

## *A prospective, comparative study of the para-aminobenzoic acid test and faecal elastase 1 in the assessment of exocrine pancreatic function*

S. A. SONWALKAR\*, I. B. HOLBROOK†, I. PHILLIPS‡ & S. M. KELLY\*

\*Department of Medicine and Gastroenterology, York Hospital, York, UK; †Department of Chemical Pathology, York Hospital, York, UK; ‡Department of Chemical Pathology, Southampton General Hospital, Southampton, UK

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### SUMMARY

**Background:** The assessment of exocrine pancreatic insufficiency is part of the routine work-up of patients with persistent diarrhoea or suspected steatorrhoea. Direct and indirect tests for the diagnosis of exocrine pancreatic insufficiency have their drawbacks. Measurement of faecal elastase 1 by enzyme-linked immunosorbent assay is a simple, non-invasive, robust test for exocrine pancreatic insufficiency.

**Methods:** We performed a prospective comparison of the para-aminobenzoic acid test and faecal elastase 1 test in 45 patients being investigated for diarrhoea or suspected steatorrhoea. Details of clinical suspicion, imaging and response to treatment were recorded.

**Results:** Exocrine pancreatic function was normal in 20 patients with normal para-aminobenzoic acid and faecal elastase 1 levels. Eight patients had exocrine pancreatic

insufficiency with low para-aminobenzoic acid and faecal elastase 1 levels, which improved with enzyme supplementation. In 14 of the 15 patients with low or borderline low para-aminobenzoic acid and normal faecal elastase 1 levels, a non-pancreatic cause was found; one patient had a false positive para-aminobenzoic acid test. Two had normal para-aminobenzoic acid but low faecal elastase 1 levels. One improved with pancreatic supplementation, and imaging revealed chronic pancreatitis. The other had a false positive faecal elastase 1 test related to profuse diarrhoea.

**Conclusions:** Faecal elastase 1 estimation is a simple, non-invasive, robust test of exocrine pancreatic insufficiency, performed on an out-patient stool sample. Its diagnostic performance is superior to that of the para-aminobenzoic acid test in investigating patients with diarrhoea or suspected steatorrhoea.

### INTRODUCTION

The diagnosis of exocrine pancreatic insufficiency is usually based on abnormal pancreatic function tests and imaging criteria.<sup>1–3</sup> Direct pancreatic function tests, such as the secretin–cholecystokinin or secretin–caerulein test, have the highest sensitivity and specificity for the detection of exocrine pancreatic insufficiency and remain the gold standard.<sup>3–6</sup> Direct tests, however, are

time consuming, invasive, expensive, uncomfortable, not standardized and require fluoroscopic tube placement. They are therefore confined to academic centres. Several indirect tests for the assessment of exocrine pancreatic insufficiency are available, such as the fluorescein dilaurate test, para-aminobenzoic acid (PABA) or bentiromide test and faecal chymotrypsin determination.<sup>1–3, 5, 7</sup> These tests have their drawbacks, however, and are subject to interference by certain drugs, diarrhoea, pH and gastrointestinal operations, which lower their specificity.<sup>1–3, 5, 8, 9</sup>

One of the indirect tests used routinely in clinical practice is the PABA or bentiromide test. The test

Correspondence to: Dr S. M. Kelly, Department of Medicine and Gastroenterology, York Hospital, Wigginton Road, York YO31 8HE, UK.  
E-mail: Sean.m.kelly@york.nhs.uk

involves the cleavage of PABA by chymotrypsin in the duodenum.<sup>10</sup> The split product of PABA is absorbed in the small bowel, metabolized in the liver and excreted by the kidneys, and is a measure of exocrine pancreatic insufficiency. However, the PABA test has its limitations and lacks sensitivity and specificity in mild diseases.

The isolation of pancreatic elastase 1, and its further characterization as a pancreas-specific enzyme which is not degraded during intestinal transport and is enriched five- to six-fold in faeces, has raised considerable interest.<sup>11–13</sup> A highly sensitive enzyme-linked immunoabsorbent assay for human faecal elastase 1 (FE1), using two monoclonal antibodies in a sandwich technique, is available commercially (Schebo, Biotech AG, Netanyastrasse 3, D-35394 Giessen, Germany).<sup>11–13</sup> Early clinical studies on FE1 gave promising results in patients with exocrine pancreatic insufficiency in comparison with the fluorescein dilaurate test<sup>14, 15</sup> and the secretin–caerulein test.<sup>16, 17</sup>

The aim of this study was to compare the results of another indirect test of pancreatic exocrine deficiency, the PABA test, with the determination of the FE1 levels in patients undergoing assessment of exocrine pancreatic function for long-standing diarrhoea or possible steatorrhoea in a routine out-patient setting.

## METHODS AND MATERIALS

Forty-five consecutive patients (23 males and 22 females; average age, 57.8 years; range, 31–77 years), with a clinical suspicion of pancreatic insufficiency because of persistent diarrhoea or suspected steatorrhoea, underwent analysis of exocrine pancreatic insufficiency by a PABA test and subsequent FE1 estimation.

In patients with a high suspicion of pancreatic insufficiency, pancreatic imaging with ultrasonography, abdominal computed tomography or endoscopic retrograde cholangiography was also performed.

### *PABA test*

The patient fasted overnight, and pancreatic supplements and all but the most essential medications were discontinued for 24 h before starting the test. The patient was given a Lundh test meal (18 g vegetable oil, 40 g glucose and 15 g casein) into which was mixed 500 mg of bentiromide (Sigma, Poole, UK), 185 kBq of <sup>14</sup>C-labelled PABA (Fluorchem, Glossop, UK) and water in the form of a 'milk shake'. All urine passed over the

following 6 h was collected. <sup>14</sup>C-PABA was measured by beta counting. The total PABA was measured by a modified colorimetric method.<sup>18</sup> The PABA excretion index (PEI) was calculated by dividing the percentage recovery of total PABA by the percentage recovery of <sup>14</sup>C-PABA. A PABA excretion index (PEI) of > 0.68 was considered to be normal.

### *FE1 estimation*

FE1 was measured using an enzyme-linked immunoabsorbent assay based on two monoclonal antibodies against human pancreatic elastase 1 (Schebo, Biotech AG, Netanyastrasse 3, D-35394 Giessen, Germany).<sup>11–13</sup> The lower detection limit of the elastase 1 assay was below 1 ng/mL.<sup>11</sup> The intra-assay variance was 5.8% and the inter-assay variance was 7.7%.<sup>11</sup> A random stool was collected in a plastic pot. The stool was homogenized with a small rod, and a portion (50–100 mg) of the homogenate was carefully weighed into a 10-mL plain plastic tube. Extraction buffer was added to the stool sample according to the mass of the sample in the proportion 10 mL of buffer to 100 mg of stool. The stool suspension was mixed thoroughly at room temperature and then left overnight at 4–8 °C. The following day the stool suspension was mixed again and allowed to settle before being diluted to 1 : 250. This diluted sample was measured in duplicate using the enzyme-linked immunoabsorbent assay for pancreatic elastase 1 with a monoclonal antibody against human pancreatic elastase 1.

## RESULTS

Paired data were obtained from 45 patients. In 20 patients, the PABA and FE1 tests were both normal. Fourteen of these subsequently had a positive diagnosis of irritable bowel syndrome. Three had alcohol excess as the cause of their diarrhoea and one each had bacterial overgrowth, Addison's disease and diverticular disease (see Table 1).

In eight patients with a high clinical index of suspicion for pancreatic insufficiency, both tests were low, and the patients improved with Creon. Six of these patients had changes suggestive of chronic pancreatitis on computed tomography scan of the pancreas. Two patients did not have a computed tomography scan performed. One had an abnormal pancreas on ultrasound scan and the other had a high clinical index of suspicion for exocrine

Table 1. Results of *para*-aminobenzoic acid (PABA) test vs. faecal elastase 1 (FE1) test

	FE1 normal, PABA normal	FE1 normal, PABA low	FE1 low, PABA low	FE1 low, PABA normal
Irritable bowel syndrome	14	9	0	0
Exocrine pancreatic insufficiency	0	0	8	1
Coeliac disease	0	2	0	0
Bacterial overgrowth	1	1	0	0
Miscellaneous	5	3	0	1
Total	20	15	8	2

pancreatic insufficiency. In 15 patients, the PABA test was low or borderline low, but the FE1 test was normal. Nine of these patients had a low clinical index of suspicion for pancreatic insufficiency and did not improve on Creon. They all fitted the Rome II criteria for irritable bowel syndrome, which was their final clinical diagnosis. In five other patients from this group, a non-pancreatic cause for their symptoms was found. There was one patient each with ulcerative colitis, thyrotoxicosis and bacterial overgrowth, and two had coeliac disease. This suggests that the FE1 test was correct in these patients. In one case, the patient vomited 4 h after taking the Lundh meal and this could explain the false positive PABA test.

Two patients had a normal PABA test, but low FE1 levels. One of these improved with Creon. Computed tomography scan confirmed chronic pancreatitis, suggesting underlying pancreatic insufficiency and that the FE1 test was the more accurate. The other patient was a diabetic with severe watery diarrhoea. A false low FE1 level can occur in patients with very watery stool samples.

## DISCUSSION

Elastase (molecular weight, 28 kDa) is a proteolytic, pancreas-specific enzyme with a specific affinity to the carboxyl group of alanine, valine and leucine.<sup>11, 13</sup> First described by Mallory and Travis in 1975 as protease F, it was further characterized as an elastolytic pancreatic enzyme by Largmann *et al.*<sup>19</sup> It forms 6% of all secreted pancreatic enzymes, with a pancreatic juice concentration of between 170 and 360 µg/L.<sup>20</sup> During intestinal passage, in contrast with other pancreatic enzymes, it is not degraded in the gut, but is in fact concentrated in human faeces compared with pancreatic juice.<sup>11, 12, 20</sup>

The PABA test was introduced in Japan in the mid- to late 1970s as an indirect test for pancreatic insufficiency.<sup>21</sup> It has been used as an alternative diag-

nostic tool for exocrine pancreatic insufficiency with reasonable sensitivity and specificity.<sup>22-25</sup> The PABA test has been compared with the other frequently used method of indirect assessment of exocrine pancreatic function, namely the pancreolauryl test, and has been found to be similar.<sup>26, 27</sup> Lankisch *et al.* concluded that the sensitivity and specificity of the PABA test were comparable with those of the pancreolauryl test in patients with pancreatic steatorrhoea.<sup>28</sup>

A number of research studies have been published on the value of FE1 measurement in the diagnosis of exocrine pancreatic insufficiency. Dominguez-Munoz *et al.* concluded that the determination of FE1 is a simple and accurate test of chronic pancreatitis, with the same specificity as chymotrypsin estimation and a higher sensitivity than other faecal enzyme estimations.<sup>14</sup> In all 11 patients with exocrine pancreatic insufficiency confirmed by the gold standard secretin-cholecystokinin test, Stein *et al.* found low FE1 levels.<sup>16</sup>

Most of these studies involved patients with an established diagnosis of chronic pancreatitis and addressed the accuracy of FE1 in this setting. Our study aimed to compare the PABA and FE1 tests in a routine out-patient setting in the investigation of patients with diarrhoea or suspected steatorrhoea. Most centres in the UK do not have simple tests for steatorrhoea available, as these tests are generally unreliable and unpopular with laboratory staff.<sup>29</sup> Clinicians therefore have to look for potential causes of diarrhoea and steatorrhoea, such as exocrine pancreatic insufficiency. The two most frequently used indirect methods are the pancreolauryl and PABA tests. Our department has been using the PABA test for several years. However, as stated above, the PABA test is cumbersome, time consuming and has drawbacks. In comparison, FE1 estimation can be performed on a single stool specimen.

We found concordance between the two tests in 28 patients (20 normal, eight low). In patients with discordant results, i.e. low PABA and normal FE1

levels, other causes of diarrhoea were found. Therefore, our study shows FE1 to be a more robust test of exocrine pancreatic insufficiency than the PABA test in the work-up of patients with persistent diarrhoea.

The PABA test is less sensitive, and false abnormal results occur with liver disease, renal impairment, intestinal malabsorption<sup>2</sup> and bacterial degradation of the tripeptide. A normal PABA test result rules out severe exocrine pancreatic insufficiency, but may produce false normal results in mild or moderate disease. Pancreatic enzyme supplements and many drugs have to be stopped at least 3 days prior to the test, and there is the risk of administering radioactive material to the patients. Accurate urine collection, vital for an accurate result, can be difficult. On the other hand, FE1 estimation requires only a random, single stool sample of more than 100 mg. It is stable at room temperature for at least 7 days, making handling and even mailing of a small stool sample easy. As monoclonal antibodies against human pancreatic elastase are used in the enzyme-linked immunoabsorbent assay kit, FE1 estimation is not affected by the simultaneous administration of pancreatic enzyme replacement therapy.

In conclusion, the data from this study suggest that FE1 is a simple, robust test with a better sensitivity than the PABA test in the detection of exocrine pancreatic insufficiency in a group of patients with unexplained diarrhoea or steatorrhoea in an out-patient setting.

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