Original Paper

Comparison of Urine Trypsinogen-2 Test Strip with Serum Lipase in the Diagnosis of Acute Pancreatitis

M-L Kylänpää-Bäck, E Kemppainen, P Puolakkainen, J Hedström[1], R Haapiainen A Korvuo[2], U-H Stenman[1]

Department of Surgery, [1]Department of Clinical Chemistry, Helsinki University Central Hospital Helsinki, Finland; [2]Medix Biochemica, Kauniainen, Finland

Corresponding Author: M-L Kylänpää-Bäck, MD, Second Department of Surgery Helsinki University Central Hospital, P.O. POX 340, 00290 Helsinki, Finland

Tel: +358 40 5862096, Fax: +358 9 47174675, E-mail: lennukylanpaa@hotmail.com

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ABBREVIATIONS:
Acute Pancreatitis (AP); Negative Predictive Value (NPV); Positive Predictive Value (PPV)

ABSTRACT

Background/Aims: The accuracy of a new rapid urinary trypsinogen-2 test strip (actim Pancreatitis) was compared with that of serum lipase for detection of acute pancreatitis in patients with acute abdominal pain.

Methodology: A prospective study was conducted which consisted of 237 consecutive patients with acute abdominal pain admitted to the emergency unit at Helsinki University Central Hospital. The patients were tested on admission with the actim Pancreatitis test strip. Serum amylase, serum lipase, and urine trypsinogen-2 concentrations were also determined quantitatively.

Results: The actim Pancreatitis test strip result was positive in 27 out of 29 patients with acute pancreatitis (sensitivity 93%) and in 16 of 208 patients with non-pancreatic abdominal pain (specificity 92%). This was superior to that of serum lipase (sensitivity 79% and specificity 88%). With a cut-off >3x the upper reference limit, the sensitivity of serum lipase was only 55% while the specificity was 99%. The high sensitivity for the actim Pancreatitis test strip resulted in a very high negative predictive value of 99%. All six patients with severe acute pancreatitis were detected by the dipstick. With a higher cut-off value (>3x upper reference limit) for lipase, two patients with severe acute pancreatitis remained undetected. Combining the actim Pancreatitis dipstick with serum lipase a positive predictive value of 94% was obtained.

Conclusions: Acute pancreatitis can be excluded with a higher probability with the actim Pancreatitis strip than with serum lipase determination, and therefore appears to be more suitable for screening of acute pancreatitis. With its high specificity with a cut-off >3x the upper reference limit, serum lipase is suitable as a confirmatory test for pancreatitis when a positive dipstick result is obtained.

INTRODUCTION

There are no physical signs that are diagnostic for acute pancreatitis (AP) (1). Atypical presentation is so frequent that the diagnosis is often delayed, and a marked proportion of the cases may be undiagnosed until autopsy (2). Serum amylase and lipase are commonly used laboratory methods in the evaluation of patients with abdominal pain (3-5) although neither of them is specific for AP (6). Several other laboratory tests (i.e., urine amylase, amylase creatinine clearance rate, serum elastase-I) have been investigated but have not shown any advantage over amylase or lipase in the diagnosis of AP (7-9). In AP, serum amylase levels increase within 2-12 hours and return to normal in 3-5 days (10). Because the concentration of serum amylase may normalize soon after the onset of symptoms it is normal in up to 19% of the cases at presentation, the assay is not considered suitable for screening of AP (11).

Pancreatic lipase is a pancreatic enzyme synthesized in the exocrine acinar cells. It catalyses the hydrolysis of triglycerides into diglycerides and fatty acids (12). In pancreatitis, serum lipase rises within 4-8 hours and remains elevated longer than serum amylase (8-14 days) (10). The sensitivity and specificity of serum lipase in the diagnosis of AP vary considerably in different studies. This may partly be due to different assay methods (6,13).

Trypsin is a 24-kDa protease, which is secreted from acinar cells into pancreatic juice as inactive proenzyme trypsinogen. Trypsinogen occurs as two major isoenzymes, trypsinogen-1 (cationic) and trypsinogen-2 (anionic) (14,15). AP is triggered by events, which are mainly thought to be of extrapancreatic origin (16), but irrespective of the etiology, premature activation of trypsin within the pancreas is considered a common feature at the acinar cell level (16,17). In AP trypsinogen-2 levels increase rapidly both in serum and urine (15,18,19). Thus, trypsinogen-2 and also the trypsin-2-α_1-antitrypsin complex are accurate diagnostic markers of AP and show a marked correlation with the disease severity (20). Ear-

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lier studies have shown that trypsinogen-2 is a more reliable marker for AP than amylase. However, comparisons with amylase are biased by the fact that amylase is routinely used as a major diagnostic criterium for AP (19).

A new urinary rapid test strip based on immunochromatographic measurement of trypsinogen-2 has been developed (15). The first version of this test strip showed very high diagnostic accuracy in retrospective (18) and prospective (19) studies. The new test strip actim Pancreatitis with modified detection limits, based on two new monoclonal antibodies, is now commercially available (Medix Biochemica, Kauniainen, Finland) and has proven to be highly sensitive (96%) and specific (21).

The present prospective study was designed to compare the novel actim Pancreatitis test strip with serum lipase for screening of AP by analyzing a consecutive series of patients with acute abdominal pain.

METHODOLOGY

Patients

We investigated prospectively 237 consecutive patients with acute abdominal pain admitted to the emergency unit at Helsinki University Central Hospital between December 1997 and April 1998. In 29 patients, a diagnosis of AP could be established. The etiology of AP was alcohol in 19 patients, biliary in six, pancreas divisum in one, and unknown in three. There were 10 female and 19 male patients (mean age: 48 years, range: 30-81 years). In 5 patients the diagnosis was based on consistent clinical findings (epigastric pain, nausea and vomiting) in combination with an elevated amylase level (above 300 IU/L in serum) and diagnostic findings on contrast enhanced computed tomography. In 24 patients the diagnosis of AP was based on clinical findings in combination with highly elevated amylase levels (serum amylase over 900 IU/L). The severity of AP was categorized by the clinically based classification of the 1992 Atlanta Symposium (22). Exclusion of AP in patients with acute abdominal pain (208 patients) 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 briefly dipping the tip of the test strip into urine. Trypsinogen-2 in the sample migrated through the strip binding to monoclonal antibody-labeled blue latex particles. 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 minutes in the catching zone. A control line was used to indicate proper function of the strip. If the control line was undetectable the assay was repeated. The detection limit of the test was approximately 50µg/L. Urine samples from all patients were obtained in the emergency unit on admission and tested immediately with the urinary dipstick. The concentration of trypsinogen-2 in the urine samples was also measured by a quantitative immunoenzymometric assay (IEMA) (inhouse assay, Medix Biochemica, Kauniainen, Finland, reference value, <50µg/L). The samples were stored at -20°C until the quantitative measurements were performed.

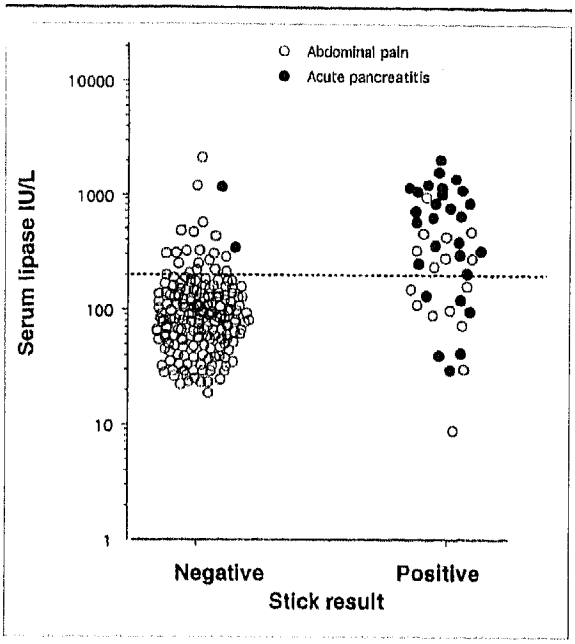

FIGURE 1 Results of the urinary trypsinogen-2 dipstick test in relation to serum lipase concentration in 29 patients with acute pancreatitis and 208 patients with abdominal pain from other causes.

The concentration of serum lipase was measured by a turbidimetric assay based on the degradation of a triolein emulsion (Boehringer - Mannheim, Germany, reference value, <200 IU/L). Serum samples for determination of lipase were taken on admission and stored at -70°C until analyzed.

Statistical Analysis

Comparison of continuous data was performed by the Mann-Whitney U test.

RESULTS

The study population consisted of 106 female and 131 male patients aged 17 to 89 years, with a mean of 50 years. Of the 237 patients with abdominal pain 29 had AP. The actim Pancreatitis test strip showed a positive result in 27 of them giving a sensitivity of 93%. Two AP patients with false negative results due to relatively low trypsinogen-2 levels (17.4µg/L and 41.8µg/L), as measured by the quantitative trypsinogen-2 assay, had a mild attack of AP. The etiology was alcohol in one and unknown in the other. The test results were also positive in 16 of the 208 control patients with abdominal pain but no evidence of AP (**Figure 1**). Thus, specificity was 92% (**Table 1**). The actim Pancreatitis test strip had a negative predictive value (NPV) of 99% and a positive predictive value (PPV) of 63%. The diagnoses of the patients with abdominal pain other than AP including the 16 false-

TABLE 1 Diagnostic Accuracy of Urinary Trypsinogen-2 Dipstick Test and Serum Lipase Activity for Detection of Acute Pancreatitis in 237 Patients with Acute Abdominal Pain

Test	Cut-off value	Acute Pancreatitis (N = 29) Positive test (N)	Sensitivity %	Other abdominal disorders (N = 208) Positive test (N)	Specificity %	PPV %	NPV %
Urinary trypsinogen-2 dipstick test (actim)	~50µg/L	27	93	16	92	63	99
Serum lipase	200 IU/L	23	79	24	88	49	97
Serum lipase	600 IU/L	16	55	3	99	84	94
Urinary trypsinogen-2 dipstick test AND serum lipase	~50µg/L 600 IU/L	16	55	1	99.5	94	94

Reference value of serum lipase activity is <200 IU/L. PPV: positive predictive value; NPV: negative predictive value.

positive cases are shown in **Table 2**. All six patients, in whom severe AP developed, had a positive actim Pancreatitis test result (sensitivity 100%).

The median urinary trypsinogen-2 concentration was significantly higher in patients with AP (930µg/L, range: 11.5-48800µg/L) than in controls with abdominal pain (1.4µg/L, range: 0.0-4500µg/L) ($P<0.001$). With a cut-off of 50µg/L the quantitative analysis of urinary trypsinogen-2 had a sensitivity of 83% and specificity of 91%. The patients with severe AP had significantly higher urinary trypsinogen-2 concentrations (median 7360µg/L, range 1010-48800µg/L) than those with mild disease (median: 530µg/L, range: 11.5-44300µg/L) ($P<0.01$). In the 16 patients with a false-positive dipstick result, the median urinary trypsinogen-2 concentration was 136µg/L (range: 35.5-4500µg/L).

The median concentration of serum lipase in patients with AP was 712 IU/L (range: 30-2070 IU/L) and in patients with abdominal pain from causes other than AP 91 IU/L (range: 9-2131 IU/L). The difference was highly significant ($P<0.001$). The sensitivity and specificity of the serum lipase with two cut-off values are presented in **Table 1**. The serum lipase concentrations were not significantly higher in patients with severe AP (median: 679 IU/L, range: 257-1172 IU/L) than in those with mild disease (median: 712 IU/L, range: 30-2070 IU/L). Two patients with a severe AP had only moderately elevated serum lipase concentrations (257 and 398 IU/L) and would have remained undetected with the higher cut-off, 600 IU/L.

A combination of the actim Pancreatitis test strip and serum lipase with a cut-off 600 IU/L improved specificity of the dipstick alone from 92% to 99.5% and PPV from 63% to 94% (**Table 1**).

DISCUSSION

The actim Pancreatitis test strip detected AP more accurately than the quantitative serum lipase determination. The high sensitivity (93%) of the dipstick resulted in a very high NPV of 99%. Thus, AP could be excluded with a very high probability. A characteristic feature of urinary trypsinogen-2 is the strong, rapid, and long-lasting elevation in AP (15). This was seen also in the present study, the median concentration of urinary trypsinogen-2 in AP being nearly 700-fold that in patients with extrapancreatic abdominal disorders. In comparison, the sensitivity of serum lipase (55-79%) was low and inadequate for screening purposes.

The levels of serum amylase or lipase bear no correlation with the prognosis in AP (5,12,23). In the present study the median concentration of serum lipase

TABLE 2 Diagnoses in 208 Patients with Abdominal Disorders other than Acute Pancreatitis

Diagnosis	Total N of patients	N with false-positive results	Diagnosis	Total N of patients	N with false-positive results
Acute appendicitis	12		Functional disorder of colon	4	
Acute gastritis or dyspepsia	12	3	Gastrointestinal bleeding	3	
Acute gastroenteritis	5	1	Hepatic disease	4	1
Biliary stones	24		Infection	7	2
Blunt trauma	4		Intestinal obstruction	7	1
Cardiac disorder or chest pain	2		Intestinal perforation	5	2
Chronic pancreatitis	3		Malignant extraabdominal tumor	2	
Colonic diverticulosis	7		Malignant abdominal tumor	8	
Crohn's disease	1	1	Other	5	
Diabetes with abdominal pain	1		Pulmonary embolus	1	1
Drug or alcohol intoxication	5		Rhabdomyolysis	1	1
Duodenal or gastric ulcer	1		Unknown	67	3
Esophagitis	5		Urinary infection, colic or retention	12	
			Total	**208**	**16**

was not higher in patients with severe AP than in those with mild disease. Thus, some patients with severe AP may be missed both by serum amylase (2) and lipase (24). This was the case in two patients in the present study, if the higher cut-off value for lipase 600 IU/L (3x the upper normal limit) was used. In contrast to amylase and lipase, a strong correlation between the concentration of trypsinogen-2 and the severity of AP has been observed (15), and this was confirmed in the present study; The actim Pancreatitis test strip detected all patients with severe AP, which we consider highly important in clinical practice.

The median urinary concentration of trypsinogen-2 in patients with abdominal pain from causes other than AP was about 1/30th the detection limit of the urinary test strip (50µg/L). Thus the specificity of the dipstick was acceptable and higher than that of serum lipase with cut-off 200 IU/L (92% *vs.* 88%, respectively). With the cut-off 600 IU/L serum lipase showed very high specificity (99%) but sensitivity was unacceptable (55%). The false-positive test strip results in 16 patients with a variety of diagnoses resulted in a relatively low PPV (63%), which was, however, higher than the PPV of lipase with a cutoff 200 IU/L (49%). Our results indicate that the diagnosis of AP cannot be established by the test strip alone but that additional enzyme measurements or radiological examinations are needed. When serum lipase >600 IU/L was combined with a positive actim Pancreatitis result, the specificity and PPV were high. Thus, we recommend the use of serum lipase as a confirmatory test for AP in cases with a positive dipstick result.

The cause of the relatively low PPV for the actim Pancreatitis test strip and lipase was that the study population included all patients with abdominal pain. Thus, the pre-test probability of AP in the present material was only 12%, which is clearly lower than the average of 21% in diagnostic studies of AP (25). With a 21% pre-test probability the PPV of the dipstick can be calculated to have been about 78%.

The sensitivity of the actim Pancreatitis test strip was higher than that of the quantitative assay for trypsinogen-2 at a cut-off of 50µg/L (93% *vs.* 83%, respectively). The better performance of the test strip was probably due to the fact that it was used immediately with fresh urine whereas the quantitative assay for trypsinogen-2 was performed on samples that had been stored at -20°C for several weeks, during which some trypsinogen-2 immunoreactivity is lost (Stenman U-H, unpublished findings).

AP still represents a major diagnostic and therapeutic challenge. The diagnosis is often unsatisfactory in an emergency setting. Computed tomography scan is the most accurate diagnostic method (26,27) but it is not always available and, because of its high costs, cannot be performed in all instances when AP is suspected clinically. The actim Pancreatitis test can be performed by medical or nursing staff at the point of care. Cases with negative results can safely await further investigations, routine laboratory tests and radiological examinations.

An early diagnosis of AP is essential to provide the patient with adequate treatment and clinical follow-up and thus reduce the risk of complications and prolonged hospitalization. The non-invasive and simple urinary screening test Actim Pancreatitis was more sensitive and specific than serum lipase, which was useful as a confirmatory test in patients with positive dipstick result.

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