Serum Tumor Markers for the Diagnosis of Cholangiocarcinoma in Primary Sclerosing Cholangitis

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Background/Aims: The diagnosis of cholangiocarcinoma in primary sclerosing cholangitis (PSC), even with the use of current imaging techniques and brush cytology, is difficult and particularly important in patients being assessed for liver transplantation. This study investigated the accuracy of serum levels of a combination of the tumor markers carcinoembryonic antigen (CEA) and carbohydrate antigen 19-9 (CA19-9) in the diagnosis of cholangiocarcinoma in patients with PSC.

Methods: Seventy-four patients with PSC were studied. Fifteen patients had tumors (11 occult on imaging), 22 had severe PSC that necessitated transplantation (with explanted liver known to be free of tumor), and 37 patients had stable PSC. Results: An index of the two serum tumor markers [using the formula CA19-9 + (CEA × 40)] gave an accuracy of 86% in diagnosis of cholangiocarcinoma, with 10 of the 15 cases of cholangiocarcinoma having an increased value compared with none in a group of 22 comparable cases with no tumor. In addition, 6 of the 11 patients with occult tumors had abnormal values. Ultrasonography, computerized tomographic scanning, and endoscopic retrograde cholangiopancreatography were poor predictors of the presence of tumor. Conclusions: A combination of serum tumor markers will identify most occult tumors and will improve selection of appropriate cases for orthotopic liver transplantation.

Cholangiocarcinoma was originally described as a complication of ulcerative colitis by Parker and Kendall1 in 1954, but it is now believed that most cholangiocarcinomas in patients with colitis are related to concomitant primary sclerosing cholangitis (PSC).2 The prevalence of the tumor in PSC varies from 7% in asymptomatic patients to 42% of autopsies.3 The natural history of PSC is such that about half of the symptomatic patients have a progressive course and are considered candidates for orthotopic liver transplantation.4,5 The patients can be stratified into low-, moderate-, and high-risk categories by a Cox regression model of clinical and biochemical values (with 5-year survivals of 91%, 55%, and 16%, respectively).6 This model was performed at a single time point and excluded patients who had known cholangiocarcinoma. Had the latter been included, survival of all three risk groups would have been worse. However, it is very difficult to distinguish patients with cholangiocarcinoma from those with tight but benign dominant strictures of PSC. Indeed, there is a significant frequency of undiagnosed cholangiocarcinoma in the explanted liver: 9% in one series7 and 4 of 11 cases in another center.8 The prognosis is poor, with recurrence of tumor in most transplant patients,8,9,10 which may be caused by spreading of tumor cells to local lymph nodes before transplantation. Current diagnostic techniques for detection of cholangiocarcinoma include ultrasonography, computerized tomographic (CT) scanning, endoscopic retrograde cholangiopancreatography (ERCP) with bile duct brush cytology11,12 or cytology of bile aspirated at percutaneous cholangiography.13

Of the two possible tumor markers available for detecting cholangiocarcinomas, carcinoembryonic antigen (CEA), is a glycoprotein tumor marker with the immunodeterminant present on the protein moiety of the molecule. The other, carbohydrate antigen 19-9 (CA19-9), is a mucin-type glycoprotein in the serum with the immunodeterminant present on the carbohydrate moiety of the molecule. Increased levels of CA19-9 may be caused either by altered gene transcription or by altered glycosyltransferase activity that could change the antigenicity of the carbohydrate portion. Both tumor markers have been investigated for the diagnosis of malignancies in the stomach, colon, pancreas, and bile duct14,15 but have not gained widespread use. In the present study, we assessed the diagnostic accuracy of these two tumor markers, alone and in combination, in the detection of cholangiocarcinoma in patients with PSC referred for orthotopic liver transplantation.

Abbreviations used in this paper: CA19-9, carbohydrate antigen 19-9; CEA, carcinoembryonic antigen; CT, computerized tomography; ERCP, endoscopic retrograde cholangiopancreatography; PSC, primary sclerosing cholangitis.
Patients and Methods

PSC was diagnosed by ERCP features of multiple strictures and dilatations together with histological features of periductual fibrosis and other biliary features as described by Chapman. Three groups of patients with PSC were studied.

Group A consisted of 15 patients with histologically proven cholangiocarcinoma, 10 of whom had undergone orthotopic liver transplant. In 4 of the 15 patients, cholangiocarcinoma was diagnosed by imaging; 2 of these 4 patients underwent transplantation (since 1990, policy has been to reject patients with known tumors). In 11 of the 15 patients, the tumors were occult (not diagnosed on ultrasonography, CT scan, or ERCP at the time of presentation); 8 of these 11 patients underwent transplantation. In the other 3 cases, evidence of tumor developed subsequently (>3 months later) as shown by imaging and biopsy or postmortem examination. In total, 5 patients did not undergo transplantation: 2 patients were considered unsuitable at the time because cholangiocarcinoma had been found during the transplant assessment; 3 patients had stage 1 or 2 disease on liver biopsy, and, in 2 patients, endoscopic stents were attempted to relieve the jaundice. The cholangiocarcinoma was diagnosed more than 3 months later on biopsy or postmortem in these cases.

Group B consisted of 22 patients with PSC severe enough to be treated by orthotopic liver transplantation but who had no cholangiocarcinoma as proven by examination of the explanted liver. This group was chosen as a direct comparison to group A, because biochemical parameters were similar.

Group C consisted of 37 patients with stable PSC who were followed up for at least 1 year and who had no clinical signs of cholangiocarcinoma clinically and carcinoma on ERCP. This group was included to assess baseline values of tumor markers to give the false positive rate in stable disease.

Serum Markers

Serum frozen at -20°C and defrosted only once was assayed for CEA and CA19-9 using commercially available kits (CIS Bioindustries, Gif-Sur Yvette, France). Both assays are solid-phase two-site immunoradiometric assays of the "sandwich" type. One monoclonal antibody to one site is used to coat the solid phase, and the second antibody, labeled with 125I, is used as the tracer, the unknown (CEA or CA19-9) molecule being "sandwiched" between.

Statistics

Variables were tested for differences between the three groups of patients by two-tailed Mann–Whitney U test; P values of <0.05 were considered significant.

Results

Comparison of the characteristics of the patients in each group (Table 1) showed that mean ages were the same but that there were relatively more men in the carcinoma group than in the other groups. Serum bilirubin and albumin levels and histological stage were not significantly different in the first two groups, although alkaline phosphatase level was significantly higher in group A than group B (P = 0.026). As expected, all parameters were less abnormal in the patients with stable PSC (group C). The number of patients with inflammatory bowel disease was not different in the three groups.

In only 1 of the 15 patients with cholangiocarcinoma, the ultrasound examination showed evidence of tumor (sensitivity, 7%). Seven of the patients had suggestive clinical signs of cholangiocarcinoma (recent weight loss, rapid increase in bilirubin level, and equivocal results on ultrasonography); subsequent CT scans showed evidence of a mass suggesting cholangiocarcinoma in only 2 of these cases (sensitivity, 29%). Seven of the 12 patients (58.3%) who underwent ERCP had a dominant stricture (which was subsequently shown to be the site of tumor) compared with 9 of 21 patients (42.8%) in group B; these frequencies were not significantly different. Two patients with cholangiocarcinoma had a mass within the bile duct; this was the only sign that had 100% specificity for tumor at ERCP examination. In 1 patient, an intraductal biopsy specimen was obtained, which conclusively diagnosed tumor.

Tumor Marker Levels

Median serum CEA levels were 7.0 ng/mL (range, 0.1–703) for group A, 2.6 ng/mL (0.5–7.0) for group B, and 2.2 ng/mL (0.5–7.9) for group C. Median values for CA19-9 were 275.4 U/mL (24.3–81,362) for group A, 56.7 U/mL (5.5–284) for group B, and 27.9 U/mL (3.1–156) for group C. The upper end of the range for both markers was very high in group A. A normal range has not been firmly established in patients with PSC; for the present analysis, values of 5 ng/mL for CEA and 200 U/mL for CA19-9 were established. Most of the levels for the patients with stable PSC fell below these values.
Figure 1. Graph of values of the combined tumor marker index in the three groups of patients.

Using these cutoff values, sensitivity of each marker in differentiating carcinoma from noncarcinoma (group A vs. group B) was 53.3% for CEA and 60% for CA19-9. Specificity was 86.3% for CEA and 91% for CA19-9.

An index combining the tests was devised using the formula CA19-9 + (CEA × 40), derived from the best discriminant value for CA19-9 (200), which was 40 times that for CEA (5.0). The cutoff value for the combined index was arbitrarily chosen as 400 U. The results of the combined index in the three groups are shown graphically in Figure 1. Ten of the 15 cases with tumor (group A) but none of those without tumor (group B) had values above 400. Only 1 of the patients with stable PSC (group C) had a value above 400; this was assumed to be a false positive because the patient remained stable for more than 1 year. The individual values for group A are shown in Table 2. In group B, there were three false positive CEA and two false positive CA19-9 results. In group C, there was one false positive CEA and none for CA19-9.

All patients with positive CEA values had diagnosis of colon cancer excluded by barium enema, colonoscopy, or laparotomy. Regression analysis of bilirubin with index levels showed no significant correlation for groups 1 and 2 (P = 0.3 and 0.2, respectively).

The accuracy of each test and the value of the combined index in detecting cholangiocarcinoma in patients assessed for transplantation (i.e., group A vs. group B) are shown in Table 3. Specificity and positive predictive value are 100%. Sensitivity is only 66% because the range for tumor patients overlaps the range for other PSC patients, but the high positive predictive value may be more important clinically because, if a positive result is found, it can be assumed that tumor is present. Analysis of the index in the 11 patients with occult tumors found only on the explanted liver showed that 6 of these patients had index values above 400 U; hence, the presumed diagnosis would have changed in 6 of a total of 15 patients with tumor if the markers had been tested at the time of presentation.

Discussion

There are limited data on the accuracy of the standard diagnostic methods of ultrasonography and CT scanning in the diagnosis of cholangiocarcinoma. In the present study, ultrasonography had a sensitivity of only 7% and CT scanning gave a positive diagnosis in only 2 cases. The reported ERCP features of cholangiocarcinoma, namely, dilatation of ducts or the presence of intraductal polypoid masses, were not observed in the majority of our patients. Also, ERCP examination could give a definitive diagnosis in only 2 cases with an additional 2 cases being diagnosed by cytology or biopsy. Of note is the finding that the presence of a dominant extrahepatic stricture did not seem to be associated with a tumor. In our series, cholangiocarcinoma was detected in only 4 of the 15 patients by imaging alone. Brush cytology has reasonable accuracy for diagnosing cholangiocarcinoma in non-PSC patients, but the only studies performed in patients with PSC were those using percutaneous aspiration of bile. Repeated samples were needed because of interpretation difficulties due to severe epithelial changes from the PSC.

The results of the present study show that combined use of the serum tumor markers CEA and CA19-9 is accurate in diagnosing cholangiocarcinoma that has developed in PSC. Retrospective studies by Safi and Ritts have shown that levels of CA19-9 are increased in 65% and 67% of cholangiocarcinoma in non-PSC patients; however, it has been suggested that these levels relate merely to degree of obstructive jaundice. Therefore, it is important to compare values of markers in groups of patients with similar characteristics, including the serum bilirubin level. The tumor markers in group A probably represent the presence of tumor because groups A and B were not significantly different in terms of bilirubin level and because regression of bilirubin with CA19-9 levels did not show a significant correlation.

In most series, the overall posttransplant prognosis in patients with PSC who have cholangiocarcinoma is very poor; this has led to a policy of rejecting these patients from the transplant program if tumor is evident before transplantation. Of the 10 patients undergoing transplantation, 8 had occult tumors before orthotopic liver transplantation; 2 of them are alive 2 years after the procedure, whereas neither of the 2 patients with nonoc-
Table 2. Details of Tumor Marker Levels, Imaging Results, and Whether Patient Underwent Transplantation in Group A

<table>
<thead>
<tr>
<th>Patient no.</th>
<th>Histological stage</th>
<th>CEA</th>
<th>CA19-9</th>
<th>DES</th>
<th>Occult</th>
<th>Transplant</th>
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<td>3</td>
<td>16.2</td>
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<td>Yes</td>
</tr>
<tr>
<td>2</td>
<td>4</td>
<td>9.0</td>
<td>334.5</td>
<td>No</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>3</td>
<td>?</td>
<td>703</td>
<td>20,314</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
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<tr>
<td>7</td>
<td>4</td>
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</table>

NOTE. Occult, tumor occult on imaging; DES, dominant extrahepatic stricture present at ERCP.

cult tumors survived more than 6 months after the procedure. Both of the longer-term survivors had a tumor marker index of <400. The implication from these limited data is that incidental tumors not detected by the tumor markers or by imaging can have long-term survival. In our series of patients, 6 of the cholangiocarcinoma cases with occult tumors detected by standard tests would have had a positive diagnosis had the tumor marker values been known at the time of transplantation. Four of these 6 cases were transplant cases and all died of tumor recurrence; hence, four inappropriate transplantations would have been prevented. The markers are now used before transplantation in our patients with PSC, because a positive index would lead to high suspicion of tumor and may necessitate further invasive diagnostic tests (e.g., endoscopic or percutaneous biopsy of a suspicious area) before transplantation is considered.

The false positive rate in patients with stable PSC (group C) is low (1 of 37), and further prospective studies are needed to assess whether the tumor markers could be applied to regular screening of this group for cholangiocarcinoma. The data in this study include only patients with cholangiocarcinoma being assessed for transplantation who by definition were severely symptomatic and all deeply jaundiced.

The index combining the two markers is a better predictor of the presence of tumor than either test alone; if a cutoff of 400 U is used, 100% specificity (and 100% predictive value of a positive test) is achieved. In non-PSC patients, the combination of CA19-9 and CEA is probably the best marker available for detecting pancreatic and biliary cancers compared with other causes of obstructive jaundice, although a lower cutoff for CA19-9 was used in these studies. The studies included a few patients with cholangiocarcinoma who had increased levels of CA19-9, CEA, or both, but the accuracy of an index has not been extensively studied with this tumor. In an immunohistochemical study, 71% of tumors of the bile duct stained positively with the monoclonal antibody to CA19-9 and 91% with CEA, indicating that the presence of the markers is due to the presence of tumor cells rather than to inflammation and local obstruction within the biliary tree. A recent study in patients with PSC showed findings similar to our findings, although the two groups may not have been comparable, providing some limitations to the study. Further prospective studies are warranted to assess the place of tumor markers in detecting cholangiocarcinoma.

Table 3. Results of Combined Index of Tumor Markers in Diagnosing the Presence of Cholangiocarcinoma by Comparing Group A With Group B

<table>
<thead>
<tr>
<th>Index</th>
<th>Group A (tumor)</th>
<th>Group B (no tumor)</th>
</tr>
</thead>
<tbody>
<tr>
<td>&gt;400</td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td>&lt;400</td>
<td>5</td>
<td>22</td>
</tr>
</tbody>
</table>

NOTE. Sensitivity, 66%; specificity, 100%; positive predictive value, 100%; negative predictive value, 81%; accuracy, 86.

References

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