Review Article

Annexin A2 versus Alpha-fetoprotein in diagnosing hepatocellular carcinoma: A diagnostic meta- analysis

Abstract.

BACKGROUND: Alpha-feto protein (AFP) remains widely used for diagnosing hepatocellular carcinoma (HCC) despite its low sensitivity and specificity. Recently, Annexin A2, a highly expressed protein in HCC and almost undetectable in normal liver cells has been studied as a potential alternative.

OBJECTIVE: To synthesize evidence for the diagnostic accuracy of annexin A2 as an alternative to AFP in the diagnosis of hepatocellular carcinoma.

METHODS: PubMed, Embase, PsycINFO and the China National Knowledge Infrastructure (chkd-cnki) databases were searched without time constraints up to 2022. Meta-analysis was conducted using Meta-Disc software

RESULTS: 6 studies were meta-analyzed. The pooled sensitivity and specificity for Annexin A2 were 84% [95% CI :(80 – 87)], and 78% [95% CI :(71 – 84)] respectively, while AFP was 70% [95% CI :(66 – 74)] and 79% [95% CI :(72 – 85)] respectively. The pooled diagnostic odds ratio was 20.35 [95% CI :(9.76 – 42.42)] for Annexin A2, and 9.71 [95% CI :(5.27 – 17.88)] for AFP. The area under the curve (AUC) was 0.88 for Annexin A2 and 0.82 for AFP.

CONCLUSIONS: Annexin A2 is significantly more sensitive than AFP for HCC diagnosis, but less specific. A combination of Annexin A2 and AFP could improve accuracy.

Key words: Annexin A2; Alpha-feto Protein; Meta-analysis, Hepatocellular carcinoma;

1 Introduction

Hepatocellular carcinoma (HCC) is a primary liver cancer that accounts for over 90% of all liver cancers and has an estimated annual mortality of 782,000 [1]. It is ranked 6th in incidence among all cancers globally, with East Asia and Africa contributing about 50% of the incidence. [2-3]. In China, the 5-year survival rate for HCC is just about 12%, attributed mainly to its insidious onset and difficulty in early-stage diagnosis, as witnessed by majority late-stage diagnoses and resultant treatment inefficacies. Similarly, its 5-year post-operative recurrence rate is high, estimated to be between 50 – 80% [4-8]. Major risk factors for HCC include chronic Hepatitis B and Hepatitis C virus (HCV) infection, liver cirrhosis, alcohol abuse, and associated metabolic diseases [9-10].

Despite the recent advances in HCC diagnosis including the discovery of molecular techniques, major challenges still exist especially with early diagnosis. Mass screening is almost impossible as all the current diagnostic techniques in clinical use are either invasive or not suitable or very expensive [11-12]. Alpha-feto protein (AFP) remains the only widely used non-invasive test for both diagnosis and prognosis follow up although its sensitivity and specificity is markedly reduced. For instance, it was noted that much as AFP levels above 500 ng/ml is highly specific for HCC, about 80% of patients with small cell HCC show no increase in AFP concentration [13]. It is thus paramount that other biomarkers are looked for to complement or replace AFP for improved early diagnosis of HCC.

Annexin A2 is an inducible, calcium-dependent phospholipid binding protein that is primarily expressed in the endothelial cells, mononuclear cells, macrophages, and marrow cells [14-15]. Its major functions include regulation of angiogenesis, cell proliferation, adhesion, migration, invasion and apoptosis [16-17]. Annexin A2 is an attractive biomarker for HCC because its

levels are almost undetectable in normal liver cells and chronic hepatitis tissues, while being highly expressed in HCC, including early-stage HCC where AFP is undetectable [18-19]. This differential expression in HCC and normal liver tissues prompted several groups to explore annexin A2 as a potential diagnostic marker for HCC albeit with conflicting results. In this study, we systematically evaluated and synthesized the results of those published articles to generate evidence for the diagnostic accuracy of annexin A2 compared to AFP in the diagnosis of hepatocellular carcinoma.

2 Materials and methods

2.1. Study design

A literature review and meta- analysis was conducted

2.2. Search strategy

Three independent investigators conducted comprehensive systematic reviews of literature on studies reporting the diagnostic accuracy of Annexin A2 in HCC. There was no time constraints on data search. PubMed, Embase, PsycINFO and the China National Knowledge Infrastructure (chkd-cnki) databases were searched without time constraints up to 2022. Additionally, references from the selected articles were manually searched for further eligible studies. The last search was conducted on 3rd January 2022. The following key words were used in the search: ANXA2: ANXA2, Annexin A2, Annexin IIAND HCC: HCC, Hepatocellular carcinoma, Liver cell carcinoma, Liver cancer. Both free text and MeSH terms search for keywords were utilized.

2.3. Inclusion and exclusion criteria:

We included studies in the meta-analysis if they fulfilled the following criteria: 1) were original articles that compared the diagnostic accuracy of annexin A2 and AFP in the same patients and used blood as the only sample type. 2) Had HCC diagnosed by either pathology slide

examination or radiologically by magnetic resonance imaging (MRI) and computer tomography (CT), and either of these techniques showed a nodule with arterial hyper-vascularization >2cm [20].3) Had sensitivity and specificity values for both annexin and AFP. 4) Their data were not part of a duplicate publication. 5) Were written and published in English.

Studies were excluded if they had the following: 1) were reviews, letters, case reports and case series, editorials or comments. 2) Had ambiguous diagnostic criteria. 3) Did not have sensitivity and specificity values for either annexin A2 or AFP or both. 4) Did not have sufficient information to make conclusive judgement on the results or were part of another publication. 5) Lacked a control group

2.4. Study selection

Based on the search strategy, we retrieved the full texts of articles whose titles and abstracts matched the search criteria and conducted further assessment. Three independent reviewers did the assessments. Any doubts that arose were settled by consensus. Where results obtained from the same patient population were reported in multiple publications, the most recent report was taken to avoid overlaps between cohorts.

2.5. Data extraction

We extracted data on the following sub-themes: First Author, Year of publication, Journal, Study design, Number of patients, Reference test, index test assay type, Cut off value, and raw data on sensitivity and specificity, *Table 1.* Data extraction was conducted by two independent reviewers.

2.6. Assessment of methodological quality

We assessed publication quality using the QUADAS (Quality Assessment of studies of Diagnostic Accuracy included in Systematic reviews) checklist recommended by the Cochrane

Collaboration. Each of the items in the QUADAS checklist was scored as “yes”, “no”, or “unclear” [21].

2.7. Representative patient spectrum

Hepatocellular carcinoma typically results from chronic liver disease and cirrhosis as such these are the target population for serum markers such as annexin A2 [2]. Studies that recruited patients with chronic liver disease or liver cirrhosis who were suspected to have HCC were scored as “Yes” while those that recruited healthy patients and those known to have HCC scored “No”. Studies with insufficient information to make conclusive judgment were scored “unclear”.

2.8. Acceptable reference standard

Histopathology slide examination under the microscopy by a pathologist is the currently acceptable reference standard for HCC diagnosis. In the absence of histopathology, radiological diagnosis is recommended using an ultrasound, CT, or MRI. Diagnosis is confirmed when either of them shows a nodule with arterial hyper vascularization>2 cm [20]. Studies that used the above reference standards were scored “yes” while those that used neither histopathology nor radiology were scored “no”. Studies with insufficient information were scored “unclear”.

2.9. Suitable time between reference standard and index test

HCC is a chronic disease and is unlikely to spontaneously disappear. Studies where samples were collected before interventions were therefore scored “Yes” while those where samples were collected after initiation of treatment were scored “No”. Studies without sufficient information were scored “unclear”.

2.10. Sample verification by reference standard

Studies where all the patients were tested with both the annexin A2 and AFP assays and whose disease statuses were confirmed by the appropriate reference standard were scored “yes”, while those where some patients missed being tested with the reference assay were scored “No”.

2.11. Consistency of reference standard

Studies where the diagnosis of hepatocellular carcinoma in all the patients were confirmed by the same reference standard (histopathology or imaging techniques) were scored “yes” while those in which the diagnosis of HCC in one group was confirmed by histopathology and the next group by radiology were scored a “No”. Studies with insufficient information to make a conclusive judgment were scored “unclear”.

2.12. Reference standard independent of index test

Studies that did not include annexin A2 and AFP in the reference standard were scored “yes”, while those that included annexin A2 and AFP in the reference standard were scored “no”.

2.13. Reference standard blinded

Studies where the annexin A2 and AFP assays were conducted by technicians blinded to the results of the reference test were scored “yes” while those that did not blind the technicians were scored “No”. Studies that didn’t provide sufficient information were scored “unclear”.

2.14. Index test blinded

Studies where confirmation of all the patients’ disease statuses by the reference standard were conducted without prior knowledge of the annexin A2 and AFP results scored “Yes”, while those where annexin A2 and AFP results were known prior to the reference test were scored “No”.

2.15. Relevant clinical information

Studies having all the relevant clinical information available during test interpretation as would have been the case in clinical practice were scored “yes”, while those that did not have relevant

clinical information during test interpretation were scored “No”. Studies without sufficient information were scored “unclear”.

2.16. Uninterpretable/intermediate test results reported

Studies where all uninterpretable or intermediate results were reported scored “yes” while those that did not report scored “No”. Studies with insufficient information to make a decision were scored “unclear”.

2.17. Explained withdrawals

Studies where detailed patient information including withdrawals from the study and reasons for withdrawal were reported scored “Yes”, while those that did not report withdrawals scored a “No”.

2.18. Diagnostic accuracy measures, meta- analysis and additional analysis

Using the Meta-Disc software, summary of pooled sensitivity, specificity and diagnostic odds ratio (DOR) were calculated for both Annexin A2 and AFP. Graphical summaries were presented in forest plots and receiver operating characteristic curves. Heterogeneity due to threshold effect was investigated using the spearman correlation coefficient, while heterogeneity due to factors other than threshold effect was investigated by: 1) Visual inspection of the forest plots for degree of deviation of sensitivity and specificity of each study from the vertical line corresponding to the pooled estimates; 2) Chi-square test and; 3) Inconsistence index calculation.

3. Results.

From database searches, 244 studies were found. After removal of duplicates and screening according to the inclusion and exclusion criteria, 28 articles were included for full text assessment. Here 23 articles got excluded for the following reasons: 1) Did not have sensitivity or specificity record [n = 8]; 2) Did not compare annexin A2 against AFP [n = 7]; 3) Had

irrelevant information or ambiguous results [n = 7]. As a result, only 6 articles remained eligible for meta-analysis. *Figure*

3.1.Quality assessment.

The QUADAS quality assessment tool was utilized to assess the studies. *Table 2*. QUADAS does not allow for calculation of summary scores as a measure of quality as they may be potentially misleading. The overall quality of the studies was average. Sample sizes were quite low in all the studies, reducing their power significantly. All the studies were retrospective case-controls and all had healthy control patients included. None of the researchers was blinded to the reference standard results and withdrawals were not reported in all but one study.

3.2.Summary of the diagnostic accuracy of Annexin A2 Vs AFP in HCC.

The pooled sensitivity and specificity for Annexin A2 was 84% [95% CI :(80 – 87)], and 78% [95% CI :(71 – 84)] respectively, while that of AFP was 70% [95% CI :(66 – 74)] and 79% [95% CI :(72 – 85)] respectively. The pooled diagnostic odds ratio was 20.35 [95% CI :(9.76 – 42.42)] for Annexin A2, and 9.71 [95% CI :(5.27 – 17.88)] for AFP. The positive likelihood ratios were 3.78 [95% CI: 2.52 – 5.68] for Annexin A2 and 3.15 [95% CI: 2.22 – 4.47] for AFP. Lastly the negative likelihood ratios were 0.23 [95% CI: 0.17 – 0.31] for Annexin A2 and 0.36 [95% CI: 0.28 – 0.46] for AFP. *Figure 2-3.*

3.3.Area under the curve

The SROC approach is the standard method of conducting meta-analysis of diagnostic accuracy test. By using the diagnostic odds ratio (DOR) as the main outcome, it eliminates the need to incorporate a threshold in the plot, since threshold varies from study to study [22, 23]. We used the DerSimonian-Laird random effect model to fit the curves. The area under the sROC curve

(AUC) was 0.88 for Annexin A2 and 0.82 for AFP while the Cochrane index (Q*) was 0.81 and 0.75 for Annexin A2 and AFP respectively. *Figure 4.*

3.4.Heterogeneity among the studies

The Spearman correlation coefficient between the logit of sensitivity and logit of 1-specificity for both Annexin A2 and AFP are presented in *table 3*. The magnitude of deviation of the sensitivity and specificity estimates from the vertical line corresponding to the pooled estimates, inconsistence index (1-squared) and the Chi-square *p*-values are presented in the forest plots for each test.

3.5.Annexin A2 vs AFP for early diagnosis of HCC

Only one study [17] assessed the diagnostic potential of Annexin A2 in early diagnosis of HCC. In this study, the sensitivity and specificity of Annexin A2 in diagnosing early-stage HCC was 83.2% and 67.5%respectively, compared to that of AFP at 54.7% sensitivity and 87.4% specificity. A combination of the two improved sensitivity 87.4%, while specificity remained 68.3%, a bit lower than that for AFP alone.

4. Discussion

This study aimed at assessing the sensitivity, specificity and diagnostic odds ratio of Annexin A2 versus AFP in the diagnosis of hepatocellular carcinoma (HCC), with the intention to evaluate if Annexin A2 determination in peripheral blood can effectively replace or augment AFP as an alternative non-invasive marker for HCC. To date, AFP remains the most widely used non-invasive biomarker for HCC diagnosis despite decades of studies demonstrating its low sensitivity. Gupta et al. [24] reviewed various studies and found out that AFP sensitivity ranged from 41- 65%, while Zhang et al. [25] in a large-scale multicenter cohort, found that AFP positivity rate was only 46% for all HCC and as low as 23.4% for small HCC (< 2cm), indicating

that nearly half of HCC patients are AFP negative especially small HCC. Annexin A2 is an attractive biomarker for HCC because its levels are almost undetectable in normal liver cells and chronic hepatitis tissues, while being highly expressed in HCC, including early-stage HCC where AFP is undetectable [18, 19].

Results of the pooled sensitivity, specificity and diagnostic odds ratio obtained demonstrate that Annexin A2 is significantly more sensitive but slightly less specific than AFP for the diagnosis of HCC. Annexin A2 having a pooled DOR of 20.35 compared to 9.71 for AFP means that Annexin A2 is much more likely that AFP to detect HCC in patients who truly have HCC than those who do not have it. However, cancer being diverse in nature and having varying etiologies and a complex pathophysiology means that one biomarker alone may not be sufficient for accurate and reliable diagnosis, and so a combination of Annexin A2 and AFP can reinforce each other. Having confirmed that Annexin A2 and AFP are not correlated and so measuring both in serum could reciprocally improve the overall diagnostic value, Sun et al. [17] assessed the diagnostic value of the combination for HCC and found out excellent result, especially for stage 0 and stage 1 HCC. The AUC of the combination was 0.85 compared to 0.79 for Annexin A2 and 0.73 for AFP individually. This is a promising finding and so further studies should explore it in a larger group of patients.

In this study, we restricted the analysis to only studies that directly compared Annexin A2 and AFP in the same group of patients. This was intentional to avoid bias, however the studies were still heterogeneous as observed in the forest plots, the Chi-square results and the inconsistence index (I^2) results. During investigation of the possible causes of heterogeneity, a relatively strong positive spearman correlation coefficient for Annexin A2, (0.6) was obtained while that for AFP was a negative spearman correlation. This meant that ‘threshold effect’ was not a possible cause

of heterogeneity in the Annexin A2 group while it could have been the cause in the AFP group. This is likely because the mean cutoffs in the Annexin A2 group was 18.47±6.14 compared to 23.45±15.61 in the AFP group. Given the low sample sizes and the generally low quality of the studies, study design and patient factors are the likely sources of heterogeneity.

We could not conduct publication bias analysis for this study because; 1) the number of studies analyzed was low and; 2) the funnel plot method used for conducting publication bias in general meta-analysis studies can be very misleading for diagnostic test accuracy studies and the alternatives aren't so goo either [26].

This study had the following limitations: 1) Only one of the studies was specifically designed to determine the diagnostic significance of Annexin A2 vs AFP for the diagnosis of HCC, and so the other could have had significant design flows that limit their power. 2) The overall sample size in the study was quite low and this equally limits the power of the study. 3) The cut off values, especially for AFP varied significantly among the different studies. This could have had an impact on the combined effect size obtained.

Conclusion

In conclusion therefore, this study found out that Annexin A2 is more sensitive than AFP for the diagnosis of HCC, including early stages of the disease. It is however slightly less specific than AFP and so a combination of the two could reinforce each other and greatly enhance accuracy in HCC diagnosis. The overall finding holds a promise for improving non-invasive HCC diagnosis and so should be studied further, in a larger trial.

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Tables

Table 1: Characteristics of the included studies.

Studies	Study design	HCC/Control	Annexin A2		AFP	
			Assay type	Cut offs	Assay type	Cut offs
Shaker 2017	Case-control	40/15 (65)	ELISA	18ng/ml	Chemiluminescence immuno assay	19.8ng/ml
Amany 2013	Case-control	70/20 (90)	ELISA	18ng/ml	Chemiluminescence immuno assay	32.0ng/ml
El-Abd 2015	Case-control	50/20 (70)	ELISA	29.3ng/ml	Chemiluminescence immuno assay	12.9ng/ml
Zhang 2012	Case-control	115/30 (145)	ELISA	18ng/ml	Radioimmunoassay	50.0ng/ml
Sun 2013	Case-control	175/49 (224)	ELISA	17.43ng/ul	Chemiluminescence immuno assay	20.0ng/ml
Hanno 2019	Case-control	40/40 (80)	ELISA	10.1ng/ml	Enzyme linked immunosorbent assay	6.0ng/ml

ELISA: Enzyme linked immunosorbent assay; HCC: Hepatocellular carcinoma; AFP: Alpha-feto protein

Table 2. A Summary of the methodological quality assessment of included studies using QUADAS checklist.

Checklist questions	**Studies**					
	Shaker et al.	Amany et al.	El-Abd et al.	Zhang et al.	Sun et al.	Hanno et al.
Representative spectrum?	N	N	N	N	N	Y
Acceptable reference standard?	Y	Y	Y	Y	Y	Y
Acceptable delay between tests?	Y	Y	Y	Y	Y	Y
Partial verification avoided?	Y	Y	Y	Y	Y	Y
Differential verification avoided?	Y	Y	Y	Y	Y	Y
Incorporation avoided?	Y	Y	Y	Y	Y	Y
Reference standard results blinded?	N	N	N	N	N	N
Index test results blinded?	Y	Y	Y	Y	Y	Y
Relevant clinical information?	Y	Y	Y	Y	Y	Y
Uninterpretable results reported?	UC	UC	UC	UC	UC	UC
Withdrawals explained?	Y	UC	UC	UC	UC	UC

Key: Y = Yes, N = No, UC = Unclear.

Table 3. Spearman correlation coefficients.

Parameters	Spearman coefficient	P-Value
Annexin A2	0.6	0.285
Alpha-feto protein	-0.103	0.870

Significance: $p<0.05$

Figures

Fig.1.

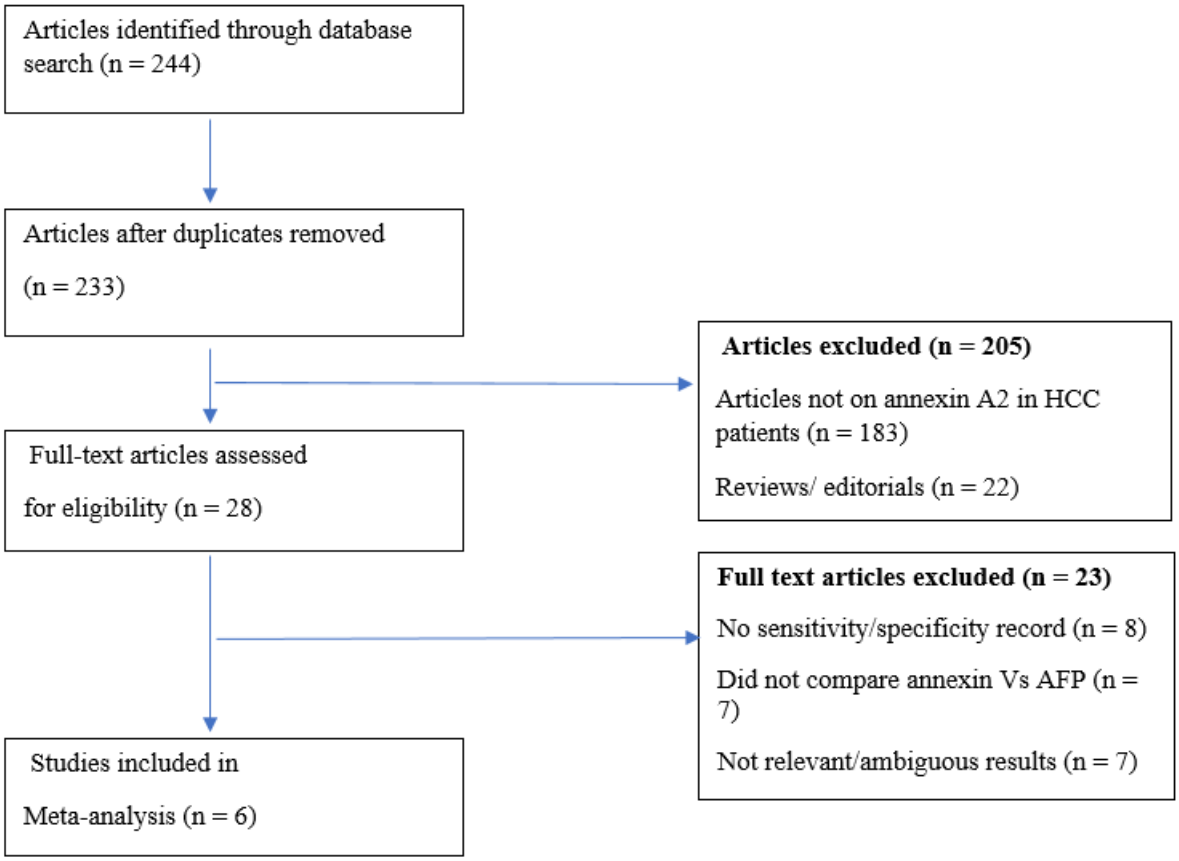


Figure 1. Study selection map showing the literature search, evaluation and inclusion and exclusion.

Fig.2.

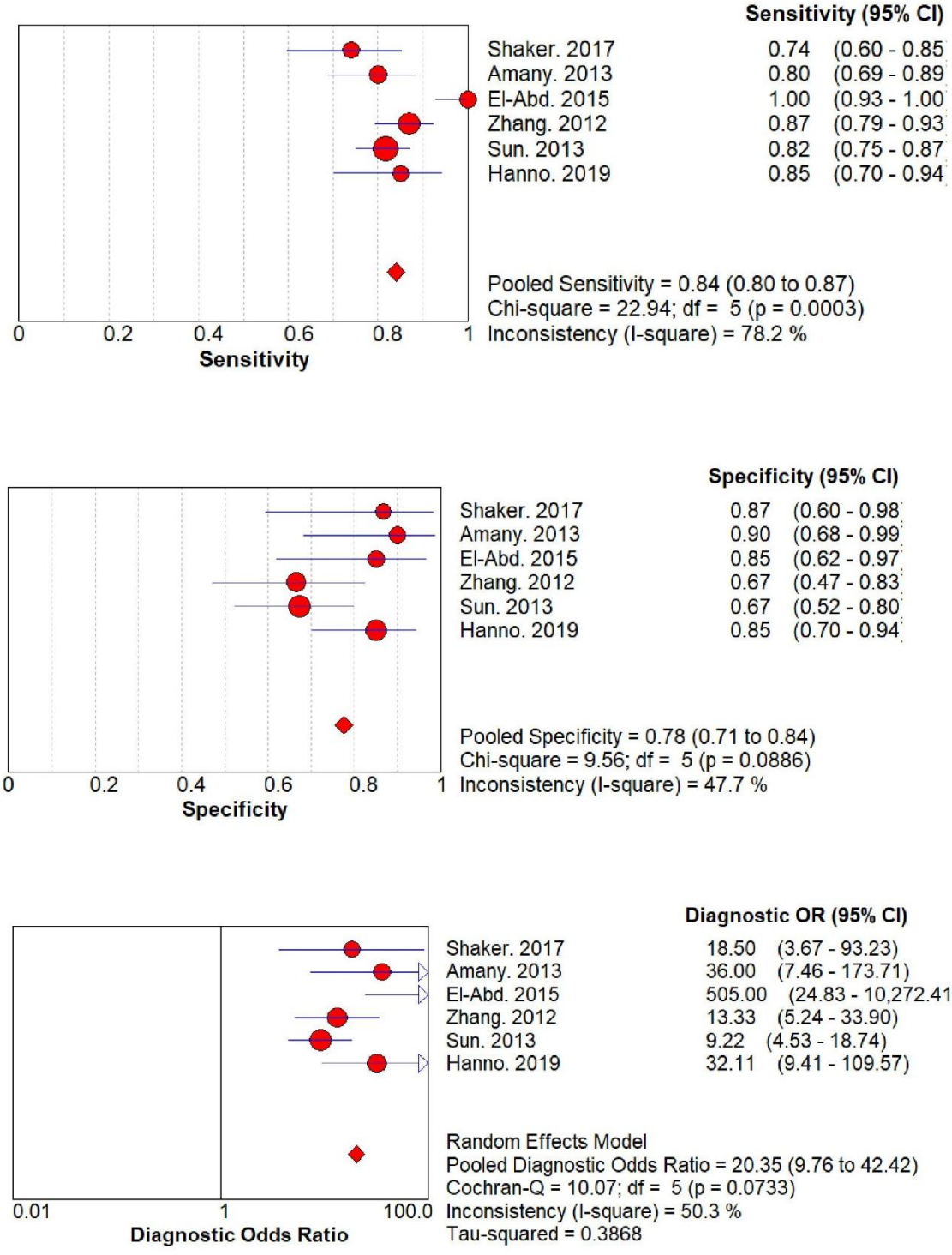


Figure 2. Sensitivity, Specificity and Diagnostic odds ratio of Annexin A2 in HCC.

Fig.3.

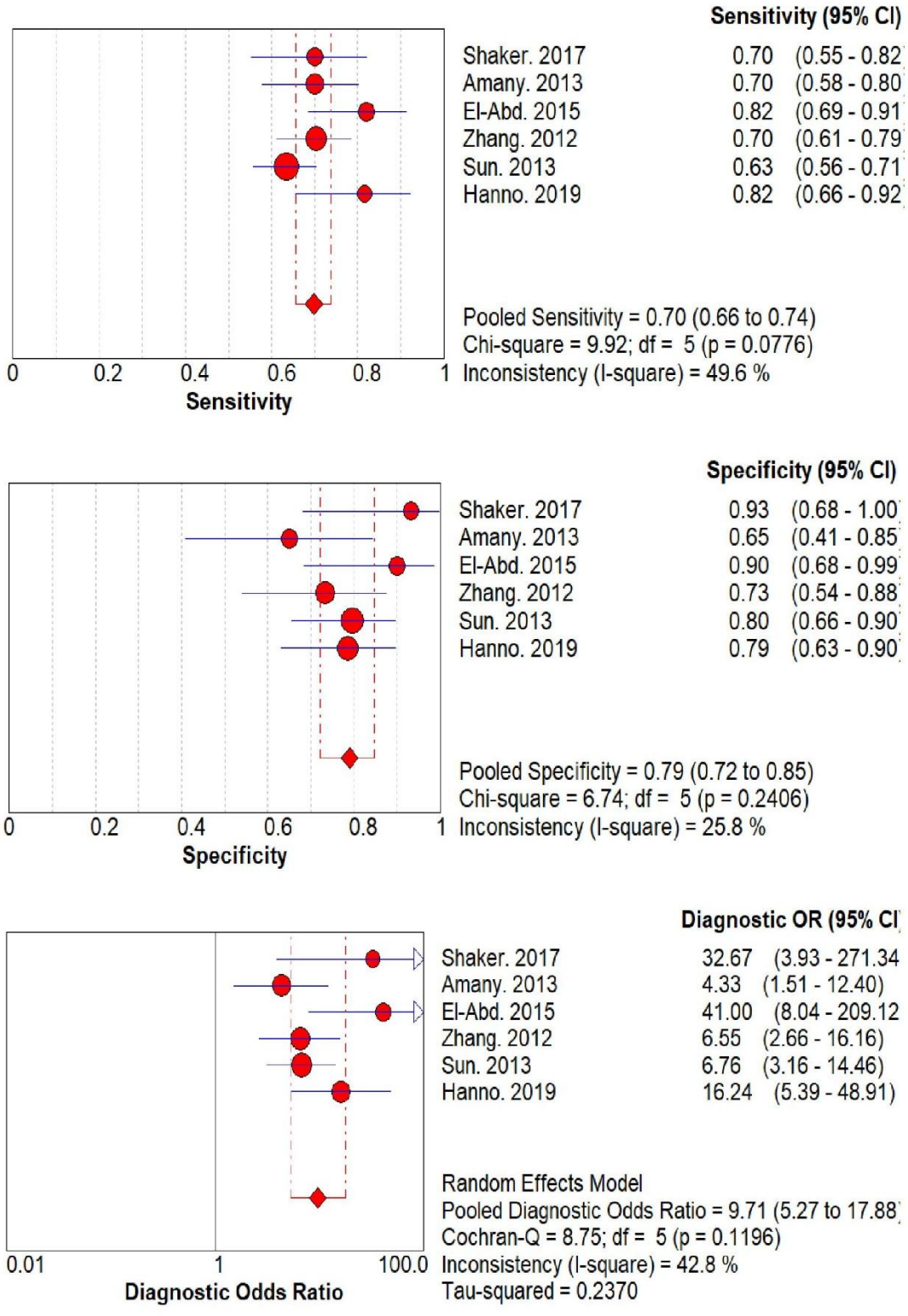
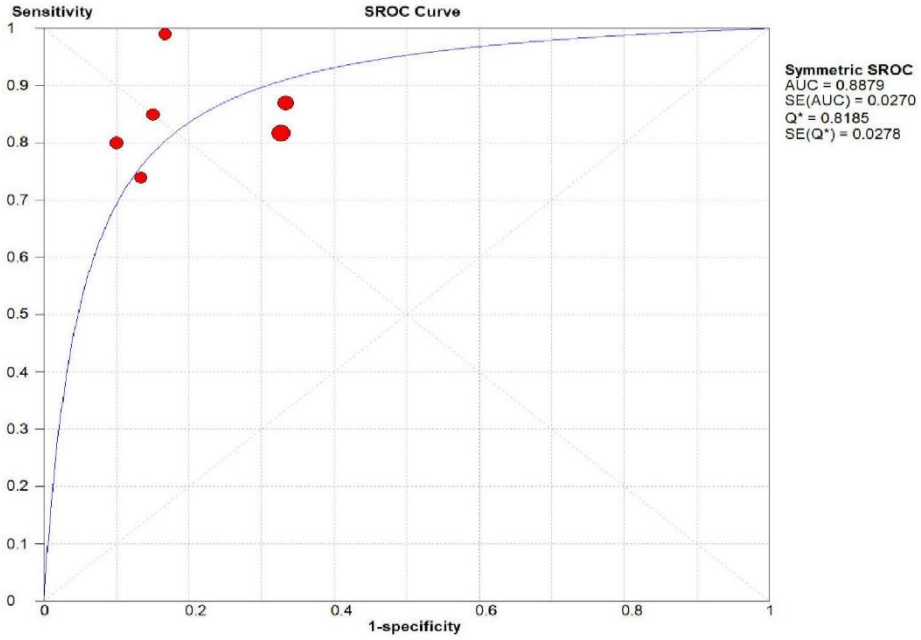
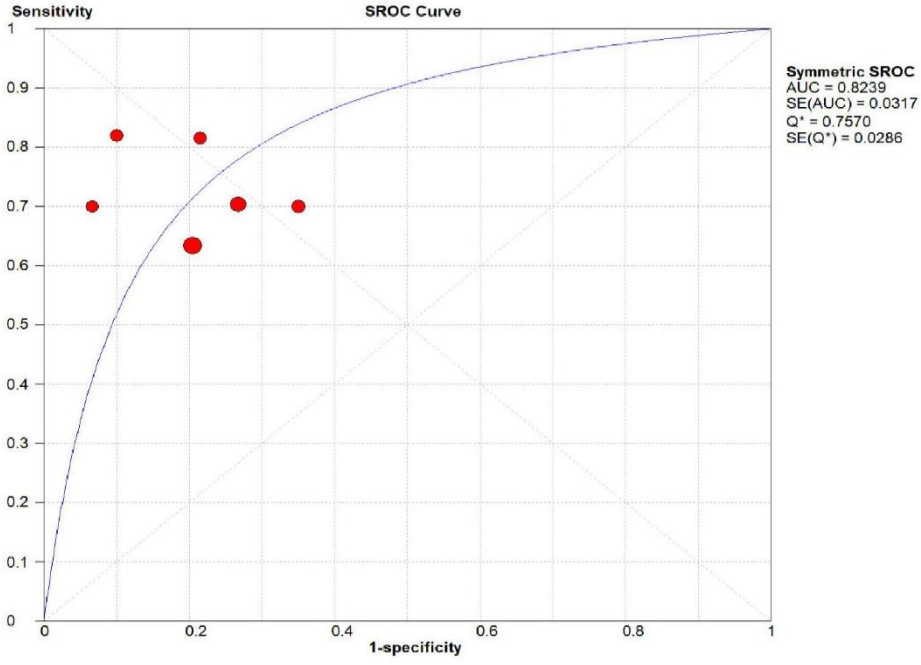


Figure 3. Sensitivity, Specificity and Diagnostic odds ratio of AFP in HCC.

Fig.4.



I.



II.

Figure 4. Annexin A2 (I) and AFP (II) receiver operating characteristic curves