Diagnostic accuracy of serum squamous cell carcinoma antigen and squamous cell carcinoma antigen-immunoglobulin M for hepatocellular carcinoma: A meta-analysis

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Abstract. A number of individual studies have evaluated the diagnostic efficiency of serum squamous cell carcinoma antigen (SCCA) and SCCA-immunoglobulin (IgM) for diagnosing hepatocellular carcinoma (HCC), but the results have been conflicting. The aim of this study was to determine the diagnostic accuracy of serum SCCA and SCCA-IgM for HCC. A systematic review of related studies was conducted and relevant data on the accuracy of serum SCCA and SCCA-IgM in the diagnosis of HCC were pooled using random-effects models. Summary receiver operating characteristic curve (SROC) analysis was used to summarize the overall test performance. A total of 12 studies were included in our meta-analysis. The summary estimates for serum SCCA and SCCA-IgM for HCC diagnosis in the included studies were as follows: Sensitivity = 0.59 (95% CI: 0.56-0.62) vs. 0.60 (95% CI: 0.56-0.63); specificity = 0.76 (95% CI: 0.73-0.79) vs. 0.70 (95% CI: 0.67-0.73); diagnostic odds ratio (DOR) = 6.68 (95% CI: 3.71-12.03) vs. 7.32 (95% CI: 3.31-16.15); and area under the SROC curve = 0.7826 vs. 0.7955. Therefore, SCCA and SCCA-IgM exhibited moderate diagnostic accuracy for HCC. Due to the design limitations, the results of published studies should be interpreted with caution. In addition, well-designed studies including larger sample sizes should be conducted to rigorously evaluate the diagnostic value of SCCA and SCCA-IgM.

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Introduction

Hepatocellular carcinoma (HCC) is the third leading cause of cancer-related mortality and the sixth most common type of cancer worldwide (1). Of the 12.7 million new cases of cancer diagnosed worldwide in 2008, HCC accounted for 5.9% (748,000), while of the 7.6 million cancer deaths worldwide in 2008, HCC accounted for 1,234,000 (9.7%) (2). As the majority of HCC patients are diagnosed at an advanced stage, the prognosis of HCC is generally poor. Therefore, early and accurate diagnosis of HCC may significantly improve the survival rate of the patients.

Squamous cell carcinoma antigen (SCCA) is a novel tumor marker recently discovered to be of diagnostic value in patients with HCC. SCCA is a serine protease inhibitor physiologically found in the spinous and granular layers of normal squamous epithelium, and typically expressed by neoplastic cells of epithelial origin (3). SCCA-immunoglobulin (Ig)M is the immunocomplex, the serpin SCCA complexed with IgM. Increased levels of SCCA have been found in epithelial cancers of the neck, cervix and lungs (4-6). Although SCCA and SCCA-IgM reportedly exhibit low sensitivity (41.9 and 52.3%, respectively), they have a high specificity (82.6 and 75.7%, respectively) for HCC (7). The aim of the present study was to determine the diagnostic performance of serum SCCA and SCCA-IgM for HCC diagnosis using a meta-analysis.

Materials and methods

Search strategy and study selection. Embase, Medline (using PubMed as the search engine), Chinese Biomedical Literature Database (CBM), Weipu, Wanfang data and CNKI databases were searched to identify relevant studies without restrictions regarding year of publication, study design or language. MeSH and keyword searches were used. A manual search was also performed of the references listed in the original articles and review articles retrieved. The keywords used for the literature search were as follows: SCCA, squamous cell carcinoma antigen, SCCA-IgM, HCC, liver cancer, liver tumor, liver neoplasm, hepatoma and hepatic carcinoma.

The inclusion criteria were as follows: i) Studies investigating the diagnostic performance of serum SCCA and SCCA-IgM for HCC diagnosis; ii) sample size of HCC and non-HCC patients, true-positive (TP), false-positive (FP), false-negative (FN) and true-negative (TN) were reported or calculable; and iii) a minimal sample size of 10 patients.

The exclusion criteria were as follows: i) Studies conducted on animals; ii) duplicate reports; iii) studies with no clearly reported outcomes of interest; iv) case reports and letters to the editors; v) reviews or systematic reviews; vi) studies investigating HCC recurrence following hepatectomy; and vii) the assay type used was not ELISA.

Data extraction and quality assessment. Two reviewers (Zhang and Zhou) independently assessed the articles. The title and abstract of each article were reviewed to identify eligible studies. Disagreements on study eligibility were resolved through discussion. The information extracted from the eligible studies included publication year, country, characteristics of the participants, test methods, reference standard and cut-off values.

Two reviewers (Zhang and Zhou) independently assessed the quality of each study, according to the Quality Assessment of Diagnostic Accuracy Studies (QUADAS-2) checklist recommended by the Cochrane Collaboration (8). Each of the items in the QUADAS-2 checklist was scored as 'yes', 'no', or 'unclear'.

Statistical analysis. In the present study, the recommended standard methods for meta-analyses of diagnostic tests was used for evaluation (9). The analyses were performed using RevMan version 5.2 and MetaDisc version 1.4 software programs (10). A random-effects model was used to pool sensitivity, specificity, diagnostic odds ratio (DOR) and their corresponding 95% confidence intervals (CIs) and forest plots were used to depict the heterogeneity of the eligible studies, as well as the sensitivity and specificity of individual studies with the corresponding 95% CIs. The summary receiver operating characteristic (SROC) curves demonstrated the overall diagnostic performance of SCCA and SCCA-IgM (11). The inconsistency index (I^2) reflected the degree of heterogeneity (12). Spearman's rank correlation coefficient was used to determine whether the heterogeneity could be explained by a threshold effect and meta-regression was performed to identify possible sources of heterogeneity caused by non-threshold effect (9).

Results

Study eligibility. An independent search identified a total of 265 articles. Following exclusion of duplicate studies, a total of 189 articles remained. After reviewing the titles and abstracts, 40 articles were considered relevant. Following full-text review, 12 articles (7,13-23) were finally included in our analysis, according to the strict inclusion and exclusion criteria mentioned above. A flowchart of the study selection process is shown in Fig. 1. The 12 studies (8 studies on the diagnostic value of SCCA, 3 studies on SCCA-IgM and 1 study on both), included a total of 2,354 subjects (1,190 HCC and 1,164 non-HCC patients). The characteristics

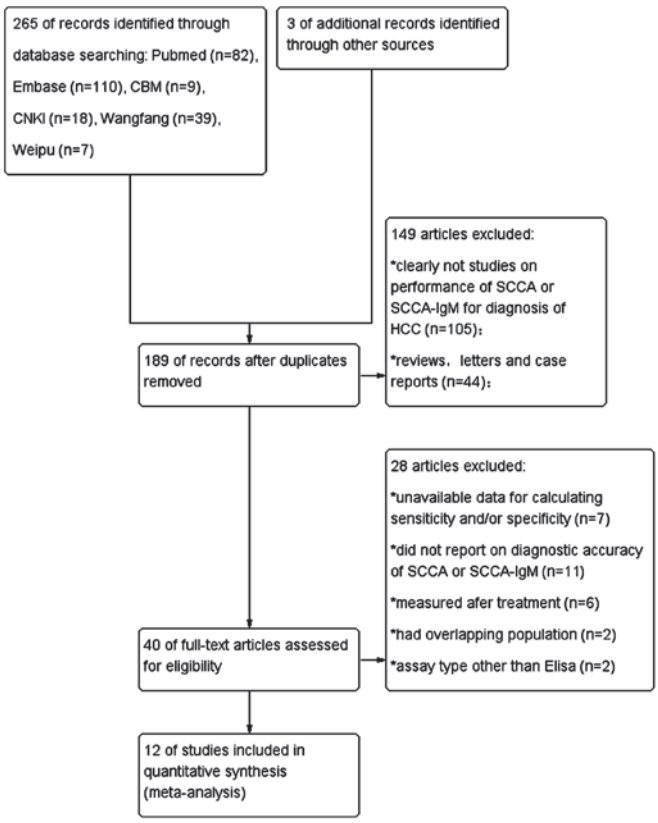

Figure 1. Flow diagram of the study selection process for the meta-analysis.

of the included articles are summarized in Tables I and II. All the eligible studies were published from 2005 onwards. The sample size ranged between 81 and 961. Five studies were performed in Asian (14,20-23), 5 in European (7,13,15,17,18) and 2 in African populations (16,19).

Quality of the studies. The quality assessment of the included studies using the QUADAS-2 tool is shown in Fig. 2. Certain design details could not be determined from the articles and, for these studies, the risk bias was labeled as 'unclear'. However, the quality was not considered to be satisfactory. All the studies used a retrospective design and in only two studies were the blood samples collected from consecutive patients. Five studies recruited healthy individuals in the control group. All the studies reported the diagnostic standard of HCC, but none of the 12 studies interpreted serum SCCA and SCCA-IgM test levels with the investigators blinded to the diagnosis. All 12 studies measured SCCA and SCCA-IgM using ELISA.

Sensitivity and specificity of SCCA and SCCA-IgM for HCC. The sensitivity in the 12 studies ranged between 41.9 and 84.2% for SCCA and between 52.3 and 89.0% for SCCA-IgM; the specificity range was 26.7-100.0% and 50.0-87.8%, respectively. Forest plots for sensitivity, specificity and their respective 95% CIs for SCCA and SCCA-IgM are shown in Figs. 3-6. The results of the pooled sensitivity and specificity and were 59.0 and 76.0%, respectively, for SCCA and 60.0 and 70.0%, respectively, for SCCA-IgM.

Table I. Characteristics of included studies on serum squamous cell carcinoma antigen.

Study (year)	Country	HCC/ controls	Gender (M/F, HCC)	Cut-off, ng/ml	TP	FP	TN	FN	AUC	Refs.
Trerotoli *et al* (2009)	Italy	55/27	44/11	1.1	40	0	27	15	0.897	(15)
Giannelli *et al* (2005)	Italy	120/90	95/25	0.368	101	47	43	19	0.705	(17)
Hussein *et al* (2008)	Egypt	49/45	39/10	1.5	38	7	38	11	0.869	(16)
Soyemi *et al* (2012)	Nigeria	60/30	40/20	0.368	45	22	8	15	0.525	(14)
Giannelli *et al* (2007)	Italy	499/462	404/95	3.8	209	80	382	290	0.656	(7)
Salman *et al* (2011)	Egypt	30/60	Unknown	0.53	24	14	46	6	Unknown	(19)
Zhai *et al* (2009)	China	50/50	41/9	0.12	40	22	28	10	Unknown	(23)
Wu (2007)	China	34/47	31/3	1.2	19	7	40	15	0.761	(20)
Chen *et al* (2010)	China	105/30	Unknown	1.5	75	4	26	30	0.91	(22)

HCC, hepatocellular carcinoma; M, male; F; female; TP/FP, true-/false-positive; TN/FN, true-/false-negative; AUC, area under the curve.

Table II. Characteristics of included studies on serum squamous cell carcinoma antigen-immunoglobulin M.

Study (year)	Country	HCC/ controls	Gender (M/F, HCC)	Cut-off, AUC/ml	TP	FP	TN	FN	AUC	Refs.
Beneduce *et al* (2005)	Italy	50/50	Unknown	120	35	13	37	15	0.741	(18)
Pozzan *et al* (2014)	Italy	81/206	63/18	89	72	102	104	9	0.66	(13)
Giannelli *et al* (2007)	Italy	499/462	404/95	104	261	112	350	238	0.675	(7)
Zhai *et al* (2014)	China	57/67	41/16	110.5	42	8	59	15	0.853	(21)

HCC, hepatocellular carcinoma; M, male; F; female; TP/FP, true-/false-positive; TN/FN, true-/false-negative; AUC, area under the curve.

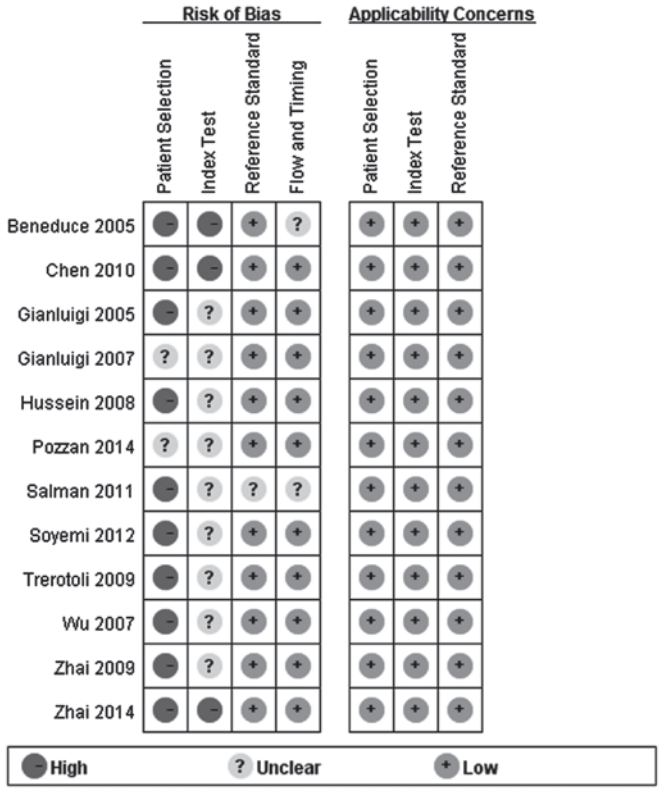

Figure 2. Summary quality assessment of the eligible studies based on the review authors' judgment on the items of Quality Assessment of Diagnostic Accuracy Studies-2 checklist for each study.

Threshold effect. The threshold effect is a significant source of between-study heterogeneity in diagnostic meta-analyses. In our analysis, the SROC curves of SCCA and SCCA-IgM demonstrated that the plane scatter plot did not exhibit the 'shoulder-arm' shape, which is characteristic of the presence of the threshold effect (Figs. 7 and 8). The Spearman's correlation coefficient was 0.577 and 0.400 and the P-value was 0.104 and 0.600 for SCCA and SCCA-IgM, respectively (Table III). These results indicated that there was no heterogeneity attributable to the threshold effect.

Meta-regression analysis for heterogeneity. We attempted to explain this heterogeneity as induced by factors other than the threshold effect, by investigating the study characteristics using meta-regression analysis. We examined race, sample size and the number of controls as possible sources of heterogeneity. Due to the small number of studies, we only tested meta-regression of the effects of methodological characteristics in the SCCA group. The P-value reflected the various test factors affecting the SCCA diagnostic efficiency (Table IV) and the differences were not found to be statistically significant.

DOR, SROC and AUC of SCCA and SCCA-IgM for HCC. We constructed the SROC curves and calculated the AUC for SCCA and SCCA-IgM (Figs. 7 and 8); the DOR was found to be 6.68 (95% CI: 3.71-12.03) for SCCA and 7.32 (95% CI: 3.31-16.15) for SCCA-IgM (Figs. 9 and 10).

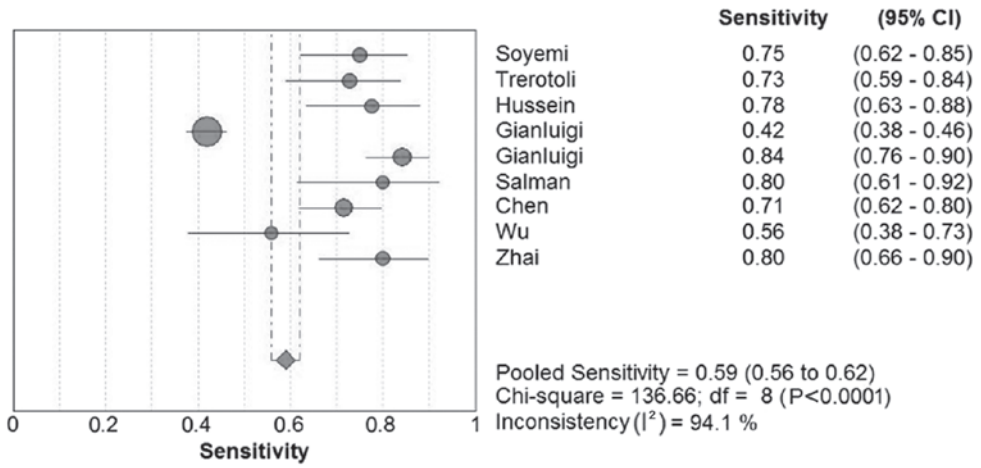

Figure 3. Forest plot of sensitivity for squamous cell carcinoma antigen in hepatocellular carcinoma diagnosis. Each solid circle represents an eligible study. The size of the solid circle reflects the sample size of each eligible study; the error bars represent 95% confidence intervals (CIs).

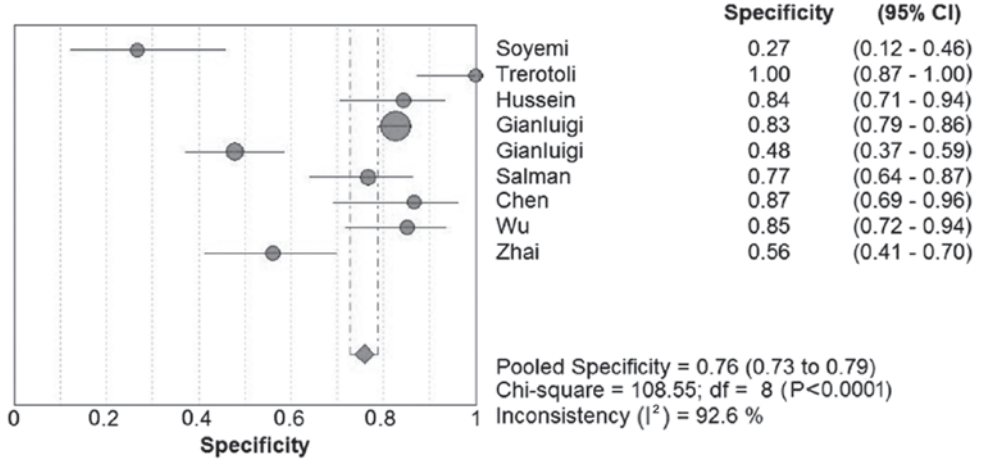

Figure 4. Forest plot of specificity for squamous cell carcinoma antigen in hepatocellular carcinoma diagnosis. Each solid circle represents an eligible study. The size of the solid circle reflects the sample size of each eligible study; the error bars represent 95% confidence intervals (CIs).

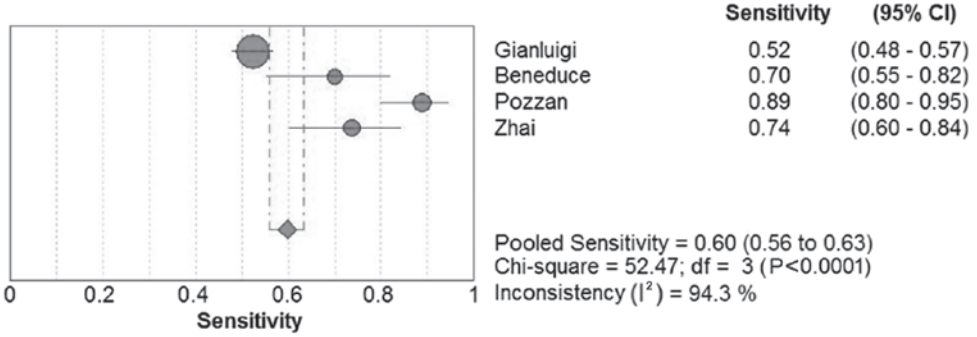

Figure 5. Forest plot of sensitivity for squamous cell carcinoma antigen-immunoglobulin M in hepatocellular carcinoma diagnosis. Each solid circle represents an eligible study. The size of the solid circle reflects the sample size of each eligible study; the error bars represent 95% confidence intervals (CIs).

Discussion

According to the present meta-analysis, serum SCCA and SCCA-IgM may be useful diagnostic biomarkers for HCC; however, the included studies had certain limitations due to their design and future well-designed studies are required to rigorously evaluate the diagnostic accuracy of SCCA and SCCA-IgM.

Serum biomarkers are crucial in HCC diagnosis and several biomarkers have been identified, including α-fetoprotein (AFP), AFP-L3, glycoprotein 73, SCCA, glypican-3, transforming growth factor-β and des-γ-carboxy prothrombin (24-31). Among these serum biomarkers, AFP is the most commonly clinically applied in the early diagnosis of HCC. However, the clinical value of AFP has been challenged over the last few

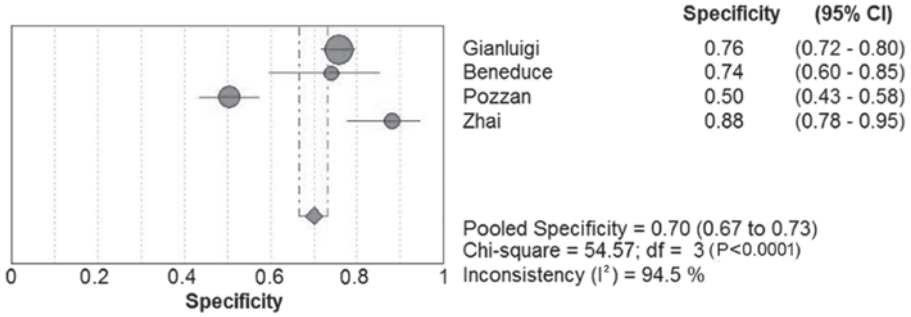

Figure 6. Forest plot of specificity for squamous cell carcinoma antigen-immunoglobulin M in hepatocellular carcinoma diagnosis. Each solid circle represents an eligible study. The size of the solid circle reflects the sample size of each eligible study; the error bars represent 95% confidence intervals (CIs).

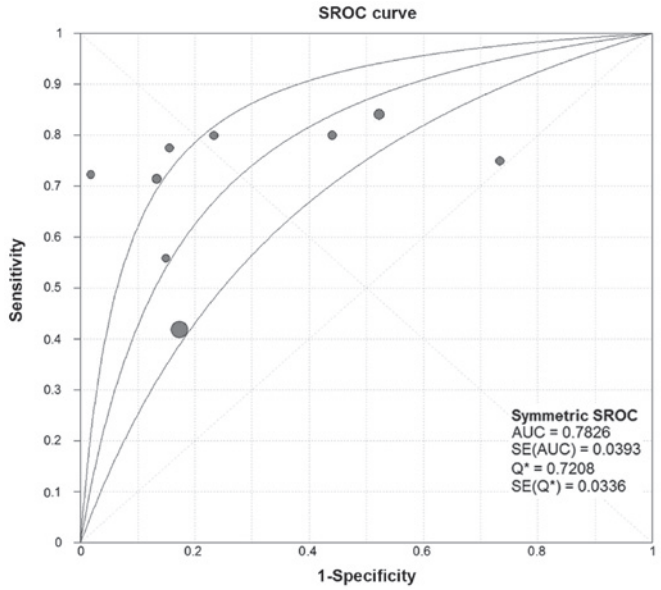

Figure 7. Summary receiver operating characteristic (SROC) curve for squamous cell carcinoma antigen. Each solid circle represents an eligible study. The size of the solid circle represents the sample size of each eligible study. The overall diagnostic efficiency is summarized by the regression curve. AUC, area under the curve; SE, standard error.

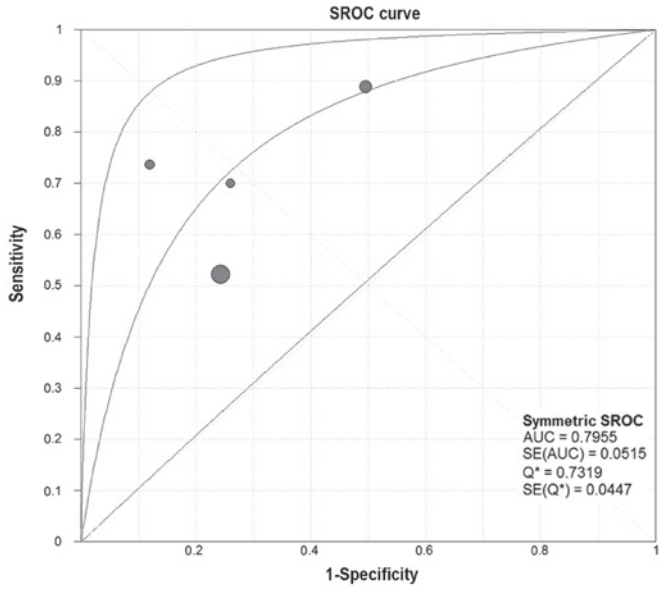

Figure 8. Summary receiver operating characteristic (SROC) curve for squamous cell carcinoma antigen-immunoglobulin M. Each solid circle represents an eligible study. The size of the solid circle represents the sample size of each eligible study. The overall diagnostic efficiency is summarized by the regression curve. AUC, area under curve; SE, standard error.

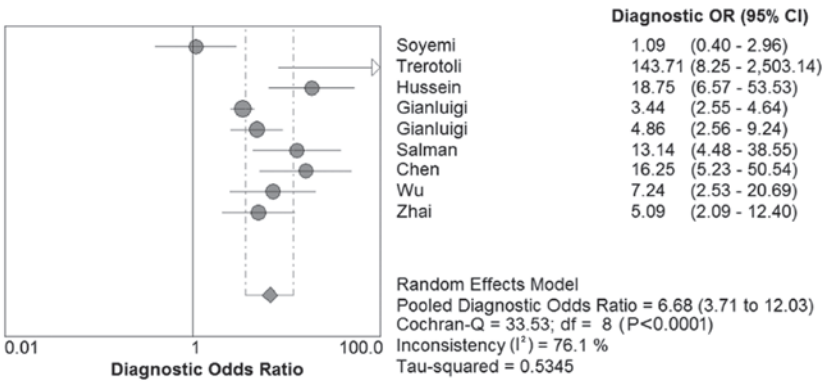

Figure 9. Diagnostic odds ratio (OR) for squamous cell carcinoma antigen. Each solid circle represents an eligible study. The size of the solid circle represents the sample size of each eligible study; the error bars represent 95% confidence intervals (CIs).

years, due to its low sensitivity and specificity (1,32-34). The latest guidelines on the management of HCC by the American Association for the Study of Liver Diseases in 2010 did not recommend AFP as a tumor marker for HCC screening (35).

In our study, we performed a meta-analysis of 12 articles investigating the diagnostic accuracy of serum SCCA and SCCA-IgM in HCC. The results indicated that the sensitivity and specificity were 59 and 76%, respectively, for SCCA,

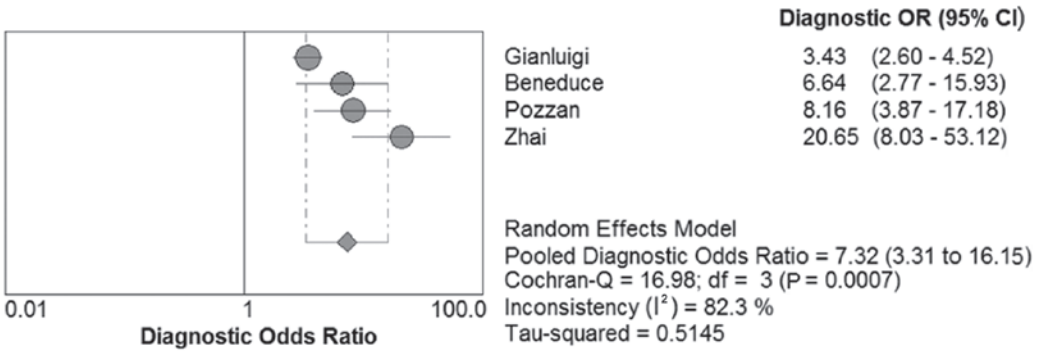

Figure 10. Diagnostic odds ratio (OR) for squamous cell carcinoma antigen-immunoglobulin M. Each solid circle represents an eligible study. The size of the solid circle represents the sample size of each eligible study; the error bars represent 95% confidence intervals (CIs).

Table III. Results of analysis of diagnostic threshold.

Markers	Spearman's correlation coefficient	P-value	No. of studies
SCCA	0.577	0.104	9
SCCA-IgM	0.400	0.600	4

SCCA, squamous cell carcinoma antigen; Ig, immunoglobulin.

Table IV. Results of various factors in meta-regression.

Variables	Coeff.	SE	P-value	RDOR	95% CI
Race	1.071	0.8790	0.2898	2.92	0.25-33.51
Sample size	-0.646	1.0383	0.5674	0.52	0.03-9.36
Controls	-0.778	1.1952	0.5505	0.46	0.02-12.68

Coeff., coefficient; SE, standard error; RDOR, relative diagnostic odds ratio; CI, confidence interval.

and 60 and 70%, respectively, for SCCA-IgM; this means that 59 and 76% of the HCC patients had elevated levels, and 60 and 70% of non-HCC patients had decreased levels of serum SCCA and SCCA-IgM, respectively. The DOR is the ratio of the odds of positive test results in patients with or without disease and a single indicator of test accuracy that incorporates sensitivity and specificity into a single index (36). In the present meta-analysis, the mean DOR was 6.68 and 7.32 for SCCA and SCCA-IgM, respectively, indicating that the odds for positivity among subjects with HCC were 6.68 and 7.32 times higher compared with the odds for positivity among non-HCC subjects. In addition, the area under the SROC curve (AUC) for SCCA was 0.7826 and for SCCA-IgM 0.7955, indicating a moderate diagnostic accuracy for HCC.

Heterogeneity was significant and could not be explained by the threshold effect. We hypothesized that the heterogeneity was due to differences in race, sample size and controls. As the number of studies was limited and certain information was unavailable, we were unable to determine the reasons for the existing heterogeneity by meta-regression.

Of note, one study reported that the SCCA levels were inversely correlated with tumor size and the AUC of smaller HCCs (<3 cm) was 0.7 (95% CI: 0.66-0.74), with a cut-off value of 3.2 ng/ml, a sensitivity of 56.1% and a specificity of 74.9% (7), suggesting that SCCA may be helpful in detecting HCC at an early stage. The Cox multivariate analysis of another study demonstrated that SCCA-IgM levels (P=0.004) was an independent predictor of survival and, combining SCCA-IgM with AFP, the sensitivity reached 94% (13). Another study also reported that the combination of AFP and SCCA yielded a correct serological diagnosis in 90.83% of HCC patients, indicating that combining the two markers may achieve a higher sensitivity (17).

There were certain limitations to the present meta-analysis. First, there were no randomized clinical trials and the number of studies included in the present study was limited. Therefore, more well-designed and large-sample sized studies are required. Second, it was not feasible to include studies with completely identical standards, particularly since the tumor and liver function characteristics were different among different patients. Third, significant heterogeneity was observed among eligible studies and the heterogeneity could not be explained by meta-regression. We used the more conservative random-effects model to address this issue. Finally, hepatitis C or B virus-infected and cirrhotic patients were at high risk of developing HCC, which represented a target population, as it was considered inappropriate to use healthy individuals as controls.

In conclusion, the present meta-analysis indicated that SCCA and SCCA-IgM exhibit moderate diagnostic accuracy as novel tumor makers of HCC, although the value of the combination of SCCA/SCCA-IgM and AFP requires further investigation. Considering the significant bias on this topic, the results of published studies and present meta-analysis should be interpreted with caution. Further studies should be undertaken to investigate the value of the SCCA and SCCA-IgM for the diagnosis of HCC. In addition, a well-designed prospective and large-sample size study is required to rigorously evaluate the diagnostic accuracy of SCCA and SCCA-IgM and confirm whether they provide an additional diagnostic benefit when replacing or combined with other widely used biomarkers.

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