Cancer Antigens 19-9 and 125 in the Differential Diagnosis of Pancreatic Mass Lesions

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Hypothesis: Accurate differentiation between inflammatory and neoplastic tumors of the pancreas remains a diagnostic dilemma for surgeons. The aim of the study was to assess the utility of 2 neoplastic markers, cancer antigen (CA) 19-9 and CA 125, in the differential diagnosis of pancreatic tumors.

Main Outcome Measures: Sensitivity, specificity, and positive and negative predictive values of each test in the differential diagnosis of pancreatic tumors were determined.

Design: The patients were assigned to a malignant or benign group based on cytological and histological evaluation of pancreatic lesion samples. The serum from each patient was tested for CA 19-9 and CA 125.

Setting and Patients: One hundred ten patients with heterogeneous pancreatic lesions (inflammatory and malignant tumors) treated at a surgical department of a university hospital were analyzed.

Interventions: Samples for cytological and histological evaluation were taken during ultrasonography-guided fine-needle aspiration biopsy or open surgery.

Results: The sensitivity, specificity, positive predictive value, and negative predictive value of the CA 19-9 test were 80.8%, 89.1%, 93.7%, and 89.2%, respectively. The sensitivity, specificity, positive predictive value, and negative predictive value of the CA 125 test were 60.8%, 83.3%, 88.2%, and 50.8%, respectively. The sensitivity and specificity of a combined evaluation of both CA 19-9 and CA 125 tests were 87.8% and 77.8%, respectively.

Conclusions: Taking into account the high but still limited sensitivity and specificity of the CA 19-9 and CA 125 tests, their results in the differential diagnosis of pancreatic tumors should be interpreted consistently and in reference to imaging techniques such as ultrasonography and computed tomography.

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Ductal pancreatic carcinoma reveals expression of mucin-related carbohydrate antigens such as carcinoembryonic antigen, cancer antigen (CA) 19-9, DU-PAN-2, Span 1, mucin MUC 1, and cytokeratins 7, 8, 18, and 19. The CA 19-9 marker is an antigen determinant, defined by murine monoclonal antibody 1116-NS-19-9, resulting from the immunization by cancer cells and is specific for the colon carcinoma–derived cell lines. An elevated CA 19-9 serum level has been widely demonstrated in pancreatic adenocarcinoma. Cancer antigen 19-9 is described as the most useful serological marker in the diagnosis of pancreatic carcinoma, with its specificity and sensitivity reported to be 63% to 93% and 78% to 98%, respectively. A markedly elevated CA 19-9 level can also be seen in extrapancreatic tumors and benign lesions, particularly with a concomitant high bilirubin level. The CA 19-9 marker is a reliable diagnostic test when it is evaluated by US, endoscopic US, or CT.

The CA 125 assay is another cancer test based on a monoclonal antibody, OC 125, that was originally raised against the epithelial ovarian carcinoma cell line. An elevated CA 125 level was found in some neoplasms, such as ovarian, breast, and lung carcinoma, and also in some nonneoplastic disorders, such as endometriosis. Recently, the utility of the CA 125 marker in the diagnosis of pancreatic carcinoma, with slightly less sensitivity than the CA 19-9 marker, has been confirmed in some articles.

A high level of the marker is usually associated with a large tumor size as well as a high stage of advancement. The mean values of the marker levels tend to increase from stage I to stage IV (according to the Union Internationale Contre le Cancer TNM classification), and they are significantly higher in patients with unresectable tumors. This allows for the use of the markers as an indicator of some limit beyond which the likelihood of unresectability of the tumor is very high. The marker levels may also be considered a prognostic factor. An increase of the marker levels indicates progression of the disease and suggests that a patient has a very small chance of surviving longer than 5 to 9 months.

The aim of this study was to evaluate the utility of serum CA 19-9 and CA 125 measurements in the differential diagnosis of pancreatic tumors.

**METHODS**

One hundred ten patients with heterogeneous pancreatic lesions were diagnosed and treated at the Second Department of General Surgery, Skubiszewski Medical University of Lublin, Lublin, Poland, between June 1, 1998, and June 30, 2002. Their ages ranged between 26 and 78 years. Abdominal US was performed in all of the patients in our US unit. For a more precise evaluation of the lesion, a spiral CT scan with 5-mm scans was carried out in 76 patients at the Radiology Department, Skubiszewski Medical University of Lublin. After a detailed analysis of US and CT presentation of the lesion, the patients were preliminarily qualified for US-guided fine-needle aspiration biopsy. The biopsy samples were taken twice from 2 distant areas (central and peripheral). In 13 patients, the biopsy was repeated owing to difficulty in cytological evaluation, the samples being taken in an inappropriate manner (cell-lacking samples), or a discrepancy between the microscopical and preliminary US and/or CT evaluation results. The cytological evaluation was carried out immediately after receiving the samples and staining with hematoxylin-eosin. Eventually, the results of the cytological evaluation were verified during open resection surgery, bypass surgery, or exploratory laparotomy. The patients with cholestasis and inoperable tumors underwent endoscopic stent placement or percutaneous biliary drainage when necessary.

Serum amylase, lipase, bilirubin, aspartate aminotransferase, alanine aminotransferase, and neoplastic marker levels were analyzed in all of the patients initially at the time of diagnosis and during the follow-up program.

**ASSAY FOR CA 19-9 AND CA 125 MARKERS**

The VIDAS (bioMérieux, Marcy L’Etoile, France) CA 19-9 test using murine 1116-NS-19-9 antibody was used for the measurement of the CA 19-9 marker level. This antibody binds reactive antigen determinants fixed to tumor glycoproteins of high molecular weight. The VIDAS CA 125 II assay, a second-generation test using monoclonal antibodies OC 125 and M11 and recognizing the remaining epitope of the OC 125 determinant antigen, was used for the measurement of the CA 125 marker level. Both tests are based on an immunoenzymatic, sandwich, dual-phase reaction with ending fluorescence recording. The test results were expressed in units per milliliter. The basic dilutions allowed for the measurements of the marker level between 0 and 900 U/mL with the possibility of further dilution and the measurement of the maximal marker level at 100 000 U/mL.

The cutoff values of the CA 19-9 and CA 125 levels that differentiated malignant and benign lesions were set at 37 U/mL and 35 U/mL, respectively. These values are characteristic for intestinal-derived adenocarcinomas and provide optimal discrimination of malignancy as it has been described previously by others.

**STATISTICAL ANALYSIS**

The diagnostic accuracy of the CA 19-9 and CA 125 tests was determined using standard definitions. Sensitivity of the tests was defined as the number of true-positive results divided by the total number of patients with confirmed pancreatic carcinoma. Specificity of the tests was defined as the number of true-negative results divided by the total number of patients without pancreatic carcinoma. Predictive value of a positive test was defined as the number of true-positive results divided by the total number of positive test results. Predictive value of a negative test was defined as the number of true-negative results divided by the total number of negative test results. In patients with benign pancreatic tumors, a positive result of the CA 19-9 or CA 125 test was regarded as false positive, and a result of the CA 19-9 or CA 125 test lower than the cutoff level was regarded as true negative. In patients with confirmed pancreatic carcinoma, a positive result of the CA 19-9 or CA 125 test was regarded as true positive, and a negative result of the CA 19-9 or CA 125 test was regarded as false negative.
the remaining 3 (2.7%). Among the 83 patients with the tumor at the head of the pancreas, an elevated bilirubin level (>2 mg/dL) was found in 56 (67.5%).

Overall, 246 US-guided fine-needle aspiration biopsies were carried out in the 110 patients. The procedure was repeated in 13 patients owing to an equivocal result. The cytological evaluation revealed carcinomatous cells or cell atypia indicating a malignant nature of the lesion in 65 patients (59.1%). After clinical and histopathological evaluation, a malignant pancreatic tumor was diagnosed in 73 (66.4%) of 110 patients, and a benign pancreatic tumor was diagnosed in the remaining 37 patients (33.6%). The malignant lesions composed a homogeneous group of pancreatic ductal adenocarcinomas. Similarly, there was a homogeneous group of patients with benign tumors who had chronic pancreatitis.

Palliative treatment owing to unresectability of the tumor or inoperability of the patient was recommended in 41 patients (56.2%) with pancreatic cancer. Thirty-two patients (43.8%) with pancreatic cancer were referred for open resection surgery. Curative resection (TNM classification R0) was possible to achieve in only 15 patients (46.9%). Palliative treatment in the form of surgical bypass procedures or endoscopic stenting was performed in the remaining 17 patients (53.1%). Fifteen patients (40.5%) without confirmation of a malignant nature of the lesion underwent surgical resection, and the remaining 22 patients (59.5%) were not referred for surgical treatment.

The CA 19-9 marker was assayed in all of the 110 patients with pancreatic tumors. The levels ranged from less than 3 to 56 000 U/mL. The cutoff level was set at 37 U/mL (dotted line). In patients with confirmed pancreatic carcinoma, the CA 19-9 level exceeded the cutoff level of 37 U/mL in the majority (59 [80.8%]) of 73 cases. However, in the remaining 14 patients (19.2%) with pancreatic carcinoma, the CA 19-9 level was lower than or approximately 37 U/mL. A CA 19-9 level higher than 500 U/mL was found in 29 (39.7%) of the 73 patients with confirmed pancreatic carcinoma, and in 23 (79.3%) of those 29 patients, advanced neoplastic disease with distant metastases, extensive lymph node involvement, or massive local infiltration was demonstrated. In this group of patients, no false-positive results were found.

In patients with benign pancreatic tumors, the CA 19-9 level ranged between 0 and 150 U/mL. In the majority (33 [89.2%]) of these 37 patients, the CA 19-9 values were low and ranged between 0 and 37 U/mL. The marker level exceeded the cutoff value in 4 patients with benign pancreatic tumors. In these patients, lesions with mass greater than 50 cm³ and significantly elevated bilirubin levels indicating a high degree of cholestasis were demonstrated on individual case analysis. A detailed distribution of the CA 19-9 level in both groups is shown in Figure 1.

The CA 125 levels ranged between 3 and 540 U/mL. The cutoff level was established as 35 U/mL. A level of CA 125 exceeding the cutoff level was found in 45 patients (61.6%) with malignant pancreatic tumors. The higher number of results indicating a malignant nature of the tumor demonstrated in the CA 19-9 evaluation suggests its higher reliability. The detailed distribution of the CA 125 level is shown in Figure 2.

Based on cytological, histopathological, and clinical evaluation, the patients were assigned to the malignant or benign group. The correlation of the tumor nature and the serum CA 19-9 level revealed true-positive results in 59 cases (53.6%) and false-positive results in 4 cases (3.6%). A 2-fold higher number of false-negative results was found in the evaluation of the CA 125 measurements (29 cases [26.4%]) than in the evaluation of the CA 19-9 measurements (14 cases [12.7%]). The detailed results are shown in Table 1.

The specificity and sensitivity of the CA 19-9 test in the assessment of the nature of the pancreatic tumor were determined to be 89.1% and 80.8%, respectively. The specificity of the CA 125 test, 83.3%, was slightly lower than the specificity of the CA 19-9 test. The main difference between the 2 tests was the low sensitivity of the CA 125 test, estimated to be 60.8%. The relatively low sensitivity of the CA 125 test resulted from an unexpectedly high number of false-negative results. The detailed statistical analysis is shown in Table 2.
A combined evaluation of the CA 19-9 and CA 125 test results in the differential diagnosis of pancreatic tumors provides a possibility of modifying the interpretation of the previous assessment resulting from the analysis of a single test. In 6 patients with negative CA 19-9 test results, a significantly elevated level of CA 125 was found. False-negative results of the CA 19-9 test were demonstrated in 14 patients, whereas in the combined evaluation of both markers, negative results were found only in 9 patients. The detailed analysis is shown in Table 3.

The combined evaluation of both marker levels revealed true-positive results (positive results for either single test) in 65 patients (59.1%), false-positive results (negative results for both tests in the case of a malignant tumor) in 9 patients (8.2%), false-negative results (positive results for both tests in the case of a benign tumor) in 8 patients (7.3%), and true-negative results (negative results for both tests in the case of a benign tumor) in the remaining 28 patients (25.4%). The sensitivity of the combined evaluation of both CA 19-9 and CA 125 levels increased from 80.8% when only the CA 19-9 level was analyzed to 87.8%. Simultaneously, the specificity of the combined evaluation of both CA 19-9 and CA 125 levels decreased to 77.8%. The statistical analysis of the combined evaluation is shown in Table 4.

A proper and effective diagnosis of pancreatic carcinoma has still remained in the scope of intensive research. Although various diagnostic imaging techniques are less or more useful, there are some serious limitations in terms of their sensitivity, specificity, and cost-effectiveness. Attempts to establish a relatively simple and reliable noninvasive diagnostic technique helpful in differentiating malignant and benign tumors quickly and cost-effectively have
been undertaken in clinical research conducted worldwide. Various serous markers of potential value in the differential diagnosis of pancreatic tumors have been evaluated, including CA 19-9 and CA 125. Among the variety of serum neoplastic markers used in pancreatic carcinoma diagnostics, CA 19-9 is considered the most sensitive.1,7,15 The serum CA 19-9 test is relatively inexpensive and accurate. Despite some limitations regarding its sensitivity, the CA 19-9 test may be applied in the diagnosis of pancreatic carcinoma owing to its high specificity. The diagnostic accuracy of the CA 19-9 test may be further augmented by a simultaneous evaluation with imaging techniques, such as US and CT. It allows for an increase in the sensitivity of the test to more than 90%.3,7,8,10 In our series, the sensitivity and specificity of the CA 19-9 test were 80.8% and 89.1%, respectively. These results are similar to those described by others,3-5,8,11,12,14,18 where the sensitivity of the CA 19-9 test ranged between 69% and 93% and its specificity between 78% and 98%. The specificity of the CA 125 test in our series was 83.3%, and this value was similar to the specificity of the CA 19-9 test. The main difference between both tests was low sensitivity (60.8%) of the CA 125 test. This value corresponds to the results described by others,1,10,17,19,20 where the sensitivity of the CA 125 test in the diagnosis of pancreatic carcinoma ranged between 40% and 74%. The main cause of the low sensitivity of the CA 125 marker is an unexpectedly large number of false-negative results. In our series, the number of false-negative results of the CA 125 test was 2-fold higher than that of the CA 19-9 test: 29 vs 14 cases, respectively.

The serum level of the CA 19-9 marker is well correlated with tumor size. This observation supports the concept that the marker level reflects the total tumor-cell burden.5,22 The bigger the tumor size and its advancement are, the higher the level of the CA 19-9 marker is. The marker level may not strictly predict the tumor volume because the tumor mass constitution varies individually and depends on the amount of non–tumor-marker–expressing cells, the volume of tumor stroma, and the association of coexistent inflammatory, degenerative, or necrotic lesions secondary to the tumor.3,5,12,15,17 Our results confirm that a high CA 19-9 level corresponds to a large tumor size. This association was observed in 79.5% of the patients. Moreover, it may also suggest the presence of distant metastases or peritoneal dissemination. Although tumor size is an important component in making a decision about surgery, its meaning as a prognostic factor may be misleading in the case of unresectable tumors. In addition, significant difficulties may appear in the assessment of the size of the neoplastic tumor, mainly owing to a large inflammatory component.10,12,15,17 Paradoxically, a large-volume carcinoma without metastases may be less malignant than an unresectable tumor of a medium size with more dynamic growth and lymph node involvement. Under these circumstances, the CA 19-9 level rather than the tumor size may appear to be a more valuable prognostic factor in the evaluation of patients with stage III pancreatic carcinoma.3,14,17

A high level of the CA 19-9 marker is usually associated with large carcinomatous tumors. Thus, the CA 19-9 test can be helpful in the assessment of resectability of pancreatic carcinoma. Tomazic and Pegan8 reported that unresectable lesions were found in 96% of patients with the marker level exceeding 1000 U/mL. However, a CA 19-9 level lower than 370 U/mL indicated a higher probability of operability of the lesion and survival longer than 10 months. The CA 19-9 level tends to increase from stage I to stage IV of a tumor (TNM classification from the Union Internationale Contre le Cancer staging system) and is higher in patients with resectable tumors than in patients with unresectable tumors. It allows for the use of the CA 19-9 marker as a determinant of some limit beyond which the probability of unresectability of the tumor is high. This limit is regarded to be in the wide range between 370 and 1000 U/mL.3,6,8,19 In our series, unresectable pancreatic carcinoma was diagnosed in 44 patients. In all of them, the CA 19-9 level was higher than 100 U/mL. Pancreatic carcinoma was regarded to be resectable in all of the patients with a CA 19-9 level between 3 and 98 U/mL.

Another equally important issue is to determine the cutoff level of the CA 19-9 marker that would allow for the differentiation of malignant and benign lesions. In the majority of articles, this value has been set at 37 U/mL. This value is also characteristic for intestinal-derived adenocarcinoma.3,9,12,13,18,21 This cutoff level, however, results in relatively low specificity and sensitivity of the test, reported to be 71% to 90% and 68% to 87%, respectively. When one increases the cutoff level to 100 U/mL, for instance, there is an increase of even more than 90% in the specificity of the test and a simultaneous decrease of 33% to 62% in the specificity.5,11,17,18,21 Decreasing the established cutoff level usually has an opposite result. Thus, in our series, the cutoff level was set at 37 U/mL. After the cytological and histological evaluations, the results of the CA 19-9 test were regarded as true positive in 59 of 110 patients as compared with all 73 patients with finally confirmed pancreatic carcinoma. In turn, the cutoff level for the CA 125 test was set at 35 U/mL. Decreasing the cutoff level to 20 U/mL resulted in an increase from 60.8% to 71.6% in the sensitivity of the test and a simultaneous decrease from 83.3% to 72.2% in its specificity.

A combined analysis of the CA 19-9 and CA 125 markers results in partial improvement of the reliability of the methods. The sensitivity usually increases from about 6% to 17%, and the specificity may even amount to 87%.4,10,20 In our series, the combined analysis of both markers increased the sensitivity of the tests from 80.8% when a single marker was analyzed to 87.8%. The specificity of the combined evaluation of the CA 19-9 and CA 125 tests decreased from 89.1% to 77.8%.

False-positive test results were found in 4 patients without malignancy with the CA 19-9 test and in 6 patients

### Table 4. Statistical Analysis of the Combined Evaluation of Both Cancer Antigens 19-9 and 125 Levels in the Differential Diagnosis of Pancreatic Tumors

<table>
<thead>
<tr>
<th>Statistic</th>
<th>Value, %</th>
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<tr>
<td>Specificity</td>
<td>77.8</td>
</tr>
<tr>
<td>Sensitivity</td>
<td>87.8</td>
</tr>
<tr>
<td>Positive predictive value</td>
<td>89.0</td>
</tr>
<tr>
<td>Negative predictive value</td>
<td>75.7</td>
</tr>
</tbody>
</table>

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Having increased levels of the CA 19-9 and CA 125 markers in patients with pancreatic pathological abnormalities usually indicates a malignant nature of the lesion. A high CA 19-9 level suggests advanced, inoperable pancreatic carcinoma. The sensitivity and specificity of the CA 19-9 test were 80.8% and 89.1%, respectively. A combined analysis of the CA 19-9 and CA 125 tests increased the sensitivity of the method to 87.8% and decreased its specificity to 77.8%. The results of the CA 19-9 test and particularly those of the CA 125 test should be interpreted in association with tumor presentation on imaging examinations such as US and CT owing to the limited sensitivity and specificity of these tests.

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Originally described as a marker for colorectal cancer, CA 19-9 has become the gold standard serologic marker for pancreatic cancer, with reported sensitivity and specificity as high as 87% and 98%, respectively. However, known limitations of CA19-9 as a biomarker include the following: (1) elevation of CA 19-9 levels with hyperbilirubinemia of both malignant and benign causes; and (2) falsely low CA 19-9 levels in individuals with the Le(a−b−) blood group who do not synthesize CA 19-9 despite having advanced pancreatic cancer. Because the CA 19-9 antigen is also a sialylated Leα blood group antigen, individuals with the Le(a−b−) blood group are deficient in a fucosyltransferase specified by the Le gene that is involved in the synthesis of the CA 19-9 antigen. About 5% to 14% of the population genotypically have the Le(a−b−) blood group and thus are unable to synthesize CA 19-9. In an attempt to improve the diagnostic accuracy of CA 19-9, the study by Cwik and colleagues evaluates the combination of CA 19-9 and CA 125, which is a serum marker that is often used to diagnose ovarian cancer.

Cwik and colleagues analyzed 110 patients with pancreatic lesions (66.4% malignant and 33.6% benign) and measured serum levels of CA 19-9 and CA 125 in all of the patients. Sensitivity and specificity of CA 19-9 in correctly diagnosing pancreatic cancer were 80.8% and 89.1%, respectively. With the combination of CA 125 and CA 19-9, sensitivity improved slightly to 87.8% (decreasing the incidence of false-negative results), yet specificity decreased to 77.8% (increasing the incidence of false-positive results). Based on their findings, Cwik and colleagues concluded that the combination of CA 19-9 and CA 125 was no better than CA 19-9 alone.

The results of this study are consistent with those in earlier studies that found no improvement in the diagnosis of pancreatic cancer when CA 125 was used in combination with CA 19-9. Clearly, the discovery of reliable biomarkers for pancreatic cancer is a critical area of research and has important implications for early detection, monitoring response to therapy, and the stratification of patients for protocol-based therapy on clinical trials. Technical advances that enable high-throughput screening and detection will hopefully facilitate this process. Moreover, the Early Detection Research Network was founded in 2000 by the National Cancer Institute, Bethesda, Md, as a collaboration between government, academia, and industry to facilitate biomarker discovery and validation (see http://edrn.nci.nih.gov). With the use of powerful bioinformatics technology and a rigorous set of criteria for biomarker development, the formidable goal of the Early Detection Research Network is to develop and validate biomarkers that will be useful in clinical practice.

Although not addressed in the article by Cwik and colleagues but of relevance to the surgeon, the serum level of CA 19-9, when elevated, is useful as an additional piece of information in the development of a treatment plan for patients with localized, nonmetastatic pancreatic cancer. For example, when we recently analyzed pretreatment CA 19-9 levels in patients with potentially resectable pancreatic adenocarcinoma (stage I, II according to the American Joint Committee on Cancer) in a clinical trial of neoadjuvant therapy, lower levels of CA 19-9 predicted both resectability and survival. In the group of patients who were without evidence of disease at last follow-up, the highest pretreatment CA 19-9 level was 1125 U/mL. Although we do not view a CA 19-9 level higher than 1125 U/mL as an absolute contraindication to surgery, the serum level of this tumor marker is used, in conjunction with other factors such as patient performance status, age, medical comorbidities, and extent of local tumor growth (likelihood of achieving a margin-negative [R0 according to the American Joint Committee on Cancer] resection), in developing an optimal treatment strategy for an individual patient. In the presence of a normal level of serum bilirubin, a marked 4-digit elevation in the CA 19-9 level should raise the concern for possible occult extrapancreatic metastatic disease, making initial treatment with systemic therapy an attractive option.

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