

Pancreatic Phospholipase A₂ Activity in Acute Pancreatitis: a Prognostic Marker for Early Identification of Patients at Risk

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Remarkably elevated levels of phospholipase A₂ (PLA₂) are measurable in human blood samples in cases of acute pancreatitis. The source of the enzyme was first thought to be exclusively the pancreas, but now it is generally accepted that two isoenzymes – the pancreatic PLA₂, group I, and the extrapancreatic PLA₂, group II – contribute to the raised activity. In contrast to the group II-PLA₂, the pancreatic PLA₂ is heat-resistant for 1 hour at 60 °C. The catalytically inactive proenzyme of the pancreatic PLA₂ can be activated by trypsin. The aim of our study was to evaluate the diagnostic value of PLA₂ isoenzyme activity measurements to identify patients with severe complications in acute pancreatitis. Blood samples from patients suffering from acute pancreatitis were analyzed for catalytically active pancreatic PLA₂ on day 1 and 2 of hospitalization with a modified radiometric *Escherichia coli*-based PLA₂ assay. In 10 of 41 patients clearly elevated values of catalytically active, heat-resistant pancreatic PLA₂ (7.2 to 81.2 U/l) were observed. This group of patients was characterized by severe complications (necrotizing pancreatitis, shock, sepsis, respiratory problems) of which two patients subsequently died. Patients with low or undetectable activity (<7 U/l) of pancreatic PLA₂ recovered rapidly. According to these results the presence of catalytically active pancreatic PLA₂ in serum is associated with severe complications of acute pancreatitis. In contrast to total serum-PLA₂, the catalytic concentration of pancreatic PLA₂ can serve as a prognostic marker in acute pancreatitis. Clin Chem Lab Med 2002; 40(3):293–297

Key words: Acute pancreatitis; Phospholipase A₂; Isoenzymes; C-reactive protein; Elastase.

Abbreviations: CRP, C-reactive protein; MAB, monoclonal antibody; Pa PLA₂, catalytically active pancreatic phospholipase A₂; PLA₂, phospholipase A₂; PMN, polymorphonuclear; Pro PLA₂, prophospholipase A₂.

Introduction

The value of serum-phospholipase A₂ (PLA₂) activity in acute pancreatitis as prognostic and diagnostic marker is controversial. Until the late 80's PLA₂ levels in acute pancreatitis were believed to be exclusively of pancreatic origin. The group I-PLA₂ concentration determined with an immunoassay correlated closely with lipase and amylase (1). Soon it became evident that the course of total serum PLA₂ activity did not correlate with the kinetics of other pancreatic enzymes but followed those of inflammatory indicators like C-reactive protein (CRP). It was of interest that the serum PLA₂ remained high after total pancreatic necrosis, and even after total pancreatectomy (2). From these observations it was concluded that two immunologically different isoenzymes are responsible for elevated serum PLA₂ levels in cases of acute pancreatitis.

The heat-resistant pancreatic PLA₂ (Pa PLA₂) group I which probably occurs only in pancreatic diseases (3). Pa PLA₂ is secreted as a zymogen which means that a cleavage from an inactive form is necessary to achieve enzymatic activity. This zymogen for Pa PLA₂ is called prophospholipase A₂ (Pro PLA₂). It is not inducible by cytokines.

A group II-PLA₂ which is released from blood cells and probably from the liver, especially during inflammation (4). This enzyme is inducible by proinflammatory cytokines (5). It is not secreted as a zymogen.

Therefore in studies on the measurements of PLA₂ activity in acute pancreatitis a differentiation between the different PLA₂ forms is necessary (6).

Until now it has not been possible to determine specifically the activity of the pancreatic PLA₂ (group I). Since it plays a very important role in the pathogenesis of acute pancreatitis, its measurement would provide helpful information on diagnosis and, above all, prognosis of the disease. We have developed a sensitive assay to measure the fraction of catalytically active pancreatic PLA₂ (Pa PLA₂) out of the total serum PLA₂. The present study is, to our knowledge, the first one comparing total PLA₂, Pa PLA₂ and Pro PLA₂ activity with the acute phase reactants CRP and polymorphonuclear elastase (PMN-elastase).

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Patients and Methods

This study included 41 patients with acute pancreatitis who were admitted to the Mannheim Department of Surgery at the University of Heidelberg. Twenty six patients were male and 15 were female. The mean age was 57 years. Twenty one patients suffered from biliary pancreatitis and 14 from alcoholic acute pancreatitis. In three cases pancreatic inflammation followed endoscopic retrograde cholangiopancreatography (ERCP) and in one case it was caused by excessive hyperlipidemia (triglycerides >11.4 mmol/l). In two patients the cause of pancreatitis remained obscure. On average, patients with mild disease had 0.3 complications (Table 1), patients with severe pancreatitis had 3.4 complications and patients who died had on average 3.5 complications. All patients with mild disease had edematous pancreatitis, whereas severe and lethal outcomes were predominantly necrotizing pancreatitis. Almost all patients of this group had to undergo laparotomy. This procedure which involves early removal of necrotic pancreatic tissue with subsequent drainage of the peripancreatic space was preferred in our department.

The diagnosis of acute pancreatitis was confirmed by typical symptoms, elevated amylase level (normal values <32 U/l) and characteristic changes in ultrasonography and computerized tomography. Once the diagnosis was established, the following laboratory parameters were determined daily: amylase, PMN-elastase, CRP and serum PLA₂. The day of hospitalization was defined as day 1. The severity of the disease was assessed by the clinical outcome and the number of complications that occurred (7). Patients were classified into two groups: mild pancreatitis, *i.e.* less than or equal to one complication (n=31) and severe/lethal pancreatitis, *i.e.* greater than or equal to two complications or lethal outcome (n=10, Table 1).

α-Amylase was determined photometrically (Beckman, Munich, Germany), CRP and PMN-elastase were determined immunologically (Merck, Darmstadt, Germany).

Total serum PLA₂ as well as Pa PLA₂ and Pro PLA₂ were measured by modifications of the radioactive *E.coli*-based assay described recently in detail (5, 8, 9). Instead of TRIS/HCl as reaction buffer, an acetate buffer (100 mmol/l; pH 6.0) was used for optimal activity of the pancreatic enzyme. Catalytic concentrations of Pa PLA₂ were determined as heat-resistant PLA₂. Serum (150 µl) was heated to 60 °C for 1 hour. The Pro PLA₂ concentration was determined as catalytic activity following activation by trypsin (10). After cooling to room tem-

perature, a 50 µl aliquot was drawn, mixed with 505 units (50 µg) of trypsin from bovine pancreas (Sigma, Deisenhofen, Germany) in 2 µl of redistilled water and incubated at 37 °C for 5 minutes. Thereafter 150 µg of trypsin inhibitor from soy bean (Sigma) in 2 µl of redistilled water was added, the reaction mixture vortexed and put on ice. Finally, the activity was measured by the *E.coli*-based assay. Stopping the reaction with antitrypsin was necessary to protect the *E.coli* membranes from digestion.

To prove that the enzymes measured as Pa PLA₂ and Pro PLA₂ were of pancreatic origin we treated samples from 10 patients with an inhibitory monoclonal antibody specific for human pancreatic PLA₂ (Roche Diagnostics GmbH, Penzberg, Germany): 25 µl sample was incubated with 25 µl of the monoclonal antibody (0.2 µg/ml) for 15 minutes at 37 °C.

The Mann-Whitney U-test was used for statistical comparison of the medians. All p-values are two-tailed. Predictive values were calculated for several parameters.

Results

The inhibition of the Pa PLA₂ and Pro PLA₂ activity by a monoclonal antibody specific for human pancreatic PLA₂ is shown in Figure 1. The antibody was able to completely inhibit the activity observed before or produced by trypsin activation (p=0.002). This confirms that the pancreas is the source of Pa PLA₂ and Pro PLA₂.

In the patient group with mild disease both Pa PLA₂ and Pro PLA₂ were mostly elevated on the first day of hospitalization but remained rather low (less than 6.8 U/l). Both correlated temporally but not with respect to the amount of increase (Figure 2). The measurement of amylase does not allow conclusions about the expected increase in Pa PLA₂ or Pro PLA₂ activity. In contrast to amylase, particularly the Pa PLA₂ activity depends on the severity of the disease. After the second day Pa PLA₂ activity was low or not detectable. In the sera from patients with severe disease much higher values of Pa PLA₂ were observed (7.2 to 81.2 U/l). Here too, the maximum was usually reached on the first day of hospitalization. In this group of patients Pro PLA₂

Table 1 Complications of acute pancreatitis in the studied patients.

	Mild (n=31)	Severe (n=8)	Lethal (n=2)	All (n=41)
Pneumonia, atelectasis, pleural effusion	2	8	0	10
Respiratory insufficiency (paO ₂ <60 mmHg)	0	6	1	7
Ascites, retroperitoneal effusion	5	5	0	10
Ileus	0	0	0	0
Diabetes mellitus	0	0	0	0
Consumptive coagulopathy	0	0	0	0
Cholangitis/cholecystitis	1	0	1	2
Renal failure	0	1	1	2
Encephalopathy	0	0	0	0
Pancreatic pseudocyst	1	2	0	3
Cholestasis	0	0	1	1
Upper gastrointestinal bleeding	1	1	0	2
Sepsis	0	2	2	4
Shock	0	2	1	3

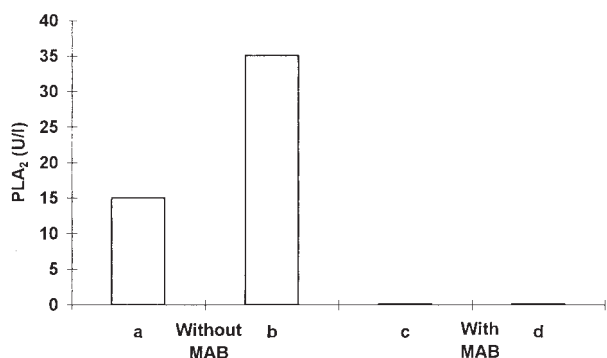


Figure 1 Inhibition of pancreatic phospholipase A₂ by a monoclonal antibody (MAB) (n=10). a: Activities measured after heating for 60 minutes (Pa PLA₂) and b: after additional incubation with trypsin for 5 minutes (Pro PLA₂). c: and d: Activities after addition of an inhibitory MAB specific for human pancreatic PLA₂. Complete inactivation was observed in all samples.

levels were somewhat higher. Further, serum PLA₂ activity reached its maximum on day 3 and did not show any correlation with other pancreatic enzymes.

We investigated five laboratory parameters to define their potential use for an individual prognosis. They were determined in samples from each patient on day 1 and 2 of hospitalization, but only the higher of the two values was used for statistical calculations. Except for Pa PLA₂, all other parameters were not reliable concerning prognosis in individual cases, although they proved to be of statistical value (Table 2). Figures 3 and 4 show an interesting comparison between total serum PLA₂ and Pa PLA₂ with regard to initial assessment of prognosis. According to the nonparametric U-test, serum PLA₂ was well able to differentiate between mild and severe/lethal pancreatitis (p=0.0061), but Figure 3 shows clearly that this has no meaning for the individual patient. For PMN-elastase the situation appears to be similar (Table 2). Its values differed significantly between the two groups (p=0.033) but did not allow reliable conclusions concerning clinical outcomes. According to this study, the CRP maxima on the first 2 days of hospitalization could not even differentiate between mild and severe pancreatitis, in contrast to PMN-elastase (p=0.476). Pro PLA₂ values can differentiate only with marginal significance (p=0.051), and sensitivity and specificity (0.60 and 0.65, respectively) suggest

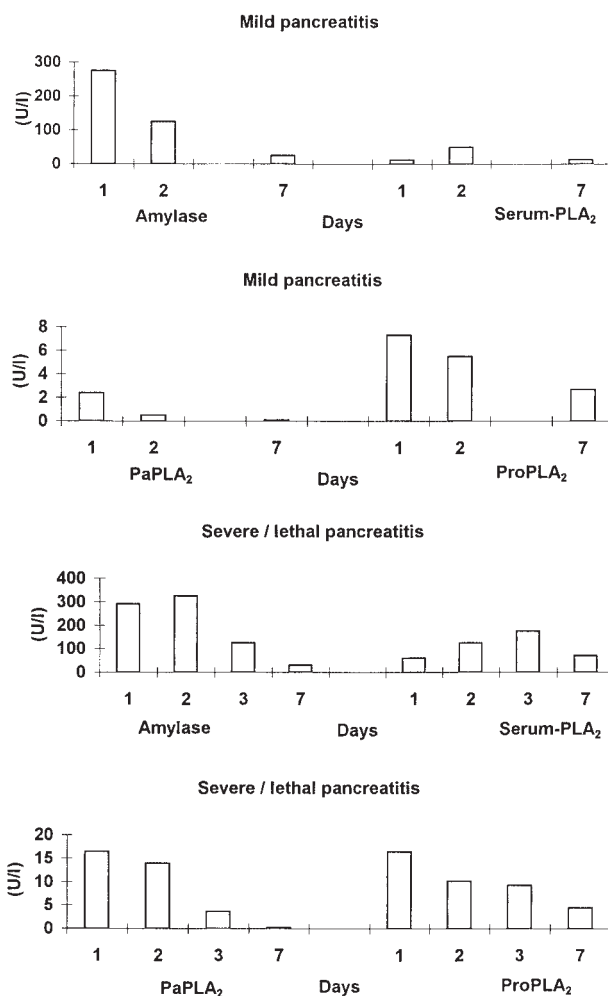


Figure 2 Time course of enzyme activities (medians) after admission to the hospital. Pa PLA₂ and Pro PLA₂ show obvious similarity to amylase. In contrast, serum PLA₂ does not correlate with pancreatic enzymes.

that this test is not suitable for clinical use. In contrast, the situation for Pa PLA₂ activity is completely different. In both groups the values did not even overlap, so that mild (edematous) and severe (necrotizing) forms of the disease could be readily distinguished (Table 2, Figure 4). The calculation of the cut-off values for the best diagnostic sensitivity and specificity for the total serum PLA₂ activity and the pancreatic PLA₂ activity was per-

Table 2 The measurement of phospholipases and inflammation statistical data.

	Serum-PLA ₂	Pa PLA ₂	Pro PLA ₂	CRP	Elastase
Cut-off	100 U/l	7 U/l	15 U/l	300 mg/l	200 µg/l
Sensitivity	60%	100%	60%	40%	60%
Specificity	83.9%	100%	65%	87.1%	74.2%
PPV ¹	54.5%	100%	37.5%	50%	42.9%
NPV ²	86.7%	100%	84%	81.8%	85.2%
p-Value ³	0.0061	<0.0001	0.051	0.476	0.033

¹positive predictive value; ²negative predictive value; ³differentiation between mild and severe disease

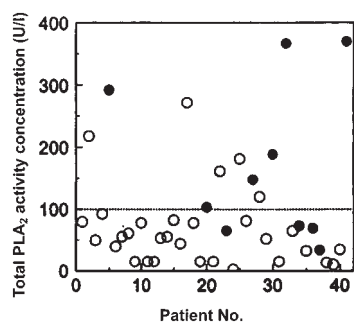


Figure 3 Maximal activity of total serum PLA₂ on days 1 or 2 of hospitalization. ● patients with severe/lethal pancreatitis (n=10); ○ patients with mild pancreatitis (n=31). The comparison between the medians and the high level of significance ($p < 0.0001$) seem to show a clear difference between mild and severe/lethal pancreatitis. However, the low sensitivity and specificity (see also Table 2) indicate that the measurement of serum PLA₂ does not allow a reliable individual prognosis.

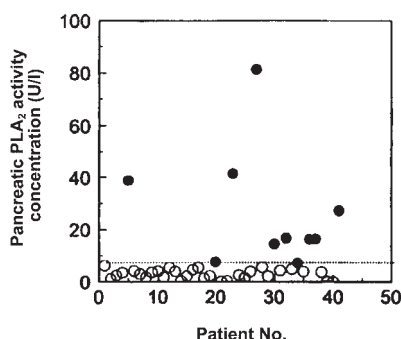


Figure 4 Maximal activity of pancreatic PLA₂ activity in serum on days 1 or 2 of hospitalization. ● patients with severe/lethal pancreatitis (n=10); ○ patients with mild pancreatitis (n=31). The plot represents same patients and in the same order as in Figure 3. The two patients showing only moderately elevated maximal activity, compared to the patients with mild pancreatitis, can be distinguished by the course of the enzyme activity over several days. Their enzyme levels were constant over 2 days, in contrast to the patients with mild disease whose Pa PLA₂ values were clearly decreased on day 2 (cut-off value=7 U/l).

formed by receiver operating characteristics (ROC) analysis and discrimination analysis.

Discussion

One of the most important questions concerning acute pancreatitis is the early differentiation between mild and severe forms of the disease. Laboratory tests such as lipase and amylase and also computerized tomography and ultrasonography serve as sufficient means to diagnose this dangerous disorder. However, the assessment of the individual prognosis by these means still remains questionable (11).

Although these results must be interpreted with caution due to the rather small number of patients, the catalytically active pancreatic PLA₂ seems to be a good in-

dicator of the pancreatic damage. Acute pancreatitis is an autodigestive disease in which digestive enzymes synthesized in the pancreatic acinar cells destroy the gland. Schmidt and Creutzfeldt came to the conclusion that pancreatic PLA₂ might be responsible for the glandular and periglandular tissue necrosis (12, 13). These findings have been confirmed by others (14, 15). It can be assumed that elevated activities of Pa PLA₂ in serum reflect necrotizing processes in the pancreas itself as well as extrapancreatic organ failures such as pulmonary or renal complications initiated by this enzyme. This is supported by the finding that Pa PLA₂ activity is detectable in peritoneal effusion and pleural fluid during acute pancreatitis (16, 17). From there, the Pa PLA₂-enriched fluids diffuse to pulmonary structures with subsequent destruction of the lungs. Furthermore, intraperitoneal Pa PLA₂ leads to enhanced translocation of bacteria through the intestinal wall into the mesenteric lymph nodes with subsequent bacteremia (18). Additionally, Pa PLA₂ causes a marked decrease in the pancreatic blood flow and damage to intra-abdominal nerve plexus followed by the inhibition of intestinal motility (19).

Interestingly, total PLA₂ serum activity kept increasing until day 3 despite decreasing Pa PLA₂ and Pro PLA₂ activities. This is due to increasing PLA₂-II activity. It is suggested that this enzyme may also be responsible for multiorgan failure in acute pancreatitis because this is the key enzyme for the eicosanoid mediator synthesis. Therefore PLA₂-II inhibitors were developed and tested (20, 21). This early increase in Pa PLA₂ followed by an increase in PLA₂-II could also be demonstrated in cerulein-induced acute pancreatitis in rats (22). Furthermore, our CRP measurements are consistent with the previous studies of other authors (23) and of our group which demonstrated that CRP is not a useful tool to distinguish between mild and severe forms of acute pancreatitis (24).

In conclusion, we demonstrated that Pa PLA₂ activity in serum is strongly elevated in severe acute pancreatitis and that it allows a discrimination between mild and severe forms of this disease in its early phase. As this enzyme may at least in part contribute to multiorgan failure, we suggest that it is worthwhile to develop and test specific inhibitors of Pa PLA₂ or proteinase inhibitors which would inhibit the cleavage of the active enzyme from Pro PLA₂.

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