Assay of Pancreatic Amylase with Use of Monoclonal Antibodies Evaluated

Martina Zaninotto, Roberta Bertorelle, Sandra Secchiero, Marlo Plebani, and Angelo Burlina

To evaluate a new method for measuring pancreatic amylase in serum, in which the salivary isoenzyme is inhibited with a specific monoclonal antibody, we determined the activity of pancreatic and salivary amylase in sera from 103 healthy subjects and from 114 hospitalized patients having a wide range of total amylase activities. CVs for the proposed method ranged from 0.8% to 5.1% (within day) and from 2.3% to 6.6% (day to day). Results correlated well with those obtained by the wheat-germ inhibition method (r = 0.998) and by electrophoresis on cellulose acetate. Analytical-recovery studies confirmed the good specificity of the monoclonal antibody for salivary amylase (97%) and its low cross-reactivity (0.6%) toward pancreatic amylase. The assay procedure presents a wide range of linearity (141–1817 U/L) and can easily be adapted to an automated kinetic system. We found the proposed method suitable for routine determinations of pancreatic amylase.

Additional Keyphrases: isoenzymes • wheat-germ inhibition and cellulose acetate electrophoresis methods compared • pancreatic disease

Despite the well-known lack of specificity of total amylase activity for pancreatic diseases, remarkable advances in its measurement have been made over the past few years (1). Efforts to achieve clinical specificity for amylase in pancreatic diseases have focused on the measurement of amylase isoenzymes (2). The two isoenzymes of human α-amylase (1,4-α-d-glucan glucohydrolase, EC 3.2.1.1), the salivary and the pancreatic α-amylase isoenzyme, are tissue specific (3). Thus it is thought that more information about pancreatic disease can be obtained by assaying pancreatic isomylase specifically.

Several techniques for this have been based on differences in net electrical charge of the isoenzymes. The chromatographic and isoelectric focusing methods are time-consuming and involve complex procedures, which limit the general use of these methods in the routine clinical laboratory (4); electrophoresis, on various supports, is also relatively time-consuming, although comparatively simple, but is difficult to use within consistently optimal resolution (5, 6). Selective inhibition of the salivary isoenzyme by wheat-germ proteins may provide a simple, rapid isomylase assay useful for routine clinical use, but this method lacks specificity (7–9).

In the immunochemical methods used, with one exception (10), the polyclonal antibodies cross-reacted by various degrees with both amylase isoenzymes (11). Recently, however, specific monoclonal antibodies directed against the salivary isoenzyme have been prepared and used in various methods, with little or no cross-reactivity with the pancreatic isoenzyme (12–15).

Here we have evaluated the method proposed by Gerber et al. (16), in which the salivary amylase is immunoprecipitated and the remaining (pancreatic) amylase activity is assayed spectrophotometrically. Some of these described results have been reported in a multicenter preliminary evaluation of the proposed method (17).

Materials and Methods

To evaluate the normal range for amylase in serum, we obtained blood samples from 103 healthy subjects, ages 25 to 50 years, who had no history of pancreatic diseases.

We used the new immunological method, the total amylase assay, and the wheat-germ inhibition test to assay samples from 114 hospitalized patients, which were submitted to the laboratory for amylase analysis because of clinically suspected pancreatic diseases.

To clinically investigate the usefulness of the new test, a group of patients affected by pancreatic diseases—including 25 with acute pancreatitis, 20 with chronic pancreatitis, and 20 with pancreatic cancer—were studied and compared with a parallel control group of 30 normal subjects. The diagnosis was made on the basis of the results of the abdominal computerized tomography and of clinical and laboratory findings.

We measured total amylase activity enzymatically, using p-nitrophenylmaltoheptaoside as substrate (Boehringer Biochemia Robin, Milan, Italy). The activity of pancreatic isoamylase was determined by using a wheat-germ inhibitor, specific for salivary isoenzyme, as a comparative method (Boehringer Biochemia Robin). The immunological method (Boehringer Mannheim Diagnostica, GmbH, Mannheim, F.R.G.) involves two monoclonal antibodies that specifically bind to MAB66C7 (18) and inhibit MAB88E8 (16) human salivary α-amylase. The assay format is "substrate start," with a pre-incubation time of 5 min, because the antibody interacts very slowly with the enzyme in the presence of substrate. To maintain comparability, we also performed total amylase and wheat-germ inhibition methods in "substrate start" versions.

All three methods were automated for the Cobas Fara centrifugal analyzer (Roche Diagnostica, Milan, Italy), and performed at 30 °C. The instrument was calibrated with the multicalibrator for automated systems (Boehringer Mannheim Diagnostica, cat. no. 759350; lot no. 155572) with a target value (402 U/L). To establish a "fixed" calculation factor the calibrator was analyzed 15 times and the mean value (8460) was calculated. Table 1 lists the Cobas Fara settings for performing the immunological method.

In addition, we assayed the samples by electrophoresis on a rigid plate of cellulose acetate (Titan III Iso-Via; Helena Laboratories, Milan, Italy), according to Burlina et al. (6), which makes visible the two principal fractions and possible alterations of isoenzymatic pattern.

Within-run precision was determined by assaying human pooled sera in three different activity ranges (normal, mid, and increased concentrations) in 20 consecutive measurements. For 20 days, we also measured aliquots of the three frozen serum pools in duplicate with the two inhibition methods, to assess day-to-day precision.
Table 1. General Conditions for Determining Pancreatic α-Amylase by the Monoclonal Antibody Inhibition Method in the Cobas Farab

<table>
<thead>
<tr>
<th>Temperature</th>
<th>30 °C</th>
</tr>
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<tbody>
<tr>
<td>Wavelength</td>
<td>405 nm</td>
</tr>
<tr>
<td>Sample</td>
<td>10 μL</td>
</tr>
<tr>
<td>Reagent</td>
<td>200 μL</td>
</tr>
<tr>
<td>Incubation</td>
<td>300 s</td>
</tr>
<tr>
<td>Start</td>
<td>20 μL</td>
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<tr>
<td>Readings:</td>
<td></td>
</tr>
<tr>
<td>First</td>
<td>80 s</td>
</tr>
<tr>
<td>Interval</td>
<td>20 s</td>
</tr>
<tr>
<td>Calibrator factor</td>
<td>8460</td>
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</table>

Table 2. Evaluation of Analytical Precision of the Monoclonal Antibody Inhibition Method

<table>
<thead>
<tr>
<th>Activity concentration</th>
<th>Normal</th>
<th>Mid</th>
<th>High</th>
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</thead>
<tbody>
<tr>
<td>Within-run (n = 20 each)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean, U/L</td>
<td>50.8*</td>
<td>120.9</td>
<td>315.5</td>
</tr>
<tr>
<td>SD, U/L</td>
<td>2.58</td>
<td>3.1</td>
<td>2.74</td>
</tr>
<tr>
<td>CV, %</td>
<td>5.11</td>
<td>2.57</td>
<td>0.87</td>
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</tbody>
</table>

| Day-to-day (n = 20 each) |        |     |        |
| Mean, U/L               | 45.9   | 126.5 | 317.87 |
| SD, U/L                 | 3.04   | 3.97 | 7.55   |
| CV, %                   | 6.62   | 3.14 | 2.37   |

* n = 10.

To verify the linearity of the evaluated method, we serially diluted a sample having a pancreatic amylase activity (1817 U/L) above the upper limit of measurement prescribed by the manufacturer, 1453 U/L, with a sample of very high salivary amylase activity (1919 U/L). We assayed each of these dilutions in duplicate and compared the results with the calculated concentration value.

Specificity studies were carried out with pure standards from human pancreas and salivary glands extracts (Boehringer Mannheim Diagnostics), which we assayed once a day for 20 days during a four-week period.

Results

Precision studies. Results for within-run and day-to-day precision are reported in Table 2.

Linearity. Figure 1 summarizes the experimental and calculated values analyzed by linear regression.

Specificity. The specificity of monoclonal antibody was evaluated by assaying two standards containing only pancreatic or salivary isoenzyme, respectively, and comparing results with the inhibition patterns of wheat-germ protein. We thus calculated the mean total and residual activity in 20 determinations and the degree of inhibition—the ratio of residual/total activity in percent (R/T%)—for both methods (Table 3).

Comparison between methods. Results for pancreatic isoamylase as determined by the monoclonal antibody method (y) correlated well by linear regression analysis with those obtained by the wheat-germ inhibition method (x): y = 1.021x - 0.401 U/L, r = 0.998, n = 114, Sxy = 26.862. Correlation with electrophoretic results was obtained by comparing the percentage of total amylase that was the pancreatic isoenzyme, (P-amyase/T-amyase) × 100 (y), and the percentages obtained by densitometric interpretation of the electrophoretic patterns (x). The correlation coefficient was 0.824 (y = 1.174x - 3.633, n = 114, Sxy = 13.593).

Reference interval. The values found in the apparently healthy subjects did not have a gaussian distribution. Therefore we based the reference interval for pancreatic amylase activity on nonparametric statistics: 20–80 U/L (2.5 and 97.5 percentiles). The confidence interval obtained in our study was comparable with those calculated by other authors (17).

Pancreatic vs total amylase. A good agreement (r = 0.928) was found between results for total amylase assay (x) and for pancreatic isoenzyme determined by the monoclonal antibody method (y). The regression equation was y = 0.603x + 47.348 (n = 114, Sxy = 186.897). The more accurate evaluation emphasized the necessity for a further thorough examination of the results in nine cases.

Discussion

The immunological method we evaluated was developed in response to a need for a reliable, specific procedure for determining the pancreatic isoenzyme of α-amylase as an aid in the diagnosis of pancreatic disease. In this new method the value for pancreatic isoenzyme is directly obtained without any of the calculations or corrections that are necessary in other automated methods in which the wheat-germ inhibition protein is used (9). Moreover, analytical times are shortened and, because of its extended linearity, predictions are rarely necessary.

Although the method involving a wheat-germ protein as inhibitor is less expensive and precise enough, its accuracy is limited by its poor specificity. In our study, in fact, the 11.6% residual activity measured in the salivary standard by the wheat-germ method demonstrates an incomplete inhibition of salivary isoenzyme, whereas the 90.9% residual activity in the pancreatic standard shows that there is a partial inhibition of the pancreatic isoenzyme.

The recently prepared monoclonal antibodies directed against the salivary isoenzyme provide a tool for a better immunochemical method: only about 3% of residual activity

Table 3. Analytical Recovery of Pancreatic and Salivary Amylase Standards

<table>
<thead>
<tr>
<th></th>
<th>Present method</th>
<th>Wheat-germ method</th>
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<tbody>
<tr>
<td>Pancreatic standard (total activity = 808.65)</td>
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</tr>
<tr>
<td>Residual</td>
<td>804.50 ± 19.95</td>
<td>735.75 ± 25.85</td>
</tr>
<tr>
<td>R/T, %</td>
<td>99.48</td>
<td>90.98</td>
</tr>
<tr>
<td>Salivary standard (total activity = 905.95)</td>
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<tr>
<td>Residual</td>
<td>28.75 ± 3.04</td>
<td>105.55 ± 5.87</td>
</tr>
<tr>
<td>R/T, %</td>
<td>3.17</td>
<td>11.64</td>
</tr>
</tbody>
</table>

n = 20.
of salivary amylase was observed after inhibition with these antibodies. Essentially, the new test measures only the human pancreatic amylase because the cross-reactivity of the antibodies toward pancreatic amylase is only 0.6%.

A good correlation was found between results by the new test and other methods widely used in laboratory routine for pancreatic isomylase determination and utilized in our study. In particular, the correlation between the immunological assay and electrophoresis is satisfactory considering the analytical and interpretive problems concerning this last method, largely described elsewhere (19).

Figure 2 shows the distribution of values for total and pancreatic amylase in serum of pancreas-disease patients selected without conscious bias, with 12 cases (10%) demonstrating a pathological pancreatic amylase activity and a normal total amylase activity. Pancreatic isomylase determination evidently provides more dependable information than does total activity. However, in our experience, the new test, if used alone, would have led us to a wrong interpretation in nine of the 114 patients we studied, for whom only the P/T% ratio indicated the correct diagnosis. Figure 3 shows results for five of these cases. In some (samples C, D, E) the absolute values for pancreatic and total amylase were in the pathological range, and only their ratio (in percent) indicated a probable extrapancreatic disease. In others (samples A and B), pancreatic disease seemed to be excluded by the observation of a normal value for pancreatic isomylase, and only comparison with the total amylase value gave more accurate information.

In our patients with chronic pancreatitis and pancreatic cancer, the values for pancreatic amylase showed a large overlap with those obtained in the control group (Figure 4), so data on pancreatic isomylase apparently are unhelpful in testing for functional failure of the exocrine pancreas.

On the other hand, in our patients with acute pancreati-

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**Fig. 2. Values for pancreatic and total amylase activities in the 114 patients**

A: subjects with pathological pancreatic amylase and normal total amylase activities

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**Fig. 3. Total (■) and pancreatic (□) amylase in five patients with extrapancreatic diseases**

A: total amylase = 187 U/L, pancreatic amylase = 32 U/L, P/T% = 17%; B: total amylase = 265 U/L, pancreatic amylase = 20 U/L, P/T% = 7%; C: total amylase = 2060 U/L, pancreatic amylase = 126 U/L, P/T% = 6%; D: total amylase = 862 U/L, pancreatic amylase = 217 U/L, P/T% = 25%; E: total amylase = 482 U/L, pancreatic amylase = 93 U/L, P/T% = 20%. Horizontal lines indicate the upper limit of normal range for pancreatic isomylase (80 U/L) and total amylase (160 U/L). The results for pancreatic amylase always exceeded the normal range, even though, as observed in Figure 5, five (20%) of them had normal values for total amylase. These data confirm that the present test is a more sensitive index than total amylase activity in differential diagnosis of this disease.

In conclusion, in keeping with the concept that monoclonal antibodies are a good analytical tool for solving problems of specificity, we found that their application in isomylase determinations provides a simple, rapid, and specific method of great promise for the diagnosis of acute pancreatitis. As for the chronic pancreatitis and pancreatic cancer, more detailed studies are necessary to verify the sensitivity of the test in monitoring the pancreatic insufficiency. Further studies involving a more representative number of patients with extrapancreatic diseases are in progress for

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**Fig. 4. Values for pancreatic isomylase in a control group and in patients with pancreatic diseases**

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evaluating the clinical value of absolute measurements of pancreatic amylase activity and of the P/T ratio; however, our results for the isoamylase activities, expressed as percent of total, show increased specificity.

References