# Pattern of lung function is not associated with prior or future morbidity in children with sickle cell anemia

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**Abstract (258 words)**

Rationale: Patient factors associated with development of abnormal lung function in children with sickle cell anemia (SCA) have not been fully characterized.

Objectives: 1) To characterize lung function abnormalities among children with SCA, and 2) to determine whether these steady state lung function results were associated with morbidity, prior to or after testing among children with SCA.

Methods: As part of the prospective NIH-funded Sleep and Asthma Cohort Study, children with HbSS or Hb Sβ$^o$ (SCA) were enrolled without regard for SCD-related co-morbidities or diagnosis of asthma. Lung function was measured by spirometry and plethysmography on the same day, when free of acute disease. Standardized asthma symptom questionnaires and review of the medical records were also performed.

Measurements and main results: A total of 149 children age 6-19 years completed lung function testing; of whom 139 participants had retrospective morbidity data from birth to the test date and 136 participants were followed prospectively for a median of 4.3 years from the test date. At baseline percentages with normal, obstructive, restrictive, non-specific, and mixed lung function patterns were 70, 16, 7, 6, and 1 respectively. Neither retrospective rates of pain nor acute chest syndrome (ACS) were associated with lung function patterns. Furthermore, baseline lung function pattern was not predictive of future pain or ACS episodes.

Conclusions: The majority of children with SCA have lung function that is within the normal range. Abnormal lung function patterns were not associated with prior vaso-occlusive pain or

ACS episodes, and baseline lung function patterns did not predict future vaso-occlusive pain or ACS episodes.

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## Introduction

The lung is often affected in sickle cell disease (SCD), acutely with acute chest syndrome (ACS) (1) and chronically with dyspnea and reduced exercise capacity (2, 3), loss of lung function (4, 5), and pulmonary hypertension (6). Studies have sought to define pulmonary function abnormalities in children and adolescents with SCD (4, 7-13) using pulmonary function evaluations conducted when children and adolescents were at their baseline status, with tests most often obtained in clinical laboratories during routine care.

In his review of lung function in SCD, Koumbourlis (14) concluded that there has been "over-estimation of the prevalence of the restrictive pattern" and "under-estimation of the obstructive pattern," which he attributed to inconsistencies across studies regarding criteria for defining those categories. In addition to discrepancies about the prevalence of lung function abnormalities, data are inconsistent about whether prior morbidity- either ACS or vaso-occlusive pain - is associated with abnormal lung function in children with SCD (7, 9, 11, 13, 15-17).

In this analysis of pulmonary function test results in children with sickle cell anemia (SCA) studied at their baseline state of health, we hypothesized that 1) prior morbidity would be associated with baseline lung function pattern and 2) abnormal lung function pattern at baseline would be associated with future rates of pain and ACS.

## Methods

*Patients and Methods*

The current study uses data collected from the prospective, observational Sleep and Asthma Cohort study. Children with SCA (who had either hemoglobin SS (HbSS) or sickle beta zero thalassemia**)** (HbSβº) phenotypes) were enrolled at 3 clinical centers from 2006-2008 and followed prospectively until the end of the study in 2013 or until they were lost to follow-up.

Children were enrolled without regard to past morbidity or diagnosis of asthma, but those on chronic transfusions or participating in a clinical trial evaluating hydroxyurea (HU) therapy were excluded. Participants included in the current analysis completed spirometry and plethysmography on the same date when they were at their baseline of health and had been followed at their clinical center since birth. Participants' medical records were available for determination of numbers of hospital admissions for pain crises and ACS episodes from birth to the dates of lung function testing and after lung function testing.

Institutional approval was obtained from participating sites: Washington University School of Medicine in St. Louis, Missouri; Case Western Reserve University School of Medicine in Cleveland, Ohio; University College London in London, UK (who recruited from three London hospitals); and from the Coordinating Center at Vanderbilt School of Medicine in Nashville, Tennessee. Informed written parental consent was obtained, and children were consented or assented according to institutional policies of each institution.

*Questionnaires*

Caregivers and participants completed the following questionnaires at the time of enrollment: 1) the American Thoracic Society Division of Lung Disease (ATS/DLD) questionnaire (18); 2) a medical history questionnaire eliciting information on demographics, participant and family history of physician-diagnosed asthma and atopy, and what medications the participant was currently using; and 3) ascertainment of exposure to environmental tobacco smoke (ETS). Start and stop dates for prescribed medications were confirmed via review of the medical record.

*Definitions*

Asthma was defined as having both a parent-reported physician diagnosis of asthma and current prescription of an asthma medication (controller and/or rescue medication). A vaso-occlusive pain episode ("pain") was defined as bone pain in the chest, extremities, or other areas (not headaches only) directly associated with SCA that required hospitalization for opioid treatment. ACS was defined as an episode of acute respiratory distress requiring a new radiodensity on chest roentgenogram, temperature greater than 38° Celsius and increased respiratory effort with a decrease in oxygen saturation or increased respiratory rate documented in the medical record. To ensure a uniform definition of pain and ACS, all ACS and pain episodes requiring hospitalization were reviewed by a single investigator at each site, with over-reading by the principal investigator (MBD).

*Pulmonary function testing*

Pulmonary function tests (PFTs) were obtained when participants were at baseline of health, i.e., without current pain, respiratory symptoms or recent illness, and at least 4 weeks after discharge from hospital for SCD complications. Height and weight measurements were performed using standard methods.

Spirometry was performed by study-certified pulmonary function technicians using a pneumotachograph-type spirometer interfaced with a personal computer system (Jaeger MasterScope, VIASYS, Hoechberg, Germany) as described in detail by Field et al. (19). Spirometry was performed at least 4 hours after the use of a short-acting bronchodilator and 12 hours after use of a long-action bronchodilator (20) according to ATS standards adapted for children (20, 21). Spirometry results from all 3 centers were over-read by a senior technician to ensure that results were valid.

Static lung volume measurements were performed on the same day as spirometry measurements using a Jaeger MasterScreen (Cleveland and London) or Sensor Medics VMAX (St. Louis) plethysmogragh per ATS/ERS standards (22). Functional residual capacity (FRC) was the mean of the technically satisfactory FRC measurements, residual volume (RV) was FRC minus the mean of the technically acceptable expiratory reserve volume (ERV) measurements, and total lung capacity (TLC) was value for RV plus the largest of the technically acceptable inspiratory vital capacity (IVC) measurements. Plethysmography results from all 3 centers were over-read by a senior technician to ensure that results were valid.

*Markers of abnormal lung function*

Predicted values were determined for each subject based on their age, sex, height, and race for $FEV_1$, FVC, $FEV_1$/FVC ratio, and $FEF_{25-75}$ using the Global Lung Function 2012 equations (23). Abnormal results for $FEV_1$, FVC, $FEV_1$/FVC ratio, and $FEF_{25-75}$ were determined by comparison to their lower limits of normal (LLN) (23). Reference equations for TLC derived by Rosenthal et al. (24) were used with an African American race adjustment of 12% (25); LLN was defined as 80% predicted per ATS/DLD recommendations (26). Percent predicted values of $FEV_1$, $FEV_1$/FVC, FVC, and TLC were used to categorize lung function patterns using the algorithm published by Pellegrino et al (26) and modified by addition of the non-specific pattern described by Hyatt et al. (27) and Iver et al. (28), as shown in Figure 1.

*Bronchodilator reactivity*

To measure bronchodilator (BD) response, technicians administered 4 inhalations of albuterol from a metered dose inhaler (90 mcg/puff at US sites, 100 mcg/puff at UK sites) using an AeroChamber (Forest Pharmaceuticals, New York, NY). Spirometry was repeated 15 minutes post-albuterol. Baseline and post-BD $FEV_1$ were compared, with percent response to albuterol

defined as [(post-BD $FEV_1$ - pre-BD $FEV_1$) / pre-BD $FEV_1$]*100. An increase of $\geq$ 12% in $FEV_1$ was considered a positive BD response (26).

*Laboratory testing*

Serum IgE was obtained from a peripheral blood draw upon study entry (Elecsys 2010 Roche Diagnostics Indianapolis, IN). Complete blood count at steady state was obtained from the medical record.

*Statistical analysis:*

Demographic and clinical factors were compared between lung function categories with a chi-square test for categorical variables and an ANOVA or Kruskal-Wallis test for continuous variables, depending on the distribution. Rates of ACS and pain episodes were separated into retrospective and prospective based on the date of the PFT session.

Logistic regression models to predict lung function pattern were constructed with the primary predictors of interest being retrospective rates of pain and ACS. In addition to unadjusted models, multivariate logistic regression models were constructed to adjust for factors potentially associated with lung function pattern among patients with SCD, including demographic, SCD-related, and respiratory-related factors including early life ETS exposure (from birth through age 2) (29-32), with a model for each factor grouping. Models were developed comparing each abnormal pattern to the normal lung function group. Our model-building approach was chosen because small numbers in the restriction and non-specific pattern groups made it statistically appropriate to build separate smaller models rather than one large screening model that included all potential confounders. Given the high degree of association between having asthma and having a history of wheezing leading to shortness of breath, models

for obstruction replaced "asthma" with "history of wheezing leading to shortness of breath" as a potential covariate.

Associations between having an abnormal lung function pattern and prospective rates of pain and ACS were tested with negative binomial regression models. Analyses for prospective rates of ACS and pain were conducted with participants who had a minimum of 3 months of follow-up after the lung function testing; we then repeated our analyses with participants who had 12 months or more of prospective follow-up. Multivariable models were built in two steps. Potential covariates were included in a screening model. In addition to lung function pattern (obstruction, restriction, or nonspecific versus normal), covariates we considered to be potentially associated with prospective rates of ACS included gender, prior history of ACS, white blood cell count, reticulocyte count, asthma, history of wheezing leading to shortness of breath, BD responsiveness, and use of inhaled corticosteroids. Covariates initially included in a screening model of the association between lung function pattern and prospective rate of pain episodes were age, sex, prior history of pain, hemoglobin, reticulocyte count, white blood cell count, BD responsiveness, wheezing leading to shortness of breath, early life ETS, and ln(IgE). All covariates with $p<0.20$ in screening models were included in the final pain and ACS models. Preliminary analyses indicated that the children on HU at the time of testing had the highest rates of ACS and pain, suggesting confounding by indication of severe disease. Initially HU was not added as a covariate in the models; models that include HU are presented in the on-line supplement.

Given recent publications examining the contribution of $FEF_{25-75}$ in lung function classification and clinical decision making (33, 34), we conducted additional analyses using both the $FEV_1/FVC$ ratio and $FEF_{25-75}$ <LLN as an alternative criteria for having obstruction.

Analyses were conducted using Stata statistical software (Version 12, College Station, TX: StataCorp LP) and IBM SPSS Statistics (Version 22, Chicago, IL, IBM).

## Results

*Baseline Characteristics*

One hundred forty nine children completed valid spirometry and plethysmography testing at a median age of 11.4 years (range 6.2 to 19.0 years); 95% had the HbSS phenotype. All had SCD morbidity data available from birth to date of the PFT session. 136 were followed prospectively for a median of 4.3 years (range 3 months to 6.7 years) after their PFT session, while 121 participants had at least 12 months of prospective follow-up. Twenty-nine percent had a physician diagnosis of asthma (see Table 1 for a complete description of the study cohort).

Using the classification scheme as shown in figure 1, 104 children (70%) had normal lung function, while 45 (30%) had an abnormal lung function pattern. Of those with an abnormal pattern, 24 had obstruction, 10 had restriction, 2 had a mixed pattern of both restrictive and obstructive abnormalities, and 9 had a non-specific pattern. When those with the non-specific pattern were assessed with the slow VC (SVC) maneuver, there was less than a 5% difference between FVC and SVC in 7 of 9 patients, with the FVC higher than SVC in 4 of 9 patients.

There were significant differences across the lung function pattern groups (Table 1) for ETS, $FEV_1$ %, FVC % predicted, $FEV_1$/FVC, $FEF_{25-75}$, having a positive BD response, and RV/TLC. However, there were no group differences for age, sex, hematologic parameters, frequency of asthma, or history of wheezing leading to shortness of breath (Table 1).

*Prior pain and ACS morbidity is not associated with lung function pattern in children with SCA*

Logistic regression models for obstruction were developed and are shown in table 2A. Neither retrospective rates of ACS nor pain prior to the lung function testing session were associated with obstruction in the unadjusted model (Table 2A, model 1) or in models adjusted for demographic factors, SCD factors, and respiratory related factors (Tables 2A, models 2-4). As with obstruction, neither retrospective rates of ACS nor pain were associated with restriction or non-specific patterns (Table 2B and C, models 1-4). Alternating asthma with wheeze causing shortness of breath in the models for obstruction, restriction, and non-specific patterns instead of wheezing did not change the results (data not shown).

We further explored whether prior morbidity was associated with lung function parameters in children with SCA. Figure 2 depicts the correlations between retrospective ACS rate (2A) and pain rate (2B) (from birth to the date of the lung function test) and percent predicted values for FVC, $FEV_1$, $FEV_1$/FVC, and TLC. There were no observable relationships between prior rates of either ACS or pain events and baseline lung function parameters in our cohort.

*Baseline lung function does not predict future morbidity in children with SCA*

Baseline lung function patterns (obstruction, restriction, and non-specific) were not associated with future ACS or pain events in screening or final models adjusted for demographic factors, SCD factors, and respiratory related factors (Table 3). When analyses were restricted to the 121 participants who had $\geq$12 months of prospective follow-up, results were unchanged: baseline lung function patterns were not associated with future rates of pain or ACS (Supplementary Table E1). As expected, taking HU at the time of testing was associated with higher rates of both ACS and pain, consistent with clinical decisions to use HU for patients with more severe disease

(please refer to Supplementary Table E3 for multivariable models examining associations between lung function pattern and prospective ACS and pain that include HU as a covariate).

The value of including $FEF_{25-75}$ as an indicator of obstruction was assessed by moving the 14 children with an $FEV_1/FVC \geq LLN$ but an $FEF_{25-75} < LLN$ from the normal group to the obstruction group. Analyses of characteristics of those with obstruction and the impact of obstruction on future morbidity were not changed from the analyses using just $FEV_1/FVC$ to define obstruction (see on-line supplement, Table E3 and 4).

**Discussion**

We found that 70% of participants in our unselected cohort of 149 children with sickle cell anemia had normal lung function at steady state. The most common abnormal pattern was obstruction (found in 16%), with fewer patients having restriction (7%). Given that lung disease is a major cause of morbidity and mortality among adults with SCA (30), we sought to establish whether sickle cell morbidity was related to abnormal lung function among children. In our retrospective cohort analysis, we found that incidence rates of neither prior pain nor ACS events were predictive of having an abnormal lung function pattern. Equally important, in a prospective cohort design, having an abnormal lung function pattern (obstructive, restrictive, non-specific) was not associated with future pain or ACS events

Prior studies have examined the prevalence and progression of lung function abnormalities in children (14). The PUSH study (29) enrolled a cohort for research purposes and used LLN criteria for spirometry and plethysmography to define abnormal patterns similar to our approach. They had similar findings to our study in terms of percentages of obstruction (19%) and restriction (9%) among the 97 children with sickle cell anemia in their cohort (29). Intzes et

al.(13) reported 44% with obstruction in a retrospective clinical cohort, but defined obstruction with a cut-off for the $FEV_1$/FVC ratio (below 0.85), rather than the ATS/ERS guideline recommendation of $FEV_1$/FVC below the LLN (26). In contrast, MacLean et al (4) reviewed longitudinal spirometry and plethysmography results obtained during routine PFT screening of their sickle cell clinic population. At age 8, 96.5% of their cohort had normal lung function, but there was progression to more abnormal lung function through adolescence such that by age seventeen, 19% of their patients had restriction defined by TLC <70% of predicted (4). They found a much lower prevalence of obstruction (0.9% at age 8 and 0% at age 17) among their patients, but their definition of obstruction was having an $FEV_1$/FVC ratio below the specific threshold of 80% *of predicted*. It should be noted that MacLean et al. reanalyzed their data using Hankinson's LLN criteria, and found that 20% of their sample had at least one $FEV_1$/FVC measurement below the LLN(35).

Nine children (6% of our cohort) were categorized as having the non-specific pattern of lung function in which the FVC was reduced, but the TLC was in the normal range. This pattern has been described in adults attending clinics for lung disease (27, 28), but not previously described in individuals with SCA. Hyatt et al. (27) found the non-specific pattern was present in adults with airway disease or obesity and speculated that it was due to volume de-recruitment, with scattered, diseased airways closing at variable rates without effect on the overall $FEV_1$/FVC ratios. Presence of non-specific patterns could develop from decreases in expiratory muscle strength that would result in mismatch between TLC measured in a body plethysmograph and forced expiratory flows. The recent findings by Ong et al. (36) of decreases in respiratory muscle force and the resultant decreases in maximal expiratory pressure in children with SCD could provide an explanation for the findings of non-specific patterns. Muscle strength was not

measured in our cohort, however the finding of slow VC not being higher than FVC does not support decreased muscle strength as a primary cause of non-specific pattern in our population. In addition, the finding of RV/TLC not being higher in the non-specific group than in the obstruction group does not provide evidence for limitation of FVC due to air trapping causing the non-specific pattern. Further understanding of this non-specific pattern in SCA could not be further explored, given the small number of children with this finding.

Prior studies have explored the association between prior history of ACS and lung function. In one of the earliest studies of lung function and morbidity, Pianosi et al. compared the lung function of 10 children with and 27 children without a history of ACS and did not find significant differences in spirometric indices or TLC between those with and without histories of ACS (9). Few investigators have examined the association between abnormal lung function and future risk of ACS or vaso-occlusive pain. Boyd et al. found that among 102 children with SCD, ATS/ERS- defined lower airway obstruction was associated with a significantly higher rate of prospective rate of vaso-occlusive pain episodes compared to those with normal lung function, however, this was a single institution cohort of children with all types of SCD referred for PFTs because of respiratory symptoms (10). As such, those children may not reflect the general population of children with SCD. It should be noted that our findings regarding a lack of association between morbidity and lung function abnormalities cannot be extrapolated to adult patients with SCD. Knight-Madden et al. studied 80 young adults with SCD (all with HbSS), ranging in age from 19-27 years, and compared lung function between those with "recurrent" (2 or more prior episodes) ACS to those with zero or one episode. They found that those with recurrent ACS had significantly lower percent predicted values of FVC, $FEV_1$, and TLC than those who did not have recurrent ACS (17).

Our study has a number of strengths, including ascertainment of lung function measures and centralized over-reading and interpretation of results using standard ATS/ERS criteria and both retrospective and prospective ascertainment of SCD morbidity. With regards to limitations, our study cohort includes children from three tertiary care academic centers whose families agreed to participation in prospective longitudinal study. We are unable to control for selection factors into the cohort such as parent willingness to participate in clinical research, severity of disease, or other unknown factors. Additionally, while we used a robust definition of asthma for our study (the commonly utilized definition of 'parent-reported physician diagnosis' plus a medical record-confirmed prescription for an asthma medication), determining which children with SCA have asthma versus isolated features of recurrent wheezing and airway hyperreactivity remains a challenge for clinicians and researchers{Cohen, 2015 #1183}. That said, the consistency of our lung function abnormality results with those of the PUSH study (29) suggest that our findings are representative of the majority of children with SCA.

In summary, more than two-thirds of children with SCA in our research cohort had normal lung function. The predominant lung function abnormality was obstruction. As in prior studies, PFT-defined lower airway obstruction was not associated with asthma. In our study lung function abnormalities were not associated with prior rates of hospitalizations for pain or ACS, nor were they associated with future risk of pain or ACS during childhood. While this is the largest study to date of children with SCA having paired spirometry and lung volumes along with longitudinal morbidity data from birth for almost 16 years, larger studies are needed to determine definitively that there is no effect of lung function abnormalities on prospective morbidity. Further research is needed to assess whether lung function abnormalities detected in childhood are associated with longer term risks of pain and ACS in adulthood. Additionally,

given current recommendations against universal PFT screening for patients with SCD (37) juxtaposed with the recently demonstrated association between lower airway obstruction and CT and echocardiographic evidence of increased pulmonary vascular volume (38, 39), future studies might explore whether lung function abnormalities are associated with other clinically relevant outcomes in adulthood, such as progressive dyspnea, vascular dysfunction, pulmonary hypertension, and early mortality.

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Table 1. Baseline characteristics of 147* study participants

| Patient characteristics | All (n=147) | Normal (n=104) | Obstruction (n=24) | Restriction (n=10) | Non-specific (n=9) | P Value† |
|---|---|---|---|---|---|---|
| Age at lung function test, yr. (median, range) | 11.4 6.2-19.0 | 10.8 6.3-19.0 | 11.8 7.1-17.4 | 13.4 8.3-18.4 | 12.1 6.2-18.1 | 0.55‡ |
| Prospective follow-up, years (median, range) | 4.3 0.3-6.7 | 4.2 0.3-6.7 | 4.4 0.6-6.4 | 4.9 1.0-6.3 | 4.5 1.7-6.3 | 0.42‡ |
| Sex (% male) | 55.8 | 56.7 | 54.2 | 50.0 | 55.6 | 0.97 |
| BMI z-score, (mean, SD) | -0.06 ± 1.15 | 0.04 ± 1.12 | -0.17 ± 1.31 | -1.08 ± 0.71 | 0.13 ± 1.07 | 0.03§ |
| Hb phenotype (% HbSS) | 94.6 | 94.2 | 95.8 | 90.0 | 100.0 | 0.70 |
| Hemoglobin (g/dl) | 8.2 ± 1.2 | 8.2 ± 1.2 | 7.9 ± 1.5 | 8.2 ± 0.8 | 8.4 ± 0.8 | 0.63 |
| WBC (k/mm$^3$) | 11.9 ± 3.8 | 12.1 ± 3.8 | 11.5 ± 3.6 | 11.2 ± 2.3 | 11.9 ± 4.8 | 0.85 |
| Reticulocyte, n=146 (%) | 11.0 ± 5.3 | 11.2 ± 5.3 | 10.2 ± 4.0 | 11.3 ± 8.1 | 11.0 ± 5.3 | 0.89 |
| Has asthma (%) | 28.6 | 23.1 | 41.7 | 50.0 | 33.3 | 0.12 |
| Early life ETS exposure, n=144 (%) | 43.1 | 42.6 | 66.7 | 20.0 | 11.1 | 0.007‖ |
| History of wheezing leading to shortness of breath, n=145 (%) | 18.6 | 16.5 | 25.0 | 22.2 | 22.2 | 0.78 |

| | | | | | | |
|---|---|---|---|---|---|---|
| On hydroxyurea n= 146 (%) | 17.8 % | 17.5 | 16.7 | 20.0 | 22.2 | 0.98 |
| On ICS (%) | 22.4 | 19.2 | 29.2 | 50.0 | 11.1 | 0.13 |
| ACS rate retrospective, n=147, events/yr. | | | | | | |
| median, (IQR); | 0.13 (0.27); | 0.11 (0.25); | 0.18 (0.41); | 0.27 (0.29); | 0.13 (0.21); | 0.27‡ |
| mean, (SD) | 0.21 (0.26) | 0.20 (0.28) | 0.23 (0.21) | 0.30 (0.30) | 0.11 (0.10) | 0.43 |
| ACS rate prospective, n=144, events/yr. | | | | | | |
| median, (IQR); | 0.00(0.31); | 0.00 (0.31); | 0.00 (0.31); | 0.20 (0.64); | 0.00 (0.18); | 0.42‡ |
| mean, (SD) | 0.23 (0.46) | 0.24 (0.49) | 0.23 (0.45) | 0.33 (0.36) | 0.76 (0.12) | 0.69 |
| Pain rate retrospective, n= 147, events/yr. | | | | | | |
| median, (IQR); | 0.29 (0.63); | 0.30 (0.69); | 0.22 (0.38); | 0.47 (0.69); | 0.11 (0.32); | 0.22‡ |
| mean, (SD) | 0.48 (0.56) | 0.50 (0.58) | 0.40 (0.54) | 0.58 (0.50) | 0.26 (0.45) | 0.48 |
| Pain rate prospective, n= 145, events/yr. | | | | | | |
| median, (IQR); | 0.47 (1.35); | 0.43 (1.54); | 0.39 (1.15); | 0.82 (1.31); | 0.49 (1.15); | 0.86‡ |
| mean, (SD) | 0.99 (1.41) | 1.07 (1.52) | 0.75 (1.14) | 1.00 (1.34) | 0.70 (0.83) | 0.68 |

[*]2 participants were categorized as having a "mixed" restrictive and obstructive pattern and are not included in this table.

[†]Chi square tests for categorical variables, ANOVA tests for continuous variables unless otherwise noted

[‡]Kruskal-Wallis test

[§]Lower in the restricted group compared to the normal group (p=0.02).

[‖]Higher frequency in obstruction compared to non-specific (p=0.02)

Table 2. Lung function parameters of the study population

| Lung Function Parameter | All (n=147) | Normal (n=104) | Obstruction (n=24) | Restriction (n=10) | Non-specific (n=9) | P Value[†] |
|---|---|---|---|---|---|---|
| $FEV_1$ % predicted, (mean, SD) | 88.3 ± 13.4 | 93.1 ± 10.4 | 80.9 ± 13.5 | 67.0 ± 7.9 | 75.3 ± 8.5 | <0.001[‡] |
| FVC % predicted, (mean, SD) | 92.8 ± 14.1 | 96 4 ± 10.9 | 94.8 ± 14.6 | 67.2 ± 8.0 | 74.1 ± 5.2 | <0.001[§] |
| FEV1/FVC (actual) | 0.84 ± 0.06 | 0.85 ± 0.04 | 0.76 ± 03 | 0.88 ± 0.04 | 0.90 ± 0.06 | <0.001[‖] |
| $FEV_1$/FVC % predicted, (mean, SD) | 94.9 ± 6.5 | 96.2 ± 4.7 | 84.9 ± 3.0 | 99.5 ± 5.4 | 101.0 ± 6.2 | <0.001[‖] |
| FEF25-75 % predicted (mean, SD) | 73.2 ± 21.1 | 79.6 ± 20.0 | 49.2 ± 26.7 | 67.3 ± 15.6 | 71.5 ± 26.7 | <0.001[¶] |
| BD response ≥12.0, n=143 (%) | 19.6 | 11.9 | 41.7 | 20.0 | 50.0 | 0.003[**] |

| | | | | | | |
|---|---|---|---|---|---|---|
| RV/TLC ratio (mean, SD) | 0.33 ± 0.07 | 0.31 ± 0.07 | 0.36 ± 0.07 | 0.34 ± 0.10 | 0.38 ± 0.06 | 0.003[††] |
| RV/TLC ratio Z-score[§§] (mean, SD) | 0.76 ± 1.23 | 0.56 ± 1.17 | 1.26 ± 1.14 | 1.08 ± 1.63 | 1.84 ± 0.86 | 0.003[‡‡] |

[*]2 participants were categorized as having a "mixed" restrictive and obstructive pattern and are not included in this table.

[†]Chi square tests for categorical variables, ANOVA tests for continuous variables unless otherwise noted

[‡] Highest in the normal group ($p<0.001$), lower in restriction versus normal group ($p=0.005$)

[§] Higher in normal and obstruction groups versus restriction and non-specific ($p<0.001$ for all comparisons);

[‖] Lowest among the obstruction group versus all other groups ($p<0.001$ for all), non-specific group higher than normal ($p=0.02$)

[¶] Lower in obstruction versus normal ($p<0.001$) and non-specific groups (0.02)

[**]Higher frequency in obstruction and non-specific groups versus normal.

[††]Higher in the obstruction ($p=0.04$) and non-specific groups ($p=0.03$) versus the normal group

[‡‡]Higher in the obstruction ($p=0.04$) and non-specific groups ($p=0.03$) versus the normal group.

[§§]Calculated based on normative data for height and gender (Mark Rosenthal, personal communication)

Tables 3A-C. Logistic regression models of the association between retrospective rates of ACS and pain and having obstruction, restriction, or non-specific lung function pattern versus normal lung function in children with sickle cell anemia.

3A. Models for Obstruction (N with complete data=23 versus 91 with normal lung function)

| | Model 1* | Model 2† | Model 3‡ | Model 4§ |
|---|---|---|---|---|
| | OR‖ (95% CI)<br>P Value | OR (95% CI)<br>P Value | OR (95% CI)<br>P Value) | OR (95% CI)<br>P Value |
| Retrospective ACS Rate | 1.91 (0.35-10.35)<br>0.45 | 2.27 (0.38-13.60)<br>0.37 | 2.75 (0.44-17.31)<br>0.28 | 1.11 (0.12-10.38)<br>0.93 |
| Retrospective Pain rate | 0.62 (0.24-1.59)<br>0.32 | 0.59 (0.23-1.55)<br>0.29 | 0.54 (0.19-1.51)<br>0.24 | 0.85 (0.32-2.21)<br>0.73 |

* Unadjusted model
† Model adjusted for age and sex
‡ Model adjusted for age, sex, and SCD factors (hemoglobin [g/dL], white blood cell count, and reticulocyte %)
§ Model adjusted for age, sex, and pulmonary factors of interest (has asthma, bronchodilator response >12%, early life ETS exposure, ln (IgE))
‖ OR=Odds Ratio, CI=Confidence Interval

3B. Models for Restriction (N with complete data=9 versus 99 with normal lung function)

| | Model 1* | Model 2† | Model 3‡ | Model 4§ |
|---|---|---|---|---|
| | OR (95% CI)<br>P Value | OR (95% CI)<br>P Value | OR (95% CI)<br>P Value) | OR (95% CI)<br>P Value |
| Retrospective ACS Rate | 2.23 (0.26-18.91)<br>0.46 | 5.12 (0.43-60.05)<br>0.19 | 5.30 (0.42-67.60)<br>0.20 | 6.94 (0.53-91.41)<br>0.14 |
| Retrospective Pain rate | 0.97 (0.29-3.24)<br>0.97 | 0.76 (0.21-2.75)<br>0.68 | 0.77 (0.19-3.06)<br>0.71 | 0.63 (0.16-2.49)<br>0.51 |

* Unadjusted model
† Model adjusted for age and sex
‡ Model adjusted for age, sex, and SCD factors (hemoglobin [g/dL], white blood cell count, and reticulocyte %)
§ Model adjusted for age, sex, and pulmonary factors of interest (history of wheezing with shortness of breath and early life ETS exposure)

3C. Models for the Non-Specific Pattern (N with complete data=9 versus 99 with normal lung function)

| | Model 1* | Model 2† | Model 3‡ | Model 4§ |
|---|---|---|---|---|
| | OR (95% CI)<br>P Value | OR (95% CI)<br>P Value | OR (95% CI)<br>P Value) | OR (95% CI)<br>P Value |
| Retrospective ACS Rate | 0.15 (0.002-13.04)<br>0.40 | 0.15 (0.002-14.34)<br>0.42 | 0.17 (0.002-14.37)<br>0.43 | 0.14 (0.001-14.22)<br>0.40 |
| Retrospective Pain rate | 0.34 (0.05-2.38)<br>0.28 | 0.33 (0.05-2.44)<br>0.28 | 0.24 (0.03-2.17)<br>0.20 | 0.33 (0.04-2.39)<br>0.27 |

* Unadjusted model
† Model adjusted for age and sex
‡ Model adjusted for age, sex, and SCD factors (hemoglobin [g/dL], white blood cell count, and reticulocyte %)
§ Model adjusted for age, sex, and wheeze with shortness of breath

Table 4. Final negative binomial regression models for prospective rates of ACS[*] and vaso-occlusive pain[†] in children with sickle cell anemia.[‡]

| | **IRR**[§] | **95% CI** | **P Value** |
|---|---|---|---|
| **3a. Prospective rates of ACS** | | | |
| Retrospective rate of ACS events per year | 14.14 | 7.38-27.07 | <0.001 |
| Obstructive pattern | 0.89 | 0.42-1.88 | 0.76 |
| Restrictive pattern | 1.01 | 0.48-2.11 | 0.98 |
| Non-specific pattern | 0.56 | 0.22-1.40 | 0.22 |
| **3b. Prospective rates of pain** | | | |
| Age (years) | 1.07 | 1.00-1.15 | 0.041 |
| Retrospective rate of pain events per year | 2.25 | 1.80-2.80 | <0.001 |
| Obstructive pattern | 0.70 | 0.36-1.37 | 0.30 |
| Restrictive pattern | 0.59 | 0.33-1.07 | 0.08 |
| Nonspecific pattern | 0.92 | 0.42-2.02 | 0.84 |

[*] Initial screening model of the association between lung function pattern and prospective ACS was adjusted for: sex, prior history of ACS, white blood cell count, reticulocyte count, asthma, history of wheezing leading to shortness of breath, bronchodilator responsiveness, and use of inhaled corticosteroids.

[†]Initial screening model of the association between lung function pattern and prospective pain was adjusted for: age, sex, prior history of pain episodes, hemoglobin, reticulocyte count, white blood cell count, bronchodilator responsiveness, wheezing leading to shortness of breath, early life ETS, and ln(IgE).

[‡] Median length of follow up 4.6 years, range 3 months – 6.7 years [‡], N=136 with complete data. Please see supplementary table E2 for analyses of the 121 participants with $\geq$ 12 months of prospective follow-up.

[§]IRR=incidence rate ratio, CI=confidence interval

**Figure Legends**

Figure 1. Algorithm used to assess lung function pattern, modified from that of Pellegrino et al. (26)

Figure 2. Scatter plots of the correlations between retrospective rates of ACS and pain on lung function parameters in children with sickle cell anemia.

2A: Spearman correlations between rates of ACS (x-axis) and lung function parameters (y-axis)

2B: Spearman correlations between rates of pain (x-axis) and lung function parameters (y axis)