Pancreatic Oncofetal Antigen and Carbohydrate Antigen 19-9 in Sera of Patients with Cancer of the Pancreas

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Pancreatic oncofetal antigen (POA) and carbohydrate antigen 19-9 (CA 19-9) were measured in the sera of 23 patients with cancer of the pancreas to determine the true positive rates of these cancer markers. In one group of unselected pancreatic cancer patients (n = 9), both tests showed above-normal results in three patients and both gave values that were within reference limits in three other patients. Two of the three remaining patients had increased CA 19-9 but normal POA values, and one patient had increased POA but normal CA 19-9 concentrations in serum. In another group of 14 pancreatic cancer patients, selected on the basis of increased concentrations of POA in serum, the CA 19-9 values were increased in eight. In four patients who had progressive disease, the concentrations of both markers increased with time in one patient, only POA in one, and only CA 19-9 concentration in another. (The fourth patient had increased but stable concentrations of POA and CA 19-9 in serum.) These data suggest that serum POA and CA 19-9 measurements should be used in combination in the evaluation of patients with cancer of the pancreas.

Additional Keyphrases: monitoring of cancer • immunoenzymometric assay • immunoradiometric assay • monoclonal antibodies

In the United States, cancer of the pancreas is the fourth leading cause of death from cancer (1). The increased incidence of this type of cancer over the past 35 years may be due to a strong association between smoking and cancer of the pancreas (2). The mortality rate during the first year after diagnosis is about 90% (3), with a five-year survival rate of <5% (1). The presenting symptoms of early pancreatic cancer are so nonspecific and ill-defined that a clinical diagnosis is usually not made in the early stages, thereby preventing a curative resection. Some 80% of patients have regional or distant metastases at initial diagnosis (2). Because metastatic disease is usually observed at initial diagnosis and most patients succumb to their disease despite therapy, monitoring of these patients with serological cancer markers is of limited clinical value. It is not clear whether this is due to late presentation of symptoms or early spread of the tumor. Current surgical and scanning procedures for the early diagnosis of pancreatic cancer are impractical as screening tests in that they are not cost-effective, and require highly trained personnel to perform and interpret them. A combination of serum markers for pancreatic cancer may aid in the early differential diagnosis of this disease.

Pancreatic oncofetal antigen (POA), first described by Banwo et al. in 1974 (4), is an 800 000–900 000 Da glycoprotein antigen (5, 6). POA is reported to be useful for preoperative differentiation between pancreatic cancer and benign diseases of the pancreas (7). The highest concentrations of POA in serum and the highest proportion of specimens with above-normal concentrations of POA have been found in patients with cancer of the pancreas (8). This, together with the increased concentrations of POA in fetal pancreas and in pancreatic cancer tissues (1, 4, 9–11), suggests the concept that POA is an oncofetal antigen that is more closely associated with the pancreas than with other organs. Increasing serum POA values generally correlate well with recurrence or progression of cancer of the pancreas (1, 5, 8, 11).

CA 19-9 is a tumor-associated antigen defined by a monoclonal antibody designated 1116 NS-19-9. The antigenic epitope that is measured is a monosialo-oligosaccharide, and in the circulation it is part of a carbohydrate-rich glycoprotein known as mucin (12–14). Development of an immunoradiometric assay (IRMA) has made it possible to measure CA 19-9 in blood and other body fluids (15). Published reports indicate that the concentration of CA 19-9 in serum is frequently increased in gastrointestinal malignancies (16–18), especially pancreatic adenocarcinomas (19–22). Assay of CA 19-9 in serum is reportedly useful in the monitoring of patients with cancer of the pancreas (21, 22), and currently it is the marker most widely used for cancer of the pancreas.

There are only two published reports on clinical usefulness of serum POA and CA 19-9 in patients with cancer of the pancreas (7, 23). In one report (7), both POA and CA 19-9 in serum were found to be markers of pancreatic cancer. Of 39 patients with cancer of the pancreas, 20 (51%) had increased values for both serum POA and CA 19-9, seven (18%) had only increased POA, and eight (21%) patients had only increased CA 19-9 values. In the other report (23), however, serum POA was not found to provide any information in addition to that provided by serum CA 19-9, which had a true-positive rate of 83% for pancreatic cancer. Here, we compare concentrations of POA and CA 19-9 in serum of 23 patients with cancer of the pancreas, assessing the frequency and extent of increase of these markers in these patients.

Materials and Methods

Patients. The 23 pancreatic cancer patients consisted of two groups. Group I (nine patients) consisted of current patients of the Kelsey-Seybold Clinic or the M. D. Anderson

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¹ Nonstandard abbreviations: POA, pancreatic oncofetal antigen; CA 19-9, carbohydrate antigen 19-9; BSA, bovine serum albumin; PBS, phosphate-buffered saline; IRMA, immunoenzymometric assay; and IRMA, immunoradiometric assay.
Cancer Center. In Group I, there was one patient with cystadenocarcinoma, one with small-cell carcinoma, and two with uncertain histology; the remaining five patients had histologically proven adenocarcinoma of the pancreas. Group II consisted of patients with adenocarcinoma of the pancreas who had increased POA concentrations in their serum. Their sera were part of a serum bank at Louisiana State University at Shreveport.

**Specimens.** Sera were prepared from blood specimens collected by venipuncture and were stored at 4°C for no longer than two weeks or at −70°C for up to two years. In our experience, POA and CA 19-9 in serum are both stable, based on their immunoreactivity, under those conditions.

**Assays.** POA was measured using a two-site, solid-phase monoclonal immunoenzymometric assay (IMMA). Antiserum was provided by Gelco Diagnostics, Inc., Shreveport, LA 71118. Polyclonal antibodies (IgG fraction) were absorbed onto 6.4-mm polystyrene beads with specular finish (Precision Plastic Ball Co., Chicago, IL 60641) as follows. A flask was thoroughly coated with a thin film of silicone (Sigma-cote, lot no. 125 P-6171; Sigma Chemical Co., St. Louis, MO 63178) according to the manufacturer's instructions. The anti-POA antibody, IgG fraction (1 g/L in a 1-g/L solution of sodium azide, lot no. BLI; Gelco Diagnostics), was then diluted 1:40 with phosphate-buffered isotonic saline (PBS; 75 mmol of sodium phosphate and 150 mmol of sodium chloride per liter, pH 7.4–7.5), and 150 μL of this solution was added per each plastic ball in the silicone-coated flask. The mixture was then shaken for 3 h at 37 ± 0.5°C in an orbital shaker at 180 rpm (Model 4730 Queue orbital shaker; Queue Systems, Inc., Parkersburg, WV 26102). The antibody-coated balls were then washed four times with equal volumes of PBS and added to an equal volume of 200 g/L bovine serum albumin (BSA; 98–99% pure albumin, lot no. 75 F-0006; Sigma Chemical Co.) in PBS. The mixture was then shaken at 180 rpm for 2 h at 37°C to block the nonspecific protein-binding sites of the beads. The excess, unbound albumin was then washed off the beads with four to six equal volumes of the PBS solution, and the beads were stored at 4–6°C in PBS. Horseradish peroxidase (EC 1.11.1.7), mostly isoenzyme C (Boehringer Mannheim Biochemicals, Indianapolis, IN 46250), was conjugated to a purified anti-POA monoclonal antibody (Gelco Diagnostics) by using the periodate method of Nakane and Kawaoi (24).

The optimized IMMA indices were as follows: sample, 200 μL of serum, diluted 1:100 in 5 g/L BSA (in PBS); peroxidase conjugate, 200 μL (1:250 dilution of our preparation of the peroxidase conjugate with 5 g/L BSA in PBS); two incubations each at 37°C for 1 h; reaction substrate, 300 μL (24 mmol/L o-phenylenediamine hydrochloride, 6 mmol/L hydrogen peroxide in 0.1 mmol/L citric acid, 0.2 mol/L sodium phosphate, pH 5.0); reaction time, 30 min at room temperature; and reaction quencher, 1.0 mL of 0.5 mol/L sulfuric acid.

Solutions containing patients’ sera or the peroxidase conjugate were washed off the beads by use of a Pentawash II multiple washer device (Abbott Laboratories, North Chicago, IL 60064) and de-ionized water. To determine POA concentrations, we measured the absorbance at 492 nm for the standard, control, and serum reaction tubes in a "Quantum II" photometer (Abbott Laboratories, Irving, TX 75015). This two-filter photometric system subtracts the absorbance at 600 nm from that at 492 nm for each reaction tube. For every assay run, we used eight standards with POA concentrations ranging from 0 to 50 mg/L (diluted 1:100 with 5 g/L BSA in PBS). The three POA control solutions, prepared from diluted pooled sera had mean POA concentrations of 8.5, 24.4, and 36.5 mg/L. Human POA standard, 65 mg/L in 1 g/L sodium azide solution, was supplied by Gelco Diagnostics (lot no. FE 3). The zero calibrator and diluent was 5 g/L BSA in PBS. The purified mouse anti-human POA monoclonal antibody was also from Gelco Diagnostics.

The described IMMA for POA had an interassay CV ranging from 5.1% to 11.3% for mean POA concentrations of 9.0 to 13.9 mg/L (n = 20). The assay showed adequate linearity in the range of 0 to 50 mg of POA per liter (diluted 1:100 for the assay); r = 0.97 on the average for four linearity curves. The detection limit, as determined by calculating the mean + 2 SD of replicate measurements of the zero calibrator, was 0.7 mg/L (n = 20). Analytical recovery ranged from 91.0% to 99.3% (mean, 94.9; n = 6).

CA 19-9 was measured by a two-site, solid-phase monoclonal IMMA (lot no. 7B0400; Centocor, Inc., Malvern, PA 19355) according to the manufacturer's procedure. We used an Abbott Pentawash II multiple washer device and an LKB 1282 Compugamma universal gamma counter set for 1-min countings of 125I and programmed according to the assay protocol (LKB Instruments, Inc., LKB Wallac, Turku, Finland). Interassay CVs ranged from 5.8% to 9.8% for mean CA 19-9 concentrations of 14.5 to 100.2 units/mL. Analytical recovery for CA 19-9 in the IMMA ranged from 85.1% to 106.0% (mean, 96.8; n = 8).

**Upper reference limits.** The upper reference limit for POA was determined by using 62 apparently healthy subjects. The upper reference limit for serum concentration of POA was 13.4 mg/L (95th percentile). We used the manufacturer's recommended upper reference limit for concentration of CA 19-9 in serum (37.0 units/mL), which agreed with our upper reference limit (determined by the 95th percentile method) of 30 units/mL.

**Results and Discussion**

**Group I.** The concentration of POA in serum was 0.2 to 2.8 times the upper reference limit; that of CA 19-9 ranged from less than 0.2 to 950 times the upper reference limit. Except for one patient (BM, Table 1), the concentrations of CA 19-9 in serum were moderately to significantly increased in the four patients (BM, GT, JS, RC) who had increased serum concentrations of POA. Two patients (TN and MD) had increased concentrations of CA 19-9, but their POA concentrations were within reference limits. The frequencies of increased values for either test alone and in combination were: POA, 4/9; CA 19-9, 5/9; POA or CA 19-9, 6/9; POA and CA 19-9, 3/9. Six of the nine patients had concordant values for POA and CA 19-9. That is, three patients had both markers increased and three had both markers within reference limits (see Table 1).

Patient BM had progressive, metastatic adenocarcinoma, which was reflected by increasing concentrations of POA in serum over a period of five months. This patient died from her cancer 3.5 months after her last marker values were obtained.

Patient GT had unresectable cancer of the pancreas diagnosed three months before her first marker values were obtained. Cancer of the pancreas was established, but the histology of adenocarcinoma was not proven. Serum concentrations of POA and CA 19-9 were stable during the one-week period between the two blood collections. This patient died from her cancer shortly thereafter. Increased concentra-
Table 1. Concentrations of POA and CA 19-9 In Serum of Group I Patients from Kelsey-Seybold Clinic (K-S) and M. D. Anderson Cancer Center (MDA)

<table>
<thead>
<tr>
<th>Patient (and institution)</th>
<th>Days after first blood collection</th>
<th>Histology (and location)</th>
<th>POA, CA 19-9- URL* x URL*</th>
</tr>
</thead>
<tbody>
<tr>
<td>BM (K-S)</td>
<td>55</td>
<td>Adenocarcinoma</td>
<td>1.0 x 0.2</td>
</tr>
<tr>
<td></td>
<td>151</td>
<td>Adenocarcinoma</td>
<td>2.8 x 0.3</td>
</tr>
<tr>
<td>EN (K-S)</td>
<td>6-11</td>
<td>Adenocarcinoma, moderately differentiated (head of pancreas)</td>
<td>0.8 x 0.4</td>
</tr>
<tr>
<td>TN (MDA)</td>
<td>7</td>
<td>Uncertain histology</td>
<td>0.4 x 185</td>
</tr>
<tr>
<td>GT (MDA)</td>
<td>7</td>
<td>Uncertain histology</td>
<td>1.2 x 123</td>
</tr>
<tr>
<td>MD (MDA)</td>
<td>7</td>
<td>Cystadenocarcinoma (tail of pancreas)</td>
<td>0.5 x 930</td>
</tr>
<tr>
<td>JS (MDA)</td>
<td>4</td>
<td>Small cell carcinoma (head of pancreas)</td>
<td>0.7 x 1.1</td>
</tr>
<tr>
<td></td>
<td>11</td>
<td></td>
<td>0.4 x 1.3</td>
</tr>
<tr>
<td></td>
<td>18</td>
<td></td>
<td>0.4 x 1.8</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td></td>
<td>1.2 x 1.7</td>
</tr>
<tr>
<td>RC (MDA)</td>
<td>11</td>
<td>Adenocarcinoma</td>
<td>1.2 x 1.7</td>
</tr>
<tr>
<td>WW (MDA)</td>
<td>3</td>
<td>Adenocarcinoma (head of pancreas)</td>
<td>0.9 x &lt;0.2</td>
</tr>
<tr>
<td>CD (MDA)</td>
<td>3</td>
<td>Adenocarcinoma</td>
<td>0.9 x &lt;0.2</td>
</tr>
</tbody>
</table>

*URL: upper reference limit.

Concentrations of POA 19-9 and POA in serum were indicative of her metastatic disease.

Patients JS and RC both died from progressive metastatic carcinoma of the pancreas within one month after their last marker values were obtained. For JS, both markers reflected disease progression over a one-month period. However, only the concentration of CA 19-9 increased over a period of 11 days for RC.

Patient WW is doing well 13 months after his markers were obtained. This patient had moderately differentiated adenocarcinoma of the pancreas metastatic to the porta hepatitis. After surgery, he has received three courses of 5-fluorouracil and adriamycin, and has responded well. Values for POA and CA 19-9 were within reference limits. Repeat analyses three days later gave similar results.

Group II. Eight of the 14 patients in this group, all of whom had adenocarcinoma of the pancreas and were selected for their increased serum concentrations of POA, had moderately to extremely increased (5.4 to 331 times the upper reference limit) concentrations of CA 19-9 (Table 2). They all had metastatic carcinomas (stages III and IV) involving the liver (eight patients) and one or more lymph nodes (all patients). Generally, the patients with increased concentrations of CA 19-9 had higher POA values: the concentration of POA (mean ± SD) was 7.1 ± 5.3 times the upper reference limit for specimens with increased CA 19-9 values (n = 6), whereas the mean POA value was 4.6 ± 2.4 times the upper reference limit for specimens with normal concentrations of CA 19-9 in the serum.

In Group I, serum CA 19-9 was a more sensitive marker than serum POA for cancer of the pancreas. Serum POA was increased, however, in one patient (BM) who had normal values for CA 19-9.

Values for CA 19-9 were increased in eight of 14 patients (57%) in Group II (who had been selected for their increased serum concentrations of POA). This observation supports the need for use of both markers to increase the clinical sensitivity for cancer of the pancreas.

In patients with serum marker values, there was a general correlation between progression of the disease and concentrations of the cancer markers (Table 1).

We have shown that both POA and CA 19-9 are frequently elevated in serum of patients with cancer of the pancreas. In agreement with the observations made by Takami et al. (7), our data indicate that these markers complement each other. In some patients only POA is increased, in others only CA 19-9. In our opinion, both CA 19-9 and POA should be used as a test profile for adenocarcinoma of the pancreas.

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Table 2. Concentrations of POA and CA 19-9 In Serum of Group II Patients from LSU Medical Center

<table>
<thead>
<tr>
<th>POA, x URL*</th>
<th>CA 19-9, x URL</th>
<th>POA, x URL</th>
<th>CA 19-9, x URL</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.9 x 2.0</td>
<td>331 x 76.5</td>
<td>2.9 x 3.2</td>
<td>15.9 x 2.1</td>
</tr>
<tr>
<td>4.4 x 5.7</td>
<td>&lt;0.2 x 5.4</td>
<td>&lt;0.2 x 8.2</td>
<td>&lt;0.2 x 5.4</td>
</tr>
<tr>
<td>2.1 x 8.2</td>
<td>284 x 158</td>
<td>7.6 x 19.1</td>
<td>16.5</td>
</tr>
</tbody>
</table>

*Each pair of values corresponds to a different patient. *URL: upper reference limit.

References