

Serum Markers in Patients with Resectable Pancreatic Adenocarcinoma: Macrophage Inhibitory Cytokine 1 versus CA19-9

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Abstract Purpose: More accurate serum markers of pancreatic cancer could improve the early detection and prognosis of this deadly disease. We compared the diagnostic utility of a panel of candidate serum markers of pancreatic cancer.

Experimental Design: We collected preoperative serum from 50 patients with resectable pancreatic adenocarcinoma, as well as sera from 50 patients with chronic pancreatitis and 50 age/sex-matched healthy controls from our institution. Sera were analyzed for the following candidate markers of pancreatic cancer: CA19-9, macrophage inhibitory cytokine 1 (MIC-1), osteopontin, tissue inhibitor of metalloproteinase 1, and hepatocarcinoma-intestine-pancreas protein levels.

Results: By logistic regression analysis, MIC-1 and CA19-9 were significant independent predictors of diagnosis. Receiver operating characteristic curve analysis showed that MIC-1 was significantly better than CA19-9 in differentiating patients with pancreatic cancer from healthy controls (area under the curve is 0.99 and 0.78, respectively; $P = 0.003$), but not in distinguishing pancreatic cancer from chronic pancreatitis (area under the curve of 0.81 and 0.74, respectively; $P = 0.63$). Hepatocarcinoma-intestine-pancreas/pancreatitis-associated protein, osteopontin, and tissue inhibitor of metalloproteinase 1 serum levels did not provide additional diagnostic power.

Conclusion: In the differentiation of patients with resectable pancreatic cancer from controls, serum MIC-1 outperforms other markers including CA19-9.

Pancreatic adenocarcinoma is the fourth leading cause of cancer death and has the lowest survival rate for any solid cancer (1, 2). One important explanation for this poor survival is that only 10% to 15% of patients present with small, resectable cancers (1). Because patients with resectable cancers can achieve a 5-year survival of 15% to 40% after pancreaticoduodenectomy (3), improved pancreatic cancer diagnosis could save lives. Indeed, screening individuals whose family history indicates an increased risk of developing pancreatic cancer can lead to the early diagnosis and treatment of their pancreatic neoplasms (4–6). Pancreatic cancer diagnosis is often delayed because patients present with nonspecific symptoms, experience delays in referral to specialized diagnosis

services, or undergo imaging tests with suboptimal sensitivity for identifying small masses (1, 7). An accurate serologic test could facilitate the rapid diagnosis of pancreatic cancer. It might also help in the screening of populations at high-risk for early pancreatic neoplasia, and improve the monitoring of patients undergoing treatment (8). The most widely used serum marker for pancreatic cancer, CA19-9, is not sufficiently accurate to be useful as a diagnostic test, especially for identifying patients with small surgically resectable cancers (8–13). CA19-9 is frequently elevated in patients with various benign pancreaticobiliary disorders, including cholestasis and pancreatitis (10–12).

Several marker candidates of pancreatic cancer have recently emerged from studies identifying genes or proteins expressed in pancreatic neoplasia and in tissues adjacent to infiltrating pancreatic ductal adenocarcinomas (14), including macrophage inhibitory cytokine 1 (MIC-1; ref. 15), osteopontin (16), tissue inhibitor of metalloproteinase 1 (TIMP-1; ref. 17), and hepatocarcinoma-intestine-pancreas protein (HIP; ref. 18). Many other candidate pancreatic cancer serum markers have been evaluated using specific assays but have not been found to be superior to CA19-9. These markers include CEA (19), amylin (islet amyloid polypeptide; ref. 20), DUPAN-2 (21), CA242 (12), CAM 17.1 (22), TPS (23), CA72-4 (19), SPan-1 (24), CA50 (25), CA195 (26), TATI (27), POA (28–30), YKL-40 (31), TUM2-PK (32). Other markers such as peptides identified by surface-enhanced laser desorption and ionization (33, 34), and other markers such as MUC4 (35) and synuclein (36) may prove useful but await confirmatory studies and assays

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Table 1. Serum levels of biomarkers in normal controls, chronic pancreatitis, and pancreatic cancer

| | Healthy controls | Chronic pancreatitis | Pancreatic cancer |
|---------------------|------------------|----------------------|-------------------|
| CA19-9 (units/mL) | | | |
| Mean | 21.8 | 27.8 | 83.5 |
| Median | 16.2 | 19.3 | 51.1 |
| SD | 18.1 | 40.6 | 75.2 |
| HIP (ng/mL) | | | |
| Mean | 11.0 | 21.4 | 23.0 |
| Median | 8.1 | 12.6 | 14.1 |
| SD | 9.7 | 21.1 | 26.9 |
| TIMP-1 (ng/mL) | | | |
| Mean | 1,102 | 1,382 | 1,784 |
| Median | 1,047 | 1,195 | 1,577 |
| SD | 231 | 740 | 711 |
| Osteopontin (ng/mL) | | | |
| Mean | 321 | 408 | 623 |
| Median | 258 | 366 | 604 |
| SD | 213 | 270 | 327 |
| MIC-1 (pg/mL) | | | |
| Mean | 759 | 2,368 | 5,388 |
| Median | 662 | 1,760 | 4,745 |
| SD | 412 | 2,431 | 3,720 |

optimized for serum analysis. We recently showed that combined serum MIC-1 and CA19-9 measurements had superior diagnostic utility compared with CA19-9 alone for differentiating patients with pancreatic cancer from those without periampullary neoplasia (15). MIC-1 (also known as placental transforming growth factor- β ; ref. 37), prostate-derived factor (38), growth/differentiation factor 15/MIC-1 (39), and placental bone morphogenetic protein (40), is a distant member of the transforming growth factor- β superfamily originally identified in the setting of macrophage activation (41). MIC-1 is overexpressed in several cancers including colon (42) and prostate cancer (43), and is elevated in the serum of many patients with colon cancer (44). MIC-1 may also have anticancer functions (45).

We evaluated the diagnostic accuracy of five candidate pancreatic cancer serum markers: MIC-1, CA19-9, TIMP-1, osteopontin, and HIP/pancreatitis-associated protein (PAP). Our previous studies indicated that MIC-1 or osteopontin might be at least as sensitive as CA19-9 in the setting of resectable pancreatic cancer. Although previous studies indicated that TIMP-1 and HIP/PAP are not as sensitive as CA19-9 in this setting, we hypothesized that use of a combination of markers derived from different compartments of the pancreas [pancreatic cancer cells (MIC-1, CA19-9), peritumoral stromal (osteopontin, TIMP-1), and the acinar compartments (HIP/PAP)] might improve the sensitivity of the marker panel.

Materials and Methods

We selected a population of patients with resectable pancreatic cancer, chronic pancreatitis, and healthy, age- and gender-matched controls. Preoperative sera were collected from 50 patients with pancreatic adenocarcinoma, 50 with chronic pancreatitis, and 50 healthy, age- and sex-matched controls attending the Johns Hopkins

Medical Institutions. These patients did not include any of the patients presented in our previous report of serum MIC-1 (15). Serum was collected from the patients with pancreatic cancer and chronic pancreatitis undergoing pancreatic surgery or endoscopic evaluation between 2002 and 2004 for the purpose of evaluating candidate serum markers of pancreatic cancer and markers were analyzed at the same time in the first half of 2004. Blood was collected from fasting patients immediately prior to surgery or endoscopy. For both groups, patients typically fasted from midnight to the day of the procedure. The control sera were collected between 1997 and 2002 after an overnight fast. None of the study subjects had been included in previous published studies of MIC-1 or the other markers. Diagnoses of pancreatic ductal adenocarcinoma were derived from histopathologic examination of the pancreaticoduodenectomy specimens. The tumor-node-metastasis stage distribution was: T₁N₀M_x, n = 1; T₁N₁M_x, n = 2; T₂N₀M_x, n = 2; T₂N₁M_x, n = 3; T₃N₀M_x, n = 9; T₃N₁M_x, n = 30; T₄N_xM_x, n = 2; T₄N₁M_x, n = 1. Chronic pancreatitis was diagnosed using histopathologic resections in 19 patients, by fine-needle aspiration cytology in 6 patients, and by endoscopic findings (endoscopic retrograde cholangiopancreatography and/or endoscopic ultrasound along with clinical information in 25 patients). Subjects in the healthy control group were taking part in a longitudinal study of aging. The mean age \pm SD and gender were: pancreatic adenocarcinoma, 67.6 \pm 10.1 years (56% female); chronic pancreatitis, 57.0 \pm 14.5 years (50% female); controls, 61.1 \pm 9.2 years (72% female). Controls were selected to match for age, and then if possible, for gender, although there were more female controls than cases, we have not observed that MIC-1 levels are affected by gender in previous studies. All sera were collected using standard procedures and stored at -80°C until analysis. A new aliquot of sera was used from patients in each group to minimize any problems related to sample degradation. The study was approved by the Johns Hopkins Committee for Clinical Investigation.

The ELISA for MIC-1 was done as previously published (46). MIC-1 measurements were done by personnel blinded to the diagnosis of each serum sample. CA19-9, TIMP-1, HIP/PAP, and osteopontin levels were measured using commercially available ELISA assays according to the manufacturer's instructions (CA19-9, Alpha Diagnostics, San Antonio, TX; TIMP-1, Amersham Biosciences, Piscataway, NJ; osteopontin, EMD Biosciences, San Diego, CA). The commercial assays were done using an EL_x50 automated ELISA plate washer (Bio-Tek Instruments, Inc., Highland Park, VT). Absorbance was read on an EL312e spectrophotometer and results calculated with the KC junior software package v 1.40.3 (Bio-Tek Instruments).

Statistics. Mean marker levels were evaluated using the entire data set. All subsequent analyses were completed after the entire data set was

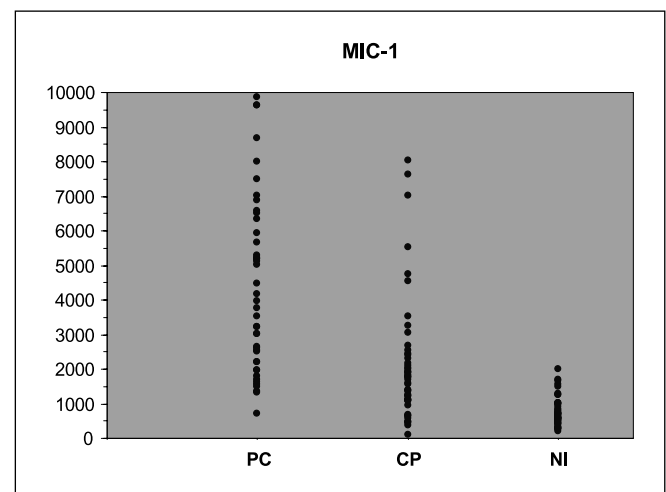


Fig. 1. Dot plot of serum MIC-1 levels. PC, pancreatic cancer; CP, chronic pancreatitis; NI, normal pancreas.

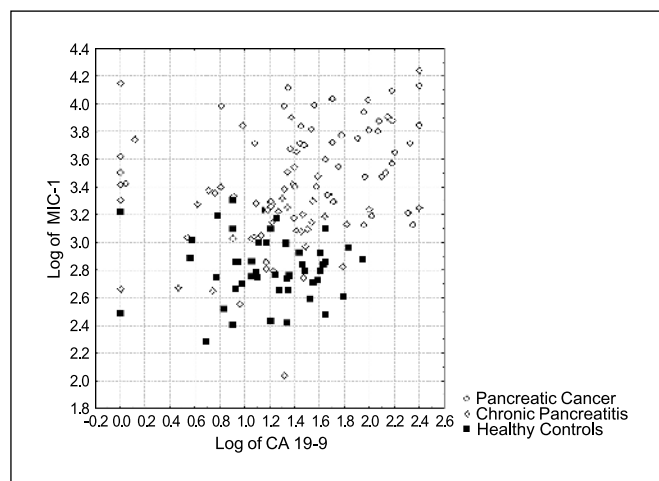


Fig. 2. A scatter plot of serum CA19-9 (horizontal axis) and MIC-1 levels (vertical axis). Most healthy controls are plotted on the lower half (low MIC-1 levels) and the left two-thirds of the scatter plot (low CA19-9 levels). In contrast, almost all patients with pancreatic cancer have elevations of one or both of these two markers (top and right corner of the scatter plot).

randomly split into training and testing data sets, each containing 50% of the samples. We hypothesized that combining multiple markers via multivariate logistic regression would improve diagnostic accuracy. We combined the markers MIC-1, CA19-9, HIP/PAP, osteopontin, and TIMP-1 to form three models comparing cancer versus normal healthy controls, cancer versus noncancer (normal healthy control and chronic pancreatitis), and cancer versus chronic pancreatitis. In order to evaluate such models, we constructed each model using the training data and tested it on the remaining samples. The testing data were used to construct receiver operating characteristic (ROC) curves and compare the area under the curves (AUC). In addition to ROC curves, we also describe diagnostic cutoffs for each marker which were chosen as 2 SD above the mean for the healthy control group apart from CA19-9. The diagnostic cutoff of 37 IU for CA19-9 is the cutoff recommended by the manufacturer. Elevated MIC-1, osteopontin, HIP/PAP, and TIMP-1 were chosen as 2 SDs above the healthy control mean. For CA19-9, a cutoff value of 37 units/mL was used according to the manufacturer's specifications for the reference range of CA19-9.

Statistical calculations were carried out using Statistica (version 7), MATLAB (release 12), and ROCKIT (version 0.9.1). The ROCKIT

program fits a binormal ROC curve by calculating maximum-likelihood estimates of the variables of a conventional binormal model. The AUC is estimated from the model. Comparison of ROC curves was done using a univariate z-score test of the difference between the areas under the two ROC curves. A scatter plot matrix was created to examine the behavior of marker combinations. Each scatter plot (box) represents the results for two markers for each individual in the study. An individual's result is a point on a graph with the point's position on the x-axis representing the result of one marker and its y-axis position, the result for a second marker (Fig. 2 displays the scatter plot of MIC-1 and CA19-9). The scatter plot matrix plots all of the scatter plots for all possible marker combinations together using the same x and y axes.

Results

The mean, medians, and SDs for each marker in each group is provided in Table 1. A plot of the individual MIC-1 results for each subject is provided in Fig. 1. A dot plot of all of the log-transformed results of each individual marker is available at <http://pathology2.jhu.edu/pancreas/serummic1paper.pdf>. Ninety percent of patients with pancreatic cancer had MIC-1 levels >2 SD above age-matched controls. Unlike CA19-9, MIC-1 elevations were independent of tumor-node-metastasis stage: six of eight patients with T₁ or T₂ cancers had elevated MIC-1, whereas only three of these eight patients had elevated CA19-9. Three of the five patients with pancreatic cancer and a normal MIC-1 level had an elevated CA19-9 level. However, the added diagnostic yield of combining both markers to distinguish healthy controls from those with pancreatic cancer was not statistically significant.

We also examined patients with pancreatic cancer or pancreatitis who did not have elevated MIC-1 levels to determine if patients with concomitant jaundice or diabetes invariably experienced elevated MIC-1 levels. We were able to identify five patients with pancreatic cancer or pancreatitis with elevated preoperative bilirubin levels and three with elevated glucose levels that had normal MIC-1 levels. Similarly, an elevated MIC-1 level was not invariably associated with preoperative jaundice or hyperglycemia. Serum osteopontin was not as sensitive for pancreatic cancer as in a previous study (16), most likely because of differences in the assay used for osteopontin measurement.

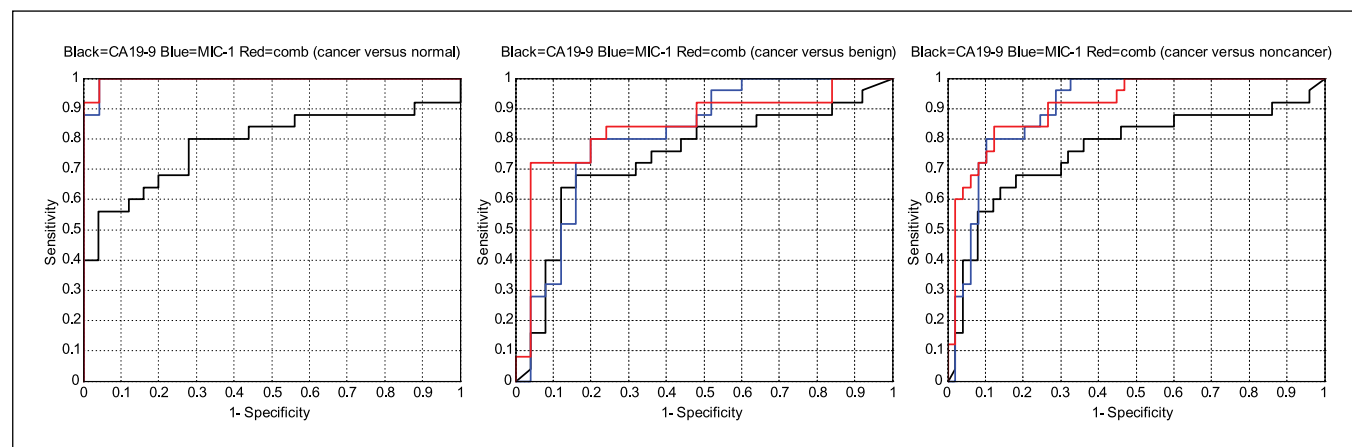


Fig. 3. ROC analysis of MIC-1 and CA19-9. Left ROC curve, pancreatic cancer versus normal controls: black, CA19-9 (AUC, 0.78); blue, MIC-1 (AUC, 0.99); red, combination (AUC, 0.99). Central curve, pancreatic cancer versus chronic pancreatitis: black, CA19-9 (AUC, 0.74); blue, MIC-1 (AUC, 0.81); red, combination (AUC, 0.84). Right curve, pancreatic cancer versus noncancer: black, CA19-9 (AUC, 0.76); blue, MIC-1 (AUC, 0.90); red, combination (AUC, 0.91).

Table 2. Diagnostic performance of individual serum markers in the testing data set

| | | Sensitivity | Specificity | Accuracy | AUC |
|---------------------------------|-----------|-------------|-------------|----------|------|
| CA19-9, cutoff (37 units/mL) | PC vs. CP | 0.62 | 0.86 | 0.74 | 0.74 |
| | PC vs. N | 0.62 | 0.80 | 0.71 | 0.78 |
| HIP/PAP, cutoff (30.4 ng/mL) | PC vs. CP | 0.22 | 0.80 | 0.51 | 0.54 |
| | PC vs. N | 0.22 | 0.96 | 0.59 | 0.75 |
| TIMP-1, cutoff (1,564 ng/mL) | PC vs. CP | 0.50 | 0.72 | 0.61 | 0.66 |
| | PC vs. N | 0.50 | 0.96 | 0.73 | 0.85 |
| Osteopontin, cutoff (747 ng/mL) | PC vs. CP | 0.34 | 0.92 | 0.83 | 0.72 |
| | PC vs. N | 0.34 | 0.94 | 0.83 | 0.86 |
| MIC-1, cutoff (1,583 pg/mL) | PC vs. CP | 0.90 | 0.44 | 0.67 | 0.81 |
| | PC vs. N | 0.90 | 0.94 | 0.92 | 0.99 |

NOTE: PC, pancreatic cancer; CP, chronic pancreatitis; N, normal controls.

To provide additional information about how markers behaved in combination in each subject, we created scatter plots of the marker data. Overall, this data indicated that the marker combination that seemed to best distinguish between disease groups was MIC-1 and CA19-9 (scatter plot shown in Fig. 2). There was little evidence that combining any other markers effectively separated the groups. Some markers such as TIMP-1 tended to correlate with other markers such as MIC-1 and osteopontin, whereas HIP/PAP correlated poorly with most of the other markers (data not shown). A scatter plot matrix of each marker pair is available at <http://pathology2.jhu.edu/pancreas/serummic1paper.pdf>.

To further determine which markers were independent predictors of diagnosis, the data were split into training and testing sets ($n = 25$ for each group). Logistic regression analysis indicated that only MIC-1 and CA19-9 were significant independent predictors in the training data for the comparison of patients with pancreatic cancer and normal healthy controls, the comparison between pancreatic cancer patients and pancreatitis patients, and the comparison between pancreatic cancer patients and noncancer (benign and healthy) patients. ROC analysis was subsequently carried out using the testing set data to evaluate the diagnostic utility of only the MIC-1 and CA19-9 results that had been identified as the independent predictors in the training set data. MIC-1 did significantly better than CA19-9 at differentiating patients with pancreatic cancer from healthy controls [AUC with 95% confidence intervals is 0.99 (0.86-1.00) versus 0.78 (0.61-0.88), $P = 0.003$, see Fig. 3A]. In the comparison between patients with chronic pancreatitis and those with pancreatic cancer, CA19-9 and MIC-1 performed equivalently [AUC, 0.74 (0.57-0.84) and 0.81 (0.68-0.92); $P =$ not significant, see Fig. 3B]. Combining both markers was not significantly superior to either marker alone [AUC, 0.84 (0.70-0.92)]. MIC-1 outperformed CA19-9 in distinguishing patients with pancreatic cancer from those without cancer [chronic pancreatitis and healthy controls: AUC, 0.90 (0.82-0.96) versus 0.76 (0.62-0.86); $P = 0.06$, see Fig. 3C]. When CA19-9 and MIC-1 were combined for this comparison, they performed significantly better than CA19-9 [AUC, 0.91 (0.82-0.96); $P = 0.01$]. No other marker provided added diagnostic value to the logistic regression model which included CA19-9 and MIC-1. The diagnostic performance

of each individual marker in the testing data set is given in Table 2.

Discussion

A useful serum marker of pancreatic cancer is urgently needed and the overall diagnostic superiority of MIC-1 over CA19-9 is promising. Most pancreatic cancers overexpress MIC-1 (15), and in this study, most patients had elevated MIC-1 levels, even patients with small early stage cancers. This group constituted only a small proportion of the patients reflecting how few patients diagnosed with pancreatic cancer present with early stage disease (~2-3%). These results suggest that serum MIC-1 could be particularly helpful in the early detection of pancreatic cancer. Recent studies indicate that populations at high-risk, such as those with a strong family history of pancreatic cancer, could benefit from pancreatic screening with endoscopic ultrasound and computed tomography (4-6). A simple, noninvasive serum test could aid in the pancreatic surveillance of these individuals at high-risk. Serum MIC-1 levels are stable over time in healthy individuals⁶ raising the possibility that rising MIC-1 levels could be used as an indicator of invasive cancer in at-risk individuals. In addition, because 90% of patients with pancreatic cancer in our study had elevated MIC-1 and 96% had elevated MIC-1 or CA19-9, the measurement of these markers could potentially be used in conjunction with pancreatic imaging and other clinical information to help rule out a diagnosis of pancreatic cancer. Although promising, MIC-1 has limitations as a serum marker for detecting pancreatic cancer. Like CA19-9, serum MIC-1 levels are often elevated in the setting of pancreatitis. Indeed, CA19-9 was less commonly elevated in patients with pancreatitis in this study than was MIC-1. These results indicate that MIC-1 is not useful for distinguishing pancreatic cancer from chronic pancreatitis. In addition, because MIC-1 is a transforming growth factor- β family cytokine expressed in macrophages, it is likely to be elevated in the serum of patients with diseases associated with macrophage activation. Despite the

⁶ Breit and coworkers, unpublished data.

findings that MIC-1 levels need not be elevated in patients with jaundice or diabetes, further study of MIC-1 performance among patients with other inflammatory diseases and diseases that can present with similar features to pancreatic cancer, such as diabetes and jaundice, is also important. In addition to patients with pancreatic cancer, patients with other periampullary cancers show elevations of MIC-1 (15) and several other cancers overexpress MIC-1 (42, 43, 47), suggesting that serum MIC-1 measurement could aid in the diagnosis of several

cancers. Large prospective studies should help provide more precise estimates of MIC-1 sensitivity and specificity for pancreatic cancer.

In conclusion, we find that serum MIC-1 is a more sensitive marker of pancreatic cancer than is CA19-9. Because serum MIC-1 is elevated in the setting of chronic pancreatitis, the goal of identifying serum markers that can accurately distinguish patients with chronic pancreatitis from those with pancreatic cancer remains.

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