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The Utility of CA 19-9 in the Diagnoses of Cholangiocarcinoma in Patients Without Primary Sclerosing Cholangitis

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OBJECTIVES: The diagnosis of cholangiocarcinoma is often difficult, making management approaches problematic. A reliable serum tumor marker for cholangiocarcinoma would be a useful additional diagnostic test. Previous studies have demonstrated that elevated serum concentrations of CA 19-9, a tumor-associated antigen, have good sensitivity and specificity for cholangiocarcinoma in patients with primary sclerosing cholangitis. However, the value of this tumor marker for cholangiocarcinoma unassociated with primary sclerosing cholangitis is unclear. Thus, the aims of this study were to determine the usefulness of a serum CA 19-9 determination in the diagnosis of *de novo* cholangiocarcinoma.

METHODS: We prospectively measured serum CA 19-9 concentrations in patients with cholangiocarcinoma (n = 36), nonmalignant liver disease (n = 41), and benign bile duct strictures (n = 26). Serum CA 19-9 concentrations were measured by an immunoradiometric assay (CIS Bio International) without knowledge of the clinical diagnosis.

RESULTS: The sensitivity of a CA 19-9 value >100 U/ml in diagnosing cholangiocarcinoma was 53%. When compared with the nonmalignant liver disease and the benign bile duct stricture groups, the true negative rates were 76% and 92%, respectively. Patients with unresectable cholangiocarcinoma had significantly greater mean CA 19-9 concentrations compared to patients with resectable cholangiocarcinoma.

CONCLUSIONS: These data suggest that the serum CA 19-9 determination is a useful addition to the available tests for the differential diagnosis of cholangiocarcinoma. (Am J Gastroenterol 2000;95:204–207. © 2000 by Am. Coll. of Gastroenterology)

INTRODUCTION

Cholangiocarcinoma is a malignant tumor arising from bile duct epithelium. Unlike most human cancers, a tissue diagnosis of cholangiocarcinoma is often extremely difficult because of tumor location, size, and desmoplastic characteristics. Percutaneous fine needle aspiration is frequently not possible because many of these tumors are located in the liver hilum amid large vascular structures. Furthermore, tumor masses are often not even identifiable by CT, ultrasound, or magnetic resonance imaging. Endoscopic approaches are also of limited usefulness in tissue diagnosis because of the desmoplastic nature of these cancers. Indeed, bile cytology obtained at endoscopic retrograde cholangiography has a sensitivity of only 30–50% (1–4), endobiliary brush cytology of 50–66% (5, 6), and endoscopic transpapillary biopsy of 53–86% for detecting cholangiocarcinoma (7–9). Because of the problems in obtaining a diagnostic tissue diagnosis, treatment and management decisions for patients with biliary strictures that may be malignant are problematic.

Diagnostic adjuncts for cholangiocarcinoma, such as a serum marker, would be useful for the clinical management of this disease. We and other investigators have reported that serum CA 19-9 determinations are useful for the diagnosis of cholangiocarcinoma in primary sclerosing cholangitis (PSC). We demonstrated that the sensitivity and specificity for CA 19-9 value >100 U/ml for cholangiocarcinoma in PSC was 89% and 86%, respectively (10). Ramage *et al.* (11) found the sensitivity to be as high as 86% when using CA 19-9 and carcinoembryonic antigen in combination in patients with cholangiocarcinoma superimposed on PSC. Although widely used as a tumor marker (12–15), the clinical value of serum CA 19-9 determinations in the diagnosis of cholangiocarcinoma in the absence of PSC is unknown. Thus, the objective of this study was to determine the clinical usefulness of CA 19-9 values for *de novo* cholangiocarcinoma. Our specific aims were to address the following questions: 1) What are the serum CA 19-9 values in patients with cholangiocarcinoma, benign bile duct strictures, and nonmalignant end-stage liver diseases? 2) Are elevated CA 19-9 levels in patients with cholangiocarcinoma related to tumor stage or liver dysfunction, or both?

PATIENTS AND METHODS

Patient Population

We prospectively obtained serum samples from patients undergoing evaluation for benign and malignant biliary strictures at the Mayo Clinic between 1994 and 1996. Serum was also prospectively obtained from patients being evaluated for liver transplantation; this cohort of patients served as the disease control group. Patients with the diagnosis of PSC were excluded from this study. Clinical information was obtained by a thorough review of the medical histories. This study included 36 patients with cholangiocarcinoma, 41 patients with nonmalignant liver disease, and 26 patients with benign biliary strictures. Of the 36 patients with cholangiocarcinoma, the diagnosis was established by surgical biopsy in 25 patients, endoscopic biopsies and brushings in six patients, and by fine needle aspiration in five patients. In patients with cholangiocarcinoma, the stage and resectability of the tumors were ascertained using information obtained by imaging studies or at the time of surgery. Unresectability was defined by Bismuth stage 4 cancer arising from the right and left hepatic ducts and extending intrahepatically. Patients with intra- and extrahepatic metastasis were also deemed unresectable.

The benign bile duct stricture group consisted of 26 patients. Eighteen patients developed ischemic strictures after orthotopic liver transplantation; none of these patients were transplanted initially for PSC. The median duration of follow-up in the nontransplanted patients was 15 months. One patient had a benign recurrent periampullary adenoma and seven patients had benign-appearing strictures with negative endoscopic brushings and biopsies; none of these patients developed metastasis, stricture progression, or died during their follow-up.

The other control group consisted of 41 patients with nonmalignant liver diseases. Twenty-seven patients from this group, who had a serum CA 19-9 measured preoperatively, eventually underwent orthotopic liver transplantation for liver failure from multiple causes including cryptogenic cirrhosis (n = 4), viral hepatitis (n = 6), autoimmune hepatitis (n = 6), primary biliary cirrhosis (n = 7), alcoholic cirrhosis (n = 2), and Wilson's disease (n = 1). The remainder of this group had liver disease secondary to alcoholic cirrhosis (n = 3), primary biliary cirrhosis (n = 1), α1-antitrypsin deficiency (n = 2), cystic liver disease (n = 3), hemachromatosis (n = 1), and cryptogenic cirrhosis (n = 4). The explanted liver specimens were evaluated by a pathologist and had no evidence of cholangiocarcinoma.

Serum concentrations of CA 19-9 were analyzed by immunoradiometric assays as directed by the manufacturer (CIS Bio International, Saclay, France). The upper sensitivity of serum CA 19-9 was 240 U/ml and necessitated routine serial dilutions of serum with greater concentrations for an accurate determination of the level of this moiety. The coefficient of variation of serum CA 19-9 in repeated measurements performed blindly on the same serum specimen is $<$10%.

Table 1. Patient Characteristics in the Three Groups

Characteristics	Cholangiocarcinoma	Nonmalignant Liver Disease	Benign Bile Duct Stricture
Number of patients	36	41	26
Sex (M:F)	22:14	13:28	11:15
Total bilirubin (mg/dl)	7.0 (9.5)	3.4 (6.6)	1.9 (2.1)
AST(U/L)	88 (84)	136 (325)	70 (56)
ALP(U/L)	803 (474)	394 (426)	837 (942)
CA 19-9 (U/ml)	7,999 (40,486)	76 (148)	44 (45)

Results are mean with SD in parentheses.
ALP = alkaline phosphatase.

Statistics

The results were expressed as mean values ± SE. Statistical significance in mean values was evaluated by the Student's *t* test. The relationship between CA 19-9 level and total bilirubin, alkaline phosphatase, and AST were determined by linear regression analysis.

RESULTS

Patient Characteristics

The mean ages of the patients with cholangiocarcinoma, nonmalignant liver disease, and benign bile duct strictures were similar (Table 1). However, the mean total serum bilirubin and serum alkaline phosphatase values were significantly higher in patients with cholangiocarcinoma, compared to the other two groups ($p < 0.01$). In contrast, the serum AST values were significantly higher in the nonmalignant liver disease group than the other two groups ($p < 0.01$). Thus, the patients with cholangiocarcinoma had a more marked cholestatic profile than the other two groups of patients.

What Are the Serum CA 19-9 Values in Patients With Cholangiocarcinoma, Benign Bile Duct Strictures, and Nonmalignant Liver Diseases?

The median CA 19-9 concentration was significantly greater in the cholangiocarcinoma group than in the nonmalignant liver disease group and the benign biliary strictures group (Fig. 1). Table 1 shows the mean serum CA 19-9 concentration in patients with cholangiocarcinoma was 15,076 U/ml (range 7–386,000 U/ml) in comparison with 76 U/ml (range 7–870 U/ml) in the benign biliary stricture group. Of the 36 patients with cholangiocarcinoma, 19 (53%) had concentrations exceeding 100 U/ml. Using a CA 19-9 concentration of 100 U/ml, the true negative rate of a CA 19-9 for cholangiocarcinoma is 76% when assessed using as the control group, patients with nonmalignant liver disease, and 92% when evaluated using the benign bile duct stricture group. Because the benign bile duct stricture group is actu-

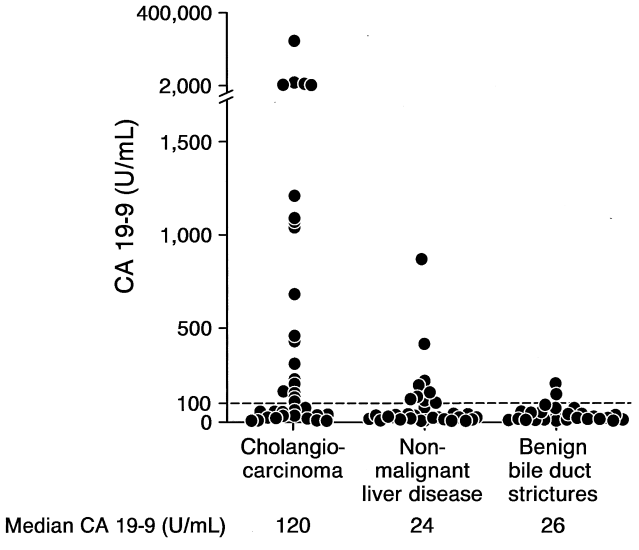

Figure 1. The serum CA 19-9 concentration is greater in the cholangiocarcinoma group compared to the nonmalignant liver disease group and the benign bile duct stricture group.

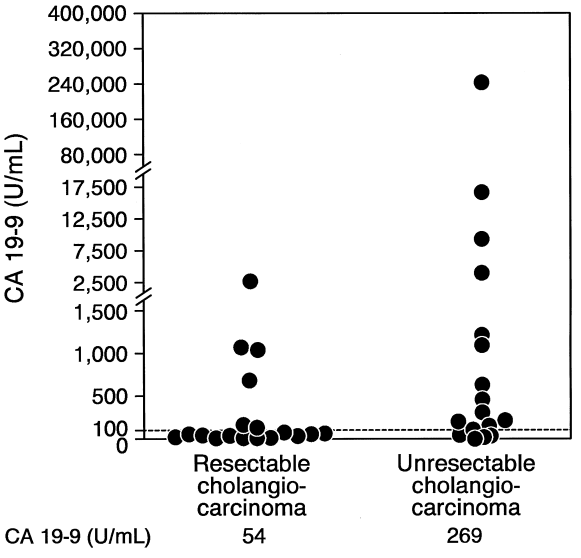

Figure 2. The serum CA 19-9 value is greater in unresectable cholangiocarcinoma than in resectable cholangiocarcinoma.

ally comprised of two groups of patients, those who have undergone liver transplants and those who have not, we also assessed the true negative rate for these two groups. The true negative rate in the nonliver transplant group with benign strictures was 100%, whereas it was 89% in the liver transplant group with benign strictures. If the reference value was increased to 200 U/ml, the true negative rate increases from 79% to 93% using the nonmalignant liver disease group as the control group, as compared with the combined benign bile duct stricture group where the true negative rate only increases from 92% to 97%.

Are Elevated Serum CA 19-9 Values in Patients With Cholangiocarcinoma Related to Liver Dysfunction?

In patients with cholangiocarcinoma, no correlation was found between the total bilirubin and the CA 19-9 concentrations ($r = 0.057$). Likewise, no correlation was found between either serum alkaline phosphatase or serum AST values, and serum CA 19-9 levels ($r = 0.147$ and $r = 0.027$, respectively). Thus, elevated serum CA 19-9 values could not be attributed to either cholestasis or hepatocellular injury.

Are Elevated Serum CA 19-9 Values in Patients With Cholangiocarcinoma Related to Tumor Stage?

Of the 36 patients with cholangiocarcinoma, 18 patients underwent curative resection and 18 had unresectable cancer. The mean CA 19-9 level in the resectable group was 344 U/ml (range 7–2,688 U/ml) as compared to 15,653 U/ml (range 7–24,6800 U/ml) in the unresectable group (Fig. 2). Thus, patients with unresectable disease had a significantly higher mean serum CA 19-9 concentration than those with resectable disease ($p < 0.05$). The sensitivities of CA 19-9 >100 U/ml for the diagnosis of cholangiocarcinoma in the resectable and unresectable groups are 33% and 72%, respectively. If we use serum CA 19-9 concentrations >200 U/ml, then the sensitivity decreases to 22% in the resectable group but does change significantly in the unresectable group (61%). Thus, serum CA 19-9 values have a limited sensitivity for identifying cholangiocarcinoma patients with early stage, surgically resectable disease. Only three patients with a CA 19-9 >1,000 had resectable disease, suggesting that CA 19-9 levels of ≥1,000 strongly suggests unresectable disease.

DISCUSSION

The observations of this study relate to the diagnostic usefulness of serum CA 19-9 determinations in the diagnosis of cholangiocarcinoma. Our results directly demonstrate that 1) serum CA 19-9 values have an acceptable sensitivity and true negative rate for the diagnosis of cholangiocarcinoma; and 2) serum CA 19-9 values are related to the tumor burden and not the magnitude of cholestatic liver dysfunction. These data suggest that serum CA 19-9 determinations can be of value in the diagnosis of cholangiocarcinoma unassociated with PSC.

We do caution against measuring serum CA 19-9 if the patient has active bacterial cholangitis; serum levels in the presence of cholangitis can be several thousand-fold higher (16). However, they decrease rapidly, and CA 19-9 determinations can be useful within weeks after the infection has resolved. In addition, it has been well established that serum CA 19-9 values can also be elevated in pancreatic cancer (17). Thus, the differential diagnosis of a distal common bile duct stricture and an elevated serum CA 19-9 includes both pancreatic cancer and cholangiocarcinoma. Further imaging studies of the pancreas are frequently necessary to differentiate between these two neoplasms (*e.g.*, CT scans of the

pancreas, endoscopic ultrasound with biopsies of the pancreas).

The sensitivity and true negative rate of the serum CA 19-9 determination for the diagnosis of cholangiocarcinoma in the absence of PSC is lower than that we previously reported for cholangiocarcinoma in the presence of PSC (10). This difference could be due to several factors including: 1) the limited number of PSC patients studied; 2) differences in the biology of the tumors; and 3) the possibility that chronic inflammation or low-grade cholangitis in PSC-associated CCA may contribute to the elevated CA 19-9 levels. Further studies are needed contrasting and comparing biological markers including genetic analysis for cholangiocarcinoma occurring *de novo* and in the presence of PSC. Such information may provide insight into the different pathogenic events arising in these two forms of cholangiocarcinoma.

In summary, serum CA 19-9 determinations are a useful adjunct in our diagnostic armamentarium for cholangiocarcinoma. However, we emphasize that like most diagnostic tests it is only helpful if positive. Indeed, our data clearly demonstrate that a negative test does not exclude cholangiocarcinoma. Additional bile- or serum-based tests are needed to help in the diagnosis of cholangiocarcinoma. Ultimately, a profile of tests analogous to liver biochemistries measuring serum or bile tumor markers and genetic analyses for cholangiocarcinoma-associated mutations will need to be developed to assist in the diagnosis of this disease.

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