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Serum tumor markers in the diagnosis of cholangiocarcinoma

Kawin Leelawat

Department of Surgery, Rajavithi Hospital, Bangkok 10400, Thailand
E-mail: Kawin.leelawat@gmail.com

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Abstract

Cholangiocarcinoma is one of the most common causes of cancer-related death in Thailand. Unlike other kinds of solid tumors, a preoperative pathological diagnosis of cholangiocarcinoma is very difficult because of the location and the desmoplastic characteristic of this disease. Only by obtaining a proper diagnosis can the optimal treatment of a cholangiocarcinoma patient be assured. Therefore, identification of tumor markers in the serum would be beneficial in the clinical diagnosis and management of this disease. Here, we review the recent data concerning serum tumor markers, including CA19-9, CEA, CYFRA21-1, IL-6, MMP-7, MUC5AC, and RCAS1, for diagnosis of cholangiocarcinoma.

Keywords**:** *Cholangiocarcinoma, CA19-9, CEA, CYFRA21-1, IL-6, MMP-7, MUC5AC, RCAS1*

Introduction

Cholangiocarcinoma is a cancer that arises from the bile duct epithelium. The incidence of and mortality rate due to cholangiocarcinoma vary considerably in different geographic regions, with the highest incidence in Southeast Asia, especially in Thailand (Sripa & Pairojkul, 2008). The lethality of this disease is due to its rapid growth combined with its tendency to invade adjacent organs and metastasize. Therapeutic options for cholangiocarcinoma have been limited due to poor responses to chemotherapy and radiation therapy. Surgery is perhaps the only effective treatment for cholangiocarcinoma. Previous studies have suggested that the most important prognostic factor is a tumor-free surgical margin. Three-year survival rates of 35% to 50% are achieved only when negative histological margins are attained at the time of surgery (Chamberlain & Blumgart, 2000). Factors associated with a poor prognosis are those related to the extent of the disease caused by cancer cell invasion, such as bi-lobar distribution, lymph node involvement, vascular invasion and distant metastases (Bridgewater & Imber, 2007). To improve the survival rate, diagnosis and treatment of patients with cholangiocarcinoma should be performed as soon as possible. In the clinical setting, most patients with cholangiocarcinoma present with obstructive jaundice. However, there are many benign biliary tract diseases that present with clinical symptoms similar to those of cholangiocarcinoma. The most important issue is to differentiate patients with cholangiocarcinoma from patients with benignbiliary tract diseases because of the differences in treatment and in prognosis between these diseases.

Unlike other kinds of solid tumors, a pathological diagnosis of hilar cholangiocarcinoma is very difficult because of the location and the desmoplastic characteristic of this disease (Blechacz & Gores, 2008; Byrnes & Afdhal, 2002). In addition, this tumor usually grows along the bile duct without expanding from the bile duct as a mass forms. Computed tomography (CT), ultrasound and magnetic resonance imaging (MRI) often miss this type of lesion (Jarnagin & Winston, 2005). In cases of resectable peripheral or intrahepatic cholangiocarcinoma, most of the hepatobiliary surgeons prefer to perform liver resection without preoperative needle biopsy of this tumor because the seeding of cancer cells along a needle tract, although rare, has been documented. In addition, it is difficult to differentiate cholangiocarcinoma from adenocarcinoma liver metastasis by pathological examination of the small biopsy specimen (Hammill & Wong, 2008). Therefore, identification of tumor markers in the serum would be beneficial in the clinical management of this disease. Here, we review the available tumor markers that have been studied for the diagnosis of cholangiocarcinoma.

Carbohydrate antigen 19-9 (CA19-9)

CA19-9 is a mucin-type serum glycoprotein with the immunodeterminant expressed on the carbohydrate moiety (Nehls, Gregor, & Klump, 2004). Serum CA19-9 is found to elevate in patients with pancreatic cancer (Hamada & Shimosegawa,

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2011), gall bladder cancer (Shukla, et al., 2006) and ovarian cancer (Engelen et al., 2000). Previously, this marker was studied for its efficacy in the diagnosis of cholangiocarcinoma occurring in primary sclerosing cholangitis (PSC) patients (Hultcrantz et al., 1999; Ramage et al., 1995). However, most of the cholangiocarcinoma cases in Thailand are not caused by PSC but rather by infection with *Opisthorchiasis viverrini* (Kaewpitoon, Kaewpitoon, & Pengsaa, 2008; Sripa & Pairojkul, 2008). In 2002, The British Society of Gastroenterology guidelines reported a lack of data regarding the diagnostic and prognostic value of CA19-9 for cholangiocarcinoma in non-PSC patients. Patel, et al. (2000) carried out a prospective diagnostic test study of the accuracy of CA19-9 in the diagnosis of cholangiocarcinoma in 36 patients with non-PSC cholangiocarcinoma, 41 patients with nonmalignant liver disease and 26 patients with benign biliary diseases. They demonstrated that the sensitivity of serum CA 19-9 (cut off point = 100 U/mL) in diagnosing cholangiocarcinoma in non-PSC patients was only 53%, when compared with nonmalignant liver disease and benign bile duct diseases (Patel, et al., 2000). Recently, we reported that the sensitivity and specificity of CA19-9 in the diagnosis of cholangiocarcinoma in Thai patients are 60.6% and 80.5%, respectively (Leelawat et al., 2009). The accuracy of CA19-9 in the diagnosis of cholangiocarcinoma from the literature are presented in Table 1.

Table 1 Performance of the biomarkers for the diagnosis of cholangiocarcinoma

Tumor markers	Type of study	Cut-off value	Sensitivity (%)	Specificity (%)	References
CEA	Case-control	5 ng/mL	53.0	86.0	Ramage et al., 1995
CEA*	Case-control	5 ng/mL	58.5	62.5	Leelawat et al., 2009
CA19-9	Case-control	100 U/mL	53.0	92.0	Patel et al., 2000
CA19-9*	Prospective consecutive	100 U/mL	68.0	87.0	Leelawat et al., 2010
MMP-7*	Prospective consecutive	5.5 ng/mL	75.0	78.0	Leelawat et al., 2010
MUC5AC*	Case-control	O.D. 0.11	87.6	89.6	Silsirivanit et al., 2011
IL-6	Case-control	25.8 pg/mL	73.0	92.0	Cheon et al., 2007
RCAS1	Case-control	10 U/mL	73.9	85.0	Enjoji et al., 2005
CYFRA22-1	Case-control	2.7 ng/mL	74.7	92.2	Uenishi et al., 2008

* The studies were performed in Thai patients

Previous studies have demonstrated that the level of serum CA19-9 depends on the severity of the bile duct obstruction and the degree of cholangitis. Increases in serum CA19-9 levels can be detected even in benign bile duct diseases (Principe et al., 2003). In addition, the level of serum CA19-9 depends on the Lewis phenotype. As much as 10% of the population has been found to be Lewis negative (Blechacz & Gores, 2008), resulting in undetectable levels of CA 19-9. Recent study performed a retrospective review of CA19-9 levels in 483 consecutive patients. They demonstrated that CA19-9 had poor clinical utility as a tumor marker for diagnosing pancreatic cancer and cholangiocarcinoma (Singh et al., 2011)

However, the previous meta-analysis study suggested that the elevation of preoperative serum CA19-9 levels were associated with the poor prognosis of cholangiocarcinoma patients (Liu et al., 2010). This evidence was confirmed by the multicenter study performed by the Study Group for Hepatic Surgery of the Japanese Society of Hepato-Biliary-Pancreatic Surgery. They studied 341 patients who underwent hepatectomy for intrahepatic cholangiocarcinoma. Using multivariate analysis, they demonstrated that preoperative CA 19-9, intrahepatic metastasis, and nodal involvement were the significant independent predictors of poor prognosis (Uchiyama et al., 2011)

From these data, we can conclude that the measurement of serum CA19-9 is not sufficient for a diagnosis of cholangiocarcinoma. However, preoperative CA19-9 may be used as a prognostic factor for cholangiocarcinoma patients who underwent hepatectomy. Therefore, novel tumor markers for the diagnosis of cholangiocarcinoma should be investigated.

Carcinoembryonic antigen (CEA)

Carcinoembryonic antigen (CEA), first described by Gold and Freeman in 1965, is a complex intracellular glycoprotein produced by about 90% of colorectal cancers (Chamberlain & Blumgart, 2000), and it can be measured quantitatively in serum. CEA levels can be elevated in other conditions such as gastric, pancreatic, lung, breast and medullary thyroid malignancies, as well as in non-neoplastic conditions such as cirrhosis, ulcerative colitis, pancreatitis and even smoking (Lim, Kam, & Eu, 2009). CA 19-9, CEA, and the combination of CA 19-9 and CEA were evaluated for their utility in the diagnosis of cholangiocarcinoma in a cohort of patients at Kings College, London. A CA 19-9 cut-off of 200 U/mL resulted in a sensitivity of 60% and specificity of 91% for the diagnosis of CCA. A CEA cut-off of 5 ng/mL resulted in a sensitivity of 53% and specificity of 86%. An index combining CEA and CA 19-9 ((40×CEA) + CA 19-9 >400 as the threshold value) achieved 100% specificity for the diagnosis of CCA (Ramage et al., 1995). However, this index has been shown to be less reliable when evaluated prospectively, with evidence of fluctuation in tumor marker levels and transient elevation in some individuals when followed over time (Chalasani, et al., 2000). Recently, we reported that the sensitivity and specificity of CEA in the diagnosis of cholangiocarcinoma in Thai patients are 58.5% and 62.5%, respectively (Leelawat, et al., 2009).

Interleukin (IL) – 6

A previous study (Goydos, et al., 1998) demonstrated that high serum IL-6 identified patients with cholangiocarcinoma and correlates with tumor burden. The 60 patients that participated in this study included 15 with cholangiocarcinoma, 14 with hepatocellular carcinoma, 26 with isolated hepatic colorectal metastasis, 5 with benign biliary tract disease and 35 healthy adult volunteers. Their sera were collected prospectively from 1992-1995. The exclusion criteria were the factors known to be associated with elevated IL-6 in serum, including the presence of acute infection, chronic inflammatory disease, recent myocardial infarction, surgical procedures within the preceding 14 days and uremia. Serum IL-6 was measured using a commercial ELISA kit (Endogen, Cambridge, MA). The results of this study demonstrated that serum IL-6 was detected in all cholangiocarcinoma cases and that the positive predictive value was 83.3%. Furthermore, the mean level of IL-6 was significantly higher in cholangiocarcinoma patients than in other groups. However, the authors did not specify the method used to measure the sensitivity and specificity of this test, nor did they state how the study was blinded. In addition, the sample size in this study was too small to test the accuracy of IL-6.

A previous study (Cheon, et al., 2007) evaluated the usefulness of serum IL-6 in the diagnosis of cholangiocarcinoma and measured changes in serum IL-6 levels following photodynamic therapy (PDT). They found that IL-6 was detected in all patients with cholangiocarcinoma and hepatocellular carcinoma and in 6 of 23 healthy adults. The level of IL-6 in the serum was higher in patients with cholangiocarcinoma than in both of the other groups (P <0.001). The diagnostic sensitivity was found to be 73% and the specificity was 92%. The positive and negative predictive values were 83% and 87%, respectively, when using a cut-off point of 25.8 pg/mL IL-6. One month after the treatment of cholangiocarcinoma with PDT, the mean IL-6 level had decreased significantly from 282.1 ± 121.8 to 38.2 ± 9.9 pg/mL (P = 0.008). The patients in this study were all in advanced stages or had unresectable tumors. Information about the value of serum IL-6 in early or resectable cases is not available. In addition, most of the hilar cholangiocarcinoma patients present with cholangitis. This condition may give a false positive result when using IL-6 as a tumor marker. Thus, we suggest that using IL-6 as a tumor marker is not appropriate for hilar cholangiocarcinoma.

Cytokeratin 19 fragment (CYFRA21-1)

Cytokeratin 19 fragment (CYFRA 21-1) is a fragment of cytokeratin 19 (CK 19). CYFRA21-1 levels are found to elevate in advanced non-small cell lung cancer (Wang et al., 2010) and head and neck squamous cell carcinoma (HNSCC) patients (Hoffmann-Fazel et al., 2003). A previous study reported that patients with large peripheral cholangiocarcinoma had high serum levels of CYFRA 21-1 (normal, < or = 2 ng/ml) (Kashihara et al., 1998).

A recent study (Uenishi et al., 2008) performed a case-control study to test the accuracy of the use of serum CYFRA21-1 for the diagnosis of intrahepatic cholangiocarcinoma (ICC). They measured the serum levels of CYFRA21-1, CA19-9 and CEA in 71 patients with ICC and 90 patients with nonmalignant liver disease. They analyzed the areas under the receiver operator characteristic (ROC) curves and found that the ROC curves for CYFRA 21-1 demonstrated better discrimination between intrahepatic cholangiocarcinoma and benign liver diseases than did the curves for CEA or CA 19-9. A cut-off at 2.7 ng/ mL for CYFRA 21-1 yielded a sensitivity of 74.7% and a specificity of 92.2%. However, we suggest that most cholangiocarcinoma

patients have high levels of serum bilirubin, which may interfere with the results of CYFRA 21-1. These authors did not report the levels of bilirubin in each patient. In addition, until now, only 2 studies of the accuracy of CYFRA21-1 in the diagnosis of cholangiocarcinoma have been reported.

Receptor-binding cancer antigen expressed on SiSo cells (RCAS1)

RCAS1, a novel tumor-associated antigen that is identified by the 22–1–1 monoclonal antibody, is highly expressed in many kinds of cancers, including uterine, ovarian, stomach, liver and colon cancer (Coban et al., 2006; Fukuda et al., 2002; Ikeguchi, Hirooka, & Kaibara, 2003; K. Leelawat et al., 2003; Nakashima, Sonoda, & Watanabe, 1999; Sonoda et al., 2006). This antigen acts as a ligand for a putative receptor present on the cells of the immune system, such as natural killer cells and activated T cells. Basic research has indicated that RCAS1 expression in cancer cells contributes to evasion from immune surveillance and progression of carcinomas. High sensitivity and specificity of serum RCAS1 in the diagnosis of cholangiocarcinoma have been reported by certain studies. (Enjoji et al., 2004; Enjoji et al., 2005). They used ROC curves to establish a threshold value of 10 U/mL for RCAS1 in collected serum, which allowed differentiation between cholangiocarcinoma and benign biliary diseases. In addition, they determined that serum RCAS1 was more sensitive for cholangiocarcinoma than CA19-9 (73.9% versus 65.2%) and was not influenced by cholestasis.

Matrix metalloproteinase (MMP)-7

Matrix metalloproteinases (MMPs) are zinc-dependent endopeptidases. They play mechanistic roles in the turnover and degradation of extracellular matrix (ECM) components and basement membranes. Previous studies have demonstrated that degradation of the extracellular matrix by MMPs is a key factor in the mechanism of tumor invasion and metastasis (Folgueras et al., 2004). MMP-7 (matrilysin) is the smallest MMP (28 kDa) that is expressed in tumor cells but not in stromal cells. Previous studies have shown that peripheral blood levels of MMP-7 are significantly elevated in ovarian cancer and renal cell carcinoma patients (Palmer et al., 2008; Ramankulov et al., 2008).

We have previously examined whether the serum level of matrix metalloproteinase-7 (MMP-7) has the potential to differentiate cholangiocarcinoma from benign biliary tract diseases (Leelawat et al., 2010). Our study was performed according to the PRoBE (a prospective-specimen-collection, retrospective-blinded-evaluation) design. A total of 187 patients with obstructive jaundice were consecutively enrolled. After the diagnostic status of these patients was ascertained, their levels of serum MMP-7 were assayed and compared with their levels of serum carbohydrate antigen 19-9 (CA19-9). The results demonstrated that MMP-7 and CA19-9 serum levels were significantly elevated in cholangiocarcinoma patients ($P < 0.001$). The area under the curve (AUC) from a receiver operating characteristic (ROC) curve analysis for the MMP-7 was more accurate for the diagnosis of cholangiocarcinoma than CA19-9 (AUC = 0.84, 95% CI: 0.778-0.903 for MMP-7 and AUC = 0.79, 95% CI: 0.708-0.868 for CA19-9). The sensitivity and specificity of serum MMP-7 (cut-off value of 5.5 ng/mL) were 75% and 78%, respectively, while the sensitivity and specificity of serum CA19-9 (cut-off value of 100 U/mL) were 68% and 87%, respectively.

In addition, a recent study examined plasma levels of MMP-7, MMP-9, TIMP-2, collagen I, and hydroxyprolene in patients with opisthorchiasis, benign biliary disease and cholangiocarcinoma. The results indicated that detection of plasma collagen I, MMP-7 and hydroxyprolene may be useful for staging the disease and discriminating benign biliary disease from cholangiocarcinoma, and prediction of the risk of opisthorchiasis-associated cholangiocarcinoma (Prakobwong et al., 2011). We therefore concluded that serum values of MMP-7 appear to be useful biomarker for differentiating cholangiocarcinoma from benign biliary tract obstructive diseases.

Mucin 5AC (MUC5AC)

Mucins (MUCs) are high molecular weight O-linked glycoproteins. The primary functions of MUC are to hydrate and protect the epithelial luminal surfaces of the ducts. MUCs have been shown to contain complex associations with various cancer pathways, impacting cancer growth, proliferation, and apoptosis. Sixteen distinct epithelial mucin genes, designated MUC1, MUC2, MUC3, MUC4, MUC5AC, MUC5B, MUC6, MUC7, MUC8, MUC9, MUC11, MUC12, MUC13, MUC15, MUC16, and MUC17, were identified. Previous studies demonstrated that serum MUC5AC can be detected in cholangiocarcinoma patients with high sensitivity and specificity (Wongkham et al., 2003; Silsirivanit et

al., 2011). Moreover, the study group from Khonkaen University analyzed the serum samples from 179 consenting cholangiocarcinoma patients (49.7% were peripheral cholangiocarcinoma, 29.4% were central or extrahepatic cholangiocarcinoma and 20.9% were a combination of both types). Most of these patients were infected with the liver fluke, *Opisthorchis viverrini*. They demonstrated that cholangiocarcinoma patients who had positive serum MUC5AC status had a 2.5-fold higher risk of death compared with cholangiocarcinoma patients who had negative serum MUC5AC status ($P < 0.001$) (Boonla et al., 2003). Therefore, we suggested that serum MUC5AC is one of the novel markers for cholangiocarcinoma.

Discussion

The need for better tests to screen for and diagnose patients with cholangiocarcinoma is an important issue that must be addressed to improve the treatment results for these patients. Unfortunately, at the present time, no specific serum tumor markers have been identified for this disease. This review demonstrates that many novel serum tumor markers are significantly elevated in cholangiocarcinoma patients. These markers have the potential to be used for discriminating cholangiocarcinoma from benign biliary tract disease but not for screening cholangio-carcinoma in healthy people. In addition, these novel markers can be elevated in other kinds of malignancy and also some benign conditions. Therefore, the diagnosis of cholangiocarcinoma requires the integration of clinical information from imaging studies of the liver and biliary systems such as computed tomography scanners with multidetector row technology with contrast-delayed imaging and magnetic resonance imaging with cholangio-pancreatography. Subsequently, in suspicious cases, the biomarkers should then be used in helping the physician's decision for diagnosis of cholangio-carcinoma. In addition, some biomarkers (CA19-9 and MUC5AC) may be used as the prognostic factor for cholangiocarcinoma patients. However, most of the studies of tumor markers were performed in referral centers, which have a high prevalence of cholangiocarcinoma. Consequently, the findings may not be broadly applicable to other hospitals that typically have a low volume of cholangiocarcinoma. We suggest that further multicenter studies, which including an increased number of cholangio-carcinoma cases, needs to be conducted before these markers may confidently be used as a diagnostic test for the detection of cholangiocarcinoma.

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