Systematic Review and Meta-Analysis

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The diagnostic performance of serum MUC5AC for cholangiocarcinoma

A systematic review and meta-analysis

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Abstract

Specific diagnostic biomarker for cholangiocarcinoma (CCA) has been lacking. This systematic review and meta-analysis was performed aiming to investigate serum MUC5AC's diagnostic performance on CCA.

Studies investigating serum MUC5AC's diagnostic value on CCA were retrieved from Pubmed, Embase, and Cochrane Library. The methodology quality of included studies was assessed according to QUADAS-2. Diagnostic 2×2 table was extracted from each eligible study, Meta-disc 1.4 was used for statistical analysis, data synthesis was done using a random-effects model. Subgroup analyses were conducted according to region and array method.

Six eligible studies were identified, a total of 1213 patients were involved in the meta-analysis. The AUC on SROC was 0.9138, and the Q^* was 8463. The pooled sensitivity, specificity, positive likelihood ratio, negative likelihood ratio, and diagnostic odds ratio (DOR) were 0.69 (95% CI: 0.65–0.73), 0.93 (95% CI: 0.91–0.95), 8.99 (95% CI: 5.65–14.30), 0.33 (95% CI: 0.24–0.46), and 33.98 (95% CI: 20.12–57.40), respectively. Targeting MUC5AC's epitope has a higher pooled sensitivity than targeting MUC5AC protein (0.77 vs 0.63). There was substantial cross-study heterogeneity.

Serum MUC5AC might be potentially used as a surrogate marker in the diagnosis of CCA. However, the appropriate array method and the optimum cut-off value are yet to be decided.

Abbreviations: AUC = area under curve, CCA = cholangiocarcinoma, DOR = diagnostic odds ratio, FN = false negative, FP = false positive, IHC = immunohistochemistry, NLR = negative likelihood ratio, PLR = positive likelihood ratio, QUADAS = quality assessment of diagnostic accuracy studies, SROC = summary receiver's operative characteristics, TN = true negative, TP = true positive.

Keywords: cholangiocarcinoma, diagnostic accuracy, meta-analysis, serum MUC5AC

1. Introduction

Although cholangiocarcinoma (CCA) is a relatively rare cancer, its incidence has been increasing in recent years.[1,2] In the clinical practice, serum carbohydrate antigen 19-9 (CA19-9) and carcinoembryonic antigen (CEA) have been widely used as tumor markers for CCA, but neither of them are of satisfactory diagnostic value. Attempts that try to identify specific serum biomarkers for CCA have been made worldwide; unfortunately, the results of these trials had been disappointing.[3–5] The diagnosis of CCA is difficult largely due to the low diagnostic accuracy of minimally invasive biopsy procedures; moreover, it sometimes takes an indolent course. Therefore, serum biomarker with high diagnostic value for CCA is in urgent need.

Because most CCA cells are mucin producing. Thus, mucin expression has become associated with CCA's carcinogenesis and development. There are various types of mucin protein, among them MUC5AC, a member of the secreted protein category, has been recently regarded as a potentially specific biomarker for CCA.[3,6] MUC5AC is rarely produced in normal conditions, whereas in pathological conditions, when there are anatomical changes of bile tract epithelial cells in presence, the expression of MUC5AC is thus up-regulated. Several recently conducted studies have shown that positive MUC5AC immunohistochemistry (IHC) staining was observed in surgically most resected CCA specimens and this finding was associated with advanced tumor stage.[5,7] There were also several studies that investigated serum MUC5AC's diagnostic value on CCA; however, there was still no consensus on whether serum MUC5AC could be used as a specific marker for this malignancy.

In that case, we performed this systematic review and meta-analysis with the aim of fully investigating serum MUC5AC's diagnostic performance on CCA.

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This research is a meta-analysis which does not need an approval from the ethnic committee board.

JX: writing of the manuscript, language polishing, quality assessments. JL: writing of the manuscript, literature search, submission. ZZ: statistical analysis, literature search. RZ: literature search. HX: quality assessments. WW: study design, quality assessments.

JX, JL and ZZ contributed equally to this work.

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2. Materials and methods

2.1. Literature search

Full-text articles published up to April 2015 were retrieved from 4 electronic databases: Pubmed, Embase, Medline, and Cochrane library. "Cholangiocarcinoma," "bile tract cancer," "serum MUC5AC," "MUC5AC," "mucin," "mucin expression," "biomarker" were used as search terms. Two investigators (JL and HX) independently conducted the literature search. When there was discrepancy occurred, agreement was reached after mutual discussion.

2.2. Inclusion and exclusion criteria

There was no restriction on published languages. Articles that met the following items were included in the meta-analysis: studies investigated serum MUC5AC's diagnostic value on CCA; studies that contain sufficient data to reconstruct a 2×2 diagnostic table; prospective cohort study, case-control studies; histological pathology serves as criterion standard. The exclusion criteria were: duplication of records; overlapping of study population; reviews, letters, case reports, abstracts or conference proceedings; studies evaluating the diagnostic value of MUC5AC's IHC expression; animal experiments or studies conducted using cell lines.

2.3. Data extraction

Two investigators independently extracted data that were needed for the meta-analysis, consensus was reached at a meeting chaired by the corresponding author (WW). Major characteristics of included studies, such as region of origin, year of publication, patients involved, male-to-female ratio, age (median or average), array method, cut-off value, were extracted. Numbers of true-positive (TP), false-positive (FP), false-negative (FN), and true-negative (TN) were retrieved from each included study.

2.4. Quality assessment

Cochrane's Quality Assessment of Diagnostic Accuracy Studies (QUADAS-2), which contain risk of bias domain and applicable concerns domain, were used to evaluate the methodology quality of each included study. Two authors (JL and HX) independently reviewed each included study, interobserver variation was solved at a meeting chaired by the corresponding author (WW).

2.5. Statistical analysis

Meta-disc (Version 1.4) was used for statistical analysis. A 0.5 value was automatically added to the cells with 0 for adjustments. Data synthesizing was done using random-effects model which is a relatively conservative approach but ensures less chance of bias. Q test was applied to assess the cross-study heterogeneity, with $I^2 > 50\%$ indicating substantial heterogeneity, whereas an $I^2 < 50\%$ was considered low heterogeneity.

Summary receiver's operative characteristics (SROC) curve was drafted to summarize serum MUC5AC's overall diagnostic performance on CCA. On SROC, area under curve (AUC) greater than 0.80 indicates good diagnostic performance, the closer it is to 1.0 the better the overall diagnostic performance is. Q point value (Q^*) was also calculated to assess the overall diagnostic performance.

Based on 2×2 diagnostic tables extracted from included studies, the pooled sensitivity, specificity, positive likelihood ratio (PLR), negative likelihood ratio (NLR), and diagnostic odds ratio (DOR) were calculated and presented with corresponding 95% confidence intervals (CI). Results of synthesized data were visualized on forest plots. Subgroup analyses were performed according to region and assay method.

3. Results

3.1. Literature search

A total of 103 articles were initially extracted, 32 records remained after duplicates were removed. Later, 11 full-text articles were potentially eligible for the meta-analysis; however, 2 of those studies that used IHC method and 2 articles that investigated serum MUC5AC's diagnostic value on other diseases were thus excluded. Seven studies met the inclusion criteria; however, among 5 studies from Thailand, 2 were excluded after screening for patients' overlapping,[8,9] so 6 studies were finally included[8,10–14] (Fig. 1).

3.2. Study characteristics

Six included studies with 1213 patients were involved in the meta-analysis. Three included studies were from Europe, while the other 3 were from Thailand. The sample sizes of 3 European studies were much smaller compared with 3 Thai studies. The documented male-to-female ratio ranged from 0.89 to 2.6, only 3 studies had documented median or average age. Four studies directly targeted serum MUC5AC while the other 2 targeted a certain epitope on MUC5AC protein using sandwich ELISA. Other details of included studies are shown in (Table 1).

All 6 included studies were of prospective cohort design, 2 Italian trials recruited healthy individuals as control group. The overall methodological quality of included studies was generally well as was measured by QUADAS-2 scores (Table 2).

3.3. The diagnostic performance of serum MUC5AC for CCA

On SROC, the AUC value was 0.9138 which was greater than 0.80 and was close to 1.0, indicating excellent overall diagnostic performance. The Q^* value calculated was 0.8463. The pooled diagnostic odds ratio (DOR) was 33.98 (95% CI: 20.12–57.40), with I^2 value at 36.7% (Fig. 2).

The pooled sensitivity was 0.69 (95% CI: 0.65–0.73), with I^2 value at 85.0%. The pooled specificity was 0.93 (95% CI: 0.91–0.95), with I^2 value at 69.1% (Fig. 3). The pooled positive likelihood ratio and negative likelihood ratio were 8.99 (95% CI: 5.65–14.30) with I^2 value at 53.0% and 0.33 (95% CI: 0.24–0.46) with I^2 value at 83.6% respectively (Fig. 4).

3.4. Subgroup analyses

According to region where the study was carried out, 6 included trials were divided into 3 Asian studies, Thailand to be more specifically, and 3 European studies. In addition, based on different array methods, 6 studies were divided into MUC5AC group that contains 4 studies and MUC5AC epitope group that contains 2 studies. Both the pooled sensitivity (0.62 vs 0.71) and specificity (0.92 vs 0.93) of European studies were slightly lower than Asian studies, but significant heterogeneity was observed in

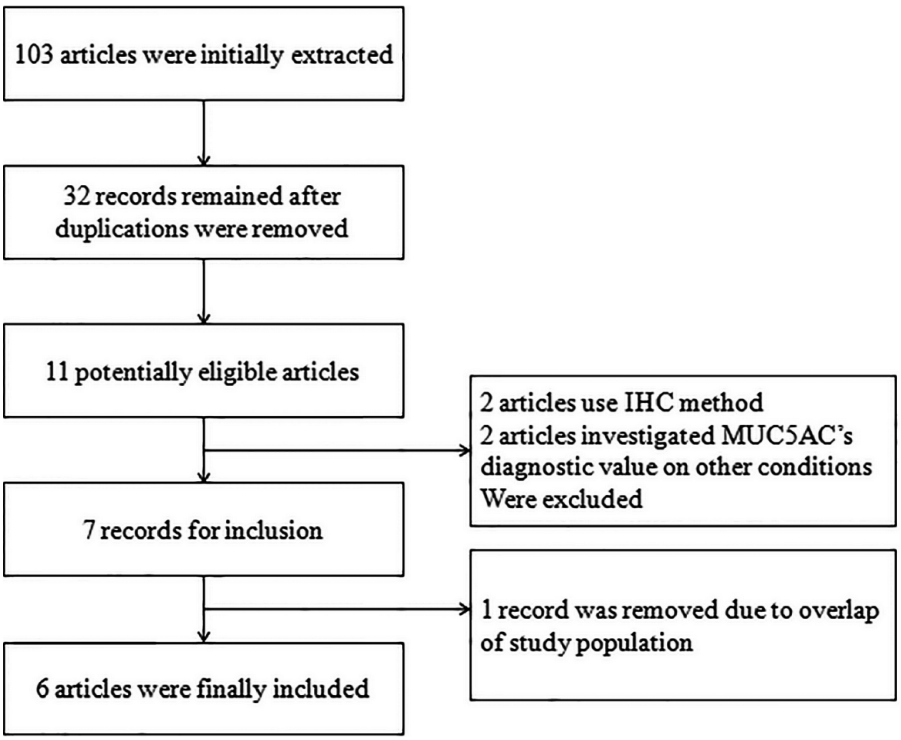

Figure 1. Flow chart illustrating literature search and screening.

those subgroups. On another subgroup analysis, the pooled sensitivity of studies targeting MUC5AC was lower than studies targeting MUC5AC's epitopes (0.63 vs 0.77), the pooled specificity of 4 studies targeting MUC5AC was slightly higher than 2 studies targeting MUC5AC's epitopes (0.96 vs 0.90) (Table 3).

4. Discussion

The early detection of CCA poses a clinical challenge because the early onset of CCA is mostly unnoticeable plus specific serum tumor marker for CCA has been lacking.[3] Efforts that search for CCA's specific serum markers have been going on in recent years; however, little advancements have been made.[4,15,16] A number of studies have shown that positive immunohistological MUC5AC expression in surgically resected CCA specimen and serum MUC5AC was associated with worse prognosis, thus serum MUC5AC was deemed as a potential specific biomarker for CCA, but the results of several diagnostic tests were heterogeneous; for this reason we performed this systematic review and meta-analysis.[5,6,17]

The results of this study indicated that serum MUC5AC might be a useful tool for the diagnosis of CCA, with the AUC on SROC at 0.9138 and the pooled DOR at 34 plus the high Q^* value. Statistically speaking, serum MUC5AC's overall diagnostic performance for CCA is excellent.

The pooled sensitivity and specificity was 0.69 and 0.93 respectively. So in clinical practice, when there is an indeterminate biliary stricture or filling defect in ERCP or MRCP images, serum MUC5AC could be otherwise used for the confirmation of CCA. On the other hand, serum MUC5AC is not an ideal approach for CCA's early detection, as the principal of diagnostic tests requires a LR- <0.05 to rule out certain disease, but the pooled negative likelihood ratio in this study was 0.33, indicating that using serum MUC5AC as a screening tool tends to miss early CCAs without clinical manifestation. Therefore, we do not

Table 1

Major characteristics of included studies.

First author	Nation	Year of publication	Assay method/target	Cut-off value	M/F ratio	Age (mean or median, Y)	Patients (n)	TP	FP	FN	TN
Wongkham[10]	Thailand	2003	Immunoblotting /MUC5AC mucin	NA	NA	NA	435	112	8	67	248
Bamrungphon[11]	Thailand	2007	Sandwich ELISA/ MoAb-22C5,MUC5AC	OD: 0.074	2.6	55.6 ± 9.8	289	120	12	49	108
Matull[12]	UK	2008	Western blot /MUC5AC	Positive expression	0.89	67 (34–90)	66	17	1	22	26
Silsirivanit[8]	Thailand	2011	Sandwich ELISA/MoAb S121,MUC5AC	NA	2.46	NA	289	85	20	12	172
Ruzzenente[13]	Italy	2014	ELISA /MUC5AC	10.5 ng/mL	1.41	68 (38–85)	88	35	2	14	37
Danese[14]	Italy	2014	ELISA /MUC5AC	10.5 ng/mL	1.19	67 ± 9	46	19	4	7	16

ELISA = enzyme linked immunosorbent assay, FN = false negative, FP = false positive, MoAb = monocolonal antibody, NA = not available, OD = optical density, TN = true negative, TP = true positive.

Table 2

Methodology quality of included studies assessed by QUADAS-2.

Study	Risk of bias				Applicable concerns		
	Patient selection	Index test	Reference standard	Flow and timing	Patient selection	Index test	Reference standard
Wongkham[10]	L	L	L	L	L	L	L
Bamrungphon[11]	L	L	?	L	L	L	L
Matull[12]	L	L	H	L	L	L	?
Silsirivanit[8]	L	L	L	L	L	L	L
Ruzzenente[13]	H	L	L	H	L	L	L
Danese[14]	H	L	L	H	L	L	L

?=unclear risk, H=high risk, L=low risk, QUADAS=Quality Assessment of Studies of Diagnostic Accuracy included in Systematic Reviews.

suggest that serum MUC5AC be used to screen for CCA in the future.

We speculate that 2 factors may be the source of cross-study heterogeneity. First, the etiology of CCA in Western countries and Asian countries might have been different. Second, the laboratory methodology that evaluates serum MUC5AC varies between studies. We conducted subgroup analyses to verify whether those 2 factors influence the overall diagnostic performance. In the first subgroup analysis, 6 studies were divided into 3 European and 3 Asian (Thailand) studies. Both the pooled sensitivity and specificity of Asian studies were slightly higher. On another subgroup analysis, the pooled sensitivity of 2 studies targeting MUC5AC epitopes was 0.77, much higher compared with studies targeting MUC5AC protein, indicating that targeting certain MUC5AC epitope may be potentially used for the early detection of CCA in the future. In

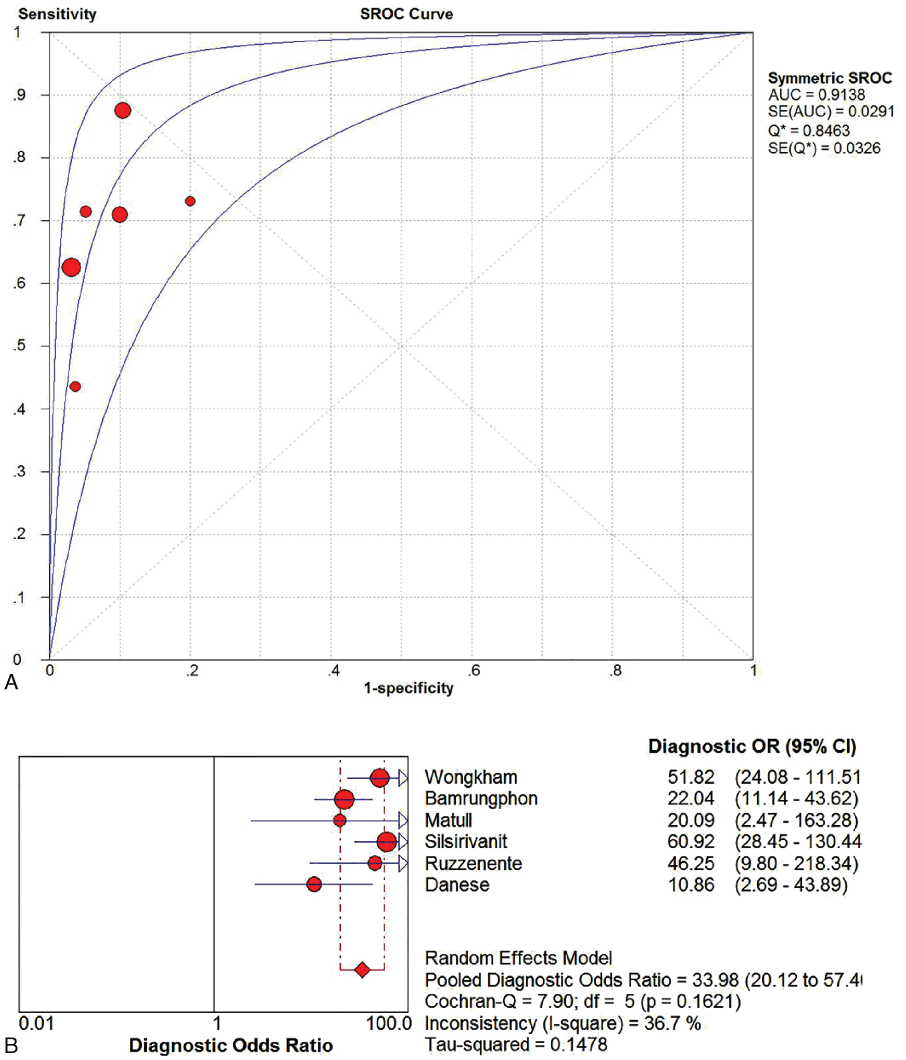

Figure 2. MUC5AC's overall diagnostic performance on CCA. A, SROC. B, Pooled DOR. AUC, area under curve; CI, confidence interval; df, degrees of freedom; SE, standard error.

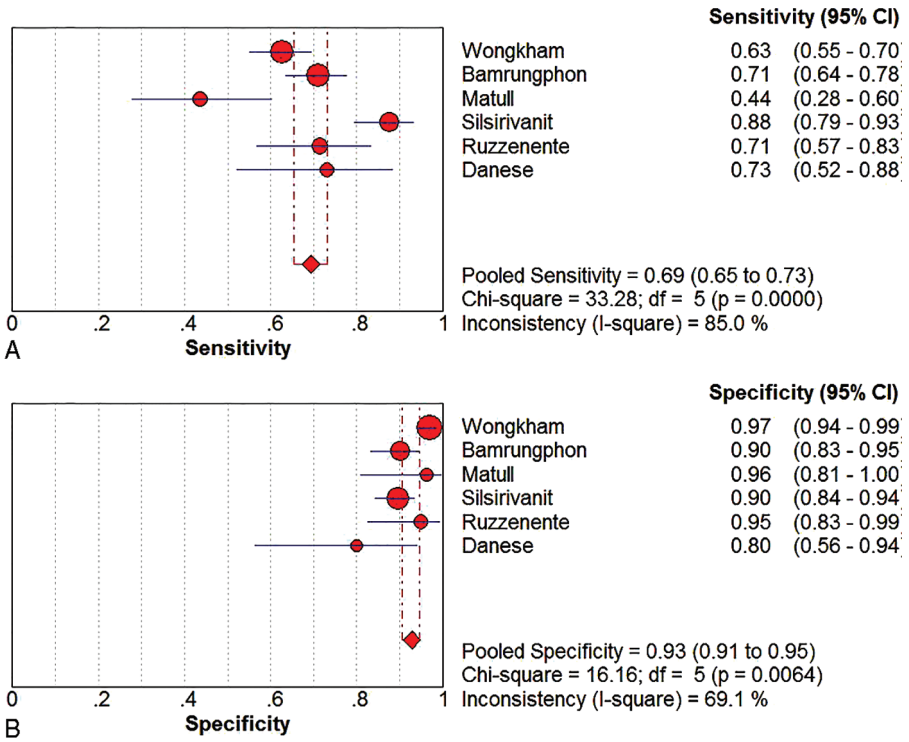

Figure 3. Pooled sensitivity and specificity of serum MUC5AC for CCA. A, Pooled sensitivity. B, Pooled specificity. CI, confidence interval; df, degrees of freedom.

contrast, targeting MUC5AC epitopes is associated with a lower pooled specificity, suggesting that targeting MUC5AC protein might be effective in confirming the diagnosis of CCA when the image studies are inconclusive. We did not see sharp decrease in I^2 value in subgroup analyses and because of small number of included studies, we were not able to perform meta-regression that requires at least 10 included trials. As a result, these findings should be interpreted with caution.

The are several limitations to note in the current study. Although we used random-effects model, in the subgroup analyses, there was still marked cross-study heterogeneity, making the results of this study less robust.[18,19] Moreover, all 3 Asian studies were from Thailand, we identified no eligible

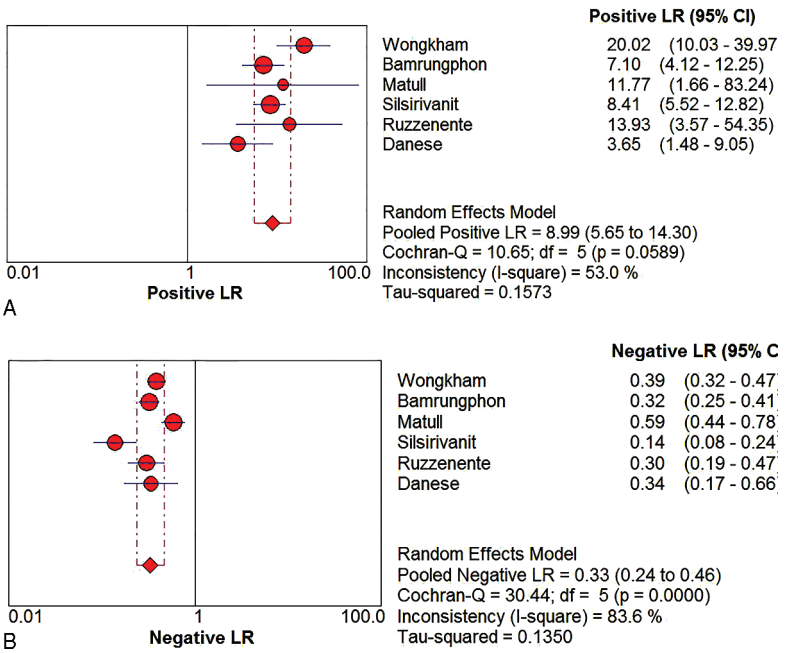

Figure 4. Pooled positive likelihood ratio and negative likelihood ratio of serum MUC5AC for CCA. CI, confidence interval; df, degrees of freedom.

Table 3

Subgroup analysis according to different regions and array methods.

Subgroup	Region/target	Studies (n)	Pooled sensitivity (95% CI)	I^2	Pooled specificity (95% CI)	I^2
Region	Europe	3	0.62 (0.53–0.71)	77.1%	0.92 (0.84–0.97)	52.2%
	Asia (Thailand)	3	0.71 (0.67–0.75)	90.6%	0.93 (0.91–0.95)	83.1%
Array method (target)	MUC5AC	4	0.63 (0.57–0.68)	65.7%	0.96 (0.93–0.98)	60.5%
	MUC5AC epitopes	2	0.77 (0.72–0.82)	90.3%	0.90 (0.86–0.93)	0%

studies from China, Japan, or Korea, regions where CCA is also endemic. Despite decent number of patients being involved in the diagnostic tests, the majority of them were from Thailand, which would inevitably make the result parochial. In addition, apart from different regions and array methods, we do not know whether other factors would have impact on the overall results, especially the liver fluke, a parasite that is closely related to CCA in Asia. In 3 Thai studies, *Opisthorchis viverrini*-infected patients and non-*Opisthorchis viverrini*-infected patients could not be separately analyzed. European studies had drawbacks too. Two Italian trials were of lower methodology quality due to selection bias.[13,14] Unfortunately, at the current level of evidence, we are not sure which array method is superior to the other one, nor could we define the appropriate cut-off value of serum MUC5AC for the diagnosis of CCA, and that could be rather useful in the practice. In that case, there is still a long way to go before this serum MUC5AC can be clinically used.

In conclusion, serum MUC5AC performs well in the diagnosis of CCA, targeting on MUC5AC using ELISA or Western blot may be an effective approach to confirm CCA when image studies are indeterminate, while targeting on certain epitopes of MUC5AC could be potentially used for the screening or early detection of CCA. Last but not least, we call for more relevant studies in the future to validate serum MUC5AC's diagnostic role for CCA.

References

[1] Malhi H, Gores GJ. Review article: the modern diagnosis and therapy of cholangiocarcinoma. Aliment Pharmacol Ther 2006;23:1287–96.

[2] Rizvi S, Gores GJ. Pathogenesis, diagnosis, and management of cholangiocarcinoma. Gastroenterology 2013;145:1215–9.

[3] Nehls O, Gregor M, Klump B. Serum and bile markers for cholangiocarcinoma. Semin Liver Dis 2004;24:139–54.

[4] Sasaki M, Tsuneyama K, Nakanuma Y. Aberrant expression of trefoil factor family 1 in biliary epithelium in hepatolithiasis and cholangiocarcinoma. Lab Invest 2003;83:1403.

[5] Park SY, Roh SJ, Kim YN, et al. Expression of MUC1, MUC2, MUC5AC and MUC6 in cholangiocarcinoma: prognostic impact. Oncol Rep 2009;22:649–57.

[6] Higashi M, Yonezawa S, Ho JJ, et al. Expression of MUC1 and MUC2 mucin antigens in intrahepatic bile duct tumors: its relationship with a new morphological classification of cholangiocarcinoma. Hepatology 1999;30:1347–55.

[7] Boonla C, Sripa B, Thuwajit P, et al. MUC1 and MUC5AC mucin expression in liver fluke-associated intrahepatic cholangiocarcinoma. World J Gastroenterol 2005;11:4939–6.

[8] Silsirivanit A, Araki N, Wongkham C, et al. A novel serum carbohydrate marker on mucin 5AC: values for diagnostic and prognostic indicators for cholangiocarcinoma. Cancer 2011;117:3393–403.

[9] Silsirivanit A, Araki N, Wongkham C, et al. CA-S27: a novel Lewis a associated carbohydrate epitope is diagnostic and prognostic for cholangiocarcinoma. Cancer Sci 2013;104:1278–4.

[10] Wongkham S, Sheehan JK, Boonla C, et al. Serum MUC5AC mucin as a potential marker for cholangiocarcinoma. Cancer Lett 2003;195:93.

[11] Bamrungphon W, Prempracha N, Bunchu N, et al. A new mucin antibody/enzyme-linked lectin-sandwich assay of serum MUC5AC mucin for the diagnosis of cholangiocarcinoma. Cancer Lett 2007;247:301–8.

[12] Matull WR, Andreola F, Loh A, et al. MUC4 and MUC5AC are highly specific tumour-associated mucins in biliary tract cancer. Br J Cancer 2008;98:1675–81.

[13] Ruzzenente A, Iacono C, Conci S, et al. A novel serum marker for biliary tract cancer: diagnostic and prognostic values of quantitative evaluation of serum mucin 5AC (MUC5AC). Surgery 2014;155:633–9.

[14] Danese E, Ruzzenente O, Ruzzenente A, et al. Assessment of bile and serum mucin5AC in cholangiocarcinoma: diagnostic performance and biologic significance. Surgery 2014;156:1218–4.

[15] Lee KT, Liu TS. Altered mucin gene expression in stone-containing intrahepatic bile ducts and cholangiocarcinomas. Dig Dis Sci 2001;46:2166–72.

[16] Lau SK, Weiss LM, Chu PG. Differential expression of MUC1, MUC2, and MUC5AC in carcinomas of various sites: an immunohistochemical study. Am J Clin Pathol 2004;122:61–9.

[17] Malaguarnera G, Paladina I, Giordano M, et al. Serum markers of intrahepatic cholangiocarcinoma. Dis Markers 2013;34:219–8.

[18] Whiting P, Rutjes AW, Reitsma JB, et al. The development of QUADAS: a tool for the quality assessment of studies of diagnostic accuracy included in systematic reviews. BMC Med Res Methodol 2003;3:25.

[19] Deeks JJ, Macaskill P, Irwig L. The performance of tests of publication bias and other sample size effects in systematic reviews of diagnostic test accuracy was assessed. J Clin Epidemiol 2005;58:882–93.