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Lung Clearance Index to Detect Early Pulmonary Changes in Children with Sickle Cell Disease

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Abstract: *Objective:* Pulmonary complications including acute chest syndrome, are leading causes of sickle cell disease (SCD) related morbidity and mortality. Prior studies have shown that patients with pulmonary complications have evidence of pulmonary involvement reflected in lung functions trends as early as childhood. Spirometry is the current standard for measuring lung function. Growing evidence suggests that lung clearance index (LCI) which is a commonly reported parameter of multiple breath washout (MBW) tests, is more sensitive than spirometry measurements in the early identification of pulmonary changes in pediatric patients. The aim of our study was to determine if this relationship between LCI and spirometry existed within the pediatric sickle cell population. *Study Design:* This is a cross-sectional, retrospective study to compare LCI to spirometry measurements in children with SCD. Charts were reviewed of clinic encounters at Phoenix Children's Hospital from March 1, 2013 – June 30, 2017. Spirometry and MBW measurements were collected from 23 patients between the ages of 5 years – 22 years. The MBW utilized sulfur hexafluoride (SF_6) as the tracer gas. Demographics and SCD variant (e.g. HbSS, HbSC, etc.) for each encounter were also collected. *Results:* Our results show that LCI correlates to FEV_1% predicted (Spearman's coefficient -0.44, $p = 0.003$) and FEF_{25-75}% (Spearman's coefficient -0.49, $p < 0.001$) over time. Based on demographics, LCI is affected by weight ($p = 0.046$) but not age or height. When comparing LCI and FEV_1% predicted, abnormal LCI results were noted to occur even in the presence of normal FEV_1% predicted measurements. *Conclusions:* Our data support LCI correlating with spirometry measurements, but more studies are necessary to explore whether LCI can be used as a screening test to detect pulmonary changes in young children with SCD. Earlier monitoring of lung function will allow for preventative therapies and delayed progression of pulmonary dysfunction.

Keywords: Spirometry, Pediatrics, Sickle Cell, Pulmonary Function Tests

1. Introduction

Sickle cell disease (SCD) is a spectrum of hematologic disorders caused by a point mutation in the β globin gene [1]. The severity of sickle cell anemia is related to complications such as vaso-occlusive crises (VOC) and acute chest syndrome (ACS). VOC and ACS are the leading causes for hospital admission in the SCD population [1-3]. Recurrent ACS is linked to processes including asthma that cause progressive pulmonary dysfunction [4]. Evidence suggests that pulmonary dysfunction can be seen several years prior to a child's first ACS episode [5]. Validating methods to detect pulmonary changes early in SCD patients is vital to reducing the morbidity and mortality that is related to these complications.

Previous studies have shown that pulmonary changes can be detected in SCD patients using spirometry [6, 7]. The studies followed several pulmonary function parameters including forced expiratory volume in one second (FEV_1) and showed a significant reduction in lung function over time. In children with SCD, both obstructive and restrictive lung patterns have been found [6, 8]. In adults, findings became

more consistent with a restrictive lung pattern [9, 10].

Currently spirometry is the accepted testing to monitor pulmonary progression in several diseases including SCD and cystic fibrosis (CF). However, there are limitations to this testing in young children. There are studies supporting the use of multiple breath washout (MBW) test in patients with CF, as a more feasible and sensitive test compared to spirometry in detecting pulmonary changes in young children [11].

The MBW test is a technique for quantifying the heterogeneity of ventilation, which is an early feature of chronic obstructive lung disease. The lung clearance index (LCI) is to date, the most commonly reported MBW parameter. LCI is defined as the cumulative expired volume where end-tidal inert gas concentration falls below $1/40^{th}$ of the original concentration, divided by the functional residual capacity (FRC). It is calculated as the number of lung volume turnovers needed to clear inert gas from the lung, with the lung volume turnovers reflecting the FRC, which is equal to the difference in end-tidal concentration of the gas measured at the start and end of the washout [12].

The advantage to this test is that tidal breathing replaces the forced respirations required for spirometry, which are often too difficult for younger children to perform [13]. CF studies demonstrate that LCI is a reliable and repeatable outcome to assess lung function in children [11, 14-16]. Evidence also suggests that LCI is a more sensitive predictor of lung disease than spirometry measurements, with a strong correlation to patient-reported symptoms [11, 15]. LCI allows indirect investigation of smaller airways (<2 mm) and has shown ability to detect early peripheral airway damage [17]. A prospective study of patients with alpha-1-antitrypsin deficiency demonstrated abnormal LCI values even in patients with normal spirometry results, which support the idea that LCI may identify peripheral airway changes earlier [18]. A prospective study of children with CF also showed a significant difference in LCI compared to healthy children even before 4 years of age [19]. These studies raise the potential for LCI to be used in the assessment of pulmonary dysfunction in other diseases.

In our study, we sought to assess the relationship between LCI and spirometry results as well as respiratory symptoms in children with SCD. We hypothesized that ventilation heterogeneity is a manifestation of ongoing pulmonary dysfunction and that LCI would reflect these changes. Additionally, we hypothesized that changes in LCI would be detected at rates comparable to changes in spirometry measurements, supporting the use of LCI to screen for pulmonary dysfunction in younger children with SCD.

2. Materials and Methods

2.1. Study Design

The study was a single center cross-sectional, retrospective chart review. IRB approval was provided by Phoenix Children's Hospital (IRB #15-069). Children routinely evaluated at the comprehensive sickle cell and pulmonary clinics were included in the study. Patient encounters occurred between March 1, 2013 and June 30, 2017. Data was collected on demographics and SCD variant (see Table 1). Spirometry and MBW results were also collected from each encounter. Spirometry measurements were already integrated into the clinic visits as standard of care during this timeframe. MBW was first introduced in the clinic for patients suspected to have lung disease but were having difficulty performing spirometry. It was subsequently implemented as standard practice in the clinic, provided availability of the equipment. This was to ensure that all patients received equal assessments that were of low risk and could potentially provide additional information regarding their pulmonary status. Spirometry and MBW tests were performed in accordance with the American Thoracic Society (ATS) guidelines and results were interpreted in accordance with the Quanjer Global Lung Initiative (GLI) 2012 equations [20].

2.1.1. Inclusion Criteria

Data was included from individuals diagnosed with any variant of SCD: HbSS, HbSC, HbS/Beta-Thal, or HbS/HPFH (hereditary persistence of fetal hemoglobin). All subjects were above the age of 4 years. The age recommendation is based on the practical limitations of spirometry.

2.1.2. Exclusion Criteria

Spirometry and MBW results within 2-3 weeks of an acute illness were not considered in the study due to the potential for confounding factors that could arise with therapies implemented during time of illness. Acute illness was defined as respiratory symptoms resulting in either hospitalization or outpatient treatment with transfusions, oral steroids and/or oral antibiotics.

2.1.3. Primary Outcome Measurements

Primary outcomes were spirometry and MBW test results. From spirometry, data retrieved included FEV_1 and FEF_{25-75} (forced expiratory flow at 25 to 75 percent of forced vital capacity (FVC)). LCI was derived from the MBW test in the manner outlined the latter portion of this section. Normal FEV_1% predicted values were defined as greater or equal to 80.00 [21]. There are very few published studies establishing reference values for LCI in young children. However, the few existing studies including those from Aurora and colleagues show similar and consistent results for LCI values in healthy preschool children. These results indicate a normal LCI value of less than 7 [22-24]. Hence, a higher value of FEV_1% predicted and a lower value of LCI indicate more favorable pulmonary function.

2.1.4. Secondary Outcome Measurements

Additional data was recorded to assess disease severity. During our chart review, evidence of pulmonary symptoms such as shortness of breath or wheezing was tracked. Pulmonary symptoms were subjectively reported by either patients or their parents during each clinic visit and documented in a review of systems. The number of hospitalizations related to ACS in the last 12 months was also

included. ACS was defined as rapid-onset fever, dyspnea and cough with corresponding radiographic findings.

We stratified individuals into groups based on the presence or absence of symptoms and/or ACS episodes.

2.2. Spirometry and MBW Testing

All methods were carried out in accordance with relevant guidelines [21]. MBW was performed using methods described by Horsley et al [13]. Participants wore a nose clip and breathed a known concentration (0.2%) of sulfur hexafluoride (SF_6), which is an inert and non-absorbed gas. Participants breathed via a mouthpiece connected to an Innocor photoacoustic gas analyzer until the expired concentration in their exhaled breath reached a steady state (wash-in phase). Participants maintained relaxed tidal breathing for the test.

Following completion of wash-in, participants were rapidly switched to breathing room air during expiration and continued tidal breathing (wash-out phase). Wash-out continued until the end-tidal concentration of expired SF_6 fell below $1/40^{th}$ of the original concentration for three consecutive breaths.

Washout curves were analyzed using software written with Innocor (CosMed – The Metabolic Company, Odense, Denmark). FRC was calculated by dividing the total volume of SF_6 expired during the washout by the difference between the SF_6 concentrations at the beginning and end of the washout period. LCI was calculated as the cumulative expired volume at the point where the end-tidal concentration of expired SF_6 fell below 1/40 of the original divided by FRC.

2.3. Statistical Analyses

Demographic and clinical characteristics were assessed using means, while standard deviations were used for continuous variables and frequencies, and proportions for categorical variables. Linear regression models compared spirometry to MBW measurements. Spearman's rank correlation coefficients (ρ) were used to assess the strength of associations between spirometry and MBW results. Statistical significance was considered a $p < 0.05$. Analyses of MBW results between patients with and without ACS and/or respiratory symptoms were performed using the Wilcoxon Rank Sum test. We also investigated associations between LCI and demographics. Box plots were also created to compare the distribution of abnormal FEV_1% predicted and LCI values over subsequent visits. Statistical analyses and figures were generated using STATA version 14 (College Station, TX).

3. Results

In our study, we had 43 encounters with corresponding spirometry and MBW measurements. The patient encounters were collected from 23 patients, many of whom were able to provide measurements at initial and follow up visits. In our data set, the average age was 12.98 years, with the youngest patient being 5 years of age and the oldest being 22. The most common sickle cell disease variant was HbSS. Table 1 includes a summary of study demographics.

Table 1. *Study Demographics.*

	Study Group Characteristics (n = 23)
Age (yrs)	12.98 ± 4.74
Male (%)	55.8
Height (cm)	146.2 ± 18.8
Weight (kg)	42.0 ± 18.8
BMI (kg/m^2)	18.64 ± 4.35
Sickle cell variant (%)	SS 86.0%
	SC 6.98%
	HbS-elevated HbF 4.65%
	HbS-βthal 2.33%

Table 1 lists the mean values for demographics in the study population. Mean ± SD is provided when applicable.

3.1. Spirometry and MBW Correlations

Within our study group, the average FEV_1% predicted was 73.85 (SD 19.12) and average LCI was 7.34 (SD 1.23). Using linear regression, Spearman's rank correlation coefficients (ρ) compared spirometry and MBW measurements. When comparing FEV_1% predicted to LCI, there was a statistically significant negative correlation ($\rho = -0.44$, $p = 0.003$). A statistically significant negative correlation was also found for FEF_{25-75}% predicted and LCI ($\rho = -0.49$, $p < 0.001$). Figure 1 illustrates the linear regression analyses.

3.2. Pulmonary Test Values Related to Disease Severity

Spirometry and MBW measurements were stratified into groups based on the presence or absence of symptoms and/or ACS episodes. FEV_1% predicted had a median value of 86 when symptoms and ACS episodes were absent (Table 2). The median value for FEV_1% predicted declined to 82 when patients had both pulmonary symptoms and the occurrence of at least one ACS episode (p=0.023). A similar trend was found for FEF_{25-75}%. In patients without symptoms or ACS episodes, the median FEF_{25-75}% was 80. The median value decreased to 71 in patients with pulmonary symptoms and at least one ACS episode (p=0.019).

Table 2. *Pulmonary Test Values and Disease Severity.*

	No Symptoms or ACS (n=15) Median (IQR)	**Symptoms and ACS present (n=7) Median (IQR)**	**p-value[1]**
FEV1% predicted	86 (80, 94.8)	82 (76, 85)	0.023
FEF 25 – 75% predicted	80 (62.4, 90)	71 (67, 75)	0.019
LCI	7 (6.2, 7.6)	6.8 (6.6, 9)	0.61

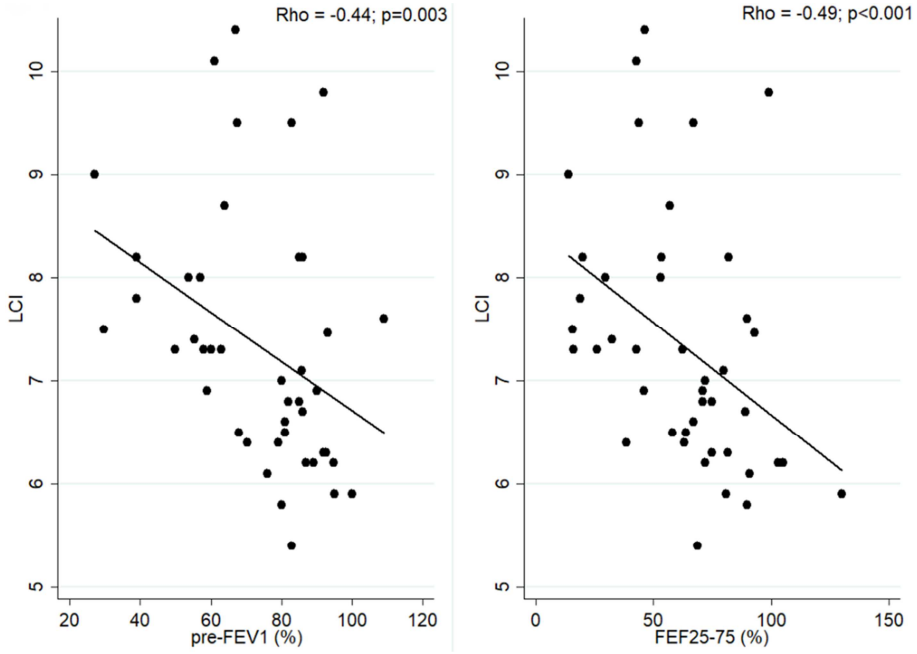

Figure 1. *Linear regression analyses between LCI and FEV_1% predicted and FEF_{25-75}% predicted.*

For LCI, the median value was 7 with the interquartile range (IQR) (6.2, 7.6) when symptoms and ACS were absent. The trend in LCI between groups with symptoms only or with ACS only, was not statistically significant. For the group containing patients with both symptoms and ACS episodes, the median value was 6.8 (6.6, 9) (p=0.61). The overall trend suggests a worsening in pulmonary function test measurements with a progression in symptoms and ACS episodes. FEV_1% predicted and FEF_{25-75}% had significant changes between asymptomatic and symptomatic groups whereas LCI did not (Table 2).

Table 2 displays the spirometry and MBW results in various patient groups based on the presence or absence of pulmonary symptoms and ACS episodes. [1]P-values calculated using the Kruskal-Wallis Test.

3.3. LCI Variations with Demographics

Based on multivariable analyses, weight was the only demographic variable with significant changes associated with LCI (β (95% CI) = -0.018 (-0.036 to -0.000), p = 0.046). Associations between LCI and height (β (95% CI) = 0.007 (-0.002 to 0.017), p = 0.132) and BMI β (95% CI) = 0.032 (-0.017 to 0.082), p = 0.195) were insignificant.

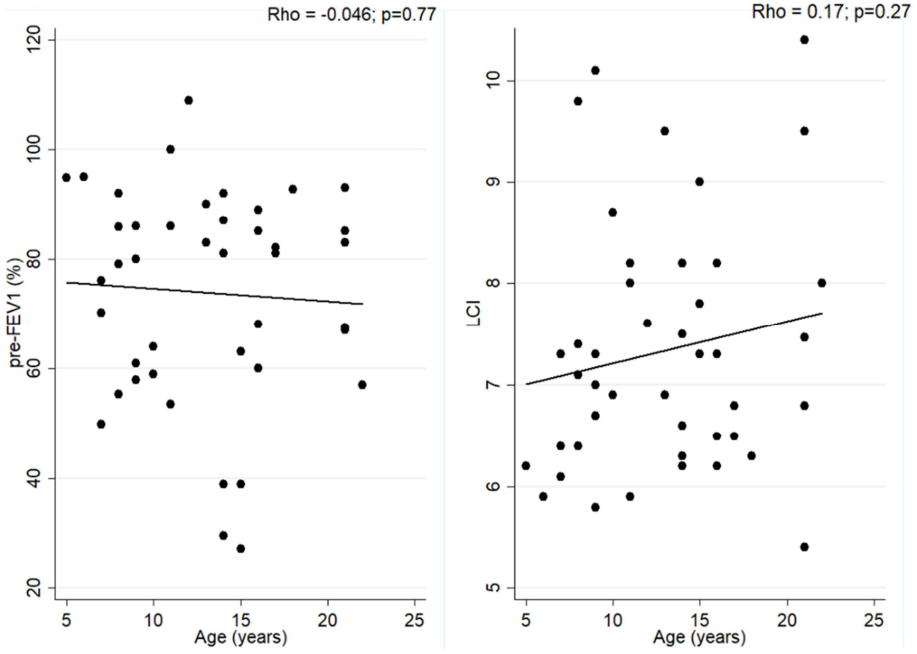

Figure 2. *FEV_1% predicted and LCI values compared to age.*

Age was also compared to trends in MBW and spirometry results. LCI had an insignificant trend with increasing age (Rho = 0.17, p = 0.27), with similar findings for FEV_1% predicted (Rho = -0.05, p=0.77) (Figure 2). The findings were inconsistent with prior literature indicating increasing pulmonary dysfunction over time and likely due to the inability to reach statistical significance in our study. Figure 2 illustrates the contrasting correlations of FEV_1% predicted and LCI with age.

3.4. Sensitivity and Specificity Comparisons

Figure 3 depicts the percentage of patients with either an abnormal FEV_1% predicted defined as <80% or an abnormal LCI defined as >7. In the box plots, a greater percentage of patients presented with an abnormal LCI on their initial visit compared to an abnormal FEV_1% predicted. By the subsequent visit(s), more patients were shown to develop an abnormal FEV_1% predicted compared to LCI. This raises the possibility that LCI can detect pulmonary changes before abnormal values emerge through spirometry. However, the differences in the percentage of patients with abnormal test values were not statistically significant between FEV_1% predicted and LCI (Figure 3).

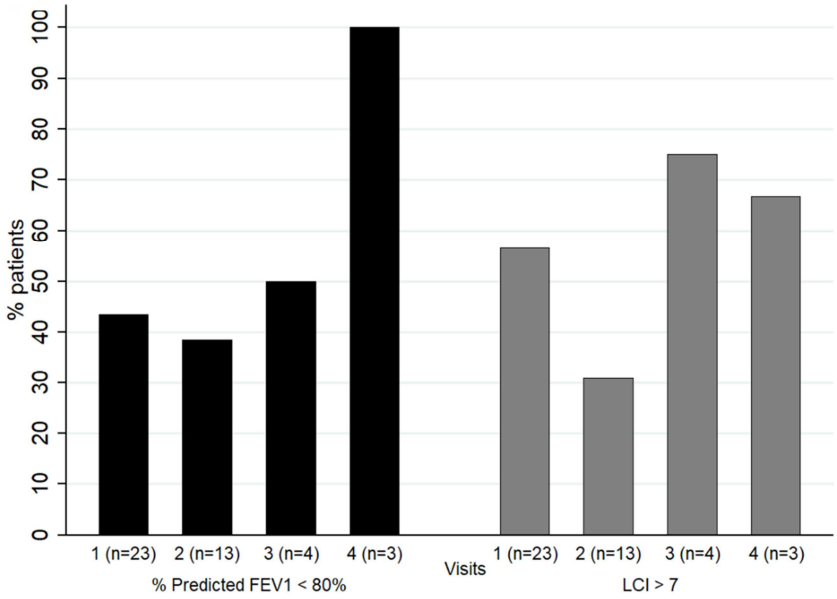

Figure 3. *Box plots displaying the percentage of patients with abnormal FEV_1% predicted (black box plots) and abnormal LCI (gray box plots) over subsequent clinic visits.*

4. Discussion

Based on our results, LCI trend is comparable to the trend of FEV_1% predicted and FEF_{25-75}% predicted. Significant negative correlations were found between LCI and each of the spirometry measurements, demonstrating that an abnormal LCI is associated with abnormal spirometry results over time. Our analyses also revealed that abnormal LCI findings can sometimes result prior to changes in FEV_1% predicted trend towards abnormality.

The recent study by Machogu et al. also looks at the utility of LCI in identifying lung disease within sickle cell patients. The study compared sickle cell patients and healthy controls and found no difference in LCI values between the two groups. They also noted a relationship between spirometry values and LCI within the sickle cell group [25]. However, the only correlation showing statistical significance was that between total lung capacity (TLC) and LCI. Our study did not look at TLC but did assess the correlation between LCI and spirometry parameters and found similar relations between FEV_1 and LCI within the sickle cell population.

Our study showed that FEV_1% predicted and FEF_{25-75}% predicted had significant associations with progressive pulmonary symptoms and ACS. However, LCI did not show such associations. Our team hypothesizes that this is due to the small sample size of the study. An additional possibility is the idea that the ventilation heterogeneity normally reflected by LCI is confounded by the presence of anemia in SCD particularly during ACS. This idea was explored by Lopes AJ et al [26]. Their study assessed the relationship between ventilation heterogeneity under exercising conditions, in adults with SCD. They proposed a few potential mechanisms to explain this, which include the reduced oxygen carrying capacity and subsequent shifts in VQ mismatch, the repeat ACS episodes contributing to infarcts that would also alter VQ mismatch, and the high cardiac output due to the anemia that resulted in changes to the cardiopulmonary vascular bed.

A recent study found variations in LCI with height [19]. Our study did not detect a significant correlation between LCI and height, but rather, an association between LCI and weight. This is potentially due to the fact that LCI is derived using the FRC which generally accounts for differences due

to height. A trend was found that both spirometry and MBW values worsen with age, however this did not reach statistical significance.

5. Study Limitations

The timeframe that LCI use was introduced as part of standard clinic visits, along with times where equipment was unavailable was a limitation to our study. As there are not many studies assessing the utility of LCI in patients with sickle cell disease, our study opted to assess the smaller subset as an initial step in determining whether or not the overall sickle cell population would benefit from this type of testing. However, in doing so, it resulted in a small sample size.

The small sample size likely accounts for the inability of the study associations to reach statistical significance. The smaller sample size also likely accounted for the inability of the study to demonstrate a significant association between LCI and symptoms as well as complications of pulmonary disease in SCD.

As mentioned previously, we incorporated a patient's symptom burden and ACS frequency in comparisons of spirometry and MBW values. Consequently, another limitation to our study is that the accuracy of our grouping depends upon the completeness of symptom reporting by patients and clinicians and the consistency of charting. Several patient encounters had to be excluded from our analyses due to incomplete records. Lack of symptom reporting would lead to an underestimation of disease severity as manifested by frequency of symptoms and hospitalization for ACS.

Despite the limitations, our study showed there is a possible use for LCI as a potential screening tool in detecting pulmonary dysfunction in young children with SCD. The testing can be used in conjunction with spirometry or as a stand-alone test for younger patients who are not yet able to perform reliable spirometry. In translating test results to patient-centered outcomes, LCI offers a chance of detecting early pulmonary disease.

In the future, with a greater understanding of the pulmonary changes associated with SCD, LCI may even be used to predict a patient's risk for ACS, leading to better detection and the opportunity for therapeutic intervention.

6. Conclusions

LCI has been validated as a screening test for early pulmonary disease in patients with other lung diseases, such as CF. Our results support similar findings from such studies which assess the associations between FEV_1 and LCI in children [11, 14-16]. Our findings also suggest that LCI can reveal pulmonary function changes earlier than FEV_1 and may be a valuable screening tool in younger children with SCD, particularly since LCI has been shown to be more feasible and consistent in this age group, where techniques for performing spirometry becomes challenging. Despite LCI's potential as a screening test, it is not at a point where it can replace spirometry.

Based on our study, future investigations are needed to evaluate trends in LCI and spirometry over time and to validate the utility of LCI in assessing pulmonary disease progression in SCD. Knowledge on the nature of pulmonary function in children with SCD and how it evolves with age would improve our understanding of the disease process. In a patient population where pulmonary disease leads to significant morbidity and mortality, finding a method to detect changes earlier is critical to delaying disease progression. This opens the possibility for clinical interventions to improve prognosis and quality of life for those with SCD. A longitudinal cohort study would allow us to characterize these changes and then be able to identify potential early therapeutic options.

Declarations

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Conflict of Interest/Competing Interests

The authors declare that they have no conflicts of interest or competing interests.

Ethics Approval

Our study was approved by the Phoenix Children's Hospital IRB Committee.

Consent to Participate

Patient informed consent for the study was waived by the Phoenix Children's Hospital IRB Committee due to the minimal risk to patients and because all data was de-identified prior to analysis.

Consent for Publication

The authors provide consent for the data to be published.

Availability of Data and Material

All data is stored on password protected computers and electronic health records at Phoenix Children's Hospital.

Author Contributions

SW was involved in the study design, IRB approval process, data analyses and publication writing. MC was involved in the study design, IRB approval process, data collection, data analyses and publication writing. PK was actively involved in the study design, data analyses, figure design and publication writing.

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