RESEARCH ARTICLE

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Hepatic Oncology

CA 19-9 in combination with abdominal CT scan for the diagnosis of mass-forming intrahepatic cholangiocarcinoma

Nilubol Raunroadroong¹, Hutchia Sriplung², Nirush Lertprasertsuke³, Suwalee Pojchamarnwiputh⁴, Witthanee Na Chiangmai⁴, Worapon Sinsuwan¹ & Sumitra Thongprasert^{*,5}

Summary points

- CA 19-9 combined with CT scan imaging (Chiang Mai CT scores) should be used as the diagnostic criteria in patients with mass-forming intrahepatic cholangiocarcinoma who had no tissue diagnosis.
- For deep seated tumor the biopsy it is difficult and will be harmful to the patient, thus in such cases the combination of Chiang Mai CT score plus CA19-9 level will be useful.

SUMMARY **Aim:** Cholangiocarcinoma (CCA) is a common form of cancer in Thailand. The diagnosis of CCA by pathology is widely accepted. Unfortunately, tissue diagnosis of mass-forming intrahepatic cholangiocarcinoma is difficult to perform due to the risk of the procedure and accessibility. The aim of this study is to assess the diagnostic utility of CA19-9 in combination with CT scan for the diagnosis of mass-forming cholangiocarcinoma. **Materials & methods:** The medical records of patients with a diagnosis of CCA and hepatocellular carcinoma (HCC) during January 2005–December 2009 were reviewed. Only cases with pathology and CT scan reports from Maharaj Nakorn Chiang Mai Hospital were included. Demographic data, clinical manifestations and laboratory results, including CA19-9 and CT scans of the liver, were carefully examined in order to establish their diagnostic utility in mass-forming CCA without a pathological diagnosis. **Results:** A total of 79 CCA patients and 66 HCC patients were included in CA19-9 cut-off level analyses. A total of 31 CCA patients and 44 HCC patients were included in the CT scan evaluation and scored according to the Chiang Mai CT score for CCA. The specificity of a CA19-9 value of 500 U/ml for the diagnosis of CCA was 95.5% with a sensitivity of 50.6%, positive predictive value of 91.8% and likelihood ratio of positive (LR+) of 11.24. A CT score greater than 2 demonstrates a positive predictive value of more than 90% in diagnosis of CCA. The CA19-9 level of 140 U/ml in combination with a CT score of 2 demonstrated LR+ as high as 57.09. A CA19-9 level of 280 U/ml in combination with a CT score of 1 demonstrated LR+ of 58.4. **Conclusion:** A CA19-9 level combined with the Chiang Mai CT score for CCA are good diagnostic tools for the diagnosis of mass-forming cholangiocarcinoma with high specificity, high positive predictive value and high likelihood ratio of positive.

KEYWORDS

- CA19-9 level
- Chiang Mai CT score for CCA
- cholangiocarcinoma
- CT scan

Cholangiocarcinoma (CCA) is an adenocarcinoma that arises from the bile duct epithelium. Parasitic infestation by *Opisthorchis viverrini* [1] and *Clonorchis sinensis* plays an important role in the cause of this cancer, especially the majority of cases from the northeastern part of Thailand where people have raw freshwater fish as their regular meals. Recently, hepatitis C viral infection has been discovered as

¹Lampang Cancer Hospital, Lampang, Thailand

²Epidemiology Unit, Faculty of Medicine, Prince of Songkla University, Songkla, Thailand

³Department of Pathology, Faculty of Medicine, Chiang Mai University, Chiang Mai, Thailand

⁴Department of Diagnostic Radiology, Faculty of Medicine, Chiang Mai University, Chiang Mai, Thailand

⁵Division of Medical Oncology, Faculty of Medicine, Chiang Mai University, Chiang Mai, Thailand

*Author for correspondence: Tel.: +66 53 945 480; Fax: +66 53 289 232; sumitra95@gmail.com

another cause of intrahepatic CCA [2]. There is large geographical variation in the incidence of this tumor, being 10-times more common in Japan and Southeast Asia than either the UK or USA. The estimated incidence of liver and bile duct cancer in Thailand in 2001–2003 was highest in Udon Thani province in the northeastern area. The incidence was 115.0 per 100,000 in males and 52.7 per 100,000 in females. In Chiang Mai, liver and bile duct cancer are responsible for the second and fourth causes of cancer with 24.9 and 10.8 per 100,000 in males and females, respectively [3].

The liver is one of the most vulnerable organs for various types of malignant tumors. In a patient presenting with an isolated malignant hepatic tumor, the diagnoses can include liver cancer (hepatocellular carcinoma, cholangiocarcinoma) or a single liver metastasis. To make the correct diagnosis among these varieties, histologic study has been well accepted in most clinical practices. Unfortunately, tissue diagnosis of intrahepatic CCA is difficult to perform according to tumor site, risk of procedure and accessibility. In the past, most CCA patients were diagnosed based on clinical characteristics, laboratory results and imaging studies altogether without pathology report.

The emergence of multi-detector computerized tomographic (CT) scanners has provided more detailed information on the hepatic parenchyma. A CT scan has been used in combination with clinical data for the diagnosis of CCA in most cases. Multidetector CT is versatile and widely

available. It depicts the level and cause of biliary obstruction, characteristics of the mass and helps survey the entire abdomen for diagnosis and disease staging. The characteristics of mass-forming CCA are thin/thick rim-enhancement at the periphery of the arterial phase, gradual centripetal enhancement on delayed phase imaging, capsular retraction and dilated bile duct peripheral to tumor [4,5].

The tumor marker, carbohydrate antigen 19-9 (CA19-9), has been widely employed as a diagnostic adjunct for CCA. CA19-9 has been used as an additional test for the diagnosis of CCA with primary sclerosing cholangitis, with a cut-off level of 100 U/ml [6]. In Chiang Mai, the study cut-off level of CA19-9 for the diagnosis of CCA was performed. With a level of 135.6 U/ml, the sensitivity and specificity in the diagnosis of CCA were 56.6 and 86.4%, respectively [7].

Due to diagnostic difficulties of mass-forming CCA and hepatocellular carcinoma (HCC), as well as no published data evaluating the accuracy of diagnosing mass-forming CCA by CT scan in combination with serum CA19-9 level, this study was conducted. The study will focus on the mass-forming type CCA and exclude other types of CCA. The primary objective of this study is the diagnostic utility of serum CA19-9 in combination with grades of the CT scan of the liver by the Chiang Mai CT score developed by the two radiologists in our hospital for mass-forming CCA.

Figure 1. Imaging characteristics of mass-forming intrahepatic cholangiocarcinoma. (A) Radiologic feature of rim-enhancing mass forming cholangiocarcinoma; **(B)** Radiologic feature of capsular retraction.

Figure 2. Trial profile.

HCC: Hepatocellular carcinoma; CCA: Cholangiocarcinoma.

Methods

• Patients

This is a retrospective review study initiated by the Division of Medical Oncology, Department of Internal Medicine, Faculty of Medicine, Chiang Mai University. All data were retrieved from a hospital-based cancer registry at Maharaj Nakorn Chiang Mai Hospital with the selection criteria of intrahepatic CCA and HCC that were histologically confirmed and diagnosed during January 2005–December 2009. Inclusion criteria included those cases with tissue pathology/cytology, serum CA19-9 and CT scans performed at Maharaj Nakorn Chiang Mai Hospital. All cases with CT report from other hospitals were excluded from the

study. Pathological reports, CA19-9 levels, liver function test, clinical data and CT scans were carefully reviewed and recorded in both groups. This study was approved by the ethics committee of Faculty of Medicine, Chiang Mai University.

• Serum CA19-9 & laboratories

The serum concentrations of CA19-9 were measured using COBAS CORE Immunoassay analyzers (laboratory reference range, 0–37 U/ml). The upper limit of CA19-9 was $\geq 10,000$ U/ml. Serum levels of CA19-9 and other laboratories (complete blood count, liver function test) were obtained within 2 months of diagnosis of CCA and HCC.

Table 1. Baseline characteristics and laboratories of cholangiocarcinoma patients and hepatocellular carcinoma patients.

Characteristics	CCA (n = 79)	HCC (n = 66)	p-value ^{††}
Male (%) [‡]	54 (68.4)	54 (81.8)	0.085
Male:female	2.1:1	4.5:1	
Median age (range)	56 (50–62)	53.5 (46.5–61.8)	0.221
Symptoms (%)[‡]			
Pain	73 (92.4)	56 (84.9)	0.186
Jaundice	9 (14.8)	4 (9.5)	0.552
Weight loss	48 (77.4)	21 (36.2)	<0.001
Anorexia	22 (61.1)	16 (36.4)	0.042
Fatigue	18 (66.7)	10 (24.4)	<0.001
Dark urine	11 (33.3)	1 (3.5)	0.003
Itching	3 (15.0)	1 (3.5)	0.291
Fever	9 (17.7)	11 (23.4)	0.617
Sign (%)[‡]			
Icteric sclera	15 (19.0)	4 (6.7)	0.046
Hepatomegaly	52 (65.8)	39 (60.9)	0.602
Ascites	12 (15.2)	3 (6.4)	0.166
Enlarged lymph node	12 (15.2)	1 (2.3)	0.031
Laboratory[†]			
CA19-9 (U/l)	556.6 (59.8–> 10,000)	17.9 (8.3–43.8)	<0.001
Hb (g/dl)	12.2 (10.7–13.3)	13.1 (11.0–14.2)	0.049
Hematocrit (mg %)	36.6 (32.8–39.5)	39.05 (32.7–42.1)	0.075
WBC (per mm ³)	9640 (8105–11,828)	7350 (5925–9025)	<0.001
Platelet (per mm ³)	300, 500 (250, 500–366, 500)	236, 500 (175, 250–342, 250)	0.009
Albumin (g/dl)	3.6 (3.2–4.1)	3.7 (3.3–4.1)	0.182
Globulin (g/dl)	4.0 (3.6–4.7)	3.9 (3.6–4.5)	0.577
AST (U/l)	42.0 (30.0–72.0)	72.0 (49.8–112.5)	<0.001
ALT (U/l)	30.0 (20.0–53.5)	53 (32.0–86.8)	<0.001
ALP (U/l)	166 (108–246)	139 (101–207)	0.243
TB (mg/dl)	0.77 (0.57–1.20)	0.88 (0.61–1.39)	0.451
DB (mg/dl)	0.20 (0.12–0.42)	0.28 (0.19–0.51)	0.065

[†]Laboratory data expressed as median (range), using Wilcoxon rank-sum test.
[‡]The remaining data expressed as number (%), using Fisher's exact test.
 ALP: Alkaline phosphatase; ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; CA19-9: Carbonic anhydrase 19–9;
 DB: Direct bilirubin; Hb: Hemoglobin; TB: Total bilirubin.

• The CT scan technique

CT examinations were performed with multidetector CT apparatus (Aquilion 16, Toshiba, Japan; SOMATOM definition, Siemens, Germany). The CT parameters were 120 kVp, 200–400 mAs, 1.0 mm × 16 and 0.6 mm × 64 section collimation with a single breath-hold helical acquisition. The unenhanced images were obtained with 5-mm-thick axial sections. The triple-phase contrast-enhanced images were obtained with a thickness of 1.5-mm and 5-mm thick sections from the dome of the diaphragm to the iliac crest.

Approximately 100–150 ml of nonionic contrast material containing 300–350 mg/ml of iodine was administered intravenously at a rate of

3 ml/s. Hepatic arterial phase image acquisition was started 25–30 s after the administration of intravenous contrast media. The portovenous phase and delayed phase were obtained 60–70 s and 5 min after the administration of intravenous contrast media, respectively.

• Imaging analysis

Two radiologists with more than 10 years of experience in abdominal imaging section evaluated the CT images in consensus for each parameter comprising 'Chiang Mai CT Score for CCA'. The presence of the radiologic features which were thin/thick rim-enhancement at the periphery on an arterial phase, gradual

centripetal enhancement on delayed phase imaging, capsular retraction and dilated bile duct peripheral to tumor were reviewed (Figure 1) [4].

• Histological evaluation

All cases diagnosed as CCA and HCC were reported by pathologists from Maharaj Nakorn Chiang Mai Hospital. The presence of adenocarcinoma features was initially treated as CCA. Medical records of these patients were reviewed until their last visit to exclude other primary sites of adenocarcinoma.

• Statistical analysis

The analysis was performed using R statistical software version 2.12.2 [8]. Wilcoxon rank-sum test was used to test the difference in the two

groups when the variables were continuous, and Fisher's exact test was used when they were dichotomous. pROC package version 1.4.1 [9] was used to analyze the receiver operator curve and also sensitivities and specificities of the CA19-9 test and CT score at different cut points. The positive predictive value (PPV) was evaluated at disease prevalence of 0.5 (50%) which is usually considered the pre-test probability when a disease is suspected, with the formula: $PPV = (Se \times P) / ((Se \times P) + ((1-Sp) \times (1-P)))$ and positive likelihood ratio (LR+) equals $Se / (1-Sp)$, where Se = sensitivity, Sp = specificity and P = prevalence rate of the disease. Sensitivity, specificity and PPV were all expressed as percentages.

The Chiang Mai CT score consisted of four characteristics evaluated on CT radiographs of

Figure 3. The receiver operator curve of CA19-9 in the diagnosis of cholangiocarcinoma among patients with suspected liver cancer. The 95% CI band is shown in gray shade.

Table 2. Diagnostic parameters of CA19-9 in the diagnosis of cholangiocarcinoma at different cut-off points.

CA19-9 threshold	Sensitivity (%)	Specificity (%)	PPV (%) (prev. = 0.5)	LR+
5	96.0	19.7	54.5	1.20
10	89.9	29.0	55.9	1.27
15	86.1	40.9	59.3	1.46
20	83.5	52.0	63.5	1.74
30	79.0	66.0	69.4	2.27
40	77.2	71.0	72.7	2.66
50	75.9	78.0	77.5	3.45
60	74.7	80.4	79.2	3.81
70	72.8	81.8	80.0	4.00
80	68.0	82.0	78.9	3.74
90	66.0	83.3	79.8	3.95
100	65.8	85.0	81.4	4.39
110	65.0	86.4	82.6	4.75
120	64.6	88.0	84.3	5.38
140	64.0	90.4	87.0	6.67
160	62.8	90.9	87.3	6.90
180	61.0	91.1	87.0	6.70
200	60.8	92.0	88.4	7.60
240	57.0	92.4	88.2	7.50
280	54.0	94.0	90.0	9.00
500	50.6	95.5	91.8	11.24
1000	48.2	98.5	97.0	32.13
2000	45.0	99.0	96.8	30.00
4000	38.5	100.0	100.0	Inf

CCA and HCC cases were as follows: thin/thick rim-enhancement at the periphery on an arterial phase, gradual centripetal enhancement on delayed phase imaging, capsular retraction and dilated bile duct peripheral to tumor. Logistic regression was used to evaluate the predictability of those four characteristics to CCA. The character that was not significant in the full model was excluded since its presence was highly related with other characters in the model. The rounded coefficients of the remaining characteristics were used as the weights of the CT scoring for CCA. When the Chiang Mai CT score was formulated, the score could be used as the predictor of CCA by the ROC method as if it were a continuous variable.

Results

• Study sample & patient characteristics

This study was conducted between October 2009 and February 2010. Figure 2 outlines the trail profile. There were 104 CCA cases and 169 cases of the HCC group registered with pathological diagnosis. Out of 104 cases in the CCA group, there were 84 cases with confirmed

pathological diagnosis and 41 cases with CT scans available for review. In 84 cases with pathological confirmation, five patients were excluded due to the absence of the CA19-9 level result. Finally, there were 79 cases left for analysis of the CA19-9 cut-off level. In 41 cases with CT scan results, 10 patients were excluded due to the absence of an image for review in two patients, one periductal-type cholangiocarcinoma, one hilar cholangiocarcinoma, one inconclusive diagnosis and five patients with incomplete CT scan protocol (no hepatic arterial and/or portovenous phase examined).

In the HCC group, 169 cases were registered with histologic confirmation. Out of these 169 cases, 66 cases had pathology reports and a CA19-9 level from Maharaj Nakorn Chiang Mai Hospital. CT scans of 45 cases were reviewed, and one patient was excluded due to an incomplete CT scan.

After excluding CCA patients whose pathology and CA 19–9 level were not tested at Maharaj Nakorn Chiang Mai Hospital, there were 79 patients left for CA19-9level evaluation compared with 66 patients in the HCC group (Figure 2).

Demographic data, clinical manifestations and laboratory results of these patients were also carefully examined in each group.

The median age of CCA group was 56 years; it was 53 years in the HCC group, which was not statistically different. Weight loss, fatigue and dark urine were present in the CCA group significantly more than in the HCC group. Icteric sclera and enlarged lymph nodes were also detected more in the CCA group. The median CA19-9 level in the CCA (556.6 U/ml) group was significantly higher than in the HCC group (17.9 U/ml) ($p < 0.001$). Hemoglobin, AST and ALT levels were lower in the CCA group than in the HCC group, while white blood count and platelet count were higher; all were statistically significant. The median

total bilirubin in the CCA and HCC group was 0.77 mg/dl and 0.20 mg/dl, respectively, without statistical significance (Table 1).

• Serum CA19-9

The median CA19-9 level in CCA group (556.6 U/l, 59.8 - >10,000) was significantly higher than in the HCC group (17.9 U/l, 8.3–43.8, $p < 0.001$). The sensitivity of serum CA19-9 decreased from 83.5 to 57.0% with increasing cut-off values from 20 to 240 U/ml, while specificity increased from 52.9 to 92.4%. With the cut-off value of 500 U/ml, specificity, PPV and LR+ were as high as 95.5, 91.8 and 11.24%, respectively. The receiver operator curve of CA19-9 was shown in Figure 3, and the

Figure 4. The receiver operator curve of Chiang Mai CT score in the diagnosis of cholangiocarcinoma among patients with suspected liver cancer. 95% CI band is shown in gray shade.

Table 3. Diagnostic characteristics of CT findings in the diagnosis of cholangiocarcinoma using CT scores.

CT score	CT score threshold	Sensitivity (%)	Specificity (%)	PPV(%) (prev. = 0.5)	LR+
0	0.5	94.4	81.8	83.9	5.19
1	1.5	91.7	84.1	85.2	5.76
2	2.5	77.8	90.9	89.5	8.56
3	3.5	66.7	97.7	96.7	29.33
4	4.5	52.8	97.7	95.9	23.22
5	5.5	50.0	97.7	95.7	22.00
6	6.5	16.7	100.0	100.0	Inf

area under the curve was 82%. The diagnostic parameters, sensitivity, specificity, PPV and LR+ of CA19-9 level at different cut points were shown in Table 2.

• CT scan

On CT evaluation, the gradual centripetal enhancement on delayed phase imaging was excluded. The weights for thin/thick rim enhancement at the periphery of the arterial phase, capsular retraction and dilated bile duct peripheral to tumor were 1, 4 and 2, respectively. Thus, the Chiang Mai CT score for CCA ranged from 0 to 7 when the absence and presence of such characteristics were 0 and 1. Capsular retraction was determined with the highest predictability for CCA by logistic model, while gradual centripetal enhancement in the delayed phase was determined to be dependent on other characters. The receiver operator curve of the CT score in prediction of CCA was shown in Figure 4. The area under the curve was as high as 93%. With the Chiang Mai CT score of 2, sensitivity and specificity were 77.8 and 90.9% respectively. The score of 3 to 5 predicted specificity of 97.7%, whereas the score of 6 produced specificity of 100% (Table 3).

• CA19-9 & CT score for diagnosis of mass-forming intrahepatic cholangiocarcinoma

When using CA19-9 level alone, the cut-off level of 500 U/ml produced sensitivity, specificity and LR+ of 50.6, 95.5, and 11.24, respectively.

While using the CT score alone, the score of 3 demonstrated sensitivity, specificity and LR+ of 66.7, 97.7 and 29.33%, respectively. The combination of CA19-9 level of 140 U/ml and Chiang Mai CT score of 2 for the diagnosis of mass-forming CCA demonstrate LR+ 57.09, whereas CA 19-9 level 280 U/ml and Chiang Mai CT score 1 show LR+ 51.84 (Tables 4 & 5).

Discussion

This is the first report demonstrating the utility of CA19-9 and the Chiang Mai CT score for the diagnosis of mass-forming CCA in advanced liver disease, non-PSC patients. Even pathological diagnosis of mass-forming CCA had been well accepted; however, with the difficulty to obtain histology, CA19-9 has been investigated for the diagnosis of CCA in primary sclerosing cholangitis (PSC) and non-PSC patients. The sensitivity and specificity for CA19-9 value of 100 U/ml for CCA in PSC was 89 and 86%, respectively [6]. In non-PSC patients, a previous study from Chiang Mai University demonstrated that sensitivity and specificity were 56.6 and 86.4% of that CA19-9 level of 135.6 U/ml [7], which was comparable with this study (at 140 U/ml; sensitivity 64.0%, specificity 90.4%, PPV 87.0%, LR+ 6.67). The CA19-9 level of 500 U/ml demonstrated 50.6% sensitivity, 95.5% specificity, PPV of 91.8 and LR+ of 11.24. The probability of being CCA increases dramatically when the level of CA19-9 reaches 40. When the level of CA19-9 is greater than 280, the PPV of being CCA reaches 90% (Figure 3).

Table 4. Chiang Mai CT score for cholangiocarcinoma.

CT score	CT scan characteristic
1	Thin/thick rim enhancement at periphery on arterial phase
2	Dilated bile duct peripheral to tumor
4	Capsular retraction
Scoring by sum CT score of each present characteristic, sum score range from 0 to 7.	

Table 5. Recommended CA19-9 level and Chiang Mai CT score in combination for the diagnosis of mass-forming cholangiocarcinoma.

CA19-9		Chiang Mai CT score		Combination
Level (U/ml)	LR+	Score	LR+	LR+
140	6.67	2	8.56	57.09
280	9.00	1	5.76	51.84
500	11.24	0	5.19	58.33
–	–	3	29.33	–

The typical appearance of mass-forming CCA was thin/thick rim enhancement at the periphery of an arterial phase, gradual centripetal enhancement on delayed phase imaging, capsular retraction and dilated bile duct peripheral to the tumor [4–5,10]. These imaging characteristics have never been evaluated for predictability of CCA diagnosis. This study demonstrated the ‘Chiang Mai CT score for CCA’ [POJCHAMARNWIPUTH S, NA CHIANG MAI W, PERS. COMM.] ranging from 0 to 7 from 3 CT scan characteristics (Table 4). Even when the CT score equals 0, PPV ($p = 0.5$) and LR+ are obviously high. It is not possible to exclude CCA even when the CT score is 0. Thus, the CT scan is a good confirmatory tool for CCA when one or more characteristics of CCA are present but not a good tool to exclude CCA since there is already high chance of being CCA when score is 0. The CA19-9 level, when used in combination with Chiang Mai CT score demonstrated very high LR+ (Table 5). The CA19-9 level of 140 U/ml with Chiang Mai CT score of 2, CA19-9 of 280 U/ml with CT score of 1 and CA19-9 of 500 U/ml with CT score of 0 demonstrated LR+ as high as 57.09, 51.84 and 58.33, respectively.

Conclusion & future perspective

Serum CA19-9 in combination with the Chiang Mai CT score are good diagnostic tools for the diagnosis of mass-forming cholangiocarcinoma. The authors recommended clinical application by using CA19-9 level of 140 U/ml with a CT score of 2, CA19-9 level of 280 U/ml with a CT score of 1, and CA19-9 level of 500 U/ml with a

CT score of 0 for the diagnosis of mass-forming cholangiocarcinoma. These criteria will be useful for the cases that have difficulty in getting histological diagnosis. Though the definite diagnosis for all cancer should be confirmed by the histology; however in some cases especially for deep seated tumor, the method to obtain histologic diagnosis may not be possible. Such cases the CT imaging and tumor marker is useful and it will be valuable if we can demonstrate the cut off value like in this report.

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Ethical conduct of research

The authors state that they have obtained appropriate institutional review board approval or have followed the principles outlined in the Declaration of Helsinki for all human or animal experimental investigations. In addition, for investigations involving human subjects, informed consent has been obtained from the participants involved.

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