Immunooreactive elastase I: clinical evaluation of a new noninvasive test of pancreatic function

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We have evaluated the diagnostic value of the fecal elastase test in comparison with the secretin–pancreozymin test in the diagnosis of exocrine pancreatic insufficiency. Pancreatic elastase was measured immunologically. Immunooreactive elastase activity in spot stools from controls ranged from 136 to 4440 µg/g; 95% of all values were within 175 to 1500 µg/g. The elastase assay CVs ranged from 3.3% to 6.3% (intraassay) and from 4.1% to 10.2% (interassay). The output of elastase correlated well with those of amylase, lipase, and trypsin, yielding respective correlation coefficients of 0.83, 0.82, and 0.84 in controls and 0.86, 0.91, and 0.91 in patients with impaired pancreatic function. In contrast to fecal chymotrypsin, the test results were unaffected by pancreatic enzyme replacement therapy. These results indicate that fecal immunooreactive elastase may be recommended as a new, noninvasive tubeless test of pancreatic function.

INDEXING TERMS: secretin–pancreozymin test • chymotrypsin • fecal fat • malabsorption • steatorrhea

Because invasive diagnostic approaches of impaired pancreatic function, such as the secretin–pancreozymin test with its several modifications or the Lundh test, are time-consuming, invasive, unpopular with patients, and expensive, numerous indirect or “tubeless” tests of pancreatic function (e.g., fecal chymotrypsin, bentisomide, and pancreolauryl tests) have been introduced as alternatives [1]. Among them, the 72-h fecal fat analysis still remains the standard test for diagnosing and quantifying fat malabsorption in chronic pancreatitis [2], even though it is both insensitive and nonspecific in the diagnosis of chronic pancreatitis [3, 4]. Furthermore, fecal fat analysis is also time consuming, requires special laboratory equipment, and is unpopular among the laboratory staff. Among tubeless pancreatic function tests, the determination of fecal chymotrypsin has been accepted as an indirect test for many years. Its sensitivity reportedly ranges from 72% to 90%, and its specificity, which appears to depend on the selection of the control subjects studied, ranges from 49% to 90% [5–13].

Previously Szegoileit detected human pancreatic elastase I [14], a member of the acidic elastase family, as a new endoproteinase and sterol-binding protein present in both human pancreatic secretions and feces. Quantitative studies by rocket immunoelectrophoresis indicated that this enzyme was unaffected during intestinal passage. Concentrations in feces were five to six times greater than those determined in pancreatic juice [15, 16]. Therefore, we propose that concentrations of this enzyme in feces reflect pancreatic function. The aim of this prospective study was to evaluate the specificity and sensitivity of fecal immunooreactive elastase as an accurate test of exocrine pancreatic function by comparing the results with direct measurement of pancreatic enzyme secretion.

Materials and Methods

Patients

Fecal elastase was measured in stool samples from 164 consecutive patients presenting between August 1992 and December 1993 (84 males, 80 females), ages 5–85 years (mean 41 years), who were admitted to the hospital (a) for the differential diagnosis of malabsorption syndromes (history of diarrhea, weight loss) or (b) with previously established causes of malabsorption or maligestion (exocrine pancreatic insufficiency due to chronic pancreatitis or cystic fibrosis, inflammatory bowel disease, celiac sprue) and from a subgroup of extensively investigated patients with functional abdominal pain, but with entirely normal markers of gastrointestinal functions, who served as controls (Table 1). In each case, final diagnoses were established after thorough investigation according to generally accepted criteria. The study was in accordance with ethical standards of the local committee.

Procedures

Enzyme measurements. Elastase was determined immunologically [16] with a new “sandwich”-type enzyme immunoassay (ScheBo-Tech, Wettenberg, Germany). Chymotrypsin activity

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in stool samples was determined according to Kaspar et al. [17], amylase according to Bernfeld [18], lipase according to Ziegenhorn et al. [19], and trypsin according to Erlanger et al. [20], all with commercial test kits manufactured by Boehringer Mannheim (Mannheim, Germany).

**Fecal fat estimation.** Fecal fat was analyzed according to van de Kamer et al. [21] or by near-infrared analysis according to Stein et al. [22].

**Sample preparation.** Stool was collected for 72 h in plastic containers, and was either analyzed the same day or stored at −20 °C until analysis. For fecal fat estimation, all stool samples were diluted, carefully homogenized (Ultraturrax-blender; Janke & Kunkel, Staufen, Germany), and analyzed by the above-mentioned standard methods. Samples were analyzed immediately after homogenization or after freezing.

**Secretin–pancreozymin test.** Studies were performed in the morning after overnight fasting [23-26]. Briefly, after a 12-h fasting period, a double-lumen Lagerlöf tube (Rüsch, Waiblingen, Germany) was placed into the duodenum under X-ray control. After 15 min of basal secretion, the subjects received intravenously secretin, 1 mg/kg body wt. (Hoechst, Frankfurt, Germany), and 30 min later cholecystokinin–pancreozymin (CCK-PZ; Ferring, Malmö, Sweden), 1 IU/kg body wt. To evaluate exocrine function, we measured the volume and concentration of bicarbonate produced 30 min after stimulation with secretin. Amylase, elastase, lipase, and trypsin output were measured 30 min after CCK-PZ.

**Stability of immunoreactive elastase.** For stability studies we used stool samples stored at room temperature for various times (see legend to Fig. 1). The degradation process was interrupted by freezing the sample at −20 °C.

The influence of enzyme replacement therapy on fecal immunoreactive elastase excretion was studied in 24 patients with cystic fibrosis.

**STATISTICS**

Because none of the data sets showed a gaussian distribution, comparison of paired differences was performed with the Wilcoxon rank test, and comparison of unpaired data with the Mann–Whitney test. Correlations were calculated with the method of least squares. Limits of significance were $P < 0.05$ in all tests.

**Results**

**Precision.** The intraassay CV of immunoreactive elastase, calculated from 10 consecutive assays of seven different fecal samples, was 3.3–6.3% (mean 4.8%). Interassay CV, calculated over 7 days from seven different fecal samples, ranged from 4.1% to 10.2% (mean 7.7%).

**Reference intervals.** Fecal immunoreactive elastase concentration in controls ranged from 136 to 4440 μg/g; 95% of all values (2.5th to 97.5th percentile) were within 175 to 2500 μg/g, and the mean ± SD was 1083 ± 193 μg/g. In contrast to other pancreatic enzymes, immunoreactive elastase concentrations were not significantly lower in children than in adults (963 ± 143 μg/g vs 1123 ± 214 μg/g). We and others [27] found an age-related increase of immunoreactive elastase only in the first 3 months after birth.

**Stability in stool samples.** To determine the effect of bacterial degradation on elastase immunoreactivity in the time between passage of stool and analysis, we analyzed the changes in immunoreactive elastase assayed in three different stools of six healthy controls and of six patients with exocrine pancreatic insufficiency during 3 days at room temperature. As shown in Fig. 1, no remarkable rate of immunoreactivity loss was detectable in stool samples at 24 °C.

**Immunological specificity.** No immunoreactive elastase could be detected in either porcine or bovine pancreatic enzyme preparations (<0.02 μg/g granulate or tablet). Substitution replacement therapy in 12 patients with exocrine pancreatic insufficiency due to cystic fibrosis resulted in a significantly greater output of chymotrypsin activity and a significantly decreased
Elastase release in duodenal aspirate. There was a good correlation (Fig. 3) between the output of elastase and that of amylase, lipase, and trypsin. The respective correlation coefficients were 0.83, 0.82, and 0.84 in normal controls (n = 25) and 0.86, 0.91, and 0.91 in subjects with impaired pancreatic function (n = 22). Furthermore, the output of elastase in duodenal aspirate correlated well with the amount of elastase in feces (r = 0.87, P < 0.01)

Figure 4 illustrates a receiver-operating characteristic (ROC) curve based on calculations of sensitivity and specificity at several cutoff values for fecal elastase [28]. The fecal elastase assay achieved optimal discrimination (defined as maximal likelihood ratio: the true-positive rate divided by the true-negative rate) at a cutoff value of 175 μg/g wet wt. (specificity 94%, sensitivity 93%). When patients with pathological secretin-pancreozymin test results were divided into groups with and without steatorrhea, correctly abnormal fecal elastase increased to 96% in severe exocrine pancreatic insufficiency. In the less severely impaired patients, without steatorrhea, the diagnostic sensitivity decreased to 88%. In the same groups of patients with chronic pancreatitis, the diagnostic sensitivity for chymotrypsin was 91%, whereas its diagnostic specificity was only 91% (with steatorrhea) and 56% (without steatorrhea) (Table 2).

Discussion

The objective of the present study was the evaluation of fecal immunoreactive elastase for the diagnosis and differential diag-
nosis of pancreatic exocrine insufficiency. This test is highly specific for human pancreatic elastase, because elastase does not interfere with pancreatic enzyme preparations. Thus, in contrast to fecal chymotrypsin tests, the results are not affected by pancreatic enzyme replacement therapy. A strong correlation was found between the duodenal secretion of elastase and that of amylase, lipase, and trypsin in the secretin–pancreozymin test. Considering the relationship between enzymatic outputs in feces and in pancreatic juice after CCK-PZ stimulation, we found a satisfactory correlation for elastase. As with fecal chymotrypsin, the immunoreactive elastase test is stable in feces for several days, which offers the possibility that stool samples from outpatients can be mailed to diagnostic centers. In contrast to chymotrypsin, however, which has a fecal recovery of only 0.5% of its activity in pancreatic juice, the degradation of immunoreactive elastase is negligible during intestinal passage, resulting in a five- to sixfold greater concentration than that in pancreatic juice [14].

Determination of fecal chymotrypsin has been accepted as an indirect test for pancreatic function. Measurement of fecal chymotrypsin can also be used to detect pancreatic insufficiency in children with cystic fibrosis. The test has some advantages over other tubeless tests: The test is simple to perform when an automatic titration is available, and stool samples from outpatients can be mailed to diagnostic centers that perform the assay, because chymotrypsin activity is very stable over several days at room temperature. However, the sensitivity of fecal chymotrypsin determination as a test for chronic pancreatitis ranges from 45% to 100%, with most studies reporting values of 72–90% [5–12]. False-positive results have been reported in a variety of nonpancreatic diseases: liver cirrhosis, Billroth II gastrectomy, celiac sprue, Crohn disease, and other gastrointestinal diseases associated with diarrhea and malabsorption [5, 29, 30]. Studies comparing results of the fecal chymotrypsin determination with those of the secretin–pancreozymin test (or analogous tests such as the secretin–cerulein test) showed that fecal chymotrypsin detects 85% of patients with advanced chronic pancreatitis but only 49% of those with mild or early manifestation of the disease [11–13].

Previously Münch and Ammann [31] used a fecal immunoreactive lipase assay as a new tubeless test in the diagnosis of pancreatic exocrine insufficiency by means of a new ELISA technique. In contrast to fecal chymotrypsin, the test results were unaffected by pancreatic enzyme replacement therapy. Despite the excellent diagnostic specificity of 98%, however, the assay had the disadvantage of very low sensitivity (34%).

In contrast, the measurement of fecal immunoreactive elastase has both high diagnostic sensitivity and specificity. All subjects with nonpancreatic diarrhea and celiac disease, except for two patients with prior gastric resection (Billroth II) and two with severe secretory diarrhea due to enzyme dilution (water content 98%), had immunoreactive elastase values within normal limits (<175 μg/g stool).

The overall sensitivity of the immunoreactive elastase test was also satisfactory, especially in patients with moderately or severely impaired pancreatic function. Of the patients with severe pancreatic insufficiency (steatorrhea) or cystic fibrosis, none had a falsely normal test result. Thus, compared with the fecal chymotrypsin or fecal lipase, the fecal elastase test showed a higher diagnostic sensitivity and specificity.

In conclusion, the simple performance, the noninvasiveness, and the diagnostic efficiency of the fecal immunoreactive elastase test make it one of the most satisfactory pancreatic function tests for screening of pancreatic insufficiency. Furthermore, the test seems to be useful not only in adults, but also in children as a screening test for cystic fibrosis. Similarly to all pancreatic function tests, the fecal test for immunoreactive elastase is unable to differentiate between pancreatic insufficiency due to chronic pancreatitis and that due to pancreatic cancer.

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**Table 2. Fecal elastase 1 compared with secretin–pancreozymin test (SPT) and fecal chymotrypsin in pancreatic insufficiency with and without steatorrhea.**

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<td>SPT</td>
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<td>Bicarbonate concentration*, enzyme output abnormal*, fecal fat normal</td>
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<td>Bicarbonate concentration*, enzyme output*, fecal fat abnormal</td>
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* Maximal concentration of bicarbonate: >70 mmol/L (normal).
* Normal output 30 min after stimulation: amylase >12 000 U/30 min, trypsin >3 U/30 min; lipase >65 000 U/30 min.
* Normal stool fat <7 g/day.
References


