stage renal disease, and (3) renal transplant patient outcomes were historically poor so that many transplant programs will not consider patients with SCD despite recent improvements in survival.[1,2,11-13] It is vital to identify high-risk patients early so that nephrology care can be established and future renoprotective trials can be developed.[11] We assessed risk factors for developing MiA, tested the hypothesis that early severe anemia is a risk factor for developing MiA, and analyzed our cohort based on the KDIGO definition of CKD.[14]

We conducted an institutional review board–approved pediatric SCD nephropathy prospective cohort of 152 patients (>5 years) with hemoglobin SS (HbSS) or HbSB$_0$ thalassemia. We recorded every spot urine albumin/creatinine level obtained since birth (n = 595) and prospectively collected annual urine measurements along with clinical and laboratory values. MiA was defined by a urine albumin/creatinine level ≥30 mg/g using a Siemens DCA Vantage Analyzer. Participants were categorized as hypertensive based on disease norms if their systolic or diastolic blood pressure was >95th percentile based on age and sex.[15] "Early severe anemia" was categorized as Hb <8.0 g/dL on their earliest complete blood count (CBC) between 12 and 24 months of age.[14]

We analyzed age, sex, blood pressure, laboratory variables, and current therapy from the visit that coincided with identification of MiA and an early CBC to identify associations with MiA using *t* tests and χ^2 tests for continuous and categorical variables, respectively. To examine the time to development of MiA and its predictors, we used Weibull parametric survival regression model, which accommodates left, right, and interval censored data. With a total of 49 cases of MiA, we included only 4 relevant clinical and laboratory variables in the regression model (Hb, white blood cell [WBC] count, systolic hypertension, and sickle-cell–modifying therapy). We excluded other variables that were highly correlated including

Table 1. MiA outcome by laboratory and clinical variables

Patient characteristics	Patients with MiA	Patients without MiA	P
Continuous variables, mean (SD)			
Age (y)	13.2 (4.4)	13.9 (4.4)	.3
Systolic blood pressure (mm Hg)	118.5 (10.7)	117.4 (10.6)	.6
Diastolic blood pressure (mm Hg)	63.8 (7.5)	62.9 (7.1)	.5
Hb (g/dL)	8.3 (1.4)	9.1 (1.5)	.003*
Fetal Hb (%)	6.7 (7.7)	10.1 (8.9)	.03*
MCV (fL)	88.1 (7.7)	87.9 (10.8)	.9
ARC ($\times 10^9$/L)	510 (220)	380 (190)	.007*
WBC count ($\times 10^9$/L)	13.5 (5.2)	10.1 (3.6)	<.0001*
ANC ($\times 10^9$/L)	7.6 (4.1)	5.1 (2.6)	.001*
Platelet count ($\times 10^9$ /L)	418 (125)	387 (134)	.2
eGFR (mL/min per 1.73 m^2)†	163.1 (34.2)	168.3 (40.2)	.5
Maximum eGFR (mL/min per 1.73 m^2)§	169.4 (35.1)	173.7 (35.3)	.5
Bilirubin (mg/dL)	4.2 (2.9)	3.1 (1.9)	.02*
Categorical variables, count/total (%total)			
Sex			
Female	27/75 (36%)	48/75 (64%)	.3
Male	22/77 (29%)	55/77 (71%)	
Disease-modifying therapy			
No therapy (n = 24)	10 (42%)	14 (56%)	.2
Transfusion (n = 53)	20 (38%)	33 (62%)	
Hydroxyurea (n = 75)	19 (25%)	56 (75%)	
Continuous variables, mean (SD) among patients on hydroxyurea			
Dose (mg/kg)	25.2 (3.6)	24.5 (3.2)	.5
HbF%	8.4 (5.8)	14.3 (8.5)	<.001*
MCV (fL)	91.3 (8.7)	93.8 (11.5)	.3

eGFR, estimated glomerular filtration rate; HbF%, percent fetal hemoglobin; MCV, mean corpuscular volume; SD, standard deviation.

*$P <$ .05 defined as statistically significant.

†Glomerular filtration rate was estimated by serum cystatin C (eGFR) using BN ProSpec System: log GFR = 1.962 + [1.123 $\times$ log (1/cystatin C)].

§Maximum eGFR obtained from highest eGFR among all recorded eGFR measurements obtained prior to developing MiA or participants' last clinic visit.

diastolic hypertension, absolute reticulocyte count (ARC), or absolute neutrophil count (ANC). To examine severe early anemia on the development of MiA, we used logistic regression. Next, we evaluated the number of patients with MiA on 1 abnormal urine microalbumin/creatinine measurement as compared with KDIGO-defined MiA (2 of 3 consecutive abnormal measurements). Finally, in descriptive manner, we recorded the therapy at time of incident MiA and all subsequent measurements to determine the potential impact of therapy on improvement in MiA levels over time. SAS 9.4. (Cary, NC) was used to perform all analyses.

Forty-nine of 152 participants (32%) in our cohort developed at least 1 episode of MiA. Participants who developed MiA had significantly lower Hb ($P =$ .003), lower percent fetal Hb ($P =$ .03), higher ARC ($P =$ .007), higher WBC count (<0.0001), and higher ANC ($P =$ .001). (Table 1) We identified no statistical differences in development of MiA by age, sex, eGFR, platelets, bilirubin, blood pressure, or therapy (hydroxyurea, transfusion therapy, no SCD-modifying therapy). Among patients on hydroxyurea, higher fetal Hb was associated with a lower incidence of MiA ($P <$ .001) despite similar doses ($P =$ .5; Table 1). Based on Weibull parametric survival model with therapy, systolic hypertension, Hb, and WBC count as variables

in the model, only decreasing Hb ($P =$ .002) and increasing WBC count ($P =$ .009) were significant. The time to develop MiA was shortened by ~10% (time ratio estimate ,0.9; 95% CI, 0.84-0.96) for every unit decrease in Hb (g/dL), and the time to develop MiA shortened by ~3% (time ratio estimate, 0.97; 95% CI, 0.94-0.99) for every unit increase in WBC count ($\times 10^9$/L).

We analyzed the earliest Hb, WBC count, and platelet count obtained at 12 to 24 months between those with and without MiA. Only lower mean Hb was associated with development of MiA (7.75 vs 8.90 g/dL, $P <$ .0001). Fifty (33%) of these 152 participants were identified with early severe anemia (Hb <8 g/dL). Thirty-one (62%) participants with early severe anemia progressed to have MiA relative to the 17 (17%) participants with Hb ≥8.0 g/dL that progressed to MiA ($P <$ .0001). Participants with early severe anemia have a 9 times higher odds of developing MiA than patients with Hb ≥8 g/dL (OR, 9.1; 95% CI, 3.8-21.7) in both the unadjusted and adjusted models (Table 2).

Finally, we compared differences between participants with 1 positive urine for MiA with those who meet KDIGO defined MiA (2 of 3 positive urine MiA). Forty-one of the 49 participants with a first episode of MiA had at least 1 confirmatory urine microalbumin/creatinine level. Thirty participants (73%) had

Table 2. Odds ratio for developing MiA based on early CBC (12-24 mo)

	Crude		Adjusted	
Variables	OR*	95% CI	OR*	95% CI
Severe anemia (Hb <8 g/dL) vs Hb ≥8 g/dL	9.01	3.98, 20.41	9.09	3.82, 21.74
WBC count ($\times 10^9$/L)	1.10	1.02, 1.12	1.06	0.97, 1.15
Platelet count ($\times 10^9$/L)	1.00	0.99, 1.00	1.02	1.00, 1.01

Early CBC defined as the earliest CBC obtained between 1.0 and 1.99 years of age.

*Based on logistic regression (n=44 MiA and n=97 non-MiA for the adjusted model).

persistent MiA as defined by KDIGO definition. Among 19 participants with KDIGO-defined MiA receiving hydroxyurea at the time of their incident MiA, 13 (68%) continued with MiA and 6 of these 13 (23%) resolved on subsequent measurements (resolution time, 11-22 months). Fifteen (83%) of 18 participants on transfusion only therapy had persistent MiA on subsequent evaluations.

This cohort study shows that the time to develop MiA is shorter in patients with lower Hb or higher WBC count. Mechanisms by which anemia could rapidly induce MiA in SCD include relative renal ischemia, ischemia-reperfusion injury, and increased medullary sickling.[16,17] Elevated WBC counts and inflammatory markers have also predicted CKD in other diseases.[18-20] A novel finding is that 1-year-old participants with severe anemia have a 9 times higher odds of developing MiA independent of any future SCD-modifying therapies or SCD complications.

Hydroxyurea therapy appeared to have no impact on the development of MiA, although patients on hydroxyurea who did not develop MiA had higher fetal Hb. Patients in the hydroxyurea group were titrated to maximum tolerated dose according to ANC, but we do not start hydroxyurea at a specific age in all patients. Important longitudinal trials of hydroxyurea, specifically Baby HUG (#NCT01783990), can better address the impact of chronic hydroxyurea on prevention of MiA.[21] One recent cohort study suggested that hydroxyurea could reverse MiA, but not proteinuria (>300 mg/g Cr), in 40% of patients after 6 months of hydroxyurea.[22] Our data are similar in that 23% of participants receiving hydroxyurea had resolved MiA. Finally, these data suggest caution in interpreting the benefit of therapy on ameliorating MiA based on 1 urine microalbumin level as only 70% of these patients met the strict KDIGO definition of abnormal levels on 2 of 3 consecutive urine microalbumin measurements.

A couple of limitations are worth noting. First, participants had urine obtained during clinic visits rather than collecting first morning voids. Therefore, a few patients with orthostatic proteinuria were likely included in our analysis.[23,24] Second, our cohort is biased to participants that attend clinic regularly. Despite these limitations, the data suggest that clinicians should closely monitor young patients with severe anemia for CKD and consider this risk when discussing therapeutic options. Therapeutic studies to prevent development of MiA are essential as are intervention trials for SCD patients that have developed CKD.

Contribution: J.D.L., T.H.H., and D.I.F. designed the cohort; J.D.L. and S.B. performed data collection; I.A., J.D.L., and S.B. performed statistical analysis; J.D.L. wrote the first draft of the manuscript; and T.H.H., I.A., L.M.H., D.I.F., and S.B. contributed to the writing and conducted critical reviews of the manuscript.

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