# A New Fecal Elastase 1 Test Using Polyclonal Antibodies for the Detection of Exocrine Pancreatic Insufficiency

***To the Editor:***

The diagnosis of chronic pancreatitis is based on the characteristic case history and a combination of abnormal results of morphologic examinations and exocrine pancreatic function tests.[1,2] The gold standard for testing exocrine pancreatic function is the secretin-pancreozymin test (SPT) or one of its modifications. This test is time-consuming, invasive, and expensive and can, therefore, only be carried out at centers specializing in gastroenterology.[3,4] There are a number of indirect pancreatic function tests that measure pancreatic enzymes in serum. These include pancreatic isoamylase and immunoreactive trypsin or split products in serum or urine, such as the NBT-PABA (bentiromide) test or the pancreolauryl test (PLT), or fecal concentrations of enzymes, such as chymotrypsin. These have been developed and evaluated for diagnosing exocrine pancreatic insufficiency.[4] However, they are rarely used because of their low sensitivity or specificity, or both. Therefore, there is not an acceptable gold standard indirect pancreatic function test that can be used in laboratories outside gastroenterologic centers.

Thus, a fecal elastase 1 measurement by a monoclonal antibody based on an enzyme-linked immunosorbent assay (ELISA), developed in Germany (Schebo-Tech, Wettenberg, Germany)[5] and now available in the United States (Genova Diagnostics, Ashville, NC) is of interest.

Fecal elastase 1 estimation significantly correlates with total volume bicarbonate output, amylase, lipase, and trypsin measurement.[6] Unfortunately, the specificity of this test may vary considerably between 57% and 96%.[6–12] The low specificity in some studies may be due to an erroneously assumed exocrine pancreatic insufficiency because of diarrhea (stool weight >250 g/day or stool water content of >85%).[13] Fischer et al[14] found a water content of ≥85% in 29% of the fecal samples sent for malabsorption investigations. In this group of patients, the elastase 1 levels were considerably higher after lyophilization. In 11% of those patients who did not have chronic pancreatitis, the elastase 1 estimation was normal after lyophilization.

The sensitivity of fecal elastase 1 is usually tested when a diagnosis of chronic pancreatitis has been made, based on the results of an ERCP examination and classified according to the Cambridge criteria as mild, moderate, or severe.[15,16] It may also be tested versus a direct pancreatic function test, preferably the SPT or one of its modifications. In the case of an abnormal direct pancreatic function test result, exocrine pancreatic insufficiency is classified as mild (reduced output of one or both enzymes, bicarbonate concentration and fecal fat excretion normal), moderate (reduced enzyme output and bicarbonate concentration, fecal fat excretion normal), or severe (reduced enzyme output and bicarbonate concentration plus steatorrhea).[1] When the 3 stages of chronic pancreatitis and exocrine pancreatic insufficiency were examined together (mild, moderate, severe), most studies showed that, in severe cases, the fecal elastase 1 estimation had a sensitivity of 100%.[6,8,10–12] However, it must be noted that other function tests have produced similar sensitivity rates in patients with pancreatic steatorrhea.[4] Unfortunately, in the mild or moderate form of the disease, the sensitivity of the fecal test varies between 0% and 89%.[6,8–12]

In view of such varying results for sensitivity and specificity, provided by the monoclonal antibody-based ELISA assay, a newly developed polyclonal ELISA assay, available in Germany (Bioserv Diagnostics, Rostock, Germany), seemed to be worth investigating.

The aim of our study was to compare these 2 fecal elastase 1 tests with the direct pancreatic function test as the gold standard, in this case the secretin-cerulein test (SCT), and to perform also a quantitative fecal fat analysis[17] in 31 patients sent to the Department of Medicine at the Municipal Hospital in Lüneburg with suspected chronic pancreatitis. Normal values were (1) SCT: after secretin administration, bicarbonate concentration >70 mol /L, bicarbonate output >6.5 mol/30 min; after cerulein administration, amyslase output >12,000 U/30 min, lipase output >21,000 U/30 min; (2) fecal fat <7 g/day. Exocrine pancreatic insufficiency was classified as mild, moderate or severe, as previously stated. Fecal elastase 1 estimations were performed in Karlsburg and according to the manufacturer's guidelines, values >200 μg/g stool were considered to be normal for both tests.

According to the results of the SCT, 11 of the 31 patients had exocrine pancreatic insufficiency that was due to chronic pancreatitis. The etiology was alcohol induced in 5, hereditary in 2, and unknown in 4 patients. Exocrine pancreatic insufficiency was mild in 2, moderate in 3, and severe in 6 patients. Exocrine pancreatic function was normal in 20 patients. Seven of these patients had experienced a first attack of acute pancreatitis more than 3 months before, whereas the remaining patients had bile acid diarrhea (3), irritable bowel disease (3), weight loss of unknown origin (2), lactase intolerance (1), trauma to the pancreas (1), cystadenoma of the gland (1), chronic diarrhea of unknown origin (1), and pancreatic carcinoma (1). The results of both fecal elastase 1 determinations are summarized in Table 1.

The sensitivity in detecting exocrine pancreatic insufficiency was 64% for both test procedures; thus, the new method for fecal elastase 1 estimation has not improved the previously reported overall sensitivity of the test.[6,8,10–12] In contrast, however, the specificity of the new test was much higher than that of the older one (95% vs. 80%). When only patients with mild to moderate exocrine pancreatic insufficiency were considered, the sensitivity for both tests was only 40% and thus unacceptable. When patients with severe exocrine pancreatic insufficiency were evaluated, the sensitivity of both tests was 83% and thus similar to those rates shown in previous studies for the monoclonal test.[6,8,10–12]

This leaves the following possibilities for the interpretation of both fecal elastase 1 estimations for the clinicians: (1) If the assay is normal and morphologic procedures such as ultrasound,

**TABLE 1.** Fecal Elastase 1 (FE1) Determination in 11 Patients With Exocrine Pancreatic Insufficiency (EPI) Due to Chronic Pancreatitis and in 20 Patients With Normal Exocrine Pancreatic Function But Other Gastrointestinal Diseases

| Fecal Elastase 1 Determination (Monoclonal Test) | | | | Fecal Elastase 1 Determination (Polyclonal Test) | | | |
|---|---|---|---|---|---|---|---|
| | SCT Result | | | | SCT Result | | |
| FE1 Test Result | Non-CP | CP | Total | FE1 Test Result | Non-CP | CP | Total |
| Normal | 16 | 4 | 20 | Normal | 19 | 4 | 23 |
| Abnormal | 4 | 7 | 11 | Abnormal | 1 | 7 | 8 |
| Sensitivity | | 64% | | Sensitivity | | 64% | |
| Specificity | | 80% | | Specificity | | 95% | |
| Positive predictive value | | 64% | | Positive predictive value | | 88% | |
| Negative predictive value | | 80% | | Negative predictive value | | 83% | |
| Total accuracy | | 74% | | Total accuracy | | 84% | |

**Mild/Moderate Exocrine Pancreatic Insufficiency**

| Fecal Elastase 1 Determination (Monoclonal Test) | | Fecal Elastase 1 Determination (Polyclonal Test) | |
|---|---|---|---|
| FE1 Test Result | Mid/moderate EPI | FE1 Test Result | Mild/moderate EPI |
| Normal | 3 | Normal | 3 |
| Abnormal | 2 | Abnormal | 2 |
| Sensitivity | 40% | Sensitivity | 40% |
| Specificity | 80% | Specificity | 95% |
| Positive predictive value | 33% | Positive predictive value | 67% |
| Negative predictive value | 84% | Negative predictive value | 86% |
| Total accuracy | 72% | Total accuracy | 84% |

**Severe Exocrine Pancreatic Insufficiency**

| Fecal Elastase 1 Determination (Monoclonal Test) | | Fecal Elastase 1 Determination (Polyclonal Test) | |
|---|---|---|---|
| FE1 Test Result | Severe EPI | FE1 Test Result | Severe EPI |
| Normal | 1 | Normal | 1 |
| Abnormal | 5 | Abnormal | 5 |
| Sensitivity | 83% | Sensitivity | 83% |
| Specificity | 80% | Specificity | 95% |
| Positive predictive value | 56% | Positive predictive value | 83% |
| Negative predictive value | 94% | Negative predictive value | 95% |
| Total accuracy | 82% | Total accuracy | 92% |

endoscopic ultrasound, computed tomography, and/or ERCP are also normal, severe exocrine pancreatic insufficiency is excluded, but mild to moderate function impairment may still be possible. (2) If the assay is normal but morphologic procedures are abnormal, the fecal test may have failed to diagnose mild to moderate exocrine pancreatic insufficiency. (3) If the assay and morphologic procedures are abnormal, the test confirms chronic pancreatitis. (4) If the fecal test is abnormal and the morphologic procedures are normal, the results are difficult to interpret. Exocrine pancreatic insufficiency may or may not be present.

In conclusion, the polyclonal test seems to have the same sensitivity as the monoclonal test but offers a higher specificity. Therefore, it is more useful for differentiating between pancreatic and nonpancreatic steatorrhea, which is a frequent clinical problem.

**Jan-Uwe Hahn, MD**
**Sabine Bochnig**
**Wolfgang Kerner, MD**
Clinic for Diabetes and Metabolic Diseases
Klinikum Karlsburg
Karlsburg, Germany

**Helma Koenig**
**Birgit Sporleder**
**Paul Georg Lankisch, MD, MRCP, FACG**
Department of Internal Medicine
Municipal Clinic of Lueneburg
Lueneburg, Germany

**Patrick Maisonneuve**
European Institute of Oncology
Milan, Italy

**Albert B. Lowenfels, MD**
New York Medical College
Valhalla, NY

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# Autoantibodies Against the Exocrine Pancreas in Fulminant Type 1 Diabetes

***To the Editor:***

Fulminant type 1 diabetes is a recently described novel subtype of type 1 diabetes characterized by a markedly acute onset of diabetes, severe metabolic disorder, and elevated serum pancreatic enzyme concentrations with lymphocytic infiltration in the exocrine pancreas.[1,2] This disease was originally proposed to be classified as idiopathic (nonautoimmune) type 1 diabetes based on the absence of islet-associated autoantibodies. It is not known, however, whether the pathogenesis of fulminant type 1 diabetes is associated with autoimmunity.[2]

We recently demonstrated the presence of autoantibodies against human carbonic anhydrase II and lactoferrin, which are distributed in the pancreatic duct cells and acinar cells, respectively, in type 1 diabetes. The prevalence of these antibodies in fulminant type 1 diabetes, however, is not known.[3]

To clarify the involvement of autoimmunity, we investigated the presence of these autoantibodies in fulminant type 1 diabetes.

Five patients (2 males and 3 females; age at onset, 46.2 $\pm$ 18.7 years; range, 23–63) at the Ohtsu Red Cross Hospital and neighboring hospitals were recruited for the study after informed consent was obtained. Diagnosis of fulminant type 1 diabetes was based on the criteria of the Japan Diabetes Society: (1) ketosis or ketoacidosis within 1 week after the onset of hyperglycemic symptoms; (2) plasma glucose >288 mg/dL and HbA1c <8.5% on the first visit; and (3) urinary C-peptide excretion <10 μg/d, fasting C-peptide <0.3 ng/mL, or serum C-peptide <0.5 ng/mL after glucagon injection or meal load soon after disease onset.[2]

Mean duration from the onset of hyperglycemic symptoms to ketosis or ketoacidosis was 4 $\pm$ 1.6 days, HbA1c was 5.6 $\pm$ 0.7%, and plasma glucose was 545 $\pm$ 145 mg/dL. Fasting C-peptide was <0.3 ng/mL in all patients. Serum amylase and/or elastase 1 was elevated in all patients (3/5 and 4/4, respectively). Anti-glutamic acid decarboxylase (GAD) antibody and anti-IA-2 antibody were negative in all patients. One patient had associated clinical acute pancreatitis, which we reported previously.[4] None of the other patients had clinical pancreatitis or swelling of the pancreas on ultrasonography. Serum levels of anti-carbonic anhydrase 2 antibody (ACA) and anti-lactoferrin antibody (ALF) were measured using the solid-phase enzyme-linked immunosorbent assay method, as described previously.[5] ACA was positive in 4 patients and ALF was positive in 5 patients. One or both antibodies was positive in all the patients.

The present data suggest that autoimmunity, at least against the exocrine pancreas, is involved in fulminant type 1 diabetes. Autoimmune mechanisms might be involved in the pathogenesis of fulminant type 1 diabetes, although islet-associated autoantibodies are negative. A small number of cases of fulminant type 1 diabetes with evidence of autoimmunity have been reported.[2,6]

Another possibility is that insult by environmental factors, eg, viruses, affects both the exocrine pancreas and the islets and triggers autoimmunity against the exocrine pancreas, whereas complete destruction of B cells does not allow the antigen-driven process to continue. Indeed, marked lymphocytic infiltration in the islets as well as the exocrine pancreas was reported in an autopsy case soon after the onset,[7] whereas lymphocytic infiltration in the exocrine pancreas but no insulitis were reported in biopsy specimens in the subacute period.[1]

In conclusion, autoantibodies against the exocrine pancreas were present in all of the patients with fulminant type 1 diabetes studied. Further studies are required to elucidate the pathogenesis of this disease.

**Takao Taniguchi, MD, PhD**
Department of Internal Medicine
Ohtsu Red Cross Hospital
Shiga, Japan

**Kazuichi Okazaki, MD, PhD**
The Third Department of Internal Medicine
Kansai Medical University
Osaka, Japan

**Motozumi Okamoto, MD, PhD**
**Shuji Seko, MD, PhD**
Department of Internal Medicine
Ohtsu Red Cross Hospital
Shiga, Japan

**Kazuaki Nagashima, MD, PhD**
**Yuichiro Yamada, MD, PhD**
Department of Diabetes and
Clinical Nutrition
Kyoto University Faculty of Medicine
Kyoto, Japan

**Tosio Iwakura, MD, PhD**
Department of Diabetology
Kobe City General Hospital
Kobe, Japan

**Yutaka Seino, MD, PhD**
Kansaidenryoku Hospital
Osaka, Japan

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