# Airway Hyperresponsiveness Does Not Predict Morbidity in Children with Sickle Cell Anemia

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*To the Editor*:

Sickle cell anemia (SCA) is one of the most common inherited blood disorders in the world, characterized by chronic hemolytic anemia and recurrent vasoocclusive pain episodes. Pulmonary complications are a major cause of morbidity and mortality. Individuals with SCA have been shown to have a high prevalence of asthma, wheezing, and airway hyperresponsiveness (AHR) (1, 2). Increased AHR has been demonstrated in individuals with SCA, both with and without a concomitant diagnosis of asthma (2, 3). Although previous studies have looked at the association between a history of acute chest syndrome (ACS) episodes and AHR to methacholine (4), investigators have not examined whether AHR to methacholine is a risk factor for future SCA-related morbidity (pain and ACS). Therefore, the aim of this study was to examine the association between AHR to methacholine and future rates of hospitalization for pain and ACS.

Children with SCA (hemoglobin SS or sickle $\beta^0$ thalassemia) aged 4 to 18 years were identified at three large clinical centers and enrolled into the Sleep and Asthma Cohort study, a prospective, observational cohort study designed to evaluate the contribution of abnormalities in sleep and asthma to SCA-related morbidity. Participants were enrolled without regard to previous morbidity or diagnosis of asthma. Methacholine airway challenge was performed as a baseline measurement when a child was well, at least 1 month after a hospitalization for pain or ACS. The 2-minute tidal breathing method was employed with an Airlife Sidestream High Efficiency nebulizer (Cardinal Health, Dublin, OH) according to American Thoracic Society standards, as previously described (2). Methacholine dose–response slopes (DRS) were calculated for all participants, as previously described (2). Briefly, the numerator was the total percentage decrease in $FEV_1$ during the challenge, and the denominator was the cumulative dose of methacholine received. A vasoocclusive pain episode was defined as pain associated with SCA requiring hospitalization and treatment with opioids. Headaches requiring hospitalization were not considered to be pain episodes. ACS was defined as a new density on chest roentgenogram and at least one of the following: increased respiratory effort (as demonstrated by a decrease in oxygen saturation or increase in respiratory rate) or temperature greater than 38°C. Pneumonia was included in this definition. ACS and pain episodes were reviewed by a single investigator at each site, with review by the principal investigator, who was blinded to methacholine results, to ensure a uniform definition of pain and ACS in this multicenter study.

Methacholine airway challenge was performed with 99 participants. The initial 98 children completed testing without a serious adverse event. However, the 99th child tested developed his first vasoocclusive pain episode requiring hospitalization within 3 days of testing;

methacholine challenge testing was subsequently removed from the study protocol. Of the 99 children who completed testing, 70 participants had complete data on all covariates included in our multivariable models (including pain and ACS episodes from birth) and were therefore included in the analysis. The median duration of prospective follow-up after methacholine testing was 5.8 years (range, 1.4–6.6 yr). There was no significant difference in methacholine response between those included and those excluded from the analysis ($P = 0.13$). Multivariable negative binomial regression models were constructed to test the association between methacholine DRS and prospective rates of ACS and pain episodes. Covariates previously shown to be associated with future rates of ACS (sex, shortness of breath, and history of ACS before age 4 yr) and pain (age and white blood cell count) were included in multivariable models (5, 6). Given a nonnormal distribution, DRS was log-transformed for all analyses. Median rates of pain and ACS per year were 0.49 (interquartile range, 0.12–1.17) and 0.16 (interquartile range, 0–0.34), respectively. As shown in our final models below, there were no statistically significant relationships between methacholine DRS and future hospitalizations for ACS or pain; coupled with the 95% confidence intervals, the relationship between methacholine response and future rates of ACS or pain is minimal, if present at all (Table 1).

Given the strong association between asthma and SCA morbidity and mortality, an important question has been whether lung function abnormalities, and specifically lower airway obstruction and/or AHR, are associated with SCA-related morbidity. A single-site study using spirometry obtained for clinical purposes found an association between lower airway obstruction and future rates of pain (7). In contrast, our group examined the association between lung function pattern and SCA-related morbidity among 149 children and found no association between obstructive pattern and future hospitalizations for pain or ACS (8). Chaudry and colleagues found no difference in the methacholine DRS between 50 children with sickle cell disease (HbSS and HbSC phenotypes) and 50 age-, sex-, and ethnically matched controls, and within the sickle cell disease group, there was no association between prior history of ACS and methacholine DRS (9). Although our results suggest that AHR, as measured by methacholine DRS, is not predictive of pain or ACS, it may nevertheless have clinical implications for pulmonary pathology in this patient population. Eiymo Mwa Mpollo and colleagues demonstrated that placental growth factor, an erythroid cell–derived growth factor that is significantly increased in individuals with SCA, augments allergen-induced airway inflammation and AHR in sickle mice as well as increased baseline AHR to methacholine through a leukotriene-dependent pathway, suggesting a novel pathway for potential therapeutic intervention (10).

Limitations of this study include our small sample size, which might affect the ability to generalize our findings as true-negative versus false-negative results. The narrow confidence interval around the incidence rates of pain and ACS episodes indicate our findings are likely a true negative result. Our study suggests that there is neither a statistically nor clinically relevant relationship between methacholine responsiveness and future morbidity in children with SCA.

In conclusion, although there is a high prevalence of AHR in patients with SCA (2), we demonstrate for the first time that AHR may not be predictive of future risk for pain or ACS. Further studies are needed to improve our understanding of associations between vascular dysfunction, pain, airway obstruction, AHR, and lung disease pathogenesis in patients with SCA.

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S.M.W. and R.T.C. had full access to all of the data in the study and take responsibility for the integrity of the data, the accuracy of the data analysis, and the work as a whole; S.M.W. interpreted the data, created the initial draft, and finalized the manuscript for submission; M.R. developed the statistical approach, performed those analyses, reviewed the integrity of those analyses, and reviewed and revised the manuscript; R.C.S. was the co–principal investigator for the SAC (Sleep and Asthma Cohort) study, conceptualized the manuscript, and contributed to the development of the SAC project, study concepts, and procedures; C.L.R. contributed to the development of the SAC project and study concepts and procedures and reviewed and helped revise the manuscript; F.J.K. was the site investigator for one site, helped develop the concepts for the SAC project, and reviewed and revised the manuscript; J.J.F. assisted with data analysis and reviewed and helped revise the manuscript; M.R.D. was the principal investigator for the SAC project, helped design the concepts for SAC and this manuscript, interpreted the results, and reviewed and revised the manuscript; R.T.C. interpreted the data, developed the initial draft, and reviewed and revised the manuscript; and all authors approved the final manuscript as submitted.

**Table 1.** Multivariable Model of Prospective Rates of ACS and Pain in 70 Children with SCA

| **Covariate** | **IRR** | **95% CI** | ***P* Value** |
|---|---|---|---|
| Multivariable model of prospective rates of ACS | | | |
| Male sex | 0.53 | 0.30–0.93 | 0.027 |
| History of an ACS episode prior to 4 yr of age | 2.88 | 1.66–4.99 | <0.001 |
| History of wheezing leading to shortness of breath | 1.50 | 0.76–2.96 | 0.24 |
| Log methacholine dose–response slope | 1.11 | 0.98–1.27 | 0.108 |
| Multivariable model of prospective rates of vasoocclusive pain episodes | | | |
| Age | 1.12 | 1.04–1.20 | 0.004 |
| White blood cell count | 1.00 | 0.93–1.08 | 0.991 |
| Log methacholine dose–response slope | 0.97 | 0.85–1.11 | 0.654 |

*Definition of abbreviations*: ACS = acute chest syndrome; CI = confidence interval; IRR = incidence rate ratio; SCA = sickle cell anemia.

Negative binomial regression models with adjustment for overdispersion, using robust SEs. Two-tailed significance values are shown.

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