Utility of Direct Pancreatic Function Testing in Children

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Objectives: Exocrine pancreatic insufficiency (EPI) can have a significant impact on a child's growth and nutrition. Our aim was to evaluate the utility of direct endoscopic pancreatic function testing (ePFT) in pediatrics.

Methods: A single-center retrospective chart review was performed of children who underwent ePFT from December 2007 through February 2015. Endoscopic pancreatic function testings were performed by 1 of 2 methods: (1) intravenous cholecystokinin, followed by the collection of a single duodenal aspirate at 10 minutes, or (2) intravenous cholecystokinin or secretin, followed by the collection of 3 duodenal aspirates at a 5, 10, and 15 minutes. Samples were tested for pH and enzyme activities.

Results: A total of 508 ePFTs were performed (481 single-sample tests, 27 multiple-sample tests). Based on the multiple-sample group, enzyme levels for chymotrypsin, amylase, and lipase peaked at 5 minutes, followed by a decrease in activity over time. Exocrine pancreatic sufficiency was identified in 373 (73.4%) and EPI in 93 (18.3%). Exocrine pancreatic sufficiency analysis found all pancreatic enzyme activities significantly increase with age: trypsin, chymotrypsin, amylase, and lipase, ($P < 0.05$).

Conclusions: Endoscopic pancreatic function testing can be used in the evaluation of EPI in children. Normative data suggest that pancreatic enzyme activities mature with age.

Key Words: pancreatic fluid, exocrine pancreatic insufficiency, pediatric

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Exocrine pancreatic insufficiency (EPI) is an uncommon condition in children but can have serious implications on a child's health and growth potential. Early diagnosis and treatment of this condition are vital to limiting patient morbidity and achieving the goal of optimal nutrition and growth.[1]

The exocrine function of the pancreas has a significant reserve such that 90% to 98% of acinar cell mass can be lost before symptoms of EPI develop.[2] Hence, advanced, irreversible pancreatic disease may exist before the recognition of EPI. The etiopathogenesis behind the development and progression of EPI is not fully understood. However, the importance of a reliable, sensitive, and specific diagnostic test for children cannot be overemphasized. The variability of pancreatic function testing methods and different thresholds for EPI detection likely are key factors that have hindered the progression of this field over the years.[3]

Diagnosis of EPI can be obtained by both direct and indirect testing. Direct pancreatic function testing (PFT) is considered the "criterion standard" and entails pancreatic stimulation and duodenal intubation for collection of pancreatic secretions (pancreatic enzyme activities or bicarbonate concentrations). Direct PFT has been shown to have a high sensitivity and specificity, 85% to 100%[4] and 93%,[5] respectively. The previously described Dreiling method for direct PFT[6,7] is not widely used, and endoscopic PFT (ePFT) is gaining greater attention during the last 2 decades because it does not require radiation and can be performed under sedation.[8] Universal application of ePFT can be restrictive because of the need for endoscopy and the associated increased costs.[2,9] Indirect, noninvasive methods for EPI testing include measurements of plasma pancreatic enzymes or hormones as well as stool sampling for fecal elastase and fecal chymotrypsin. These indirect tests have the advantage of being simple, noninvasive, and inexpensive. However, their variable sensitivity and specificity preclude their widespread usage.[10–13] Moreover, in children, data are limited, and consensus is lacking on the most appropriate test that can be performed with accurate results.

The aims of our study are to evaluate the utility of direct ePFT and to identify the optimal process for sample collection.

MATERIALS AND METHODS

The study was approved by the institutional review board of Cincinnati Children's Hospital Medical Center (2014-4213). Patients were identified by obtaining the list of all consecutive sample results historically sent to 1 laboratory (Women and Children's Hospital of Buffalo, Buffalo, NY), as well as the database that was prospectively collected starting January 2013 for children who underwent ePFT in the last year of the study. Charts were retrospectively reviewed of children who underwent ePFT at Cincinnati Children's Hospital Medical Center (Cincinnati, Ohio), from December 2007 through February 2015. Based on our standing internal hospital protocol, ePFT was performed under general anesthesia with patients nil per os 4 hours before scheduled procedure time for liquids and 8 hours for solids. Our protocol included the following steps: bolus intravenous (IV) administration of human secretin (ChiRhoClin, Burtonswille, Md) 0.2 μg/kg or CCK (KINEVAC, Bracco, Monroe Township, NJ) 0.04 μg/kg at a time point 10 minutes before the collection of the first duodenal fluid aspirate. From December 2007 through December 2012, our ePFT protocol was limited to a single duodenal aspirate of 1 to 2 mL, with these patients characterized as the "single-sample group" for the purpose of the study. From January 2013 through February 2015, the ePFT protocol was modified as a quality improvement measure to include 3 duodenal aspirates using separate syringes at 5-minute intervals beginning from 5 minutes after CCK/secretin administration, with these patients characterized as the "multiple-sample group."

Stomach was not suctioned before the collection; pH was tested to ensure that it is basic greater than 7, before the samples got collected in the tubes. All duodenal fluid aspirates were collected using a 5-4-3 tapered catheter with point-of-care pH testing of each sample. Aspirates were transferred to Eppendorf tubes and immediately placed on ice for shipment to the reference laboratory

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TABLE 1. Patient Who Underwent ePFT Demographics

	N = 466	EPS (n = 373)	EPI (pH ≥ 7, n = 93)	*P*
Sex, male, n (%)	281 (60.3)	223 (59.8)	58 (62.4)	0.65
Age, y	3.4 (1.7–8.0) [0.01–21.7]	3.4 (1.8–8.3) [0.1–21.7]	2.9 (1.7–6.3) [0.01–20.7]	0.13
Indications, n (%)				0.35
FTT or poor weight	170 (36.5)	139 (37.3)	31 (33.3)	
Diarrhea	106 (22.7)	88 (23.6)	18 (19.4)	
FTT/diarrhea	50 (10.7)	41 (11.0)	9 (9.7)	
Other	54 (11.6)	38 (10.2)	16 (17.2)	
Unknown	86 (18.5)	67 (18.0)	19 (20.4)	
Fecal elastase available, n (%)	165 (35.4)	121 (32.4)	44 (47.3)	0.01

Age presented as median (25th–75th percentile) [min-max].
FTT indicates failure to thrive.

(Women and Children's Hospital of Buffalo). The enzyme activities for trypsin,[14] amylase,[15] lipase,[16] and chymotrypsin[17] along with the total protein level were measured on each sample[18] according to previously described methods. Normal pancreatic enzyme activity values were reported as trypsin level of greater than 55.4 nmol/mL per minute, amylase level of greater than 32 μmol/mL per minute, lipase level of greater than 146 μmol/mL per minute, and chymotrypsin level of greater than 2.5 μmol/mL per minute.

Samples above the normal reference range threshold on all 4 enzymes were categorized as exocrine pancreatic sufficiency (EPS). If a patient's measured levels were below the normal reference range threshold on only 1 enzyme and the child was younger than 2 years, they were also categorized as EPS. Exocrine pancreatic insufficiency was defined as below the normal reference range on at least 1 enzyme for patients 2 years or older or if results found more than 1 enzyme to be below the normal reference range for patients younger than 2 years.

Data were analyzed using SAS, Version 9.3 (SAS Institute, Cary, NC). Categorical data were summarized as frequency counts with percentages, and group comparisons were analyzed using χ^2 tests. Age was skewed, so a Wilcoxon-Mann-Whitney test was used to compare the medians between the 2 groups. Because of the skewed data, $\log^{10}$ transformations were applied to all 4 enzymes. To account for some patients having multiple encounters, generalized linear models with random effects were used to assess whether the 4 enzymes changed with age. Kappa tests of agreement were used to compare the PFT and the fecal elastase results as well as to test whether the 15-minute sample results differed from the 10-minute sample results for the multiple-sample data. Statistical significance was set a priori at an α value of 0.05.

RESULTS

Patient Demographics and Test Indications

Our patient population had a median age of 3.4 years (interquartile range, 1.7–8.0 years). Sex distribution did not significantly differ between the EPS and EPI groups (patient demographic data

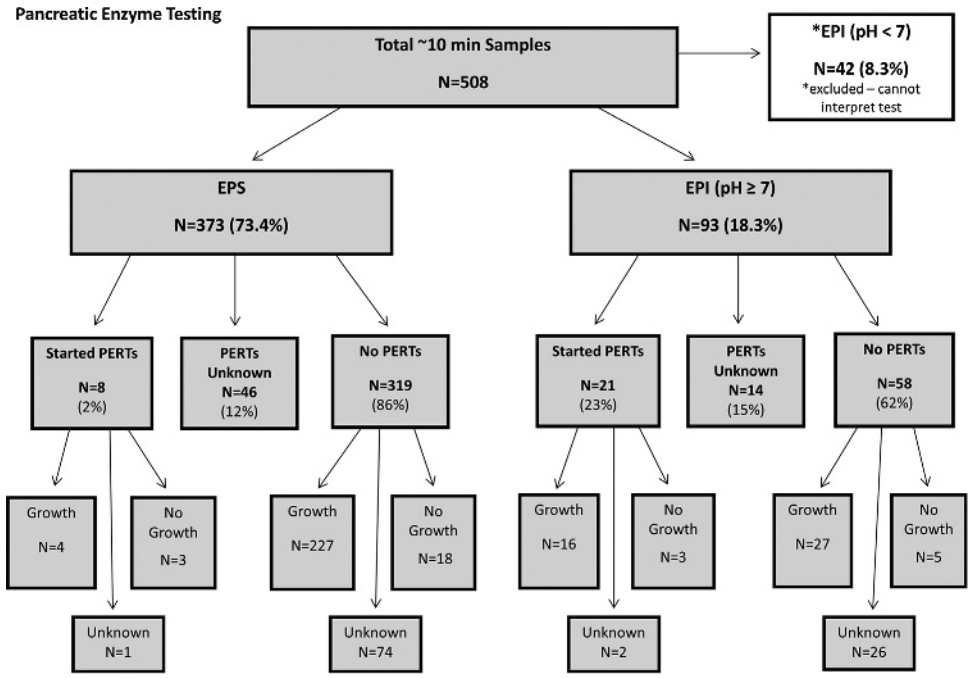

FIGURE 1. Tree diagram representing patients who underwent ePFT in the study period.

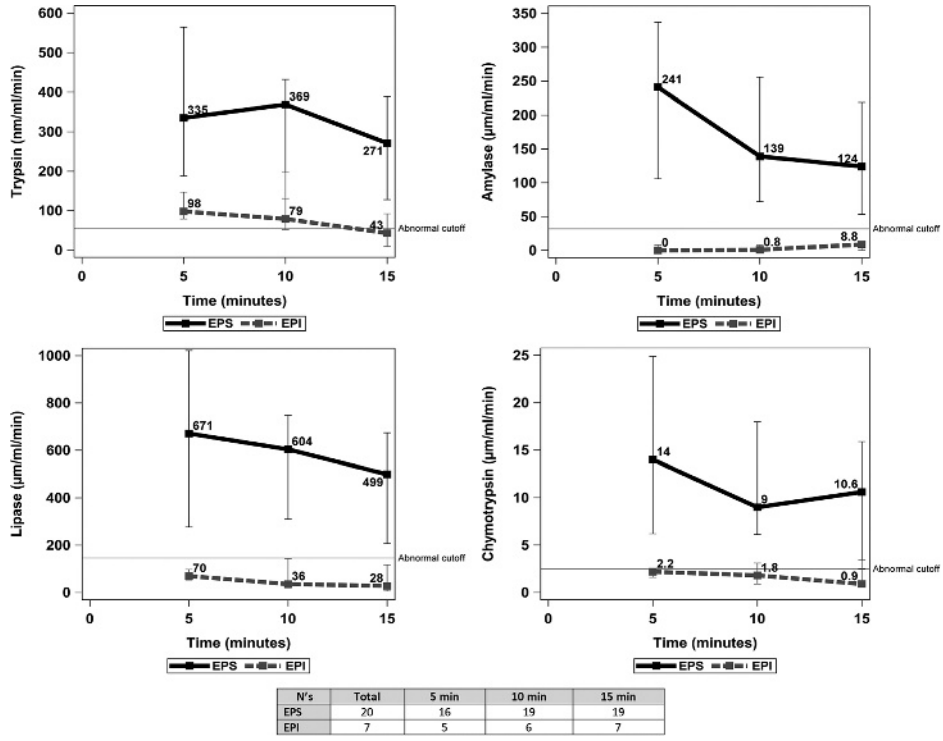

FIGURE 2. Pancreatic enzyme measurements curves for pancreatic sufficient and pancreatic insufficient patients.

shown in Table 1). The most common indication for ePFT was failure to thrive or poor weight gain, followed by chronic diarrhea (Table 1). Additional indications for ePFT included abdominal pain, chronic pancreatitis, and acute recurrent pancreatitis and were listed as "other."

Endoscopic PFT Results

A total of 508 ePFTs were performed on 493 patients (481 were single-sample testing and 27 multiple-sample testing). Forty-two tests (8.3%) all from single sample testing were considered suboptimal samples (pH < 7 resulting in uninterpretable enzyme activities) and excluded from the study analysis. These excluded samples did not significantly differ in patient age or sex from the remaining samples. The remaining 466 ePFTs (from 454 unique patients) were used for analysis. Protein concentrations did not significantly differ between the single- and multiple-sample groups at 10 minutes ($P = 0.19$) nor did any of the enzymes (trypsin [$P = 0.65$], amylase [$P = 0.66$], lipase [$P = 0.82$], or chymotrypsin [$P = 0.33$]). A total of 373 (73.4%) were found to be EPS and 93 (18.3%) were EPI in 1 or more of the enzymes tested (Fig. 1). Only a small number of patients had diagnoses known to be associated with EPI. Of those with pancreatic insufficiency, 1.1% (1/93) had CF and 7.5% (7/93) had chronic pancreatitis. None of the insufficient patients had Shwachman–Diamond syndrome. None of these disorders were present in the pancreatic-sufficient group.

Pancreatic Enzyme Levels

Analysis of ePFT from the multiple-sample group found that EPS enzyme levels for chymotrypsin, lipase, and amylase peaked at 5 minutes, followed by a decrease in activity with time (Fig. 2). Although trypsin activity level peaked at 10 minutes, the trypsin values did not significantly differ between 5 and 10 minutes. Data for EPS and EPI samples for all 4 enzymes tested are shown in Table 2. We also assessed the necessity of the 15-minute sample and found that the result at 15 minutes did not significantly differ from the values obtained at 10 minutes ($P = 0.16$).

Pancreatic Enzyme Maturation

Based on the data of EPS patients, we found that normal pancreatic enzyme activity matures with patient age. All 4 enzymes

TABLE 2. Multiple-Sample Enzyme Values for the Pancreatic Groups

	EPS	EPI	*P*
Trypsin, nmol/mL/min			
5 min	335.2 (188.2–565.3)	97.6 (78.7–146.6)	0.004
10 min	369.4 (131.2–432.3)	79.0 (51.5–197.3)	0.014
15 min	271.2 (128.3–388.7)	43.0 (8.5–91.7)	0.004
Amylase, μmol/mL/min			
5 min	240.7 (105.6–337.0)	0.0 (0.0–8.1)	0.0002
10 min	139.1 (72.2–255.8)	0.8 (0.0–7.5)	<0.0001
15 min	124.4 (53.4–219.3)	8.8 (0.0–11.7)	<0.0001
Lipase, μmol/mL/min			
5 min	671.0 (276.5–1021.5)	70.0 (53.0–98.0)	<0.0001
10 min	604.0 (310.0–748.0)	35.5 (20.0–143.0)	<0.0001
15 min	499.0 (207.0–674.0)	28.0 (6.0–117.0)	0.0004
Chymotrypsin, μmol/mL/min			
5 min	14.0 (6.2–24.9)	2.2 (1.6–2.5)	0.0003
10 min	9.0 (6.1–18.0)	1.8 (0.9–3.1)	0.001
15 min	10.6 (2.5–15.9)	0.9 (0.6–3.4)	0.0005

Data are presented as median (25th–75th percentile).

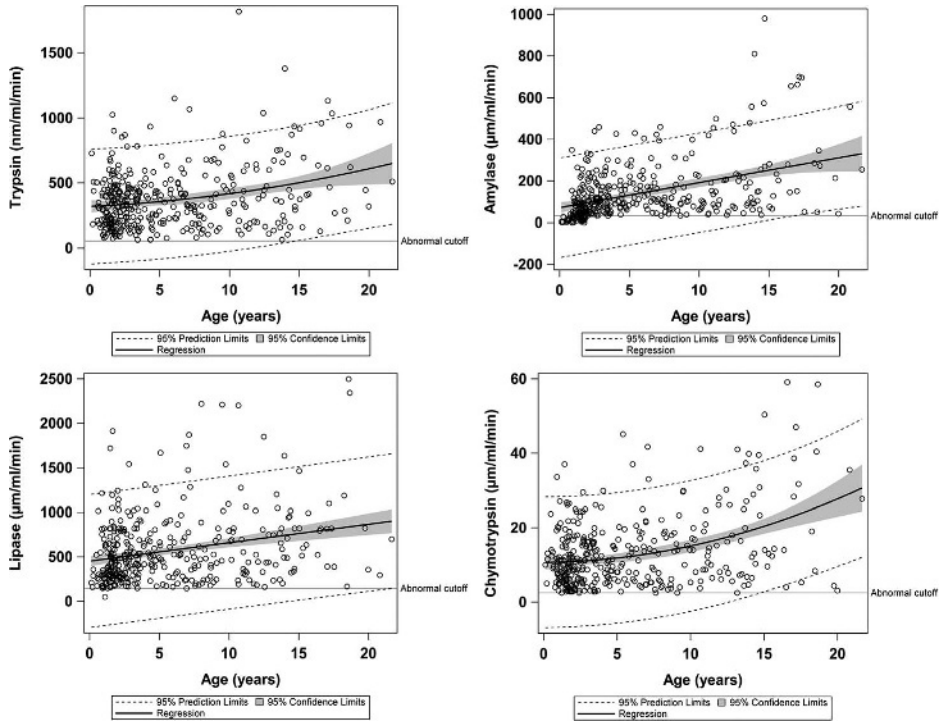

FIGURE 3. Pancreatic enzymes mature with age.

were found to significantly increase with age: trypsin ($P = 0.008$), amylase ($P = 0.0003$), lipase ($P = 0.005$), and chymotrypsin ($P = 0.003$, Fig. 3).

Endoscopic PFT and Fecal Elastase Concordance

A total of 165 patients (35%) had a fecal elastase measured within 6 months of ePFT. Fecal elastase values were available for 31%, 47%, 47%, 50%, and 42% of those with 0, 1, 2, 3, or 4 enzyme deficiencies, respectively. For the 31 patients with multiple enzyme deficiencies on ePFT, the fecal elastase was normal for 19 (61%), abnormal for 8 (26%), and indeterminate (100–200 μg/g) for 4 (13%). For those with no enzyme deficiencies on ePFT, 81 (77%) of 105 had a normal fecal elastase (Table 3).

When comparing the fecal elastase results with the ePFT, the sensitivity of fecal elastase for detecting EPI was 26.3% with a specificity of 88.7%. The positive predictive value of the fecal elastase was 45.5% with a negative predictive value of 77.0%. There was significant discordance between ePFT and fecal elastase results ($P = 0.01$).

Outcomes of Testing

Of the 93 patients with EPI by ePFT, 21 patients (22.6%) were started on pancreatic enzyme replacement therapy (PERT), 58 patients (62.4%) were not started on PERT and 14 (15.1%) patients had incomplete information as to whether PERT was initiated. Among the 21 patients who were started on PERT, 16 (76.2%) demonstrated normal growth within 6 months of starting therapy as reflected in their weight for age. Three patients (14.3%) had inadequate growth or weight loss, and 2 (9.5%) had unknown growth outcomes. From the patients who did not receive PERT, 27 (46.6%) showed growth within a 6-month period, 5 (8.6%) no growth, and 26 (44.8%) unknown. Of the 373 patients who had normal testing, 8 (2.1%) were started on PERT of which 4 (50.0%) showed normal growth, 3 (37.5%) inadequate growth, and 1 (12.5%) growth unknown (Fig. 1).

DISCUSSION

Exocrine pancreatic insufficiency in childhood is a rare condition, and patients may present with a diversity of signs and

TABLE 3. Number of Insufficient Enzymes and Fecal Elastase Results

	No. Abnormal Enzymes					
	0	**1**	**2**	**3**	**4**	**Total**
EPS	336 (90.1)	37 (9.9)	0	0	0	373
EPI, pH ≥ 7	0	25 (26.9)	34 (36.6)	8 (8.6)	26 (28.0)	93
Fecal elastase	105 (31)	29 (47)	16 (47)	4 (50)	11 (42)	165 (35)
Normal	81 (77.1)	22 (75.8)	11 (68.7)	1 (25)	7 (63.6)	122 (73.9)
Abnormal	10 (9.5)	4 (13.8)	4 (25)	1 (25)	3 (27.2)	22 (13.3)
100-200 μg/g	14 (13.3)	3 (10.3)	1 (6.2)	2 (50)	1 (9.1)	21 (12.7)

Data are presented as n (%).

symptoms such as poor weight gain, steatorrhea, or other symptoms of malabsorption that may raise concerns and prompt further investigation.[2,3] Having a high index of suspicion combined with a robust test for exocrine pancreatic function is critical to early detection and intervention that can prevent the sequelae of malnutrition. Endoscopic PFT with pancreatic fluid analysis to assess pancreatic function can be conducted by measuring bicarbonate or enzyme activities.[5,8] Previously published PFTs used different protocols, medication doses, test duration, and sample collection methods and even the result of what they measure (some measured bicarbonate and other studies measured enzyme activities) make each of them unique and not easy to compare with one another.

Comparison of these methods has not been conducted in pediatrics. In our study, we demonstrate that ePFT with different protocols has clinical utility in the pediatric population. By measuring the activity of amylase, lipase, chymotrypsin, and trypsin from multiple-sample duodenal aspirate collections can provide reliable results within 15 minutes of pancreatic stimulation.

Currently, there is limited information on how pancreatic exocrine function matures with age in humans. Studies in animal models[19–21] have demonstrated that the pancreas passes through different postnatal transitions until the functional maturation that includes adequate production of digestive enzymes.[22] Human studies have shown that chymotrypsin and trypsin activity levels vary in children older than 2 years,[23] suggesting that the pancreas may respond to different stimuli in the diet or vary in hormone levels in early versus late infancy.[23] Our study is the first to show that levels of all 4 pancreatic enzymes tested progressively increase after birth. This can set the foundation in the attempt to identify factors that influence the maturation and may provide therapeutic targets to overcome pancreatic exocrine insufficiency.

Reliably diagnosing EPI continues to be a challenge in the pediatric population. Despite several previous attempts to identify an accurate, rapid, and noninvasive test, direct ePFT remains the criterion standard.[1,3] However, a standardized protocol for ePFT has yet to be validated in children.[24–26] In adult studies, infusing CCK for an hour and collecting samples to determine lipase levels at 20, 40, 60, and 80 minutes have found that lipase peaks between 40 and 50 minutes after stimulation and can provide an accurate and reproducible diagnosis of EPI.[27] Our institution has recently adapted the protocol for ePFT similar to the one described by Del Rosario et al[25] who used a bolus administration of CCK or secretin, followed by the collection of duodenal aspirates within 15 minutes from pancreatic stimulation. Our current results suggest that by obtaining multiple samples, we are less likely to misdiagnose conditions of patients with EPI as normal because the results from 1 sample may be inadequate or inconclusive. This is evident by the fact that all the patients who had EPI on the multiple collection ePFT were treated with PERTs and that 62% of the EPI result on single collection group were not treated with PERTs. Moreover, we found no statistically significant difference between a 10-minute sample and a 15-minute sample, when both samples were obtained, supporting an abbreviated sample collection time requiring only 2 pancreatic fluid samples to provide a reliable assessment of exocrine pancreatic function in children. Interpretation of test results should take into consideration the EPS and EPI curves because there is some overlap in enzyme activities 25th and 75th percentiles, but this can be better interpreted when patient age is accounted for, because the maturation affects those enzyme activities as the patients grow in age.

Because stool sampling for fecal elastase measurement is noninvasive and readily available, it has continued to be a commonly used test in clinical practice. However, its sensitivity and specificity are questionable when mild forms of EPI are present.[9,12,28] From our results, we show that fecal elastase and ePFT lacked strong agreement with 7 of 11 EPI patients insufficient in all 4 enzymes having a normal fecal elastase (Table 3 and Supplementary Table 1, http://links.lww.com/MPA/A528). However, because fecal elastase continues to be widely used in clinical practice, its validity to accurately detect EPI or exclude it necessitates further study to identify the appropriate clinical setting for its use.

Our study represents one of the largest pediatric cohorts with PFTs; however, there are limitations to address. Given the retrospective nature of the study, a proportion of patients included were lost to follow up, and data results for fecal elastase and growth outcomes were incomplete. Not all patients had fecal elastase values, and the test was not sent at the exact time PFTs were performed. In addition, the clinical reasoning underlying a physician's choice for not starting PERT that was encountered in a subset of patients with EPI and abnormal ePFT results is undiscernable. For future studies, such limitations can be overcome by a prospective study with simultaneous acquisition of PFT data, anthropometrics, and defined growth and nutritional outcomes to derive a consensus on ePFT and its utility in the pediatric population.

CONCLUSIONS

Endoscopic PFT is one of the most useful tests to diagnose EPI in the appropriate clinical setting and should be considered to come to a diagnosis when other causes of malnutrition or malabsorption have been considered and ruled out. Optimal ePFT in pediatrics can be obtained with an abbreviated collection time through a multiple-sample collection protocol. The interpretation of ePFT results in children should be referenced according to patient age, because enzyme maturation occurs through the early years of a child's life. Future pediatric studies are needed to validate normative ePFT values across all age groups and assess the effect of ePFT on management and nutritional outcomes in children.

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