Clinical Chemistry 42:5
685–690 (1996)

Urine trypsinogen-2 as marker of acute pancreatitis

JOHAN HEDSTRÖM,[1]* VESA SAINIO,[2] ESKO KEMPPAINEN,[2] PAULI PUOLAKKAINEN,[2] REIJO HAAPIAINEN,[2] EERO KIVILAAKSO,[2] KNUT-OLOF SCHAUMAN,[3] and ULF-HÅKAN STENMAN[1]

We examined the clinical utility of urine trypsinogen-2 as a marker of acute pancreatitis (AP). Fifty-nine patients with AP, 42 with acute abdominal diseases of extrapancreatic origin, and 63 without evidence of acute abdominal disease were studied. Urine trypsinogen-2 was determined by a time-resolved immunofluorometric assay. As reference methods we used serum trypsinogen-2, urine amylase, and serum amylase. The diagnostic accuracy of the markers was evaluated by receiver-operating characteristic (ROC) analysis. At admission, urine trypsinogen-2 differentiated patients with AP from controls with high accuracy. The area under the ROC curve (AUC) was 0.978, which was equal to that of serum trypsinogen-2 (0.998) and serum amylase (0.969) and significantly larger than that of urine amylase. For differentiation between severe and mild AP, urine trypsinogen-2 (0.730) was equal to serum trypsinogen-2 (0.721), and clearly better than amylase in serum and urine. These results suggest that determination of urine trypsinogen-2 is a useful test to detect AP and to evaluate disease severity.

INDEXING TERMS: urine marker • proenzymes • proteases • amylase • trypsinogen activation peptides • time-resolved immunofluorometric assay

Earlier studies suggest a role of proteolytic enzymes in the pathophysiology of pancreatitis *[1–3]*, and the concentration of trypsinogen in serum appears to reflect pancreatic damage *[4]*. Trypsin is the main protease in human pancreatic fluid. It is secreted by exocrine cells of the pancreas as a proenzyme, trypsinogen, which is activated in the intestine by enterokinase *[5]*. The two major isoenzymes of trypsinogen are trypsinogen-1 and -2. The ratio of trypsinogen-1 in plasma of healthy subjects is fourfold that of trypsinogen-2 *[6]*. However, in acute pancreatitis (AP) the serum concentrations of trypsinogen-2 are more strongly increased *[4, 7]*, and trypsinogen-2 has been shown to be a useful marker for AP *[4, 8]*.[4]

As a test sample, urine has the advantage of being easily available. Furthermore, it is suitable for rapid and sensitive immunological determinations, as exemplified by pregnancy tests. Determination of amylase in urine has sometimes been considered even more accurate than serum amylase, which is still the most widely used test to support a diagnosis of AP *[9, 10]*. Although increasing somewhat later and remaining increased for a longer time, amylase concentrations in urine parallel those in serum, and the same limitations of specificity and sensitivity therefore apply *[11, 12]*. Another potential urine marker for AP is the trypsinogen activation peptides (TAP) *[13]*. A previously developed assay for TAP has been successfully used to assess the severity of AP *[14]*.

Trypsinogen-2 can be specifically measured with a time-resolved immunofluorometric assay (IFMA) developed in our laboratory *[4]*. In the present study we evaluated the accuracy of urine trypsinogen-2 in the early diagnosis and prediction of severity of AP by measuring the concentrations in urine from healthy subjects, patients with acute abdominal disorders of extrapancreatic origin, and patients with mild and severe AP. As reference methods we used serum trypsinogen-2 and serum and urine amylase.

Methods

PATIENTS

A total of 59 patients with a diagnosis of AP and 42 controls with acute upper abdominal disease of extrapancreatic origin admitted to the Second Department of Surgery at Helsinki University Central Hospital from March 1992 through November 1993 were studied. Serum and spot urine samples were obtained from these patients within 24 h after admission . The diagnosis of AP was established on the basis of consistent clinical findings

[1] Department of Clinical Chemistry and [2] Second Department of Surgery, University of Helsinki, Helsinki, Finland.
[3] Helsinki City Hospital, Helsinki, Finland.
*Address correspondence to this author at: Department of Clinical Chemistry, Helsinki University Central Hospital, FI-00290 Helsinki, Finland. Fax Int + 358-0-4714804; e-mail ulf-hakan.stenman@hyks.mailnet.fi.
Received November 10, 1995; accepted February 7, 1996.

[4] Nonstandard abbreviations: AP, acute pancreatitis; TAP, trypsinogen activation peptides; IFMA, immunofluorometric assay; CT, computed tomography; and AUC, area under the curve.

supported by an increased serum amylase concentration (>300 U/L) and the typical appearance of AP in computed tomography (CT). If severe pancreatitis was clinically suspected, the patients underwent contrast-enhanced CT to detect necrosis of the pancreas *[15]*. Ultrasonography was used to detect possible gallstones or underlying biliary disease, and the findings were considered diagnostic for AP in 12 patients. In 5 patients the diagnosis of AP was based on clinical findings and serum amylase only (Table 1). Three patients with a diagnosis of AP had normal amylase values, but showed clinical and CT findings characteristic for AP on admission.

The patients with AP were classified according to their clinical outcome into two groups: mild AP (group I, n = 40) and severe AP (group II, n = 19). AP was defined as severe if one or more major local or systemic disease complications were present. Median age of patients with AP was 44 years (range 21–91 years); 80% were men and the etiology was due to alcohol in 74% (Table 1). Pancreatitis was regarded as alcoholic on the basis of a history of recent alcohol abuse and by exclusion of other causes. A biliary origin was deduced when gallstones were shown by ultrasonography, endoscopic retrograde pancreatography, laparotomy, or necropsy.

The patients in group I improved spontaneously with conservative treatment and none developed local pancreatic or systemic complications. Median hospital stay was 6 days (Table 1). The three patients with normal serum amylase on admission belonged to this group. Complications occurring in the 19 patients with severe AP were respiratory failure (13 patients), renal failure (5 patients), septicemia (2 patients), abscesses (2 patients), and necrosis of the pancreas (15 patients). Four patients died of hemorrhagic pancreatitis verified at autopsy. Four patients underwent surgery as their condition deteriorated despite intensive conservative therapy. The operative procedures were necrosectomy and distal pancreatic resection. Necrosis of the pancreas was confirmed by contrast-enhanced CT, laparotomy, or at autopsy. Median hospital stay in group II was 23 days (Table 1).

Table 1. Clinical features of patients.

	Patients, n (%)		
	Mild AP	Severe AP	All AP
Clinical features			
With AP	40 (68)	19 (32)	59 (100)
Age[a]	46	40	44
Sex ratio M/F	31/9	16/3	47/12
Episodes of pancreatitis			
First attack	15 (38)	18 (95)	33 (56)
Recurrence	25 (62)	1 (5)	26 (44)
Etiology			
Alcohol	27 (68)	17 (89)	44 (74)
Biliary disease	6 (15)	1 (5)	7 (12)
Unknown	7 (18)	1 (5)	8 (14)
Methods of diagnosing AP			
Ultrasonography	30 (75)	16 (84)	46 (78)
CT	14 (35)	17 (89)	31 (53)
Contrast-enhanced CT	12 (30)	14 (74)	26 (44)
Serum amylase >300 U/L	37 (92)	19 (100)	56 (100)
Clinical course			
Treatment in intensive care unit	0	17 (89)	17 (29)
Conservative treatment	40 (100)	15 (79)	55 (93)
Surgical treatment	0	4 (21)	4 (7)
Days of hospitalization[a]	6	23	9
Surgical procedure			
Necrosectomy	0	2 (11)	2 (3)
Distal pancreatic resection	0	2 (11)	2 (3)

[a] Median value.

Patients with acute abdominal disease of extrapancreatic origin were categorized into the control group on the basis of clinical, radiographic, endoscopic, or surgical findings. Median age was 41 years (range 20–99 years) and 48% were men. The final diagnoses are shown in Table 2. Serum and urine samples were drawn within 24 h of admission. The procedures followed were in accordance with the Helsinki Declaration of 1975.

HEALTHY CONTROLS

Urine samples from 63 control patients without evidence of acute abdominal disease undergoing minor orthopedic intervention were obtained from the City Hospital of Helsinki. All samples were stored at −20 °C until assayed.

LABORATORY TESTS

Serum and urine amylase were measured by an enzymatic method with a Hitachi 705E analyzer and SYS 1 α-Amylase EPS reagents (Boehringer Mannheim, Mannheim, Germany). Calibration was according to the Scandinavian Committee on Enzymes *[16]*. The upper reference limit of serum amylase was 300 U/L and for urine amylase 2000 U/L.

Trypsinogen-1 and 2 were determined by a time-resolved IFMA *[4]*. The assays involve monoclonal antibodies produced by immunization with trypsinogen isolated from mucinous ovarian cyst fluid. For assay of trypsinogen in urine, 25 μL of sample was used. The reference range for trypsinogen-2 in serum was 18–90 μg/L (median 39 μg/L). The detection limit of trypsinogen-2 was 0.3 μg/L and that of trypsinogen-1 0.1 μg/L *[4, 8]*.

STATISTICAL ANALYSIS

The ability of various tests to differentiate between mild and severe AP and nonpancreatic disease was estimated on the basis

Table 2. Diagnosis of 42 patients with acute abdominal disorders of extrapancreatic origin.

Diagnosis	No. of cases
Acute appendicitis	9
Intestinal obstruction	7
Peptic ulcer/esophagogastritis	8
Acute biliary disease	5
Ureterolithiasis	4
Acute gastroenteritis	3
Acute sigmoid diverticulitis	2
Nonspecific abdominal pain	4

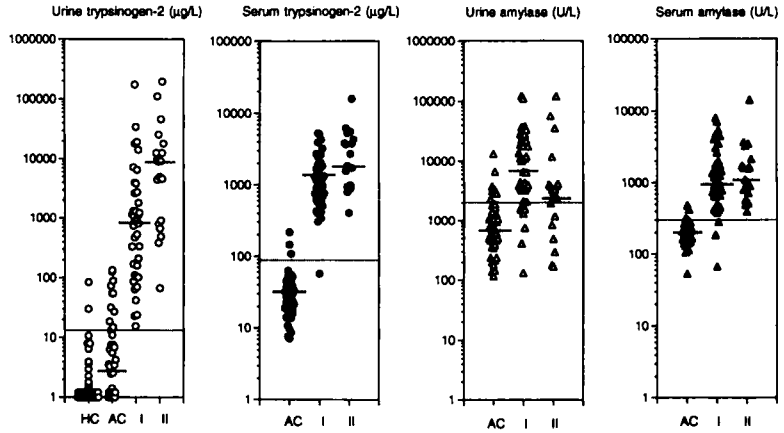

Fig. 1. Concentrations of trypsinogen-2 and amylase in urine and serum samples from 40 patients with mild (I) and 19 patients with severe AP (II). Controls were 42 patients with acute abdominal disorders of extrapancreatic origin (AC) and 63 patients without acute abdominal disorders (HC). The *dashed horizontal line* indicates the upper reference limit; the *smaller dashed horizontal lines* represent the median concentrations for each of the groups.

of sensitivity and specificity at various cutoff concentrations. The validity of the tests was further evaluated by receiver-operating characteristic (ROC) curve analysis. The area under the curve (AUC) of the ROC plot describes the accuracy of the test: 1 indicates 100% sensitivity and specificity and 0.5 no discriminatory power *[17]*. A univariate *z*-score test was performed with the CLABROC program (C.E. Metz, Department of Radiology, The University of Chicago Medical Center, Chicago, IL) to estimate the significance of the difference between the areas under the ROC curves. The reference range for trypsinogen-2 in urine was determined on the basis of the 2.5 and 97.5 percentiles in urine from 63 patients without acute abdominal disorders. The correlation between serum and urine values was calculated with the least-squares method and the equation for the regression line with the standardized principal component method *[18]*.

Results

The reference range for urine trypsinogen-2 was 0.3–11 μg/L (median 1.0). All patients with AP had urine trypsinogen-2 values above the upper reference limit (11 μg/L), whereas serum trypsinogen-2 was normal in 1 patient, serum amylase in 3 patients, and urine amylase in 14 patients with AP (Fig. 1). The median concentration of urine trypsinogen-2 in all patients with AP was 102-fold that of the upper reference limit. For comparison, the concentration of trypsinogen-2 in serum was 13-fold, that of urine amylase twofold, and that of serum amylase 3.4-fold (Table 3). The median concentration of urine trypsinogen-2 in AP group II was 10-fold that in group I, whereas the differences for serum trypsinogen-2 and serum and urine amylase were 1.8-fold, 1.1-fold, and 0.4-fold, respectively. The much greater increase in urine than in serum trypsinogen-2 in AP is shown by Fig. 2. The correlation between the logarithms of urine (y) and serum (x) is highly significant ($y = 2.4x - 4.2$, $r = 0.570$, $P < 0.0001$).

When comparing all AP patients with controls with acute abdominal extrapancreatic disorders (Fig. 3), the differences between the AUCs for urine trypsinogen-2 (AUC = 0.978, SD = 0.012) and for serum trypsinogen-2 (AUC = 0.998, SD = 0.003) or serum amylase (AUC = 0.969, SD = 0.018) were not statistically significant ($P = 0.085$ and 0.660, respectively). However, the difference between AUC for urine trypsinogen-2 and urine amylase (AUC = 0.845, SD = 0.038) was significantly different ($P < 0.001$).

When comparing severe with mild AP (Fig. 4), the differences between the AUCs for urine trypsinogen-2 (AUC = 0.730, SD = 0.069) and for serum amylase (AUC = 0.535, SD = 0.076) or urine amylase (AUC = 0.332, SD = 0.078) were statistically significant ($P = 0.040$ and <0.001, respectively). The difference between AUC for urine trypsinogen-2 and serum trypsinogen-2 (AUC = 0.721, SD = 0.072) was not statistically significant ($P = 0.790$) (Fig. 4). Medians and ranges for the various markers in controls and in patients with AP are shown in Table 3. The specificities, sensitivities, and predictive values of the various tests at selected cutoffs are shown in Tables 4 and 5.

Table 3. Urine (U) and serum (S) trypsinogen-2 and amylase in controls and patients.

	Median (and range), μg/L				
	Healthy controls (n = 63)	Acute abdominal controls (n = 42)	Mild AP (n = 40)	Severe AP (n = 19)	All AP (n = 59)
U-Trypsinogen-2	1.0 (0.2–84)	2.9 (1.0–130)	890 (15–170 000)	9000 (67–190 000)	1100 (15–190 000)
S-Trypsinogen-2		38 (7.2–220)	1000 (57–5300)	1800 (400–16 000)	1200 (57–16 000)
U-Amylase		700 (120–13 000)	8200 (130–120 000)	3300 (170–120 000)	40 000 (130–120 000)
S-Amylase		200 (53–490)	990 (67–8000)	3300 (390–14 000)	4000 (67–14 000)

Values represent the first determination within 24 h after admission.

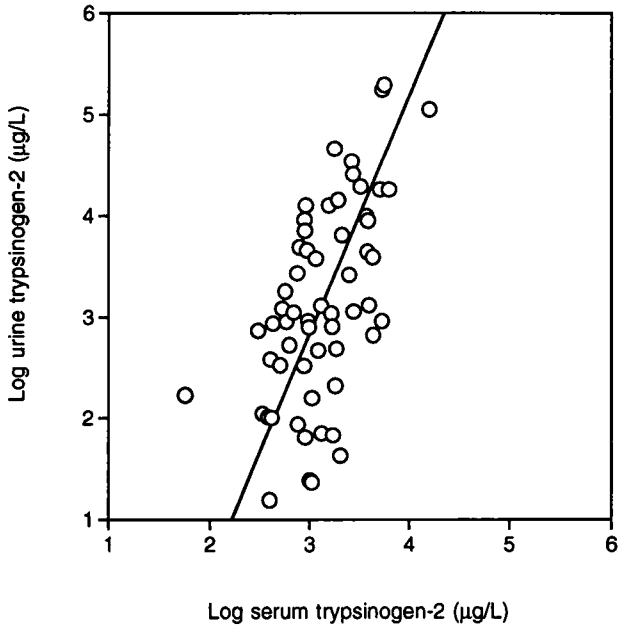

Fig. 2. Correlation between serum and urine concentrations of trypsinogen-2 in patients with AP.

The concentrations of trypsinogen-1 in urine were very low in patients with AP (median 0.8 μg/L, range 0.14–4 μg/L) and close to the detection limit of the assay. Therefore this assay was not investigated further.

Discussion

The present study shows that trypsinogen-2 in urine is a marker with high accuracy for AP. All cases of AP had increased urine concentrations of trypsinogen-2 at presentation; thus a concentration below the upper reference value excluded this diagnosis.

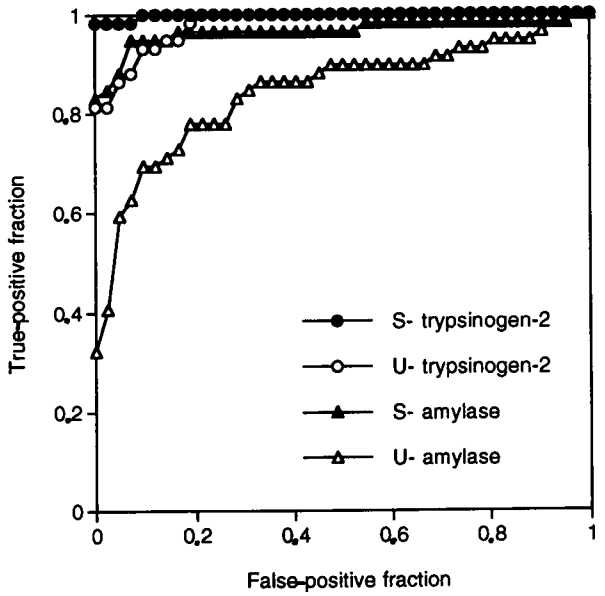

Fig. 3. ROC plots showing the accuracy of the various serum (S) and urine (U) tests in differentiating between AP and acute abdominal extrapancreatic disease.

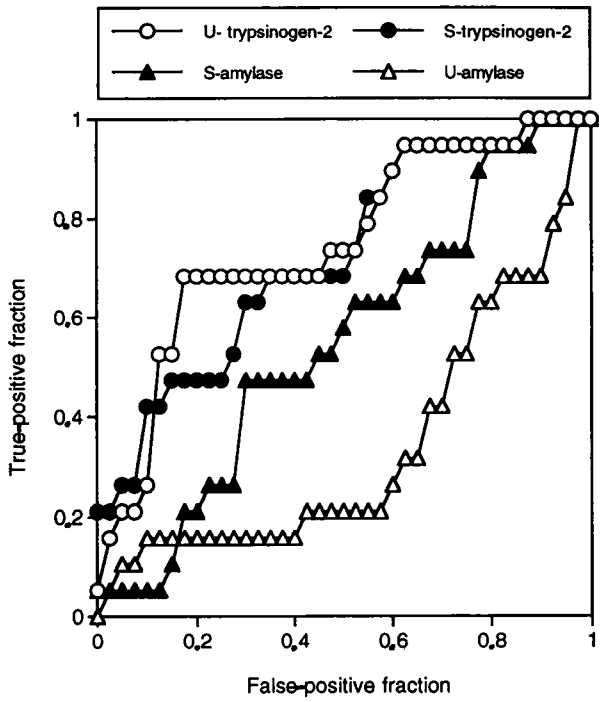

Fig. 4. ROC plots showing the accuracy of the various serum (S) and urine (U) tests in differentiating between severe and mild AP.

The lowest trypsinogen-2 value in AP (15 μg/L) was 1.4-fold the upper reference limit in healthy controls. The median urine trypsinogen-2 concentration of patients with AP was 390-fold that of patients with acute abdominal disease of extrapancreatic origin, whereas the difference for serum trypsinogen-2 was 32-fold (Table 3). ROC curve analysis showed that serum assay of trypsinogen-2 was slightly better than the urine assay for separation between AP and acute abdominal disease of extrapancreatic origin, but the difference was small and not statistically significant. Serum amylase had only slightly lower accuracy than

Table 4. Specificity, sensitivity, positive predictive value (PPV), and negative predictive value (NPV) of the various tests to differentiate AP from other acute gastrointestinal diseases of extrapancreatic origin.

Cutoff	Specificity, %	Sensitivity, %	PPV	NPV
Urine trypsinogen-2, μg/L				
55	90	93	0.93	0.90
89	95	86	0.96	0.83
Serum trypsinogen-2, μg/L				
56	90	100	0.93	1.00
110	95	98	0.96	0.97
Urine amylase, U/L				
3200	90	69	0.91	0.67
3700	95	59	0.94	0.62
Serum amylase, U/L				
310	90	95	0.93	0.93
420	95	88	0.96	0.85

Values are given for specificity levels of 90% and 95%.

Table 5. Specificity, sensitivity, positive predictive value (PPV), and negative predictive value (NPV) of the various tests to differentiate severe AP from mild AP.

Cutoff	Specificity, %	Sensitivity, %	PPV	NPV
Urine trypsinogen-2, μg/L				
3800	80	68	0.62	0.84
14 000	90	26	0.55	0.72
Serum trypsinogen-2, μg/L				
2100	80	47	0.53	0.76
3200	90	42	0.67	0.77
Urine amylase, U/L				
24 000	80	16	0.28	0.67
33 000	90	16	0.43	0.69
Serum amylase, U/L				
2600	80	21	0.33	0.68
4500	90	5	0.19	0.67

Values are given for specificity levels of 80% and 90%.

trypsinogen in serum and urine, whereas urine amylase had significantly lower accuracy.

In severe AP the median concentration of urine trypsinogen-2 was 10-fold that of mild AP, whereas the difference was 1.8-fold for serum trypsinogen-2. For differentiation of severe and mild AP, urine trypsinogen-2 had the highest accuracy of the markers studied (AUC = 0.730). Interestingly, it had slightly better AUC than serum trypsinogen-2 (AUC = 0.721), although the difference was not statistically significant. A low urine trypsinogen-2 concentration on admission indicates that the patient does not need the full range of supportive treatment that patients with AP and high trypsinogen-2 require. Amylase in serum and urine had poor ability to differentiate severe from mild AP. AUC of serum and urine amylase was 0.535 and 0.332, respectively, i.e., close to 0.5, which indicates no discriminative power. Urine amylase was actually slightly lower in severe than in mild disease (Fig. 1). This is in agreement with earlier studies *[19]*. The difference in AUC between urine trypsinogen-2 and serum or urine amylase was significant.

An interesting finding was that some patients with AP had extremely high concentrations of urine trypsinogen-2 (>100 000 μg/L), whereas the highest concentration of serum trypsinogen-2 exceeded 10 000 μg/L in only one case. A possible explanation for the much greater increase of urinary trypsinogen-2 in AP is that the severe inflammatory reaction in combination with free proteolytic activity causes protein breakdown and release of peptides. Because some amino acids are known to inhibit protein reabsorption in the renal tubules *[20]*, the tissue and protein breakdown occurring in pancreatitis could contribute to the very high urine concentrations of trypsinogen-2. It has been shown that in AP the increased renal clearance for amylase and many other low-molecular-mass proteins found in the urine may be due to tubular insufficiency *[21]*. In spite of this, there was a good correlation between serum and urine concentrations.

Determination of trypsinogen in serum and in urine have earlier been used for diagnosis of pancreatic disease *[22–24]*. However, most earlier immunoassays preferentially measure cationic trypsinogen, i.e., trypsinogen-1. We have previously shown a much greater increase in the serum concentrations of trypsinogen-2 than trypsinogen-1 in AP *[4]*. We measured urine trypsinogen-1 in 19 patients with AP, but the concentrations were very low and often close to the detection limit. This suggests that trypsinogen-1 is efficiently reabsorbed in the kidneys or degraded in urine.

The determination of TAP in urine of patients with AP has been shown to be useful for evaluation of disease severity *[14]*. This assay reflects trypsin activation. However, 30% of patients with hyperamylasemia and the classical clinical features of AP had normal TAP values on admission, suggesting that this assay may not act as a primary diagnostic test for AP.

Although the concentration of trypsinogen-2 in urine may be influenced by dehydration and impaired renal function, such effects are likely to be small in the early stages of pancreatitis *[14]*. In spite of this potential limitation, trypsinogen-2 in urine showed good accuracy for evaluation of the severity of the disease. We have previously shown that serum trypsinogen-2 is increased in dialysis patients *[8]*, but the effect of moderately impaired renal function on urine trypsinogen-2 requires further study.

On the basis of the promising results in this study, determination of urine trypsinogen-2 could replace amylase as a routine test for pancreatitis. However, this would require that the determination could be performed on a stat basis with an automated immunoanalyzer. Although such analyzers already are available, their stat use is still rare.

Because early diagnosis of AP is important *[9, 11, 25, 26]*, there is a need for a rapid test that could be used at points of care lacking laboratory facilities. Two such tests have been described, one for urine amylase and the other for serum lipase, but these have been found not to have sufficient sensitivity for screening AP *[26, 27]*. We are therefore investigating the possibility of developing a rapid semiquantitative immunological test for trypsinogen-2 in urine.

This work was supported by grants from The Finska Läkaresällskapet, The Sigrid Juselius Foundation, and The Finnish Academy of Sciences.

References

1. Ohlsson K, Eddeland A. Release of proteolytic enzymes in bile-induced pancreatitis in dogs. Gastroenterology 1975;69:668–75.
2. Lasson A, Ohlsson K. Protease inhibitors in acute human pancreatitis. Correlation between biochemical changes and clinical course. Scand J Gastroenterol 1984;19:779–86.
3. Ohlsson K. Acute pancreatitis. Biochemical, pathophysiological and therapeutical aspects. Acta Gastroenterol Belg 1988;51:3–12.
4. 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.
5. Figarella C, Amouric M, Carrere J, Miszczuk Jamska B, Guy Crotte O. Pancreatic proteins in normal state and pancreatic diseases in pancreatic juice and blood. Ric Clin Lab 1984;14:349–59.
6. Durie PR, Gaskin KJ, Geokas MC, O'Rourke MO, Largman C.

Plasma immunoreactive anionic pancreatic trypsin in cystic fibrosis. J Pediatr Gastroenterol Nutr 1982;1:337–43.
7. Kimland M, Russick C, Marks WH, Borgström A. Immunoreactive anionic and cationic trypsin in human serum. Clin Chim Acta 1989;184:31–46.
8. Hedström J, Leinonen J, Sainio V, Stenman U-H. Time-resolved immunofluorometric assay of trypsin-2 complexed with α_1-antitrypsin in serum. Clin Chem 1994;40:1761–5.
9. Moossa AR. Diagnostic tests and procedures in acute pancreatitis. N Engl J Med 1984;311:639–43.
10. Salt WB, Schenker S. Amylase—its clinical significance: a review of the literature. Medicine 1976;55:269–89.
11. Clavien PA, Burgan S, Moossa AR. Serum enzymes and other laboratory tests in acute pancreatitis. Br J Surg 1989;76:1234–43.
12. Saxon EI, Hinkley WC, Vogel WC, Zieve L. Comparative value of serum and urinary amylase in the diagnosis of acute pancreatitis. Arch Intern Med 1957;99:607–21.
13. Hurley PR, Cook A, Jehanli A, Austen BM, Hermon Taylor J. Development of radioimmunoassays for free tetra-L-aspartyl-L-lysine trypsinogen activation peptides (TAP). J Immunol Methods 1988;111:195–203.
14. Gudgeon AM, Heath DI, Hurley P, Jehanli A, Patel G, Wilson C, et al. Trypsinogen activation peptides assay in the early prediction of severity of acute pancreatitis. Lancet 1990;335:4–8.
15. Kivisaari L, Somer K, Standertskjöld-Nordenstam CG, Schröder T, Kivilaakso E, Lempinen M. A new method for the diagnosis of acute hemorrhagic-necrotizing pancreatitis using contrast-enhanced CT. Gastrointest Radiol 1984;9:27–30.
16. 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.
17. Zweig MH, Campbell G. Receiver-operating characteristic (ROC) plots: a fundamental evaluation tool in clinical medicine [Review]. Clin Chem 1993;39:561–77.
18. Feldmann U, Schneider B, Klinkers H. A multivariate approach for the biometric comparison of analytical methods in clinical chemistry. J Clin Chem Clin Biochem 1981;19:121–37.
19. Albo R, Silen W, Goldman L. A critical analysis of acute pancreatitis. Arch Surg 1963;86:1032–5.
20. Mogensen CE, Solling K. Studies on renal tubular protein reabsorption: partial and near complete inhibition by certain amino acids. Scand J Clin Lab Invest 1977;37:477–86.
21. Johnson SG, Ellis CJ, Levitt MD. Mechanism of increased renal clearance of amylase/creatinine in acute pancreatitis. N Engl J Med 1976;295:1214–7.
22. Elias E, Redshaw M, Wood T. Diagnostic importance of changes in circulating concentrations of immunoreactive trypsin. Lancet 1977;ii:66–8.
23. Brodrick JW, Geokas MC, Largman C, Fassett M, Johnson JH. Molecular forms of immunoreactive pancreatic cationic trypsin in pancreatitis patient sera. Am J Physiol 1979;237:474–80.
24. Largman C, Brodrick JW, Geokas MC, Johnson JH. Demonstration of human pancreatic anionic trypsinogen in normal serum by radioimmunoassay. Biochim Biophys Acta 1978;543:450–4.
25. Corfield AP, Cooper MJC, Williamson RCN. Acute pancreatitis: a lethal disease of increasing incidence. Gut 1985;26:724–9.
26. Thomson HJ, Brydon WG, Obekpa PO, Smith AN. Screening for acute pancreatitis. J R Coll Surg Edinb 1987;32:348–51.
27. Gunn IR, Faye S, Clayton MGG. Prospective evaluation of urinary amylase test strip [Letter]. Lancet 1986;ii:1161.