Published in final edited form as:
*Eur J Haematol*. 2010 September ; 85(3): 257–263. doi:10.1111/j.1600-0609.2010.01471.x.

# Urinary Albumin Excretion is Associated with Pulmonary Hypertension in Sickle Cell Disease: Potential Role of Soluble Fms-Like Tyrosine Kinase-1

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## Abstract

**Background—**Pulmonary hypertension (PHT) is reported to be associated with measures of renal function in patients with sickle cell disease (SCD). The purpose of this exploratory study was to determine the relationship between albuminuria and both clinical and laboratory variables in SCD.

**Design and Methods—**This cross-sectional study was performed using a cohort of adult patients with SCD and control subjects without SCD. Spot urine for microalbumin/creatinine ratio, measures of hemolysis, inflammation and other laboratory studies were obtained. Pulmonary artery systolic pressure was determined by Doppler echocardiography, and the diagnosis of PHT was defined using age, sex and body mass index-adjusted reference ranges.

**Results—**73 patients with SCD and 21 healthy, race-matched control subjects were evaluated. In patients with SCD, normoalbuminuria was observed in 34 patients (46.6%), microalbuminuria in 24 patients (32.9%) and macroalbuminuria in 15 patients (20.5%). There was a significant correlation between urine albumin excretion and age. In HbSS and $S\beta^0$ thalassemia patients, the levels of sFLT-1, soluble VCAM and NT pro-BNP were significantly higher in those with macroalbuminuria, compared to patients with microalbuminuria and normoalbuminura, but no significant differences were observed in the levels of laboratory measures of hemolysis. Urine albumin excretion was associated with PHT and a history of stroke.

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**Conclusions—**Our study confirms the high prevalence of albuminuria in SCD. The association of urine albumin excretion with sFLT-1 suggests that this VEGFR family member may contribute to the development of albuminuria in SCD. By inducing endothelial activation and endothelial dysfunction, sFLT-1 appears to be a link between glomerulopathy and PHT in SCD.

### Keywords

Sickle Cell Disease; Albuminuria; Pulmonary Hypertension; sFLT-1; Endothelial Activation

## Introduction

Pulmonary hypertension (PHT) is a common complication in patients with sickle cell disease (SCD) (1,2) and is associated with an increased risk of mortality in these patients (1-4). While the pathogenesis of SCD-associated PHT is probably multifactorial, there is evidence that chronic intravascular hemolysis, with subsequent destruction of nitric oxide by cell-free plasma hemoglobin, plays a central role in this complication (1,5,6). More recently, there have been numerous reports that suggest a role for inflammation in the pathophysiology of SCD-associated PHT (7-11).

Albuminuria, a manifestation of glomerular injury, is also common in both children and adults with SCD (12-14). Multiple studies report associations between various measures of renal function and SCD-associated PHT (1,2,4), suggesting that both glomerulopathy and PHT may share a similar pathophysiology in SCD.

The purpose of this exploratory study was to investigate the relationship between albuminuria and clinical complications, as well as laboratory measures of hemolysis, inflammation, and renal function in patients with SCD.

## Design and Methods

### Patients and Study Design

The patients evaluated in this cross-sectional study represent a cohort followed at the Adult Sickle Cell Clinic at The University of North Carolina, Chapel Hill. The data were collected as part of an ongoing study to investigate the pathophysiology of PHT in SCD. Consecutive patients seen in clinic, as well as healthy, race-matched control subjects, who agreed to participate in this study, were enrolled. Seventy-three patients with SCD were included in the analyses that assessed the associations between urine albumin excretion (also referred to as albuminuria) and other clinical and laboratory variables. Each enrolled patient was studied while in the non-crisis, steady state; had not experienced an episode of acute chest syndrome in the 4 weeks preceding enrollment; and had no clinical evidence of congestive heart failure. No patient on chronic red cell transfusion was studied. All subjects gave written informed consent to participate and the study was approved by the Institutional Review Board.

All of the study evaluations were performed during the course of a single visit to the Clinical and Translational Research Center. Urine albumin excretion was assessed by measuring spot urine microalbumin/creatinine ratios. The urine albumin excretion, expressed as mg/ g creatinine, was defined as normoalbuminuria (< 30 mg/ g creatinine), microalbuminuria (30 – 299 mg/ g creatinine) or macroalbuminuria (≥ 300 mg/ g creatinine) (12). Commercially available ELISA kits (R&D Systems, Minneapolis, Minnesota) were used according to the manufacturers' recommendations to measure levels of cystatin C, placenta growth factor (PlGF), human soluble vascular cell adhesion molecule-1 (soluble VCAM-1), and soluble fms-like tyrosine kinase 1 (sFLT-1). Measurements of N-terminal pro-brain natriuretic

peptide (NT proBNP) and other routine laboratory studies were obtained in the clinical laboratories at UNC Hospitals. Values of NT proBNP that were below the measurable limit (<50 pg/dL) were assigned a value of 49 pg/dL, and values of urine microalbumin that were below the measurable limit (0.6 mg/dL) were assigned a value of 0.5 mg/dL. The estimated glomerular filtration rate (eGFR) was calculated using the abbreviated "Modification of Diet in Renal Disease" (MDRD) study (GFR, in mL/min per 1.73 $m^2$ = 186.3 × serum Cr (exp[-1.154]) × Age (exp[- 0.203]) × (0.742 if female) × (1.21, if black) (15). The presence of PHT was assessed by transthoracic Doppler echocardiography using a Hewlett-Packard 2500 Ultrasound System, with a 2·5/2·0 MHz ultrasound probe (Model 21215A) for recording continuous wave signals. The pulmonary artery systolic pressure (PASP) was calculated using the modified Bernoulli equation ($PASP = 4V^2$ + right atrial pressure), with the right atrial pressure assumed to be 10 mmHg (16). The diagnosis of PHT in our study was based on adjusted PASP values for age, sex, and body mass index (16). The presence or history of other clinical complications was ascertained from a history at the time of evaluation, combined with a detailed review of the medical records.

### Statistical Analysis

Albuminuria was analyzed as both a continuous and a trichotomized categorical variable. We explored correlations between continuous covariates of interest using Spearman's Rank Correlation Coefficient. When urine albumin excretion was categorized into normo-, micro-, or macroalbuminuria, we used the Kruskal-Wallis method to test for significant differences between the three categories and continuous covariates. Medians, with their distribution-free 95% confidence intervals, have been presented for these continuous covariates. Fisher's exact tests were used to test for significant differences between this categorized urine albumin excretion variable and categorical covariates of interest. All tests were two-sided ($\alpha = 0.05$). Reported p-values are considered 'nominal' and are for individual tests, unadjusted for multiple comparisons because of the exploratory nature of this study. Statistical analyses were performed using SAS software, version 9.2, SAS Institute, Cary, NC, USA.

## Results

### Demographics and Laboratory Characteristics

Seventy three subjects with SCD (SS = 61, SC = 8, $S\beta^0$ = 3, $S\beta^+$ = 1; 47 women) and 21 healthy, race-matched control subjects were evaluated in this study. The baseline demographic and laboratory characteristics are shown in Table 1. Normoalbuminuria was observed in 34 patients with SCD (46.6%), microalbuminuria in 24 patients with SCD (32.9%) and macroalbuminuria in 15 patients with SCD (20.5%). There was a modest correlation between age and albuminuria (r = 0.32, 95% CI: 0.09 – 0.51; p = 0.006), although there was no significant difference when the ages of SCD patients with macroalbuminuria, microalbuminuria and normoalbuminuria were compared (43 yrs vs. 40 yrs vs. 35 yrs; p = 0.12). There were no differences in the systolic and diastolic blood pressures when patients with macroalbuminuria were compared to those with microalbuminuria and normoalbuminuria. Although the level of lactate dehydrogenase appeared to be higher in patients with macroalbuminuria compared to those with microalbuminuria and normoalbuminuria (1136 U/L vs. 846 U/L vs. 773.5 U/L, p = 0.15), there were no significant differences in the levels of chemical measures of hemolysis, including both total and indirect bilirubin (Table 2). Patients with macroalbuminuria had lower hemoglobin values compared with those patients with microalbuminuria and normoalbuminuria (7.2 g/dL vs. 9.05 g/dL vs. 8.85 g/dL, p = 0.09), but this difference was not statistically significant.

As expected, the estimated glomerular filtration rate, assessed by the MDRD method was lowest in patients with macroalbuminuria compared to patients with microalbuminuria and normoalbuminuria (80.5 ml/min vs. 141.2 ml/min vs. 128.2 ml/min; p = 0.001). Furthermore, the level of cystatin C was significantly higher in patients with macroalbuminuria compared to other albuminuria categories (856.6 ng/mL vs. 565.5 ng/mL vs. 494.7 ng/mL; p = 0.03).

When only those patients with sickle cell anemia (HbSS) and sickle $\beta^0$ thalassemia (HbS$\beta^0$) were evaluated, we observed similar results, with no significant differences in the levels of lactate dehydrogenase (1175 U/L vs. 901 U/L vs. 914.5 U/L; p = 0.25), total bilirubin (2.3 mg/dL vs. 2.5 mg/dL vs. 1.9 mg/dL; p = 0.28) and indirect bilirubin (2.2 mg/dL vs. 2.3 mg/dL vs. 1.8 mg/dL; p = 0.39) when patients with macroalbuminuria were compared to those with both microalbuminuria and normoalbuminuria. However, patients with macroalbuminuria had significantly lower hemoglobin values compared to those patients with microalbuminuria and normoalbuminuria (7.1 g/dL vs. 9.1 g/dL vs. 8.7 g/dL, p = 0.033).

## Association of Albuminuria with Biologic and Inflammatory Markers

There were no significant differences in the white blood cell counts ($9.1 \times 10^3$/μL vs. $10.7 \times 10^3$/μL vs. $8.75 \times 10^3$/μL; p = 0.08), absolute neutrophil counts ($5.1 \times 10^3$/μL vs $5.25 \times 10^3$/μL vs. $4.65 \times 10^3$/μL; p = 0.57) or absolute monocyte counts ($0.60 \times 10^3$/μL vs $0.65 \times 10^3$/μL vs. $0.40 \times 10^3$/μL; p = 0.11) when patients with macroalbuminuria were compared to patients with microalbuminuria and normoalbuminuria (Table 2). NT-proBNP was significantly higher in patients with macroalbuminuria compared with those patients with microalbuminuria and normoalbuminuria (440 pg/mL vs. 68.5 pg/mL vs. 135 pg/mL; p = 0.01). The level of sFLT-1 was also significantly higher in patients with macroalbuminuria, compared to patients with microalbuminuria and normoalbuminura (120.1 pg/mL vs. 99.7 pg/mL vs. 85.4 pg/mL; p = 0.016). Although the level of soluble VCAM-1 appeared to be higher in patients with macroalbuminuria compared with patients with microalbuminuria and normoalbuminura, the difference was not statistically significant (1337.9 ng/mL vs. 668.9 ng/mL vs. 753.4 ng/mL; p = 0.10). No significant difference in the level of PlGF was observed in patients with macroalbuminuria, compared to patients with microalbuminuria and normoalbuminura.

When only HbSS and HbS$\beta^0$ patients were evaluated, we observed no significant differences in the white blood cell counts, absolute neutrophil counts or absolute monocyte counts when patients with macroalbuminuria were compared to those with microalbuminuria and normoalbuminuria. There were significantly higher levels of NT-proBNP (593 pg/mL vs. 68.5 pg/mL vs. 135 pg/mL; p = 0.005), sFLT-1 (124.6 pg/mL vs. 99.7 pg/mL vs. 86.3 pg/mL; p = 0.035) and soluble VCAM-1 (1347 ng/mL vs. 765.4 ng/mL vs. 753.4 ng/mL; p = 0.033) in patients with macroalbuminuria compared to those with microalbuminuria and normoalbuminuria. No significant difference in the level of PlGF was observed in patients with macroalbuminuria, compared to patients with microalbuminuria and normoalbuminura.

## Clinical Correlates of Albuminuria

In patients with measurable tricuspid regurgitant (TR) jet velocities, the TR jet velocities were significantly higher in those with macroalbuminuria compared to patients with microalbuminuria and normoalbuminuria (3.15 m/s vs. 2.45 m/s vs. 2.45 m/s; p = 0.00068). We also observed significant associations between urine albumin excretion and both the presence of PHT (p = 0.04) and a history of stroke (p = 0.002). No significant associations were observed between urine albumin excretion and the number of acute pain episodes,

history of acute chest syndrome, history of leg ulcers, history of priapism, retinopathy or history of hypertension.

When only HbSS and HbS$\beta^0$ patients were evaluated, significant associations were observed between urine albumin excretion and the presence of PHT ($p = 0.046$), stroke ($p = 0.0018$) and history of hypertension ($p = 0.021$). We also observed trends towards association between urine albumin excretion and use of hydroxyurea ($p = 0.085$) and presence of retinopathy ($p = 0.082$).

## Discussion

The increasing survival into adulthood of patients with SCD is associated with an increased incidence of multi-organ dysfunction. Renal abnormalities are common in patients with SCD. Like pulmonary hypertension, renal failure is a risk factor for death in SCD patients (17). In our study of adult SCD patients, we observed that increased urine albumin excretion is common, occurring in approximately 53% of patients. Approximately 33% of the study patients had microalbuminuria, a value lower than the 42% prevalence reported by Guasch and colleagues in a cohort of adult patients (12), but higher than the reported prevalence of between 16% and 19% in children with SCD (13,14). The lower prevalence in children compared with adults is consistent with our finding and that of others that albuminuria is associated with increasing age (12-14,18).

Our finding that sFLT-1 is associated with urine albumin excretion, combined with data associating sFLT-1 with albuminuria in other disease states suggests that sFLT-1 may play a role in the development of albuminuria in SCD. The level of sFLT-1 is reported to be elevated in SCD patients during the clinically asymptomatic state (19,20). sFLT-1 is a member of the vascular endothelial growth factor receptor (VEGFR) family (21) and is a splice variant of the VEGFR1. VEGF plays a critical role in glomerular development and function, and is important in maintaining the glomerular filtration barrier. VEGF has also been implicated in glomerular healing by facilitating glomerular capillary repair (22). sFLT-1 lacks transmembrane and cytoplasmic domains, and acts by adhering to the receptor-binding domains of VEGF and PlGF, preventing their interaction with endothelial receptors on the cell surface and thereby inducing endothelial dysfunction. Significant associations have been reported between serum levels of both sFLT-1 and VEGF with urinary albumin excretion in normal pregnant women (22). High levels of sFLT-1 in the serum and amniotic fluid of pregnant women, as well as excess placental sFLT-1 have been found to contribute to endothelial dysfunction, hypertension and proteinuria in preeclampsia by blocking the effects of VEGF and PlGF (23-25). Urinary and serum levels of VEGF and sFLT-1 have also been reported to be significantly increased in microalbuminuric and proteinuric patients with diabetes and essential hypertension, suggesting that VEGF and sFLT-1 may play a role in the pathogenesis of the nephropathy associated with these conditions (26,27). The association of urine albumin excretion with soluble VCAM in this study, combined with the association of sFLT-1 with soluble VCAM (20) suggests that sFLT-1 may contribute to the pathogenesis of albuminuria in SCD by promoting endothelial activation and possibly, endothelial dysfunction.

Hemolysis appears to predispose SCD patients to a vasculopathy that may manifest as one or more of several complications including PHT, leg ulceration, priapism, and possibly stroke (28). Similar to a previous report by Guasch et al (12), we did not observe any significant association between measures of hemolysis and albuminuria in our study. The lower hemoglobin levels in our patients with macroalbuminuria compared to those with microalbuminuria and normoalbuminuria is similar to the findings in previous reports that showed associations of both albuminuria and proteinuria with lower hemoglobin

(4,13,14,29). Although no significant associations between urine albumin excretion and chemical markers of red cell destruction were observed in our relatively small study, the level of lactate dehydrogenase was higher in patients with macroalbuminuria compared to those with both microalbuminuria and normoalbuminuria, and as a result we cannot rule out a role for hemolysis in the pathogenesis of albuminuria in SCD. The lower hemoglobin level in patients with macroalbuminuria may also be due to the decreased renal function and consequent decreased production of erythropoietin in these patients (30,31).

Multiple recent reports show an association of PHT with laboratory measures of renal function in patients with SCD (1,2,4). In addition, De Castro and colleagues reported an association of proteinuria with PHT (4). The reason for this association was previously uncertain, although it was suspected that both PHT and glomerulopathy in SCD share a similar pathophysiology. Our present study suggests that the association of albuminuria with PHT may occur, at least in part, due to the increased endothelial activation and endothelial dysfunction that follow increased levels of sFLT-1 in these patients. Indeed, we have recently reported that sFLT-1 is associated with PHT in SCD (19). The observed association of albuminuria with NT-proBNP, a laboratory marker of cardiac dysfunction and an established predictor of PHT in SCD (32), appears to provide additional evidence for the relationship between albuminuria and PHT. However, as NT-proBNP is significantly cleared by the kidney (33,34), the higher level of NT-proBNP in patients with macroalbuminuria may also be due to the reduced renal clearance in these patients compared with those patients with both normoalbuminuria and microalbuminuria.

The association of albuminuria with both PHT and a history of stroke suggest that albuminuria may be a risk factor for these complications in SCD. Furthermore, as proteinuria is associated with glomerulopathy (35), our finding highlights the importance of early screening for albuminuria in SCD patients. However, other studies are required to determine the natural history of microalbuminuria in SCD. Furthermore, longitudinal studies are required to determine whether the presence of microalbuminuria is predictive of specific SCD-associated complications. Treatment with hydroxyurea has been reported to prevent the development and progression of microalbuminuria in some patients with SCD (14). Other studies suggest that angiotensin converting enzyme inhibitors may be beneficial in SCD patients with microalbuminuria (36,37). A randomized, double-blind, placebo-controlled study of captopril in 22 normotensive HbSS patients showed significant differences in the mean absolute change (-45 mg per 24 hours in captopril group vs. +18 mg per 24 hours in the placebo group, $P < 0.01$) and the mean percentage change (37% in captopril group vs. +17% in the placebo group, $P < 0.01$) in microalbuminuria between the two treatment groups at 6 months (36). In addition, a single institution retrospective analysis reported that the combination of hydroxyurea and enalapril was associated with a near normal urine protein/creatinine ratio in 3 patients with sickle cell anemia following $3.5 \pm 1.2$ years of treatment (37). Pharmacologic interventions such as angiotensin converting enzyme inhibitors, angiotensin receptor blockers and aldosterone antagonists may have anti-inflammatory properties that are independent of their hemodynamic effect (38) and may prove to be beneficial in SCD patients with microalbuminuria.

In conclusion, our study confirms the high prevalence of albuminuria in patients with SCD. Although limited by its exploratory nature, the association of urine albumin excretion with sFLT-1 suggests that this VEGFR family member may contribute to the development of albuminuria in SCD. Furthermore, by inducing endothelial activation and endothelial dysfunction, sFLT-1 appears to be a link between glomerulopathy and PHT in patients with SCD. Prospective studies are needed to further evaluate the association of albuminuria with clinical complications of SCD, as well as assess the risk factors for progression of

albuminuria in these patients. Finally, additional studies are needed to determine the role of sFLT-1 in the pathogenesis of albuminuria in SCD.

## Acknowledgments

This work was supported in part by NIH grants RR00046, UL1RR025747, and HL79915. Support for this work was also provided by an award from the North Carolina State Sickle Cell Program.

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**Table I**

**Demographics and Laboratory Characteristics of Study Subjects**

| Variable | SCD (N = 73) Median (95% CI) | Control (N = 21) Median (95% CI) | p Value |
|---|---|---|---|
| Age (years) | 39 (34,42) | 34 (24, 44) | 0.15 |
| Systolic blood pressure (mm Hg) | 119 (115, 124) | 120.5 (114, 127) | 0.82 |
| Diastolic blood pressure (mm Hg) | 71 (68,72) | 72.5 (64,77) | 0.99 |
| White blood cell count ($\times 10^9$/L) | 9.4 (8.6, 10) | 7.2 (6.1, 9.3) | 0.001 |
| Hemoglobin (g/dL) | 8.7 (8.2, 9.4) | 12.4 (12.1,13.4) | P<0.0001 |
| Platelet count (x $10^9$/L) | 425.5 (374, 463) | 286.5 (269, 322) | P<0.0001 |
| Reticulocyte count (%) | 7.2 (6.6, 9.0) | 1.55 (1.4, 1.8) | P<0.0001 |
| Hemoglobin F (%) | 7.2 (5.7, 10.4) | 0.45 (0.4, 0.6) | P<0.0001 |
| Microalbumin/Creatinine ratio | 33 (20.5, 58.3) | 6.1 (4.1 11.5) | P<0.0001 |
| Serum creatinine (mg/dL) | 0.7 (0.7, 0.8) | 0.8 (0.75, 0.9) | 0.30 |
| Blood urea nitrogen (mg/dL) | 9.0 (8, 10) | 12.0 (10, 14) | 0.03 |
| Lactate dehydrogenase (U/L) | 922.5 (846, 1068) | 486.0 (413, 515) | P<0.0001 |

* Data shown as median with 95% distribution- free confidence interval.

**Table II**

**Association of Urinary Albumin Excretion with Laboratory Variables**

| | Albuminuria Category | | | |
|---|---|---|---|---|
| **Variable** | **Normoalbuminuria (N=34)** | **Microalbuminuria (N=24)** | **Macroalbuminuria (N=15)** | **p value** |
| White blood count (× $10^9$/L) | 8.75 (7.4 – 9.9) | 10.7 (9.6 – 12.2) | 9.1 (7.9 – 12.5) | 0.08 |
| Hemoglobin (g/dL) | 8.85 (8.3 – 9.8) | 9.05 (8.6 – 10.0) | 7.2 (6.2 – 9.5) | 0.09 |
| Platelet count (x $10^9$/L) | 378 (325 – 489) | 454 (410 – 529) | 430 (355 – 494) | 0.12 |
| Reticulocyte count (%) | 6.45 (4.9 – 9.0) | 8.4 (5.9 – 11.0) | 6.6 (4.5 – 8.6) | 0.48 |
| Absolute neutrophil count (× $10^9$/L) | 4.65 (4.2 – 5.6) | 5.25 (4.3 – 6.0) | 5.1 (4.4 – 7.7) | 0.57 |
| Absolute monocyte count (× $10^9$/L) | 0.4 (0.3 – 0.5) | 0.65 (0.4 – 0.9) | 0.6 (0.3 – 0.8) | 0.11 |
| Hemoglobin F (%) | 6.3 (4.9 – 12.9) | 6.5 (5.0 – 10.7) | 5.0 (2.7 – 9.5) | 0.84 |
| Lactate dehydrogenase (U/L) | 773.5 (672 – 992) | 846 (669 – 1136) | 1136 (873 – 1367) | 0.15 |
| Total bilirubin (mg/dL) | 1.75 (1.5 – 2.4) | 2.35 (2.0 – 3.0) | 2.2 (1.0 – 2.8) | 0.2 |
| Indirect bilirubin (mg/dL) | 1.66 (1.4 – 2.3) | 2.16 (1.9 – 2.8) | 2.1 (0.9 – 2.7) | 0.3 |
| Serum creatinine (mg/dL) | 0.70 (0.70 – 0.80) | 0.70 (0.6 – 0.8) | 1.20 (0.8 – 1.8) | 0.0007 |
| Blood urea nitrogen (mg/dL) | 8.50 (7.0 – 10) | 8.0 (6.0 – 11.0) | 13.0 (10.0 – 18.0) | 0.003 |
| Soluble vascular cell adhesion molecule-1 (ng/mL) | 753.3 (558.7- 969.4) | 668.9 (516.9-885.0) | 1337.9 (660.5 - 1772) | 0.10 |
| Soluble fms-like tyrosine kinase-1 (pg/mL) | 85.4 (70.2 – 98.2) | 99.3 (83.1 – 121.6) | 120.1 (111.9 – 165.7) | 0.02 |
| Placenta growth factor (pg/ml) | 9.3 (7.8 – 11.2) | 8.6 (6.0 – 10.5) | 10.3 (7.7 – 15.9) | 0.32 |
| N-terminal pro-brain natriuretic peptide (pg/ml) | 135 (99 – 197) | 68.5 (54 – 155) | 440 (54 – 1597) | 0.009 |

* Data shown as median, with 95% distribution-free confidence interval.