Simultaneous Evaluation of a Pancreas-specific Antigen and a Pancreatic Cancer-associated Antigen in Pancreatic Carcinoma

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ABSTRACT

A pancreas cancer-associated antigen (PCAA) and a pancreas-specific antigen (PaA) were simultaneously quantitated by enzyme-linked immunosorbent assays in serum specimens from 51 normal controls, 76 pancreatic cancers, 194 nonpancreatic cancers, and 22 benign pancreatic diseases. Primary immunological reagents used in the enzyme-linked immunosorbent assays were our polyclonal antibodies produced in rabbits against purified PCAA and PaA. Results revealed discordance of these two markers in pancreatic cancer, suggesting that the presence of these two biochemically and immunologically distinct pancreas proteins in patients’ serum may reflect different biological aspects of cancer. The combination test resulted in a better sensitivity and specificity for pancreatic cancer, 90 and 85%, respectively, than either PCAA or PaA alone. This study demonstrated that the combination of serum PCAA and PaA tests yields an additive clinical value and may be a useful adjunctive aid for the immunodiagnosis of the pancreatic cancer.

INTRODUCTION

Early diagnosis of pancreatic cancer is still difficult, primarily due to the fact that the initial symptoms are frequently vague and nonspecific, and that sufficiently sensitive and specific diagnostic procedures are not available (5, 8). In search for tumor marker antigens which may be used in the immunodiagnostic test, several pancreas tumor-associated antigens have been reported, such as oncofetal antigen (6) (M, 40,000), pancreatic oncofetal antigen (4) (M, 8 to 9 x 10^6), oncofetal pancreatic antigen (16) (M, 36,000), tumor-associated pancreatic antigen (14) (M, 9 to 10 x 10^6), and pancreas asctes glycoprotein (2) (M, 1.8 x 10^6).

Recently, this laboratory isolated 2 pancreatic antigens, designated as PCAA (15) and PaA (9, 10), which are biochemically different from those antigens described above. PCAA is a glycoprotein with a molecular weight of approximately 1 to 1.5 x 10^6, pl 4.7, with microheterogeneity and expressed maximally by pancreas carcinoma. PaA is a single peptide protein with a molecular weight of 44,000, pl 4.9, and expressed specifically by exocrine pancreatic cell, both normal and malignant. In addition, PCAA is different from PaA in biochemical characteristics and immunological property. Since these 2 distinct proteins are associated with human pancreatic cancer, their expressions may represent different biological aspects of pancreatic cancer and, therefore, may be useful in a combination test for serological detection of pancreatic cancer. This paper describes our initial effort on the simultaneous test of serum PCAA and PaA from patients with pancreatic and nonpancreatic cancers, as well as with benign pancreatic diseases along with normal control subjects.

MATERIALS AND METHODS

Specimens. Blood samples from patients with histologically confirmed pancreatic and nonpancreatic cancers were obtained from Roswell Park Memorial Institute and the University Hospital of Amsterdam. The pathology of pancreatitis, cholelithiasis, and pancreatic cancer was determined by laparoscopy, computerized tomography, and endoscopic retrograde cholangiopancreatography. Each case of pancreatic cancer and benign pancreatic disease was histologically confirmed by biopsy. There were 76 patients in the pancreatic cancer group: 10 patients with Stage I (resectable); 18 Stage II (local disease not resectable and no liver metastasis); and 48 Stage III (liver metastasis) at the time of blood specimen was drawn. All patients were with disease, although some patients were receiving radiation and/or chemotherapy at the time of the blood test. The benign pancreatic disease group consisted of 6 cases of acute pancreatitis, 10 of chronic pancreatitis, and 8 of cholelithiasis. Other cancer groups included 56 lung, 50 colorectal, 39 breast, and 49 prostate carcinomas. Most of these patients with cancer other than pancreas were with advanced disease. Each case was histologically confirmed. Fifty-one control sera were drawn from apparently healthy blood donors with the kind cooperation of the American Red Cross, Buffalo Chapter. Serum specimens were stored at -70°C until simultaneously assayed for both PCAA and PaA.

ELISA of PCAA. The ELISA of PCAA, using purified PCAA, anti-PCAA IgG, CNBr-activated Sepharose 4B-conjugated anti-PCAA IgG, and anti-PCAA IgG-conjugated peroxidase, were described previously (15). Anti-PCAA IgG was isolated from polyclonal antiserum produced in rabbit against purified PCAA as reported (15). PCAA in specimens was quantitated from a standard curve which was constructed by measuring various concentrations of purified PCAA in an identical manner. The ELISA of PCAA was sensitive to detect 0.1 μg of PCAA/ml and reproducible as shown by coefficients of variation of 4 and 6% for within and between assays, respectively. In the present study, serum samples were simultaneously assayed for PCAA according to this ELISA method along with serum PaA test.

ELISA of PaA. The purification of PaA and production and specificity of rabbit polyclonal anti-PaA antiserum were reported previously (10). The ELISA of PaA, using purified PaA, anti-PaA IgG, peroxidase-conjugated anti-PaA IgG, and anti-PaA IgG-coated polystyrene tubes, was described recently (9). Serum samples were diluted 5 times for the assay.

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8 The abbreviations used are: PCAA, pancreatic cancer-associated antigen; PaA, pancreas-specific antigen; ELISA, enzyme-linked immunosorbent assay.

Received February 13, 1984; accepted May 7, 1984.
RESULTS

**Single Assay of PCAA.** Circulating PCAA levels in patients with pancreatic and nonpancreatic cancers as well as those with benign pancreatic diseases and normal control subjects are shown in Chart 1 and Table 1. The means and medians of serum PCAA from patients with nonpancreatic cancers, including the lung, colorectum, breast, and prostate cancers, were similar to those from normal controls. However, the means and medians of serum PCAA were shown to be higher in patients with pancreatic cancer and benign pancreatic diseases (pancreatitis and cholelithiasis) than those of normal controls.

In comparison with the normal group, pancreatic cancer, pancreatitis, and cholelithiasis revealed highly significant differences \((p < 0.005)\) as analyzed by the Wilcoxon test. However, there was no significant difference among pancreatic cancer, pancreatitis, and cholelithiasis. Also, no significant difference was noted between the groups of normal and nonpancreatic cancers.

As shown in Chart 1 and Table 1, sera from 51 healthy donors had PCAA levels ranging from 3.6 to 21.0 \(\mu g/ml\). From this normal group, the upper cut-off point of normal range was determined as 19.0 \(\mu g/ml\) (the upper 97.5 percentile). Using this upper limit of normal range, an elevated PCAA was found in 40 of 76 pancreatic cancer (53%), 7 of 14 pancreatitis (50%), and 3 of 8 cholelithiasis (38%). A few patients with other cancers were also found to have an elevated serum PCAA: 8 of 56 (14%) of lung cancer; 6 of 50 (12%) of colorectal cancer; 2 of 39 (5%) of breast cancer; and 5 of 49 (10%) of prostate cancer. The overall sensitivity and specificity of a single PCAA test for pancreatic cancer were calculated to be 53 and 91%, respectively.

Among 14 pancreatitis patients, 5 of 6 (83%) acute pancreatitis and 2 of 8 (25%) chronic pancreatitis patients had an elevated serum PCAA level. An elevated PCAA level was found in 5 of 10 (50%) Stage I, 9 of 18 (50%) Stage II, and 26 of 48 (54%) Stage III pancreatic cancer patients.

**Single Assay of PaA.** Serum PaA levels in patients and normal controls are revealed in Chart 2. As shown, PaA levels obtained from normal controls and patients with carcinomas of the lung, colorectum, breast, and prostate were almost identical, ranging from <4 \(\mu g/ml\) to 34 \(\mu g/ml\) for the normals and 36 \(\mu g/ml\) for the nonpancreatic cancer. In comparison with the normal controls, serum PaA levels in pancreatic cancer were significantly higher \((p < 0.0001)\) as were those in patients with benign pancreatic disease \((p < 0.005)\). There was also a significant difference between the pancreatic cancer group and the benign pancreatic cancers.

### Chart 1
Percentage of distribution of serum PCAA levels in normal controls (○), benign pancreatic diseases (+), pancreatic cancer (●), and nonpancreatic cancers (X) as assayed by ELISA. The vertical broken line, located at 19.0 \(\mu g/ml\), represents the upper 97.5% of PCAA as derived from the control group of 51 healthy blood donors. Number of patients in each patient group: normal control, 51; benign pancreatic diseases, 22; pancreatic cancer, 76; and nonpancreatic cancers, 194.

### Table 1
Serum PCAA levels in various diseases

<table>
<thead>
<tr>
<th>Groups</th>
<th>Range</th>
<th>Mean</th>
<th>Median</th>
<th>Mode</th>
<th>No. elevated&lt;sup&gt;8&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>3.6–21</td>
<td>8.9</td>
<td>9.0</td>
<td>12.5</td>
<td>51</td>
</tr>
<tr>
<td>Nonpancreatic cancer</td>
<td>1.4–34</td>
<td>12.1</td>
<td>9.9</td>
<td>16.0</td>
<td>50</td>
</tr>
<tr>
<td>Lung</td>
<td>3.8–36</td>
<td>10.8</td>
<td>8.0</td>
<td>18.0</td>
<td>56 (14)</td>
</tr>
<tr>
<td>Colorectum</td>
<td>2.3–36</td>
<td>10.8</td>
<td>8.0</td>
<td>18.0</td>
<td>56 (14)</td>
</tr>
<tr>
<td>Breast</td>
<td>1.4–34</td>
<td>12.1</td>
<td>9.9</td>
<td>16.0</td>
<td>50 (12)</td>
</tr>
<tr>
<td>Prostate</td>
<td>3.8–31</td>
<td>9.4</td>
<td>4.6</td>
<td>4.6</td>
<td>39 (5)</td>
</tr>
<tr>
<td>Pancreatitis</td>
<td>4.5–50</td>
<td>22.4</td>
<td>23.0</td>
<td>13.0</td>
<td>7 (50)</td>
</tr>
<tr>
<td>Cholelithiasis</td>
<td>3.3–52</td>
<td>22.4</td>
<td>18.0</td>
<td>8</td>
<td>3 (38)</td>
</tr>
<tr>
<td>Pancreatic cancer</td>
<td>1.9–81</td>
<td>20.0</td>
<td>20.0</td>
<td>36.0</td>
<td>76 (53)</td>
</tr>
</tbody>
</table>

<sup>8</sup> The elevated level was the value higher than the upper normal cut-off point, 19.0 \(\mu g/ml\) (the upper 97.5%), of PCAA derived from the normal controls.

<sup>4</sup> Numbers in parentheses, percentages of those with elevated PCAA levels.

<sup>5</sup> There is no mode available in the cholelithiasis group.

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disease group ($p < 0.005$).

The upper 97.5% of PaA levels in 51 healthy controls (mean, 8.2 ng/ml) was determined to be 21.5 ng/ml. Using this value as an upper normal limit, 50 of 76 (66%) patients with pancreatic cancer (mean, 43.4 ng/ml) and 7 of 24 (29%) with benign pancreatic disease (4 of 16 with pancreatitis and 3 of 8 with cholelithiasis) (mean, 24.3 ng/ml) were shown to have an elevated serum PaA level, along with a very few of patients (11 of 194) with nonpancreatic carcinomas (mean, 8.5 ng/ml), including 4 of 56 lung, 2 of 50 colorectum, 1 of 39 breast, and 4 of 49 prostate cancer patients. The sensitivity and specificity of the PaA test for pancreatic cancer were 66 and 95%, respectively.

An elevated serum PaA level was found in 6 of 10 (60%) Stage I, 11 of 18 (61%) Stage II, and 33 of 48 (69%) Stage III pancreatic cancers. In 16 patients with pancreatitis, 3 of 6 (50%) with acute pancreatitis and one of 10 (10%) with chronic pancreatitis exhibited an elevated PaA level.

Simultaneous Determination of PCAA and PaA. The results on simultaneous measurements of serum PCAA and PaA are compiled in Chart 3 and Table 2. Analysis of linear regression revealed that there was no correlation between PCAA and PaA values (Chart 3) in pancreas carcinoma patients. As shown in Table 2, pancreatic cancer, with a total of 76 specimens, was revealed as 28% (21 of 76) both positive, 41% (31 of 76) PaA positive, 21% (16 of 76) PCAA positive, and 10% (8 of 76) both negative. In comparison with the pancreatic cancer group, the group of benign pancreatic diseases had a higher percentage of both negative (41%) and lower percentage of both positive (18%). Normals and nonpancreatic cancers registered a very high percentage (84 and 92%) of both negative and an extremely low percentage (0 and 1%) of both positive. The sensitivity and

<table>
<thead>
<tr>
<th>Total no. of patients or controls</th>
<th>PCAA- PaA-</th>
<th>PCAA+ PaA-</th>
<th>PCAA- PaA+</th>
<th>PCAA+ PaA+</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>51</td>
<td>47 (92)%</td>
<td>2 (4)</td>
<td>2 (4)</td>
</tr>
<tr>
<td>Nonpancreatic cancers</td>
<td>194</td>
<td>162 (84)%</td>
<td>20 (10)</td>
<td>11 (6.5)</td>
</tr>
<tr>
<td>Benign pancreatic cancer</td>
<td>22</td>
<td>9 (41)%</td>
<td>6 (27)</td>
<td>3 (14)</td>
</tr>
<tr>
<td>Pancreatic cancer</td>
<td>76</td>
<td>8 (10)%</td>
<td>16 (21)</td>
<td>31 (41)</td>
</tr>
</tbody>
</table>

$^a$ PCAA + or - represents the levels higher or lower than 19.0 ${\mu}$g/ml (the upper 97.5%), respectively, of PCAA derived from the normal controls. PaA + or - represents the levels of higher or lower than 21.5 ng/ml (the upper 97.5%), respectively, of PaA derived from the normal controls.

$^b$ Numbers in parentheses, percentage of total number of serum PaA and PCAA determinations.

$^c$ The nonpancreatic cancers included 56 lung, 50 colorectum, 39 breast, and 49 prostate cancers.

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specificity of combined tests (PCAA and/or PaA) for pancreatic cancer were calculated to be 90 and 85%, respectively.

DISCUSSION

Results obtained from this study reveal that, in single assay of PCAA and PaA, the sensitivity and specificity are 53 and 91% and 66 and 95%, respectively. When comparing single assay alone, the PaA test thus appears to provide a better discrimination between pancreatic cancer and benign pancreatic disease, other cancers, and normal individuals than does the PCAA test. In the simultaneous determinations of serum PCAA and PaA for pancreatic cancer, the sensitivity and specificity are 90 and 85%, respectively. The resulting sensitivity from the combined test is 37% higher than that of the PCAA test and 24% higher than that of the PaA test. Therefore, an overall evaluation indicates that the combined tests of PCAA and PaA are superior to either assay alone, although either test alone has a slightly higher specificity.

Some pancreatic tumor-associated antigens have been clinically evaluated. Hobbs et al. (6) demonstrated that the pancreatic oncofetal antigen ($M, 40,000$) had a 97% sensitivity and a 98% specificity for pancreatic cancer as assayed by rocket immunnoassay and 4 mg/ml as a cut-off limit. By using rocket immunnoelectrophoresis and 20 mg/ml as an upper limit, Gelder et al. (4) showed that pancreatic oncofetal antigen ($M, 8$ to $9 \times 10^5$) had a 48% sensitivity and a 90% specificity for pancreatic cancer. As determined by counterimmunoelectrophoresis in a limited number of patients, pancreatic tumor-associated antigen (14) ($M, 225,000$) had a sensitivity of 100% for pancreatitis and 44% for chronic alcoholics with a 93% specificity as assayed by radioimmunoassay. However, no clinical evaluation on pancreatic cancer was performed. The serum test of galactosyltransferase isoenzyme II was found to have a sensitivity of 67% and a specificity of 98% for pancreatic cancer by Podolsky et al. (13). The recently reported CA-19-9, a monoclonal antibody-defined tumor marker, yields a sensitivity of 79% and a specificity of 62% for the pancreatic cancer test (3). Thus, in comparison with the serodiagnostic tests available for pancreatic cancer, results of the combined test of PCAA and PaA are quite encouraging.

As shown in Chart 1, the degree of discrimination between pancreatic cancer and benign pancreatic disease by PCAA test is very poor. The reason for this result perhaps is due to the broad cross-reactivity of anti-PCAA antibody used in the test. Our recent study, using the immunoperoxidase technique, revealed that, after absorption with normal colonie mucosa extract, only pancreatic tumor and a few colonie and stomach cancers were positively stainable by the absorbed anti-PCAA. None of the nonneoplastic pancreatic lesions (7 normal, 5 benign) was positive (11). It is probable that an improved serum assay based upon the treated anti-PCAA reagent may result in a better clinical value. This approach is being investigated in our laboratory.

In conclusion, a simultaneous test of serum PCAA and PaA has been shown to yield an additive clinical value for serodiagnosis of pancreatic cancer. This paper also presents the difficulty in developing a specific serum test that will differentiate pancreatic cancer from benign pancreatic disease. Hopefully, combined with either ultrasound, computerized tomography, or endoscopic retrograde cholangiopancreatography (13), the simultaneous test of serum PCAA and PaA may provide a better diagnostic procedure for pancreatic carcinoma, which currently has the most dismal prognosis of all.

ACKNOWLEDGMENTS

The authors thank Joan Ogledzinski for secretarial assistance and Dr. Nelson Stack for statistical consultation.

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