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Invited Review

Indirect Pancreatic Function Tests in Children

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CHRONIC PANCREATITIS AND PANCREATIC EXOCRINE INSUFFICIENCY

Cystic fibrosis (CF) is the most frequent cause of severe exocrine pancreatic insufficiency in childhood. Other, less common, disorders causing exocrine pancreatic insufficiency in children are listed in Table 1 (1,2). The pathophysiologic basis of exocrine pancreatic dysfunction in childhood has been well characterized (3).

Chronic pancreatitis in children is not as rare as is generally thought. The improved sensitivity of imaging procedures has increased the previous estimates of frequency in adults (4–6), and may do so in children. Recognition of high-risk groups with genetic predisposition but less evident clinical manifestations may also result in more diagnosed cases (2,7–9). The diagnosis and staging of chronic pancreatitis can be made by histology or morphologic criteria alone or by a combination of morphologic, functional and clinical findings (10–14). Abnormal pancreatic function tests are almost diagnostic of chronic pancreatitis when clinically suspected, but also occur in other conditions including pancreatic agenesis, pancreatic resection, Shwachman-Diamond syndrome, Pearson syndrome, intestinal atrophy, kwashiorkor and gastrinoma. Normal pancreatic function tests results do not exclude chronic pancreatitis, especially mild and moderate forms. Functional tests give no information about the underlying pancreatic process, and therefore the use of structural imaging or functional assessment of the pancreas should be dependent on the specific aim of the evaluation.

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An assessment of pancreatic function is a major diagnostic procedure routinely used in CF, and it also creates the basis for proper treatment of other pancreatic disorders in children.

PANCREATIC FUNCTION TESTS

Chronic diarrhea, steatorrhea, abdominal pain, failure to thrive and weight loss are the main indications for pancreatic function testing in pediatrics.

Exocrine pancreatic function can be assessed by direct or indirect procedures (15,16). Direct pancreatic function tests such as the secretin-cholecystokinin test have the highest sensitivity and specificity. Because of its reliability, the secretin-cholecystokinin test remains the accepted standard procedure for the assessment of exocrine pancreatic function (17). However, it has major disadvantages that make it unsuitable for routine use in pediatric patients. Direct tests are time-consuming, expensive, uncomfortable and not well standardized in children.

Indirect tests are used routinely in clinical practice because they are noninvasive and simple, and they are less time-consuming and less expensive than direct tests. The development of new indirect tests has improved the diagnostic approach. However, indirect tests have limited sensitivity and specificity, especially in patients with mild to moderate exocrine pancreatic insufficiency. Indirect pancreatic function tests are summarized in Table 2. Three main categories of indirect tests can be described:

1) Assessment of the absorption of markers that are hydrolyzed from conjugates by pancreatic enzymes and subsequently appearing in the urine or serum (NBT-PABA test, pancreolauryl test) (18–23),
2) Analysis of undigested and unabsorbed food components in feces (fecal fat excretion, efficiency of fat/nitrogen absorption, fecal fat concentration,

TABLE 1. *Main causes of exocrine pancreatic insufficiency in childhood*

Anatomical abnormalities	Pancreatic agenesis Pancreatic hypoplasia
Inherited diseases	Cystic fibrosis Shwachman-Diamond syndrome Johanson-Blizzard syndrome Pearson syndrome Hereditary pancreatitis Isolated enzyme deficiences (lipase, colipase)
Pancreatitis	Idiopathic Traumatic Viral Drug-induced Nutritional Auto-immune
Insufficiency secondary to other disorders	Pancreatic resection Celiac disease and other cases of villous atrophy Malnutrition Primary sclerosing cholangitis Allagile's syndrome Diabetes Enterokinase deficiency
Neoplastic diseases	

steatocrit, acid steatocrit) (24–31) or the analysis of oxidation products of digested and absorbed fat in expired air (breath tests) (32–35),

3) Measurement of pancreatic enzymes in the serum (amylase, isoamylase, lipase, trypsinogen, elastase-1) (36–39) or stool (chymotrypsin, lipase, elastase-1). (40–48).

The measurement of serum enzymes contributes to assessment of exocrine pancreatic function with poor quantitative precision about residual function. Fecal enzymes provide a better indication of likely functional adequacy. The other tests measure the biologic effect of pancreatic enzymes (i.e., digestion and consequent absorption). The presence of steatorrhea is the most frequently used criterion for the confirmation of exocrine pancreatic insufficiency. However, steatorrhea, especially in CF patients, is influenced by many factors in addition to pancreatic insufficiency, including fat intake, abnormalities of gastrointestinal motility, duodenal acidity secondary to diminished pancreatic bicarbonate secretion and gastric hypersecretion, inactivation of pancreatic enzymes by low pH and loss of bile salts (49). As shown in CF patients (50), the correlation between fecal fat excretion and the secretin-cholecystokinin test is less significant than the correlation between fecal elastase-1 concentration and the secretin-cholecystokinin test. Thus, fecal fat excretion seems to be less precise than the other tests in defining real exocrine pancreatic function. In addition, the presence of steatorrhea may be also related to other gastrointestinal diseases such as celiac disease. On the other hand, fecal fat excretion reflects the biologic effects of pancreatic insufficiency and can be very important when considering the adequacy of enzyme supplementation.

TABLE 2. *Major indirect pancreatic function tests*

	Invasiveness/ patient rejection	Time-consumption	Sensitivity/ specificity	Costs
NBT-PABA test*	−−	−−	++	−−
Pancreolauryl test†	−/−−	−−	++/+++	−−
Fecal fat excretion	−−	−−	+/++	−−
Fecal fat concentration	−	−	+	−
Steatocrit	−	−	+	−
Acid steatocrit	−	−	+/++	−
Breath tests	−/−−	−−	++/+++	−−
Fecal chymotrypsin	−	−	++/+++	−
Fecal elastase-1	−	−	+++	−

More pluses-value of the test more positive; more minuses-value of the test more negative/higher costs.

*Not available in Europe; †standard test (without secretin stimulation).

CLASSIFICATION OF PANCREATIC EXOCRINE INSUFFICIENCY

According to the results of secretin-cholecystokinin test and normal/abnormal fecal fat excretion, exocrine pancreatic insufficiency is divided into three categories (51):

I) Mild: pathologic volume and bicarbonate secretion, normal secretion of enzymes, no steatorrhea;
II) Moderate: pathologic volume and bicarbonate secretion as well as pathologic enzyme secretion, no steatorrhea;
III) Severe: as II with steatorrhea.

Patients with pancreatic involvement are divided into two categories according to their fecal fat excretion:

1) pancreatic sufficient (PS): without steatorrhea;
2) pancreatic insufficient (PI): with steatorrhea.

The term ''pancreatic sufficient'' is not equivalent to normal pancreatic function. The choice of the test should be based on the specific aim of the assessment: detection of exocrine pancreatic insufficiency or assessment of fecal fat losses. Pancreatic function tests answer two questions: ''does a subject have exocrine pancreatic insufficiency?'' and ''does exocrine pancreatic insufficiency result in steatorrhea?''

Does a Subject Have Exocrine Pancreatic Insufficiency?

Considering time, expense, proven clinical value of the test in the assessment of pancreatic function and patient acceptance (Table 2), four possible choices were selected: fecal chymotrypsin, fecal elastase-1,

pancreolauryl test and mixed triglyceride breath test. The major description of selected tests is summarized in Table 3.

The choice of the test used in the assessment of exocrine pancreatic function in children should be based on sensitivity/specificity and noninvasiveness. The tests described in Table 3 have high sensitivity in the assessment of severe exocrine pancreatic insufficiency and limited sensitivity in mild pancreatic dysfunction (41,42,50,52–57). However, the diagnosis of exocrine pancreatic insufficiency in patients with severe steatorrhea is not a clinical problem. In the subgroup of adult patients with chronic pancreatitis and severe steatorrhea (>15 g/day), Lankisch et al. (58,59) demonstrated excellent sensitivity of most tests and suggested that the diagnosis could be even made visually (60). However, the study of Borowitz et al. (61) challenged the common opinion often expressed at international congresses and in research papers that the subjective assessment of pancreatic insufficiency in CF is easy and obvious.

The diagnosis of mild exocrine pancreatic dysfunction is more difficult. There are few studies directly comparing the usefulness of different tests in the assessment of mild exocrine pancreatic insufficiency. In any comparison, it is important to take into account both sensitivity and specificity (i.e., improved sensitivity with at least equal specificity). Moreover, such comparison should be made in the same patient group at the same time.

Fecal elastase-1 is more sensitive than fecal chymotrypsin, fecal lipase or breath test in the assessment of exocrine pancreatic function in chronic pancreatitis (53,56,62) and cystic fibrosis (63,64). Fecal elastase-1 is at least as sensitive as the pancreolauryl test in chronic pancreatitis (52,54). However, the urininary collection for the pancreolauryl test lasts for 2 days and has not found wide acceptance among patients and physicians. In addition, there are no reliable standards in children for the pancreolauryl test dependent on serum samples. Therefore, the use of fecal elastase-1 test seems to be the most reasonable choice. Although the test has the highest sensitivity among indirect tests, the practical value of the measurement of fecal elastase-1 concentration in the assessment of mild pancreatic dysfunction is also limited. The use of the test for long-term observation of declining exocrine pancreatic function in individual subjects seems promising (65). In doubtful cases, the only reliable tool is the secretin-cholecystokinin test. However, its routine or repeated application in children is not acceptable. An algorithm based on the measurement of fecal elastase-1 is suggested (Fig. 1).

Determination of fecal chymotrypsin has been an accepted indirect test in pediatric practice for several years (40–42). However, as a result of the colorimetric method used, it is affected by enzyme substitution therapy (44). Thus, pancreatic enzyme supplementation must be stopped at least for 3 days before the test. In contrast, the fecal elastase-1 test (ELISA) is specific for the human enzyme and not influenced by exogenous enzyme supplementation (44,45). On the other hand, the interference by exogenous enzymes creates the possibility to check adherence to recommended pancreatic enzyme supplementation by the measurement of chymotrypsin activity.

An important disadvantage of the fecal elastase-1 test is the lack of full differentiation between primary exocrine pancreatic insufficiency and exocrine pancreatic dysfunction secondary to intestinal villous atrophy. Carrocio et al. (66) and Nousia-Arvanitakis et al. (67) found decreased fecal elastase-1 concentrations in celiac disease. Walkowiak and Herzig (68) proved that fecal elastase-1 concentrations might be decreased in villous atrophy regardless of the underlying disease. Schappi et al (69) documented significant correlation between decreased fecal elastase-1 concentrations and duodenal morphology and inflammation in primary gastrointestinal diseases. However, reduced pancreatic secretory capacity in celiac disease patients was also reported with the use of other tests: fecal chymotrypsin test (70), pancreolauryl test (71), breath test (72) and secretin-cholecystokinin test (67,70,73) in a smaller percentage of patients. Thus, villous atrophy generally seems to influence indirect pancreatic function tests. After mucosal regeneration on gluten-free diet, fecal elastase-1 specificity is again high (67,68). According to available data (66,68), in cases of villous atrophy and low fecal elastase-1 concentrations, the assays should be repeated

TABLE 3. *Selected indirect pancreatic function tests*

	Basic description	The interference with enzyme supplements
Pancreolauryl test	Standard meal, urine collection (2-day procedure) or blood sampling (1-day procedure), colorimetic method	Yes
Breath tests	Standard meal, 5-hour procedure, carbon isotopes – mass spectroscopy	Yes
Fecal elastase-1	Single stool sample, ELISA	No
Fecal chymotrypsin	Single stool sample, colorimetric method	Yes

ELISA = enzyme-linked immunosorbent assay.

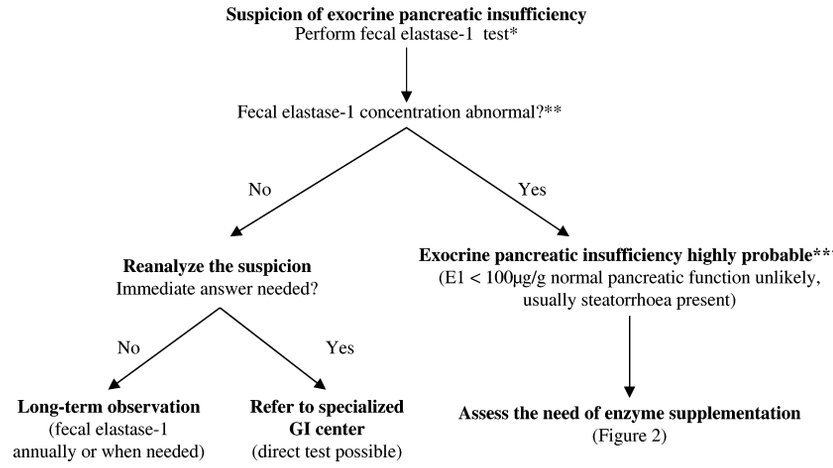

FIG. 1. Algorithm for assessment of exocrine pancreatic function. *If not available use other test. Perform proper imaging procedure of the pancreas (appropriate to the expected underlying disease). **In the case of borderline values we suggest to perform an assay with the use of three independent samples. ***Conduct differential diagnosis (especially consider villous atrophy and dilution effect of watery stool).

after 6 weeks of gluten-free diet and at 3, 6 or 12 months, if necessary.

The fecal elastase-1 test may also give false positive results during acute episodes of diarrhea (47). This was also observed with other indirect function tests. Salvatore et al. (74) documented transient pancreatic insufficiency associated with both bacterial and viral infections. As shown previously (47), the measurements should be repeated 2 weeks after recovery and later if necessary. The other disadvantage of indirect tests is the inability to detect rare cases of pancreatic exocrine insufficiency such as isolated lipase and colipase deficiency (75).

The measurement of serum immunoreactive trypsinogen (IRT) is another test applied in the assessment of exocrine pancreatic function. IRT levels are elevated in CF infants (76,77), and this finding creates the basis for the neonatal CF screening. However, the applicability of the test in the assessment of pancreatic function is limited. IRT levels in PI infants fall sharply in the first years of life so that more than 95% patients have subnormal values by the age of 7 years (38). By contrast, there is no age-related decline in serum IRT among PS patients (37) who maintain IRT levels within or above the normal range (38). Under the age of 7 years, IRT fails to distinguish PS from PI patients (37). Therefore, it seems that the measurement of IRT in the assessment of exocrine pancreatic function in PI patients is less useful than the fecal elastase-1 test. In PS patients with normal fecal elastase-1 concentrations, however, IRT could bring additional information. As the increased enzyme activity reflects the obstructive process in pancreatic ducts and secondarily the function of the parenchyma, the serum lipase activity does not adequately reflect pancreatic function. The use of serum enzymes as a measure of pancreatic function in chronic pancreatitis has low sensitivity and specificity (72,78).

The assessment of exocrine pancreatic function in PS patients creates a diagnostic challenge. Indirect tests lack sensitivity and direct tests are invasive. Therefore, there is a need for a new noninvasive method directed to mild cases of pancreatic insufficiency. Leodolter et al. (79) documented very high sensitivity, exceeding that of fecal elastase-1 test, of a modified serum pancreolauryl test in a small group of adult patients having mild and moderate forms of chronic pancreatitis. However, the control group was extremely small and the specificity of the test was unclear. In addition, the test is rather complicated and there are no data concerning children. Although preliminary results are promising, the test demands further studies. Similarly, it has been shown that the measurement of basal and secretin-stimulated lipase levels (80) have higher sensitivity than fecal elastase-1 test in the assessment of pancreatic involvement in older PS CF patients. The test could be an alternative to the direct tests in pancreatic sufficient patients, at least in some cases. Other possible diagnostic methods should also be evaluated in the future.

Does Exocrine Pancreatic Insufficiency Result in Steatorrhea?

The pancreas has a huge functional reserve capacity and the presence of steatorrhea indicates total/subtotal pancreatic damage (81). The digestion and absorption of fat are complicated processes. They demand the presence of pancreatic enzymes (lipase, colipase, phospholipase A2), bile salts, adequate intestinal milieu and normal intestinal mucosa. Fecal fat excretion, especially in CF patients, is influenced by many factors (49). Because only patients with severe exocrine pancreatic insufficiency need enzyme supplementation, the low sensitivity of fecal fat determination in the assessment of exocrine pancreatic function plays a minor role. In such cases we measure fecal fat losses, the biologic effect of pancreatic enzyme deficiency.

The analysis of daily fecal fat excretion over a period of 72 hours is the accepted standard procedure. The diet

TABLE 4. *Type of fecal fat assessed with the use of different methods*

	Triglycerides	Medium chain triglycerides	Phospholipids	Cholesterolester	Cholesterol	Fatty acids
Gravimetric analysis	+	+	+	+	+	+
Chemical analysis	+	−	+	+	−	+
Near-infrared spectroscopy	+	−	+	+	−	+
Steatocrit	+	+	+	+	+	+
Microscopy	+	+	−	−	+	+

regimens should be standardized, respectively, for age, weight and sex before and during the collection of stool. The following methods are used for the quantitative determination of fecal fat: gravimetric analysis (82), chemical analysis (24) and near-infrared spectroscopy (29,83). Chemical analysis is the standard procedure, but it is a time-consuming procedure. Near-infrared spectroscopy offers a good alternative because it has very good correlation with the chemical method and an additional possibility to measure water, nitrogen and protein content, but it requires special equipment. The range of normal values for fecal fat excretion in children has been established (25,84,85). As mentioned earlier, according to fecal fat excretion patients can be defined as PS (fecal fat excretion: 2–10 years <4–5 g/day; older than 10 years <7 g/day); or PI (fecal fat excretion: 2–10 years ≥4–5 g/day; above 10 years >7 g/day). In younger children and in infants, especially those younger than the age of 3 months, the available data are sparse and contradictory. The assessment of relative fecal fat losses is more accurate not being dependent on fat intake.

The effectiveness of fat absorption is measured by the coefficient of fat absorption.

$$\text{Coefficient of fat absorption (CFA)}(\%) = \frac{\textit{Fat intake}\ (g) - \textit{fecal fat losses}\ (g)}{\textit{Fat intake}\ (g)} \times 100$$

In adults, adolescents, school age and preschool children, the coefficient of fat absorption should exceed 93%. In younger children and in infants, especially those younger than 3 months, the available data are sparse. In neonates the mean coefficient of fat absorption is approximately 85% and it increases with age (86). The 85% cut-off level is suggested for infant younger than 6 months (87).

Because fecal fat balance studies are cumbersome, expensive and unpleasant for all involved, simple tests are often preferred (microscopy with Sudan staining, fecal fat concentration, steatocrit, acid steatocrit) (27,28,30,88). Among these, acid steatocrit seems to be the most reliable (30). In experienced hands, fecal microscopy, a very inexpensive method, is also useful

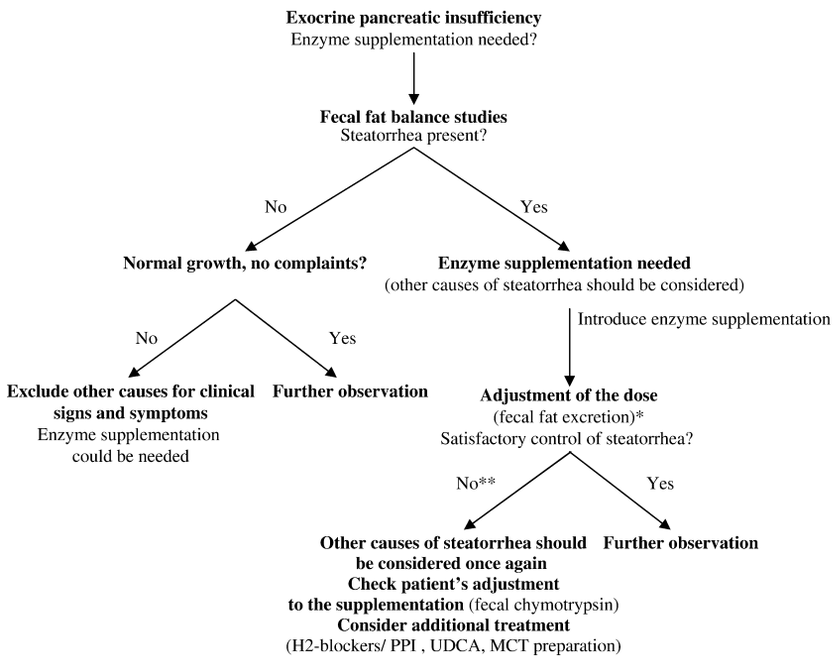

FIG. 2. Algorithm for assessment of the need for enzyme supplementation. *If not available/possible, use acid steatocrit, fecal fat concentration or microscopy. **Dose of 10,000 FIP lipase/kg body weight should not be exceeded. UDCA = ursodeoxycholic acid.

(27). When selecting the test, one should have in mind the differences in the assessment of fecal fat with the use of different methods (Table 4).

Breath tests are potential but expensive alternative for the assessment of fat digestion and absorption. The mixed triglyceride breath test is very sensitive in detecting severe exocrine pancreatic insufficiency (89). The fact that the digestion of the substrate used is dependent exclusively on pancreatic lipase is the advantage of this test (90,91). Cholesteryl octanoate (used in another breath test) hydrolyzed by cholesterol esterase also requires pancreatic lipase, phospholipase and bile salts, and it is not hydrolyzed by preduodenal lipase (92,93). This test could also be more suitable for the assessment of exocrine pancreatic function in young infants in whom preduodenal lipase is more active and efficient for the assessment of fat digestion and absorption in all children. However, neither test has been adequately studied in children. A suggested algorithm for the assessment of the need of enzyme supplementation in children is presented in Figure 2.

In conclusion, fecal pancreatic elastase-1 is the most suitable test for the detection of exocrine pancreatic insufficiency and for the follow-up of PS patients with chronic pancreatitis. Fecal chymotrypsin is useful to check patient compliance with exogenous pancreatic enzyme supplementation. For the routine assessment of the need for pancreatic supplementation as well as for monitoring efficiency in routine clinical practice, fecal fat excretion, acid steatocrit or stool microscopy may be applied.

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