

# Pancreas

## Early prediction of severe acute pancreatitis by urinary trypsinogen activation peptide

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**Objective:** To investigate the value of urinary trypsinogen activation peptide (TAP) in the early prediction of severe acute pancreatitis and to compare it with acute physiology and chronic health evaluation II (APACHE II).

**Methods:** We assessed the predictive value of urinary TAP concentrations measured by a competitive enzyme-linked immunosorbent assay. Urine samples were collected for detecting TAP concentrations at admission, and 24, 48, and 72 h from 41 patients with acute pancreatitis (12 with severe disease, 29 with mild disease) who presented within 48 h the onset of symptoms and from 11 control patients, while APACHE II scores were recorded at 48 h after admission.

**Results:** The peak median urinary TAP concentration was seen at admission. The median urinary TAP concentration at admission for severe pancreatitis (95 nmol/L) was significantly higher than the median for patients with mild pancreatitis (20 nmol/L,  $P < 0.005$ ) and controls (15 nmol/L,  $P < 0.005$ ). TAP concentrations were significantly higher in patients with severe acute pancreatitis than the median in patients with mild pancreatitis ( $P < 0.05$ ) and controls ( $P < 0.05$ ) on days 2 to 3. The median APACHE II scores of severe patients were significantly different from those of mild patients (10.5 vs 6.0,  $P < 0.01$ ). The sensitivity, specificity, positive predictive, and negative predictive values of an admission urinary TAP  $\geq 35$  nmol/L for severe pancreatitis were 91.7%, 89.7%, 78.6% and 96.3%, whereas 48 h after admission the values for APACHE II scores ( $\geq 9$ ) were 75.0%, 72.7%, 52.9% and 87.5%. In prediction of disease severity, the urine TAP concentration was much better than APACHE II at 48 h.

**Conclusions:** Urinary TAP obtained at the first 48 h of the onset of symptoms can predict severe acute

pancreatitis. In prediction of disease severity, the urinary TAP is much better than APACHE II score.

(*HBPD Int* 2002; 1: 285–289)

**Key words:** acute pancreatitis; trypsinogen activation peptide; APACHE II

### Introduction

About 20% of patients with acute pancreatitis run a severe clinical course and they must be identified as quickly as possible.<sup>[1]</sup> Pancreatic interstitial and circulating trypsinogen activation is essential to the development of acute necrosis and multiple organ dysfunction syndrome. Measurement of trypsinogen activation peptide (TAP) is of predictive value since it is a single marker specifically related to the onset and development of severe acute pancreatitis.<sup>[2–4]</sup> We investigated prospectively the predictive value of TAP assay compared with acute physiology and chronic health evaluation II (APACHE II) scoring system.

### Methods

#### Definitions

Acute pancreatitis was defined as the presence of amylase and/or lipase > three times the upper lim-

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it of the normal in association with a compatible clinical picture of the disease and typical findings on computed tomography or macroscopic changes in the gland seen at laparotomy.<sup>[5]</sup> Severe acute pancreatitis was diagnosed when acute pancreatitis was associated with organ failure and/or local complications such as necrosis, abscess or pseudocyst.

### Patients

Forty-one patients with acute pancreatitis who presented within the first 48 h the onset of symptoms and admitted to one of four hospitals in the same area were enrolled into the study. Eleven patients with acute abdominal pain of other causes served as controls. Disease severity was graded by the Atlanta criteria.<sup>[6]</sup> In the 41 patients, the disease was severe in 12 patients and mild in 29. Each patient was subjected to ultrasound scanning at admission and CT scanning after admission.

### Samples

Urinary samples were collected for measuring TAP concentration at admission, 24, 48, and 72 h after admission in containers with ethylene diamine tetraacetic acid (EDTA) at a final concentration of 10 mmol. If not for immediate analysis, the samples were frozen immediately at  $-20^{\circ}\text{C}$ . They were avoided for repeated freeze-thawing to prevent possible loss of TAP immunoreactivity.

### APACHE II determination

Blood was taken daily for analysis of amylase, electrolytes, liver function, and gas. Physiological data (temperature, heart rate, mean blood pressure, and respiratory rate) were recorded every 6 hours. The abnormal variables in a 24-h period were used for calculation of APACHE II scores.<sup>[7]</sup>

### Assay

Serum amylase was subjected to an enzymatic colorimetric test with EPS-G7 as a substrate (CL-7200 Clinical Chemistry Analyzer).

Urinary TAP concentrations were determined

by enzyme immunoassay (TAP Kit, Biotrin International Ltd., Dublin, Ireland). The solid-phase ELISA used was based on the competition between free and immobilized peptide binding to antibody to TAP. The procedure was performed according to the manufacturer's instructions. In brief, TAP linked to a carrier protein is immobilized on the solid phase, to which the peptide calibrator or diluted (1:4) urine sample plus the TAP antibody is added. After the competition reaction, anti-rabbit Ig-horseradish peroxidase conjugate is added, followed by TMB substrate. Absorption was measured with an ELISA plate reader at 450 nm using 630 nm as reference. The assay range of Biotrin TAP EIA was 0.56–800 nm.

### Statistical analysis

Parametric data were compared by Student's *t* test. Nonparametric data were analyzed using medians, Kruskal-Wallis test. The number of patients with complications in relation to the cut-off values for the various tests was compared by  $\chi^2$  tests. *P* values  $<0.05$  were considered significant.

### Results

#### Serum amylase

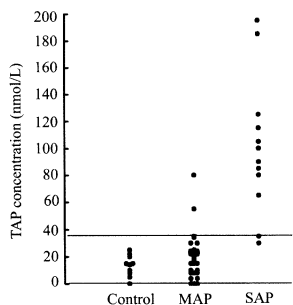
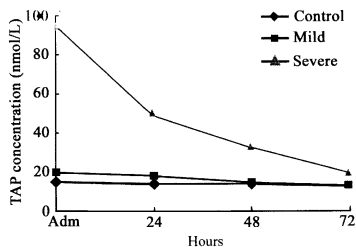
The mean serum amylase for the severe group was 2516 (standard deviation  $\pm 1451$ ) U/L, and for the mild group 2037 ( $\pm 1259$ ) U/L; the difference between the two groups was not significant ( $P > 0.05$ ).

#### APACHE II

The median APACHE II scores at 48 h after admission in patients with severe acute pancreatitis were significantly different from those in patients with mild acute pancreatitis (10.5 vs 6.0,  $P < 0.01$ ). For prediction of severe pancreatitis 48 h after admission, a score of 9 had a sensitivity of 75% and a specificity of 72.7% while correctly categorizing 73.2% of the patients. 52.9% of the patients with a score  $\geq 9$  had at least one complication, whereas 87.5% of those with a score  $< 9$  experienced an uncomplicated recovery.

**Table.** Comparison of APACHE II score and TAP for prediction of severity (%)

	Cut-off value	Sensitivity	Specificity	Correctness	Positive predictive value	Negative predictive value
APACHE II score	9.0	75.0	72.7	73.2	52.9	87.5
TAP	25.0	100.0	75.9	83.9	63.2	100.0
Concentration (nmol/L)	35.0	91.7	89.7	90.2	78.6	96.3

**Fig. 1.** TAP for each patient (MAP: mild acute pancreatitis; SAP: severe acute pancreatitis).**Fig. 2.** Median TAP concentrations on first 3 days after admission.

### Urinary TAP

The median urinary TAP at admission for patients with severe pancreatitis (95 nmol/L) was significantly higher than the median for both patients with mild pancreatitis (20 nmol/L;  $P < 0.005$ ) and controls (15 nmol/L;  $P < 0.005$ ). A cut-off value of 35 nmol/L was found to show

an optimum sensitivity and specificity for the TAP test in predicting severe disease (Fig. 1). The assay on admission predicted disease severity with a sensitivity of 91.7% and a specificity of 89.7%. The outcome, mild or severe disease, was correctly predicted in 90.2% of patients (Table). 78.6% of those patients with a TAP concentration of  $\geq 35$  nmol/L had one or more serious complications, whereas 96.3% of those with a value of  $< 35$  nmol/L experienced an uncomplicated recovery. The variation of urinary TAP was noted in a 3-day period of sampling (Fig. 2). The peak median urinary TAP was seen at admission. In the following days, the concentration of TAP decreased gradually. The concentrations of TAP were significantly higher in patients with SAP than the median in both patients with mild pancreatitis ( $P < 0.05$ ) and controls ( $P < 0.05$ ) on days 2 to 3. The median urinary TAP was not significantly different in the three groups at 72 h after admission ( $P > 0.05$ ).

### Discussion

It is very important to establish the severity of acute pancreatitis on admission since patients at risk of serious or life-threatening complications may benefit from intensive monitoring and specific therapy. Researchers have attempted to differentiate between mild and severe forms of acute pancreatitis by using so-called indicators in blood or urine. Examples of these parameters are C-reactive protein, PMN elastase, phospholipase A2, antiproteases, hyaluronic acid,  $\text{NH}_2$ -terminal propeptide of type III procollagen (p III p), and cytokines.<sup>[8-10]</sup> C-reaction protein is helpful in

clinical practice, but its use is hampered by the fact that the differentiation between mild and severe disease is best on three to four days after the onset of the disease.

In acute pancreatitis, large amounts of trypsinogen are present in the pancreatic interstitium and drained via the portal and lymphatic circulation. Pancreatic interstitial and circulating trypsinogen induces acute hemorrhagic necrosis in a setting of mild edematous pancreatitis. Active trypsin induces macrophages to produce TNF- $\alpha$  and IL-1 $\beta$ , which correlate with the local and systemic complications of AP. Some studies demonstrate that the occurrence and severity of pancreatitis-associated lung injury correspond to the levels of circulating trypsinogen and its activation to trypsin.<sup>[11-13]</sup> TAP is the amino-terminus peptide released from the activation of trypsinogen. The development of immunoassays to TAP has provided investigators with a more stable and accurate method for measuring trypsinogen activation.<sup>[14]</sup> Antibody developed against TAP is highly specific and does not recognize antigen binding sites on parent trypsinogen or trypsin molecules. In addition, TAP does not bind to protease inhibitors, e. g., alpha-1 protease inhibitor and alpha-2 macroglobulin. This makes TAP an attractive method for measuring trypsinogen activation. Serum, urinary, and peritoneal TAP concentrations have been shown to correlate directly with the severity of experimental acute pancreatitis.<sup>[15-17]</sup> Wang et al<sup>[18]</sup> identified that plasma TAP increased immediately after the induction of pancreatitis, and the excretion of TAP into urine was delayed several hours in rats with pancreatitis. Urine TAP concentration has a good correlation with the TAP output and TAP/creatinine ratio in urine and can reflect sufficiently the amount of TAP at the initial stage of acute pancreatitis.

Our study shows that in early prediction of severe acute pancreatitis, the urine TAP concentration at admission is better than APACHE II score at 48 h. The TAP concentration in urine at admission can reflect trypsinogen activation in a few hours before admission. We compared TAP measurement with APACHE II system only from admission, since data are not available to assess

these from symptom onset. The median urinary TAP at admission was significantly higher for patients with severe acute pancreatitis than for those with mild acute pancreatitis and controls. Urinary TAP remained higher in patients with severe acute pancreatitis up to 48 h after admission. These results differ from those measured at 24 h and 48 h after admission.<sup>[2]</sup> The reason for this may be due to the different percentage of patients with severe acute pancreatitis. However, in both studies peak urinary TAP concentration occurred at admission, showing a striking difference between mild and severe disease. In our study, 7 of 29 patients with mild acute pancreatitis and all patients with severe acute pancreatitis showed urinary TAP concentration  $\geq 25$  nmol/L at admission. Given a cutoff of 35 nmol/L for urinary TAP, the sensitivity and specificity was 91.7% and 89.7%, respectively. In accordance with previous studies,<sup>[2,19]</sup> urinary TAP obtained within the first 48 h of the onset of symptoms provided a good discrimination between mild acute pancreatitis and severe acute pancreatitis. Urinary TAP has a high negative predictive value on admission for patients with severe acute pancreatitis. This finding agrees with Tenner's study. In recent years, APACHE II score system has been used widely in early prediction of outcome in acute pancreatitis. In this study, APACHE II score of  $\geq 9$  at 48 h after admission provided a sensitivity of 75% and a specificity of 72.7%. In prediction of disease severity, urinary TAP is much better than APACHE II score. In addition, TAP has several advantages over APACHE II score. First, TAP is specific to acute pancreatitis. Second, its simplicity and applicability make it an extremely valuable severity marker. The present study provides strong evidence that urinary TAP is an accurate early predictor of severity in acute pancreatitis.

## Competing interest

No benefits in any form have been received or will be received from a commercial party related directly or indirectly to the subject of this article.

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*Received November 19, 2001*

*Accepted after revision March 16, 2002*