Global Pediatric Health
Volume 6: 1–8

DOI: 10.1177/2333794X20938931
journals.sagepub.com/home/gph
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A Liver Biopsy Validation Pilot Study of Shear Wave Elastography, APRI, FIB-4, and Novel Serum Biomarkers for Liver Fibrosis Staging in Children With Chronic Viral Hepatitis

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Abstract

As liver biopsy in children poses inherent risks, noninvasive measures of liver fibrosis are needed. This was a cross-sectional, liver biopsy validation pilot study of 16 participants evaluating the ability of shear wave elastography, aspartate transaminase to platelet ratio index (APRI), fibrosis index based on the 4 factors, and novel serum biomarkers to stage liver fibrosis in children with chronic hepatitis B or C. There was very high intrasegmental shear wave speed variation in our participants and little correlation with fibrosis. APRI and monocyte chemoattractant protein (MCP-1) were higher in fibrosis stage F2-3 versus F0-1 ($P = .02$, $P = .06$, respectively). Soluble Fas (sFas) was lower in F2-3 versus F0-1 ($P = .046$). A logistic regression analysis calculated by (APRI $\times$ MCP-1)/sFas demonstrated an area under the receiver operating characteristic curve of 0.92 ($P < .001$), suggesting that this combination can differentiate fibrosis stage F0-1 from F2-3 in children with chronic viral hepatitis.

Keywords

Shear wave elastography, APRI, pediatric chronic viral hepatitis, hepatitis B, hepatitis C, liver stiffness, MCP-1, sFas

Received January 7, 2020. Received revised March 12, 2020. Accepted for publication May 26, 2020.

Introduction

Hepatitis B virus (HBV) and hepatitis C virus (HCV) continue to have a significant global impact, infecting approximately 400 million and 170 million people worldwide, respectively.[1-3] Both lead to chronic liver disease and cirrhosis, accounting for 80% of hepatocellular carcinoma worldwide with high mortality rates.[4-6] At higher rates than adults, 90% and 60% of infants infected with HBV and HCV, respectively, will develop chronic infection.[3,7] Unfortunately, most children remain asymptomatic with minimal to no abnormal clinical and laboratory findings even with advanced fibrosis, making it difficult to monitor disease progression. In addition, children are at higher risk for progression to cirrhosis and hepatocellular carcinoma given their long exposure to these viruses.[3,8,9] Close monitoring of fibrosis progression in children with viral hepatitis is important for implementing early therapeutic interventions to slow progression or reverse fibrosis with antiviral therapy.[10,11] Liver biopsy has long been the gold standard for staging liver fibrosis in children, but is an invasive and costly procedure with limitations and risks including sampling error, interobserver variability, pain, hemorrhage, anesthesia complications, and infection.[12-15]

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Real-time shear wave elastography (SWE) is a readily available noninvasive ultrasound technique to evaluate the elasticity of tissue measured in kilopascals or meters/second (m/s), though less well studied in children. SWE has been studied in adult populations with chronic HBV and HCV, showing potential to replace liver biopsy as the gold standard for staging liver fibrosis, but there is limited biopsy-validated data in pediatric populations with chronic viral hepatitis.[16-18]

Noninvasive fibrosis scores including aspartate transaminase to platelet ratio index (APRI) and fibrosis index based on the 4 factors (FIB-4) utilize a combination of standard of care laboratory tests and have been studied extensively in both adults and children with good accuracy in staging liver fibrosis.[19-26] Furthermore, many novel serum biomarkers are involved in extracellular matrix formation or degradation, inflammation, or hepatocellular damage. Studies of these biomarkers in pediatric patients with biliary atresia, nonalcoholic fatty liver disease, cystic fibrosis–associated liver disease, and adults with chronic viral hepatitis show promise for accurately staging liver fibrosis.[27-43] It is unclear if these markers are age-dependent or affected by growth. In this study, we evaluate the utility of 3 noninvasive modalities to stage liver fibrosis in children with chronic viral hepatitis validated by liver biopsy using SWE, fibrosis scores, and a panel of 10 novel serum biomarkers.

Methods

Study Population

In this single-center, prospective study, participants were enrolled based on the following inclusion criteria: 2 to 17 years of age; diagnosis of chronic HBV or HCV based on the Centers for Disease Control and Prevention, American Association for the Study of Liver Diseases, or North American Society of Pediatric Gastroenterology, Hepatology, and Nutrition guidelines; elevated transaminases for >6 months; and planned, clinically indicated liver biopsy. Exclusion criteria included the following: known concomitant fibrosing liver disease (ie, primary sclerosing cholangitis, autoimmune, metabolic, biliary atresia, or other hepatic infection) and/or any medical contraindication to sedation or anesthesia.

Serum was collected and SWE was performed ±7 days of liver biopsy. Each biopsy was staged for fibrosis using the Metavir classification (F0 = no fibrosis, F1 = portal fibrosis without septa, F2 = portal fibrosis with few septa, F3 = numerous septa without cirrhosis, and F4 = cirrhosis).[44] SWE was performed by 2 experienced sonographers trained in SWE using an iU22 ElastPQ (Philips Healthcare) with the C5-1 PureWave transducer. All patients were fasted overnight and studies were performed in a supine position with the right arm elevated. Images were obtained after a small breath hold when feasible and with free breathing in younger patients. Scans were obtained using an intercostal approach, with sampling greater than 1 cm from the liver surface and away from major vessels. The standard machine region of interest (ROI) was used. At least 2 measurements were obtained from each of segments 5 to 8 of the right lobe of the liver. Images were interpreted by a single, blinded senior radiologist.

Using Young's modulus, a liver stiffness measurement (LSM) in kilopascals can be calculated using SWE generated shear waves and measuring the shear wave speed (SWS) in meters/second through the liver.[45] There is a direct correlation between SWS and LSM. Using an aspartate transaminase (AST) upper limit of normal (ULN) of 40 U/L, APRI was calculated (AST/AST ULN)/platelet count and FIB-4 was calculated (age × AST)/(platelet count × $\sqrt{}$ALT). The serum biomarker panel included tissue inhibitor of metalloproteinase 1 (TIMP-1), prolyl hydroxylase (PH), hyaluronic acid (HA), monocyte chemoattractant protein 1 (MCP-1), collagen IV, interleukin 8 (IL-8), platelet-derived growth factor (PDGF-BB), soluble Fas (sFas), soluble intercellular adhesion molecule (sICAM), and lysyl hydroxylase (LH) assayed via sandwich enzyme linked immunoassays.

Data and Statistical Analysis

Data was grouped by liver biopsy fibrosis stage F0-1 versus F2-3 for analysis. Wilcoxon rank sum tests were used for comparing continuous variables between the 2 cohorts. Fisher's exact test was used to compare nominal variables. A multivariable logistic regression analysis and receiver operating characteristic (ROC) curve analysis were performed for APRI, sFas, and MCP-1 to predict liver fibrosis stage F2-3. The area under the ROC (AUROC) curves was estimated with 95% confidence intervals.

Ethical Approval and Informed Consent

This study was approved by the Baylor College of Medicine Institutional Review Board (approval protocol number H-30472). Written informed consent was obtained prior to enrollment in the study from parents/legal guardians and assent from children of appropriate age. Consent was obtained for the addition of SWE to routine liver ultrasound, collection of extra blood for serum biomarkers at the time of routine blood sample

Table 1. Clinical Characteristics of Participants.

Clinical characteristics	All fibrosis stages (min, max)	F0-1 (min, max)	F2-3 (min, max)
N	16	6	10
Median age at enrollment, years	12.6 (3.4, 17.9)	13.0 (4.4, 17.1)	8.9 (3.4, 17.9)
Median BMI, kg/m^2	18.4 (14.6, 22.5)	19.2 (14.6, 21.2)	18.1 (15.4, 22.5)
Median BMI *Z* score	0.1 (−0.9, 2.9)	0 (−0.9, 2.9)	0.6 (−0.9, 2.0)
Female, n (%)	10 (62.5)	3 (50)	7 (70)
Hepatitis B, n (%)	9 (56.3)	5 (83.3)	4 (40)
Hepatitis C, n (%)	7 (43.8)	1 (16.7)	6 (60)
Ethnicity, n (%)			
Asian	10 (62.5)	5 (83.3)	5 (50)
White	5 (31.3)	1 (16.7)	4 (40)
Other	1 (6.3)	0 (0)	1 (10)

Abbreviation: BMI, body mass index.

Table 2. Median Shear Wave Speed (m/s) per Segment for F0-1 Versus F2-3.

Liver segments	N	All fibrosis stages, (min, max)	N	F0-1 (min, max)	N	F2-3 (min, max)	*P*
All segments	15	1.17 (0.87, 1.69)	6	1.18 (1.13, 1.43)	9	1.14 (0.87, 1.69)	.46
Segment 5	15	1.26 (0.20, 1.46)	6	1.28 (1.18, 1.36)	9	1.23 (0.20, 1.46)	.75
Segment 6	15	1.09 (0.81, 2.14)	6	1.28 (0.81, 1.50)	9	1.06 (0.83, 2.14)	.69
Segment 7	15	1.14 (0.46, 1.89)	6	1.38 (1.05, 1.67)	9	1.06 (0.46, 1.89)	.09
Segment 8	14	1.24 (0.22, 1.64)	6	1.19 (0.22, 1.62)	8	1.30 (0.85, 1.64)	.57

collection, and use of aggregated data (demographics, clinical characteristics, SWE, biopsy, and laboratory values) in this research study.

Results

A total of 17 participants were screened, and all 17 were enrolled in the study although one was later excluded due to lack of data collection. Data from 16 participants were used in the analysis. The median age of the 16 study participants was 12.6 years, and 62.5% (n = 10) were female (Table 1). Fifty percent of participants with a liver biopsy had stage 2 fibrosis and none had stage 4 fibrosis (F0 = 4, F1 = 2, F2 = 8, and F3 = 2). Thirteen (13/15; 86.7%) participants had a gray scale ultrasound score of 1 (heterogeneous echogenicity). Participants with F2-3 fibrosis tended to be younger than those with F0-1 (9 years vs 13 years, $P = .64$).

There was poor reproducibility of SWE within segments, and no significant difference in the SWS between F0-1 and F2-3 in all segments (1.18 m/s vs 1.14 m/s, $P = .46$). Seventy percent of the variance in SWS was intrasegmental, 17% was intersegmental, and 13% due to variation between participants. Segments 6 and 7 demonstrated higher SWS in F0-1 than F2-3. Only segment 8 had higher SWS trends in F2-3 versus F0-1 (1.30 m/s vs 1.19 m/s, $P = .57$; Table 2).

APRI was significantly higher in F2-3 versus F0-1 (0.5 vs 0.3, $P = .02$). SFas level was significantly lower in F2-3 versus F0-1 (5.6 vs 6.9, $P = .046$). MCP-1 was higher in F2-3 versus F0-1 (751.5 vs 559.5, $P = .06$). There were no significant differences in FIB-4, HA, TIMP-1, PH, collagen IV, IL-8, PDGF-BB, sICAM, or LH between F0-1 and F2-3 (Table 3). There were no significant differences in serum biomarker levels when divided by age groups (0-4, 5-9, ≥10 years), but there were differences in AST, alanine aminotransferase (ALT), and platelets by age ($P = .01$, $P = .03$, $P = .003$, respectively; Table 4). There was only 1 participant in the 5- to 9-year age group. AST and ALT were lowest in participants >10 years of age, and platelets decreased with increasing age.

A multiple logistic regression model predicting odds of F2-3 included APRI, sFas, and MCP-1 as predictors (N = 14). A 0.1-unit increase in APRI score was associated with a 1.86-fold (95% CI = 0.48-7.25; $P = .37$) increased odds of a participant having F2-3. A 1-unit increase in sFas was associated with a 0.37-fold (95% CI = 0.07-1.98; $P = .24$) decreased odds of a participant having F2-3. A 1-unit increase in MCP-1 was associated with a 1.01-fold (95% CI = 0.99-1.02; $P = .37$) increased odds of a participant having F2-3. The AUROC curve for simple logistic regression models for APRI, sFas, and MCP-1 to predict F2-3

Table 3. Median Biomarker Scores of F0-1 Versus F2-3.

Biomarker	N	All fibrosis stages (min, max)	N	F0-1 (min, max)	N	F2-3 (min, max)	*P*
APRI	16	0.5 (0.2, 0.8)	6	0.3 (0.2, 0.5)	10	0.5 (0.3, 0.8)	.02
FIB-4	16	0.3 (0.1, 0.5)	6	0.3 (0.1, 0.4)	10	0.3 (0.1, 0.5)	.71
TIMP-1	14	79.5 (49.8, 171.8)	6	84.8 (53.7, 161.2)	8	79.5 (49.8, 171.8)	.85
PH	14	5.3 (1.7, 12.7)	6	3.6 (1.9, 10.3)	8	5.8 (1.7, 12.7)	.18
HA	14	35.1 (11.0, 72.2)	6	38.6 (18.0, 72.2)	8	34.6 (11.0, 52.6)	.76
MCP-1	14	650.7 (470.5, 1095.0)	6	559.5 (470.5, 675.8)	8	751.5 (472.8, 1095.0)	.06
Collagen IV	14	692.4 (445.5, 1939.5)	6	692.4 (458.3, 863.3)	8	751.5 (445.5, 1939.5)	.66
IL-8	14	7.5 (5.1, 14.0)	6	8.1 (5.3, 14.0)	8	7.5 (5.1, 10.3)	.66
PDGF-BB	14	70.0 (38.1, 83.7)	6	59.2 (38.1, 77.6)	8	73.6 (41.3, 83.7)	.23
SFas	14	6.1 (3.3, 7.9)	6	6.9 (4.9, 7.9)	8	5.6 (3.3, 6.3)	.046
sICAM	14	217.4 (171.2, 348.2)	6	204.1 (189.9, 348.2)	8	242.0 (171.2, 300.4)	.66
LH	14	104.7 (40.5, 174.7)	6	88.8 (40.5, 172.5)	8	123.3 (55.1, 174.7)	.66

Abbreviations: APRI, aspartate transaminase to platelet ratio index; FIB-4, fibrosis index based on 4 factors; TIMP-1, tissue inhibitor of metalloproteinase 1; PH, prolyl hydroxylase; HA, hyaluronic acid; MCP-1, monocyte chemoattractant protein 1; IL-8, interleukin 8; PDGF-BB, platelet-derived growth factor; SFas, soluble Fas; sICAM, soluble intercellular adhesion molecule; LH, lysyl hydroxylase.

Table 4. Median Biomarker and Noninvasive Fibrosis Scores Among Different Age Groups.

Biomarker	N	0-4 years (min, max)	N	5-9 years (min, max)	N	≥10 years (min, max)	*P*
APRI	6	0.5 (0.3, 0.8)	1	0.7 (0.7, 0.7)	9	0.5 (0.2, 0.7)	.29
FIB-4	6	0.1 (0.1, 0.1)	1	0.1 (0.1, 0.1)	9	0.4 (0.3, 0.5)	.003
AST	6	66.0 (50.0, 85.0)	1	76.0 (76.0, 76.0)	9	37.0 (14.0, 62.0)	.01
ALT	6	77.5 (52.0, 112.0)	1	113.0 (113.0, 113.0)	9	42.0 (17.0, 78.0)	.03
Platelets	6	322.0 (269.0, 536.0)	1	276.0 (276.0, 276.0)	9	201.0 (171.0, 238.0)	.003
TIMP-1	5	91.1 (49.8, 171.8)	1	76.5 (76.5, 76.5)	8	77.8 (53.7, 171.8)	.98
PH	5	5.5 (3.0, 12.7)	1	4.6 (4.6, 4.6)	8	4.9 (1.7, 11.6)	.67
HA	5	33.3 (18.0, 52.6)	1	29.0 (29.0, 29.0)	8	39.0 (11.0, 72.2)	.81
MCP-1	5	659.2 (480.5, 927.8)	1	1066.6 (1066.6, 1066.6)	8	630.0 (470.5, 1095.0)	.37
Collagen IV	5	837.0 (458.3, 890.3)	1	543.8 (543.8, 543.8)	8	692.4 (445.5, 1939.5)	.82
IL-8	5	10.0 (5.4, 14.0)	1	9.5 (9.5, 9.5)	8	6.0 (5.1, 11.8)	.36
PDGF-BB	5	73.9 (44.1, 77.6)	1	68.9 (68.9, 68.9)	8	66.9 (38.1, 83.7)	.94
SFas	5	4.9 (3.3, 7.5)	1	6.0 (6.0, 6.0)	8	6.2 (5.0, 7.9)	.11
sICAM	5	258.2 (184.1, 348.2)	1	299.6 (299.6, 299.6)	8	199.7 (171.2, 342.5)	.50
LH	5	129.2 (55.1, 167.9)	1	60.5 (60.5, 60.5)	8	104.7 (40.5, 174.7)	.81

Abbreviations: APRI, aspartate transaminase to platelet ratio index; FIB-4, fibrosis index based on 4 factors; TIMP-1, tissue inhibitor of metalloproteinase 1; PH, prolyl hydroxylase; HA, hyaluronic acid; MCP-1, monocyte chemoattractant protein 1; IL-8, interleukin 8; PDGF-BB, platelet-derived growth factor; SFas, soluble Fas; sICAM, soluble intercellular adhesion molecule; LH, lysyl hydroxylase.

separately were 0.88 (95% CI = 0.68-1.00), 0.82 (95% CI = 0.57-1.00), and 0.81 (95% CI = 0.57-1.00), respectively (Figure 1). A logistic regression analysis combining APRI, sFas, and MCP-1 as calculated by (APRI × MCP-1)/sFas was performed (N = 14). A 1-unit increase in the combined measure was associated with a 1.1-fold increased odds of F2-3 (95% CI = 0.98-1.22; $P = .10$). AUROC curve for this model was 0.92 ($P < .001$, 95% CI = 0.75-1.00; Figure 2). A cut-off value of 49.8 for the calculated value provided a sensitivity of 87.5% (95% CI = 47-100) and specificity of 100% (95% CI = 54-100).

Discussion

Progressive liver fibrosis and cirrhosis are serious complications of chronic pediatric viral hepatitis.[6] The ability to accurately monitor the progression of fibrosis noninvasively would better inform the timing of initiating highly effective nucleos(t)ide analogues or direct-acting

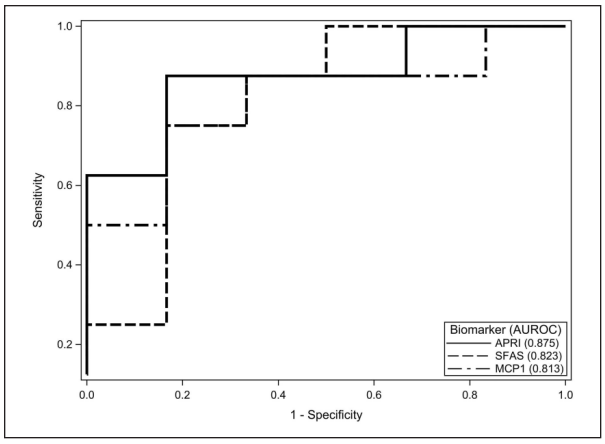

Figure 1. Individual AUROC of APRI, sFas, and MCP-1. AUROC, area under the receiver operating characteristic curve; APRI, aspartate transaminase to platelet ratio index; sFas, soluble Fas; MCP-1, monocyte chemoattractant protein 1.

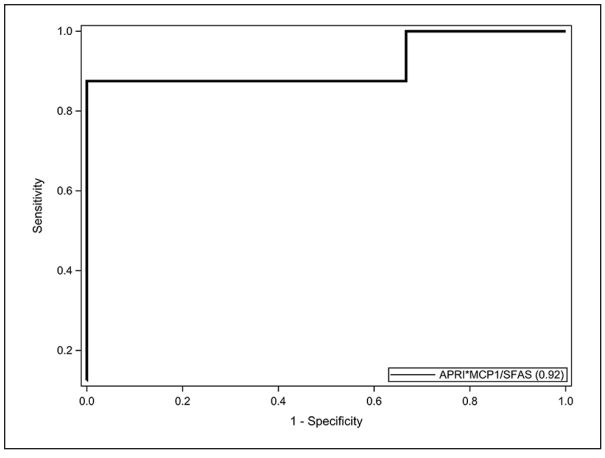

Figure 2. AUROC of multiple logistic regression analysis combining (APRI × MCP-1)/sFas. AUROC, area under the receiver operating characteristic curve; APRI, aspartate transaminase to platelet ratio index; sFas, soluble Fas; MCP-1, monocyte chemoattractant protein 1.

antivirals.[3,10] While some studies have shown greater correlation between SWE and fibrosis stage in the right upper lobe, the optimal segment for reproducibility within the right upper lobe has not been established in children.[46,47] In our study, measurements were taken in multiple segments of the right liver lobe, in order to compare reproducibility and identify if one provided more consistent measurements correlating with liver biopsy fibrosis stage. In our cross-sectional liver biopsy validation study, SWE demonstrated poor reproducibility within segments of the right upper lobe and poor correlation with fibrosis among pediatric participants with chronic viral hepatitis, as seen in previous studies.[46,48,49] Factors such as steatosis and inflammation early in the process of viral hepatitis may have confounded SWS.[50] Only segment 8 consistently demonstrated a positive correlation between fibrosis stage and SWS as a surrogate for LSM, as reported by adult studies. We identified very high intrasegmental SWS variation in our participants, suggesting either poor compliance during acquisition of measurements or operator inconsistency. Variation between segments in a single participant may reflect heterogeneous fibrosis and/or inflammation. SWE does not appear as reliable, reproducible, or standardized as transient elastography (ie, Fibroscan) in children with viral hepatitis.[51-53]

While the primary limitation to this study is its sample size, liver biopsy validation was performed for each participant. Collection of 20% and 33% of serum samples and SWE, respectively, were performed >3 months from the time of biopsy; however, significant change in liver remodeling or fibrosis during this time is unlikely. None of the participants had stage 4 fibrosis, eliminating an important population for SWE measurement analysis. However, stage 4 fibrosis (cirrhosis) from hepatitis B and C in children is uncommon with an estimated prevalence of 0.2% to 3.8% and 2%, respectively.[54-57] Since the median age of our study cohort was only 12.6 years, the likelihood of identifying a study participant with F4 or cirrhosis was very small.

Most liver biomarker analyses typically compare difference between F0-2 (not severe) versus F3-4 (severe); however, given the lack of F4 in our participants, we compared F0-1 (minimal to none) versus F2-3 (mild to moderate fibrosis).[58,59] These findings are clinically important for providers as patients with F2-3 are often prioritized for earlier antiviral treatment. Importantly, our finding suggests that a few of the novel serum biomarkers, namely, sFas and MCP-1 both individually and in combination with APRI can differentiate between minimal (F0-1) and moderate fibrosis (F2-3), which we believe is even more clinically useful as it may inform sooner clinical management before progression to F3-4 (severe fibrosis), which may be harder to reverse. In particular, APRI and MCP-1 were increased in F2-3 versus F0-1. MCP-1 is an inflammatory chemokine, which attracts monocytes and leads to increased inflammation and later fibrosis.[35-37] Interestingly, sFas, known to trigger apoptosis leading to hepatocyte damage, was lower in F2-3 versus F0-1. While the literature in adults suggests sFas concentrations are higher in more advanced fibrosis, we hypothesize that our finding of low sFas levels among F2-3 may reflect immunoregulation of inflammation once moderate fibrosis has formed.[60-62] Our biomarker concentrations were not affected by age,

which would be an important confounder for which to control, as markers associated with collagen may be influenced by growth. The logistic regression analysis combining APRI, sFas, and MCP-1 was able to predict F2-F3 fibrosis with high sensitivity. When examining the AUROC for each of the variables individually, they all had a significant AUROC, which shows that the combined regression analysis is not driven by one single factor, but the combined impact of them all.

Conclusion

In this pilot study, we conclude that APRI, sFas, and MCP-1 show promise as noninvasive markers of the progression of liver fibrosis in chronic pediatric viral hepatitis. Further studies of APRI and serum biomarkers in a larger cohort of children with viral hepatitis may inform thresholds predicting specific stages of fibrosis. Our SWE data highlight the importance of reliability and reproducibility of this testing modality, which may be more difficult in a pediatric population.

Author Contributions

Rebecca Mercedes contributed to analysis and interpretation of the data and drafting of the manuscript. Jameisha Brown contributed to the conception and design of the project and acquisition of the data. Charles Minard contributed to design of the project, analysis and interpretation of the data, and drafting of the manuscript. Cynthia M. Tsai contributed to acquisition of data. Sridevi Devaraj contributed to conception and design of the project and analysis and interpretation of the data. Marthe Munden contributed to conception and design of the project, acquisition, analysis and interpretation of the data. Daniel Leung contributed to conception and design of the project, acquisition, analysis and interpretation of the data, and drafting of the manuscript. All authors critically revised the manuscript for important intellectual content.

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding

The author(s) disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: This work was supported by a grant funded by the Texas Children's Hospital Pediatric Pilot Research Fund.

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