Diagnostic Value of Measuring Pancreatic Lipase and the P₃ Isoform of the Pancreatic Amylase Isoenzyme in Serum of Hospitalized Hyperamylasemic Patients

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We assayed amylase (AMY) isoenzymes by cellulose acetate electrophoresis and determined pancreatic lipase (LPS) activity by a turbidimetric colipase-supplemented method in 54 hospitalized hyperamylasemic patients (32 men and 22 women; mean age 61.5, SD 16, years). In AMY isoenzyme analysis, use of a value for P₃ isofrom >14 U/L as a positive test for acute pancreatitis gave a diagnostic efficiency of 92.6%, a predictive value of a positive test result of 90.5%, and a predictive value of a negative test of 100%. Four of 12 patients with other, nonpancreatic abdominal diseases had false-positive test results. LPS activity (cutoff limit, 700 U/L) was as effective as P₃ isofrom in distinguishing patients with acute pancreatitis (sensitivity, 100%) from those without acute pancreatitis (specificity, 81.3%). Thus, P₃ isofrom and LPS appear to be interchangeable markers of pathological release of pancreatic enzymes into the bloodstream during acute pancreatitis. This finding decreases the need for the expensive radiological procedures currently required to confirm this diagnosis. In particular, negative results virtually exclude acute pancreatitis.

Additional Keyphrases: acute pancreatitis • electrophoresis, cellulose acetate • turbidimetry • receiver-operating characteristic curves • emergency procedure • pancreatic disease

Several organs contribute to the α-amylase (AMY) activity in serum, and a wide variety of extrapancreatic disorders may be associated with increased total AMY activity in serum (1, 2). From a practical viewpoint, the nonspecificity of increases in total serum AMY limits its usefulness in the differential evaluation of abdominal pain (2–4). Increasing the test's specificity by increasing the cutoff value, considered advisable by some (5), obviously leads to decreases in sensitivity. Recent new laboratory approaches could probably add specificity to the assay of total AMY activity in serum (4). In particular, analyses for pancreatic lipase (LPS) and amylase isoenzymes in serum have added a new dimension to the laboratory detection and differentiation of pancreatic disease.²

Measurement of the activity of LPS, an enzyme that is anticipated to be specific for the pancreas (6), in serum has been hampered by incorrect results related to nonenzymatic methodological artifacts (7). Recently, however, a reliable turbidimetric method involving colipase supplementation has been developed that circumvents these problems (8).

Investigators generally agree (9–13) that the AMY in human serum can be separated into six distinct forms, consistent with those of pancreatic (P₁, P₂, P₃) and salivary origin (S₁, S₂, S₃). Except for P₁, which is considered a genetic isoenzymatic variant, the multiple forms (isoforms) in each family are generated by post-translational deglycosidation or deamidation of asparagine or glutamine residues of the AMY gene products (P₃ and S₃), producing more negatively charged forms (14, 15). The classical finding in a patient with acute pancreatitis is a dominant increase in the P-type isoenzyme activity in serum (14, 16–22)—a characteristic that is manifested in all the separation methods. However, some investigators claim (4, 9, 10, 13, 23, 24) that the so-called P₃ isofrom, detected electrophoretically, is increased in a high percentage of patients with acute pancreatitis, presumably because this isofrom originates from inflammatory intra-pancreatic proteolytic breakdown of pancreatic AMY (23), and thus may serve as a more specific marker for that disorder.

Results of a previous comparison of LPS assay with a electrophoretic separation of P₃ isomylase in patients with hyperamylasemia suggested that the latter technique diagnosed acute pancreatitis more reliably (23). However, only six cases in that study did not have acute pancreatitis, so the relative specificities of the two procedures could not be adequately compared. The present study was designed to further evaluate the diagnostic utility of serum LPS and P₃ isofrom in patients with hyperamylasemia.

Materials and Methods

Analytical Procedures

Total serum AMY activity was determined at 30 °C by a kinetic method in which maltotetraose is the substrate for AMY, and the NADH produced after a series of enzymatic reactions is measured (25).

We determined LPS activity in serum at 30 °C by colipase-supplemented turbidimetry according to Neumann et al. (8), using the commercially available test kit from Boehringer, Mannheim, F.R.G. (Monotest Lipase, no. 159697). The assay is based on the specific de-emulsification of a triolein emulsion by LPS in the presence of sodium deoxycholate, colipase, and calcium chloride. The decrease in turbidity is measured at 340 nm. The activity of the porcine LPS calibrator was determined with the titrimetric method, considered by many to be the candidate reference method for measuring LPS in serum (26).

Total AMY and LPS measurements were carried out with a Cobas Bio analyzer (F. Hoffmann-La Roche and Co., Ltd., Basle, Switzerland). Isomylases were separated with cellulose acetate electrophoresis as previously described (12), summarized as follows. Apply 3 μL of sample and electrophorese it in a discontinuous buffer system (4 °C, 90 min, 250 mV). The
anod buffer is 0.15 mol/L Tris–borate, pH 9.15, and the
cathodal buffer is 0.03 mol/L sodium barbital, pH 8.6.
Prepare the substrate-containing gel used to develop
the electrophoretograms by dissolving Phadebas Amylase Test
tables (Pharmacia Diagnostics, Piscataway, NJ 08854) in
Tris, 50 mmol/L, pH 7.1, and adding 20 g of agarose gel per
liter. At the completion of the electrophoresis, remove the
membrane, invert it, and place it on the Blue Starch–
agarose-gel plate, which is then incubated at 37 °C for 50
min.

This method yields results that are linearly related to
activity concentration up to 300 U/L. Samples with values
greater than this were diluted with isotonic saline and re-
 assayed. The lower limit of analysis for each isomylase was
5 U/L. Densitometric tracings of the isomylase zymograms
were obtained, and the different isomylase fractions were
quantified with a "Cliniscan" densitometer (Helena Labs.,
Beaumont, TX 77704) equipped with a 610-nm filter. The
electrophoretic mobility of the patient’s isomylase fractions
was compared with that of saliva and pancreatic juice
samples run on the same cellulose acetate membrane.

Precision data for isomylase fractionation were obtained
by using human sera with normal (35 U/L) and high (272 U/
L)AMY activities. The CV (between-day analysis, n = 10)
ranged from 2.4% to 12.7%.

Pancreatitis isomylase nomenclature followed accepted
practice (9, 13).

Previously established upper reference limits were 45 U/L
for total serum AMY and 150 U/L for serum LPS. The P3
isofrom was not detected in normal serum samples (12).

Patients

Enzyme determinations were carried out on sera from 54
consecutive hospitalized hyperamylasemic patients (32 men
and 22 women, mean age 61.5 years, SD 16). These samples
were initially analyzed in our clinical laboratory for total
AMY activity because of clinically suspected acute pancrea-
atitis. Concurrently, patients’ sera were aliquoted and
stored at −20 °C until analyzed for LPS and isomylases
(within one week after sample collection). In particular,
under these conditions, there was no significant in vitro
modification of migratory characteristics of isomylases.
The hospital charts of these patients were reviewed with
regard to all blood-chemistry results obtained during the
present admission, past history of acute pancreatitis, and
any recent history of alcoholism, biliary disease, salivary
gland disease, or surgery.

A diagnosis of acute pancreatitis was made on the basis of
clinical presentation (unequivocal history of acute pain in
the upper abdomen) and ultrasound and computed-tomogra-
phy scan examinations of the pancreas. In eight cases there
was additional diagnostic support from exploratory laparot-
omy or autopsy.

A diagnosis of chronic pancreatitis was based on the
presence of steatorrhea, radiological evidence of pancreatic
calciﬁcation, and (or) typical duct alterations at endoscopic
retrograde pancreatography. The results of the analysis for
the enzyme activities evaluated were not available to the
attending clinicians at the time of the diagnosis.

Data Analysis

Partial statistical analysis of the data was done with a
"PVC" statistical software package (27). In particular, we
used receiver-operating characteristic (ROC) curves and the
Hartz overlap index (OI) to discriminate between the ability
of LPS and P3 to separate the patients with acute pancreati-
is from the remaining patients. Use of ROC curves make it
possible to compare the diagnostic performance of different
tests under the same conditions (equal true-positive rates or
equal false-positive rates) and over the entire range of
possible discrimination values of the different tests by using
discrimination values corresponding to observed test results
(28). The calculation of the Hartz OI, however, does not
depend on defining a cutoff limit. This nonparametric rank-
order statistic can be used to quantify the utility of a
laboratory test independently of a decision level, the test
with the lowest OI value having the greatest potential for
differentiating between the groups under consideration (29).

Results

Review of the charts showed that virtually all of the
hyperamylasemic patients had some abdominal pain, which
led to the serum total AMY determination. Table 1 summa-

izes the clinical diagnoses. In 38 cases (70.4%) the final
diagnosis was acute pancreatitis. Of these, 24 cases were
associated with biliary-tract disease, six with alcohol con-
sumption, two with hyperlipidemic status (type IV hyperlip-
idemia), and one with carcinoma of the head of the pancreas,
but for five the etiology was unknown.

Figure 1 shows the distribution of LPS and P3 isofrom
activities in the serum of hyperamylasemic patients. All 38
patients diagnosed as having acute pancreatitis had in-
creased serum LPS (median 2545, range 710–12 620 U/L)
and P3 isofrom (median 54, range 15–162 U/L) activities.
Conversely, the median LPS activity was within normal
limits in the patients with chronic pancreatitis (105, range
61–1 150 U/L), and the P3 isofrom activity was always not
detectable. In the patients with nonpancreatic diseases,
seven of 12 patients (58.3%) had LPS activity exceeding the
upper reference limit, whereas five patients (41.7%) had
detectable P3 isofrom activities in serum. Five of these
(three of them with increased P3) had acute biliary disease.

Table 1. Groupings of Hyperamylasemic Patients Based on Diagnosis

<table>
<thead>
<tr>
<th>Diagnosis (and sex)</th>
<th>Number</th>
<th>Age, y</th>
<th>Total serum AMY, U/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pancreatic diseases</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acute pancreatitis</td>
<td>38 (24M, 14F)</td>
<td>60 (27–84)*</td>
<td>221 (101–900)*</td>
</tr>
<tr>
<td>Chronic pancreatitis in clinical remission</td>
<td>4 (2M, 2F)</td>
<td>49 (40–66)</td>
<td>118 (99–156)</td>
</tr>
<tr>
<td>Nonpancreatic diseases</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acute cholecystitis and (or) cholangitis</td>
<td>8 (5M, 3F)</td>
<td>76 (32–81)</td>
<td>133 (105–263)</td>
</tr>
<tr>
<td>Peritonitis after perforated colonic diverticula</td>
<td>1 (F)</td>
<td>82</td>
<td>272</td>
</tr>
<tr>
<td>Intestinal obstruction</td>
<td>1 (F)</td>
<td>55</td>
<td>143</td>
</tr>
<tr>
<td>Nonspeciﬁc abdominal pain</td>
<td>1 (F)</td>
<td>68</td>
<td>118</td>
</tr>
<tr>
<td>Acute ethanol abuser</td>
<td>1 (M)</td>
<td>54</td>
<td>134</td>
</tr>
</tbody>
</table>

*Median value and range.
Furthermore, there was one patient with peritonitis after perforated colonic diverticula and one with small-bowel obstruction, both without macroscopic pancreatic injury (as proved by surgery), who had false-positive results for both enzymes.

Figure 2 shows the ROC curve analysis for the two pancreatic enzymes for diagnosing acute pancreatitis in the patients we studied. Predictive values were calculated for LPS and P₃ isofrom activity for selected decision levels (Table 2). No patient had a false-negative test result. Therefore, the negative predictive values were 100% for both LPS and P₃ isofrom. There were three false-positive results for LPS and four false-positive results for P₃ isofrom determination, two of them in the same patient. Table 3 gives details about these patients. The positive predictive values were therefore 92.7% and 90.5% for LPS and P₃ isofrom activity, respectively. Thus, using the best cutoff value, the P₃ isofrom activity measurement was slightly inferior in specificity to LPS activity. Measurement of both LPS and P₃ isofrom activities in serum moderately improved diagnostic accuracy (Table 2). The discriminant value, defined as the level that gives the highest numerical efficiency, both for LPS (700 U/L) and P₃ isofrom (14 U/L), was considerably higher than the respective upper reference limits. Mainly for LPS the most nonspecific increases (five of eight) were those between a twofold and about a fivefold increase in activity above the upper reference limit.

On the basis of enzyme activity data, we calculated the O₁ to further compare the utility of each enzyme for differentiating between the patient with or without acute pancreatitis. The LPS (O₁ = 0.15) and P₃ isofrom (O₁ = 0.18) in serum provided a similar discrimination between the two groups of patients.

Discussion

Knowledge of the origin of hyperamylasemia may have an important influence on treatment, hospitalization, and the extent of laboratory and radiological investigation. In particular, a backup assay is needed to help corroborate or rule out the diagnosis of acute pancreatitis. The present study was undertaken to clarify the role of serum LPS and P₃ isofrom activity in hospitalized patients with hyperamylasemia. A serious drawback to investigation of any laboratory test intended to clarify this clinical problem is the establishment of clear-cut criteria for the diagnosis. Unfortunately, there currently is no practical "gold standard" (30). In the design of this study, we established clinical criteria for the diagnosis of acute pancreatitis and required objective confirmation of the clinical diagnosis by ultrasonography, computed tomography, or laparotomy.

Usually, acute pancreatitis accounts for about 10% of hospital admissions for acute abdominal pain (4). The high-
er prevalence (70%) of acute pancreatitis in the present study may reflect in part the way the patients were entered into this study: they were initially analyzed for total AMY activity only when there was a clinical suspicion of possible acute pancreatitis and were definitively entered into the study if an increased value for total AMY was recorded. The criterion of hyperamylasemia significantly increased the a priori probability (the prevalence of acute pancreatitis in our study) of acute pancreatitis. Because the predictive value of a positive test result increases (and the predictive value of a negative test result decreases) with increasing prevalence, regardless of the relationship between sensitivity and specificity, our conclusions are valid only for populations with similar prevalence of acute pancreatitis. However, we believe that this makes our study specifically helpful, because clinicians frequently are confronted with the differential diagnosis of hyperamylasemia in patients with clinical signs similar to those observed in acute pancreatitis (31).

Measurement of LPS and P3 isoform activity in serum seems to be useful in confirming or excluding acute pancreatitis in patients with equivocal hyperamylasemia. The importance of selecting an appropriate cutoff test value has been emphasized by Steinberg et al. (5) and by Moller-Petersen et al. (21), who described for laboratory tests used in diagnosing acute pancreatitis a "gray zone of nonspecificity" above the upper reference limit. Mainly for LPS activity, in the present study, there was a "gray area" between the upper limit of the reference interval and 4.5 times that level (150–700 U/L). Most nonspecific increases fell within this "gray area" and the diagnostic value of a moderate increase in serum LPS is therefore limited.

In agreement with previous studies (9, 10, 13, 23, 24), the P3 isoform was present in the sera of all patients with acute pancreatitis. False-positive results were observed in biliary disease, in agreement with the findings of Frost (10) and Collins et al. (24), and in patients with necrosis of the bowel. We are not precisely sure of the mechanism of these false-positives. Collins et al. (24) suggest that the P3 isoform, produced from organs other than the pancreas, could be liberated and modified to form an isoform identical with or similar to P3. Because all our cases of acute pancreatitis had P3 activity exceeding 14 U/L, and despite the fact that the test does not absolutely confirm the diagnosis of acute pancreatitis, we believe that an absence of the P3 isoform excludes this diagnosis. The test in its present form does not lend itself readily to the emergency situation, its usefulness being limited by the tediousness of the electrophoretic procedure. This limitation does represent the major obstacle to the use of the test in the initial diagnosis of acute pancreatitis and in management decisions. Thus, the development of new emergency techniques for assaying P3 will still be of great diagnostic aid. Assays in which specific monoclonal antibodies are used could offer important advantages of simplicity and speed. However, at present, electrophoretic methods are likely to remain the most practical means of obtaining information about AMY isofoms (15).

If LPS is compared with P3, it is found that the incidence of increased values is nearly identical, and in general the enzymes supply the same diagnostic indications. When evaluated by means of ROC curves and OR, no differences were found in the diagnostic performance of the two enzymes. Only for one patient with chronic pancreatitis did the LPS activity not agree with the P3 result. The reason for this disagreement is not readily apparent, but may, in part, be due to insufficient sensitivity of the electrophoretic method for P3 determination. Important aspects, already mentioned, when considering the superiority of one of these two pancreatic enzymes in clinical routine are the analytical characteristics of the assays, their methodological handling, and local laboratory capabilities. The automated turbidimetric LPS procedure used in this study proved to be fast (less than 8 min to obtain the answer), allowing its application as an emergency procedure.

Our present results on LPS determination support previous reports (5, 19–21, 25). The test is not entirely specific for acute pancreatitis, because high values for LPS were found also in patients with acute cholecystitis, small-bowel obstruction, and chronic pancreatitis. Abnormal results relative to the upper reference limit were obtained by Tietz et al. (20) in 62.5% of their patients with biliary and bowel disease, a finding very similar to ours (58.3%). Tietz (31) suggests several mechanisms by which such increase may occur: silent invasion of the pancreas by microorganisms, reflux of duodenal juice containing enterokinase that activates pancreatic enzymes, synthesis of pancreatic trypsinogen in excess and its intraductal activation, and regurgitation of bile into the pancreas.

In conclusion, our results demonstrate that, in the differential diagnosis of hyperamylasemia, LPS and P3 isofoms have the same diagnostic value. However, the use of a combination of the two enzymes had little advantage over single enzyme measurement, and the serum LPS determination is the more convenient because of its simplicity and rapidity. As has also been described elsewhere (26), in light of the demonstrably improved performance of the colipase-supplemented test and its amenability to automation, LPS assay should be requested more often when acute pancreatitis is suspected. LPS activity exceeding fivefold the upper reference limit, in the context of suggestive clinical signs, appears to recommend a diagnosis of acute pancreatitis. In particular, we suggest that a sequence of biochemical investigations such as that outlined in Figure 3 should be used in the study of cases of hyperamylasemia.

The skillful technical assistance of Mrs. Olga Alebardi is gratefully acknowledged.

References