BMJ Open

Meta-analysis of the diagnostic value of wisteria floribunda agglutinin-sialylated mucin1 and the prognostic role of mucin1 in human cholangiocarcinoma

Journal:	*BMJ Open*
Manuscript ID	bmjopen-2018-021693
Article Type:	Research
Date Submitted by the Author:	12-Jan-2018
Complete List of Authors:	Tang, Zengwei; The First Clinical Medical School of Lanzhou University, Department of Special Minimally Invasive Surgery, The first Hospital of Lanzhou University Yang, Yuan; The First clinical medical School of Lanzhou University, Department of Internal Medicne, The First hospital of Lanzhou University Wang, Xiaolu; West China Medical School, Sichuan University, Department of General Surgery, West China Hospital/West China Medical School, Sichuan University Zhao, Zhonghong; Department of Integrated traditional Chinese and Western Medicine, People's Hospital of Zhengning County Meng, Wenbo; Department of Special Minimally Invasive Surgery, The First Hospital of Lanzhou University Li, Xu; The second department of General Surgery, The First Hospital of Lanzhou University
Keywords:	Cholangiocarcinoma, prognosis, dignosis, mucin1, meta-analysis

Meta-analysis of the diagnostic value of wisteria floribunda agglutinin-sialylated mucin1 and the prognostic role of mucin1 in human cholangiocarcinoma

Authors and affiliations:

Zengwei Tang [1]

Yuan Yang [1]

Xiaolu Wang [3]

Zhonghong Zhao [4]

Wenbo Meng [1,2]

Xun Li [1,5]

Correspondence:

Professor **Wenbo Meng**

Department of Special Minimally Invasive Surgery, The First Hospital of Lanzhou University, Lanzhou 730000, China.

Tel:+86 139 1917 7177

Fax:+96 0931-8622275

E-mail: mengwb@lzu.edu.cn

[1]The First Clinical Medical School of Lanzhou University, Lanzhou, China.

[2]Department of Special Minimally Invasive Surgery, The First Hospital of Lanzhou University, Lanzhou, China.

[3]Department of General Surgery, West China Hospital/West China Medical School, Sichuan University, Chengdu, China.

[4]People's Hospital of Zhengning County, Qingyang, China.

[5]The second department of General Surgery, The First Hospital of Lanzhou University, Lanzhou, China.

Keywords: cholangiocarcinoma, prognosis, diagnosis, mucin1, meta-analysis

Word count: 3,942

Financial support: The study was funded by West Light Foundation of The Chinese Academy of Science [(2015) 90]. The funding sources had no role in the design conduct of the study, collection, development of methodology,analysis, management and interpretation of the data, or preparation, review, or approval of the manuscript.

Author Contributions: Zengwei Tang–study concept and design; development of methodology, collection and extraction of data, statistical analysis and interpretation of data, and drafting of the manuscript; critical revision of the manuscript for significant intellectual content.

Yuan Yang, Xiaolu Wang and Zhonghong Zhao–collection and extraction of data, analysis and interpretation of data.

Wenbo Meng and Xun Li–critical revision of the manuscript for significant intellectual content, and study supervision.

Disclosures: Zengwei Tang, Yuan Yang, Xiaolu Wang, Zhonghong Zhao, Wenbo Meng and Xun Li have no conflicts of interest to declare.

Patient consent: Consent is not required when conducting a meta-analysis.

Ethics approval: This study did not require ethical approval as the data used have been published previously, and hence are already in the public domain.

Data sharing statement: Extracted data are available upon request to the corresponding author.

Meta-analysis of the diagnostic value of wisteria floribunda agglutinin-sialylated mucin1

and the prognostic role of mucin1 in human cholangiocarcinoma

Abstract

Objective: To evaluate the diagnostic and prognostic value of wisteria floribunda agglutinin-sialylated mucin1(WFA-MUC1) / mucin1(MUC1) in human cholangiocarcinoma (CCA).

Materials and Methods: Four electrical databases were searched systematically to identify studies investigating the diagnostic and prognostic value of WFA-MUC1/MUC1 in CCA. The diagnostic sensitivity and specificity of WFA-MUC1were extracted and analyzed as bivariate data. Pooled hazard ratio (HRs) and its 95% confidence interval (CI) of MUC1 were calculated with a random-effects model meta-analysis on overall survival of resectable CCA.

Results: Sixteen reports were included. The pooled sensitivity and specificity of WFA-MUC1 were 0.76 (0.71 to 0.81) and 0.72 (0.59 to 0.83) in serum, 0.85 (0.81 to 0.89) and 0.72 (0.64 to 0.80) in bile, and 0.72 (0.50 to 0.87) and 0.85 (0.70 to 0.93) in tissue. The SROC were 0.77 (0.73 to 0.81) in serum, 0.88 (0.85 to 0.90) in bile, and 0.86(0.83-0.89) in tissue. The pooled HRs of MUC1 in CCA was 2.20 (1.57 to 3.01), and in mass-forming intrahepatic CCA was 4.17 (1.71-10.17).

Conclusions: Compared to the CA19-9, WFA-MUC1 has shown an increased diagnostic capability. Furthermore, MUC1 can be served as a poor prognosis factor of CCA, particularly in mass-forming intrahepatic CCA.

Keywords: cholangiocarcinoma, prognosis, diagnosis, mucin1, meta-analysis

Strengths and limitations of this study

- The present meta-analysis evaluated the diagnostic and prognostic role of WFA-MUC1/MUC1 in cholangiocarcinoma.
- The diagnostic capability of WFA-MUC1 is superior to that of CA19-9.
- The diagnostic capability biliary level of WFA-MUC1 outweigh that in serum.
- Positive expression of MUC1 in biliary duct cancer tissue was a poor prognosis factor for resectable cholangiocarcinoma.
- The majority subjects including in this meta-analysis were from Asian hospitals, there may be biological differences in tumor behavior among different region populations.

Introduction

Cholangiocarcinoma (CCA) is an epithelial malignancy arising from varying anatomic locations in the biliary tree.[1] The median survival rates of patients with unresectable CCA is less than a year.[2, 3] The prognosis of subjects with CCA undergoing the radical resection is considerably higher, with five year-survival rates of 20%-40%.[4, 5] While surgical resection at the early stage of CCA is not feasible in most cases since the beginning of detection of these types of carcinoma is difficult even with an currently advanced imaging technology and a complete diagnostic workup, which limits the benefits of surgery therapy and curative treatment options and contributes to the poor outcome of patients with CCA.

There is a vast amount of literature on reported that numerous molecular biomarkers with limited diagnostic or prognostic capability of CCA have been certified and use for guiding clinical diagnosis and treatment world widely, such as mucin2~6,[6-15] carbohydrate antigen 19-9 (CA19-9),[16-18]

minterleukin6,[19, 20] Serum cytokeratin19 fragments[21, 22] and carbohydrate antigen125 (CA125),[16, 23, 24] et al. Traditionally, serum CA19-9's research focus has always been a biomarker for CCA. However, CA19-9 is not satisfied with an overall sensitivity and specificity, and does not contribute to the detection of the progression of CCA.[5, 17, 24] Moreover, CA19-9 is elevated in up to 85% suspected CCA.[17, 25, 26] The limited diagnostic utility in account of the influence of co-existing inflammatory conditions of the biliary tract and the negative lewis gene phenotypes theoretically never produce CA19-9.[17, 18, 27]

Mucin1, cell surface associated (MUC1), one of the member of mucin family, also known as, polymorphic epithelial mucin is a mucin encoded by the MUC1 gene in humans[28], a high molecular weight membrane-associated glycoprotein with an extracellular domain consisting of a variable number of highly conserved tandem repeats of 20 amino acids, a transmembrane domain, and a cytoplasmic tail of 69 amino acids,[28, 29] was known as a highly glycosylated mucin associated with malignancy in many other organs.[30] Matsuda et al[31] have indicated that wisteria floribunda agglutinin-sialylated (WFA) could employed as the best probe to detect alteration of glycan structure in biliary tract cancer and distinguish it from normal specimens, and they identified sialylated MUC1 as a potential cholangiocarcinoma-specific glycoprotein marker that carries WFA-positive glycans. Therefore, wisteria floribunda agglutinin sialylated-mucin1(WFA-MUC1) has been certified as a sensitive molecular biomarker of CCA.[9, 31-35] While the optimal diagnostic capability of WFA-MUC1 remains unclear (the reported area under receiver operating curve (AUC) of WFA-MUC1 discriminating CCA from benign biliary diseases differed greatly (0.74~0.87 in serum, 0.72~0.90 in bile).[9, 31-35] In addition, the correlation between the positive MUC1 expressed in biliary duct cancer

tissue and overall survival (OS) rates of resectable CCA have been evaluated by Kaplan-Meier method in several clinical trials still remain unclear, moreover, lingering questions about whether the positive expression of MUC1 indicating the poor prognosis of CCA and associating the progression of CCA.[7, 9, 10, 12-15, 36, 37]

We, therefore, conducted a systematic review and meta-analysis to investigate the candidate key indicator molecular, MUC1, in determining the cumulative OS CCA and to evaluate the diagnostic capability of WFA-MUC1 in discriminating CCA from benign biliary diseases.

Methods

Search strategy

The comprehensive literature search (up to 18 Mar. 2017) was performed using PubMed, Web of Science, The Cochrane Library and the China National Knowledge Infrastructure, restricted to articles published in English or Chinese. Searching keywords entered as “mucin1/MUC1”, “cholangiocarcinoma/CCA,” “cholangiocellular carcinoma,” “intrahepatic cholangiocarcinoma,” “extrahepatic cholangiocarcinoma,” “Klatskin tumor/hilar cholangiocarcinoma/perihilar cholangiocarcinoma,” “prognosis/prognostic/prognoses/survival” and “diagnosis/diagnostic/diagnoses”. The reference lists of any studies meet the inclusion criteria were also reviewed manually to identify additional relevant publications.

Eligibility criteria

Published studies were included if they met the following criteria: (i) the published projects were focused on CCA; (ii) all subjects with CCA were diagnosed by pathologist postoperatively; (iii) the positive tissue MUC1 expression was tested by immunohistochemistry staining and the level of biliary and serum WFA-MUC1 was tested by sandwich enzyme-linked immunosorbent assay(ELISA); (iv) the receiver operating characteristic curve (ROC) of WFA-MUC1 and/or CA19-9 was described and the true positives, false positives, false negatives, and true negatives can be calculated; and (v) the hazard ratios (HRs) including 95% confidence interval (95%CI) and the corresponding P value can be extracted. The excluded studies based on following criteria: (1) animal studies;(2) review articles, case reports or letters; (3) duplicated publication; (4) non-English or non-Chinese papers; and (5) insufficient data on the HRs including 95%CI or that could not be extracted from Kaplan-Meier curve.

Data extraction

Data extraction was carried out by two investigators independently (Zengwei Tang and Yuan Yang), with the discrepancies resolved by the consensus of these two investigators. Data relating to study characteristics were extracted for the following variables: the first author of the study, study design and duration, year of publication, institution, the number of subjects in each study with mean age and gender, the selected antibody for the MUC1 immunochemical staining, sandwich enzyme-linked immunosorbent assay kits testing the level of biliary and /or serum WFA-MUC1 and the level of serum CA-19-9, the AUC of WFA-MUC1, the cut-off value of MUC1, essay's sensitivity and specificity, HRs and their 95% CI and data on follow-up time. Three studies did not provide the value of HRs and their 95%CI, we digitized and extracted the data from the Kaplan–Meier curve by using the software designed by Jayne F Tierney and matthew R Sydes.[38] The optimal sensitivity and

specificity were reported graphically in one study with two cohorts and were extracted using Plot Digitizer software 2.6.8 (provided by source forge.net, found online at http://plot digitizer source forge.net/) to convert data points on the graphs into numerical data.[39, 40] Repeated data points were isolated using nonparametric bootstrap sampling[41] guided by the descriptive statistics provided in the supporting text, the possible repeated data points were repeatedly sampled until the set that matched the descriptive statistics have been found. All the data was extracted from papers published.

.

Quality assessment across studies and publication Bias

Study quality assessment of studies included in the prognostic meta-analysis was assessed by using the modified risk of bias tool recommended by the Cochrane Collaboration as described previously.[42-44] Furthermore, Begg's funnel plot and the Egger's linear regression test were applied to evaluate potential publication bias for eligible studies using OS as an endpoint. Quality assessment of studies evaluating the diagnostic capability of MUC1 was assessed using the QUADAS(Quality Assessment of Studies of Diagnostic Accuracy included in Systematic reviews) checklists.[45, 46] However, We did not calculate the summary scores of each study investigating the diagnostic capability of MUC1 not merely because their interpretation is problematic, and has reported for potentially misleading.[47] Moreover, seven of the best differentiating items have been selected from the QUADAS checklists (box).

Statistical analyses

The statistical analysis was performed according to the guidelines proposed by the Meta-Analysis of Observational Studies in Epidemiology group (MOOSE).[48] The pooled HRs with 95% CI were

calculated with a random-effect model according to the DerSimonian-Laird method to estimate the association between the positive expression of MUC1 and overall survival (OS).[49, 50] We calculated sensitivity and specificity for each study evaluating the diagnostic capability of WFA- MUC1 and analyzed this datum as bivariate data according to methods for diagnostic meta-analysis.[51] An aggregated bivariate data meta-analysis with the generation of forest plots and summary receiver-operating characteristic curve (SROC) was performed. Forest plots display the diagnostic probabilities of individual studies, the corresponding 95% CI, and squares with area proportional to study weight in the meta-analysis. The SROC show individual study data point as circles, with size proportion to study weight and 95% prediction contour and 95% confidence contour around the pooled estimate. The heterogeneity among studies was measured using the Q tests and I^2 statistic to assess the extent of the inconsistency. A probability value of $P<0.1$ and I^2 >50% indicated the existence of significant heterogeneity.[52] Publication bias was evaluated for OS analysis by Egger's and Begg's test. Moreover, a P <0.05 for Egger's test was considered representative of significant publication bias.[49] All statistical analyses were performed with Stata/MP 14.0 (StataCorp, Parallel Edition).

Results

Study selection

The study includes results of electronic searches from date/month/year, up to 18 March 2017. A total of 341 papers were identified, of which 148 were retrieved from full-text review. Among these publications, according to the inclusion and exclusion criteria, 16 studies were eligible for the meta-analyses. Of these, nine studies[7, 9, 10, 12-15, 36, 37] using OS as endpoint, and eight studies[6, 9, 31, 32, 34, 35, 53, 54] using sensitivity and specificity rate(one study reported by Huang et al[9] also provided the data on

diagnostic value of MUC1 in tissue). The detailed screening process was shown in **Fig1**.

Study and participants characteristics

Table 1 and Supplementary Table 1, 2 were presenting the characteristics of eligible studies and their participants. Nine studies evaluating the prognostic value of MUC1 for resectable CCA which were conducted in 4 countries (Korea, Japan, China and Thailand), other seven studies[6, 31, 32, 34, 35, 53, 54] investigating the diagnostic capability of WFA-MUC1were undertaken in 5 countries (Japan, UK, Brazil, Thailand, China). A retrospective study design has been applied to prognostic meta-analysis for all selected studies. Seven investigations which are demonstrating the diagnostic capability of WFA-MUC1 discriminating the CCA from benign biliary diseases used a prospective study design. All individuals diagnosed with CCA were based on histopathology as reported in the manuscripts. The sample size of eligible studies for evaluating the prognostic value of MUC1 differed greatly, ranged from 27 to 87 (median=56), the studies investigating the diagnostic capability of WFA-MUC1 ranged from 30 to 303 (median=80) in biliary tract carcinoma group and from 20-287 (median=69) in benign biliary diseases.

Evaluation of studies were investigating the prognostic value of MUC1 for CCA, three studies[9, 14, 36] has provided the Kaplan-Meier curve and we digitized and extracted the data of HRs including their 95%CI from the curve using the methods described above. The cut-off value of positive expression of MUC1(2 trials[12, 37]>25%, one trial[14] >20%, 2 trials[9, 10]>10% and 4 trials[7, 13, 14, 36] >5%), the follow-up time (7 trials[7, 10, 13-15, 36, 37]>50 months, a trail[9] >20 months and another one[12]>15 months), and the antibody of MUC1 were selected for immunochemistry (mAb DF3, Clone Mab DF3,Clone

Ma695,Clone Ma689 and mAb HMPV) were inconsistent (As shown in **Supplementary Table 2**). The level of WFA-MUC1 in biliary and serum were tested by the approach of ELISA using mAb WFAMY.1E12. The concentration of serum CA19-9 was tested by CA19-9 ELISA kits similarly. The sensitivity, specificity and AUC of each study included in the diagnostic meta-analysis were shown in **Supplementary Table 2**.

Primary endpoint: the outcomes of diagnostic meta-analysis

Three trials[35, 54] including 414 biliary tract carcinoma (59 gall bladder carcinomas and 355 CCA) and 405 subjects with benign biliary diseases investigated the diagnostic capability of testing the serum level of WFA-MUC1. **Fig2a** presents the diagnostic parameters in a summary receiver operating characteristic (SROC) graph of serum WFA-MUC1. The pooled optimal sensitivity (true positive rate) was 0.76(0.71 to 0.81) and specificity (true negative rate) was 0.72(0.59 to 0.83). the AUC of SROC was 0.77(0.73 to 0.81).

As a comparison, four trials[34, 35, 54] with 588 subjects with biliary tract carcinoma (73 subjects with gall bladder carcinoma and 515 CCA) and 432 subjects with benign biliary disease assessed the diagnostic capability of testing the serum level of CA19-9. **Fig2b** presents the diagnostic parameters in a SROC graph of serum level of CA19-9. The pooled optimal sensitivity was 0.67(0.61 to 0.72) and specificity was 0.86(0.75 to 0.93). The AUC under SROC was 0.75(0.71 to 0.79).

Four trials[31, 32, 34, 35] including 209 subjects with benign biliary disease and 416 biliary tract carcinomas (73 gall bladder carcinomas) assessed the diagnostic capability of biliary level of WFA-MUC1. SROC

of biliary WFA-MUC1 is showed **in Fig2c**. The pooled sensitivity was 0.85(0.81 to 0.89) and specificity was 0.72(0.64 to 0.80). The AUC under SROC was 0.88(0.85-0.90). Furthermore, three trials[6, 9, 53] with 72 subjects with CCA and 119 benign biliary disease, using the positive expression of MUC1 in tissue to discriminate CCA from benign biliary disease. The diagnostic parameters of positive expression of MUC1 in biliary duct cancer tissue were shown in **Fig2d**. The pooled sensitivity was 0.72(0.50 to 0.87) and specificity 0.85(0.70-0.93). The AUC of SROC was 0.86(0.83-0.89).

Secondary endpoint: the outcome of prognostic meta-analysis

Nine studies with a total of 511 individuals diagnosed with CCA were eligible for the pooled analysis of OS. As shown in the **Fig3**, the overall pooled HRs of MUC1 was 2.20 (1.57 to 3.01). No heterogeneity among these studies was found ($\mathbf{I^2}$=0;**P**=0.869). Subgroup analyses stratified by the histopathological morphology of CCA, the pooled HRs of mass-forming intrahepatic CCA was 4.17(1.71 to 10.17). The pooled HRs of CCA was 1.98(1.37 to 2.85).

Risk of bias within studies

Supplementary table 3 presents the details of the risk of bias assessment of studies included in the prognostic meta-analysis. All besides one study[12] showed a high risk of bias, six showed[7, 9, 10, 13, 15, 37] a low risk of bias and two[14, 36] were presenting the unclear risk of bias. Moreover, as **Fig 4** shown, Begg's funnel plots of OS showed no clear indication of publication bias (Egger's test, P>0.134). Selection bias of diagnostic analyses may be caused by two trials including 73 subjects diagnosed with gall bladder carcinoma.[34, 35] **Supplementary Table 4** shows the detailed items selected for quality assessment of studies included in diagnostic meta-analysis.

Additional analysis

Several researchers have concludes that the OS of patients with mass-forming intrahepatic CCA or periductal infiltrating has a worse prognosis than other types, with higher rates of recurrence after resection.[55, 56] In prognostic meta-analysis, subgroup analysis stratified by the histopathological morphology of CCA was conducted to reduce the inconsistency caused by the type of CCA. We found the OS of patients with positive expression of MUC1 was significantly more decreased than that of negative group (The overall pooled HRs=2.20, especially for subjects with mass-forming intrahepatic CCA (HRs=4.17). In addition, a sensitivity analysis was performed to investigate the stability of the pooled HRs. As shown in **Supplementary Fig1**, the results of pooled HRs were not affected significantly by each individual study.

DISCUSSION

Serum CA19-9 has been widely used as a tumor marker of CCA. However, the diagnostic accuracy is limited since the serum level of CA19-9 can be strongly influenced by the co-existing inflammatory conditions of the biliary tract and this antigen could not be detected in Lewis gene negative individuals[16, 18]. In addition, biliary cytology is the most commonly performed diagnostic method of CCA by testing the bile sample from a biliary drainage catheter, but the sensitivity of biliary cytology is extremely low (20.7 ± 3.5%) as reported in published studies[57]. In the individual participant data (CCA) diagnostic meta-analysis, seven prospective trials[12, 31, 32, 34, 35, 54] [and a retrospective study[9] were eligible for diagnostic analysis which shows that the diagnostic capability of CA19-9 was inferior to

other molecules, such as WFA-MUC1.

In the diagnostic meta-analysis, the diagnostic role of WFA-MUC1 in serum, bile and biliary duct cancer tissue were evaluated in subgroup analysis. Two studies[35, 54] with 3 trials (studies reported by Matsuda et.al[54] included two cohorts) assessed the diagnostic accuracy of the serum level of WFA-MUC1, the pooled sensitivity of WFA-MUC1 was 0.76 (0.71 to 0.81) and specificity 0.72 (0.59 to 0.83), and the AUC of SROC was 0.77 (0.73 to 0.81). While in three studies[34, 35, 54] with four trials assessing the diagnostic accuracy of serum level of CA19-9, the pooled sensitivity of CA19-9 was 0.67 (0.61 to 0.72) and specificity was 0.86 (0.75 to 0.93) and AUC of SROC was 0.75 (0.71 to 0.79), which means it would bring a severe error into clinical diagnosis.

The diagnostic capability of serum WFA-MUC1 was superior to that of CA19-9 (as the data shows, $AUC_{WFA\text{-}MUC1}$ vs. $AUC_{CA19\text{-}9}$: 0.77(0.73 to 0.81) vs. 0.75(0.71 to 0.79)). The sensitivity rate of WFA-MUC1 was higher than that of CA19-9 ((0.76(0.71-0.81) vs. 0.67(0.61-0.72)), nevertheless, the specificity rate of serum WFA-MUC1 was less than that of CA19-9 ((0.72 (0.59 to 0.83) vs. 0.86 (0.75 to 0.93)). In order to discriminate CCA from benign biliary disease, the combination of these two biomarkers may be applied to improve the diagnostic capability of WFA-MUC1 or CA19-9, as reported by previously published trails.

In four prospective studies[31, 32, 34, 35] with 343 CAA and 73 gall bladder carcinomas and 209 benign biliary disease, the diagnostic accuracy of WFA-MUC1 in bile was also assessed. The pooled sensitivity of WFA-MUC1 testing was 0.85 (0.81 to 0.89) and specificity was 0.72 (0.64 to 0.80) and

AUC of SROC was 0.86 (0.83-0.89). The diagnostic capability of bile WFA-MUC1 was better than that of serum WFA-MUC1 ($AUC_{MUC1\ in\ bile}$ vs $AUC_{MUC1\ in\ serum}$: 0.86 (0.83 -0.89) vs 0.77(0.73 to 0.81)), which is keep consistent in most of diseases the elevated diagnostic molecules different expressed between locally and systemically.

As described above, the level of WFA-MUC1 has extremely higher diagnosis accuracy level than CA19-9. Nevertheless, the diagnostic accuracy of testing the biliary level of WFA-MUC1 was better than which detected in the serum as well. Therefore, the diagnostic capability of the combined serum CA19-9 and biliary WFA-MUC1 was better than that of the combination serum CA19-9 and serum level of WFA-MUC1 in discriminating the CCA from the benign bilary disease. Such combined measurement would represent a superior diagnostic testing assay for the detection of CCA in daily clinical practice. Unfortunately, as one study[54] included in the diagnostic meta-analyses did not provided the detailed cut-off value of serum WFA-MUC1 level, nor CA19-9 level, the optimal cut-off value of SROC cannot be estimated by this meta-analysis.

It has been demonstrated that MUC1 expression in various human tumors is related to invasive tumor proliferation and a poor patient outcome.[10, 36, 58, 59] In the prognostic meta-analysis, the pooled analysis of nine retrospective studies[7, 9, 10, 12-15, 36, 37] has showing that positive expression of MUC1 in tissue was a poor prognosis factor for resectable CCA (the pooled HRs was 2.20, 95%CI: 1.57 to 3.01), especially for patients with mass-forming intrahepatic CCA (the pooled HRs was 4.17, 95%CI: 1.71-10.17), showed by the subgroup analysis stratified by the morphology of CCA.

The publications has indicated that around 50-60% are identified as perihilar CCA, up to 20% of CCA are distal, and 5% of tumours are multifocal, whereas up to 20% of all CCA are intrahepatic.[5,60] Different type of CCA demonstrates various epidemiological, morphological and clinical features. A previous meta-analysis[43] found several prognostic biomarkers (EGFR, MUC1, MUC4, and p27) of resected CCA, with a small number of subjects in each synthesis group (four studies with 265 subjects with resected CCA were included in the analysis of the prognostic value of the MUC1 expression in tissue). The sample size of our prognostic meta-analysis was doubled (9 studies including 511 patients with resectable CCA), provided more explicit description and analysis, subgroup analysis and sensitivity analysis were conducted to get a a more credible result. Results of pooled HRs showed that the overexpression of MUC1 in tissue was a poor prognostic index of resectable CCA, in particular for patients with mass-forming intrahepatic CCA.

Predictive biomarkers could serve as tallow for personalized cancer treatment, such as verifying the chemosensitivity of CCA and giving birth to the vaccine of CCA development. Up to now, VEGFR, EGFR, HER2, MEK, and BRAF have been a focus on evaluating molecularly targeted therapies for CCA.[61] Furthermore, with the understanding of the MUC1 pathogenesis of CCA increasing, MUC1 may become a new focus of targeted therapy for CCA.

The strength and limitation of this study

This meta-analysis, to our best knowledge, is the first paper to evaluate the diagnostic value of WFA-MUC1 and prognostic value of MUC1 in human CCA in human CCA; We obtained data on prognostic and /or diagnostic capability of WFA-MUC1/MUC1 in patients with CCA from16 trails

identified by systematically researching four electronic data bases; All subjects with CCA was diagnosed by pathologist postoperatively, to compensate the bias of including only studies with reported HRs that may skew the analysis towards statistically significant results, we digitized and extracted the data of HRs of three studies[9, 14, 36] from Kaplan-Meier curve, in addition, subgroup analysis stratified by the morphology of CAA and sensitivity analysis made the results of the pooled HRs more stable; in the analysis the diagnostic capability of WFA-MUC1, we separately assessed the diagnostic accuracy of testing the level of WFA-MUC1 in serum, in bile and in tissue separately, and we also conducted a comparison of the diagnostic accuracy of between serum level of WFA-MUC1 and that of CA19-9 and a comparison between serum level of WAF-MUC1 and bile WFA-MUC1 as previously clinical trials described.

While the present study has supplied much useful information, however our study has some limitations that should be considered. Firstly, the majority subjects including in this meta-analysis were from Asian hospitals (data on prognostic meta-analysis were retrieved from Japan, China, Korea and Thailand; data on diagnostic meta-analysis were from Japan, Thailand, China, Brazil and the UK), there may be biological differences in tumor behavior among different region populations, as reported the observed difference in mortality from stomach cancer between Eastern countries and Western.[62] Secondly, four different cut-off value of positive MUC1 immunostaining (> 5% of carcinoma cells stained was defined as the cut-off point by four studies, >10% defined by 2 studies, >20% identified by a study and >25% defined by another 2 studies) and four different antibody of MUC1 (mAb DF3, Clone Ma689, Clone Ma695 and mAb HMPV) were used among nine studies including in the prognostic meta-analysis. Lacking consistent definition of cut-off value and the type of antibody of

MUC1 resulted in considerable heterogeneity. Third, in the diagnostic meta-analysis, although the majority of subjects diagnosed with CCA in the group of biliary tract carcinoma, a total of 73 subjects with gall bladder carcinomas was included in assessing the diagnostic capability of biliary level of MUC1 and serum level of CA19-9, and 59 patients with gall bladder carcinomas was placed in the evaluation of the diagnostic capability of serum level of WFA-MUC1. The heterogeneity caused by the inconsistency of participants cannot be underestimated since WFA-MUC1 can serve as an independent predictor of hepatocellular carcinoma recurrence.[63] It may be useful for discriminating gall bladder carcinoma from benign gall bladder disease. Fourth, given that only seven trials with a small number of patients were eligible for the diagnostic meta-analysis and two trials did not provide the cut-off value of the level of serum WFA-MUC1 and CA19-9, we cannot give an estimated optimal cut-off value of the level of serum WFA-MUC1. Finally, all data retrieved from subjects with resectable CCA or gall bladder carcinoma, there may be some difference in the pathogenesis between resectable and unresectable CCA.

Conclusions

This paper has highlighted the importance of the WFA-MUC1 has an increased diagnostic capability than CA19-9, And the diagnostic capability of testing the biliary level of WFA-MUC1 was superior to that in the serum. Furthermore, MUC1 was served as a poor prognosis factor of resectable CCA, particularly in mass-forming intrahepatic CCA.

Future large multicenters studies need continue to focus on enhancing the understanding of the molecular pathogenesis of CCA, developing combined kits of testing the serum/ biliary level of MUC1 and serum level of CA19-9 for clinical use conveniently in routine clinical practice, and

providing an optimal cut-off value with higher diagnostic accuracy of CCA and benefiting the populations from different regions.

References

1. Razumilava N, Gores GJ. Cholangiocarcinoma. *Lancet* 2014; 383(9935):2168-79.
2. Eckel F, Schmid RM. Chemotherapy in advanced biliary tract carcinoma: a pooled analysis of clinical trials. *Br J Cancer* 2007; 96(6):896-902.
3. Park J, Kim MH, Kim KP, et al. Natural History and Prognostic Factors of Advanced Cholangiocarcinoma without Surgery, Chemotherapy, or Radiotherapy: A Large-Scale Observational Study. *Gut Liver* 2009; 3(4):298-305.
4. DeOliveira ML, Cunningham SC, Cameron JL, et al. Cholangiocarcinoma: thirty-one-year experience with 564 patients at a single institution. *Ann Surg* 2007; 245(5):755-62.
5. Khan SA, Davidson BR, Goldin RD, et al. Guidelines for the diagnosis and treatment of cholangiocarcinoma: an update. *Gut* 2012; 61(12):1657-69.
6. Esperança ABT, Camacho AHDS, Monteiro JBM, et al. Mucins and NCAM (CD56) in intrahepatic cholangiocarcinogenesis. *Jornal Brasileiro De Patologia E Medicina Laboratorial* 2014; 50(3):216-220.
7. Higashi M, Yamada N, Yokoyama S, et al. Pathobiological implications of MUC16/CA125 expression in intrahepatic cholangiocarcinoma-mass forming type. *Pathobiology* 2012; 79(2):101-6.
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(15):3393-403.
9. Huang F, Zhou QB, Chen RF. Expression and significance of MUC1 in hepatolithiasis associated with intrahepatic cholangiocarcinoma. *Chinese Archives of General Surgery* 2010.
10. Park SY, Roh SJ, Kim YN, et al. Expression of MUC1, MUC2, MUC5AC and MUC6 in cholangiocarcinoma: prognostic impact. *Oncol Rep* 2009; 22(3):649-57.
11. 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(10):1675-81.
12. Boonla C, Sripa B, Thuwajit P, et al. MUC1 and MUC5AC mucin expression in liver fluke-associated intrahepatic cholangiocarcinoma. *World J Gastroenterol* 2005; 11(32):4939-46.
13. Shibahara H, Tamada S, Higashi M, et al. MUC4 is a novel prognostic factor of intrahepatic cholangiocarcinoma-mass forming type. *Hepatology* 2004; 39(1):220-9.
14. Tamada S, Goto M, Nomoto M, et al. Expression of MUC1 and MUC2 mucins in extrahepatic bile duct carcinomas: its relationship with tumor progression and prognosis. *Pathol Int* 2002; 52(11):713-23.
15. Matsumura N, Yamamoto M, Aruga A, et al. Correlation between expression of MUC1 core protein and outcome after surgery in mass-forming intrahepatic cholangiocarcinoma. *Cancer* 2002; 94(6):1770-6.
16. Chen CY, Shiesh SC, Tsao HC, et al. The assessment of biliary CA 125, CA 19-9 and CEA in diagnosing cholangiocarcinoma--the influence of sampling time and hepatolithiasis. *Hepatogastroenterology* 2002; 49(45):616-20.

17. Patel AH, Harnois DM, Klee GG, et al. The utility of CA 19-9 in the diagnoses of cholangiocarcinoma in patients without primary sclerosing cholangitis. *Am J Gastroenterol* 2000; 95(1):204-7.
18. Narimatsu H, Iwasaki H, Nakayama F, et al. Lewis and secretor gene dosages affect CA19-9 and DU-PAN-2 serum levels in normal individuals and colorectal cancer patients. *Cancer Res* 1998; 58(3):512-8.
19. Xu H, Inagaki Y, Tang W, et al. Elevation of serum KL-6 mucin levels in patients with cholangiocarcinoma. *Hepatogastroenterology* 2008; 55(88):2000-4.
20. Cheon YK, Cho YD, Moon JH, et al. Diagnostic utility of interleukin-6 (IL-6) for primary bile duct cancer and changes in serum IL-6 levels following photodynamic therapy. *Am J Gastroenterol* 2007; 102(10):2164-70.
21. Lumachi F, Lo Re G, Tozzoli R, et al. Measurement of serum carcinoembryonic antigen, carbohydrate antigen 19-9, cytokeratin-19 fragment and matrix metalloproteinase-7 for detecting cholangiocarcinoma: a preliminary case-control study. *Anticancer Res* 2014; 34(11):6663-7.
22. Uenishi T, Yamazaki O, Tanaka H, et al. Serum cytokeratin 19 fragment (CYFRA21-1) as a prognostic factor in intrahepatic cholangiocarcinoma. *Ann Surg Oncol* 2008; 15(2):583-9.
23. Valle J, Wasan H, Palmer DH, et al. Cisplatin plus gemcitabine versus gemcitabine for biliary tract cancer. *N Engl J Med* 2010; 362(14):1273-81.
24. Abbas G, Lindor KD. Cholangiocarcinoma in primary sclerosing cholangitis. *J Gastrointest Cancer* 2009; 40(1-2):19-25.
25. Hultcrantz R, Olsson R, Danielsson A, et al. A 3-year prospective study on serum tumor markers used for detecting cholangiocarcinoma in patients with primary sclerosing cholangitis. *J Hepatol* 1999; 30(4):669-73.
26. Gores GJ. Early detection and treatment of cholangiocarcinoma. *Liver Transpl* 2000; 6(6 Suppl 2):S30-4.
27. Hamada E, Taniguchi T, Baba S, et al. Investigation of unexpected serum CA19-9 elevation in Lewis-negative cancer patients. *Ann Clin Biochem* 2012; 49(Pt 3):266-72.
28. Gendler SJ, Lancaster CA, Taylor-Papadimitriou J, et al. Molecular cloning and expression of human tumor-associated polymorphic epithelial mucin. *J Biol Chem* 1990; 265(25):15286-93.
29. Lan MS, Batra SK, Qi WN, et al. Cloning and sequencing of a human pancreatic tumor mucin cDNA. *J Biol Chem* 1990; 265(25):15294-9.
30. Brockhausen I. Pathways of O-glycan biosynthesis in cancer cells. *Biochim Biophys Acta* 1999; 1473(1):67-95.
31. Matsuda A, Kuno A, Kawamoto T, et al. Wisteria floribunda agglutinin-positive mucin 1 is a sensitive biliary marker for human cholangiocarcinoma. *Hepatology* 2010; 52(1):174-82.
32. Matsuda A, Kuno A, Matsuzaki H, et al. Glycoproteomics-based cancer marker discovery adopting dual enrichment with Wisteria floribunda agglutinin for high specific glyco-diagnosis of cholangiocarcinoma. *J Proteomics* 2013; 85:1-11.
33. Xu F, Liu F, Zhao H, et al. Prognostic Significance of Mucin Antigen MUC1 in Various Human Epithelial Cancers: A Meta-Analysis. *Medicine (Baltimore)* 2015; 94(50):e2286.
34. Yamaguchi T, Yokoyama Y, Ebata T, et al. Verification of WFA-Sialylated MUC1 as a Sensitive Biliary Biomarker for Human Biliary Tract Cancer. *Ann Surg Oncol* 2016; 23(2):671-7.
35. Shoda J, Matsuda A, Shida T, et al. Wisteria floribunda agglutinin-sialylated mucin core polypeptide 1 is a sensitive biomarker for biliary tract carcinoma and intrahepatic cholangiocarcinoma: a multicenter study. *J Gastroenterol* 2017; 52(2):218-228.
36. 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(6):1347-55.

37. Takao S, Uchikura K, Yonezawa S, et al. Mucin core protein expression in extrahepatic bile duct carcinoma is associated with metastases to the liver and poor prognosis. *Cancer* 1999; 86(10):1966-75.
38. Tierney JF, Stewart LA, Ghersi D, et al. Practical methods for incorporating summary time-to-event data into meta-analysis. *Trials* 2007; 8:16.
39. Kelsey TW, Anderson RA, Wright P, et al. Data-driven assessment of the human ovarian reserve. *Mol Hum Reprod* 2012; 18(2):79-87.
40. Iliodromiti S, Kelsey TW, Anderson RA, et al. Can anti-Mullerian hormone predict the diagnosis of polycystic ovary syndrome? A systematic review and meta-analysis of extracted data. *J Clin Endocrinol Metab* 2013; 98(8):3332-40.
41. Ciaccio AD. Bootstrap and Nonparametric Predictors to Impute Missing Data, 2011.
42. Rahbari NN, Aigner M, Thorlund K, et al. Meta-analysis shows that detection of circulating tumor cells indicates poor prognosis in patients with colorectal cancer. *Gastroenterology* 2010; 138(5):1714-26.
43. Ruys AT, Groot Koerkamp B, Wiggers JK, et al. Prognostic biomarkers in patients with resected cholangiocarcinoma: a systematic review and meta-analysis. *Ann Surg Oncol* 2014; 21(2):487-500.
44. Higgins JP, Green S. Cochrane Handbook For Systematic Reviews Of Interventions [internet]. *Naunyn-Schmiedebergs Archiv für experimentelle Pathologie und Pharmakologie* 2009; 2011(14):S38.
45. 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.
46. van Rheenen PF, Van de Vijver E, Fidler V. Faecal calprotectin for screening of patients with suspected inflammatory bowel disease: diagnostic meta-analysis. *BMJ* 2010; 341:c3369.
47. Whiting P, Harbord R, Kleijnen J. No role for quality scores in systematic reviews of diagnostic accuracy studies. *BMC Med Res Methodol* 2005; 5:19.
48. Stroup DF, Berlin JA, Morton SC, et al. Meta-analysis of observational studies in epidemiology: a proposal for reporting. Meta-analysis Of Observational Studies in Epidemiology (MOOSE) group. *JAMA* 2000; 283(15):2008-12.
49. DerSimonian R, Laird N. Meta-analysis in clinical trials revisited. *Contemp Clin Trials* 2015; 45(Pt A):139-45.
50. Xie S, Wang K, Xu H, et al. PRISMA-Extracapsular Dissection Versus Superficial Parotidectomy in Treatment of Benign Parotid Tumors: Evidence From 3194 Patients. *Medicine (Baltimore)* 2015; 94(34):e1237.
51. Harbord RM, Deeks JJ, Egger M, et al. A unification of models for meta-analysis of diagnostic accuracy studies. *Biostatistics* 2007; 8(2):239-51.
52. Higgins JP, Thompson SG, Deeks JJ, et al. Measuring inconsistency in meta-analyses. *BMJ* 2003; 327(7414):557-60.
53. Zen Y, Britton D, Mitra V, et al. Tubulin beta-III: a novel immunohistochemical marker for intrahepatic peripheral cholangiocarcinoma. *Histopathology* 2014; 65(6):784-92.
54. Matsuda A, Kuno A, Nakagawa T, et al. Lectin Microarray-Based Sero-Biomarker Verification Targeting Aberrant O-Linked Glycosylation on Mucin 1. *Anal Chem* 2015; 87(14):7274-81.
55. Yamamoto Y, Shimada K, Sakamoto Y, et al. Clinicopathological characteristics of intrahepatic cholangiocellular carcinoma presenting intrahepatic bile duct growth. *J Surg Oncol* 2009; 99(3):161-5.
56. Shimada K, Sano T, Sakamoto Y, et al. Surgical outcomes of the mass-forming plus periductal infiltrating types of intrahepatic cholangiocarcinoma: a comparative study with the typical mass-forming type of

intrahepatic cholangiocarcinoma. *World J Surg* 2007; 31(10):2016-22.

57. Tsuchiya T, Yokoyama Y, Ebata T, et al. Randomized controlled trial on timing and number of sampling for bile aspiration cytology. *J Hepatobiliary Pancreat Sci* 2014; 21(6):433-8.
58. Yonezawa S, Goto M, Yamada N, et al. Expression profiles of MUC1, MUC2, and MUC4 mucins in human neoplasms and their relationship with biological behavior. *Proteomics* 2008; 8(16):3329-41.
59. Utsunomiya T, Yonezawa S, Sakamoto H, et al. Expression of MUC1 and MUC2 mucins in gastric carcinomas: its relationship with the prognosis of the patients. *Clin Cancer Res* 1998; 4(11):2605-14.
60. Khan SA, Emadossadaty S, Ladep NG, et al. Rising trends in cholangiocarcinoma: is the ICD classification system misleading us? *J Hepatol* 2012; 56(4):848-54.
61. Zhu AX, Hezel AF. Development of molecularly targeted therapies in biliary tract cancers: reassessing the challenges and opportunities. *Hepatology* 2011; 53(2):695-704.
62. Strong VE, Song KY, Park CH, et al. Comparison of gastric cancer survival following R0 resection in the United States and Korea using an internationally validated nomogram. *Ann Surg* 2010; 251(4):640-6.
63. Tamaki N, Kuno A, Matsuda A, et al. Serum Wisteria Floribunda Agglutinin-Positive Sialylated Mucin 1 as a Marker of Progenitor/Biliary Features in Hepatocellular Carcinoma. *Sci Rep* 2017; 7(1):244.

Figure legends:

Figure 1. Search flow diagram.

Figure 2. Summary Receiver operating characteristic curve (SROC) of WFA-MUC1 and that of CA19-9.

Figure 2a. SROC of serum level of MUC1.

Figure 2b. SROC of serum level of CA19-9.

Figure 2c. SROC of biliary level of MUC1.

Figure 2d. SROC of MUC1 in biliary duct cancer tissue.

Figure 3. Forest plot of hazards ratio evaluating overall survival of patients with resectable CCA.

Figure 4. Begg's funnel plot for overall survival.

Supplementary Figure 1. Sensitivity analysis of studies evaluating the prognostic value of MUC1 in biliary cancer tissue.

1

Table 1. Characteristics of studies included in this meta-analysis

Author	Year	Country	No. BTC	No. BBD	Mean Age		Male/Female)		Type of BTC				Specimen Source	Association	Study Design
					BTC	BBD	BTC	BBD	iCCA	pCCA	dCCA	GC			
Shoda et al[35]	2017	Japan	303	287	71 (33-101)	68 (19-92)	193/110	153/134	59	117	71	59	Bile,Serum	Diagnosis	P
Yamaguchi et al[34]	2016	Japan	174	27	69 (36-85)	64 (27-82)	108/66	19/8	9	133	18	14	Bile,Serum	Diagnosis	P
Matsuda et al[54]	2015	Thailand (cohort1)	78	78	56±8.25 (57–90)	54 ±10.42 (32–73)	23/55	23/55	CCA				Plasma	Diagnosis	P
Matsuda et al[54]	2015	Japan (cohort2)	33	40	77 ± 8.25 (57–90)	76 ± 9.50 (56–93)	20/13	19/21	28	1	4	0	Serum	Diagnosis	P
Zen et al[53]	2014	UK	28	20	67 (42–83)	61 (38–77)	17/11	15/5	28	0	0	0	Tissue	Diagnosis	R
Esperança et al[6]	2014	Brizal	11	67	NA	NA	NA	NA	CCA				Tissue	Diagnosis	R
Matsuda et al[32]	2013	Japan	29	29	NA	-	NA	-	CCA				Bile,Serum	Diagnosis	P
Higashi et al[7]	2012	Japan	63	-	67.4 (41-85)	-	33/30	-	iCCA				Tissue	Prognosis	R
Huang et al[9]	2010	China	33	32	56.41± 13.14 (32-75)	55.41±13.45 (33-77)	18/15	17/15	iCCA				Tissue	Prognosis, Diagnosis	R
Matsuda et al[31]	2010	Japan	30	38	NA	NA	NA	NA	iCCA				Bile	Diagnosis	P
Park et al[10]	2009	Korea	85	-	63.8 (44-82)	-	58/27	-	34	51	0	0	Tissue	Prognosis	R
Boonla et al[12]	2005	Thailand	87	-	56.7±8.6 (36-73)	-	59/28	-	iCCA				Tissue	Prognosis	P
Shibahara et al[13]	2004	Japan	27	-	65.3 (45-79)	-	16/11	-	iCCA				Tissue	Prognosis	R

Matsumura et al[15]	2002	Japan	50	-	60.3±10.3 (30-75)	-	33/17	-	iCCA				Tissue	Prognosis	R
Tamada et al[14]	2002	Japan	60	-	61.9(41-88)	-	41/19	-	CCA				Tissue	prognosis	R
Higashi et al[36]	1999	Japan	39	-	NA	-	-	-	30	0	7	0	Tissue	Prognosis	R
Takao et al[37]	1999	Japan	73	-	65.9(39-85)	-	50/23	-	0	37	36	0	Tissue	Prognosis	R

BTC, biliary tract carcinoma; BBD, benign biliary tract diseases; GC, gallbladder carcinoma; CCA, cholangiocarcinoma; iCCA, intrahepatic cholangiocarcinoma; pCCA perihilar cholangiocarcinoma; dCCA, distal cholangiocarcinoma; P, prospective; R, retrospective; NA, not available

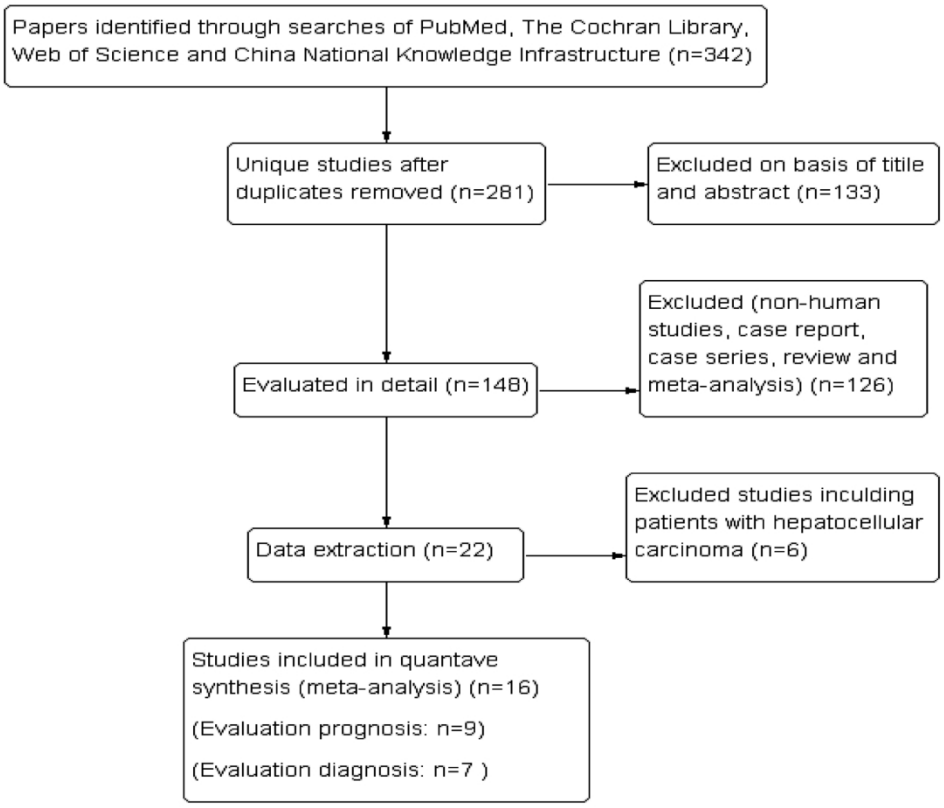


Figure 1. Search flow diagram.

169x144mm (300 x 300 DPI)

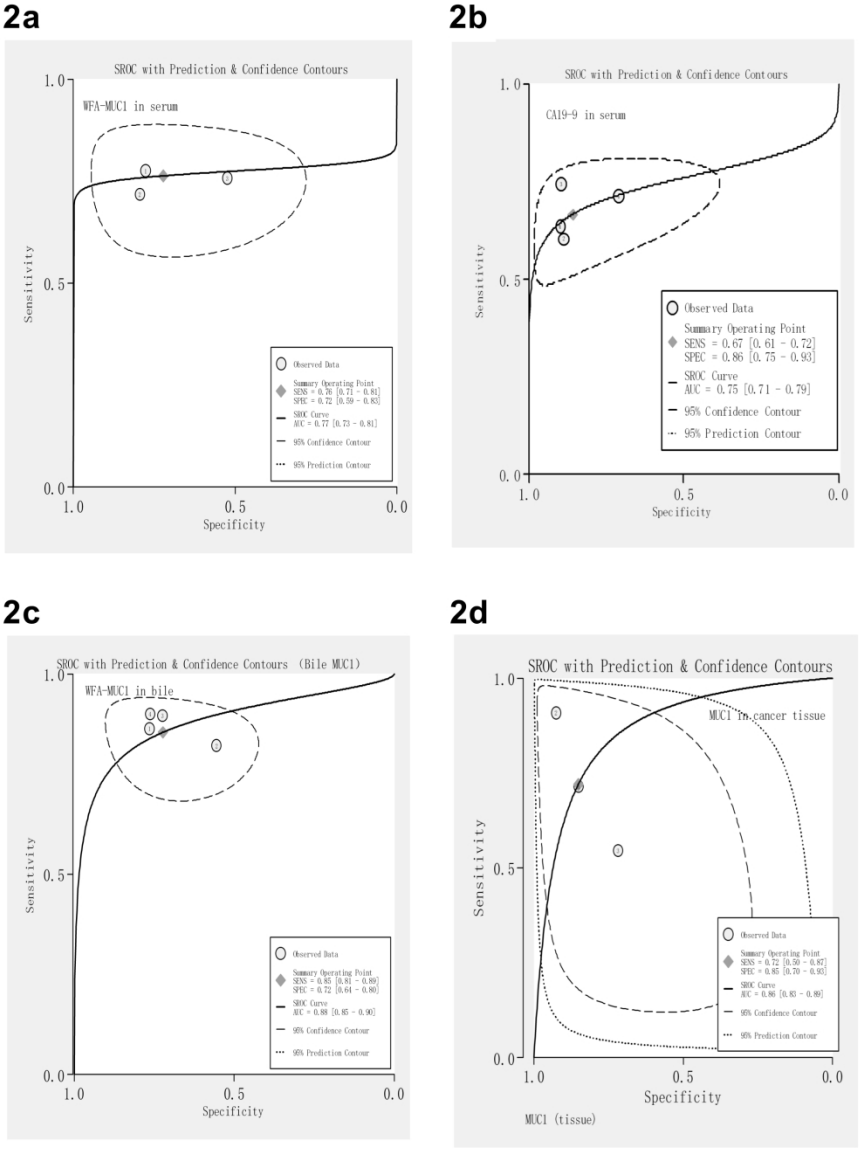


Figure 2. Summary Receiver operating characteristic curve (SROC) of WFA-MUC1 and that of CA19-9.

195x258mm (300 x 300 DPI)

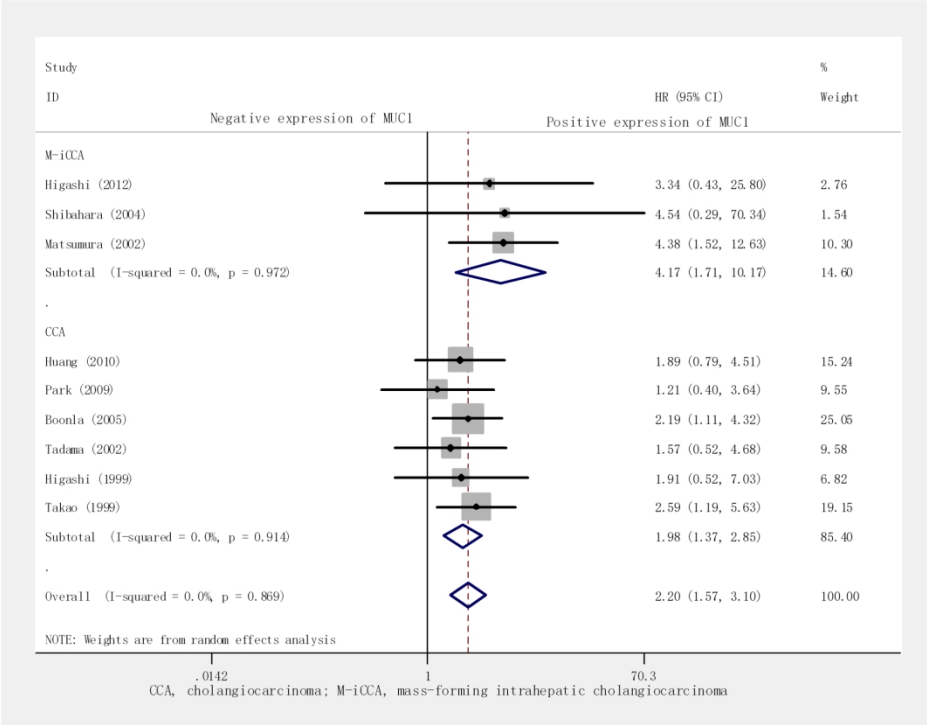


Figure 3. Forest plot of hazards ratio evaluating overall survival of patients with resectable CCA.

194x152mm (300 x 300 DPI)

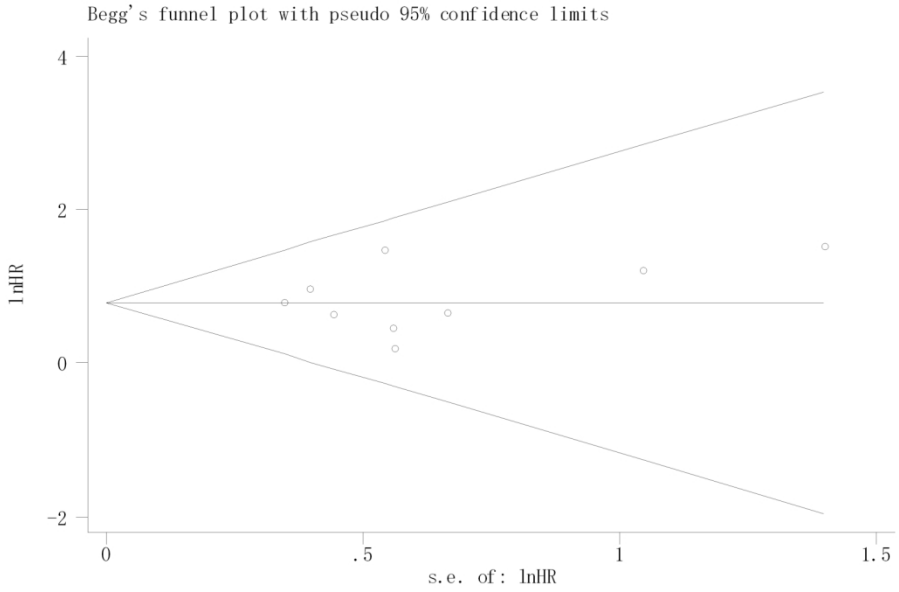
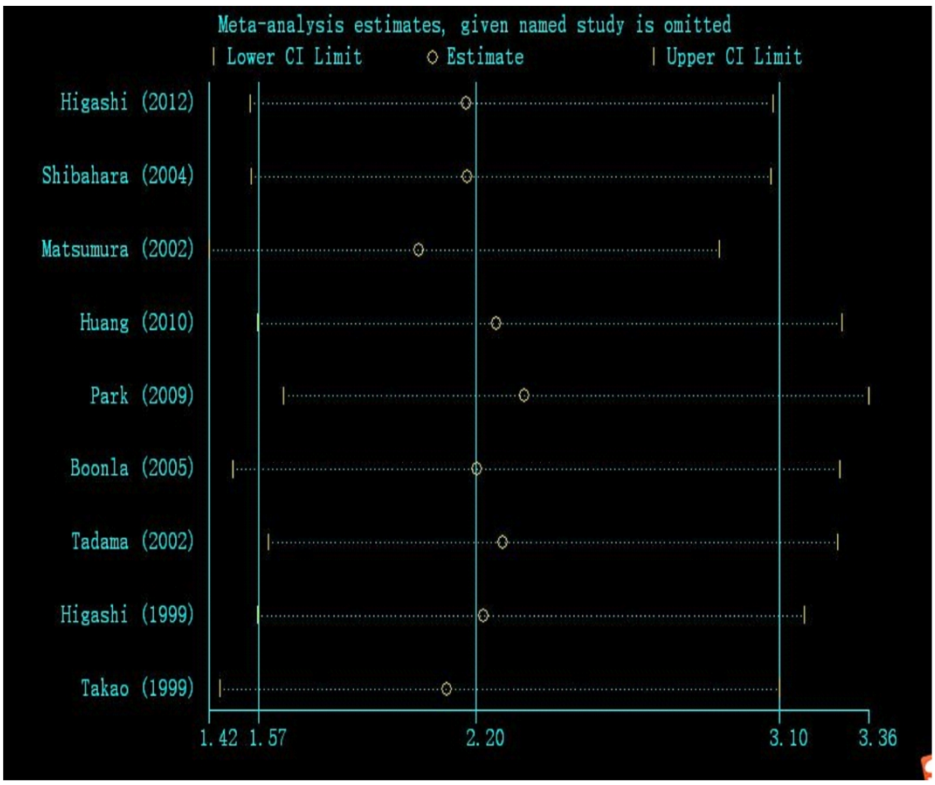


Figure 4. Begg's funnel plot for overall survival.

155x106mm (300 x 300 DPI)



Supplementary Figure 1. Sensitivity analysis of studies evaluating the prognostic value of MUC1 in biliary cancer tissue.

198x180mm (300 x 300 DPI)

Supplementary Table 1. Characteristics of studies included in the diagnostic meta-analysis

2.1. Characteristics of eligible studies evaluating the diagnostic accuracy of WFA-MUC1 in serum samples

Author	Year	country	Optimal Se	Optimal Sp	Reference Standard	Antibody	AUC	Cut-Off Value	TP	FP	TN	FN
Shoda et al[35]	2017	Japan	0.776	0.780	Histopathology	WFA-MY.1E12	0.873	214.2 uL/ml	235	64	223	68
Matsuda et al[54]	2015	Thailand (cohort 1)	0.722	0.748	Histopathology	WFA-MY.1E12	0.841	NA	56	16	62	22
Matsuda et al[54]	2015	Japan (cohort 2)	0.766	0.643	Histopathology	WFA-MY.1E12	0.738	NA	25	19	21	8

2.2. Characteristics of eligible studies evaluating the diagnostic accuracy of CA19-9 in serum samples

Shoda et al[35]	2017	Japan	0.713	0.711	Histopathology	CA19-9 ELISA Kits	0.753	27.6 U/ml	216	83	204	87
Yamaguchi et al[34]	2016	Japan	0.603	0.889	Histopathology	NA	0.761	38 U/ml	105	3	24	69
Matsuda et al[54]	2015	Thailand (cohort1)	0.743	0.887	Histopathology	CA19-9 ELISA kits	0.849	NA	58	8	70	20
Matsuda et al[54]	2015	Japan (cohort2)	0.637	0.896	Histopathology	CA19-9 ELISA kits	0.759	NA	21	4	36	12

2.3. Characteristics of eligible studies evaluating the diagnostic accuracy of MUC1 in bile samples

Shoda et al[35]	2017	Japan	0.863	0.765	Histopathology	MY.1E12 (mAb)	0.896	13.5 nL/ug	158	27	88	25
Yamaguchi et al[34]	2016	Japan	0.822	0.556	Histopathology	Antibody for MUC1	0.715	10.5 nl/ug	143	12	15	31
Matsuda et al[32]	2013	Japan	0.90	0.72	Histopathology	WFA-MY.1E12	0.85	7 nl/ug	26	8	21	3
Matsuda et al[31]	2010	Japan	0.90	0.763	Histopathology	WFA-MY.1E12	0.86	6.64 nl/ug	27	9	29	3

2.4. Characteristics of eligible studies evaluating the diagnostic accuracy of MUC1 in tissue samples

Zen et al[53]	2014	UK	0.71	0.85	Histopathology	clone DF3	-	>5% (positive)	20	3	17	8
Esperança et al[6]	2014	Brizal	0.909	0.925	Histopathology	Clone Ma695	-	>10% (positive)	10	5	62	1
Huang et al[9]	2010	China	0.545	0.719	Histopathology	Ma689	-	>10%	18	9	23	15

Se, sensitivity; Sp, specificity; AUC, area under the receiver-operating characteristic curve; TP, true positive; FP, false positive; TN, true negative; FN, false negative; NA, not available

Supplementary Table 2. Characteristics of eligible studies included in prognostic meta-analysis

Author	Year	Country	Type of CCA	No.Patients	Anti-MUC1	Cut- Off (Positive/High Expression)	Follow-Up (Months)	HR For Overall Survival (95%CI)	P Values
Higashi et al[7]	2012	Japan	M-iCCA	63	mAb DF3	> 5% (58)	>50	3.34 (0.43-25.8)	0.168
Huang et al[9]	2010	China	iCCA	33	Clone Ma689	>10% (18)	>20	1.89 (0.79-4.511)*	<0.01
Park et al[10]	2009	Korea	CCA	85	Clone Ma695	>10% (56）	>50	1.211 (0.403-3.640)	0.733
Boonla et al[12]	2005	Thailand	iCCA	87	Clone Ma695	>25% (34)	>15	2.19 (1.11-4.32)	0.026
Shibahara et al[13]	2004	Japan	M-iCCA	27	Mab DF3	>5% (22)	>50	4.536 (0.292–70.336)	0.2797
Matsumura et al[15]	2002	Japan	M-iCCA	50	mAb HMPV	>5% (38)	>50	4.377 (1.517–12.629)	0.0063
Tamada et al[14]	2002	Japan	CCA	60	MAb DF3	>20%(46)	>50	1.57（0.52-4.68)*	<0.05
Higashi et al[36]	1999	Japan	CCA	39	mAb DF3	>5% (23)	>50	1.91 (0.52-7.03)*	<0.05
Takao et al[37]	1999	Japan	CCA	67	Mab DF3	>25% (47)	>50	2.59 (1.19–5.63)	0.016

CCA, cholangiocarcinoma; iCCA, intrahepatic cholangiocarcinoma; M-iCCA, mass-forming intrahepatic cholangiocarcinoma; HR, hazard ratio; * the data was digitized and extracted from the Kaplan–Meier curve using the software designed by Jayne F Tierney and matthew R Sydes.

Supplementary Table 3. Assessment of risk of bias for studies evaluating the prognostic value of MUC1

Author (Year)	Were adequate eligibility criteria developed and applied	Was the measurement of both exposure and outcome adequate?	Was confounding adequately controlled for?	Was the follow-up complete and adequate in duration?	Are reports of the study free of suggestion of selective outcome reporting?	Was the study free of other problems that put it at a high risk of bias?	Risk of bias
Higashi (2012)	YES	YES	YES	Unclear	YES	YES	Low
Huang (2010)	YES	YES	NO	YES	YES	YES	Low
Park (2009)	YES	YES	YES	YES	YES	YES	Low
Boonla(2005	YES	YES	NO	YES	YES	YES	High
Shibahara (2004)	YES	YES	YES	YES	YES	YES	Low
Matsumura (2002)	YES	YES	YES	YES	YES	YES	Low
Tamada (2002)	YES	YES	YES	Unclear	YES	YES	Unclear
Higashi (1999)	YES	YES	YES	Unclear	YES	YES	Unclear
Takao(1999)	YES	YES	YES	YES	YES	YES	Low

Supplementary Table 4. Assessment of risk of bias for studies evaluating the diagnostic capability of WFA-MUC1

Author (Year)	Representative spectrum?	Acceptable reference standard?	Acceptable delay between tests?	Partial verification avoided?	Differential verification avoided?	Index test results blinded?	Withdrawals explained?
Shoda (2017)	YES	YES	YES	NO	YES	Unclear	YES
Yamaguchi (2016)	YES	YES	YES	NO	YES	Unclear	YES
Matsuda[+] (2015)	YES	YES	Unclear	NO	YES	YES	YES
Matsuda [ǂ](2015)	YES	YES	Unclear	NO	YES	YES	YES
Zen (2014)	YES	YES	YES	YES	YES	YES	YES
Esperança (2014)	YES	YES	YES	YES	YES	YES	YES
Matsuda (2013)	YES	YES	YES	NO	YES	Unclear	YES
Matsuda (2010)	YES	YES	YES	NO	YES	Unclear	YES
Huang (2010)	YES	YES	YES	YES	YES	YES	YES

[+], Cohort1; [ǂ], cohort2

Items chosen to score from QUADAS checklist

1# **Was the spectrum of patients representative of those who will receive the test in practice?**

2# **Was the reference standard likely to correctly classify patients cholangiocarcinoma?**

3# **Was the time period between reference standard and index test short enough to be reasonably sure that the target condition did not change between the two tests?**

4# **Did the whole sample or a random selection of the sample receive verification using a reference standard?**

5# **Did patients receive the same reference standard regardless of the index test result?**

6# **Were the reference standard results interpreted without knowledge of the results of the index test?**

7# **Were withdrawals from the study explained?**

MOOSE Checklist

Meta-analysis of the diagnostic value of wisteria floribunda agglutinin-sialylated mucin1 and the prognostic role of mucin1 in human cholangiocarcinoma

Criteria		Brief description of how the criteria were handled in the meta-analysis
Reporting of background should include		
√	Problem definition	*See Page 5, 6.*
√	Hypothesis statement	*See Page 5, 6.*
√	Description of study outcomes	*See Page 3, 9-12.*
√	Type of exposure or intervention used	*See Page 7,8.*
√	Type of study designs used	*See Page 10.*
√	Study population	*See Page 7.* Subjects with cholangiocarcinima and subjects with benign biliary disease.
Reporting of search strategy should include		
√	Qualifications of searchers	*See Page 7.*
√	Search strategy, including time period included in the synthesis and keywords	*See Page 6.*
√	Databases and registries searched	*See Page 6.*
√	Search software used, name and version, including special features	*See Page 7, 8.* We did not employ a search software. EndNote X7(BId 7072) was used to merge retrieved citations and eliminate duplications.
√	Use of hand searching	*See Page 6.* We hand-searched bibliographies of retrieved papers for additional references.
√	List of citations located and those excluded, including justifications	*See Page 7, 9, 10.* Details of the literature search process are outlined in the flow chart(figure1). The citation list is available upon request.
√	Method of addressing articles published in languages other than English	*See Page 7.* No studies published in English or in Chinese.
√	Method of handling abstracts and unpublished studies	*See Page 9.* We did not include studies only published as abstracts.
√	Description of any contact with authors	*See Page 7, 8, 10.* We did not contact authors. Potentially, data is available just from published studies data.
Reporting of methods should include		
√	Description of relevance or	*See Page 7.*

	appropriateness of studies assembled for assessing the hypothesis to be tested	Detailed inclusion and exclusion criteria were described in the methods section.
√	Rationale for the selection and coding of data	*See Page 7, 8.* Data extracted from each of the studies were relevant to the population characteristics, study design, exposure, outcome, and possible effect modifiers of the association.
√	Assessment of confounding	*See Page 7, 8, 12.* We included only studies where populations were representative of the subjects with cholangiocarcinoma or benign biliary disease. And sensitivity analyses was conducted to give a more stable results of the prognostic value of MUC1 in cholangicarcinoma.
√	Assessment of study quality, including blinding of quality assessors; stratification or regression on possible predictors of study results	*See Page 8, 12, 13.* Study quality assessment of studies included in the prognostic meta-analysis was assessed by using the modified risk of bias tool recommended by the Cochrane Collaboration as described previously.Quality assessment of studies evaluating the diagnostic capability of MUC1 was assessed using the QUADAS(Quality Assessment of Studies of Diagnostic Accuracy included in Systematic reviews) checklists. As described in **Supplementary Table 3, 4.**
√	Assessment of heterogeneity	*See Page 9.*
√	Description of statistical methods in sufficient detail to be replicated	*See Page 8, 9.*
√	Provision of appropriate tables and graphics	*See Page 11-13.* The characteristics of studies included in this meta-analysis were outlined in Table 1, and Supplementary Table 1, 2, 3,4. The results of primary and secondary outcomes were presented in Figures and Supplementary Figures.
Reporting of results should include		
√	Graph summarizing individual study estimates and overall estimate	*See Page 11, 12.* Figures 2-4 and supplementary Figure 1.
√	Table giving descriptive information for each study included	*See Page 10, 11.* Table 1 and Supplementary Table 1, 2.
√	Results of sensitivity testing	*See Page 13.*
√	Indication of statistical uncertainty of findings	*See Page 9, 11-13.* 95% confidence intervals were presented with all summary estimates, I^2 values and results of sensitivity analyses
Reporting of discussion should include		
√	Quantitative assessment of bias	*See Page 12, 17.*
√	Justification for exclusion	*See Page 7, 9, 10.* We excluded the studies if they included patients with other malignant biliary diseases or hepatocellular carcinoma.
√	Assessment of quality of included studies	*See Page 12, 13* As described in Supplementary Table 3, 4.

Reporting of conclusions should include		
√	Consideration of alternative explanations for observed results	*See Page 13, 17, 18.* We discussed that potential unmeasured confounders may have caused residual confounding. We noted that the variations in the strengths of association may be due to the differences in quality of studies the locations.
√	Generalization of the conclusions	*See Page 18.*
√	Guidelines for future research	*See Page 19.*
√	Disclosure of funding source	*See Page 2.*

BMJ Open

Meta-analysis of the diagnostic value of wisteria floribunda agglutinin-sialylated mucin1 and the prognostic role of mucin1 in human cholangiocarcinoma

Journal:	*BMJ Open*
Manuscript ID	bmjopen-2018-021693.R1
Article Type:	Research
Date Submitted by the Author:	03-Jul-2018
Complete List of Authors:	Tang, Zengwei; The First Clinical Medical School of Lanzhou University, Department of Special Minimally Invasive Surgery, The first Hospital of Lanzhou University Yang, Yuan; The First clinical medical School of Lanzhou University, Department of Internal Medicne, The First hospital of Lanzhou University Wang, Xiaolu; West China Medical School, Sichuan University, Department of General Surgery, West China Hospital/West China Medical School, Sichuan University Zhao, Zhonghong; Department of Integrated traditional Chinese and Western Medicine, People's Hospital of Zhengning County Meng, Wenbo; Department of Special Minimally Invasive Surgery, The First Hospital of Lanzhou University Li, Xun; The second department of General Surgery, The First Hospital of Lanzhou University
Primary Subject Heading:	Gastroenterology and hepatology
Secondary Subject Heading:	Gastroenterology and hepatology, Diagnostics, Evidence based practice, Oncology
Keywords:	Cholangiocarcinoma, prognosis, dignosis, Mucin1, meta-analysis

Meta-analysis of the diagnostic value of wisteria floribunda agglutinin-sialylated mucin1 and the prognostic role of mucin1 in human cholangiocarcinoma

Authors and affiliations:

Zengwei Tang [1,2]

Yuan Yang [1]

Xiaolu Wang [3]

Zhonghong Zhao [4]

Wenbo Meng [1,2]

Xun Li [1,5]

Correspondence:

Professor **Wenbo Meng**

Department of Special Minimally Invasive Surgery, The First Hospital of Lanzhou University, Lanzhou 730000, China.

Tel:+86 139 1917 7177

Fax:+96 0931-8622275

E-mail: mengwb@lzu.edu.cn

[1]The First Clinical Medical School of Lanzhou University, Lanzhou, China.

[2]Department of Special Minimally Invasive Surgery, The First Hospital of Lanzhou University, Lanzhou, China.

[3]Department of General Surgery, West China Hospital/West China Medical School, Sichuan University, Chengdu, China.

[4]People's Hospital of Zhengning County, Qingyang, China.

[5]The second department of General Surgery, The First Hospital of Lanzhou University, Lanzhou, China.

Keywords: cholangiocarcinoma, prognosis, diagnosis, Mucin1, meta-analysis

Word count: 4,136

Financial support: This study was supported by the West Light Foundation of the Chinese Academy of Science [2015(90)]. The funding sources played no roles in the study design, experiment method development, data collection, organization, analysis and interpretation, nor in the manuscript drafting, reviewing or approval.

Author contributions: Zengwei Tang–study concept and design; development of methodology, collection and extraction of data, statistical analysis and interpretation of data, and drafting of the manuscript; critical revision of the manuscript for significant intellectual content.

Yuan Yang–development of methodology, collection and extraction of data, statistical analysis and interpretation of data.

Xiaolu Wang and Zhonghong Zhao–collection and extraction of data; analysis and interpretation of data.

Wenbo Meng and Xun Li–funding application and study supervision; critical revision of the manuscript for significant intellectual content.

Disclosures: Zengwei Tang, Yuan Yang, Xiaolu Wang, Zhonghong Zhao, Wenbo Meng and Xun Li have no conflicts of interest to declare.

Patient consent: Consent is not required when conducting a meta-analysis.

Ethics approval: This study did not require ethical approval as the data used have been published previously, and hence are already in the public domain.

Data sharing statement: Extracted data are available upon request to the corresponding author.

Abstract

Objective: The purpose of this study is to evaluate the diagnostic value of wisteria floribunda agglutinin-sialylated Mucin1(WFA-MUC1) and the prognostic role of Mucin1(MUC1) in human cholangiocarcinoma (CCA).

Design: Meta-analysis.

Data sources: Studies published in PubMed, Web of Science, The Cochrane Library and the China National Knowledge Infrastructure up to 11 Oct 2017.

Eligibility criteria: We included reports assessing the diagnostic capacity of WFA-MUC1 and the prognostic role of MUC1 in CCA. The receiver operating characteristic curve (ROC) of WFA-MUC1 and/or CA19-9 was described and the hazard ratios (HRs) including 95% confidence interval (95%CI) and the corresponding P value for MUC1 can be extracted.

Data extraction and synthesis: Two independent researchers extracted data and assessed risk of bias. The diagnostic sensitivity and specificity data of WFA-MUC1were extracted and analyzed as bivariate data. Pooled hazard ratio (HRs) and its 95%CI for MUC1 were calculated with a random-effects meta-analysis model on overall survival of resectable CCA.

Results: Sixteen reports were included in this study. The pooled sensitivity and specificity of WFA-MUC1 were 0.76 (0.71 to 0.81) and 0.72 (0.59 to 0.83) in serum, 0.85 (0.81 to 0.89) and 0.72 (0.64 to 0.80) in bile, and 0.72 (0.50 to 0.87) and 0.85 (0.70 to 0.93) in tissue, respectively. The summary receiver-operating characteristic curve (SROC) were 0.77 (0.73 to 0.81) in serum, 0.88 (0.85 to 0.90) in bile, and 0.86(0.83-0.89) in tissue respectively. Furthermore, The pooled sensitivity and specificity and the SROC of CA19-9 in serum were 0.67(0.61 to 0.72), 0.86(0.75 to 0.93) and

0.75(0.71 to 0.79) respectively. The pooled HRs for MUC1 was 2.20 (1.57 to 3.01) in CCA, and 4.17 (1.71-10.17) in mass-forming intrahepatic CCA.

Conclusions: Compared to CA19-9, WFA-MUC1 was shown to possess stronger diagnostic capability. MUC1 could serve as a prognosis factor for poor outcomes of CCA, particularly, mass-forming intrahepatic CCA.

Keywords: cholangiocarcinoma, prognosis, diagnosis, Mucin1, meta-analysis

Strengths and limitations of this study

- This meta-analysis evaluated the diagnostic capability of WFA-MUC1 and prognostic role of MUC1 in cholangiocarcinoma.
- The diagnostic capability of WFA-MUC1 is superior to that of CA19-9.
- The diagnostic capability of WFA-MUC1 in bile is better that in serum.
- Expression of MUC1 in biliary duct cancer tissues is a prognosis factor for poor outcomes of resectable cholangiocarcinoma..
- Majority of the subjects included in this meta-analysis were from Asia. More participants from different regions other than Asia are needed to better evaluate the roles of Mucin1 in the diagnosis and prognosis of cholangiocarcinoma worldwide.

Introduction

Cholangiocarcinoma (CCA) is a malignancy arising from epithelia at various anatomic locations in the biliary tree.[1] The median survival time for patients with unresectable CCA is less than a year.[2, 3] The prognosis is considerably better for patients who undergo radical resection of CCA, with a five year-survival rates ranging from 20% to 40%.[4, 5] .However, it is hard to detect CCA at the early stage, even with the advanced imaging technology and the complete diagnosis protocol currently. This situation limits the benefits of surgery therapy and curative treatment options to CCA patients and contributes to the poor outcome of patients with CCA.

Currently, a huge amount of literature reporting numerous molecular biomarkers with limited diagnostic or prognostic capability for CCA have been published. Some of the reported biomarkers have been used for guiding clinical diagnosis and treatment of CCA worldwide, such as Mucin2 to Mucin6,[6-15] carbohydrate antigen 19-9 (CA19-9),[16-18] interleukin6,[19, 20] serum cytokeratin19 fragments[21, 22] and carbohydrate antigen125 (CA125)[16, 23, 24]. Among these biomarkers, CA19-9 in serum has been the focus of related research and always been used as a biomarker for CCA. However, the overall sensitivity and specificity of CA19-9 is not satisfying and CA19-9 is not capable of detecting CCA progression.[5, 17, 24] In addition, although CA19-9 expression is elevated in up to 85% suspected CCA, [17, 25, 26] the capability of CA19-9 as a diagnostic marker is still limited due to influence of co-existing inflammation in biliary tract and the fact that cancer cells from Lewis gene negative subtype of CCA doesn't produce CA19-9 theoretically.[17, 18, 27]

Mucin1 (MUC1), also known as polymorphic epithelial Mucin, is cell surface associated and belongs

to Mucin family. It is a mucin encoded by the MUC1 gene in humans[28]. MUC1 is a high molecular weight, membrane-associated glycoprotein with a 69 amino acids cytoplasmic tail, a transmembrane domain and an extracellular domain consisting of a variable number of highly conserved tandem repeats of 20 amino acids[28, 29]. Highly glycosylated MUC1 has been reported to be associated with malignancies in many other organs.[30] Matsuda et al[31] reported that wisteria floribunda agglutinin-sialylation(WFA) could be employed as the best probe to detect alterations of glycan structure in biliary tract derived cancer cells and distinguish it from normal tissues. They also identified sialylated MUC1 as a potential cholangiocarcinoma-specific glycoprotein marker. From then on, wisteria floribunda agglutinin sialylated-Mucin1(WFA-MUC1) has been regarded as a sensitive molecular biomarker for CCA.[9, 31-35] However, the diagnostic capability of WFA-MUC1 remains unclear since the reported range of WFA-MUC1 distinguishing CCA from benign biliary diseases varied greatly (0.74~0.87 in serum, 0.72~0.90 in bile).[9, 31-35] In addition, although the correlation between the expression of MUC1 in biliary duct derived cancer and the overall survival(OS) rate for patients with resectable CCA has been analyzed with Kaplan-Meier plot in several clinical trials, the result still remains inconclusive. Besides, more questions about MUC1 in CCA still need to be answered such as whether expression of MUC1 suggests a poor prognosis for CCA patients and whether expression level of MUC1 associates with CCA progression.[7, 9, 10, 12-15, 36, 37]

Therefore, we conducted this meta-analysis to evaluate the diagnostic capability of WFA-MUC1 in discriminating CCA patients from benign biliary diseases and to investigate the prognostic role of MUC1 in CCA patients.

Methods

Search strategy

The initial comprehensive literature search through 11 March 2017 was performed in database of PubMed, Web of Science, The Cochrane Library and the China National Knowledge Infrastructure. Our latest search was completed on 11 October 2017. The publication language was restricted to articles published in English or Chinese. Searching keywords used are "Wisteria floribunda agglutinin sialylated-mucin1(WFA-MUC1)" "Mucin1/MUC1", "cholangiocarcinoma/CCA", "cholangiocellular carcinoma", "intrahepatic cholangiocarcinoma", "extrahepatic cholangiocarcinoma" or "Klatskin tumor/hilar cholangiocarcinoma/perihilar cholangiocarcinoma" combined with "prognosis/prognostic/prognoses/survival" or "diagnosis/diagnostic/diagnoses". The reference lists of every study that met the inclusion criteria were also manually reviewed to identify additional relevant publications.

Patient and public involvement

Patients and public were not involved as all the data used have been published previously, and hence are already in the public domain.

Eligibility criteria

Published studies were included if they met the following criteria: (1) the published studies were focused on CCA; (2) all studied subjects with CCA were diagnosed by pathologist postoperatively; (3) the expression of MUC1 in tissues was detected by immunohistochemistry staining and the level of WFA-MUC1 in bile or serum was tested by sandwich enzyme-linked immunosorbent assay(ELISA);

(4) the receiver operating characteristic curve (ROC) of WFA-MUC1 and/or CA19-9 was described and the rates of true positives, false positives, false negatives, and true negatives can be calculated; and (5) the hazard ratios (HRs)including 95% confidence interval (95%CI) and the corresponding P value can be extracted. Studies were excluded based on following criteria: (1) animal studies;(2) review articles, case reports or letters; (3) duplicated publication; (4) non-English or non-Chinese papers; and (5) insufficient data on the HRs or that could not be extracted from Kaplan-Meier analysis result.

Data extraction

Data extraction was carried out by two investigators independently (Zengwei Tang and Yuan Yang). If discrepancies occurred, it would be resolved by the consensus of these two investigators. Data related to the study characteristics were extracted with the following variables: the first author of the study, study design and duration, year of publication,institution, the number of subjects in the study with mean age and gender, the selected antibody for the MUC1 immunochemical staining, ELISA assay kits testing the level of biliary and /or serum WFA-MUC1 and the level of serum CA-19-9, the AUC for WFA-MUC1, the cut-off value of MUC1, assay's sensitivity and specificity, HRs and their 95% CI and case follow-up time. For the three studies that did not provide the value of HRs and their 95%CI, we digitized and extracted the data from the Kaplan–Meier curve in the publications by using the software designed by Jayne F Tierney and Matthew R Sydes.[38] The optimal sensitivity and specificity were reported graphically in one study with two cohorts and were extracted using Plot Digitizer software 2.6.8 (provided by source forge.net, found online at http://plot digitizer source forge.net/) to convert data points on the graphs into numerical data.[39, 40] Repeated data points were isolated using nonparametric bootstrap sampling[41] guided by the descriptive statistics provided in the supporting

document. The possible repeated data points were repeatedly sampled until the sets matching the descriptive statistics was found. All the data was extracted from published literature.

.

Quality assessment across studies

Quality assessment of the studies in the prognostic meta-analysis was performed by using the modified risk of bias tool recommended by the Cochrane Collaboration as described previously.[42-44] Quality assessment of studies evaluating the diagnostic capability of WFA-MUC1 was performed using the QUADAS(Quality Assessment of Studies of Diagnostic Accuracy included in Systematic reviews) checklists.[45, 46] However, we did not calculate the summary scores for each study investigating the diagnostic capability of WFA-MUC1 not only because their interpretation was problematic, but also because their report was potentially misleading.[47] Moreover, seven of the best differentiating items have been selected from the QUADAS checklists.

Statistical analyses

The statistical analysis was performed according to the guidelines proposed by the Meta-Analysis of Observational Studies in Epidemiology group (MOOSE).[48] The pooled HRs with 95% CI were calculated with a random-effect model according to the DerSimonian-Laird method to evaluate the correlation between the positive expression of MUC1 and overall survival (OS).[49, 50] Sensitivity and specificity for each study evaluating the diagnostic capability of WFA-MUC1 were calculated and and analyzed this datum as bivariate data according to methods for diagnostic meta-analysis.[51] An aggregated bivariate data meta-analysis with the generation of forest plots and summary receiver-operating characteristic curve (SROC) was performed. Forest plots displayed the diagnostic

probabilities of individual studies, the corresponding 95% CI, and squares with area proportional to study weight in the meta-analysis. The SROC demonstrated individual study data point with circles, with size proportion to study weight and 95% prediction contour and 95% confidence contour around the pooled estimate. The heterogeneity among studies was measured using the Q tests and I^2 statistic to assess the extent of the inconsistency. A probability value of $P<0.1$ and I^2 >50% indicated the existence of significant heterogeneity.[52] Furthermore, Funnel plot and the Egger's linear regression test were applied to evaluate potential publication bias for eligible studies using OS as an endpoint.[53] A P <0.1 for Egger's test was considered statistically significant. All statistical analyses were performed with Stata/MP 14.0 (StataCorp, Parallel Edition).

Results

Study selection

The study includes results of electronic searches up to 11 October 2017. A total of .341 papers were identified, of which 148 were retrieved for full-text review. Among these 148 publications, 16 studies[6, 7, 9, 10, 12-15, 31, 32, 34-37, 54, 55] were eligible for the meta-analyses according to the inclusion and exclusion criteria. Nine studies[7, 9, 10, 12-15, 36, 37] out of 16 studies used OS as endpoint, and eight studies[6, 9, 31, 32, 34, 35, 54, 55] used the sensitivity and specificity rate as the endpoint (One study reported by Huang et al also provided the data on diagnostic value of MUC1 in tissue). The detailed literature searching process was shown in **Fig1**.

Characteristics of the included studies and participants

Characteristics of eligible studies and their participants were listed in **Table 1, Table 2 and Table 3.** Nine studies[7, 9, 10, 12-15, 36, 37] evaluating the prognostic value of MUC1 for resectable CCA which were

conducted in 4 countries (Korea, Japan, China and Thailand), the other seven studies[6, 31, 32, 34, 35, 54, 55] investigating the diagnostic capability of WFA-MUC1were undertaken in 5 countries (Japan, UK, Brazil, Thailand, China). Retrospective study design was applied to perform the meta-analysis of prognostic value by all selected studies. The seven studies investigating the diagnostic capability of WFA-MUC1, meaning that discriminating CCA from benign biliary diseases, used prospective study design. All CCA diagnosis included in this study were based on histopathology as reported in the include publications. The sample size of eligible studies evaluating the prognostic value of MUC1 varied greatly, ranging from 27 to 87 with a median size of 56. The sample size of studies investigating the diagnostic capability of WFA-MUC1 ranged from 30 to 303 (median=80) and 20 to 287(median=69) for biliary tract carcinoma group and benign biliary diseases group, respectively.

The level of WFA-MUC1 in bile and serum were tested by the approach of ELISA using mAb WFAMY.1E12. The concentration of serum CA19-9 was tested by CA19-9 ELISA kits. The sensitivity, specificity and AUC of each study included in the diagnostic meta-analysis were shown in **Table 2**. Three studies[9, 14, 36] investigating the prognostic value of MUC1 for CCA provided the Kaplan-Meier curve and we digitized and extracted the data of HRs including their corresponding 95%CI from the curve by using the methods described above. The cut-off value to define positive expression of MUC1(2 trials[12, 37]>25%, one trial[14] >20%, 2 trials[9, 10]>10% and 4 trials[7, 13, 14, 36] >5%), the follow-up time (7 trials[7, 10, 13-15, 36, 37]>50 months, 1 trail[9] >20 months and another one[12]>15 months), and the antibody of MUC1 were selected for immunochemistry (mAb DF3, Clone Mab DF3,Clone Ma695,Clone Ma689 and mAb HMPV) were inconsistent (As shown in **Table 3**).

Primary endpoint: the outcomes of diagnostic meta-analysis

Three trials[35, 55] including 414 cases of biliary tract carcinoma (59 gall bladder carcinomas and 355 CCA) and 405 subjects with benign biliary diseases investigated the diagnostic capability of WFA-MUC1 level in serum. **Fig2a** presented the diagnostic parameters s for serum WFA-MUC1 in a summary receiver operating characteristic graph. The pooled optimal sensitivity (true positive rate) was 0.76(0.71 to 0.81) and specificity (true negative rate) was 0.72(0.59 to 0.83). the AUC of SROC was 0.77(0.73 to 0.81).

As a comparison, three trials[34, 35, 55] with 588 subjects with biliary tract carcinoma (73 subjects with gall bladder carcinoma and 515 CCA) and 432 subjects with benign biliary disease evaluated the diagnostic capability of CA19-9 level in serum. **Fig2b** presented the diagnostic parameters for serum level of CA19-9 in a SROC graph. The pooled optimal sensitivity was 0.67(0.61 to 0.72) and specificity was 0.86(0.75 to 0.93). The AUC under SROC was 0.75(0.71 to 0.79).

Four trials[31, 32, 34, 35] including 209 subjects with benign biliary disease and 416 biliary tract carcinomas (73 gall bladder carcinomas) evaluated the diagnostic capability of biliary level of WFA-MUC1. SROC of biliary WFA-MUC1 was shown **in Fig2c**. The pooled sensitivity was 0.85(0.81 to 0.89) and specificity was 0.72(0.64 to 0.80). The AUC under SROC was 0.88(0.85-0.90). Furthermore, three trials[6, 9, 54] inculding 72 subjects with CCA and 119 benign biliary disease used the positive expression of MUC1 in tissue as a criterium to discriminate CCA from benign biliary disease. The diagnostic

parameters of positive expression of MUC1 in biliary duct cancer tissue were shown in **Fig2d**. The pooled sensitivity was 0.72(0.50 to 0.87) and specificity 0.85(0.70-0.93). The AUC of SROC was 0.86(0.83-0.89).

Secondary endpoint: The outcome of prognostic meta-analysis

Nine studies[7, 9, 10, 12-15, 36, 37] with a total of 511 individuals diagnosed with CCA were eligible for the pooled analysis of OS. As shown in the **Fig3**, the overall pooled HRs of MUC1 was 2.20 (1.57 to 3.01). No heterogeneity among these studies was found ($\mathbf{I^2}$=0; ***P***=0.869). Subgroup analyses stratified by the histopathological morphology of CCA reveal that the pooled HRs of mass-forming intrahepatic CCA was 4.17(1.71 to 10.17). The pooled HRs of CCA was 1.98(1.37 to 2.85).

Risk of bias within studies

Detailed results of the risk of bias assessment for included studies in prognostic meta-analysis were shown in **Supplementary Table 1.** Except one study[12] showed a high risk of bias, six showed[7, 9, 10, 13, 15, 37] a low risk of bias and two[14, 36] were shown with the unclear risk of bias. Moreover, as demonstrated in **Fig4**, the result of funnel plots of OS showed no clear indication of publication bias (Egger's test, ***P***=0.661). Selection bias of diagnostic analyses may be caused by two trials including 73 subjects diagnosed with gall bladder carcinoma.[34, 35] Detailed items selected for quality assessment of studies included in diagnostic meta-analysis was shown in **supplementary Table 2**.

Additional analysis

Studies conducted by several research groups have concluded that the patients with mass-forming intrahepatic CCA or periductal infiltrating CCA had a worse prognosis than patients with other types of CCA regarding the OS. These types of CCA have higher rates of recurrence after resection.[56, 57] In our meta-analysis for prognosis, subgroup analysis stratified by the histopathological morphology of CCA was conducted to reduce the inconsistency caused by the type of CCA. We found that the OS for patients with positive expression of MUC1 was significantly shorter than that of MUC1 negative group. The overall pooled HRs=2.20. For subjects with mass-forming intrahepatic CCA, HRs=4.17. In addition, a sensitivity analysis was performed to investigate the stability of the pooled HRs. As shown in **Supplementary Figure1**, the results of pooled HRs were not affected significantly by each individual study.

Molecular Function (MF), Biological Process (BP) and Reactome Pathways of MUC1 in cancer

we searched the GO classification system (http://www.pantherdb.org/) to found the Molecular Function, Biological Process and Reactome Pathways of MUC1 in cancer, the search results was summarized in **Table 4**.

DISCUSSION

As we all known, serum CA19-9 has been widely used as a tumor marker for CCA. However, it's diagnostic accuracy is limited since the serum level of CA19-9 can be strongly influenced by the co-existing inflammatory conditions of the biliary tract and this antigen could not be detected in Lewis gene negative individuals[16, 18]. The most commonly performed diagnostic method for CCA is biliary

cytology which tests the bile sample from a biliary drainage catheter. But the sensitivity of biliary cytology is extremely low (20.7 ± 3.5%) as reported in published study[58]. In our meta-analysis of the diagnostic capability of markers for CCA, seven prospective trials[12, 31, 32, 34, 35, 55] [and a retrospective study[9] were eligible for diagnostic analysis which showed that the diagnostic capability of CA19-9 was inferior to other molecules, such as WFA-MUC1.

In the meta-analysis for diagnosis, the diagnostic value of WFA-MUC1 in serum, bile and biliary duct cancer tissue was evaluated stratified by subgroups of CCA. Two studies[35, 55] with 3 trials (studies reported by Matsuda et.al[55] included two cohorts) assessed the diagnostic accuracy of WFA-MUC1 level in serum, the pooled sensitivity of WFA-MUC1 was 0.76 (0.71 to 0.81). The specificity was 0.72 (0.59 to 0.83), and the AUC of SROC was 0.77 (0.73 to 0.81). While in three studies[34, 35, 55] with four trials assessing the diagnostic accuracy of CA19-9 level in serum, the pooled sensitivity of CA19-9 was 0.67 (0.61 to 0.72), the specificity was 0.86 (0.75 to 0.93) and the AUC of SROC was 0.75 (0.71 to 0.79), which means it would bring a severe error into clinical diagnosis.

The diagnostic capability of serum WFA-MUC1 was superior to that of CA19-9 (as the data showed, $AUC_{WFA\text{-}MUC1}$ vs. $AUC_{CA19\text{-}9}$: 0.77(0.73 to 0.81) vs. 0.75(0.71 to 0.79)). The sensitivity rate of WFA-MUC1 was higher than that of CA19-9 ((0.76(0.71-0.81) vs. 0.67(0.61-0.72)), nevertheless, the specificity rate of serum WFA-MUC1 was less than that of CA19-9 ((0.72 (0.59 to 0.83) VS 0.86 (0.75 to 0.93)). In order to discriminate CCA from benign biliary disease, the combination of these two biomarkers may be applied to improve the diagnostic capability of WFA-MUC1 or CA19-9, as reported by previously published trials.

In the four prospective studies[31, 32, 34, 35] with 343 CAA and 73 gall bladder carcinomas and 209 benign biliary diseases, the diagnostic accuracy of WFA-MUC1 in bile was also assessed. The pooled sensitivity of WFA-MUC1 testing was 0.85 (0.81 to 0.89) and specificity was 0.72 (0.64 to 0.80) and AUC of SROC was 0.86 (0.83-0.89). The diagnostic capability of bile WFA-MUC1 was better than that of serum WFA-MUC1 ($AUC_{MUC1\ in\ bile}$ vs $AUC_{MUC1\ in\ serum}$: 0.86 (0.83 -0.89) vs 0.77(0.73 to 0.81)), which is consistent with the concept that for most of diseases, the diagnostic molecule levels are different between locally and systemically.

As described above, the level of WFA-MUC1 has significantly higher diagnosis accuracy than CA19-9. Furthermore, the diagnostic accuracy of biliary WFA-MUC1 level was better than that in serum. Therefore, the diagnostic capability of the combined serum CA19-9 and biliary WFA-MUC1 would be better than that of the combination of serum CA19-9 and serum WFA-MUC1 level in discriminating CCA from the benign biliary disease. Such combined measurement would represent a superior diagnostic test for the detection of CCA in daily clinical practice. Unfortunately, as one study[55] included in the diagnostic meta-analyses did not provided the detailed cut-off value of serum WFA-MUC1 level, nor CA19-9 level, the optimal cut-off value of SROC cannot be estimated by this meta-analysis.

It has been demonstrated that MUC1 expression in various human tumors is related to invasive tumor progression and a poor patient outcome.[10, 36, 59, 60] In the prognostic meta-analysis, pooled analysis of nine retrospective studies[7, 9, 10, 12-15, 36, 37] has shown that positive MUC1 expressed of tissue was a poor

prognosis factor for resectable CCA (the pooled HRs was 2.20, 95%CI: 1.57 to 3.01), especially for patients with mass-forming intrahepatic CCA (the pooled HRs was 4.17, 95%CI:1.71-10.17), which was demonstrated by the subgroup analysis stratified by the morphology of CCA.

It has been reported by publications that around 50-60% of CCA are identified as perihilar CCA, up to 20% of CCA are distal, 5% of tumors are multifocal and up to 20% of all CCA are intrahepatic.[5, 61] Different type of CCA demonstrates various epidemiological, morphological and clinical features. A previous meta-analysis[43] identified several prognostic biomarkers (EGFR, MUC1, MUC4, and p27) for resectable CCA, with a small number of subjects in the subgroup of evaluating the prognostic role of MUC1 (Four studies including 265 subjects with resectable CCA were included in the analysis evaluating the prognostic value of MUC1 expression in tissue). The sample size of the prognostic meta-analysis in our study was doubled (9 studies including 511 patients with resectable CCA) and our study provided more explicit description and analysis. Subgroup analysis and sensitivity analysis were conducted to get more reliable results. The pooled HRs result in our study showed that overexpression of MUC1 in tissue was a poor prognostic index for resectable CCA, in particular for patients with mass-forming intrahepatic CCA.

Predictive biomarkers could serve as the key point for personalized cancer treatments such as verifying the chemosensitivity of CCA and developing vaccines to CCA. Up to now, VEGFR, EGFR, HER2, MEK, and BRAF have been the focus for the studies evaluating molecular targeting therapies for CCA.[62] Along with the better understanding of the pathogenesis of CCA mediated by MUC1, MUC1 may become a new focus of targeted therapies for CCA.

The strength and limitation of this study

This meta-analysis, to our best knowledge, is the first study to evaluate the diagnostic value of WFA-MUC1 and prognostic role of MUC1 for human CCA; We obtained data about the prognostic and /or diagnostic capability of WFA-MUC1/MUC1 for CCA from16 trials, which were identified by systematically searching four databases; All subjects with CCA included in this study were diagnosed by pathologist postoperatively. To avoid the possible bias brought by including studies only with reported HRs which may affect the significance of the statistical analysis, we digitized and extracted the HR data from Kaplan-Meier curves in three studies[9, 14, 36] In addition, sensitivity analysis and subgroup analysis which was stratified by the morphology of CAA, made our results of the pooled HRs more stable. To analyze the diagnostic capability of WFA-MUC1, we separately assessed the diagnostic accuracy of WFA-MUC1 level in serum, in bile and in tissue. A comparison of diagnostic accuracy between WFA-MUC1 level and CA19-9 level in serum, as well as a comparison of diagnostic accuracy between the WFA-MUC1 level in serum and in bile were also conducted in our study, as previously clinical trials described.

While our present study could provide a great amount of useful information, limitations of our study should be keep in mind. Firstly, majority of the subjects included in this meta-analysis were from Asian hospitals (data on prognostic meta-analysis were retrieved from Japan, China, Korea and Thailand; data on diagnostic meta-analysis were from Japan, Thailand, China, Brazil and the UK). There may be biological differences in terms of tumor behaviors among populations from different regions worldwide. The phenomenon has been reported on the mortality of stomach cancer between eastern countries and western countries.[63] Secondly, four different cut-off values of positive MUC1

immunostaining (> 5% of carcinoma cells stained was defined as the cut-off point by four studies, >10% defined by 2 studies, >20% identified by one study and >25% defined by another 2 studies) and four different MUC1 antibodies (mAb DF3, Clone Ma689, Clone Ma695 and mAb HMPV) were used in the nine included studies in our prognostic meta-analysis. Lack of consistency on cut-off value and the type of MUC1 antibody used resulted in considerable heterogeneity. Thirdly, in the diagnostic meta-analysis, although majority of subjects in the biliary tract carcinoma group were diagnosed with CCA, a total of 73 subjects with gall bladder carcinomas were included in this group to evaluate the diagnostic capability of biliary level of MUC1 and serum level of CA19-9. 59 patients with gall bladder carcinomas were included in the evaluation of the diagnostic capability of serum level of WFA-MUC1. The heterogeneity caused by the inconsistency of participants cannot be underestimated since WFA-MUC1 can serve as an independent predictor of hepatocellular carcinoma recurrence.[64] It may be useful for discriminating gall bladder carcinoma from benign gall bladder disease. Fourthly, given that only seven trials with a small number of patients were eligible for the diagnostic meta-analysis and two of them did not provide the cut-off value of WFA-MUC1 and CA19-9 in serum, we cannot give an estimated optimal cut-off value for WFA-MUC1 level in serum. Finally, all data in our study was retrieved from subjects with resectable CCA or gall bladder carcinoma, there may be some difference in the pathogenesis between resectable and unresectable CCA.

Conclusions

This paper highlighted the importance of WFA-MUC1. It has a better diagnostic capability than CA19-9, and the diagnostic capability of the biliary level of WFA-MUC1 was superior to that in the

serum. Furthermore, MUC1 could served as a prognosis factor for poor outcomes of resectable CCA, particularly in mass-forming intrahepatic CCA.

Larger, multi-center studies are still needed for better understanding of the molecular pathogenesis of CCA, developing combined kits to conveniently test the serum/ biliary level of MUC1 and serum level of CA19-9 in routine clinical practice, providing an optimal cut-off value of WFA-MUC1 with higher diagnostic accuracy for CCA and benefiting the populations from different regions worldwide.

References

1. Razumilava N, Gores GJ. Cholangiocarcinoma. *Lancet* 2014; 383(9935):2168-79.
2. Eckel F, Schmid RM. Chemotherapy in advanced biliary tract carcinoma: a pooled analysis of clinical trials. *Br J Cancer* 2007; 96(6):896-902.
3. Park J, Kim MH, Kim KP, et al. Natural History and Prognostic Factors of Advanced Cholangiocarcinoma without Surgery, Chemotherapy, or Radiotherapy: A Large-Scale Observational Study. *Gut Liver* 2009; 3(4):298-305.
4. DeOliveira ML, Cunningham SC, Cameron JL, et al. Cholangiocarcinoma: thirty-one-year experience with 564 patients at a single institution. *Ann Surg* 2007; 245(5):755-62.
5. Khan SA, Davidson BR, Goldin RD, et al. Guidelines for the diagnosis and treatment of cholangiocarcinoma: an update. *Gut* 2012; 61(12):1657-69.
6. Esperança ABT, Camacho AHDS, Monteiro JBM, et al. Mucins and NCAM (CD56) in intrahepatic cholangiocarcinogenesis. *Jornal Brasileiro De Patologia E Medicina Laboratorial* 2014; 50(3):216-220.
7. Higashi M, Yamada N, Yokoyama S, et al. Pathobiological implications of MUC16/CA125 expression in intrahepatic cholangiocarcinoma-mass forming type. *Pathobiology* 2012; 79(2):101-6.
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(15):3393-403.
9. Huang F, Zhou QB, Chen RF. Expression and significance of MUC1 in hepatolithiasis associated with intrahepatic cholangiocarcinoma. *Chinese Archives of General Surgery* 2010.
10. Park SY, Roh SJ, Kim YN, et al. Expression of MUC1, MUC2, MUC5AC and MUC6 in cholangiocarcinoma: prognostic impact. *Oncol Rep* 2009; 22(3):649-57.
11. 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(10):1675-81.
12. Boonla C, Sripa B, Thuwajit P, et al. MUC1 and MUC5AC mucin expression in liver fluke-associated intrahepatic cholangiocarcinoma. *World J Gastroenterol* 2005; 11(32):4939-46.

13. Shibahara H, Tamada S, Higashi M, et al. MUC4 is a novel prognostic factor of intrahepatic cholangiocarcinoma-mass forming type. *Hepatology* 2004; 39(1):220-9.
14. Tamada S, Goto M, Nomoto M, et al. Expression of MUC1 and MUC2 mucins in extrahepatic bile duct carcinomas: its relationship with tumor progression and prognosis. *Pathol Int* 2002; 52(11):713-23.
15. Matsumura N, Yamamoto M, Aruga A, et al. Correlation between expression of MUC1 core protein and outcome after surgery in mass-forming intrahepatic cholangiocarcinoma. *Cancer* 2002; 94(6):1770-6.
16. Chen CY, Shiesh SC, Tsao HC, et al. The assessment of biliary CA 125, CA 19-9 and CEA in diagnosing cholangiocarcinoma--the influence of sampling time and hepatolithiasis. *Hepatogastroenterology* 2002; 49(45):616-20.
17. Patel AH, Harnois DM, Klee GG, et al. The utility of CA 19-9 in the diagnoses of cholangiocarcinoma in patients without primary sclerosing cholangitis. *Am J Gastroenterol* 2000; 95(1):204-7.
18. Narimatsu H, Iwasaki H, Nakayama F, et al. Lewis and secretor gene dosages affect CA19-9 and DU-PAN-2 serum levels in normal individuals and colorectal cancer patients. *Cancer Res* 1998; 58(3):512-8.
19. Xu H, Inagaki Y, Tang W, et al. Elevation of serum KL-6 mucin levels in patients with cholangiocarcinoma. *Hepatogastroenterology* 2008; 55(88):2000-4.
20. Cheon YK, Cho YD, Moon JH, et al. Diagnostic utility of interleukin-6 (IL-6) for primary bile duct cancer and changes in serum IL-6 levels following photodynamic therapy. *Am J Gastroenterol* 2007; 102(10):2164-70.
21. Lumachi F, Lo Re G, Tozzoli R, et al. Measurement of serum carcinoembryonic antigen, carbohydrate antigen 19-9, cytokeratin-19 fragment and matrix metalloproteinase-7 for detecting cholangiocarcinoma: a preliminary case-control study. *Anticancer Res* 2014; 34(11):6663-7.
22. Uenishi T, Yamazaki O, Tanaka H, et al. Serum cytokeratin 19 fragment (CYFRA21-1) as a prognostic factor in intrahepatic cholangiocarcinoma. *Ann Surg Oncol* 2008; 15(2):583-9.
23. Valle J, Wasan H, Palmer DH, et al. Cisplatin plus gemcitabine versus gemcitabine for biliary tract cancer. *N Engl J Med* 2010; 362(14):1273-81.
24. Abbas G, Lindor KD. Cholangiocarcinoma in primary sclerosing cholangitis. *J Gastrointest Cancer* 2009; 40(1-2):19-25.
25. Hultcrantz R, Olsson R, Danielsson A, et al. A 3-year prospective study on serum tumor markers used for detecting cholangiocarcinoma in patients with primary sclerosing cholangitis. *J Hepatol* 1999; 30(4):669-73.
26. Gores GJ. Early detection and treatment of cholangiocarcinoma. *Liver Transpl* 2000; 6(6 Suppl 2):S30-4.
27. Hamada E, Taniguchi T, Baba S, et al. Investigation of unexpected serum CA19-9 elevation in Lewis-negative cancer patients. *Ann Clin Biochem* 2012; 49(Pt 3):266-72.
28. Gendler SJ, Lancaster CA, Taylor-Papadimitriou J, et al. Molecular cloning and expression of human tumor-associated polymorphic epithelial mucin. *J Biol Chem* 1990; 265(25):15286-93.
29. Lan MS, Batra SK, Qi WN, et al. Cloning and sequencing of a human pancreatic tumor mucin cDNA. *J Biol Chem* 1990; 265(25):15294-9.
30. Brockhausen I. Pathways of O-glycan biosynthesis in cancer cells. *Biochim Biophys Acta* 1999; 1473(1):67-95.
31. Matsuda A, Kuno A, Kawamoto T, et al. Wisteria floribunda agglutinin-positive mucin 1 is a sensitive biliary marker for human cholangiocarcinoma. *Hepatology* 2010; 52(1):174-82.
32. Matsuda A, Kuno A, Matsuzaki H, et al. Glycoproteomics-based cancer marker discovery adopting dual enrichment with Wisteria floribunda agglutinin for high specific glyco-diagnosis of cholangiocarcinoma.

J Proteomics 2013; 85:1-11.

33. Xu F, Liu F, Zhao H, et al. Prognostic Significance of Mucin Antigen MUC1 in Various Human Epithelial Cancers: A Meta-Analysis. *Medicine (Baltimore)* 2015; 94(50):e2286.
34. Yamaguchi T, Yokoyama Y, Ebata T, et al. Verification of WFA-Sialylated MUC1 as a Sensitive Biliary Biomarker for Human Biliary Tract Cancer. *Ann Surg Oncol* 2016; 23(2):671-7.
35. Shoda J, Matsuda A, Shida T, et al. Wisteria floribunda agglutinin-sialylated mucin core polypeptide 1 is a sensitive biomarker for biliary tract carcinoma and intrahepatic cholangiocarcinoma: a multicenter study. *J Gastroenterol* 2017; 52(2):218-228.
36. 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(6):1347-55.
37. Takao S, Uchikura K, Yonezawa S, et al. Mucin core protein expression in extrahepatic bile duct carcinoma is associated with metastases to the liver and poor prognosis. *Cancer* 1999; 86(10):1966-75.
38. Tierney JF, Stewart LA, Ghersi D, et al. Practical methods for incorporating summary time-to-event data into meta-analysis. *Trials* 2007; 8:16.
39. Kelsey TW, Anderson RA, Wright P, et al. Data-driven assessment of the human ovarian reserve. *Mol Hum Reprod* 2012; 18(2):79-87.
40. Iliodromiti S, Kelsey TW, Anderson RA, et al. Can anti-Mullerian hormone predict the diagnosis of polycystic ovary syndrome? A systematic review and meta-analysis of extracted data. *J Clin Endocrinol Metab* 2013; 98(8):3332-40.
41. Ciaccio AD. Bootstrap and Nonparametric Predictors to Impute Missing Data, 2011.
42. Rahbari NN, Aigner M, Thorlund K, et al. Meta-analysis shows that detection of circulating tumor cells indicates poor prognosis in patients with colorectal cancer. *Gastroenterology* 2010; 138(5):1714-26.
43. Ruys AT, Groot Koerkamp B, Wiggers JK, et al. Prognostic biomarkers in patients with resected cholangiocarcinoma: a systematic review and meta-analysis. *Ann Surg Oncol* 2014; 21(2):487-500.
44. Higgins JP, Green S. Cochrane Handbook For Systematic Reviews Of Interventions [internet]. *Naunyn-Schmiedebergs Archiv für experimentelle Pathologie und Pharmakologie* 2009; 2011(14):S38.
45. 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.
46. van Rheenen PF, Van de Vijver E, Fidler V. Faecal calprotectin for screening of patients with suspected inflammatory bowel disease: diagnostic meta-analysis. *BMJ* 2010; 341:c3369.
47. Whiting P, Harbord R, Kleijnen J. No role for quality scores in systematic reviews of diagnostic accuracy studies. *BMC Med Res Methodol* 2005; 5:19.
48. Stroup DF, Berlin JA, Morton SC, et al. Meta-analysis of observational studies in epidemiology: a proposal for reporting. Meta-analysis Of Observational Studies in Epidemiology (MOOSE) group. *JAMA* 2000; 283(15):2008-12.
49. DerSimonian R, Laird N. Meta-analysis in clinical trials revisited. *Contemp Clin Trials* 2015; 45(Pt A):139-45.
50. Xie S, Wang K, Xu H, et al. PRISMA-Extracapsular Dissection Versus Superficial Parotidectomy in Treatment of Benign Parotid Tumors: Evidence From 3194 Patients. *Medicine (Baltimore)* 2015; 94(34):e1237.
51. Harbord RM, Deeks JJ, Egger M, et al. A unification of models for meta-analysis of diagnostic accuracy studies. *Biostatistics* 2007; 8(2):239-51.
52. Higgins JP, Thompson SG, Deeks JJ, et al. Measuring inconsistency in meta-analyses. *BMJ* 2003;

327(7414):557-60.

53. Egger M, Davey Smith G, Schneider M, et al. Bias in meta-analysis detected by a simple, graphical test. *Bmj* 1997; 315(7109):629-34.

54. Zen Y, Britton D, Mitra V, et al. Tubulin beta-III: a novel immunohistochemical marker for intrahepatic peripheral cholangiocarcinoma. *Histopathology* 2014; 65(6):784-92.

55. Matsuda A, Kuno A, Nakagawa T, et al. Lectin Microarray-Based Sero-Biomarker Verification Targeting Aberrant O-Linked Glycosylation on Mucin 1. *Anal Chem* 2015; 87(14):7274-81.

56. Yamamoto Y, Shimada K, Sakamoto Y, et al. Clinicopathological characteristics of intrahepatic cholangiocellular carcinoma presenting intrahepatic bile duct growth. *J Surg Oncol* 2009; 99(3):161-5.

57. Shimada K, Sano T, Sakamoto Y, et al. Surgical outcomes of the mass-forming plus periductal infiltrating types of intrahepatic cholangiocarcinoma: a comparative study with the typical mass-forming type of intrahepatic cholangiocarcinoma. *World J Surg* 2007; 31(10):2016-22.

58. Tsuchiya T, Yokoyama Y, Ebata T, et al. Randomized controlled trial on timing and number of sampling for bile aspiration cytology. *J Hepatobiliary Pancreat Sci* 2014; 21(6):433-8.

59. Yonezawa S, Goto M, Yamada N, et al. Expression profiles of MUC1, MUC2, and MUC4 mucins in human neoplasms and their relationship with biological behavior. *Proteomics* 2008; 8(16):3329-41.

60. Utsunomiya T, Yonezawa S, Sakamoto H, et al. Expression of MUC1 and MUC2 mucins in gastric carcinomas: its relationship with the prognosis of the patients. *Clin Cancer Res* 1998; 4(11):2605-14.

61. Khan SA, Emadossadaty S, Ladep NG, et al. Rising trends in cholangiocarcinoma: is the ICD classification system misleading us? *J Hepatol* 2012; 56(4):848-54.

62. Zhu AX, Hezel AF. Development of molecularly targeted therapies in biliary tract cancers: reassessing the challenges and opportunities. *Hepatology* 2011; 53(2):695-704.

63. Strong VE, Song KY, Park CH, et al. Comparison of gastric cancer survival following R0 resection in the United States and Korea using an internationally validated nomogram. *Ann Surg* 2010; 251(4):640-6.

64. Tamaki N, Kuno A, Matsuda A, et al. Serum Wisteria Floribunda Agglutinin-Positive Sialylated Mucin 1 as a Marker of Progenitor/Biliary Features in Hepatocellular Carcinoma. *Sci Rep* 2017; 7(1):244.

Figure legends:

Figure 1. Diagram showing the literature searching work flow.

Figure 2. Summary Receiver Operating Characteristic curve (SROC) for WFA-MUC1 and CA19-9.

2a. SROC of serum level of MUC1.

2b. SROC of serum level of CA19-9.

2c. SROC of biliary level of MUC1.

2d. SROC of MUC1 in biliary duct cancer tissue.

Figure 3. Forest plot of hazards ratio evaluating overall survival of patients with resectable CCA.

Figure 4. Funnel plot for overall survival.

Supplementary Figure 1. Sensitivity analysis of studies evaluating the prognostic value of MUC1 in biliary cancer tissue.

Table 1. Characteristics of studies included in this meta-analysis

Author	Year	Country	No. BTC	No. BBD	Mean Age		Male/Female)		Type of BTC				Specimen Source	Association	Study Design
					BTC	BBD	BTC	BBD	iCCA	pCCA	dCCA	GC			
Shoda et al[35]	2017	Japan	303	287	71 (33-101)	68 (19-92)	193/110	153/134	59	117	71	59	Bile,Serum	Diagnosis	P
Yamaguchi et al[34]	2016	Japan	174	27	69 (36-85)	64 (27-82)	108/66	19/8	9	133	18	14	Bile,Serum	Diagnosis	P
Matsuda et al[54]	2015	Thailand (cohort1)	78	78	56±8.25 (57–90)	54 ±10.42 (32–73)	23/55	23/55	CCA				Plasma	Diagnosis	P
Matsuda et al[54]	2015	Japan (cohort2)	33	40	77 ± 8.25 (57–90)	76 ± 9.50 (56–93)	20/13	19/21	28	1	4	0	Serum	Diagnosis	P
Zen et al[53]	2014	UK	28	20	67 (42–83)	61 (38–77)	17/11	15/5	28	0	0	0	Tissue	Diagnosis	R
Esperança et al[6]	2014	Brizal	11	67	NA	NA	NA	NA	CCA				Tissue	Diagnosis	R
Matsuda et al[32]	2013	Japan	29	29	NA	-	NA	-	CCA				Bile,Serum	Diagnosis	P
Higashi et al[7]	2012	Japan	63	-	67.4 (41-85)	-	33/30	-	iCCA				Tissue	Prognosis	R
Huang et al[9]	2010	China	33	32	56.41± 13.14 (32-75)	55.41±13.45 (33-77)	18/15	17/15	iCCA				Tissue	Prognosis, Diagnosis	R
Matsuda et al[31]	2010	Japan	30	38	NA	NA	NA	NA	iCCA				Bile	Diagnosis	P
Park et al[10]	2009	Korea	85	-	63.8 (44-82)	-	58/27	-	34	51	0	0	Tissue	Prognosis	R
Boonla et al[12]	2005	Thailand	87	-	56.7±8.6 (36-73)	-	59/28	-	iCCA				Tissue	Prognosis	P
Shibahara et al[13]	2004	Japan	27	-	65.3 (45-79)	-	16/11	-	iCCA				Tissue	Prognosis	R

Matsumura et al[15]	2002	Japan	50	-	60.3±10.3 (30-75)	-	33/17	-	iCCA				Tissue	Prognosis	R
Tamada et al[14]	2002	Japan	60	-	61.9(41-88)	-	41/19	-	CCA				Tissue	prognosis	R
Higashi et al[36]	1999	Japan	39	-	NA	-	-	-	30	0	7	0	Tissue	Prognosis	R
Takao et al[37]	1999	Japan	73	-	65.9(39-85)	-	50/23	-	0	37	36	0	Tissue	Prognosis	R

BTC, biliary tract carcinoma; BBD, benign biliary tract diseases; GC, gallbladder carcinoma; CCA, cholangiocarcinoma; iCCA, intrahepatic cholangiocarcinoma; pCCA perihilar cholangiocarcinoma; dCCA, distal cholangiocarcinoma; P, prospective; R, retrospective; NA, not available

1

Table 2. Characteristics of studies included in the diagnostic meta-analysis

2.1. Characteristics of eligible studies evaluating the diagnostic accuracy of WFA-MUC1 in serum samples

Author	Year	country	Optimal Se	Optimal Sp	Reference Standard	Antibody	AUC	Cut-Off Value	TP	FP	TN	FN
Shoda et al[35]	2017	Japan	0.776	0.780	Histopathology	WFA-MY.1E12	0.873	214.2 uL/ml	235	64	223	68
Matsuda et al[54]	2015	Thailand (cohort 1)	0.722	0.748	Histopathology	WFA-MY.1E12	0.841	NA	56	16	62	22
Matsuda et al[54]	2015	Japan (cohort 2)	0.766	0.643	Histopathology	WFA-MY.1E12	0.738	NA	25	19	21	8

2.2. Characteristics of eligible studies evaluating the diagnostic accuracy of CA19-9 in serum samples

Shoda et al[35]	2017	Japan	0.713	0.711	Histopathology	CA19-9 ELISA Kits	0.753	27.6 U/ml	216	83	204	87
Yamaguchi et al[34]	2016	Japan	0.603	0.889	Histopathology	NA	0.761	38 U/ml	105	3	24	69
Matsuda et al[54]	2015	Thailand (cohort1)	0.743	0.887	Histopathology	CA19-9 ELISA kits	0.849	NA	58	8	70	20
Matsuda et al[54]	2015	Japan (cohort2)	0.637	0.896	Histopathology	CA19-9 ELISA kits	0.759	NA	21	4	36	12

2.3. Characteristics of eligible studies evaluating the diagnostic accuracy of MUC1 in bile samples

Shoda et al[35]	2017	Japan	0.863	0.765	Histopathology	MY.1E12 (mAb)	0.896	13.5 nL/ug	158	27	88	25
Yamaguchi et al[34]	2016	Japan	0.822	0.556	Histopathology	WFA-MY.1E12	0.715	10.5 nl/ug	143	12	15	31
Matsuda et al[32]	2013	Japan	0.90	0.72	Histopathology	WFA-MY.1E12	0.85	7 nl/ug	26	8	21	3
Matsuda et al[31]	2010	Japan	0.90	0.763	Histopathology	WFA-MY.1E12	0.86	6.64 nl/ug	27	9	29	3

2.4. Characteristics of eligible studies evaluating the diagnostic accuracy of MUC1 in tissue samples

Zen et al[53]	2014	UK	0.71	0.85	Histopathology	clone DF3	-	>5% (positive)	20	3	17	8
Esperança et al[6]	2014	Brizal	0.909	0.925	Histopathology	Clone Ma695	-	>10% (positive)	10	5	62	1

2

Huang et al[9]	2010	China	0.545	0.719	Histopathology	Ma689	-	>10%	18	9	23	15

Se, sensitivity; Sp, specificity; AUC, area under the receiver-operating characteristic curve; TP, true positive; FP, false positive; TN, true negative; FN, false negative; NA, not available

Table 3. Characteristics of eligible studies included in prognostic meta-analysis

Author	Year	Country	Type of CCA	No.Patients	Anti-MUC1	Cut- Off (Positive/High Expression)	Follow-Up (Months)	HR For Overall Survival (95%CI)	P Values
Higashi et al[7]	2012	Japan	M-iCCA	63	mAb DF3	> 5% (58)	>50	3.34 (0.43-25.8)	0.168
Huang et al[9]	2010	China	iCCA	33	Clone Ma689	>10% (18)	>20	1.89 (0.79-4.511)*	<0.01
Park et al[10]	2009	Korea	CCA	85	Clone Ma695	>10% (56）	>50	1.211 (0.403-3.640)	0.733
Boonla et al[12]	2005	Thailand	iCCA	87	Clone Ma695	>25% (34)	>15	2.19 (1.11-4.32)	0.026
Shibahara et al[13]	2004	Japan	M-iCCA	27	Mab DF3	>5% (22)	>50	4.536 (0.292–70.336)	0.2797
Matsumura et al[15]	2002	Japan	M-iCCA	50	mAb HMPV	>5% (38)	>50	4.377 (1.517–12.629)	0.0063
Tamada et al[14]	2002	Japan	CCA	60	MAb DF3	>20%(46)	>50	1.57（0.52-4.68)*	<0.05
Higashi et al[36]	1999	Japan	CCA	39	mAb DF3	>5% (23)	>50	1.91 (0.52-7.03)*	<0.05
Takao et al[37]	1999	Japan	CCA	67	Mab DF3	>25% (47)	>50	2.59 (1.19–5.63)	0.016

CCA, cholangiocarcinoma; iCCA, intrahepatic cholangiocarcinoma; M-iCCA, mass-forming intrahepatic cholangiocarcinoma; HR, hazard ratio; * the data was digitized and extracted from the Kaplan–Meier curve using the software designed by Jayne F Tierney and Matthew R Sydes.

Table 4. The Molecular Function (MF), Biological Process (BP) and Reactome Pathways of MUC1 in cancer	
GO MF Complete	p53 binding, transcription coregulator activity, protein binding, RNA polymerase II proximal promoter sequence-specific DNA binding
GO BP Complete	DNA damage response, signal transduction by p53 class mediator resulting in transcription of p21 class mediator, negative regulation of cell adhesion mediated by integrin, positive regulation of transcription from RNA polymerase II promoter in response to stress, DNA damage response, signal transduction by p53 class mediator resulting in cell cycle arrest, negative regulation of transcription by competitive promoter binding, regulation of transcription from RNA polymerase II promoter in response to stress, cytokine-mediated signaling pathway, negative regulation of intrinsic apoptotic signaling pathway in response to DNA damage by p53 class mediator, O-glycan processing, positive regulation of histone H4 acetylation, stimulatory C-type lectin receptor signaling pathway
Reactome Pathways	O-linked glycosylation of mucins, Metabolism of proteins, O-linked glycosylation, Defective C1GALT1C1 causes Tn polyagglutination syndrome (TNPS), Diseases of glycosylation, Termination of O-glycan biosynthesis, Defective GALNT3 causes familial hyperphosphatemic tumoral calcinosis (HFTC), Defective GALNT12 causes colorectal cancer 1 (CRCS1), Post-translational protein modification, Disease, Diseases associated with O-glycosylation of proteins

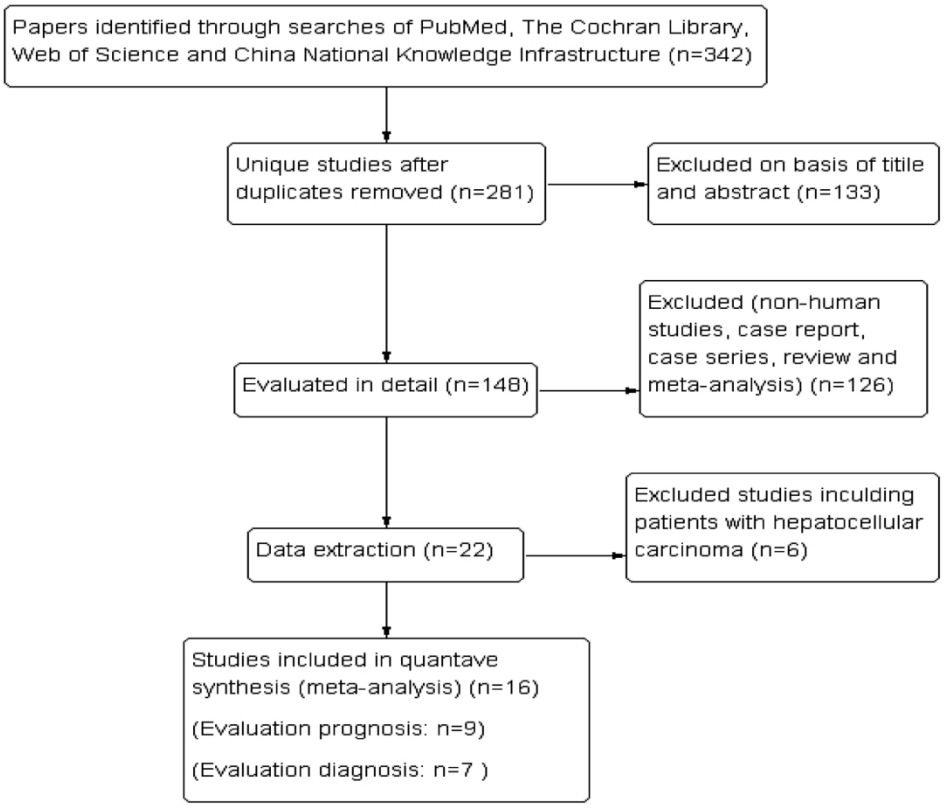


Figure 1. Search flow diagram.

169x144mm (300 x 300 DPI)

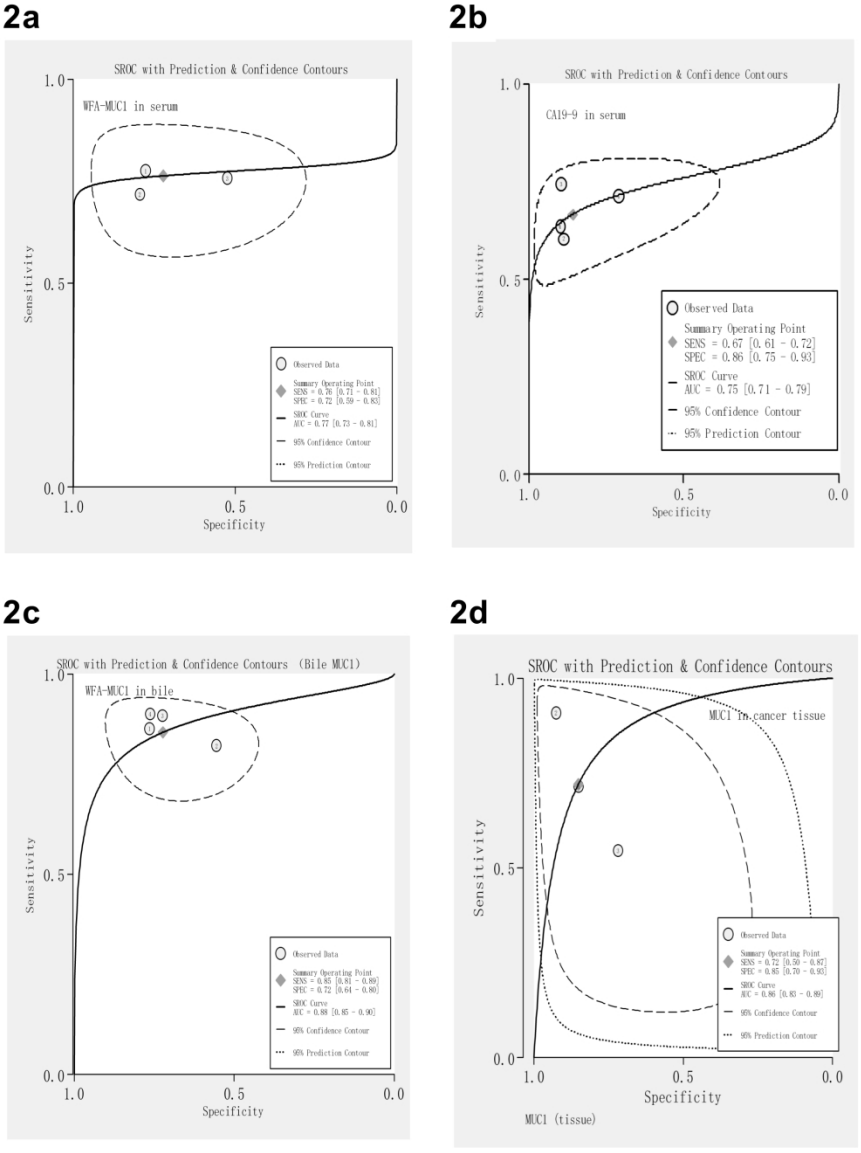


Figure 2. Summary Receiver operating characteristic curve (SROC) of WFA-MUC1 and that of CA19-9.

195x258mm (300 x 300 DPI)

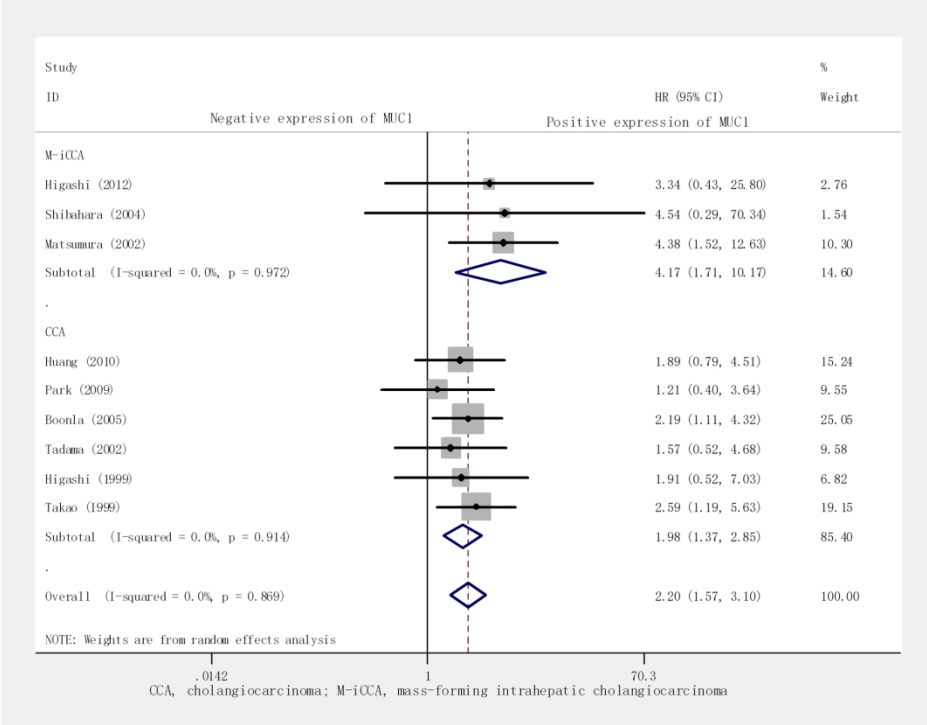


Figure 3. Forest plot of hazards ratio evaluating overall survival of patients with resectable CCA.

194x152mm (300 x 300 DPI)

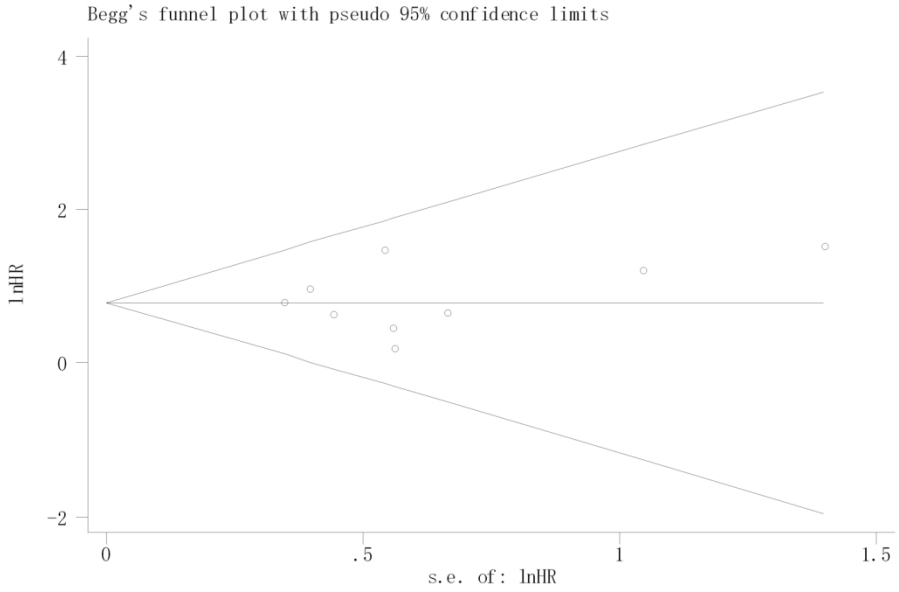
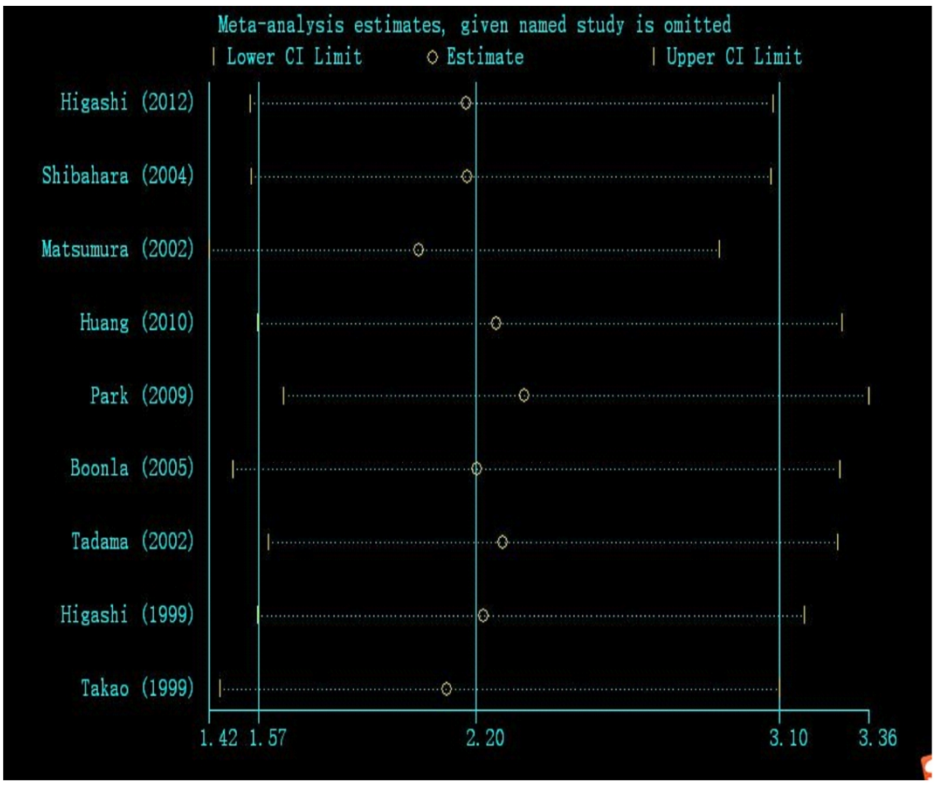


Figure 4. Begg's funnel plot for overall survival.

155x106mm (300 x 300 DPI)



Supplementary Figure 1. Sensitivity analysis of studies evaluating the prognostic value of MUC1 in biliary cancer tissue.

198x180mm (300 x 300 DPI)

Supplementary Table 1. Assessment of risk of bias for studies evaluating the prognostic value of MUC1

Author (Year)	Were adequate eligibility criteria developed and applied	Was the measurement of both exposure and outcome adequate?	Was confounding adequately controlled for?	Was the follow-up complete and adequate in duration?	Are reports of the study free of suggestion of selective outcome reporting?	Was the study free of other problems that put it at a high risk of bias?	Risk of bias
Higashi (2012)	YES	YES	YES	Unclear	YES	YES	Low
Huang (2010)	YES	YES	NO	YES	YES	YES	Low
Park (2009)	YES	YES	YES	YES	YES	YES	Low
Boonla(2005	YES	YES	NO	YES	YES	YES	High
Shibahara (2004)	YES	YES	YES	YES	YES	YES	Low
Matsumura (2002)	YES	YES	YES	YES	YES	YES	Low
Tamada (2002)	YES	YES	YES	Unclear	YES	YES	Unclear
Higashi (1999)	YES	YES	YES	Unclear	YES	YES	Unclear
Takao(1999)	YES	YES	YES	YES	YES	YES	Low

Supplementary Table 2. Assessment of risk of bias for studies evaluating the diagnostic capability of WFA-MUC1

Author (Year)	Representative spectrum?	Acceptable reference standard?	Acceptable delay between tests?	Partial verification avoided?	Differential verification avoided?	Index test results blinded?	Withdrawals explained?
Shoda (2017)	YES	YES	YES	NO	YES	Unclear	YES
Yamaguchi (2016)	YES	YES	YES	NO	YES	Unclear	YES
Matsuda[+] (2015)	YES	YES	Unclear	NO	YES	YES	YES
Matsuda [ǂ](2015)	YES	YES	Unclear	NO	YES	YES	YES
Zen (2014)	YES	YES	YES	YES	YES	YES	YES
Esperança (2014)	YES	YES	YES	YES	YES	YES	YES
Matsuda (2013)	YES	YES	YES	NO	YES	Unclear	YES
Matsuda (2010)	YES	YES	YES	NO	YES	Unclear	YES
Huang (2010)	YES	YES	YES	YES	YES	YES	YES

[+], Cohort1; [ǂ], cohort2

Items chosen to score from QUADAS checklist

1# **Was the spectrum of patients representative of those who will receive the test in practice?**

2# **Was the reference standard likely to correctly classify patients cholangiocarcinoma?**

3# **Was the time period between reference standard and index test short enough to be reasonably sure that the target condition did not change between the two tests?**

4# **Did the whole sample or a random selection of the sample receive verification using a reference standard?**

5# **Did patients receive the same reference standard regardless of the index test result?**

6# **Were the reference standard results interpreted without knowledge of the results of the index test?**

7# **Were withdrawals from the study explained?**

MOOSE Checklist

Meta-analysis of the diagnostic value of wisteria floribunda agglutinin-sialylated mucin1 and the prognostic role of mucin1 in human cholangiocarcinoma

Criteria		Brief description of how the criteria were handled in the meta-analysis
Reporting of background should include		
√	Problem definition	*Page 6.*
√	Hypothesis statement	*Page 6.*
√	Description of study outcomes	*Page10-14.*
√	Type of exposure or intervention used	*Page 7, 8.*
√	Type of study designs used	*Page 10, 11.*
√	Study population	*Page 7, 8.*
Reporting of search strategy should include		
√	Qualifications of searchers	*Page 8.*
√	Search strategy, including time period included in the synthesis and keywords	*Page 7.*
√	Databases and registries searched	*Page 7.*
√	Search software used, name and version, including special features	We did not employ a search software. EndNote X7(BId 7072) was used to merge retrieved citations and eliminate duplications.
√	Use of hand searching	*Page 7.*
√	List of citations located and those excluded, including justifications	*Page 7, 8, 10.*
√	Method of addressing articles published in languages other than English	*Page 8.*
√	Method of handling abstracts and unpublished studies	*Page 8, 10.*
√	Description of any contact with authors	We did not contact authors. Potentially, data is available just from published studies data.
Reporting of methods should include		
√	Description of relevance or appropriateness of studies assembled for assessing the hypothesis to be tested	*Page 7, 8.*

√	Rationale for the selection and coding of data	*Page 7, 8.*
√	Assessment of confounding	*Page 9, 10.*
√	Assessment of study quality, including blinding of quality assessors; stratification or regression on possible predictors of study results	*Page 9,10.*
√	Assessment of heterogeneity	*Page 10.*
√	Description of statistical methods in sufficient detail to be replicated	*Page 9, 10.*
√	Provision of appropriate tables and graphics	*Page 9-11.*
Reporting of results should include		
√	Graph summarizing individual study estimates and overall estimate	*Page 11-14.*
√	Table giving descriptive information for each study included	*Page 10-12.*
√	Results of sensitivity testing	*Page 13, 14.*
√	Indication of statistical uncertainty of findings	*Page 11-13.*
Reporting of discussion should include		
√	Quantitative assessment of bias	*Page 17, 18.*
√	Justification for exclusion	*Page 18, 19.*
√	Assessment of quality of included studies	*Page 17, 19.*
Reporting of conclusions should include		
√	Consideration of alternative explanations for observed results	*Page 14-17.*
√	Generalization of the conclusions	*See Page 18.*
√	Guidelines for future research	*See Page 18, 19.*
√	Disclosure of funding source	*See Page 2.*

BMJ Open

Meta-analysis of the diagnostic value of wisteria floribunda agglutinin-sialylated mucin1 and the prognostic role of mucin1 in human cholangiocarcinoma

Journal:	*BMJ Open*
Manuscript ID	bmjopen-2018-021693.R2
Article Type:	Research
Date Submitted by the Author:	10-Nov-2018
Complete List of Authors:	Tang, Zengwei; The First Clinical Medical School of Lanzhou University, Department of Special Minimally Invasive Surgery, The first Hospital of Lanzhou University Yang, Yuan; The First clinical medical School of Lanzhou University, Department of Internal Medicne, The First hospital of Lanzhou University Wang, Xiaolu; West China Medical School, Sichuan University, Department of General Surgery, West China Hospital/West China Medical School, Sichuan University Meng, Wenbo; Department of Special Minimally Invasive Surgery, The First Hospital of Lanzhou University Li, Xun; The second department of General Surgery, The First Hospital of Lanzhou University
Primary Subject Heading:	Gastroenterology and hepatology
Secondary Subject Heading:	Gastroenterology and hepatology, Diagnostics, Evidence based practice, Oncology
Keywords:	Cholangiocarcinoma, prognosis, dignosis, Mucin1, meta-analysis

Meta-analysis of the diagnostic value of wisteria floribunda agglutinin-sialylated mucin1 and the prognostic role of mucin1 in human cholangiocarcinoma

Authors and affiliations:

Zengwei Tang [1,2]

Yuan Yang [1]

Xiaolu Wang [3]

Wenbo Meng [1,2]

Xun Li [1,4]

Correspondence:

Professor **Wenbo Meng**

Department of Special Minimally Invasive Surgery, The First Hospital of Lanzhou University, Lanzhou 730000, China.

Tel:+86 139 1917 7177

Fax:+96 0931-8622275

E-mail: mengwb@lzu.edu.cn

[1]The First Clinical Medical School of Lanzhou University, Lanzhou, China.

[2]Department of Special Minimally Invasive Surgery, The First Hospital of Lanzhou University, Lanzhou, China.

[3]Department of General Surgery, West China Hospital/West China Medical School, Sichuan University, Chengdu, China.

[4]The second department of General Surgery, The First Hospital of Lanzhou University, Lanzhou, China.

Keywords: cholangiocarcinoma, prognosis, diagnosis, Mucin1, meta-analysis

Word count: 4,136

Financial support: This study was supported by the West Light Foundation of the Chinese Academy of Science [2015(90)]. The funding sources played no roles in the study design, experiment method development, data collection, organization, analysis and interpretation, nor in the manuscript drafting, reviewing or approval.

Author contributions: Zengwei Tang–study concept and design; development of methodology, collection and extraction of data, statistical analysis and interpretation of data, and drafting of the manuscript; critical revision of the manuscript for significant intellectual content.

Yuan Yang and Xiaolu Wang–development of methodology, collection and extraction of data and statistical analysis.

Wenbo Meng and Xun Li–funding application and study supervision; critical revision of the manuscript for significant intellectual content.

Disclosures: Zengwei Tang, Yuan Yang, Xiaolu Wang, Wenbo Meng and Xun Li have no conflicts of interest to declare.

Patient consent: Consent is not required when conducting a meta-analysis.

Ethics approval: This study did not require ethical approval as the data used have been published previously, and hence are already in the public domain.

Data sharing statement: Extracted data are available upon request to the corresponding author.

Abstract

Objective: Serum carbohydrate antigen 19-9 (CA19-9) is a widely used tumor marker for cholangiocarcinoma (CCA). However, it is not a necessarily good CCA marker in terms of diagnostic

accuracy. The purpose of this study is to evaluate the diagnostic value of wisteria floribunda agglutinin-sialylated Mucin1(WFA-MUC1) and the prognostic role of Mucin1(MUC1) in human CCA.

Design: Meta-analysis.

Data sources: Studies published in PubMed, Web of Science, The Cochrane Library and the China National Knowledge Infrastructure up to 11 Oct 2017.

Eligibility criteria: We included reports assessing the diagnostic capacity of WFA-MUC1 and the prognostic role of MUC1 in CCA. The receiver operating characteristic curve (ROC) of WFA-MUC1 and/or CA19-9 was described and the hazard ratios (HRs) including 95% confidence interval (95%CI) and the corresponding P value for MUC1 can be extracted.

Data extraction and synthesis: Two independent researchers extracted data and assessed risk of bias. The diagnostic sensitivity and specificity data of WFA-MUC1were extracted and analyzed as bivariate data. Pooled hazard ratio (HRs) and its 95%CI for MUC1 were calculated with a random-effects meta-analysis model on overall survival of resectable CCA.

Results: Sixteen reports were included in this study. The pooled sensitivity and specificity of WFA-MUC1 were 0.76 (0.71 to 0.81) and 0.72 (0.59 to 0.83) in serum, 0.85 (0.81 to 0.89) and 0.72 (0.64 to 0.80) in bile, and 0.72 (0.50 to 0.87) and 0.85 (0.70 to 0.93) in tissue, respectively. The summary receiver-operating characteristic curve (SROC) were 0.77 (0.73 to 0.81) in serum, 0.88 (0.85 to 0.90) in bile, and 0.86(0.83-0.89) in tissue respectively. Furthermore, The pooled sensitivity and specificity and the SROC of CA19-9 in serum were 0.67(0.61 to 0.72), 0.86(0.75 to 0.93) and 0.75(0.71 to 0.79) respectively. The pooled HRs for MUC1 was 2.20 (1.57 to 3.01) in CCA, and 4.17 (1.71-10.17) in mass-forming intrahepatic CCA.

Conclusions: Compared to CA19-9, WFA-MUC1 was shown to possess stronger diagnostic capability. MUC1 could serve as a prognosis factor for poor outcomes of CCA, particularly, mass-forming intrahepatic CCA.

Keywords: cholangiocarcinoma, prognosis, diagnosis, Mucin1, meta-analysis

Strengths and limitations of this study

- This meta-analysis evaluated the diagnostic capability of WFA-MUC1 and prognostic role of MUC1 in cholangiocarcinoma.
- The diagnostic capability of WFA-MUC1 is superior to that of CA19-9.
- The diagnostic capability of WFA-MUC1 in bile is better that in serum.
- Expression of MUC1 in biliary duct cancer tissues is a prognosis factor for poor outcomes of resectable cholangiocarcinoma..
- Majority of the subjects included in this meta-analysis were from Asia. More participants from different regions other than Asia are needed to better evaluate the roles of Mucin1 in the diagnosis and prognosis of cholangiocarcinoma worldwide.

Introduction

Cholangiocarcinoma (CCA) is a malignancy arising from epithelia at various anatomic locations in the

biliary tree.[1] The median survival time for patients with unresectable CCA is less than a year.[2, 3] The prognosis is considerably better for patients who undergo radical resection of CCA, with a five year-survival rates ranging from 20% to 40%.[4, 5] However, it is hard to detect CCA at the early stage, even with the advanced imaging technology and the complete diagnosis protocol currently. This situation limits the benefits of surgery therapy and curative treatment options to CCA patients and contributes to the poor outcome of patients with CCA.

Currently, a huge amount of literature reporting numerous molecular biomarkers with limited diagnostic or prognostic capability for CCA have been published. Some of the reported biomarkers have been used for guiding clinical diagnosis and treatment of CCA worldwide, such as Mucin2 to Mucin6,[6-15] carbohydrate antigen 19-9 (CA19-9),[16-18] interleukin6,[19, 20] serum cytokeratin19 fragments[21, 22] and carbohydrate antigen125 (CA125)[16, 23, 24]. Among these biomarkers, CA19-9 in serum has been the focus of related research and always been used as a biomarker for CCA. However, the overall sensitivity and specificity of CA19-9 is not satisfying and CA19-9 is not capable of detecting CCA progression.[5, 17, 24] In addition, although CA19-9 expression is elevated in up to 85% suspected CCA, [17, 25, 26] the capability of CA19-9 as a diagnostic marker is still limited due to influence of co-existing inflammation in biliary tract and the fact that cancer cells from Lewis gene negative subtype of CCA doesn't produce CA19-9 theoretically.[17, 18, 27]

Mucin1 (MUC1), also known as polymorphic epithelial Mucin, is cell surface associated and belongs to Mucin family. It is a mucin encoded by the MUC1 gene in humans[28]. MUC1 is a high molecular weight, membrane-associated glycoprotein with a 69 amino acids cytoplasmic tail, a transmembrane

domain and an extracellular domain consisting of a variable number of highly conserved tandem repeats of 20 amino acids[28, 29]. Highly glycosylated MUC1 has been reported to be associated with malignancies in many other organs.[30] Matsuda et al[31] reported that wisteria floribunda agglutinin-sialylation(WFA) could be employed as the best probe to detect alterations of glycan structure in biliary tract derived cancer cells and distinguish it from normal tissues. They also identified sialylated MUC1 as a potential cholangiocarcinoma-specific glycoprotein marker. From then on, wisteria floribunda agglutinin sialylated-Mucin1(WFA-MUC1) has been regarded as a sensitive molecular biomarker for CCA.[9, 31-35] However, the diagnostic capability of WFA-MUC1 remains unclear since the reported range of WFA-MUC1 distinguishing CCA from benign biliary diseases varied greatly (0.74~0.87 in serum, 0.72~0.90 in bile).[9, 31-35] In addition, although the correlation between the expression of MUC1 in biliary duct derived cancer and the overall survival(OS) rate for patients with resectable CCA has been analyzed with Kaplan-Meier plot in several clinical trials, the result still remains inconclusive. Besides, more questions about MUC1 in CCA still need to be answered such as whether expression of MUC1 suggests a poor prognosis for CCA patients and whether expression level of MUC1 associates with CCA progression.[7, 9, 10, 12-15, 36, 37]

Therefore, we conducted this meta-analysis to evaluate the diagnostic capability of WFA-MUC1 in discriminating CCA patients from benign biliary diseases and to investigate the prognostic role of MUC1 in CCA patients.

Methods

Search strategy

The initial comprehensive literature search through 11 March 2017 was performed in database of PubMed, Web of Science, The Cochrane Library and the China National Knowledge Infrastructure. Our latest search was completed on 11 October 2017. The publication language was restricted to articles published in English or Chinese. Searching keywords used are "Wisteria floribunda agglutinin sialylated-mucin1(WFA-MUC1)" "Mucin1/MUC1", "cholangiocarcinoma/CCA", "cholangiocellular carcinoma", "intrahepatic cholangiocarcinoma", "extrahepatic cholangiocarcinoma" or "Klatskin tumor/hilar cholangiocarcinoma/perihilar cholangiocarcinoma" combined with "prognosis/prognostic/prognoses/survival" or "diagnosis/diagnostic/diagnoses". The reference lists of every study that met the inclusion criteria were also manually reviewed to identify additional relevant publications.

Patient and public involvement

Patients and public were not involved as all the data used have been published previously, and hence are already in the public domain.

Eligibility criteria

Published studies were included if they met the following criteria: (1) the published studies were focused on CCA; (2) all studied subjects with CCA were diagnosed by pathologist postoperatively; (3) the expression of MUC1 in tissues was detected by immunohistochemistry staining and the level of WFA-MUC1 in bile or serum was tested by sandwich enzyme-linked immunosorbent assay(ELISA); (4) the receiver operating characteristic curve (ROC) of WFA-MUC1 and/or CA19-9 was described and the rates of true positives, false positives, false negatives, and true negatives can be calculated; and

(5) the hazard ratios (HRs)including 95% confidence interval (95%CI) and the corresponding P value can be extracted. Studies were excluded based on following criteria: (1) animal studies;(2) review articles, case reports or letters; (3) duplicated publication; (4) non-English or non-Chinese papers; and (5) insufficient data on the HRs or that could not be extracted from Kaplan-Meier analysis result.

Data extraction

Data extraction was carried out by two investigators independently (Zengwei Tang and Yuan Yang). If discrepancies occurred, it would be resolved by the consensus of these two investigators. Data related to the study characteristics were extracted with the following variables: the first author of the study, study design and duration, year of publication,institution, the number of subjects in the study with mean age and gender, the selected antibody for the MUC1 immunochemical staining, ELISA assay kits testing the level of biliary and /or serum WFA-MUC1 and the level of serum CA-19-9, the AUC for WFA-MUC1, the cut-off value of MUC1, assay's sensitivity and specificity, HRs and their 95% CI and case follow-up time. For the three studies that did not provide the value of HRs and their 95%CI, we digitized and extracted the data from the Kaplan–Meier curve in the publications by using the software designed by Jayne F Tierney and Matthew R Sydes.[38] The optimal sensitivity and specificity were reported graphically in one study with two cohorts and were extracted using Plot Digitizer software 2.6.8 (provided by source forge.net, found online at http://plot digitizer source forge.net/) to convert data points on the graphs into numerical data.[39, 40] Repeated data points were isolated using nonparametric bootstrap sampling[41] guided by the descriptive statistics provided in the supporting document. The possible repeated data points were repeatedly sampled until the sets matching the descriptive statistics was found. All the data was extracted from published literature.

.

Quality assessment across studies

Quality assessment of the studies in the prognostic meta-analysis was performed by using the modified risk of bias tool recommended by the Cochrane Collaboration as described previously.[42-44] Quality assessment of studies evaluating the diagnostic capability of WFA-MUC1 was performed using the QUADAS(Quality Assessment of Studies of Diagnostic Accuracy included in Systematic reviews) checklists.[45, 46] However, we did not calculate the summary scores for each study investigating the diagnostic capability of WFA-MUC1 not only because their interpretation was problematic, but also because their report was potentially misleading.[47] Moreover, seven of the best differentiating items have been selected from the QUADAS checklists.

Statistical analyses

The statistical analysis was performed according to the guidelines proposed by the Meta-Analysis of Observational Studies in Epidemiology group (MOOSE).[48] The pooled HRs with 95% CI were calculated with a random-effect model according to the DerSimonian-Laird method to evaluate the correlation between the positive expression of MUC1 and overall survival (OS).[49, 50] Sensitivity and specificity for each study evaluating the diagnostic capability of WFA-MUC1 were calculated and and analyzed this datum as bivariate data according to methods for diagnostic meta-analysis.[51] An aggregated bivariate data meta-analysis with the generation of forest plots and summary receiver-operating characteristic curve (SROC) was performed. Forest plots displayed the diagnostic probabilities of individual studies, the corresponding 95% CI, and squares with area proportional to study weight in the meta-analysis. The SROC demonstrated individual study data point with circles,

with size proportion to study weight and 95% prediction contour and 95% confidence contour around the pooled estimate. The heterogeneity among studies was measured using the Q tests and I^2 statistic to assess the extent of the inconsistency. A probability value of P<0.1 and I^2 >50% indicated the existence of significant heterogeneity.[52] Furthermore, Funnel plot and the Egger's linear regression test were applied to evaluate potential publication bias for eligible studies using OS as an endpoint.[53] A P <0.1 for Egger's test was considered statistically significant. All statistical analyses were performed with Stata/MP 14.0 (StataCorp, Parallel Edition).

Results

Study selection

The study includes results of electronic searches up to 11 October 2017. A total of .341 papers were identified, of which 148 were retrieved for full-text review. Among these 148 publications, 16 studies[6, 7, 9, 10, 12-15, 31, 32, 34-37, 54, 55] were eligible for the meta-analyses according to the inclusion and exclusion criteria. Nine studies[7, 9, 10, 12-15, 36, 37] out of 16 studies used OS as endpoint, and eight studies[6, 9, 31, 32, 34, 35, 54, 55] used the sensitivity and specificity rate as the endpoint (One study reported by Huang et al also provided the data on diagnostic value of MUC1 in tissue). The detailed literature searching process was shown in **Fig1**.

Characteristics of the included studies and participants

Characteristics of eligible studies and their participants were listed in **Table 1, Table 2 and Table 3.** Nine studies[7, 9, 10, 12-15, 36, 37] evaluating the prognostic value of MUC1 for resectable CCA which were conducted in 4 countries (Korea, Japan, China and Thailand), the other seven studies[6, 31, 32, 34, 35, 54, 55] investigating the diagnostic capability of WFA-MUC1were undertaken in 5 countries (Japan, UK,

Brazil, Thailand, China). Retrospective study design was applied to perform the meta-analysis of prognostic value by all selected studies. The seven studies investigating the diagnostic capability of WFA-MUC1, meaning that discriminating CCA from benign biliary diseases, used prospective study design. All CCA diagnosis included in this study were based on histopathology as reported in the include publications. The sample size of eligible studies evaluating the prognostic value of MUC1 varied greatly, ranging from 27 to 87 with a median size of 56. The sample size of studies investigating the diagnostic capability of WFA-MUC1 ranged from 30 to 303 (median=80) and 20 to 287(median=69) for biliary tract carcinoma group and benign biliary diseases group, respectively.

The level of WFA-MUC1 in bile and serum were tested by the approach of ELISA using mAb WFAMY.1E12. The concentration of serum CA19-9 was tested by CA19-9 ELISA kits. The sensitivity, specificity and AUC of each study included in the diagnostic meta-analysis were shown in **Table 2**.

Three studies[9, 14, 36] investigating the prognostic value of MUC1 for CCA provided the Kaplan-Meier curve and we digitized and extracted the data of HRs including their corresponding 95%CI from the curve by using the methods described above. The cut-off value to define positive expression of MUC1(2 trials[12, 37]>25%, one trial[14] >20%, 2 trials[9, 10]>10% and 4 trials[7, 13, 14, 36] >5%), the follow-up time (7 trials[7, 10, 13-15, 36, 37]>50 months, 1 trail[9] >20 months and another one[12]>15 months), and the antibody of MUC1 were selected for immunochemistry (mAb DF3, Clone Mab DF3,Clone Ma695,Clone Ma689 and mAb HMPV) were inconsistent (As shown in **Table 3**).

Primary endpoint: the outcomes of diagnostic meta-analysis

Three trials[35, 55] including 414 cases of biliary tract carcinoma (59 gall bladder carcinomas and 355 CCA) and 405 subjects with benign biliary diseases investigated the diagnostic capability of WFA-MUC1 level in serum. **Fig2a** presented the diagnostic parameters s for serum WFA-MUC1 in a summary receiver operating characteristic graph. The pooled optimal sensitivity (true positive rate) was 0.76(0.71 to 0.81) and specificity (true negative rate) was 0.72(0.59 to 0.83). the AUC of SROC was 0.77(0.73 to 0.81).

As a comparison, three trials[34, 35, 55] with 588 subjects with biliary tract carcinoma (73 subjects with gall bladder carcinoma and 515 CCA) and 432 subjects with benign biliary disease evaluated the diagnostic capability of CA19-9 level in serum. **Fig2b** presented the diagnostic parameters for serum level of CA19-9 in a SROC graph. The pooled optimal sensitivity was 0.67(0.61 to 0.72) and specificity was 0.86(0.75 to 0.93). The AUC under SROC was 0.75(0.71 to 0.79).

Four trials[31, 32, 34, 35] including 209 subjects with benign biliary disease and 416 biliary tract carcinomas (73 gall bladder carcinomas) evaluated the diagnostic capability of biliary level of WFA-MUC1. SROC of biliary WFA-MUC1 was shown **in Fig2c**. The pooled sensitivity was 0.85(0.81 to 0.89) and specificity was 0.72(0.64 to 0.80). The AUC under SROC was 0.88(0.85-0.90). Furthermore, three trials[6, 9, 54] inculding 72 subjects with CCA and 119 benign biliary disease used the positive expression of MUC1 in tissue as a criterium to discriminate CCA from benign biliary disease. The diagnostic parameters of positive expression of MUC1 in biliary duct cancer tissue were shown in **Fig2d**. The

pooled sensitivity was 0.72(0.50 to 0.87) and specificity 0.85(0.70-0.93). The AUC of SROC was 0.86(0.83-0.89).

Secondary endpoint: The outcome of prognostic meta-analysis

Nine studies[7, 9, 10, 12-15, 36, 37] with a total of 511 individuals diagnosed with CCA were eligible for the pooled analysis of OS. As shown in the **Fig3**, the overall pooled HRs of MUC1 was 2.20 (1.57 to 3.01). No heterogeneity among these studies was found ($\mathbf{I^2}$=0; ***P***=0.869). Subgroup analyses stratified by the histopathological morphology of CCA reveal that the pooled HRs of mass-forming intrahepatic CCA was 4.17(1.71 to 10.17). The pooled HRs of CCA was 1.98(1.37 to 2.85).

Risk of bias within studies

Detailed results of the risk of bias assessment for included studies in prognostic meta-analysis were shown in **Supplementary Table 1.** Except one study[12] showed a high risk of bias, six showed[7, 9, 10, 13, 15, 37] a low risk of bias and two[14, 36] were shown with the unclear risk of bias. Moreover, as demonstrated in **Fig4**, the result of funnel plots of OS showed no clear indication of publication bias (Egger's test, ***P***=0.661). Selection bias of diagnostic analyses may be caused by two trials including 73 subjects diagnosed with gall bladder carcinoma.[34, 35] Detailed items selected for quality assessment of studies included in diagnostic meta-analysis was shown in **supplementary Table 2**.

Additional analysis

Studies conducted by several research groups have concluded that the patients with mass-forming intrahepatic CCA or periductal infiltrating CCA had a worse prognosis than patients with other types of CCA regarding the OS. These types of CCA have higher rates of recurrence after resection.[56, 57] In our meta-analysis for prognosis, subgroup analysis stratified by the histopathological morphology of CCA was conducted to reduce the inconsistency caused by the type of CCA. We found that the OS for patients with positive expression of MUC1 was significantly shorter than that of MUC1 negative group. The overall pooled HRs=2.20. For subjects with mass-forming intrahepatic CCA, HRs=4.17. In addition, a sensitivity analysis was performed to investigate the stability of the pooled HRs. As shown in **Supplementary Figure1**, the results of pooled HRs were not affected significantly by each individual study.

Molecular Function (MF), Biological Process (BP) and Reactome Pathways of MUC1 in cancer

we searched the GO classification system (http://www.pantherdb.org/) to found the Molecular Function, Biological Process and Reactome Pathways of MUC1 in cancer, the search results was summarized in **Table 4**.

DISCUSSION

As we all known, serum CA19-9 has been widely used as a tumor marker for CCA. However, it's diagnostic accuracy is limited since the serum level of CA19-9 can be strongly influenced by the co-existing inflammatory conditions of the biliary tract and this antigen could not be detected in Lewis gene negative individuals[16, 18]. The most commonly performed diagnostic method for CCA is biliary cytology which tests the bile sample from a biliary drainage catheter. But the sensitivity of biliary cytology is extremely low (20.7 ± 3.5%) as reported in published study[58]. In our meta-analysis of the

diagnostic capability of markers for CCA, seven prospective trials[12, 31, 32, 34, 35, 55] [and a retrospective study[9] were eligible for diagnostic analysis which showed that the diagnostic capability of CA19-9 was inferior to other molecules, such as WFA-MUC1.

In the meta-analysis for diagnosis, the diagnostic value of WFA-MUC1 in serum, bile and biliary duct cancer tissue was evaluated stratified by subgroups of CCA. Two studies[35, 55] with 3 trials (studies reported by Matsuda et.al[55] included two cohorts) assessed the diagnostic accuracy of WFA-MUC1 level in serum, the pooled sensitivity of WFA-MUC1 was 0.76 (0.71 to 0.81). The specificity was 0.72 (0.59 to 0.83), and the AUC of SROC was 0.77 (0.73 to 0.81). While in three studies[34, 35, 55] with four trials assessing the diagnostic accuracy of CA19-9 level in serum, the pooled sensitivity of CA19-9 was 0.67 (0.61 to 0.72), the specificity was 0.86 (0.75 to 0.93) and the AUC of SROC was 0.75 (0.71 to 0.79), which means it would bring a severe error into clinical diagnosis.

The diagnostic capability of serum WFA-MUC1 was superior to that of CA19-9 (as the data showed, $AUC_{WFA\text{-}MUC1}$ vs. $AUC_{CA19\text{-}9}$: 0.77(0.73 to 0.81) vs. 0.75(0.71 to 0.79)). The sensitivity rate of WFA-MUC1 was higher than that of CA19-9 ((0.76(0.71-0.81) vs. 0.67(0.61-0.72)), nevertheless, the specificity rate of serum WFA-MUC1 was less than that of CA19-9 ((0.72 (0.59 to 0.83) VS 0.86 (0.75 to 0.93)). In order to discriminate CCA from benign biliary disease, the combination of these two biomarkers may be applied to improve the diagnostic capability of WFA-MUC1 or CA19-9, as reported by previously published trials.

In the four prospective studies[31, 32, 34, 35] with 343 CAA and 73 gall bladder carcinomas and 209 benign

biliary diseases, the diagnostic accuracy of WFA-MUC1 in bile was also assessed. The pooled sensitivity of WFA-MUC1 testing was 0.85 (0.81 to 0.89) and specificity was 0.72 (0.64 to 0.80) and AUC of SROC was 0.86 (0.83-0.89). The diagnostic capability of bile WFA-MUC1 was better than that of serum WFA-MUC1 ($AUC_{MUC1\ in\ bile}$ vs $AUC_{MUC1\ in\ serum}$: 0.86 (0.83 -0.89) vs 0.77(0.73 to 0.81)), which is consistent with the concept that for most of diseases, the diagnostic molecule levels are different between locally and systemically.

As described above, the level of WFA-MUC1 has significantly higher diagnosis accuracy than CA19-9. Furthermore, the diagnostic accuracy of biliary WFA-MUC1 level was better than that in serum. Therefore, the diagnostic capability of the combined serum CA19-9 and biliary WFA-MUC1 would be better than that of the combination of serum CA19-9 and serum WFA-MUC1 level in discriminating CCA from the benign biliary disease. Such combined measurement would represent a superior diagnostic test for the detection of CCA in daily clinical practice. Unfortunately, as one study[55] included in the diagnostic meta-analyses did not provided the detailed cut-off value of serum WFA-MUC1 level, nor CA19-9 level, the optimal cut-off value of SROC cannot be estimated by this meta-analysis.

It has been demonstrated that MUC1 expression in various human tumors is related to invasive tumor progression and a poor patient outcome.[10, 36, 59, 60] In the prognostic meta-analysis, pooled analysis of nine retrospective studies[7, 9, 10, 12-15, 36, 37] has shown that positive MUC1 expressed of tissue was a poor prognosis factor for resectable CCA (the pooled HRs was 2.20, 95%CI: 1.57 to 3.01), especially for patients with mass-forming intrahepatic CCA (the pooled HRs was 4.17, 95%CI:1.71-10.17), which

was demonstrated by the subgroup analysis stratified by the morphology of CCA.

It has been reported by publications that around 50-60% of CCA are identified as perihilar CCA, up to 20% of CCA are distal, 5% of tumors are multifocal and up to 20% of all CCA are intrahepatic.[5, 61] Different type of CCA demonstrates various epidemiological, morphological and clinical features. A previous meta-analysis[43] identified several prognostic biomarkers (EGFR, MUC1, MUC4, and p27) for resectable CCA, with a small number of subjects in the subgroup of evaluating the prognostic role of MUC1 (Four studies including 265 subjects with resectable CCA were included in the analysis evaluating the prognostic value of MUC1 expression in tissue). The sample size of the prognostic meta-analysis in our study was doubled (9 studies including 511 patients with resectable CCA) and our study provided more explicit description and analysis. Subgroup analysis and sensitivity analysis were conducted to get more reliable results. The pooled HRs result in our study showed that overexpression of MUC1 in tissue was a poor prognostic index for resectable CCA, in particular for patients with mass-forming intrahepatic CCA.

Predictive biomarkers could serve as the key point for personalized cancer treatments such as verifying the chemosensitivity of CCA and developing vaccines to CCA. Up to now, VEGFR, EGFR, HER2, MEK, and BRAF have been the focus for the studies evaluating molecular targeting therapies for CCA.[62] Along with the better understanding of the pathogenesis of CCA mediated by MUC1, MUC1 may become a new focus of targeted therapies for CCA.

The strength and limitation of this study

This meta-analysis, to our best knowledge, is the first study to evaluate the diagnostic value of WFA-MUC1 and prognostic role of MUC1 for human CCA; We obtained data about the prognostic and /or diagnostic capability of WFA-MUC1/MUC1 for CCA from16 trials, which were identified by systematically searching four databases; All subjects with CCA included in this study were diagnosed by pathologist postoperatively. To avoid the possible bias brought by including studies only with reported HRs which may affect the significance of the statistical analysis, we digitized and extracted the HR data from Kaplan-Meier curves in three studies[9, 14, 36] In addition, sensitivity analysis and subgroup analysis which was stratified by the morphology of CCA, made our results of the pooled HRs more stable. To analyze the diagnostic capability of WFA-MUC1, we separately assessed the diagnostic accuracy of WFA-MUC1 level in serum, in bile and in tissue. A comparison of diagnostic accuracy between WFA-MUC1 level and CA19-9 level in serum, as well as a comparison of diagnostic accuracy between the WFA-MUC1 level in serum and in bile were also conducted in our study, as previously clinical trials described.

While our present study could provide a great amount of useful information, limitations of our study should be keep in mind. Firstly, majority of the subjects included in this meta-analysis were from Asian hospitals (data on prognostic meta-analysis were retrieved from Japan, China, Korea and Thailand; data on diagnostic meta-analysis were from Japan, Thailand, China, Brazil and the UK). There may be biological differences in terms of tumor behaviors among populations from different regions worldwide. The phenomenon has been reported on the mortality of stomach cancer between eastern countries and western countries.[63] Secondly, four different cut-off values of positive MUC1 immunostaining (> 5% of carcinoma cells stained was defined as the cut-off point by four studies, >10% defined by 2 studies, >20% identified by one study and >25% defined by another 2 studies) and

four different MUC1 antibodies (mAb DF3, Clone Ma689, Clone Ma695 and mAb HMPV) were used in the nine included studies in our prognostic meta-analysis. Lack of consistency on cut-off value and the type of MUC1 antibody used resulted in considerable heterogeneity. Thirdly, in the diagnostic meta-analysis, although majority of subjects in the biliary tract carcinoma group were diagnosed with CCA, a total of 73 subjects with gall bladder carcinomas were included in this group to evaluate the diagnostic capability of biliary level of MUC1 and serum level of CA19-9. 59 patients with gall bladder carcinomas were included in the evaluation of the diagnostic capability of serum level of WFA-MUC1. The heterogeneity caused by the inconsistency of participants cannot be underestimated since WFA-MUC1 can serve as an independent predictor of hepatocellular carcinoma recurrence.[64] It may be useful for discriminating gall bladder carcinoma from benign gall bladder disease. Fourthly, given that only seven trials with a small number of patients were eligible for the diagnostic meta-analysis and two of them did not provide the cut-off value of WFA-MUC1 and CA19-9 in serum, we cannot give an estimated optimal cut-off value for WFA-MUC1 level in serum. Finally, all data in our study was retrieved from subjects with resectable CCA or gall bladder carcinoma, there may be some difference in the pathogenesis between resectable and unresectable CCA.

Conclusions

This paper highlighted the importance of WFA-MUC1. It has a better diagnostic capability than CA19-9, and the diagnostic capability of the biliary level of WFA-MUC1 was superior to that in the serum. Furthermore, MUC1 could served as a prognosis factor for poor outcomes of resectable CCA, particularly in mass-forming intrahepatic CCA.

Larger, multi-center studies are still needed for better understanding of the molecular pathogenesis of CCA, developing combined kits to conveniently test the serum/ biliary level of MUC1 and serum level of CA19-9 in routine clinical practice, providing an optimal cut-off value of WFA-MUC1 with higher diagnostic accuracy for CCA and benefiting the populations from different regions worldwide.

References

1. Razumilava N, Gores GJ. Cholangiocarcinoma. *Lancet* 2014; 383(9935):2168-79.
2. Eckel F, Schmid RM. Chemotherapy in advanced biliary tract carcinoma: a pooled analysis of clinical trials. *Br J Cancer* 2007; 96(6):896-902.
3. Park J, Kim MH, Kim KP, et al. Natural History and Prognostic Factors of Advanced Cholangiocarcinoma without Surgery, Chemotherapy, or Radiotherapy: A Large-Scale Observational Study. *Gut Liver* 2009; 3(4):298-305.
4. Tang Z, Yang Y, Zhao Z, et al. The clinicopathological factors associated with prognosis of patients with resectable perihilar cholangiocarcinoma: A systematic review and meta-analysis. Medicine, 2018, 97(34):e11999.
5. Khan SA, Davidson BR, Goldin RD, et al. Guidelines for the diagnosis and treatment of cholangiocarcinoma: an update. *Gut* 2012; 61(12):1657-69.
6. Esperança ABT, Camacho AHDS, Monteiro JBM, et al. Mucins and NCAM (CD56) in intrahepatic cholangiocarcinogenesis. *Jornal Brasileiro De Patologia E Medicina Laboratorial* 2014; 50(3):216-220.
7. Higashi M, Yamada N, Yokoyama S, et al. Pathobiological implications of MUC16/CA125 expression in intrahepatic cholangiocarcinoma-mass forming type. *Pathobiology* 2012; 79(2):101-6.
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(15):3393-403.
9. Huang F, Zhou QB, Chen RF. Expression and significance of MUC1 in hepatolithiasis associated with intrahepatic cholangiocarcinoma. *Chinese Archives of General Surgery* 2010; 4(5):424-7.
10. Park SY, Roh SJ, Kim YN, et al. Expression of MUC1, MUC2, MUC5AC and MUC6 in cholangiocarcinoma: prognostic impact. *Oncol Rep* 2009; 22(3):649-57.
11. 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(10):1675-81.
12. Boonla C, Sripa B, Thuwajit P, et al. MUC1 and MUC5AC mucin expression in liver fluke-associated intrahepatic cholangiocarcinoma. *World J Gastroenterol* 2005; 11(32):4939-46.
13. Shibahara H, Tamada S, Higashi M, et al. MUC4 is a novel prognostic factor of intrahepatic cholangiocarcinoma-mass forming type. *Hepatology* 2004; 39(1):220-9.
14. Tamada S, Goto M, Nomoto M, et al. Expression of MUC1 and MUC2 mucins in extrahepatic bile duct carcinomas: its relationship with tumor progression and prognosis. *Pathol Int* 2002; 52(11):713-23.
15. Matsumura N, Yamamoto M, Aruga A, et al. Correlation between expression of MUC1 core protein

and outcome after surgery in mass-forming intrahepatic cholangiocarcinoma. *Cancer* 2002; 94(6):1770-6.

16. Chen CY, Shiesh SC, Tsao HC, et al. The assessment of biliary CA 125, CA 19-9 and CEA in diagnosing cholangiocarcinoma--the influence of sampling time and hepatolithiasis. *Hepatogastroenterology* 2002; 49(45):616-20.
17. Patel AH, Harnois DM, Klee GG, et al. The utility of CA 19-9 in the diagnoses of cholangiocarcinoma in patients without primary sclerosing cholangitis. *Am J Gastroenterol* 2000; 95(1):204-7.
18. Narimatsu H, Iwasaki H, Nakayama F, et al. Lewis and secretor gene dosages affect CA19-9 and DU-PAN-2 serum levels in normal individuals and colorectal cancer patients. *Cancer Res* 1998; 58(3):512-8.
19. Xu H, Inagaki Y, Tang W, et al. Elevation of serum KL-6 mucin levels in patients with cholangiocarcinoma. *Hepatogastroenterology* 2008; 55(88):2000-4.
20. Cheon YK, Cho YD, Moon JH, et al. Diagnostic utility of interleukin-6 (IL-6) for primary bile duct cancer and changes in serum IL-6 levels following photodynamic therapy. *Am J Gastroenterol* 2007; 102(10):2164-70.
21. Lumachi F, Lo Re G, Tozzoli R, et al. Measurement of serum carcinoembryonic antigen, carbohydrate antigen 19-9, cytokeratin-19 fragment and matrix metalloproteinase-7 for detecting cholangiocarcinoma: a preliminary case-control study. *Anticancer Res* 2014; 34(11):6663-7.
22. Uenishi T, Yamazaki O, Tanaka H, et al. Serum cytokeratin 19 fragment (CYFRA21-1) as a prognostic factor in intrahepatic cholangiocarcinoma. *Ann Surg Oncol* 2008; 15(2):583-9.
23. Valle J, Wasan H, Palmer DH, et al. Cisplatin plus gemcitabine versus gemcitabine for biliary tract cancer. *N Engl J Med* 2010; 362(14):1273-81.
24. Abbas G, Lindor KD. Cholangiocarcinoma in primary sclerosing cholangitis. *J Gastrointest Cancer* 2009; 40(1-2):19-25.
25. Hultcrantz R, Olsson R, Danielsson A, et al. A 3-year prospective study on serum tumor markers used for detecting cholangiocarcinoma in patients with primary sclerosing cholangitis. *J Hepatol* 1999; 30(4):669-73.
26. Gores GJ. Early detection and treatment of cholangiocarcinoma. *Liver Transpl* 2000; 6(6 Suppl 2):S30-4.
27. Hamada E, Taniguchi T, Baba S, et al. Investigation of unexpected serum CA19-9 elevation in Lewis-negative cancer patients. *Ann Clin Biochem* 2012; 49(Pt 3):266-72.
28. Gendler SJ, Lancaster CA, Taylor-Papadimitriou J, et al. Molecular cloning and expression of human tumor-associated polymorphic epithelial mucin. *J Biol Chem* 1990; 265(25):15286-93.
29. Lan MS, Batra SK, Qi WN, et al. Cloning and sequencing of a human pancreatic tumor mucin cDNA. *J Biol Chem* 1990; 265(25):15294-9.
30. Brockhausen I. Pathways of O-glycan biosynthesis in cancer cells. *Biochim Biophys Acta* 1999; 1473(1):67-95.
31. Matsuda A, Kuno A, Kawamoto T, et al. Wisteria floribunda agglutinin-positive mucin 1 is a sensitive biliary marker for human cholangiocarcinoma. *Hepatology* 2010; 52(1):174-82.
32. Matsuda A, Kuno A, Matsuzaki H, et al. Glycoproteomics-based cancer marker discovery adopting dual enrichment with Wisteria floribunda agglutinin for high specific glyco-diagnosis of cholangiocarcinoma. *J Proteomics* 2013; 85:1-11.
33. Xu F, Liu F, Zhao H, et al. Prognostic Significance of Mucin Antigen MUC1 in Various Human Epithelial Cancers: A Meta-Analysis. *Medicine (Baltimore)* 2015; 94(50):e2286.

34. Yamaguchi T, Yokoyama Y, Ebata T, et al. Verification of WFA-Sialylated MUC1 as a Sensitive Biliary Biomarker for Human Biliary Tract Cancer. *Ann Surg Oncol* 2016; 23(2):671-7.
35. Shoda J, Matsuda A, Shida T, et al. Wisteria floribunda agglutinin-sialylated mucin core polypeptide 1 is a sensitive biomarker for biliary tract carcinoma and intrahepatic cholangiocarcinoma: a multicenter study. *J Gastroenterol* 2017; 52(2):218-228.
36. 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(6):1347-55.
37. Takao S, Uchikura K, Yonezawa S, et al. Mucin core protein expression in extrahepatic bile duct carcinoma is associated with metastases to the liver and poor prognosis. *Cancer* 1999; 86(10):1966-75.
38. Tierney JF, Stewart LA, Ghersi D, et al. Practical methods for incorporating summary time-to-event data into meta-analysis. *Trials* 2007; 8:16.
39. Kelsey TW, Anderson RA, Wright P, et al. Data-driven assessment of the human ovarian reserve. *Mol Hum Reprod* 2012; 18(2):79-87.
40. Iliodromiti S, Kelsey TW, Anderson RA, et al. Can anti-Mullerian hormone predict the diagnosis of polycystic ovary syndrome? A systematic review and meta-analysis of extracted data. *J Clin Endocrinol Metab* 2013; 98(8):3332-40.
41. Ciaccio AD. Bootstrap and Nonparametric Predictors to Impute Missing Data, 2011.
42. Rahbari NN, Aigner M, Thorlund K, et al. Meta-analysis shows that detection of circulating tumor cells indicates poor prognosis in patients with colorectal cancer. *Gastroenterology* 2010; 138(5):1714-26.
43. Ruys AT, Groot Koerkamp B, Wiggers JK, et al. Prognostic biomarkers in patients with resected cholangiocarcinoma: a systematic review and meta-analysis. *Ann Surg Oncol* 2014; 21(2):487-500.
44. Higgins JP, Green S. Cochrane Handbook For Systematic Reviews Of Interventions [internet]. *Naunyn-Schmiedebergs Archiv für experimentelle Pathologie und Pharmakologie* 2009; 2011(14):S38.
45. 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.
46. van Rheenen PF, Van de Vijver E, Fidler V. Faecal calprotectin for screening of patients with suspected inflammatory bowel disease: diagnostic meta-analysis. *BMJ* 2010; 341:c3369.
47. Whiting P, Harbord R, Kleijnen J. No role for quality scores in systematic reviews of diagnostic accuracy studies. *BMC Med Res Methodol* 2005; 5:19.
48. Stroup DF, Berlin JA, Morton SC, et al. Meta-analysis of observational studies in epidemiology: a proposal for reporting. Meta-analysis Of Observational Studies in Epidemiology (MOOSE) group. *JAMA* 2000; 283(15):2008-12.
49. DerSimonian R, Laird N. Meta-analysis in clinical trials revisited. *Contemp Clin Trials* 2015; 45(Pt A):139-45.
50. Xie S, Wang K, Xu H, et al. PRISMA-Extracapsular Dissection Versus Superficial Parotidectomy in Treatment of Benign Parotid Tumors: Evidence From 3194 Patients. *Medicine (Baltimore)* 2015; 94(34):e1237.
51. Harbord RM, Deeks JJ, Egger M, et al. A unification of models for meta-analysis of diagnostic accuracy studies. *Biostatistics* 2007; 8(2):239-51.
52. Higgins JP, Thompson SG, Deeks JJ, et al. Measuring inconsistency in meta-analyses. *BMJ* 2003; 327(7414):557-60.
53. Egger M, Davey Smith G, Schneider M, et al. Bias in meta-analysis detected by a simple, graphical test.

Bmj 1997; 315(7109):629-34.

54. Zen Y, Britton D, Mitra V, et al. Tubulin beta-III: a novel immunohistochemical marker for intrahepatic peripheral cholangiocarcinoma. *Histopathology* 2014; 65(6):784-92.
55. Matsuda A, Kuno A, Nakagawa T, et al. Lectin Microarray-Based Sero-Biomarker Verification Targeting Aberrant O-Linked Glycosylation on Mucin 1. *Anal Chem* 2015; 87(14):7274-81.
56. Yamamoto Y, Shimada K, Sakamoto Y, et al. Clinicopathological characteristics of intrahepatic cholangiocellular carcinoma presenting intrahepatic bile duct growth. *J Surg Oncol* 2009; 99(3):161-5.
57. Shimada K, Sano T, Sakamoto Y, et al. Surgical outcomes of the mass-forming plus periductal infiltrating types of intrahepatic cholangiocarcinoma: a comparative study with the typical mass-forming type of intrahepatic cholangiocarcinoma. *World J Surg* 2007; 31(10):2016-22.
58. Tsuchiya T, Yokoyama Y, Ebata T, et al. Randomized controlled trial on timing and number of sampling for bile aspiration cytology. *J Hepatobiliary Pancreat Sci* 2014; 21(6):433-8.
59. Yonezawa S, Goto M, Yamada N, et al. Expression profiles of MUC1, MUC2, and MUC4 mucins in human neoplasms and their relationship with biological behavior. *Proteomics* 2008; 8(16):3329-41.
60. Utsunomiya T, Yonezawa S, Sakamoto H, et al. Expression of MUC1 and MUC2 mucins in gastric carcinomas: its relationship with the prognosis of the patients. *Clin Cancer Res* 1998; 4(11):2605-14.
61. Khan SA, Emadossadaty S, Ladep NG, et al. Rising trends in cholangiocarcinoma: is the ICD classification system misleading us? *J Hepatol* 2012; 56(4):848-54.
62. Zhu AX, Hezel AF. Development of molecularly targeted therapies in biliary tract cancers: reassessing the challenges and opportunities. *Hepatology* 2011; 53(2):695-704.
63. Strong VE, Song KY, Park CH, et al. Comparison of gastric cancer survival following R0 resection in the United States and Korea using an internationally validated nomogram. *Ann Surg* 2010; 251(4):640-6.
64. Tamaki N, Kuno A, Matsuda A, et al. Serum Wisteria Floribunda Agglutinin-Positive Sialylated Mucin 1 as a Marker of Progenitor/Biliary Features in Hepatocellular Carcinoma. *Sci Rep* 2017; 7(1):244.

Figure legends:

Figure 1. Diagram showing the literature searching work flow.

Figure 2. Summary Receiver Operating Characteristic curve (SROC) for WFA-MUC1 and CA19-9.

2a. SROC of serum level of MUC1.

2b. SROC of serum level of CA19-9.

2c. SROC of biliary level of MUC1.

2d. SROC of MUC1 in biliary duct cancer tissue.

Figure 3. Forest plot of hazards ratio evaluating overall survival of patients with resectable CCA.

Figure 4. Funnel plot for overall survival.

Supplementary Figure 1. Sensitivity analysis of studies evaluating the prognostic value of MUC1 in biliary cancer tissue.

Table 1. Characteristics of studies included in this meta-analysis

Author	Year	Country	No. BTC	No. BBD	Mean Age		Male/Female)		Type of BTC				Specimen Source	Association	Study Design
					BTC	BBD	BTC	BBD	iCCA	pCCA	dCCA	GC			
Shoda et al[35]	2017	Japan	303	287	71 (33-101)	68 (19-92)	193/110	153/134	59	117	71	59	Bile,Serum	Diagnosis	P
Yamaguchi et al[34]	2016	Japan	174	27	69 (36-85)	64 (27-82)	108/66	19/8	9	133	18	14	Bile,Serum	Diagnosis	P
Matsuda et al[55]	2015	Thailand (cohort1)	78	78	56±8.25 (57–90)	54 ±10.42 (32–73)	23/55	23/55	CCA				Plasma	Diagnosis	P
Matsuda et al[55]	2015	Japan (cohort2)	33	40	77 ± 8.25 (57–90)	76 ± 9.50 (56–93)	20/13	19/21	28	1	4	0	Serum	Diagnosis	P
Zen et al[54]	2014	UK	28	20	67 (42–83)	61 (38–77)	17/11	15/5	28	0	0	0	Tissue	Diagnosis	R
Esperança et al[6]	2014	Brizal	11	67	NA	NA	NA	NA	CCA				Tissue	Diagnosis	R
Matsuda et al[32]	2013	Japan	29	29	NA	-	NA	-	CCA				Bile,Serum	Diagnosis	P
Higashi et al[7]	2012	Japan	63	-	67.4 (41-85)	-	33/30	-	iCCA				Tissue	Prognosis	R
Huang et al[9]	2010	China	33	32	56.41± 13.14 (32-75)	55.41±13.45 (33-77)	18/15	17/15	iCCA				Tissue	Prognosis, Diagnosis	R
Matsuda et al[31]	2010	Japan	30	38	NA	NA	NA	NA	iCCA				Bile	Diagnosis	P
Park et al[10]	2009	Korea	85	-	63.8 (44-82)	-	58/27	-	34	51	0	0	Tissue	Prognosis	R
Boonla et al[12]	2005	Thailand	87	-	56.7±8.6 (36-73)	-	59/28	-	iCCA				Tissue	Prognosis	P
Shibahara et al[13]	2004	Japan	27	-	65.3 (45-79)	-	16/11	-	iCCA				Tissue	Prognosis	R

Matsumura et al[15]	2002	Japan	50	-	60.3±10.3 (30-75)	-	33/17	-	iCCA				Tissue	Prognosis	R
Tamada et al[14]	2002	Japan	60	-	61.9(41-88)	-	41/19	-	CCA				Tissue	prognosis	R
Higashi et al[36]	1999	Japan	39	-	NA	-	-	-	30	0	7	0	Tissue	Prognosis	R
Takao et al[37]	1999	Japan	73	-	65.9(39-85)	-	50/23	-	0	37	36	0	Tissue	Prognosis	R

BTC, biliary tract carcinoma; BBD, benign biliary tract diseases; GC, gallbladder carcinoma; CCA, cholangiocarcinoma; iCCA, intrahepatic cholangiocarcinoma; pCCA perihilar cholangiocarcinoma; dCCA, distal cholangiocarcinoma; P, prospective; R, retrospective; NA, not available

Table 2. Characteristics of studies included in the diagnostic meta-analysis

Author	Year	country	Optimal Se	Optimal Sp	Reference Standard	Antibody	AUC	Cut-Off Value	TP	FP	TN	FN
2.1. Characteristics of eligible studies evaluating the diagnostic accuracy of WFA-MUC1 in serum samples												
Shoda et al[35]	2017	Japan	0.776	0.780	Histopathology	WFA-MY.1E12	0.873	21.2 uL/ml	235	64	223	68
Matsuda et al[55]	2015	Thailand (cohort 1)	0.722	0.748	Histopathology	WFA-MY.1E12	0.841	NA	56	16	62	22
Matsuda et al[55]	2015	Japan (cohort 2)	0.766	0.643	Histopathology	WFA-MY.1E12	0.738	NA	25	19	21	8
2.2. Characteristics of eligible studies evaluating the diagnostic accuracy of CA19-9 in serum samples												
Shoda et al[35]	2017	Japan	0.713	0.711	Histopathology	CA19-9 ELISA Kits	0.753	21.6 U/ml	216	83	204	87
Yamaguchi et al[34]	2016	Japan	0.603	0.889	Histopathology	NA	0.761	38 U/ml	105	3	24	69
Matsuda et al[55]	2015	Thailand (cohort1)	0.743	0.887	Histopathology	CA19-9 ELISA kits	0.849	NA	58	8	70	20
Matsuda et al[55]	2015	Japan (cohort2)	0.637	0.896	Histopathology	CA19-9 ELISA kits	0.759	NA	21	4	36	12
2.3. Characteristics of eligible studies evaluating the diagnostic accuracy of MUC1 in bile samples												
Shoda et al[35]	2017	Japan	0.863	0.765	Histopathology	MY.1E12 (mAb)	0.896	15.5 nL/ug	158	27	88	25
Yamaguchi et al[34]	2016	Japan	0.822	0.556	Histopathology	WFA-MY.1E12	0.715	15.5 nl/ug	143	12	15	31
Matsuda et al[32]	2013	Japan	0.90	0.72	Histopathology	WFA-MY.1E12	0.85	6 nl/ug	26	8	21	3
Matsuda et al[31]	2010	Japan	0.90	0.763	Histopathology	WFA-MY.1E12	0.86	6.64 nl/ug	27	9	29	3
2.4. Characteristics of eligible studies evaluating the diagnostic accuracy of MUC1 in tissue samples												
Zen et al[54]	2014	UK	0.71	0.85	Histopathology	clone DF3	-	>5% (positive)	20	3	17	8
Esperança et al[6]	2014	Brizal	0.909	0.925	Histopathology	Clone Ma695	-	>10% (positive)	10	5	62	1

Huang et al[9]	2010	China	0.545	0.719	Histopathology	Ma689	-	10%	18	9	23	15

Se, sensitivity; Sp, specificity; AUC, area under the receiver-operating characteristic curve; TP, true positive; FP, false positive; TN, true negative; FN, false negative; NA, not available

Table 3. Characteristics of eligible studies included in prognostic meta-analysis

Author	Year	Country	Type of CCA	No.Patients	Anti-MUC1	Cut- Off (Positive/High Expression)	Follow-Up (Months)	HR For Overall Survival (95%CI)	P Values
Higashi et al[7]	2012	Japan	M-iCCA	63	mAb DF3	> 5% (58)	>50	3.34 (0.43-25.8)	0.168
Huang et al[9]	2010	China	iCCA	33	Clone Ma689	>10% (18)	>20	1.89 (0.79-4.511)*	<0.01
Park et al[10]	2009	Korea	CCA	85	Clone Ma695	>10% (56）	>50	1.211 (0.403-3.640)	0.733
Boonla et al[12]	2005	Thailand	iCCA	87	Clone Ma695	>25% (34)	>15	2.19 (1.11-4.32)	0.026
Shibahara et al[13]	2004	Japan	M-iCCA	27	Mab DF3	>5% (22)	>50	4.536 (0.292–70.336)	0.2797
Matsumura et al[15]	2002	Japan	M-iCCA	50	mAb HMPV	>5% (38)	>50	4.377 (1.517–12.629)	0.0063
Tamada et al[14]	2002	Japan	CCA	60	MAb DF3	>20%(46)	>50	1.57（0.52-4.68)*	<0.05
Higashi et al[36]	1999	Japan	CCA	39	mAb DF3	>5% (23)	>50	1.91 (0.52-7.03)*	<0.05
Takao et al[37]	1999	Japan	CCA	67	Mab DF3	>25% (47)	>50	2.59 (1.19–5.63)	0.016

CCA, cholangiocarcinoma; iCCA, intrahepatic cholangiocarcinoma; M-iCCA, mass-forming intrahepatic cholangiocarcinoma; HR, hazard ratio; * The data was digitized and extracted from the Kaplan–Meier curve using the software designed by Jayne F Tierney and Matthew R Sydes.

Table 4. The Molecular Function (MF), Biological Process (BP) and Reactome Pathways of MUC1 in cancer	
GO MF Complete	p53 binding, transcription coregulator activity, protein binding, RNA polymerase II proximal promoter sequence-specific DNA binding
GO BP Complete	DNA damage response, signal transduction by p53 class mediator resulting in transcription of p21 class mediator, negative regulation of cell adhesion mediated by integrin, positive regulation of transcription from RNA polymerase II promoter in response to stress, DNA damage response, signal transduction by p53 class mediator resulting in cell cycle arrest, negative regulation of transcription by competitive promoter binding, regulation of transcription from RNA polymerase II promoter in response to stress, cytokine-mediated signaling pathway, negative regulation of intrinsic apoptotic signaling pathway in response to DNA damage by p53 class mediator, O-glycan processing, positive regulation of histone H4 acetylation, stimulatory C-type lectin receptor signaling pathway
Reactome Pathways	O-linked glycosylation of mucins, Metabolism of proteins, O-linked glycosylation, Defective C1GALT1C1 causes Tn polyagglutination syndrome (TNPS), Diseases of glycosylation, Termination of O-glycan biosynthesis, Defective GALNT3 causes familial hyperphosphatemic tumoral calcinosis (HFTC), Defective GALNT12 causes colorectal cancer 1 (CRCS1), Post-translational protein modification, Disease, Diseases associated with O-glycosylation of proteins

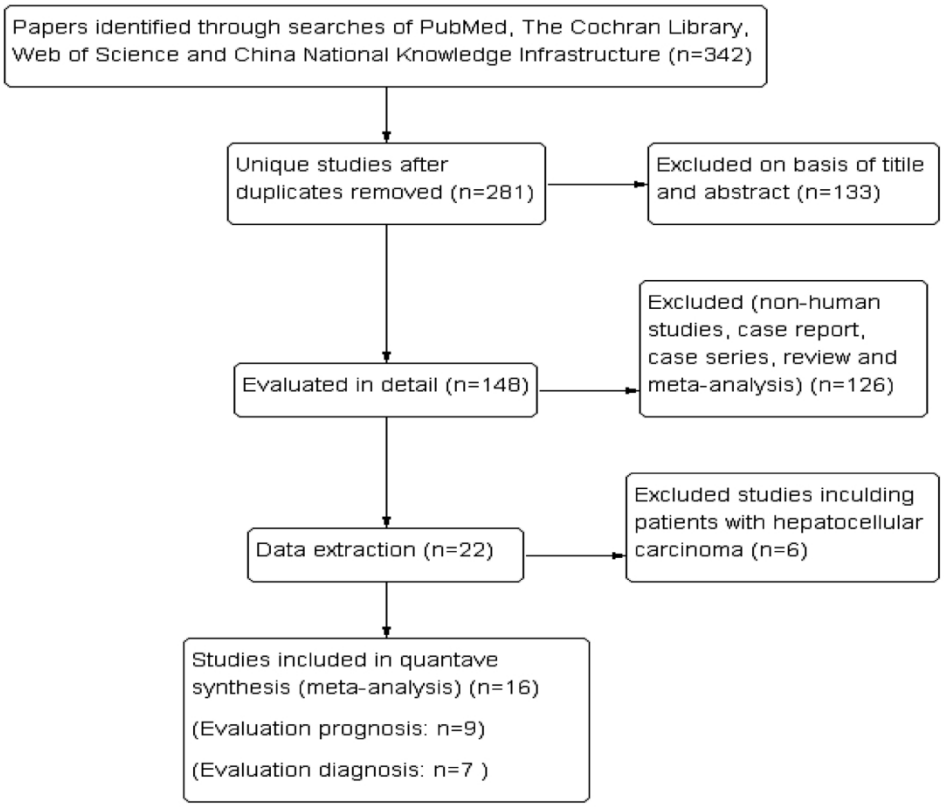


Figure 1. Search flow diagram.

169x144mm (300 x 300 DPI)

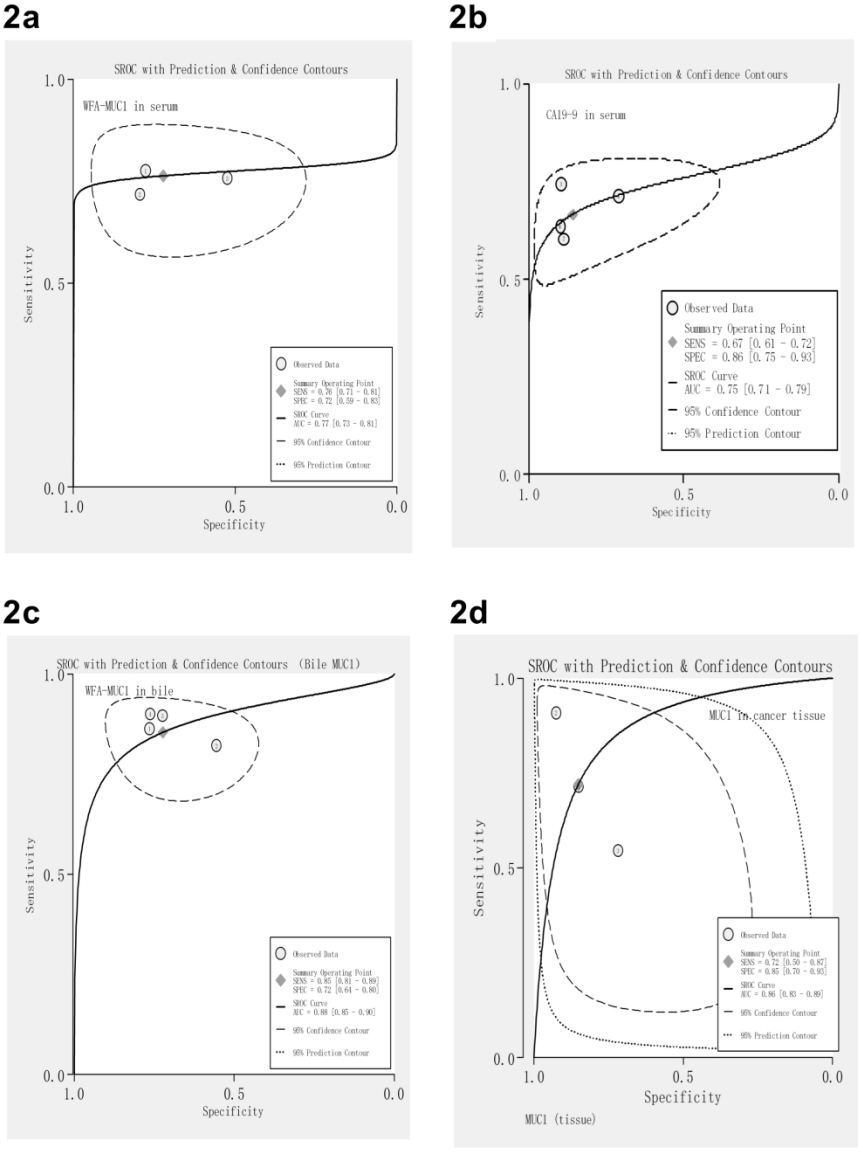


Figure 2. Summary Receiver operating characteristic curve (SROC) of WFA-MUC1 and that of CA19-9.

195x258mm (300 x 300 DPI)

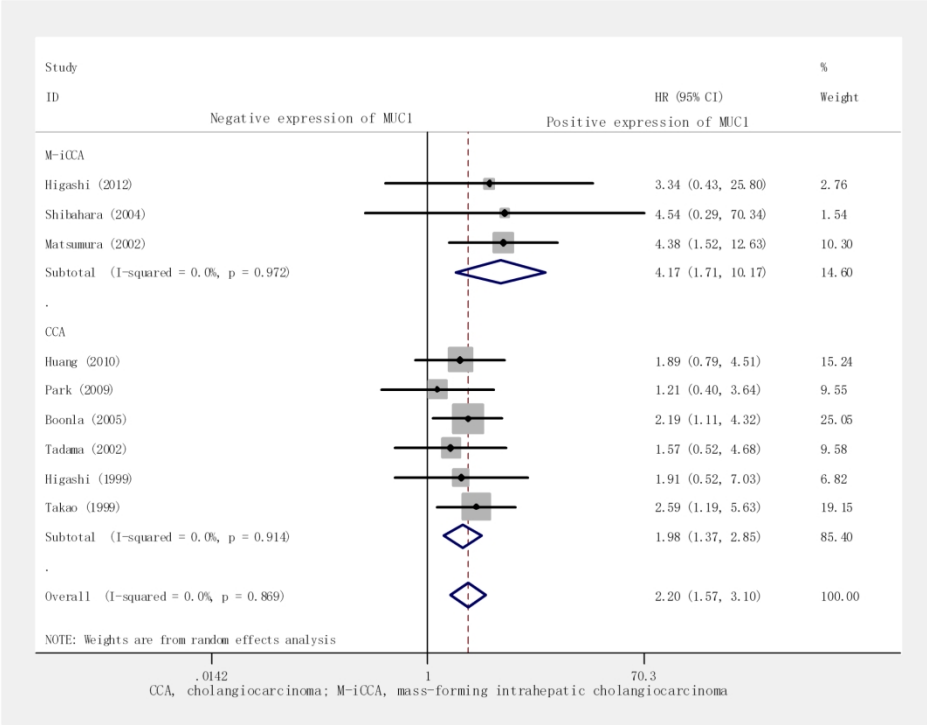


Figure 3. Forest plot of hazards ratio evaluating overall survival of patients with resectable CCA.

194x152mm (300 x 300 DPI)

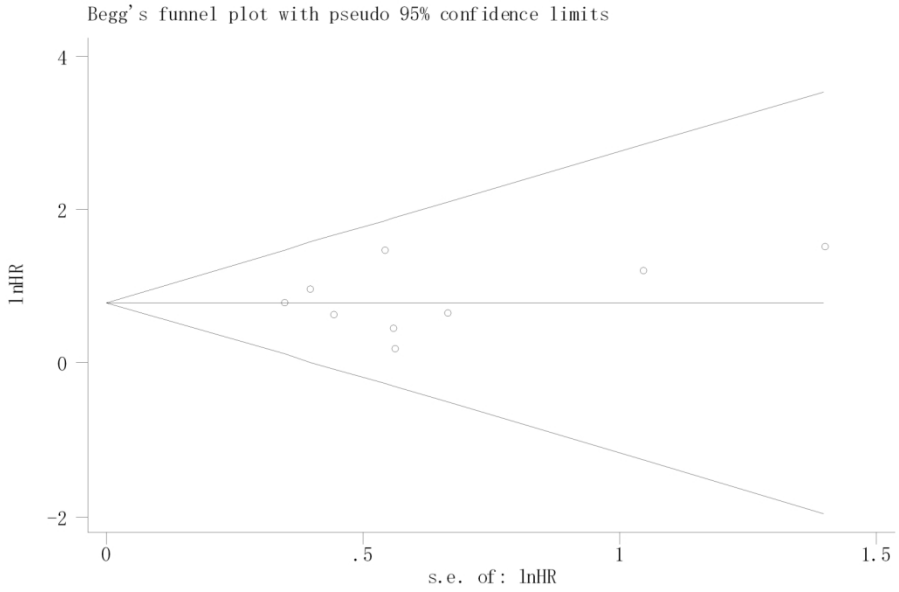
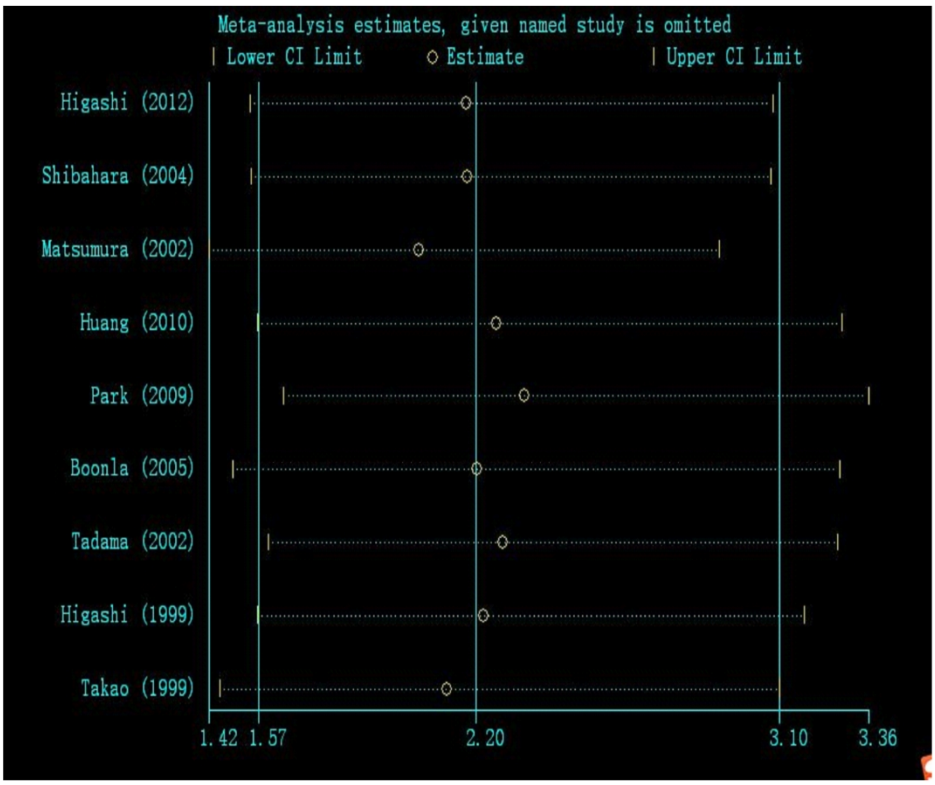


Figure 4. Begg's funnel plot for overall survival.

155x106mm (300 x 300 DPI)



Supplementary Figure 1. Sensitivity analysis of studies evaluating the prognostic value of MUC1 in biliary cancer tissue.

198x180mm (300 x 300 DPI)

Supplementary Table 1. Assessment of risk of bias for studies evaluating the prognostic value of MUC1

Author (Year)	Were adequate eligibility criteria developed and applied	Was the measurement of both exposure and outcome adequate?	Was confounding adequately controlled for?	Was the follow-up complete and adequate in duration?	Are reports of the study free of suggestion of selective outcome reporting?	Was the study free of other problems that put it at a high risk of bias?	Risk of bias
Higashi (2012)	YES	YES	YES	Unclear	YES	YES	Low
Huang (2010)	YES	YES	NO	YES	YES	YES	Low
Park (2009)	YES	YES	YES	YES	YES	YES	Low
Boonla(2005	YES	YES	NO	YES	YES	YES	High
Shibahara (2004)	YES	YES	YES	YES	YES	YES	Low
Matsumura (2002)	YES	YES	YES	YES	YES	YES	Low
Tamada (2002)	YES	YES	YES	Unclear	YES	YES	Unclear
Higashi (1999)	YES	YES	YES	Unclear	YES	YES	Unclear
Takao(1999)	YES	YES	YES	YES	YES	YES	Low

Supplementary Table 2. Assessment of risk of bias for studies evaluating the diagnostic capability of WFA-MUC1

Author (Year)	Representative spectrum?	Acceptable reference standard?	Acceptable delay between tests?	Partial verification avoided?	Differential verification avoided?	Index test results blinded?	Withdrawals explained?
Shoda (2017)	YES	YES	YES	NO	YES	Unclear	YES
Yamaguchi (2016)	YES	YES	YES	NO	YES	Unclear	YES
Matsuda[+] (2015)	YES	YES	Unclear	NO	YES	YES	YES
Matsuda [ǂ](2015)	YES	YES	Unclear	NO	YES	YES	YES
Zen (2014)	YES	YES	YES	YES	YES	YES	YES
Esperança (2014)	YES	YES	YES	YES	YES	YES	YES
Matsuda (2013)	YES	YES	YES	NO	YES	Unclear	YES
Matsuda (2010)	YES	YES	YES	NO	YES	Unclear	YES
Huang (2010)	YES	YES	YES	YES	YES	YES	YES

[+], Cohort1; [ǂ], cohort2

Items chosen to score from QUADAS checklist

1# **Was the spectrum of patients representative of those who will receive the test in practice?**

2# **Was the reference standard likely to correctly classify patients cholangiocarcinoma?**

3# **Was the time period between reference standard and index test short enough to be reasonably sure that the target condition did not change between the two tests?**

4# **Did the whole sample or a random selection of the sample receive verification using a reference standard?**

5# **Did patients receive the same reference standard regardless of the index test result?**

6# **Were the reference standard results interpreted without knowledge of the results of the index test?**

7# **Were withdrawals from the study explained?**

MOOSE Checklist

Meta-analysis of the diagnostic value of wisteria floribunda agglutinin-sialylated mucin1 and the prognostic role of mucin1 in human cholangiocarcinoma

Criteria		Brief description of how the criteria were handled in the meta-analysis
Reporting of background should include		
√	Problem definition	*Page 6.*
√	Hypothesis statement	*Page 6.*
√	Description of study outcomes	*Page10-14.*
√	Type of exposure or intervention used	*Page 7, 8.*
√	Type of study designs used	*Page 10, 11.*
√	Study population	*Page 7, 8.*
Reporting of search strategy should include		
√	Qualifications of searchers	*Page 8.*
√	Search strategy, including time period included in the synthesis and keywords	*Page 7.*
√	Databases and registries searched	*Page 7.*
√	Search software used, name and version, including special features	We did not employ a search software. EndNote X7(BId 7072) was used to merge retrieved citations and eliminate duplications.
√	Use of hand searching	*Page 7.*
√	List of citations located and those excluded, including justifications	*Page 7, 8, 10.*
√	Method of addressing articles published in languages other than English	*Page 8.*
√	Method of handling abstracts and unpublished studies	*Page 8, 10.*
√	Description of any contact with authors	We did not contact authors. Potentially, data is available just from published studies data.
Reporting of methods should include		
√	Description of relevance or appropriateness of studies assembled for assessing the hypothesis to be tested	*Page 7, 8.*

√	Rationale for the selection and coding of data	*Page 7, 8.*
√	Assessment of confounding	*Page 9, 10.*
√	Assessment of study quality, including blinding of quality assessors; stratification or regression on possible predictors of study results	*Page 9,10.*
√	Assessment of heterogeneity	*Page 10.*
√	Description of statistical methods in sufficient detail to be replicated	*Page 9, 10.*
√	Provision of appropriate tables and graphics	*Page 9-11.*
Reporting of results should include		
√	Graph summarizing individual study estimates and overall estimate	*Page 11-14.*
√	Table giving descriptive information for each study included	*Page 10-12.*
√	Results of sensitivity testing	*Page 13, 14.*
√	Indication of statistical uncertainty of findings	*Page 11-13.*
Reporting of discussion should include		
√	Quantitative assessment of bias	*Page 17, 18.*
√	Justification for exclusion	*Page 18, 19.*
√	Assessment of quality of included studies	*Page 17, 19.*
Reporting of conclusions should include		
√	Consideration of alternative explanations for observed results	*Page 14-17.*
√	Generalization of the conclusions	*See Page 18.*
√	Guidelines for future research	*See Page 18, 19.*
√	Disclosure of funding source	*See Page 2.*