Original article

Reliable screening for acute pancreatitis with rapid urine trypsinogen-2 test strip

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Background: This study was designed to evaluate the validity of a new rapid urinary trypsinogen-2 test strip (Actim Pancreatitis) for detection of acute pancreatitis in patients with acute abdominal pain.

Methods: A total of 525 consecutive patients presenting with abdominal pain at two emergency units was included prospectively and tested with the Actim Pancreatitis test strip. Urine trypsinogen-2 concentrations were also determined by a quantitative method. The diagnosis and assessment of severity of acute pancreatitis was based on raised serum and urinary amylase levels, clinical features and findings on dynamic contrast-enhanced computed tomography.

Results: In 45 patients the diagnosis of acute pancreatitis could be established. The Actim Pancreatitis test strip result was positive in 43 of them resulting in a sensitivity of 96 per cent. Thirty-seven false-positive Actim Pancreatitis test strips were obtained in patients with non-pancreatic abdominal pain resulting in a specificity of 92 per cent. Nine patients with severe acute pancreatitis were all detected by the dipstick.

Conclusion: A negative Actim Pancreatitis strip result excludes acute pancreatitis with high probability. Positive results indicate the need for further evaluation, i.e. other enzyme measurements and/or radiological examinations. The test is easy and rapid to perform, unequivocal in its interpretation and can be used in healthcare units lacking laboratory facilities.

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Introduction

Acute pancreatitis is a common abdominal disorder. To date, there is no 'gold standard' for the diagnosis of acute pancreatitis and the diagnosis may be problematic, especially in an emergency setting. The clinical signs of acute pancreatitis, such as upper abdominal pain, epigastric dullness and nausea, are non-specific and may be absent in about 10 per cent of cases[1,2]. Determination of amylase in serum or urine is the principal laboratory method for diagnosing acute pancreatitis[3–5] although it is far from perfect. Hyperamylasaemia may be absent in 19 per cent of cases, so this assay is not sensitive enough as a screening test[6]. The need for more sensitive tests for acute pancreatitis has been pointed out[7]. Presently, contrast-enhanced computed tomography (CT) is the most accurate non-invasive single method of evaluating patients with acute pancreatitis and to assess the severity of the disease[8,9]. However, CT cannot always be performed when acute pancreatitis is suspected because of high costs and limited availability. Thus it too is not suitable as a screening test.

Trypsinogen, which is the inactive precursor of trypsin, is secreted from acinar cells into pancreatic juice. Trypsinogen occurs as two major isoenzymes, trypsinogen-1 (cationic) and trypsinogen-2 (anionic)[10,11]. Premature activation of trypsin within the pancreas is thought to play a crucial role in the pathogenesis of acute pancreatitis[12]. The inflammatory process also leads to leakage of pancreatic enzymes into the circulation, and trypsinogen-2 levels are increased in both serum and urine[11,13]. Trypsinogen-2 and the complex between trypsin-2 and α_1-antitrypsin are therefore accurate diagnostic markers and are of predictive value in assessing the severity of acute pancreatitis[14].

The authors have evaluated a new rapid test strip to detect trypsinogen-2 in urine. The first version of this test strip showed very high diagnostic accuracy in retrospective[13] and prospective[15] studies. The new test strip, Actim Pancreatitis, which is based on new monoclonal antibodies

with modified detection limits, is now available commercially (Medix Biochemica, Kauniainen, Finland).

The purpose of the present study was to evaluate prospectively the clinical validity of the novel Actim Pancreatitis test strip in screening for acute pancreatitis by analysing a consecutive series of patients with acute abdominal pain.

Patients and methods

Patients

The prospective study population consisted of 525 consecutive patients admitted to the emergency units at Helsinki University Central Hospital and Helsinki City Hospital between December 1997 and April 1998. All patients presenting with abdominal pain potentially caused by acute pancreatitis were included.

In 45 patients, a diagnosis of acute pancreatitis could be established. The aetiology of acute pancreatitis was alcohol in 32 patients, biliary in seven, drugs in one and unknown in five. There were 16 women and 29 men of mean age 50 (range 30–81) years. In 29 patients the diagnosis was based on consistent clinical findings (epigastric pain, nausea and vomiting) in combination with a raised amylase level (above 300 units/l in serum and/or above 2000 units/l in urine) and diagnostic findings on contrast-enhanced CT or ultrasonography. In 15 patients the diagnosis of acute pancreatitis was based on clinical findings and highly raised amylase levels (serum amylase over 900 units/l, urinary amylase over 6000 units/l). The diagnosis of acute pancreatitis was confirmed by contrast-enhanced CT in one patient without a raised amylase level on admission. In patients with marginally raised amylase levels (300–900 units/l in serum and 2000–6000 units/l in urine) and abdominal pain, pancreatitis was excluded or confirmed with repeated amylase measurements, clinical follow-up and radiological examinations.

The severity of acute pancreatitis was categorized by the clinically based classification of the 1992 Atlanta symposium[16]. Exclusion of acute pancreatitis in 480 patients with acute abdominal disease of extrapancreatic origin was based on clinical, radiographic, endoscopic and surgical findings.

Biochemical measurements

The Actim Pancreatitis test strip is based on the immunochromatography principle. The test was carried out by dipping the tip of the test strip into urine. Trypsinogen-2 in the sample was absorbed and bound to monoclonal antibody-labelled blue latex particles. Then the sample fluid with the latex–antibody–trypsinogen-2 complex migrated across the nitrocellulose membrane with a catching zone containing another antibody specific for a different epitope on trypsinogen-2. The test was considered positive when a clear blue line was detected within 5 min. A control line was used to indicate proper functioning of the strip. If the control line was undetectable the assay was repeated. The detection limit of the test was approximately 50 μg/l.

Urine samples from all patients were obtained in the emergency unit and tested immediately with the urinary dipstick. The concentration of trypsinogen-2 in the samples was also measured by a quantitative immunoenzymometric assay (in-house assay; Medix Biochemica). The samples were stored at −20°C until the quantitative measurements were performed.

Statistical analysis

Comparison of continuous data was performed with the Mann–Whitney *U* test. Agreement between quantitative trypsinogen-2 concentration and the test strip result was evaluated with the kappa statistic ($\kappa < 0{\cdot}20$ indicates poor agreement and $\kappa > 0{\cdot}81$ very good agreement)[17].

Results

There were 244 women and 281 men, with a mean age of 50 (range 16–90) years. In 45 of the 525 patients with abdominal pain the diagnosis of acute pancreatitis could be established. In 43 of these patients the Actim Pancreatitis test strip showed a positive result, giving a sensitivity of 96 per cent. There were two false-negative results due to relatively low trypsinogen-2 levels (below 50 μg/l) as measured by the quantitative trypsinogen-2 assay. Both patients had a mild attack of acute pancreatitis. The aetiology was alcohol in one and unknown in the other. Thirty-seven of 480 control patients with acute abdominal disease of extrapancreatic origin tested positive (*Fig. 1*). Thus, the specificity of the test strip was 92 per cent. The positive predictive value was 54 per cent and the negative predictive value 99·6 per cent.

The diagnoses of the patients with abdominal pain other than acute pancreatitis, including the 37 false-positive cases, are shown in *Table 1*. Only two of these patients had CT for further diagnostic evaluation, but the diagnoses were achieved by other methods (endoscopy, operation). There were 172 patients with no abdominal diagnosis (*Table 1*), 14 of whom had CT.

Among the 45 patients with acute pancreatitis there were nine with a severe form of the disease. All of these patients showed a positive Actim Pancreatitis test resulting in a sensitivity of 100 per cent. In one patient with severe acute pancreatitis, confirmed by contrast-enhanced CT, the amylase level was not raised whereas the urinary concentra-

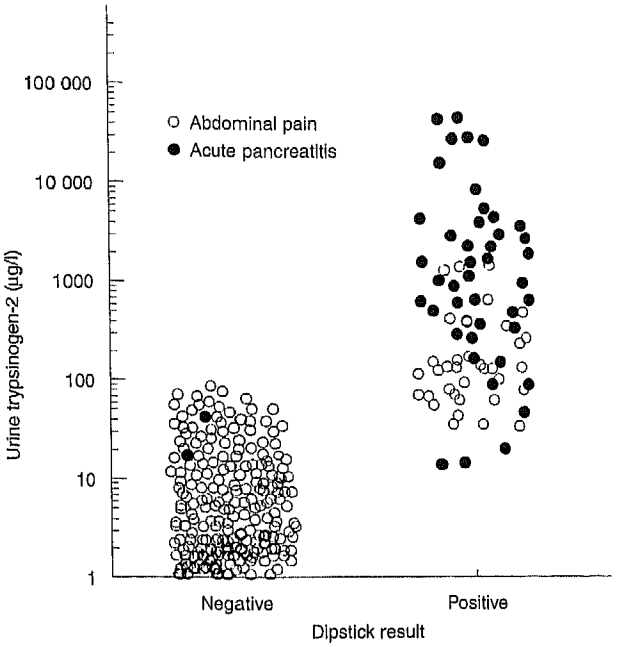

Fig. 1 Results of the urinary trypsinogen-2 dipstick test in relation to quantitative urinary trypsinogen-2 concentration in 45 patients with acute pancreatitis and 480 patients with abdominal pain from other causes

tion of trypsinogen-2 was greatly increased (2330 μg/l) and the test strip result was positive.

The median urinary concentration of trypsinogen-2 in patients with acute pancreatitis was 1030 (range 11·5–48 800) μg/l and in patients with abdominal pain other than acute pancreatitis it was 1·5 (range 0–4500) μg/l. The difference was highly significant ($P < 0.0001$, Mann–Whitney U test). The sensitivity and specificity of the quantitative analysis of urinary trypsinogen-2 with a cut-off of 50 μg/l was 91 and 92 per cent respectively. Urinary trypsinogen-2 concentrations were significantly higher in patients with severe acute pancreatitis (median 5640 (range 1010–48 800) μg/l) than in those with mild disease (median 649 (range 11·5–44 300) μg/l) ($P < 0.0015$, Mann–Whitney U test). The median urinary concentration of trypsinogen-2 in the nine patients with severe acute pancreatitis was nearly nine times higher than that in patients with a mild course of the disease. In the 37 patients with a false-positive dipstick result, the median urinary trypsinogen-2 concentration was 138 (range 35·5–4500) μg/l. The kappa value was 0·86 indicating very good agreement between the test strip result and the quantitative analysis.

Discussion

The Actim Pancreatitis test strip detected patients with acute pancreatitis accurately. The sensitivity (96 per cent) of the new test strip was slightly higher than that of the original test strip (94 per cent) and the specificity a little lower (92 *versus* 95 per cent). The high sensitivity resulted in a very high negative predictive value of more than 99 per cent. Thus, acute pancreatitis could be excluded with a very high probability, which is considered to be of prime importance for a screening test. Another valuable feature was the ability to detect all patients with severe acute pancreatitis. This is to be expected because of the strong correlation between release of trypsinogen-2 and the severity of acute pancreatitis[14]. The probable explanation for the high clinical sensitivity of the test strip is the steep increase in urinary trypsinogen-2 level in acute pancreatitis[14]. Thus the median concentration in patients with pancreatitis was nearly 700-fold that in patients with extrapancreatic abdominal disorders.

False-positive test strip results were obtained in 37 patients with a variety of diagnoses. Thus the positive predictive value was relatively low (54 per cent) indicating that further diagnostic procedures are needed to clarify the

Table 1 Diagnosis in 480 patients with abdominal disorders other than acute pancreatitis

Diagnosis	Total no. of patients	No. with false-positive results
Acute appendicitis	20	
Acute gastritis or dyspepsia	35	3
Acute gastroenteritis	18	2
Biliary stones	47	1
Blunt trauma	4	
Cardiac disorder or chest pain	11	
Chronic pancreatitis	7	
Colitis	2	
Colonic diverticulosis	14	1
Crohn's disease	1	1
Diabetes with abdominal pain	9	1
Drug or alcohol intoxication	14	4
Duodenal or gastric ulcer	2	
Oesophagitis	6	
Functional disorder of colon	8	
Gastrointestinal bleeding	10	
Hepatic disease	14	4
Hepatorenal syndrome	1	1
Infection	16	5
Intestinal obstruction	11	1
Intestinal perforation	4	3
Malignant extra-abdominal tumour	4	
Malignant abdominal tumour	10	1
Pancreatic pseudocyst	1	
Pulmonary embolus	1	1
Rhabdomyolysis	1	1
Urinary infection, colic or retention	28	1
Other	9	
Unknown	172	6
Total	480	37

cause of a positive result. False-positive results were seen in patients who were seriously ill and therefore needed further diagnostic evaluation and follow-up. In general, the differential diagnosis between acute pancreatitis and patients with false-positive results was relatively simple. The authors recommend that a patient with a positive result undergoes further laboratory evaluation (amylase/lipase) and comprehensive ultrasonography or CT if needed.

In the present study the median urinary concentration of trypsinogen-2 in patients with abdominal pain other than acute pancreatitis was 1·5 μg/l. The detection limit of the urinary test strip (50 μg/l) is approximately 30-fold higher, providing sufficient specificity. The specificity could be somewhat underestimated since subclinical mild acute pancreatitis could not be excluded in all patients with abdominal pain. Raised trypsinogen-2 levels have been reported in hepatobiliary and pancreatic malignancies, in colonic cancer and in chronic pancreatitis[18]. It is likely that the test strip will also detect these conditions.

In general, the correspondence between the test strip result and quantitative analysis of urinary trypsinogen-2 was very good, supporting the use of the trypsinogen-2 test strip. The minor differences between the results of the Actim Pancreatitis test strip and the quantitative urinary trypsinogen-2 assay possibly resulted from the fact that the samples were stored frozen before quantitative analysis; partial activation of trypsinogen-2, leading to degradation, and complex formation between trypsin and its inhibitors may have occurred during storage.

A missed diagnosis of acute pancreatitis leads to inadequate treatment, frequent complications and increased costs. The non-invasive simple urinary screening test evaluated in the present study could be useful in an effort to decrease the number of misdiagnosed cases of acute pancreatitis in an emergency setting.

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