Diagnostic and Prognostic Utility of Phospholipase A Activity in Patients with Acute Pancreatitis: Comparison with Amylase and Lipase

Steven C. Kazmierczak, Frederick Van Lente, and Edna D. Hodges

We compared the diagnostic and prognostic utility of phospholipase A (PLA; EC 3.1.1.4) for acute pancreatitis with that of amylase and lipase by analysis of sera from 151 consecutive patients presenting with abdominal pain in whom assays of serum amylase and (or) lipase had been ordered. We determined the diagnostic accuracy for both the initial and the peak enzyme activities. Maximal diagnostic accuracy obtained for the initial activities of amylase, lipase, and PLA was 0.83, 0.83, and 0.76 at cutoff values of 650, 650, and 41 U/L, respectively. Use of peak enzyme activities showed maximal diagnostic accuracy of 0.85, 0.86, and 0.73 at cutoff values of 650, 1050, and 42 U/L, respectively. Receiver-operator characteristic curve analysis revealed the diagnostic performance of amylase and lipase to be similar, whereas that of PLA was almost random and not incremental. As with amylase and lipase, PLA activities in sera showed no relation to patients' survival; three patients who died after an attack of acute pancreatitis failed to demonstrate the dramatic increases in PLA activity previously described. We conclude that assessing the severity of acute pancreatitis by using enzyme activities still remains problematical. Measurements of amylase or lipase activities provide similar diagnostic discrimination when appropriate cutoff values are used and remain the methods of choice for diagnosis of acute pancreatitis.

Additional Keyphrases: cutoff value • receiver-operator characteristic curves • initial vs peak enzyme activity compared

The enzyme diagnosis of pancreatic injury is usually accomplished by measurement of amylase (1,4-alpha-D-glucan glucohydrolase; EC 3.2.1.1) and (or) lipase (triacylglycerol acylhydrolase; EC 3.1.1.3) activities in serum. Release of phospholipase A (PLA; lecithinase A, phosphatid 2-acylhydro-lase; EC 3.1.1.4) by injured pancreas tissue has been suggested to play an important role in the pathogenesis of acute pancreatitis and may facilitate the diagnosis of this disease (1, 2). In contrast to amylase and lipase, activity of PLA in serum has also been advocated as a marker of the severity of pancreatitis, with greatly increased activities of PLA being associated with severe hemorrhagic forms of the disease and lesser increases being found in the mild edematous type (1, 3).

Recently, several studies have reported increased PLA activities in association with a wide variety of nonpancreatic disorders, including sepsis, malignancy, myocardial infarction, and hematological diseases (4–7). These studies raise concern regarding the clinical utility of PLA in the diagnosis of pancreatitis. To assess the diagnostic and prognostic utility of PLA, we measured amylase, lipase, and PLA activities in sera of patients for whom either amylase or lipase assays had been requested for the evaluation of abdominal pain. We attempted to evaluate PLA in an appropriate clinical context as a marker of acute pancreatitis by choosing study subjects representative of the clinical population to which the test might be applied.

We developed receiver-operator characteristic (ROC) curves to assess the effects of choosing different decision thresholds for PLA, amylase, and lipase and compared the predictive value of initial and peak enzyme activities.

Materials and Methods

Patients. We investigated 151 consecutively presenting patients who had assays of amylase or lipase requested because of abdominal pain. Patients were assigned to a control group or to an acute pancreatitis group, based on the final discharge diagnosis. The control group comprised 109 patients (59 males, 50 females), the acute pancreatitis group 42 (22 males, 20 females). The median age (and range) in years for the control group and acute pancreatitis group was 53 (11–92) and 49 (15–81), respectively.

Samples. Serum was obtained from blood samples drawn for measurement of amylase or lipase activity (as requested by the physician). Any subsequent samples were collected only if amylase or lipase was again requested by the physician. Using this protocol, we obtained 477 serum samples from 151 patients. Amylase and lipase activities were determined within 2 h of receipt of the specimen by the laboratory. An aliquot of serum from these same samples was then frozen at −70 °C for analysis of PLA activity. None of the sample aliquots was frozen for more than four weeks before PLA activity was measured. As has been previously shown, PLA activity is not influenced by freezing for up to one month, provided the thawed sera is recentrifuged and mixed thoroughly to ensure homogeneity of the specimen (8).

Procedures. Total amylase and lipase activities in serum were measured in a Cobas-Fara centrifugal analyzer (Roche Diagnostic Systems, Nutley, NJ 07110). The assay for amylase, based on a coupled enzymatic reaction sequence with maltotetraose as substrate, was performed according to the manufacturer's protocol.
(Roche Diagnostic Systems). Lipase activities in serum were measured by monitoring the rate of decrease in the turbidity of a triolein suspension (Boehringer Mannheim Diagnostics, Indianapolis, IN 46250).

We measured PLA activities according to the method of Hoffmann et al. (8, 9). The assay is based on the liberation of long-chain fatty acids from an emulsion of soybean phosphatidylcholine. The initial part of the assay requires a manual timed incubation. In brief, the patient's serum is added to two tubes containing reagent with phosphatidylcholine substrate prewarmed to 37 °C. The tubes are immediately placed in a 37 °C shaking water bath. After 5 min of incubation, stop reagent (EDTA, 6 mmol/L in 0.05 mol/L phosphate buffer, pH 5.5) is added to one of the tubes, which serves as the blank. After an additional 60-min incubation, stop reagent is added to the remaining tube. The difference in free fatty acid concentrations in the two tubes is proportional to PLA activity. The concentration of free fatty acids formed by the action of PLA on phosphatidylcholine is determined by use of an enzymatic procedure we adapted to the Cobas-Fara analyzer (Table 1 lists the centrifugal analyzer settings for this assay). One unit (1 U) of PLA activity was defined as that amount of enzyme liberating 1 μmol of free fatty acids per minute at 37 °C. Analytical imprecision is relatively high because of the low absorbance changes involved. Therefore, we assayed patients' samples in duplicate and used the mean value.

We determined the linearity and precision of our automated PLA method as described in NCCLS guidelines EP6 (10) and EP5 (11), respectively.

The upper limits of the reference interval for amylase and lipase activities in clinical use are 135 and 190 U/L, respectively. The reference interval for PLA activity in serum was determined by analysis of serum samples from 25 men and 25 women who underwent routine health examination and appeared to be in good health.

We compared means by Student's t-test, and determined reference intervals by using the REFVAL protocol (12). We compared reference value groups by the Mann–Whitney U-test. The 95% confidence limit was used to assess statistical significance.

**Results**

The semiautomated procedure for PLA exhibited a linear response with increasing enzyme activity up to 92 U/L. Samples with activity exceeding this range gave the expected values after dilution. The lower detection limit of the automated method was evaluated by analysis of replicate reagent blanks. The enzyme activity corresponding to an absorbance change that exceeded by 3SD the mean absorbance change of reagent alone was 2.18 U/L.

The precision of the amylase, lipase, and PLA procedures was evaluated by analyzing two separate pools of patients' sera with normal and increased enzyme activities. The patients' serum pools were aliquoted into individual tubes, stored frozen at −20 °C, and analyzed in duplicate aliquots, once daily for 20 consecutive days. Table 2 summarizes the results of the precision studies.

PLA activity was measured in serum from 50 apparently normal individuals. The central 95 percentile interval was 7.3–9.7 U/L by nonparametric analysis. Analysis of the data by the Mann–Whitney U-test revealed no significant gender-related differences.

We used the final discharge diagnosis to classify patients into one of two groups. Group I comprised 42 patients with clinically or surgically documented pancreatitis; Group II comprised 109 control patients without pancreatitis. Fifty-one of the patients had had tests performed on an inpatient basis; the other 100 patients had had tests performed after presentation to the Emergency Department. Ten (20%) of the inpatients and 32 (32%) of those who presented to the Emergency Department were found to have experienced an attack of acute pancreatitis. Thirty-one (74%) of the patients with pancreatitis and 57 (52%) of the control patients had their diagnoses confirmed by sonogram, computerized tomography, or laparoscopy.

Peak activities of amylase and lipase in serum of patients with acute pancreatitis were significantly correlated ($r = 0.56, P < 0.05$). Peak PLA and amylase activities correlated poorly ($r = 0.08, P > 0.10$), as did peak PLA and lipase activities ($r = 0.12, P > 0.10$).

The temporal change in PLA activity in patients with

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**Table 1. Cobas-Fara Centrifugal Analyzer Settings for Assay of PLA**

<table>
<thead>
<tr>
<th>Measurement mode</th>
<th>Reaction mode</th>
<th>Calibration mode</th>
<th>Reagent blank</th>
<th>Wavelength</th>
<th>Temperature</th>
<th>Decimal</th>
<th>Units</th>
<th>Sample vol</th>
<th>Diluent vol</th>
<th>Reagent vol</th>
<th>Incubation time</th>
<th>Start reagent vol</th>
<th>Time of first reading</th>
<th>Time interval</th>
<th>No. of readings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Absorb</td>
<td>P-10-SR1-AO</td>
<td>Factor</td>
<td>Reag/Dil</td>
<td>546 nm</td>
<td>37 °C</td>
<td>1</td>
<td>U/L</td>
<td>25 μL</td>
<td>5 μL</td>
<td>45 μL</td>
<td>600 s</td>
<td>90 μL</td>
<td>0.5 s</td>
<td>180 s</td>
<td>2</td>
</tr>
</tbody>
</table>

**Table 2. Precision Data for Amylase, Lipase, and PLA Activity in Serum**

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Mean enzyme activity, U/L</th>
<th>CV, %</th>
<th>Within-run</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amylase</td>
<td>58</td>
<td>1.8</td>
<td>2.5</td>
<td></td>
</tr>
<tr>
<td>Lipase</td>
<td>79</td>
<td>2.6</td>
<td>6.2</td>
<td></td>
</tr>
<tr>
<td>PLA</td>
<td>11.0</td>
<td>5.3</td>
<td>8.3</td>
<td></td>
</tr>
</tbody>
</table>

n = 40 each.
acute pancreatitis was similar to that of amylase and lipase. The time [mean (SD)] from onset of pain to peak PLA activity was 62 (31) h vs 50 (26) and 58 (26) h for amylase and lipase, respectively.

The mean peak activities of PLA were significantly greater (*P* <0.05) in patients with acute pancreatitis than in the control patients, but there was substantial overlap between both groups (Table 3). PLA activities were >30 U/L in 11 (26%) patients with acute pancreatitis, but >50 U/L in only three. These three patients had experienced acute abdominal pain for three or four days before seeking medical attention. The significance of greatly increased PLA activities in patients with acute pancreatitis who delay in seeking treatment is unclear. The highest PLA activity was observed in a patient who had experienced acute abdominal pain for three consecutive days after consuming a substantial quantity of ethanol. His serum PLA and lipase activities were significantly increased at the initial presentation: 109 and 1520 U/L, respectively; his amylase activity was mildly increased, 263 U/L. This patient's hospital course was unremarkable and he was discharged 10 days later.

In 13 (12%) of the control patients, PLA activities exceeded 30 U/L. Five of these patients were noted to have some form of gastrointestinal disturbance, including gastritis and perforate and imperforate gastric ulcer. The highest PLA activity seen in a control patient was 53 U/L in an individual with massive head trauma after a motor vehicle accident. The association of head trauma and extremely increased PLA activities has also been previously reported (13).

We did not find PLA activities in serum to be an indicator of patients' survival. A total of 13 (9%) patients died during the course of this study, three after an attack of acute pancreatitis. PLA activities in these three patients, measured within 48 h of death, were <15 U/L. Of the 10 nonsurvivors in the control group, one patient with a perforated gastric ulcer demonstrated PLA activities of 34 U/L before death. The remaining nine patients showed PLA activities <25 U/L in the last sample taken.

The diagnostic efficiency of each enzyme was assessed by use of ROC curves derived from initial enzyme findings obtained when the physician first ordered amylase or lipase because of suspected pancreatitis, and from peak enzyme activities retrospectively obtained from patients with two or more serial enzyme measurements (Figure 1). For 30 patients, all in the control group, enzyme activities were determined only once. Based on the initial enzyme activities, the maximal diagnostic accuracy (efficiency) achieved by adjustment of the diagnostic cutoff value was 0.83, 0.83, and 0.76 for amylase, lipase, and PLA at cutoff values of 650, 650, and 41 U/L, respectively. Use of peak enzyme activities revealed similar diagnostic accuracy. The diagnostic accuracies obtained with use of peak enzyme activities were 0.85, 0.86, and 0.73 for amylase, lipase, and PLA at cutoff values of 650, 1050, and 42 U/L, respectively. The sensitivities and specificities associated with each diagnostic cutoff value are shown in Table 4. The ROC curves clearly demonstrate that the diagnostic performance of PLA for acute pancreatitis is almost random and not incremental.

**Discussion**

The recent development of a semiautomated assay for PLA activity has stimulated investigations of the utility of PLA as a diagnostic and prognostic marker for acute pancreatitis (8, 9). Previous methods for measurement

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**Table 3. Diagnostic Groups**

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>No. patients</th>
<th>Amylase (mean, SD)</th>
<th>Lipase (mean, SD)</th>
<th>PLA (mean, SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Acute pancreatitis</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gallstone-induced</td>
<td>12</td>
<td>1661 (1186)</td>
<td>3485 (5451)</td>
<td>17.9 (9.0)</td>
</tr>
<tr>
<td>Alcohol-induced</td>
<td>10</td>
<td>894 (1077)</td>
<td>1145 (431)</td>
<td>22.5 (32.1)</td>
</tr>
<tr>
<td>Cardiac surgery</td>
<td>4</td>
<td>771 (594)</td>
<td>772 (527)</td>
<td>17.8 (9.1)</td>
</tr>
<tr>
<td>G.I. and liver disease</td>
<td>3</td>
<td>1515 (1868)</td>
<td>1063 (652)</td>
<td>20.9 (5.1)</td>
</tr>
<tr>
<td>Pancreatic pseudocyst</td>
<td>2</td>
<td>146 (52)</td>
<td>323 (34)</td>
<td>25.2 (11.2)</td>
</tr>
<tr>
<td>Unknown etiology</td>
<td>11</td>
<td>846 (718)</td>
<td>943 (408)</td>
<td>25.0 (17.1)</td>
</tr>
<tr>
<td><strong>Controls</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>G.I. disease</td>
<td>29</td>
<td>241 (197)</td>
<td>385 (404)</td>
<td>15.2 (12.5)</td>
</tr>
<tr>
<td>Liver disease</td>
<td>10</td>
<td>214 (181)</td>
<td>342 (208)</td>
<td>11.3 (6.1)</td>
</tr>
<tr>
<td>Renal disease</td>
<td>11</td>
<td>410 (411)</td>
<td>747 (594)</td>
<td>15.4 (9.1)</td>
</tr>
<tr>
<td>Multigorgan failure</td>
<td>16</td>
<td>302 (183)</td>
<td>313 (251)</td>
<td>13.6 (10.9)</td>
</tr>
<tr>
<td>Trauma</td>
<td>20</td>
<td>294 (227)</td>
<td>371 (327)</td>
<td>15.6 (13.1)</td>
</tr>
<tr>
<td>Surgery</td>
<td>5</td>
<td>257 (72)</td>
<td>388 (242)</td>
<td>13.8 (6.1)</td>
</tr>
<tr>
<td>Cardiac disease</td>
<td>9</td>
<td>325 (171)</td>
<td>553 (355)</td>
<td>21.8 (12.4)</td>
</tr>
<tr>
<td>Pancreatic cancer</td>
<td>4</td>
<td>185 (220)</td>
<td>414 (251)</td>
<td>18.7 (19.3)</td>
</tr>
<tr>
<td>Cancer</td>
<td>5</td>
<td>114 (67)</td>
<td>342 (98)</td>
<td>23.2 (7.5)</td>
</tr>
<tr>
<td>Upper reference limit</td>
<td></td>
<td>135</td>
<td>190</td>
<td>10.0</td>
</tr>
</tbody>
</table>

G.I., gastrointestinal.
of PLA activity were both labor intensive and expensive (14, 15). As a result, the number of patients included in these studies was often small. We have shown that an inexpensive, semiautomated assay for PLA can be readily adapted for routine laboratory use with the Cobas-Fara centrifugal analyzer.

We assessed the diagnostic performance of amylase, lipase, and PLA for acute pancreatitis. Many evaluations of diagnostic markers for acute pancreatitis are deficient with respect to preselection of patient populations. Our patient population consisted of individuals who had amylase or lipase ordered by a physician because of suspected pancreatitis. Thus, we evaluated amylase, lipase, and PLA in the appropriate clinical context in which these markers are used for the diagnosis of acute pancreatitis. We also evaluated the diagnostic performance of each enzyme by use of initial and peak enzyme activities. The use of peak enzyme activities selected retrospectively from serial measurements for the evaluation of the diagnostic capabilities of markers for acute pancreatitis is unrealistic; the immediate diagnosis of a life-threatening event, such as acute pancreatitis, forces the physician to rely on initial enzyme findings.

The precision, linearity, and analytical sensitivity of our assay were comparable with that of the previously reported assay (8, 9). The total precision of the assay varied with PLA activity. The imprecision in samples with PLA activities <10 U/L was relatively high. Duplicate analysis of all specimens is probably warranted.

The upper reference interval for PLA activity in sera of normal men and women (9.7 U/L) was essentially identical to that previously reported (10 U/L) for a similar semiautomated method (9).

We did not find PLA useful for diagnosing acute pancreatitis. Values were also markedly increased in a wide variety of conditions not involving the pancreas. Although PLA may contribute to the necrosis and autodigestion of the pancreas in patients with acute pancreatitis, increased PLA activity was not specific for dysfunction of any one organ system or for any clinical syndrome, and recent observations indicate that PLA may enter the blood circulation from sources other than the pancreas. Substantial concentrations of PLA have been found in leukocytes (16). Activation of these cells, with release of PLA into the circulation, may explain the increased PLA activities seen in sera of patients with nonpancreatic inflammatory diseases (17, 18).

Although others have reported an increase in mortality associated with increasing PLA activities in serum (13, 19, 20), we could not confirm such findings. Patients who died after an attack of acute pancreatitis had PLA activities well below those values seen in patients with mild, uncomplicated cases of acute pancreatitis. We conclude that assessment of the severity of acute pancreatitis by using PLA activities is not possible.

Determination of lipase activity with use of colipase supplementation has been advocated as an efficient predictor of pancreatitis (21, 22). We found no significant difference between amylase and lipase as markers for acute pancreatitis when appropriate diagnostic cutoff values are used. Use of peak enzyme activities obtained from serial enzyme measurements did not significantly improve discrimination of acute pancreatitis compared with that based on initial enzyme values. Werner et al. (23) have reported similar findings. Initial values for lipase activity showed diagnostic cutoff values different from those for peak values. However, for amylase and PLA, similar cutoff values were obtained whether initial or peak activities were used. The difference for lipase is probably the result of lipase's ability to exhibit much greater increases above the upper reference limit in patients with acute pancreatitis than do amylase and PLA (24).

We conclude that PLA activity in serum is a poor marker of pancreatitis when tested in patients with
other diseases that cause abdominal pain and mimic pancreatitis. PLA activities in serum show no correlation with patients' survival and are therefore of no help in assessing prognosis. Assays of amylase or lipase provide similar diagnostic discrimination when appropriate cutoff values are used and remain the methods of choice for the diagnosis of acute pancreatitis.

References