

## RAPID MEASUREMENT OF URINARY TRYPSINOGEN-2 AS A SCREENING TEST FOR ACUTE PANCREATITIS

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### ABSTRACT

**Background** Acute pancreatitis can be difficult to diagnose. We developed a rapid dipstick screening test for pancreatitis, based on the immunochromatographic measurement of urinary trypsinogen-2.

**Methods** We prospectively compared the urinary trypsinogen-2 dipstick test with a quantitative urinary trypsinogen-2 assay, a urinary dipstick test for amylase, and serum and urinary amylase assays in 500 consecutive patients with acute abdominal pain at two emergency departments. Acute pancreatitis was diagnosed according to standardized criteria.

**Results** The urinary trypsinogen-2 dipstick test was positive in 50 of the 53 patients with acute pancreatitis (sensitivity, 94 percent), including all 7 with severe pancreatitis. Two patients with urinary trypsinogen-2 concentrations below the sensitivity threshold of the test (50 ng per milliliter) and one with a very high concentration had false negative results. The test was also positive in 21 of the 447 patients without pancreatitis (specificity, 95 percent), including 7 with abdominal cancers, 3 with cholangitis, and 2 with chronic pancreatitis. The sensitivity and specificity of the dipstick test were similar to those of the quantitative urinary trypsinogen-2 assay and higher than those of the urinary amylase dipstick test. The serum amylase assay had a sensitivity of 85 percent (with a cutoff value of 300 U per liter for the upper reference limit) and a specificity of 91 percent. The sensitivity and specificity of the urinary amylase assay (cutoff value, 2000 U per liter) were 83 and 88 percent, respectively.

**Conclusions** In patients with acute abdominal pain seen in the emergency department, a negative dipstick test for urinary trypsinogen-2 rules out acute pancreatitis with a high degree of probability. A positive test usually identifies patients in need of further evaluation. (N Engl J Med 1997;336:1788-93.)

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**M**OST patients with acute pancreatitis have mild or occasionally subclinical disease that resolves spontaneously. About 20 percent of patients have a severe necrotizing disease with systemic or local complications.<sup>1,2</sup> Mortality associated with severe acute pancreatitis can be as high as 40 percent, especially if bacterial contamination of the necrotic pancreas develops.<sup>3</sup>

Immediate diagnosis of severe acute pancreatitis is

important, since early intensive therapy is likely to be beneficial.<sup>2</sup> In an emergency setting, however, the diagnosis of acute pancreatitis is often problematic.<sup>4</sup> The clinical signs may be nonspecific, and the presentation atypical. In many patients with severe disease, the diagnosis is not made until autopsy.<sup>5</sup>

Measurement of amylase or lipase is the principal laboratory method for diagnosing acute pancreatitis,<sup>1</sup> but the sensitivity and specificity of the assays for these enzymes are considered unsatisfactory.<sup>6</sup> Contrast-enhanced computed tomography (CT) is the most accurate method for diagnosing and assessing the severity of acute pancreatitis. Because of its cost and limited availability and the potential side effects of the contrast material, however, CT cannot always be performed.<sup>1,7</sup> A screening test for pancreatitis with the use of a test strip that detects urinary amylase (Rapignost-Amylase, Boehringer Mannheim, Marburg, Germany) is available in Europe. The detection limit of about 2000 U of amylase per liter has been considered to result in a fairly low sensitivity.<sup>8,9</sup>

Trypsinogen is a 25-kd pancreatic proteinase. The two main isoenzymes, (cationic) trypsinogen-1 and (anionic) trypsinogen-2, are secreted at high concentrations into pancreatic fluid, but a small proportion escapes into the circulation.<sup>10</sup> Because of their relatively small size, trypsinogens are readily filtered through the glomeruli. For unknown reasons, the tubular reabsorption of trypsinogen-2 is lower than that of trypsinogen-1, and consequently, the urinary concentration of trypsinogen-2 is higher. Measurement of trypsinogen is considered useful in diagnosing acute pancreatitis and assessing its severity.<sup>4,11</sup> However, previous methods have generally measured trypsinogen-1.<sup>12</sup> Healthy people have higher concentrations of trypsinogen-1; those with acute pancreatitis have a preferential elevation of trypsinogen-2 concentrations, making this isoenzyme a more accurate diagnostic marker.<sup>12,13</sup> Intrapancreatic activation

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of trypsinogen to trypsin is thought to have a pivotal role in the pathophysiology of acute pancreatitis.<sup>14</sup> A mutation was recently identified in the trypsinogen-1 gene in patients with hereditary pancreatitis. The mutation is thought to make trypsin-1 resistant to proteolytic inactivation and may thus trigger an intrapancreatic protease cascade that leads to acute pancreatitis.<sup>15</sup>

We have developed a rapid dipstick test for the detection of elevated trypsinogen-2 concentrations in urine. The results of a preliminary retrospective study of the test were encouraging.<sup>16</sup> We report on a prospective evaluation of the clinical use of the dipstick test to screen for acute pancreatitis. The results of the dipstick test were compared with those of amylase measurements and a quantitative immunofluorometric assay for urinary trypsinogen-2.

## METHODS

### Patients

The study group consisted of 500 consecutive patients (306 men and 194 women) with acute abdominal pain seen in the emergency department at Helsinki University Central Hospital or Helsinki City Hospital between October and December 1995. The mean duration of pain was 1.1 days (range, 0.1 to 4). No patients were excluded because of other symptoms. The mean age was 45 years (range, 17 to 90).

### Study Design

Urine samples were obtained from all patients in the emergency department and immediately tested with the strips for trypsinogen-2 and amylase. The tests were performed by laboratory staff without knowledge of the patients' clinical presentations. Trypsinogen-2 and amylase concentrations in urine were also determined in all patients by quantitative methods. After the patients had undergone clinical evaluation, serum amylase concentrations were determined in all patients except 12 with diagnoses other than acute pancreatitis. In patients with marginally elevated serum amylase concentrations (300 to 900 U per liter) and abdominal pain, pancreatitis was ruled out or confirmed on the basis of repeated serum and urinary amylase measurements and clinical follow-up, as well as abdominal CT and ultrasonographic studies. Contrast-enhanced CT was performed when we suspected the presence of severe disease or acute pancreatitis in a patient with normal serum amylase concentrations. The severity of the disease was classified according to the 1992 Atlanta Symposium criteria.<sup>17</sup> Acute pancreatitis was classified as severe if one or several local or systemic complications were present (e.g., shock, renal failure, respiratory insufficiency, disseminated intravascular coagulation, pancreatic necrosis, an abscess, a pseudocyst, intestinal perforation, or bleeding).

The criteria for a diagnosis of acute pancreatitis were characteristic clinical findings (typical epigastric pain, nausea, and vomiting) and very high amylase concentrations (serum amylase concentration, >900 U per liter; and urinary amylase concentration, >6000 U per liter) (19 patients); characteristic clinical findings combined with an elevated amylase concentration (>300 U per liter in serum or >2000 U per liter in urine) and CT or ultrasonographic findings typical of acute pancreatitis (28 patients); or a characteristic clinical presentation and positive findings on contrast-enhanced CT<sup>18</sup> in patients without an elevated amylase concentration at presentation (6 patients). Other acute abdominal diseases were diagnosed on the basis of clinical, imaging, endoscopic, surgical, and autopsy findings.

### Trypsinogen-2 Measurements

The dipstick test for urinary trypsinogen-2 is an immunochromatographic test. After the test strip has been dipped into the urine sample, trypsinogen-2 is bound to monoclonal-antibody-labeled blue latex particles, which migrate across a nitrocellulose membrane with a zone containing another antibody specific for another epitope on trypsinogen-2. At trypsinogen-2 concentrations higher than 50 ng per milliliter, a blue line develops in this zone. A positive result on the test strip remains visible for at least one year. The test strip is produced by Medix Biochemica, Kauniainen, Finland. As of June 1997, it was not commercially available.

The dipstick test was considered positive if a clear blue line was detected within three minutes. In 53 subjects without gastrointestinal disease, 1 had a positive test result.<sup>16</sup> The variability was studied by analyzing aliquots of three samples with trypsinogen-2 concentrations around the cutoff level at weekly intervals for eight weeks. A sample with a concentration of 50 ng per milliliter by the quantitative immunoassay was positive according to the dipstick test six times out of eight. Samples containing 10 or 30 ng per milliliter were consistently negative.

The concentration of trypsinogen-2 in urine was also measured by a quantitative immunofluorometric assay.<sup>12</sup> The reference range for urinary trypsinogen-2 is 0.3 to 11 ng per milliliter.<sup>11</sup> In patients with acute pancreatitis, the median concentration is 1100 ng per milliliter (range, 15 to 19,000).<sup>11,12</sup> The intraassay variation is 9 percent and the interassay variation is 12 percent at a concentration of 94 ng per milliliter. The assay has been calibrated with pure trypsinogen-2.<sup>12</sup>

### Amylase Measurements

The Rapiagnost-Amylase test was performed by dipping the test strip into urine and determining the color reaction after one minute. Amylase activity in serum and urine was measured enzymatically ( $\alpha$ -Amylase EPS, Boehringer Mannheim) with a Hitachi 717 analyzer. The upper reference limit is 300 U per liter for amylase in serum and 2000 U per liter for amylase in urine.<sup>19</sup> The intraassay variability is 0.4 to 1.0 percent and the interassay variability is 3.5 to 4.1 percent at concentrations of 193, 255, 514, 759, and 1870 U per liter, each estimated from 20 aliquots.<sup>19</sup>

### Statistical Analysis

Continuous data were compared by the Mann-Whitney U test. Test-strip results were compared by McNemar's test.<sup>20</sup> All P values are two-tailed, and those less than 0.05 were considered to indicate statistical significance. The validity of the tests was evaluated by an analysis of receiver-operating-characteristic curves. The area under the receiver-operating-characteristic curve indicates the accuracy of the test, with a value of 1.0 representing 100 percent sensitivity and specificity, and a value of 0.5 no discriminatory power.<sup>21</sup> Agreement between quantitative measurements of trypsinogen-2 and the test-strip results was estimated with the kappa statistic (<0.20 indicates poor agreement, and >0.81 very good agreement).<sup>22</sup>

## RESULTS

Acute pancreatitis was diagnosed in 53 patients (12 women and 41 men) with a mean age of 42 years (range, 24 to 65). The results of the urinary trypsinogen-2 dipstick test were positive in 50 patients (sensitivity, 94 percent). The results were also positive in 21 of the 447 patients with abdominal pain but no evidence of acute pancreatitis (specificity, 95 percent). Of these 21 patients, 7 had abdominal cancers, 3 had purulent cholangitis, 2 had chronic pancreatitis, and 7 had miscellaneous other

**TABLE 1.** DIAGNOSES IN 447 PATIENTS WITH ABDOMINAL DISORDERS OTHER THAN ACUTE PANCREATITIS, INCLUDING 21 PATIENTS WITH FALSE POSITIVE RESULTS ON THE DIPSTICK TEST FOR URINARY TRYPSINOGEN-2.

DIAGNOSIS	NO. OF PATIENTS	NO. WITH FALSE POSITIVE RESULTS
Acute gastritis	60	1
Biliary stones	47	3*
Urinary infection, colic, or retention	33	
Acute gastroenteritis	32	
Intestinal obstruction	29	
Malignant abdominal tumor	22	7
Duodenal or gastric ulcer	17	
Drug or alcohol intoxication	17	
Infection	16	1
Acute appendicitis	16	1
Chronic pancreatitis	15	2
Functional disorder of the colon	14	
Gastrointestinal bleeding	14	
Esophagitis	12	
Cardiac disorder	11	
Acute hepatic disease	10	1
Blunt trauma	9	
Vascular abdominal disorder	7	1
Gynecologic disorder	5	
Diabetes mellitus with abdominal pain	5	
Acute diverticulitis	5	
Intestinal perforation	5	
Intraabdominal abscess	4	1
Abdominal hernia	4	
Low back pain	3	
Jaundice	3	
Ulcerative colitis	2	
Other	6	1
Unknown	24	2
Total	447	21

\*These three patients had purulent cholangitis.

diseases; in 2 patients the cause of pain was not determined (Table 1). None of the patients with chronic pancreatitis had superimposed acute disease, according to the diagnostic criteria we used.

The urinary trypsinogen-2 dipstick test had a negative predictive value of 99 percent and a positive predictive value of 68 percent; it had the best combination of sensitivity and specificity of the five tests evaluated (Table 2). The performance of the quantitative urinary trypsinogen-2 assay was similar (sensitivity, 92 percent; specificity, 93 percent). The accuracy of the Rapignost-Amylase test was significantly lower (sensitivity, 79 percent; specificity, 89 percent;  $P=0.04$ ), mainly because of its lower sensitivity.

All seven patients in whom severe acute pancreatitis developed had positive results on the urinary

trypsinogen-2 test strip, whereas only five had positive results on the amylase test strip. Six patients had acute pancreatitis with normal serum and urinary amylase concentrations and a negative Rapignost-Amylase test. The diagnosis in these patients was confirmed by contrast-enhanced CT. All six had positive results with the trypsinogen-2 test strip and elevated urinary trypsinogen-2 concentrations as determined by the quantitative assay. Among the other 40 patients with mild disease, the diagnosis was based on the clinical presentation combined with amylase concentrations that were more than three times the upper reference limit (in 19 patients) or on elevated amylase concentrations combined with the clinical presentation and imaging findings (in 21).

The median urinary trypsinogen-2 concentration was 800 ng per milliliter (range, 2 to 402,000) in the patients with acute pancreatitis and 2 ng per milliliter (range, 0 to 8110) in those with abdominal pain from causes other than acute pancreatitis ( $P<0.001$ ). All but 1 of the 53 patients with acute pancreatitis had trypsinogen-2 concentrations higher than 25 ng per milliliter. In the three patients with false negative results on the test strip, the quantitative trypsinogen-2 concentrations were 2, 25, and 402,000 ng per milliliter.

The correlation between the results of the urinary trypsinogen-2 dipstick test and those of the serum amylase assay is shown in Figure 1. The results of the quantitative assay were closely correlated with the results of the dipstick test ( $\kappa=0.92$ ). The quantitative tests were compared by receiver-operating-characteristic analysis (Fig. 2). The area under the curve was 0.96 for trypsinogen-2, 0.94 for serum amylase, and 0.93 for urinary amylase. Quantitative determinations of amylase in serum and urine had a specificity of 97 percent when high cutoff values (900 and 6000 U per liter, respectively) were used, but their sensitivity (70 and 51 percent, respectively) was unsatisfactory. Lowering the cutoff levels to the upper reference limits (300 U per liter for serum amylase and 2000 U per liter for urinary amylase) increased the sensitivity of these tests (85 and 83 percent, respectively), but the sensitivity of the urinary trypsinogen-2 dipstick test remained superior.

## DISCUSSION

The urinary trypsinogen-2 dipstick test detected acute pancreatitis more accurately than quantitative serum or urinary amylase determinations, and its accuracy was similar to that of the quantitative assay for urinary trypsinogen-2. The detection limit of the trypsinogen-2 test strip, about 50 ng per milliliter, appears to provide a good balance between sensitivity (94 percent) and specificity (95 percent). The false positive results in 21 patients with abdominal pain may in some instances have reflected subclinical

**TABLE 2.** SENSITIVITY AND SPECIFICITY OF FIVE SCREENING TESTS FOR ACUTE PANCREATITIS IN 500 PATIENTS.

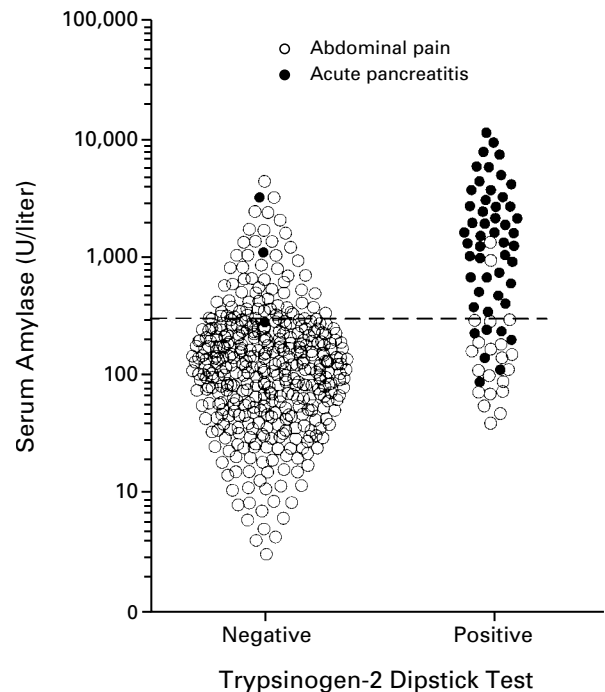
TEST AND CUTOFF VALUE*	ACUTE PANCREATITIS (N=53)		OTHER ABDOMINAL DISORDERS (N=447)	
	POSITIVE TEST	SENSITIVITY	POSITIVE TEST	SPECIFICITY
	no. of patients	%	no. of patients	%
Urinary trypsinogen-2 dipstick test ~50 ng/ml	50	94	21	95
Quantitative urinary trypsinogen-2 assay 50 ng/ml	49	92	30	93
Rapignost-Amylase dipstick test ~2000 U/liter	42	79	49	89
Quantitative serum amylase assay 900 U/liter	37	70	13	97
359 U/liter	44	83	22	95
300 U/liter	45	85	40	91
Quantitative urinary amylase assay 6000 U/liter	27	51	14	97
3140 U/liter	39	74	23	95
2000 U/liter	44	83	53	88

\*For the serum and urinary amylase tests, the first and third cutoff values for a positive result are one and three times the upper reference limit, and the second cutoff value is that which results in a specificity of 95 percent, which is the specificity of the trypsinogen-2 dipstick test. The serum amylase assay was performed in 488 patients; the other tests were performed in all 500 patients.

pancreatic irritation or tumor-derived trypsinogen-2. Trypsinogen-2 has been reported to be a tumor marker for gastrointestinal and ovarian cancers,<sup>23</sup> and it is also expressed in the epithelium of bile ducts and peribiliary glands,<sup>24</sup> which may explain the elevations in the three patients with cholangitis. We consider this frequency of false positive results acceptable, because a high level of sensitivity is essential for a screening test.

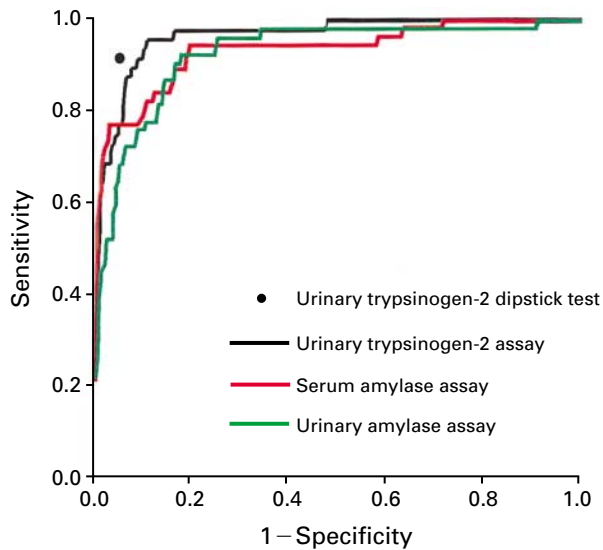
There were three false negative results with the trypsinogen-2 test strip. In two cases, the false negative results were due to low trypsinogen levels as measured by the quantitative trypsinogen-2 assay. One false negative result was caused by the inability of the test strip to detect an extremely high concentration (402,000 ng per milliliter), which was probably due to a "hook effect" (i.e., an excess of antigen saturating the antibodies). In general, the results of the dipstick test and the quantitative analysis were in agreement.

Intrapancreatic activation of trypsin is believed to play an essential part in acute pancreatitis, especially in the necrotizing form of the disease.<sup>25,26</sup> Markedly increased levels of trypsinogen-2 and trypsin-2- $\alpha_1$ -antitrypsin are associated with severe disease.<sup>11,26</sup> The increased proteolytic activity in acute pancreatitis causes the breakdown of protein and the release of peptides, which inhibit the capacity of the renal tubuli to reabsorb proteins. For this reason, the concentration of trypsinogen increases much more steeply in urine than in serum.<sup>11,12</sup> Thus, a high de-



**Figure 1.** Results of the Urinary Trypsinogen-2 Dipstick Test in Relation to Serum Amylase Concentrations in 53 Patients with Acute Pancreatitis and 435 Patients with Abdominal Pain from Other Causes.

The upper reference limit for serum amylase (300 U per liter) is indicated by the dashed line.



**Figure 2.** Receiver-Operating-Characteristic Curves for the Urinary Trypsinogen-2 Dipstick Test and Quantitative Assays of Trypsinogen-2 and Serum and Urinary Amylase.

The curves show the ability of the four tests to differentiate acute pancreatitis (in 53 patients) from acute extrapancreatic abdominal disease (in 435). The solid circle denotes the single value for the specificity and sensitivity of the dipstick test.

tection limit (i.e., 50 ng per milliliter, which is more than four times the upper reference limits for urinary trypsinogen-2) can be used for the test strip with little loss of sensitivity.

The most valuable clinical feature of the dipstick test was its ability to detect all cases of severe acute pancreatitis. Amylase concentrations typically return to normal within three to five days after the onset of acute pancreatitis.<sup>27</sup> The rapid return to normal values may indicate the resolution of the disease or extensive destruction of the pancreas, with the cessation of amylase production.<sup>4,28</sup> Normal serum amylase values are not uncommon in patients with acute pancreatitis; the incidence may exceed 19 percent.<sup>28</sup> This fairly high incidence probably explains why some of our patients with severe disease had normal amylase values.

We have found that the concentration of trypsinogen-2 remains elevated for 4 to 30 days in both urine and serum (data not shown). Mero et al. have reported that the value for total trypsinogen immunoreactivity in serum remains elevated for more than nine days.<sup>29</sup> These findings may help explain the high sensitivity of the trypsinogen-2 tests.

Measurement of serum or urinary amylase is currently the cornerstone of the diagnosis of acute pancreatitis. Despite their simplicity, these tests are usually used selectively in patients with abdominal symptoms. Systematic measurement of serum amylase, lipase, and other enzymes in all such patients is

difficult to justify. A urinary screening test could help reduce the risk of misdiagnosing acute pancreatitis in patients seen in the emergency department. Our results suggest such a role for the urinary trypsinogen-2 dipstick test. A negative test result rules out acute pancreatitis with a high probability, and a positive result usually identifies patients in need of further evaluation.

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Dr. Hedström and Dr. Stenman have applied for a patent for the method of measuring trypsinogen-2 in urine. Dr. Stenman is the sole owner of the potential patent rights.

## REFERENCES

- Steinberg W, Tenner S. Acute pancreatitis. *N Engl J Med* 1994;330:1198-210.
- Corfield AP, Cooper MJ, Williamson RCN, et al. Prediction of severity in acute pancreatitis: prospective comparison of three prognostic indices. *Lancet* 1985;2:403-7.
- Beger HG, Bittner R, Block S, Buchler M. Bacterial contamination of pancreatic necrosis: a prospective clinical study. *Gastroenterology* 1986;91:433-8.
- Clavien PA, Burgan S, Moossa AR. Serum enzymes and other laboratory tests in acute pancreatitis. *Br J Surg* 1989;76:1234-43.
- Wilson C, Imrie CW, Carter DC. Fatal acute pancreatitis. *Gut* 1988;29:782-8.
- Wong ECC, Butch AW, Rosenblum JL, Ladenson JH, Scott MG. The clinical chemistry laboratory and acute pancreatitis. *Clin Chem* 1993;39:234-43.
- Balthazar EJ. CT diagnosis and staging of acute pancreatitis. *Radiol Clin North Am* 1989;27:19-37.
- Gunn IR, Faye S, Clayton MG. Prospective evaluation of urinary amylase test strip. *Lancet* 1986;1:1161.
- Burkitt DS. The Rapignost-Amylase test in acute pancreatitis. *Br J Surg* 1987;74:1063.
- Rinderknecht H, Geokas MC. Anionic and cationic trypsinogens (trypsins) in mammalian pancreas. *Enzyme* 1972;73;14:116-30.
- Hedström J, Sainio V, Kempainen E, et al. Urine trypsinogen-2 as marker of acute pancreatitis. *Clin Chem* 1996;42:685-90.
- Itkonen O, Koivunen E, Hurme M, Alfthan H, Schröder T, Stenman U-H. Time-resolved immunofluorometric assays for trypsinogen-1 and 2 in serum reveal preferential elevation of trypsinogen-2 in pancreatitis. *J Lab Clin Med* 1990;115:712-8.
- Kimland M, Russick C, Marks WH, Borgström A. Immunoreactive anionic and cationic trypsin in human serum. *Clin Chim Acta* 1989;184:31-46.
- Marshall JB. Acute pancreatitis: a review with an emphasis on new developments. *Arch Intern Med* 1993;153:1185-98.
- Whitcomb DC, Gorry MC, Preston RA, et al. Hereditary pancreatitis is caused by a mutation in the cationic trypsinogen gene. *Nat Genet* 1996;14:141-5.
- Hedström J, Korvuo A, Kenkimäki P, et al. Urinary trypsinogen-2 test strip for acute pancreatitis. *Lancet* 1996;347:729-30.
- Bradley EL III. A clinically based classification system for acute pancreatitis. *Arch Surg* 1993;128:586-90.
- Kempainen E, Sainio V, Haapiainen R, Kivisaari L, Kivilaakso E, Puolakkainen P. Early localization of necrosis by contrast-enhanced computed tomography can predict outcome in severe acute pancreatitis. *Br J Surg* 1996;83:924-9.
- Gerhardt W, Waldenström J, Hörder M, et al. SCE Nordic alpha-amylase study. II. Assessment of proposed calibration procedure: a report by the Scandinavian Committee on Enzymes (SCE). *Scand J Clin Lab Invest* 1986;46:465-9.
- Armitage P, Berry G. *Statistical methods in medical research*. 2nd ed. Oxford, England: Blackwell Scientific, 1987.
- Zweig MH, Campbell G. Receiver-operating characteristic (ROC) plots: a fundamental evaluation tool in clinical medicine. *Clin Chem* 1993;39:561-77. [Erratum, *Clin Chem* 1993;39:1589.]
- Landis JR, Koch GG. The measurement of observer agreement for categorical data. *Biometrics* 1977;33:159-74.

23. Hedström J, Haglund C, Haapiainen R, Stenman U-H. Serum trypsinogen-2 and trypsin-2-alpha<sub>1</sub>-antitrypsin complex in malignant and benign digestive tract diseases: preferential elevation in patients with cholangiocarcinomas. *Int J Cancer* 1996;66:326-31.
24. Terada T, Nakanuma Y. Immunohistochemical demonstration of pancreatic alpha-amylase and trypsin in intrahepatic bile ducts and peribiliary glands. *Hepatology* 1991;14:1129-35.
25. Ohlsson K, Eddeland A. Release of proteolytic enzymes in bile-induced pancreatitis in dogs. *Gastroenterology* 1975;69:668-75.
26. Hedström J, Sainio V, Kempainen E, et al. Serum complex of trypsin 2 and alpha 1 antitrypsin as diagnostic and prognostic marker of acute pancreatitis: clinical study in consecutive patients. *BMJ* 1996;313:333-7.
27. Brooks FP. Testing pancreatic function. *N Engl J Med* 1972;286:300-3.
28. Clavien PA, Robert JH, Meyer P, et al. Acute pancreatitis and normoamylasemia: not an uncommon combination. *Arch Surg* 1989;210:614-20.
29. Mero M, Schröder T, Tenhunen R, Lempinen M. Serum phospholipase A<sub>2</sub>, immunoreactive trypsin, and trypsin inhibitors during human acute pancreatitis. *Scand J Gastroenterol* 1982;17:413-6.