ORIGINAL ARTICLE

The diagnostic efficacy of CYFRA21-1 on intrahepatic cholangiocarcinoma: A meta-analysis

He Guowei, Liao Yuan, Li Ma, Liu Zhongyang, Song Zhixing, Li Lin, Luo Minqi*

Department of clinical laboratory, Third affiliated hospital of Sun Yat-sen University, No. 600, Tianhe road, Guangzhou, Guangdong, PR China

Summary

Objective: To evaluate the reliability and accuracy of Cytokeratin-19-fragment (CYFRA21-1) in the diagnosis of intrahepatic cholangiocarcinoma (ICC) based on literature meta-analysis and the diagnostic efficacy and clinical application of CYFRA21-1 in ICC.

Methods: MEDLINE, China National Knowledge Infrastructure Library and other databases were used to base the inclusion and exclusion criteria. In addition, relevant data from studies on CYFRA21-1 were used to diagnose ICC individually or in combination was retrieved for meta-analysis. Research papers were manually screened by two independent researchers. The selected papers were evaluated by QUADAS-2 standard. The SROC was plotted according to the extracted data combined with the results of diagnosis to evaluate the diagnostic efficiency of CYFRA21-1 in ICC.

Results: Six articles with an overall sample size of 731 cases, including 217 cases in the positive group and 514 cases in the control group, met the inclusion criteria and were included for the systematic review. When CYFRA21-1 was used for ICC diagnosis, the pooled diagnostic indices were as follows: sensitivity 0.81 (95%CI: 0.75—0.86); specificity, 0.86 (95%CI: 0.82—0.89); positive likelihood rate, 4.72 (95%CI: 2.02—11.02); negative likelihood ratio, 0.25 (95%CI: 0.19—0.33); diagnostic odds ratio, 27.43 (95%CI: 13.20—57.00); and area under the ROC curve, 0.904 (SE = 0.0171).

Conclusion: CYFRA21-1 is of certain value in the diagnosis of intrahepatic cholangiocarcinoma.
© 2018 Elsevier Masson SAS. All rights reserved.

Introduction

Intrahepatic cholangiocarcinoma (ICC) is the second most common primary liver cancer. Some of noticeable dif-

* Corresponding author.
  E-mail address: luominqi@mail.sysu.edu.cn (L. Minqi).

ferences between intrahepatic cholangiocarcinoma (ICC) and hepatocellular carcinoma (HCC) lie in the pathology, pathogenesis of molecular mechanisms, epidemiology, and treatment [1]. Although some progress has been made in the study of ICC in recent years, its therapeutic effect and prognosis remain unclear [2−8]. This could be attributed to the fact that a timely accurate diagnosis of ICC is not available due to the obscure symptoms and the lack of effective laboratory diagnostic methods in the early stage of ICC [3]. Consequently, it is critical to identify the serum markers with high sensitivity and high specificity for early diagnosis of ICC and treatment.

Cytokerantin-19-fragment (CYFRA21-1) is an important tumor marker with high sensitivity and specificity for the diagnosis of non-small cell lung cancer [9,10,11]. Studies have showed that CYFRA21-1 has some value in the diagnosis of biliary tumors [12]. However, these conclusions have not yet been supported by large-scaled multi-center evidence-based clinical trials. This study analyzed and evaluated the reliability and accuracy of previous studies on the use of CYFRA21-1 in the diagnosis of ICC to explore its efficiency in the early diagnosis of ICC and to evaluate its diagnostic efficacy.

Methods

Literature collection

A systematic literature search was performed using the following databases: PubMed, Scopus, Web of Science, Clinical Evidence, SciFinder, and Clinical key and some other English literature databases as well as Chinese literature database including SinoMed, China National Knowledge Infrastructure, Wanfang Data, and CQVIP. The keywords, CYFRA21-1 and ''Intrahepatic cholangiocarcinoma'' together with their Chinese and English extension were included in the search. Literature retrieval was conducted from the establishment time of the above database to January 2017. All articles that have claimed to use CYFRA21-1 alone or in combination with other markers for diagnosis of human ICC were collected. In order to collect as comprehensively as possible, we manually searched for references that were included in the literature or reviews for possible prospective and retrospective studies on the diagnostic accuracy of CYFRA21-1 in ICC. If there is no direct access to the literature, an e-mail was sent to the corresponding author to gain access to full text.

Inclusion criteria

Types of research

The articles that demonstrated that CYFRA21-1 was used for diagnosis of human ICC, either alone or in combination with other markers, regardless of whether the studies included were blind or randomized designed were collected. Pathological diagnosis must be used as the gold standard in those researches. The language of the article was restricted to Chinese and English.

Research objective

In all the cases, ICC was diagnosed by pathological examination, the general pathological features of these tumors includes: mass forming type, periductal infiltrating type and intraductal growth type. Histopathological classification of these tumors include adenocarcinoma, adenosquamous carcinoma, squamous cell carcinoma, mucoepidermoid carcinoma and other types. ICC patients confirmed by pathological diagnosis were included in the positive group. Non-ICC patients were assigned to the control group. All patient samples and data were collected at enrollment or before treatment.

Exclusion criteria

Exclusion criteria included: the control group of study was only composed of healthy people; complete four-fold table data of the research could not be obtained; redundant publication or retrieved with duplicate data; non-original studies; research without or only partial adapted the pathological diagnostic gold standard; and animal experiments or in vitro research.

Literature screening and data extraction

Two independent researchers conducted a preliminary screening of the literature based on the inclusion and exclusion criteria, followed by manual secondary screening and cross-validation. In case of disagreement, collective discussions were used for resolution. The data was extracted according to the self-made data extraction table: first author, study period, sample size, ICC detection protocol, true positive rate, false positive rate, true negative rate, false negative rate, area under the ROC curve (AUROC), standard error, cutoff point, and its 95% confidence interval.

Data analysis and quality evaluation

This study used Review Manager 5.3 software to assess the quality of all included studies according to QUADAS-2 methodology [18]. We used the GRADE approach for the quality evaluation of the included studies.

Data was inserted into MetaDiSc 1.4 software. Heterogeneity was examed by Q-test and I^2-test, while in the Q-test, homogeneity among the studies was assumed (significance level was set to $\alpha = 0.10$). Spearman correlation analysis was used to determine whether or not heterogeneity was caused by the threshold effect. Significance level was set to $\alpha = 0.05$ assuming no apparent threshold effect exists. Indicators such as the pooled diagnostic sensitivity, specificity, positive likelihood rate, negative likelihood ratio, and diagnostic odds ratio were calculated. Forest and summary receiver operating characteristic curve (SROC) plots were produced. In this study, Egger's linear regression test was used to test whether publication bias exists, test level $\alpha = 0.05$.

Results

Basic characteristics of the selected studies

A total of 97 articles were obtained from the initial search, of which 59 were in Chinese and 38 in English. After carefully reviewing the title, abstract, and the full text of the articles, the relevant articles demonstrating the role of CYFRA21-1 alone or in combination with other markers in the diagnosis of ICC were included; while reviews, articles that failed in obtaining full data, or redundant publications were excluded. A total of six articles were chosen, of which one was in Chinese and the other five in English. All six articles were published between 1998 and 2014, with an overall sample size of 731 cases, including 217 cases in the positive group and 514 cases in the control group. In all these studies, pathological diagnosis was chosen as the gold standard for diagnosis of ICC. The basic features of the selected studies are shown in Table 1.

Methodological quality assessment of included research literature

As shown in Fig. 1, after comprehensive consideration of the following four factors: case selection and exclusion methods; the reliability of the test mentioned; the diagnostic criteria; and the research process and measurement, we could think of only one tenable risk that could lead to bias in the systematic review. Most biases were either low-risk or unknown; however, the risk of possible selection bias was higher than the others, thereby suggesting a high possibility of bias.

Most of the early-selected documents were of high quality, and the quality of all included studies reached medium to high according to our evaluation, with no study at high risk of bias (Fig. 2).

Heterogeneity assessing

Our test for heterogeneity in the current analysis: $Q = 35.2870$, $P < \alpha = 0.10$, $I^2 = 85.83\%$, the 95% CI was 71.20–93.03%; therefore, a remarkable heterogeneity was observed among the included papers. Spearman's correlation coefficient = 0.657, $P = 0.156$ indicated no threshold effect.

Publication bias

Egger's linear regression method was used to determine publish publication bias of the included literature, and no publication bias among the literature was detected ($P > 0.05$).

Results of meta-analysis

After combining the clinical data from all six studies, the pooled diagnostic sensitivity of CYFRA21-1 in the diagnosis of ICC was found to be 0.81 (95%CI: 0.75-0.86, $I^2 = 54.9\%$, $P = 0.0498$). However, a more obvious heterogeneity was observed for the pooled specificity (Spe = 0.86, 95% CI: 0.82–0.89, $I^2 = 92.8\%$, $P < 0.0001$).

When positive likelihood ratios of the studies were combined, the pooled positive likelihood rate, LR+ = 4.72 (95% CI:2.02–11.02, $I^2 = 93.6\%$, $P < 0.0001$) indicating the existence of a noticeable heterogeneity. Negative likelihood ratio in the combination is 0.25 (95% CI:0.19–0.33), indicating that CYFRA21-1 tested negative to exclude ICC. On the other hand, the combined diagnostic odds ratio, DOR = 27.43, (95% CI 13.20–57.00) indicated that CYFRA21-1 has a strong discriminating effect on ICC. The ROC curves for each study were integrated, area under the combined SROC curve was 0.904, with a Q-index = 0.8355 (Figs. 3–6).

Discussion

Although there are rigorous literature analysis methods [18] and complete research report formats [19] for diagnostic accuracy research, there are still many influencing factors that could significantly impact the analysis of the literature on diagnostic accuracy. These factors include experimental design, literature collection, and inclusion criteria for literature review. The effects can be directly manifested as heterogeneity of the study. In this study, based on the QUADAS-2 research quality system evaluation, we observed that in the six selected articles, the control group had different clinical inclusion criteria, so there was a large clinical heterogeneity. Due to the paucity of clinical research of ICC both at home and abroad, most of the researches we screened were concentrated in East Asia. There is a high possibility of regional bias in these study (of the six research included in our study, two were from China, three were from Japan, and one was from Italy).

Of the six studies included, four of them included the same experimental methodology for cytokeratin 19, using Roche's electrochemiluminescence immunoassay kits (Switzerland) [14,16,17]. One study used radioimmunoassay to detect CYFRA21-1 [15], whereas the last study used a chemiluminescent detection kit (Maglumi, China). The methodology for cytokeratin 19 fragment detection was nearly identical in all studies. However, the interpretation of the results of cytokeratin 19 fragment test was inconsistent in each study. Most of the studies [14,16,17] did not adopt the reference range established by the reagent manufacturer, but set the reference range by themselves, which could lead to a review bias regarding methodology in the current meta-analysis.

Summary

Of the six studies included, the overall quality of the literature was at the medium to high level, according to QUADAS-2's evaluation criteria. The inconsistencies between the study population and the interpretation of the test methods were the important factors influencing the quality of our research on the diagnostic efficacy of CYFRA21-1.

Through this meta-analysis, using cytokeratin 19 fragment to diagnose ICC, the pooled diagnostic sensitivity Sen = 0.81, specificity Spe = 0.86, positive likelihood ratio LR + = 4.72, negative likelihood ratio LR- = 0.25, diagnos-

Table 1 Summary of the basic features of the selected studies.

| Studies	Author	Research design	Gold-standard	Positive grou (case number)	Control group (case number)	Detection method	Reagent source	Cut-off point (ng/mL)	True positive (case number)	False positive (case number)	False negative (case number)	True negative (case number)	QUADAS-2 Quality evaluation
1	FRANCO LUMACHI 2014 [13]	Retrospective study	pathologic	24	25	Chemilumi-nescence CLIA	Maglumi, China	2.7	18	5	6	20	medium
2	Hongbo Ma 2012 [14]	Retros-pective study	pathologic	54	42	Electrochemil-uminescence ECLIA	ROCHE, Switzerland	5	50	21	4	21	medium
3	Kashihara T 1998 [15]	Retros-pective study	pathologic	4	13	Solid-phase Radioim-munoassay RIA	Centocor, Diagnostic, USA	2	4	9	0	4	high
4	Li Huang 2015 [16]	Retros-pective study	pathologic	41	93	Electrochemil-uminescence ECLIA	ROCHE, Switzerland	3.3	31	3	10	90	high
5	Takahiro Uenishi 2003 [12]	Retros-pective study	pathologic	23	251	Electrochemil-uminescence ECLIA	ROCHE, Switzerland	3	20	8	3	156	high
6	Takahiro Uenishi 2007 [17]	Retros-pective study	pathologic	71	90	Electrochemil-uminescence ECLIA	ROCHE, Switzerland	2.7	53	7	18	83	high
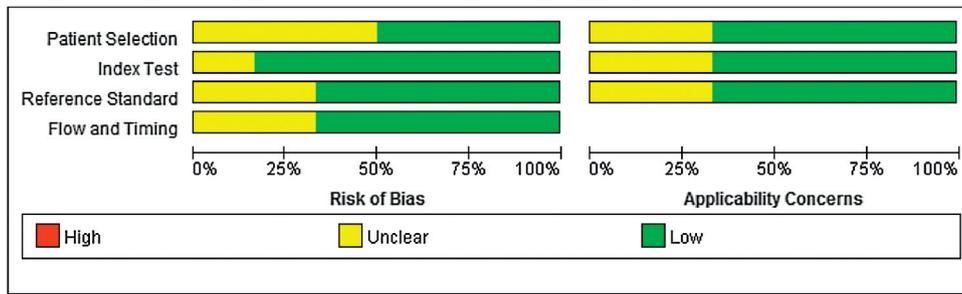
Figure 1   QUADAS-2 evaluation of the selected studies.
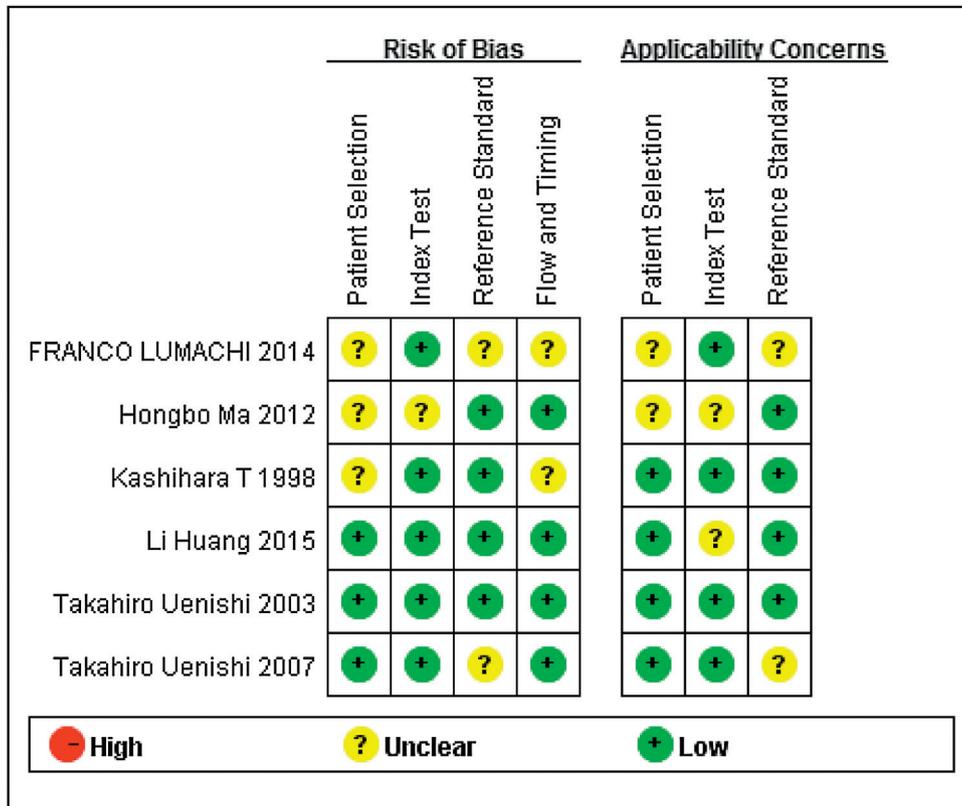

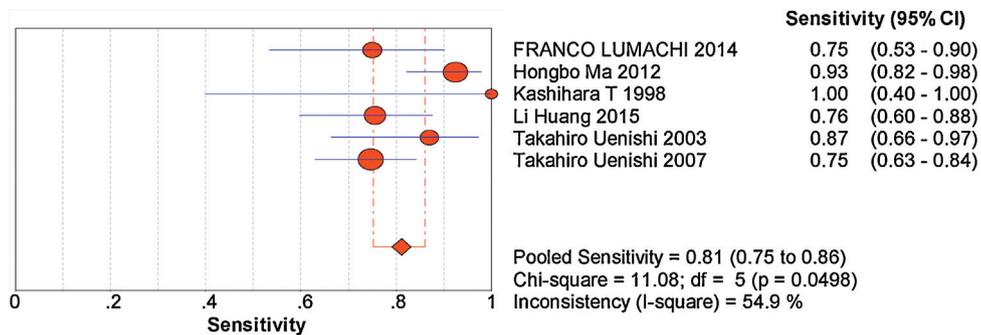**Figure 2**   Quality evaluation of each literature.

Figure 3   Pooled diagnostic indices of CYFRA21-1 to intrahepatic cholangiocarcinoma: Sensitivity.
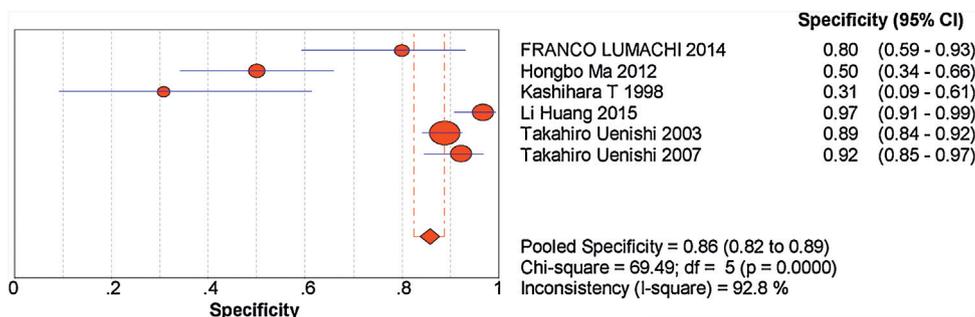
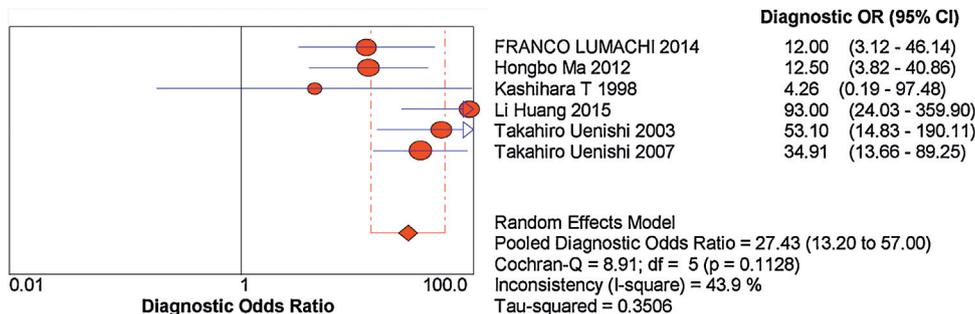**Figure 4**   Pooled diagnostic indices of CYFRA21-1 to intrahepatic cholangiocarcinoma: Specificity.

Figure 5   Pooled diagnostic indices of CYFRA21-1 to intrahepatic cholangiocarcinoma: Diagnostic ratio.
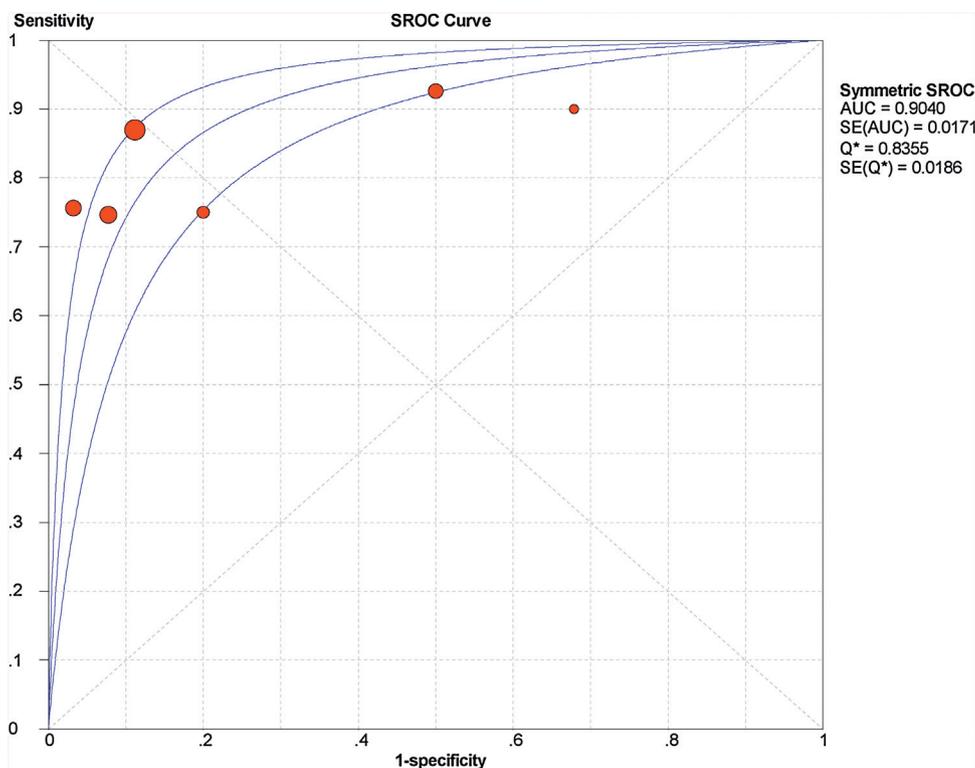

Figure 6   Pooled diagnostic indices of CYFRA21-1 to intrahepatic cholangiocarcinoma: SROC.

tic odds ratio DOR = 27.43, and area under the ROC curve AUROC = 0.904 indicated that CYFRA21-1 has a higher diagnostic value for ICC; diagnostic sensitivity is even higher than that of carcinoembryonic antigen [20—23]. We also observed that the diagnostic specificity of CYFRA21-1 for ICC is higher compared to CA199 with a similar sensitivity to that of CA199 [24]. Compared with CA125, the diagnostic specificity of CYFRA21-1 is similar to that of CA125, but

its sensitivity is much higher than CA125 (sensitivity 37—72%) [25—28]. Therefore, CYFRA21-1 is a promising serum marker for ICC. Therefore, we opined that CYFRA21-1 is a promising serum marker for the clinical diagnosis of ICC.

Disclosure of interest

The authors declare that they have no competing interest.

References

[1] Moeini A, Sia D, Bardeesy N, et al. Molecular pathogenesis and targeted therapies for intrahepatic cholangiocarcinoma. Clin Cancer Res 2016;22(2):291—300.

[2] Shaib YH, Davila JA, McGlynn K, et al. Rising incidence of intrahepatic cholangiocarcinoma in the United States: a true increase. J Hepatol 2004;40(3):472—7.

[3] Shaojun Z, Zhiyong H. Prognostic factors for tumor recurrence and metastasis of intrahepatic cholangiocarcinoma after radical resection. Chinese J General Surg 2014;23(08):1024—9.

[4] Fanghua L, Xiaoqin C, Huiyan L, et al. Prognosis of 84 intrahepatic cholangiocarcinoma patients. Chinese J Cancer 2009;28(05):528—32.

[5] Fu X-H, Tang Z-H, Zong M, et al. Clinic pathologic features, diagnosis and surgical treatment of intrahepatic cholangiocarcinoma in 104 patients. Hepatobiliary Pancreat Dis Int 2004;02:279—83.

[6] Shen W-F, Zhong W, Xu F, et al. Clinic pathological and prognostic analysis of 429 patients with intrahepatic cholangiocarcinoma. World J Gastroenterol 2009;15(47):5976—82.

[7] Ma KW, Cheung TT, She WH, et al. The effect of wide resection margin in patients with intrahepatic cholangiocarcinoma: A single-center experience. Agrawal. S, ed. Medicine 2016;95(28):e4133.

[8] Chen Q, Yang LX, Li XD, et al. The elevated preoperative neutrophil-to-lymphocyte ratio predicts poor prognosis in intrahepatic cholangiocarcinoma patients undergoing hepatectomy. Tumor Biol 2015;36:5283.

[9] Cui C, Sun X, Zhang J, et al. The value of serum Cyfra21-1 as a biomarker in the diagnosis of patients with non-small cell lung cancer: a meta-analysis. J Cancer Res Ther 2014;10:C131—4.

[10] Wang J, Yi Y, Li B, et al. CYFRA21-1 can predict the sensitivity to chemo radiotherapy of non-small-cell lung carcinoma. Biomarkers 2010;15(7):594—601.

[11] Zhang ZH, Han YW, Liang H, et al. Prognostic value of serum CYFRA21-1 and CEA for non-small-cell lung cancer. Cancer Med 2015;4(11):1633—8.

[12] Uenishi T, Kubo S, Hirohashi K, et al. Cytokeratin-19 fragments in serum (CYFRA 21-1) as a marker in primary liver cancer. Br J Cancer 2003;88(12):1894—9.

[13] 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;34:6663—8 [6663-6669].

[14] Hongbo M, Tao H, Feng H, et al. Clinical significance of combined detection of CA19-9, CA125 and CK19 in diagnosing cholangiocarcinoma. Chinese J Chongqing Med 2012;41(25):263—4.

[15] Kashihara T, Ohki A, Kobayashi T. Intrahepatic cholangiocarcinoma with increased serum CYFRA 21-1 level. J Gastroenterol 1998;33(3):447—53.

[16] Huang Li, Chen Wei, Liang Peiwen, et al. Serum CYFRA 21-1 in biliary tract cancers: a reliable biomarker for gallbladder carcinoma and intrahepatic cholangiocarcinoma. Dig Dis Sci 2015;60:1273—83.

[17] Yamazaki T, Yamazaki O, Tanaka H, et al. Serum cytokeratin 19 fprognostic factor in intrahepatic c. Ann Surg Oncol 2008;15:583.

[18] Whiting PF, Rutjes AW, Westwood ME, et al. QUADAS-2: a revised tool for the quality assessment of diagnostic accuracy studies. Ann Intern Med 2011;155:529—36.

[19] Bossuyt PM, Reitsma JB, Bruns DE, et al. The STARD statement for reporting studies of diagnostic accuracy: explanation and elaboration. Ann Intern Med 2003;138(1):W1—12.

[20] Li Y, Tian L, Feng Z, et al. The clinical analysis of tumor marker CA19-9 and CEA in diagnosis of cholangiocarcinoma. Chinese J Med Info 2010;23:5—6.

[21] Jiang H, Liu P, Pan L. The diagnosis value of evaluating tumor marker CA19-9, CA242, CA125 and CEA in cholangiocarcinoma. J Hunan Normal Univ 2011;8:59—61.

[22] Liling Q, Qingfeng S, Weijia L, et al. The diagnostic value of combined detection three of serum tumor marker CEA, CA19-9 and sICAM-1 for cholangiocarcinoma. Chin J Lab Diagn 2010;14:413—5.

[23] Yang C, Zhang J, Lin YX, et al. Risk factors of hepatolithasis-associated intrahepatic cholangiocarcinoma and the value of serum tumor-related makers in its diagnosis. Sichuan Da Xue Xue Bao Yi Xue Ban 2014;45(4):628—32.

[24] Jie Y, Wu Y, Hao J, et al. The role of MSCT and CA199 in diagnosis of intrahepatic peripheral cholangiocarcinoma. Chinese J CT MRI 2015;07:72—4 [+83].

[25] Ren S, Deng G, Zhao Y. Significance of the detection of tumor markers CA19-9, CA125, AFP and CEA in the diagnosis of cholangiocarcinoma. Acta Medicine Sinica 2013;26(2):242—5.

[26] Guan C, Cui F, Su H. Differential diagnosis between hepatocellular carcinoma and cholangiocarcinoma. Chinese Hepatol 2015;20(11):864—6.

[27] Wu W-R, Shi X-D, Zhang R, et al. Clinicopathological significance of aberrant Notchreceptors in intrahepatic cholangiocarcinoma. Int J Clin Exp Pathol 2014;7(6): 3272—9.

[28] Yong Li, Da-Jiang Li, Jian Chen, et al. Application of joint detection of AFP, CA19-9, CA125 and CEA in identification and diagnosis of cholangiocarcinoma. Asian Pac J Cancer Prev, 16 (8), 3451-3455.