

ARTICLE

Low Serum Pancreatitis-Associated Protein Does not Exclude Complications in Mild Acute Pancreatitis

Janja POLANEC¹, Zlatko P PAVELIC², Igor KRIZMAN³, Joe OSREDKAR¹

¹Institute for Clinical Chemistry and Biochemistry, ²Gastroenterology Clinic, Ljubljana, Slovenia; ³Department of Otolaryngology-Head and Neck Surgery, University of Cincinnati, Cincinnati, USA

Normal serum PAP levels on admission to the hospital in patients with acute pancreatitis has been proposed to help select the patients who are not going to develop complications. The aims of this study were, first, to assess the specificity of serum pancreatitis-associated protein (PAP) serology test and second, to evaluate the usefulness of the test for predicting complications in acute pancreatitis on admission to the hospital. The sensitivity of the PAP ELISA in patients with acute pancreatitis on admission to the hospital was 70% and the serum PAP levels significantly higher than in healthy controls ($p < 0.0001$). However, the serum PAP levels in

patients with acute pancreatitis were not significantly different from values in patients with various abdominal diseases ($p < 0.58$). Serum PAP levels gave good correlation to APACHE II ($p = 0.02$) and CRP ($p = 0.01$). Two patients with local complications (necrotizing pancreatitis, pancreatic fluid collection) had elevated serum PAP levels on admission to the hospital (> 100 ng/ml). The diagnostic specificity of PAP ELISA is low. Patients, who develop local complications in acute pancreatitis can not be excluded by normal serum PAP levels on admission to the hospital. (Pathology Oncology Research Vol 3, No 1, 30–33, 1997)

Key words: pancreatitis-associated protein, ELISA, acute pancreatitis

Introduction

Acute pancreatitis is disease of variable severity, ranging from mild edema to severe fulminant necrosis.¹ Several multifactorial prognostic criteria for evaluation of the initial severity of disease have been proposed.²⁴ Measurements of single biochemical factor like interleukin-6⁵ or C-reactive protein⁶ was also reported. Recently, monitoring pancreatitis-associated antigen (PAP) in patients' serum has been used as an indicator of the course of acute pancreatitis.⁷

PAP belongs to a calcium-dependent group of lectins with the molecular weight of 17500 Da and isoelectric point of 7.2.⁸ It is an exocrine protein, which has no enzymatic activity and does not inhibit any enzyme in pancreatic juice but has the affinity to bacterial surfaces and acts as an endogenous antibiotic factor that prevents the bacterial

infection of the inflamed pancreas.^{8,9} The expression of PAP is absent in healthy pancreas, however, appears in patient's sera a few hours after the onset of the disease, lasts for several days and shows a strong correlation to the severity of the disease.^{7,10} It was reported that evaluation of serum PAP levels could predict the severity of the disease at the admission to the hospital.⁷

In the present study we assess the fullness clinical use of PAP assay in patients with mild acute pancreatitis.

Materials and Methods

Patients

Thirty patients with acute pancreatitis were included in this study. The diagnosis was based on the presence of a suggestive clinical picture, elevated serum amylase and lipase and consistent morphological findings confirmed by ultrasonography. The median age of the patients was 47 years (range 19–87 years) with a male predominance (23 men and 7 women). The etiology of acute pancreatitis was alcoholism in 13 patients, gallstones in 14 patients,

Received: Nov 25, 1996; accepted: April 25, 1997

Correspondence: Igor KRIZMAN, M.D., Ph.D., Gastroenterology Clinic, Japljeva 2, Ljubljana 1000, Slovenia. Telephone: (061) 1313113 ext. 2639; Fax: (061) 1334190.

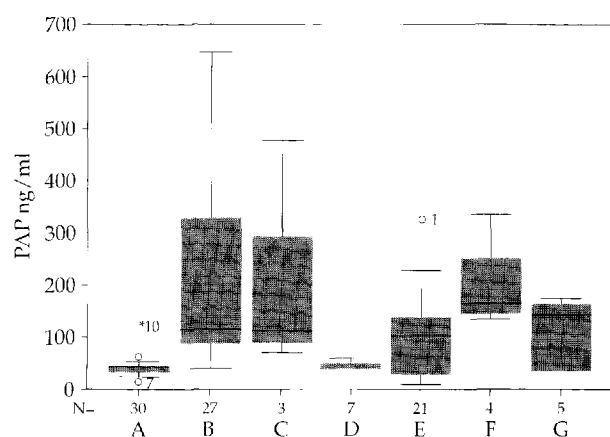


Figure 1. Quantification of serum PAP levels: A: healthy subjects; B: patients with mild acute pancreatitis without complications; C: patients with fluid collections or necrotizing pancreatitis; D: patients with chronic pancreatitis; E: patients with Crohn's disease, ulcerative colitis, pancreatic cancer, abdominal sepsis, perforated ulcer duodeni, non-tropical sprue, pseudomembranous enterocolitis; F: patients with alcoholic liver cirrhosis; G: patients with cancer of bile duct. ⁰, * Extreme serum PAP values out of the range of the group are indicated separately.

hypertriglyceridemia, hypercalcemia and unknown etiology in 1-1 patient, respectively. Seven patients with relapsing chronic pancreatitis were also included in the study. The diagnosis was confirmed by proved exocrine insufficiency and changes in the pancreas (fibrosis and calcifications) by ultrasonography. Thirty three patients with various abdominal diseases were also included in this study: Crohn's disease (5 cases), ulcerative colitis (4), alcoholic liver cirrhosis (5), cholangiocarcinoma (1), cancer of the main bile duct (2), pancreatic cancer (2), diverticulosis of the colon (1), abdominal sepsis (1), perforated duodenal ulcer (1), pseudomembranous enterocolitis (1), non-tropical sprue (9) and cholelithiasis (1). A group of 30 healthy blood donors, median age 29,5 years (range 22-57 years) were used as a control.

ELISA

Serum PAP was measured by enzyme-linked immunosorbent assay according to the instructions (Dynabio, La Gaude, France). Briefly, serum samples, diluted 1:100 were deposited into the microtitration plates and coated with monoclonal anti-PAP antibodies. Bounded PAP was recognized by polyclonal anti-PAP antibody coupled to biotin, detected by avidin-peroxidase complex and visualized by the addition of a chromogenic substrate. The concentration of PAP in each serum was determined by extrapolation from the standard curve. The proposed upper limit for normal values was 100 ng/ml, according to the instructions.

Laboratory analysis

Measurement of serum *alfa amylase* was done on HITACHI 717 random access discrete analyser (Boehringer, Mannheim), using an enzymatic colorimetric assay (alfa amylase PNP, Boehringer; normal value is <2.5 ukat/l). *Lipase* was measured in the same way (Boehringer; normal value is <3.9 ukat/l). C-reactive protein (CRP) concentrations in serum were determined by Boehringer Turbidimeter (TurbiQuant CRP, Boehringer Werke AG, Warburg, Germany, normal value is <5 mg/ml). Blood samples were taken on admission and on days 2, 4 and 10 of the hospitalization. Specimens were stored at -20°C.

Grading of disease severity

Ranson's prognostic signs (range 0-11) were determined at the time of diagnosis and after 48 h.⁴ Also, APACHE II prognostic criteria (range 0-71) were determined on days 1, 2 and 3 of the hospitalization.³ Nine patients were graded with 3 or 4 points on Ranson's scale (moderate severity) and 21 patients got less than 3 points (mild disease). Two patients developed pancreatic fluid collections and one patient had necrotizing pancreatitis. None of the patients died.

Statistical methods

Descriptive analysis for all variables was performed. Data are given in medians with quartile ranges, where indicated. Mann-Whitney U test was used to determine the statistical differences between the study groups ($p < 0.05$). Spearman rank correlation test was used to evaluate the correlation between the clinical severity criteria and biological markers.

Results

On admission to the hospital, 9 patients (30%) with acute pancreatitis showed normal serum PAP levels (less than 100 ng/ml). The median was 63 ng/ml (range 37-80 ng/ml). Peak serum PAP levels in these patients were reached around the fourth day with median value of 415 ng/ml (range 400-1500 ng/ml). Twenty one patients (70%) had elevated serum PAP levels on admission with the median value of 200 ng/ml (range 100-650 ng/ml) and the peak values reached also around the fourth day of hospitalization (median value of 550 ng/ml; range 50-2300 ng/ml).

In the majority of the patients (22) an increase in serum PAP was observed during the first 5 days of the hospitalization, and then the value started to decrease. In 3 patients the peak PAP levels appeared at the admission and in 3

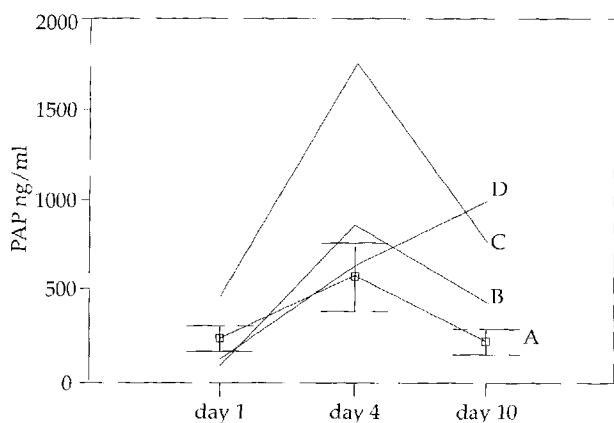


Figure 2. Profiles of serum PAP levels in patients with acute pancreatitis during the hospitalization. A: patients with mild acute pancreatitis and no complications ($n=27$); B, C: patients with pancreatic fluid collections ($n=2$); D: patient with necrotizing acute pancreatitis.

patients' serum PAP levels remained low (<100 ng/ml) throughout the illness, although serum amylase and lipase were elevated at the same time.

Increased serum PAP values were found in 1 of 5 patients with Crohn's disease (130 ng/ml), 3 of 5 patients with ulcerative colitis (median 200 ng/ml, range 140-330 ng/ml), 1/2 patients with the cancer of the main bile duct (170 ng/ml), 2/2 patients with pancreatic cancer (150 and 170 ng/ml), 3/5 patients with alcoholic liver cirrhosis (median 140 ng/ml, range 140-180 ng/ml), 1/1 patient with abdominal sepsis (800 ng/ml), 1/1 patient with perforated duodenal ulcer (140 ng/ml), 1/1 patient with pseudomembranous enterocolitis (120 ng/ml) and 6/9 patients with non-tropical sprue (median 140 ng/ml, range 110-230 ng/ml). Serum PAP levels were under 100 ng/ml in 1/1 patient with colonic diverticulosis, 1/1 patient with cholangiocarcinoma and 1/1 patient with cholelithiasis. The median serum PAP levels in 7 patients with chronic pancreatitis was 40 ng/ml (range 38-62 ng/ml) and 40 ng/ml (range: 30-60 ng/ml) in 30 healthy controls. Serum PAP levels in patients with acute pancreatitis and different abdominal diseases were significantly higher than in the healthy controls ($p<0.0001$ and $p<0.009$, respectively). However, serum PAP levels in patients with different abdominal disease did not differ from the patients with acute pancreatitis on admission to the hospital ($p=0.58$, Fig. 1).

Two patients developed pancreatic fluid collections, which resolved spontaneously and one patient had necrotizing pancreatitis and was admitted to the hospital with still normal PAP level (100 ng/ml) which rose to 550 ng/ml on the fourth day of the hospitalization but was still in the range of patients with mild disease (100-650 ng/ml). However, PAP levels continued to rise to 1000 ng/ml on the tenth day (Fig. 2). The first patient with pancreatic fluid

collection was admitted with normal PAP level (70 ng/ml), which rose to 800 ng/ml on the fourth day which is slightly higher than the values for mild acute pancreatitis. The second patient with fluid collection, however, showed elevated values on admission (480 ng/ml) and high peak values (1700 ng/ml) on day 4 (Fig. 2).

Comparison of Ranson's scores and serum PAP levels gave no correlation. When serum PAP levels were compared to APACHE II, the correlation coefficient was 0.46 ($p=0.02$) and 0.43 ($p=0.03$) for day 1 and 3 of the hospitalization. The comparison of APACHE II and Serum CRP gave correlation coefficient 0.56 ($p=0.01$) for the first day of hospitalization. Correlation between lipase and alpha amylase values subsequently decreased from $r=0.44$ ($p<0.003$) on admission to less significant on day 3, $r=0.44$ ($p<0.03$), to nonsignificant on day 10, $r=0.39$ ($p=0.06$).

Discussion

Selection of patients with acute pancreatitis, who might develop complications, is important for choosing the right management. Monitoring serum PAP levels on admission to the hospital was reported to help identify the patients who will not develop complications and those who are severely ill.⁷ In this study we assess the usefulness of monitoring serum PAP levels on admission to the hospital to exclude development of complications in the course of mild acute pancreatitis. The serum PAP levels were elevated in 70% of the patients with acute pancreatitis, which is in agreement to the previous report⁷ and they were not significantly different from values in patients with different abdominal diseases ($p=0.58$). Therefore the diagnostic specificity of this test in patients with acute pancreatitis on the admission to the hospital is questionable.

Three patients in our study developed local pancreatic complications. In one patient with pancreatic fluid collection serum PAP levels on admission to the hospital did not differ from the values of the healthy group. The values on the fourth day were slightly above the upper range value for the patients with mild disease and the fluid collection was detected by routine sonography. This is contrary to the report by Iovanna et al⁷ in which patients with normal serum PAP levels on admission to the hospital never developed complications. In the second patient with pancreatic fluid collection, the ultrasonography showed a lesion that could develop into a cyst. The high serum PAP level (450 ng/ml) supported the finding. Also, confirmation of pancreatic fluid collection on day 4 by sonography was accompanied with high serum PAP levels (1700 ng/ml). This is in agreement with the previous report,¹⁰ where PAP levels on admission to the hospital were higher than 200 ng/ml and presented a higher possibility of complication. However, twelve of our patients had serum PAP levels higher than 200 ng/ml (220-650 ng/ml) and

developed no complications. In patients with localized complication, peak values, rather than serum PAP levels on admission to the hospital showed the real clinical status of the patient.

In a patient with necrotizing pancreatitis the necrosis was detected by CT. Serum PAP levels on admission and on the day 4 of hospitalization were not different from the values in patients with mild acute pancreatitis, who had no complications. However, serum PAP levels in the patient continued to rise for 10 days, which supports the previous reports that elevated serum PAP indicates a still active process in the pancreas.⁷ It is also known from the previous study, that serum PAP levels in necrotizing acute pancreatitis are not characteristic and that the peak values overlap with the values in oedemic acute pancreatitis. Since the peak value (1000 ng/ml) in this patient occurred on day 10 of hospitalization the ultrasound and CT were necessary for the confirmation of the diagnosis and monitoring of the disease.

In conclusion, this study shows that serum PAP levels can not be used for establishing the diagnosis of acute pancreatitis, because 30% of the patients do not have elevated serum PAP levels on admission to the hospital. Also, elevated serum PAP levels appear in numerous abdominal diseases and in patients with serum PAP levels under 200 ng/ml on admission, a coexisting pathological process in abdomen must be excluded. We also found that normal serum PAP levels on admission do not exclude possible local pancreatic complications in mild acute pancreatitis. Monitoring of disease progression in mild acute pancreatitis by ultrasound or CT is still unavoidable can not be replaced with measurements of serum PAP levels.

References

1. Agarwal N and Pitchumani CS: Assessment of severity in acute pancreatitis. *Am J Gastroenterol* 86:1385-1391, 1991.
2. Bank S, Wise L and Gersten M. Risk factors in acute pancreatitis. *Am J Gastroenterol* 78:637-640, 1983.
3. Knaus WA, Draper EA, Wagner DP and Zimmerman JE: APACHE II: A severity of disease classification system. *Crit Care Med* 13:818-829, 1985.
4. Ranson JHC, Rifkind KM, Roses DF et al: Prognostic signs and role of operative management in acute pancreatitis. *Surg Gynecol Obstet* 139:69-81, 1974.
5. Heath DI, Cruickshank A, Gudgeon M, et al: Role of interleukin-6 in mediating the acute phase protein response and potential as an early means of severity assessment in acute pancreatitis. *Gut* 34:41-45, 1993.
6. Puolakkainen P, Valtonen V, Paananen A and Schroder T: C-reactive protein (CRP) and serum phospholipase A2 in the assessment of the severity of the acute pancreatitis. *Gut* 28:764-771, 1987.
7. Iovanna JL, Keim V, Nordback I, et al: Serum concentrations of pancreatitis-associated protein (PAP) correlate with the severity of the acute pancreatitis. *Gastroenterology* 105:309-311, 1993.
8. Keim V, Iovanna JL, Rohr G, et al: Purification and partial characterization of a rat pancreatic secretory protein associated with acute pancreatitis. *Gastroenterology* 100:775-781, 1991.
9. Keim V, Iovanna JL and Dagorn JC: The acute phase reaction of the exocrine pancreas. *Digestion* 55:65-72, 1994.
10. Iovanna JL, Keim V, Nordback I, et al: Serum concentrations of pancreatitis-associated protein (PAP) correlate with the severity of the acute pancreatitis. *Gastroenterology* 106:728-734, 1994.