

# Fecal Elastase-1 Is Superior to Fecal Chymotrypsin in the Assessment of Pancreatic Involvement in Cystic Fibrosis

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**ABSTRACT.** *Objective.* Exocrine pancreatic function in patients with cystic fibrosis (CF) can be evaluated by direct and indirect tests. In pediatric patients, indirect tests are preferred because of their less invasive character, especially in CF patients with respiratory disease. Fecal tests are noninvasive and have been shown to have a high sensitivity and specificity. However, there is no comparative study in CF patients. Therefore, the aim of the present study was to compare the sensitivity and the specificity of the fecal elastase-1 (E1) test with the fecal chymotrypsin (ChT) test in a large cohort of CF patients and healthy subjects (HS).

*Design.* One hundred twenty-three CF patients and 105 HS were evaluated. In all subjects, E1 concentration and ChT activity were measured. In the CF group, fecal fat excretion was also determined. The sensitivity and specificity of the fecal E1 test and ChT test were compared.

*Results.* With a cutoff level of 3 U/g, ChT specificity in HS was similar to that of E1, but E1 sensitivity in CF patients was significantly higher (90.2% vs 81.3%). With a cutoff level of 6 U/g, ChT and E1 sensitivity in CF patients was identical, but E1 specificity in HS was again significantly higher (98.1% vs 90.5%). In all CF patients with severe steatorrhea (>15 g/d), E1 concentrations were abnormal and ChT activity was lower than 3 U/g. In contrast, in pancreatic-sufficient patients and patients with mild steatorrhea ( $\leq$ 15 g/d), the E1 sensitivity was significantly higher compared with ChT (69.2% vs 41.0%).

*Conclusions.* The fecal E1 test is superior to fecal ChT determination in the assessment of CF pancreatic involvement in pancreatic-sufficient patients and those patients with mild steatorrhea. *Pediatrics* 2002;110(1). URL: <http://www.pediatrics.org/cgi/content/full/110/1/e7>; fecal elastase-1, fecal chymotrypsin, exocrine pancreatic function, cystic fibrosis.

ABBREVIATIONS. CF, cystic fibrosis; PI, pancreatic insufficient; SPT, secretin-pancreozymin test; E1, elastase-1; ChT, chymotrypsin; PS, pancreatic sufficient; ELISA, enzyme-linked immunosorbent assay; HS, healthy subjects.

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Cystic fibrosis (CF) is the most common cause of exocrine pancreatic insufficiency in childhood. Approximately 85% of CF patients are pancreatic insufficient (PI).<sup>1,2</sup> Thus, the assessment of exocrine pancreatic function in CF patients is of great clinical importance. For the evaluation, both direct and indirect tests are used.<sup>3,4</sup> The gold standard is the secretin-pancreozymin test (SPT) or one of its modifications. However, this test is invasive, time consuming, expensive, and not well standardized in children. Therefore, its use is limited to qualified gastroenterologic centers.

Several indirect tests, such as serum tests—amylase, lipase, trypsin, and the pancreolauryl or bentiromide test, 72-hour fecal fat analysis, fecal elastase-1 (E1) and chymotrypsin (ChT) analysis, and breath tests—have been developed. The sensitivity of these tests is limited in pancreatic-sufficient (PS) CF patients.<sup>3,4</sup> The use of the SPT in children, on the other hand, is controversial because of its invasive character, especially in CF patients with pulmonary disease. Thus, in pediatric CF patients, indirect tests are commonly used. Among the indirect tests, because of their low invasiveness and high sensitivity and specificity, the measurement of pancreatic enzymes in feces seems to be the most appropriate one.

Determination of fecal ChT has been an accepted indirect test in the pediatric routine for several years.<sup>5-8</sup> However, because of the colorimetric method, there is an interference with the enzyme substitution therapy.<sup>9</sup> Thus, pancreatic enzyme supplementation should be stopped for at least 3 days. On the contrary, fecal E1 test (enzyme-linked immunosorbent assay [ELISA]) is specific for the human enzyme and not influenced by exogenous enzyme supplementation.<sup>9-11</sup> In adult chronic pancreatitis patients, the determination of E1 and ChT were usually performed in small groups of patients and healthy subjects (HS).<sup>9,12-15</sup> Moreover, the statistical analysis was conducted only in 1 of them.<sup>15</sup> Therefore, the aim of the study was to compare the sensitivity and the specificity of the fecal E1 test with the fecal ChT test in a large cohort of CF patients and HS.

## MATERIALS AND METHODS

One hundred twenty-three patients with CF (60 females and 63 males), aged from 7 months to 25 years (mean±SEM: 8.8±0.4 years), were evaluated during 1997-2000. The diagnosis of the disease was based on clinical manifestation, chloride sweat concentration, and confirmed by CFTR gene analysis (29 most com-

mon mutations). In all patients, fecal E1 concentration, ChT activity, and fecal fat excretion were measured. During the study, no patient suffered an acute episode of diarrhea. The control group (HS) consisted of 105 children and adolescents (55 females and 50 males) aged from 7 months to 40 years (mean±SEM: 13.3±0.5 years) without any gastrointestinal diseases. In all HS, E1 concentrations were determined. The protocol of the investigation was approved by the Ethical Committee of the Medical Faculty, Karol Marcinkowski University of Medical Sciences in Poznan, Poland.

Fecal E1 was measured by an ELISA (ScheBo·Tech, Giessen, Germany).<sup>16</sup> Fecal ChT was measured by colorimetric method (Roche Diagnostics GmbH, Mannheim, Germany).<sup>17</sup> For additional analysis, the mean value from 3 nonrelated measurements was taken.

Fecal fat was analyzed according to van de Kamer et al.<sup>18</sup> The diet regimens were standardized for age, weight, and sex before and during the 3-day collection of stool.<sup>19</sup> Daily fecal fat excretion was defined as a mean of a 72-hour collection period. According to fecal fat excretion, CF patients were defined as PI (7 month-10 years: ≥5 g/d; above 10 years ≥7 g/d) or PS (7 month-10 years: <5 g/d; above 10 years <7 g/d).<sup>20-22</sup> In addition, steatorrhea was defined as a mild (<15 g/d) and severe (≥15 g/d). Fecal E1 concentrations below 200 μg/g of feces and ChT activities lower than 3 U/g were considered to be abnormal. Because fecal ChT activity between 3 U/g and 6 U/g of feces is indicative of exocrine pancreatic insufficiency, the analysis for cutoff level of 6 U/g was also performed. The sensitivity and specificity of the fecal E1 test and ChT test were compared. For the statistical evaluation of the results of fecal tests, the McNemar test was used.<sup>23</sup>

## RESULTS

Fecal E1 concentrations were abnormal in 2 (1.9%) out of 105 HS and in 111 (90.2%) of 123 CF patients (Table 1). Fecal ChT activity was abnormal in 3 (2.9%) HS and 100 (81.3%) CF patients with 3 U/g as a lower limit of normal, and in 10 (9.5%) HS and 111 (90.2%) CF patients with 6 U/g as a lower limit of normal. With a cutoff level of 3 U/g, ChT specificity in HS was similar to that of E1, but E1 sensitivity in CF patients was significantly higher compared with ChT (90.2% vs 81.3%;  $P < .006$ ). With a cutoff level of 6 U/g, ChT and E1 sensitivity in CF patients was identical, but E1 specificity in HS was significantly higher (98.1% vs 90.5%;  $P < .014$ ).

According to fecal fat excretion, 107 CF patients were classified as PI and 16 as PS. In PI subgroup, 106 (99.1%) patients had abnormal E1 concentration and ChT activity lower than 6 U/g, whereas 98 (91.6%) patients presented with ChT activity lower than 3 U/g. In the PS subgroup, 5 (31.2%) patients had abnormal E1 concentration and ChT activity lower than 6 U/g, and only 2 (12.5%) patients presented with ChT activity lower than 3 U/g.

In the subgroup of CF patients with fecal fat ex-

cretion lower than 15 g/d, fecal E1 and fecal ChT tests were compared in a similar manner. In 27 (69.2%) of 39 selected patients, fecal E1 concentration was lower than 200 μg/g and ChT activity was lower than 6 U/g. With a cutoff level of 3 U/g, ChT was abnormal in 16 (41.0%) patients. The E1 sensitivity in PS patients and patients with mild steatorrhea (≤15 g/d) was significantly higher than that of ChT with a cutoff level of 3 U/g ( $P < .006$ ). In all patients with severe steatorrhea (>15 g/d), fecal E1 concentration was abnormal, and ChT activity was lower than 3 U/g.

## DISCUSSION

Fecal ChT activity in randomly collected stool specimen of CF patients poorly correlates to pancreatic output measured by direct stimulation by SPT.<sup>7</sup> In contrast, the correlation between fecal E1 concentration and single parameters of secretin-cholecystokinin has been shown to be highly significant, both in CF patients<sup>24</sup> and HS.<sup>9</sup> The day-to-day variation in fecal ChT activity is a disadvantage of this method,<sup>7,14</sup> while fecal E1 concentrations seem to be more stable.<sup>14</sup> Moreover, with the use of the fecal E1 test (ELISA) exclusively endogenous enzyme release is measured. In the ChT test (colorimetric method), both endogenous and exogenous enzymes are evaluated.<sup>9,11</sup> The sensitivity of ChT test in CF patients has been shown to be high in PI patients and poor in those who are PS.<sup>6-8</sup> More recently, fecal E1 test has been demonstrated to have very good sensitivity in PI CF patients.<sup>11,25-27</sup> Yet, in milder forms of CF pancreatic insufficiency, the sensitivity of the test was reduced.<sup>24,27</sup> Therefore, one could expect a higher sensitivity and specificity of fecal E1 test. However, there is no study comparing the diagnostic value of the fecal E1 test with the fecal ChT test in CF.

In the present study, we have shown that, depending on the cutoff level for ChT (3 or 6 U/g), sensitivity or specificity of fecal E1 in CF patients is superior compared with fecal ChT in the assessment of exocrine pancreatic function in CF patients. The sensitivity and specificity of these tests have been compared in adult patients with chronic pancreatitis and in HS.<sup>5,12-15</sup> These studies differ in the diagnostic standards with which fecal enzymes concentrations were compared. In addition, patients differed in their degree of chronic pancreatitis and exocrine pan-

**TABLE 1.** Distribution of Fecal E1 and Fecal ChT Results in CF Patients and HS

Group (Number)	Abnormal Fecal E1		Abnormal Fecal ChT				Statistical Significance	
			Cutoff 3 U/g		Cutoff 6 U/g		E1 Versus ChT (3 U/g)	E1 Versus ChT (6 U/g)
	n	%	n	%	n	%		
HS (105)	2	1.9	3	2.9	10	9.5	NS	$P < 0.014$
CF (123)	111	90.2	100	81.3	111	90.2	$P < 0.006$	NS
PI (107)	106	99.1	98	91.6	106	99.1	$P < 0.014$	NS
PS (16)	5	31.2	2	12.5	5	31.2	NS	NS
ST <sub>S</sub> (84)	84	100	84	100	84	100	NS	NS
ST <sub>M</sub> (39)	27	69.2	16	41.0	27	69.2	$P < .006$	NS

NS indicates not significant.

ST<sub>M</sub> = PS + PI with steatorrhea ≤15 g/d.

ST<sub>S</sub> = PI with steatorrhea >15 g/d.

**TABLE 2.** Sensitivity and Specificity of Fecal E1 and Fecal ChT Tests in Patients With Exocrine Pancreatic Insufficiency and HS—Comparative Analysis

Study	Number		Sensitivity (%)			Specificity (%)			Statistical Significance
	EPI	HS	E1	ChT (3 U/g)	ChT (6 U/g)	E1	ChT (3 U/g)	ChT (6 U/g)	
Dominguez-Munoz 1995*	20	—	70	40	—	—	—	—	—
Glasbrenner 1996*	63	57	41	24	—	93	91	—	—
Loser 1996†	44	50	93	64	—	93	89	—	—
Stein 1996†	29	—	93	83	—	—	—	—	—
Lankisch 1998†	30	34	53	37	57	94	91	79	NS
Luth 2001†	62	65	84	35	64	57	54	77	SS
Carroccio 2001‡§	23	45	78.3	—	73.9§	100	—	100§	NS
Walkowiak 2002‡	123	105	90.2	81.3	90.2	98.1	97.1	90.5	SS

EPI indicates exocrine pancreatic insufficiency; NS, not significant; SS, statistically significant (see Table 1).

\* Chronic pancreatitis.

† Chronic pancreatitis (direct function test also performed).

‡ CF.

§ Cutoff level of 7.5 U/g for ChT.

atic insufficiency. The number of patients investigated also varied. In 4 of the studies, a higher sensitivity of fecal E1 test has been found (Table 2). However, except the latest study by Lankisch et al,<sup>15</sup> no statistical analysis was performed. In this study, the sensitivity and specificity of fecal E1 and fecal ChT tests were not statistically different.

In a recent study by Luth et al<sup>28</sup> analyzing 127 patients with clinical signs of malassimilation, higher sensitivity and specificity of the fecal E1 test in comparison to the fecal ChT test was found (Table 2). However, this study differs significantly in terms of “control group,” which comprised patients with gastrointestinal disorders and normal pancreatic function evaluated by the SPT. As shown previously, the specificity of indirect tests in gastrointestinal diseases is limited.<sup>11,29,30</sup>

In a recent multicenter Italian study,<sup>31</sup> fecal E1 determination was not found to be superior to fecal ChT measurement in the assessment of pancreatic function in CF (Table 2). Fecal E1 and fecal ChT levels were compared in 49 CF patients (40 PI and 9 PS) and 45 children without any history of gastrointestinal disease. However, in 26 out of 40 PI patients, pancreatic supplementation was not discontinued. Because in the fecal ChT test, exogenous enzymes are also assessed, this subgroup was excluded from the comparison. Therefore, the final comparison comprised a much smaller group of 23 CF patients (9 PS and 14 PI). Fecal E1 concentrations and fecal ChT

activities were abnormally low in all PI patients and in 4 (or 44.4%) and 3 (or 33.3%) of the PS patients, respectively. In addition, a higher cutoff level of 7.5 U/g for the ChT test was used.

In the present study, the sensitivity of the fecal E1 test was clearly higher than that found for the fecal ChT test (cutoff level of 3U/g) in PI patients, while in PS subgroup this was not the case. However, this finding seems to be related to the smaller number of PS patients. In most studies on patients with chronic pancreatitis, the difference between fecal E1 and fecal ChT sensitivity was larger for PS than PI patients (Table 3). In the selected subgroup of our CF patients with severe steatorrhea, both tests had 100% sensitivity. 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 presenting with severe steatorrhea (>15 g/d), Lankisch et al<sup>3,32</sup> have shown that different tests have an excellent sensitivity, and the diagnosis could be even made visually.<sup>33</sup> In our CF patient group—with no/mild steatorrhea, the fecal E1 test has been shown to be superior to the fecal ChT test. In fact, the observed difference in the sensitivity of these tests in CF is related to the higher sensitivity in the subgroup of patients with pancreatic sufficiency or mild steatorrhea.

For a cutoff level of 6 U/g for ChT, both tests had the same sensitivity in all defined subgroups. However, it is impossible in such a comparison to exclude

**TABLE 3.** Sensitivity of Fecal E1 and Fecal ChT in PS and PI Patients—Comparative Analysis

Study	PS				PI			
	Number	Sensitivity (%)			Number	Sensitivity (%)		
		E1	ChT (3 U/g)	ChT (6 U/g)		E1	ChT (3 U/g)	ChT (6 U/g)
Loser 1996*	22	82	41	—	22	100	86	—
Stein 1996*	7	88	56	—	22	96	91	—
Lankisch 1998*	19	37	16	37	11	82	73	91
Luth 2001*	37	73.0	—	56.8	25	100	—	76.0
Carroccio 2001‡	9	44.4	—	33.3‡	14	100	—	100‡
Walkowiak 2002‡	16	31.2	12.5	31.2	107	99.1	91.6	99.1

\* Chronic pancreatitis.

† CF.

‡ Cutoff level of 7.5 U/g for ChT.

the specificity, which was significantly higher for the fecal E1 test. Otherwise, the cutoff level for fecal E1 could be increased, thereby decreasing the specificity to that found for the fecal ChT test with a cutoff level of 6 U/g. This would result in a better sensitivity of the fecal E1 test.

## CONCLUSION

Fecal E1 test is superior to fecal ChT determination in the assessment of CF pancreatic involvement in PS patients and those with mild steatorrhea.

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