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AFP-L3 for the diagnosis of early hepatocellular carcinoma
A meta-analysis

Jian-Ming Zhou, PhD [iD], Ting Wang, PhD, Kun-He Zhang, PhD*

Abstract

Background: The present study aimed to systematically evaluate the diagnostic value of an isoform of alpha-fetoprotein (AFP), AFP-L3, for early hepatocellular carcinoma (HCC) by a meta-analysis.

Methods: Diagnostic reports of AFP-L3% in early HCC were searched in the PubMed, Web of Science, Cochrane Library, and Embase databases up to December 2019. The retrieved literature was reviewed, and eligible articles were selected. Data were extracted from the eligible articles, and the risk of bias was evaluated according to the Quality Assessment of Diagnostic Accuracy Studies scale. Statistical analyses were conducted by MetaDiSc1.4 and RevMan5.3 software. The sensitivities, specificities, and diagnostic odds ratios were pooled. The summary receiver operating characteristic curve was drawn, and the area under the curve was calculated.

Results: Six studies with acceptable quality were included in the meta-analysis involving 2447 patients. No threshold effect was observed among the 6 studies, but there was obvious heterogeneity. The pooled sensitivity, specificity, and positive and negative likelihood ratios of AFP-L3% for the diagnosis of early HCC were 0.34 (95% CI 0.30–0.39, $P < .0001$), 0.92 (95% CI 0.91–0.93, $P < .0001$), 4.46 (95% CI 2.94–6.77, $P = .0033$), and 0.71 (95% CI 0.61–0.82, $P = .0004$), respectively. The diagnostic odds ratio was 6.78 (95% CI 4.02–11.44, $P = .0074$). The the area under the curve of the summary receiver operating characteristic was 0.755 (95% CI 0.57–0.94).

Conclusion: AFP-L3% has high specificity but low sensitivity for diagnose early HCC, suggesting that AFP-L3% is more valuable for excluding HCC in conditions with elevated AFP than for diagnosing early HCC. In addition, a hypersensitive detection method can improve the diagnostic accuracy of AFP-L3% for early HCC.

Abbreviations: μTAS = micro total analysis systems, AFP = alpha-fetoprotein, AUC = the area under the curve, DCP = descarboxy prothrombin, HCC = hepatocellular carcinoma, ROC = receiver operating characteristic, SROC = summary receiver operating characteristic.

Keywords: alpha-fetoprotein isoform L3, biomarker, diagnostic performance, early diagnosis, hepatocellular carcinoma, meta-analysis

1. Introduction

Liver cancer is a common malignant tumor of the digestive system. According to the Global Cancer Report 2018 released by the International Agency for Research on Cancer (IARC), it is estimated that there were approximately 841,000 new liver cancer cases and 782,000 deaths in 2018, ranking liver cancer as the fourth leading cause of cancer death worldwide.[1] Most of the new cases occurred in underdeveloped countries, especially in China. Hepatocellular carcinoma (HCC) is a major type of primary liver cancer. The prognosis of patients with HCC is related to the tumor stage at the time of diagnosis.[2] Because of the lack of obvious clinical symptoms and signs in the early stages, most HCC is already in advanced stages when diagnosed, and thus, the prognosis is poor. Therefore, one of the key strategies to improving the prognosis of HCC is to diagnose the tumor in the early stages when curative treatment is still possible.

Currently, the most frequently used diagnostic method for HCC is imaging combined with tumor marker evaluations.[3–5] Tumor markers, as simple and inexpensive indicators, have been widely used in clinical practice. Alpha fetoprotein (AFP), which was discovered in 1963, has become the most important serum

marker for diagnose HCC and is also widely used in postoperative recurrence monitoring.[6,7] Unfortunately, the positive rate of AFP expression in HCC is only approximately 70%,[8] and nearly 40% of HCC is AFP-negative.[9–11] Due to the elevation of AFP in some active liver diseases, reproductive system tumors and pregnancy, the diagnostic specificity of AFP for HCC is impacted.

In addition to AFP, several new serum biomarkers such as des-carboxy prothrombin (DCP), glypican-3, osteopontin have been proposed for early HCC detection. Serum DCP exhibited sensitivities of 48–62%, specificities of 81–98%, and accuracies of 59–84% for diagnosing HCC.[12,13] Other, more recent studies show glypican-3 levels play a potential role for early HCC detection that can be used for HCC screening. Liu et al.[14] found that the proportion of GPC3-positive was significantly higher in early HCC than AFP (76.4% vs. 64.3%). The similar result also found at Shang et al.[15] study, for early stage HCC, osteopontin had a better sensitivity than AFP (75% vs 46%), but the specificity is lower than AFP (62% vs 93%). Among these new biomarkers, none of them has both high sensitivity and high specificity for early diagnosis of liver cancer. We therefore need to continue efforts to find new serum markers complementary to AFP.

A subtype of AFP, lens culinary agglutinin-reactive fraction of fetoprotein (AFP-L3), is derived from cancerous hepatocytes and considered to be specific to HCC.[16] The proportion of AFP-L3 to total AFP (AFP-L3%) can be used to diagnose early HCC. Early studies reported that the diagnostic sensitivity of AFP-L3% for HCC ranged from 75.0% to 96.9% with a specificity of 90.0–92.0%.[17,18] A few months or even years before the diagnosis of HCC, serum AFP-L3 levels could increase in the blood; a sensitivity of 39% was observed in small HCC (<2 cm).[19] Recent studies showed that newer and simpler methods could be more sensitive in detecting serum AFP-L3%, thus enhancing the potential value of this marker in the diagnosis of early liver cancer.[20,21]

At present, AFP-L3% has not been widely used as a conventional indicator for the clinical diagnosis of HCC. A previous study of AFP-L3% for diagnosing HCC showed a low pooled sensitivity (0.483), but the diagnostic performance of AFP-L3% for early HCC was not evaluated.[22] Hence, we performed a systematic review and meta-analysis of the diagnostic value of AFP-L3% for early HCC.

2. Materials and methods

2.1. Literature retrieval and screening

There is no need for ethical approval because the data used in this study have been published. Articles on AFP-L3% for the diagnosis of early liver cancer were searched from January 1960 to December 2019 in the PubMed, Embase, Cochrane Library, and Web of Science databases. The search terms were liver cancer subject words and free words and AFP-L3 subject words and free words. Two researchers independently screened the eligible literature according to the following inclusion and exclusion criteria.

2.1.1. Inclusion criteria for articles. Studies that are involved in the evaluation of AFP-L3% for diagnose early HCC (defined as HCC patients at Barcelona clinic liver cancer stage 0/A or TNM stage I); and articles that contain the number of true-positive,

false-positive, true-negative, and false-negative diagnoses of early liver cancer.

2.1.2. Exclusion criteria for articles. Publications that are considered case reports, reviews, meta-analyses, abstracts, and editorials, animal studies, or cell experiments; articles that do not apply the definition of early HCC mentioned above; articles without the complete data needed to obtain the sensitivity and specificity; and duplicate articles.

2.2. Data extraction and quality evaluation

Two researchers independently reviewed the eligible articles and extracted the following data: basic information (paper title, publication year, first author, sample size, research type), cutoff value of AFP-L3%, true positives, true negatives, false positives, and false negatives. A third party was available to resolve any discrepancies between the sets of extracted data.

The quality of all eligible studies was evaluated and analyzed by 2 authors according to the Quality Assessment of Diagnostic Accuracy Studies scale,[23] and disagreements were discussed and resolved. A third author assisted in the evaluation if the discussion failed.

2.3. Statistical analyses

Review Manager 5.3 software (The Cochrane Collaboration, Copenhagen, Denmark), MetaDisc1.4 software (XI Cochrane Colloquium, Barcelona, Spain) were used for analysis. Spearman correlation analysis and a distribution map of the receiver operating characteristic (ROC) curve were obtained to determine whether there was a threshold effect in the eligible studies. If the correlation coefficient was insignificant (with $P > .05$) or the ROC curve did not show a shoulder-arm distribution, then there was no threshold effect, and the sensitivity, specificity, and positive and negative likelihood ratios were pooled by Meta-DiSc1.4 software, and the area under the summary ROC (SROC) curve (AUC) was obtained by SROC fitting analysis; otherwise, only SROC fitting analysis was performed. There was no heterogeneity when $P > .10$ and $I^2 \leq 50\%$, moderate heterogeneity when $P \leq .10$ and $50\% \leq I^2 \leq 70\%$, and high heterogeneity when $P \leq .10$ and $I^2 > 70\%$. All statistical tests were 2-sided, and $P < .05$ was considered statistically significant.

3. Results

3.1. Results of literature retrieval and screening and quality of the studies

One thousand two hundred and twenty published records were retrieved. After screening according to the inclusion and exclusion criteria, 6 articles were eligible,[24–29] and a total of 2447 patients were included in the analysis. The literature screening process and results are shown in Fig. 1. The basic characteristics of the 6 eligible studies are shown in Table 1, and the quality of the 6 eligible articles evaluated by using the Quality Assessment of Diagnostic Accuracy Studies tool is shown in Fig. 2.

3.2. Meta-analysis results
3.2.1. Threshold effect. The Spearman correlation coefficient was 0.771, and the P value was .072, indicating that there was no threshold effect. Based on the distribution of each eligible study,
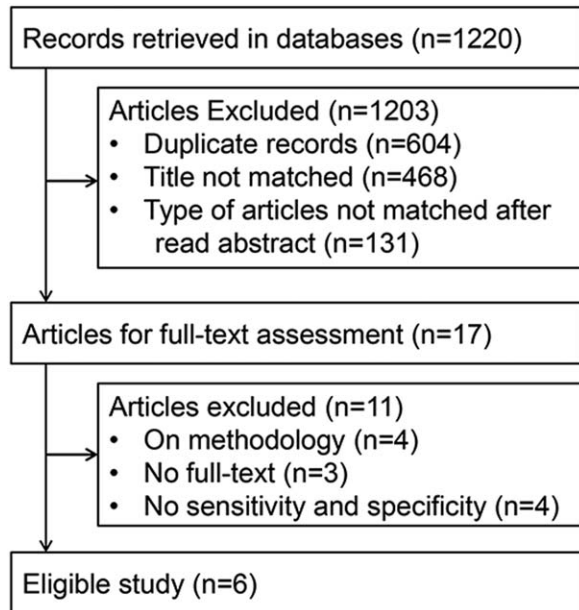
Figure 1. Flow diagram of the article selection process for meta-analysis.

Figure 2. Risk of bias and applicability concerns of the 6 articles in the meta-analysis.

there were no obvious "shoulder-arm" distributions, which further indicate that there was no threshold effect.

3.2.2. Heterogeneity test. The sensitivity, specificity, and diagnostic odds ratio of each eligible study were combined with MetaDisc1.4 software and yielded I^2 values of 83.5%, 89.4%, and 68.4% (Fig. 3), respectively, indicating the presence of obvious heterogeneity.

3.2.3. Quantitative analysis of the merger effect. The random effect model was used to analyze the pooled diagnostic performance because of the high heterogeneity among the studies, and the results are shown in Figures 3 and 4 and summarized in Table 2.

4. Discussion

In the present study, 6 articles involving 2447 patients were included. The results showed that AFP-L3% has a high pooled specificity (92%), low pooled sensitivity (34%), and moderate AUC of the summary ROC curve (0.755) for diagnose early HCC, with a pooled diagnostic odds ratio of 6.78.
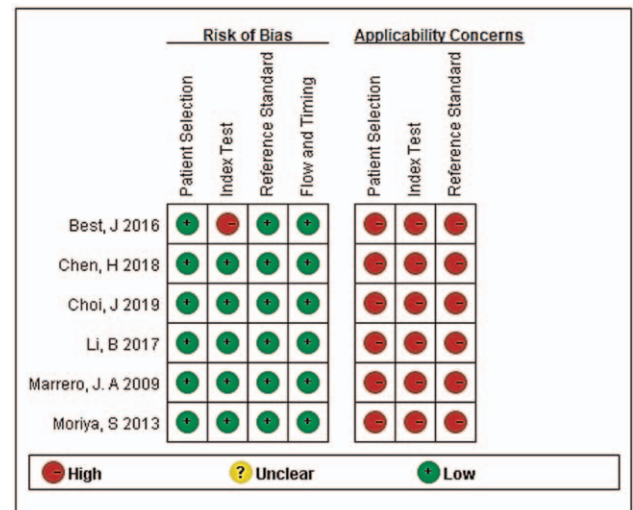
Most of the articles had a low risk of bias and high applicability of concerns, but obvious heterogeneity was found among the studies, so we chose the random effect model to perform the pooled analysis. To identify the source of heterogeneity, a subgroup analysis was carried out based on the different methods of detecting AFP-L3%. The results showed that the micro total analysis systems (μTAS) method group had no obvious heterogeneity, while the non-μTAS methods still had high heterogeneity, suggesting that the heterogeneity mainly comes from different detection methods. Indeed, compared with other methods, the μTAS method can improve the diagnostic sensitivity and specificity of AFP-L3% for early HCC.[30]

Abdominal ultrasound combined with AFP is widely used in the diagnosis of HCC. However, AFP has not been recommended as an essential indicator for screening early HCC given its suboptimal sensitivity and specificity when used alone. AFP was found to have a sensitivity ranging from 49% to 71% and specificity ranging from 49% to 86% in detecting tumors <5 cm in size when the cutoff value set at 20 ng/mL.[31] Hence, European guidelines recommend ultrasonography alone for HCC screening.[32] Although abdominal ultrasound is recommended for the screening of early HCC, it is not an ideal method in practice and is especially unsatisfactory for the detection of liver lesions in obese patients. A large difference in the sensitivity of abdominal

Table 1

The basic characteristics of the 6 studies on AFP-L3% for the diagnosis of early hepatocellular carcinoma (HCC).

							Patient number				
First author	Year	Country	Study design	Method	Cut-off	HCC stage	HCC/Control	TP	FP	FN	TN
Best, J	2016	Germany	Case control	μTAS	10%	BCLC 0/A	61/402	29	35	32	367
Chen, H	2018	China	Case control	ELISA	NA	TNM I	94/644	22	64	72	580
Choi, J	2019	Korea	Case control	uTAS	4%	BCLC 0/A	38/152	20	17	18	135
Li, B	2017	China	Case control	CLIA	3.84%	BCLC A	81/152	40	24	41	128
Marrero, J. A	2009	USA	Case control	NA	10%	BCLC 0/A	208/417	58	13	150	404
Moriya, S	2013	Japan	Case control	ELISA	10%	pTNM I	15/183	2	3	13	180

μTAS = micro total analysis systems, BCLC = Barcelona clinic liver cancer, CLIA = chemiluminescence immunoassay, ELISA = enzyme linked immunosorbent assay, FN = false negative, FP = false positive, NA = not available, TN = true negative, TP = true positive.
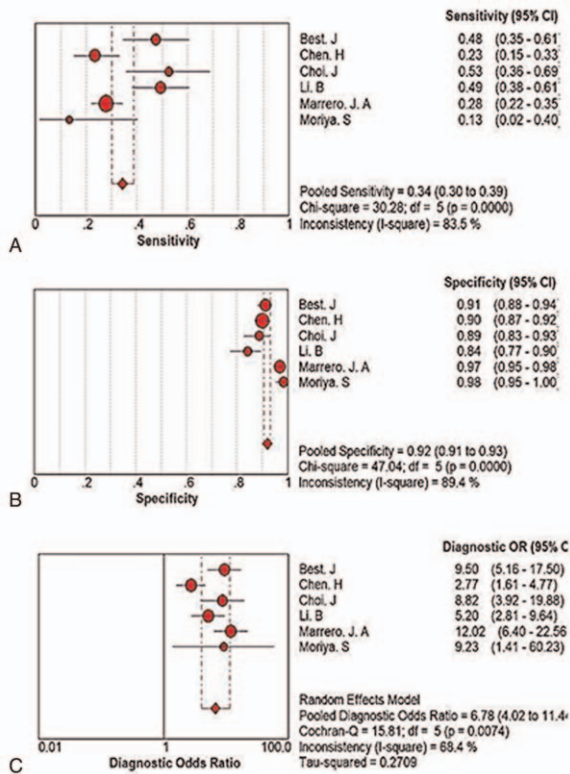
Figure 3. Forest plots of the diagnostic performance of AFP-L3% for early hepatocellular carcinoma. A: Sensitivity and related indicators. B: Specificity and related indicators. C: Diagnostic odds ratio and related indicators. AFP = alpha-fetoprotein.

The merger effects of AFP-L3 for the diagnosis of early hepatocellular carcinoma.

	Value (95% CI)	P
Pooled sensitivity	0.34 (0.30–0.39)	<.001
Pooled specificity	0.92 (0.91–0.93)	<.001
Pooled positive likelihood ratio	4.46 (2.94–6.77)	.003
Pooled negative likelihood ratio	0.71 (0.61–0.82)	<.001
Pooled diagnostic odds ratio	6.78 (4.02–11.44)	.007
Area under summary ROC curve	0.755 (0.57–0.94)	

ROC = receiver operating characteristic.

ultrasound for HCC detection (77% vs 21%) was found between BMI groups (<30 vs >30).[33] Therefore, continuous efforts have been made towards the discovery of new serum biomarkers; in the past decades, due to the minimally invasive nature, ease of repetition, and high consistency of these methods, many new serum biomarkers have been reported, although few have been used in clinics.[34,35] Serum chromogranin A and microRNA-107 expression levels could be potential diagnostic markers in early stage HCC.[36,37] Deng et al[38] found that serum pentraxin 3 was an independent risk factor of HCC and could identify hepatitis B virus-associated HCC with an AUC of 0.920 for early-stage HCC. However, further studies are needed to confirm these potential biomarkers.

Because of its low pooled sensitivity in the diagnosis of early HCC, AFP-L3% does not seem suitable for first-line screening of early HCC. However, AFP-L3% has been used as a reliable tumor biomarker for HCC and recommended by the Japanese Society of Hepatology.[39] Continuous efforts have been made to improve the performance of AFP-L3% in diagnose HCC,
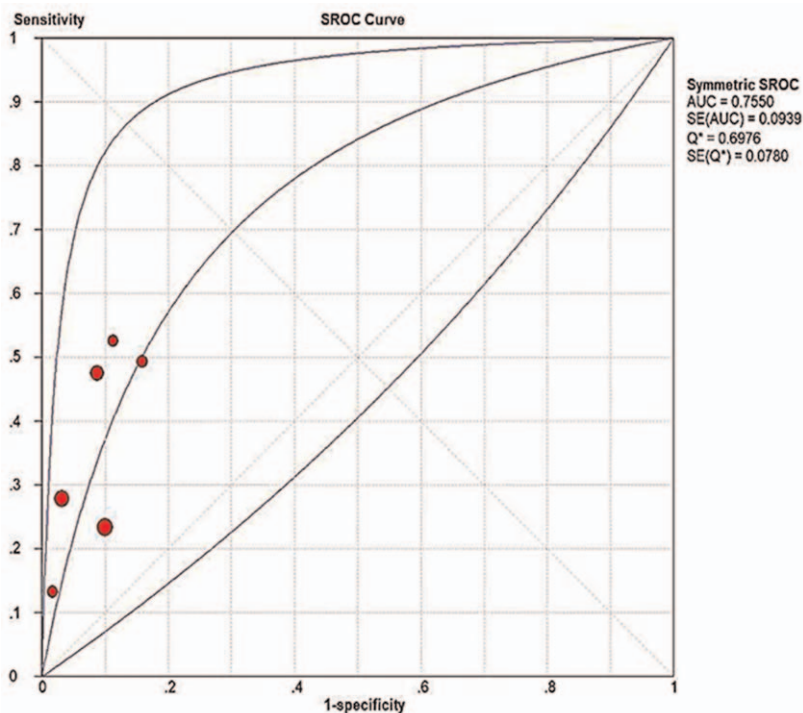
Figure 4. Summary receiver operating characteristic (SROC) curve of AFP-L3% for the diagnosis of early hepatocellular carcinoma. AFP = alpha-fetoprotein.

including the development of new methods and combining AFP-L3% with other biomarkers. Some hypersensitive methods have been reported, one of which has a lower detection limit of 12 pg/mL for AFP-L3 detection.[40–42] The GALAD score, based on the combination of sex, age, AFP, AFP-L3, and DCP, had a significantly improved diagnostic performance for early-stage HCC patients that was defined using the Milan criteria, with an AUC of 0.90 (sensitivity 68%; specificity 95.2%).[43,44]

Although AFP-L3% is not highly sensitive for early HCC, it has high specificity that cannot be surpassed by total AFP, with the advantage of differentiating HCC from benign liver diseases in patients with elevated serum AFP. In the differentiation of hepatitis C virus-related HCC and cirrhosis, the high specificity of AFP-L3% persisted in patients with low-level AFP elevation,[45] and a higher incidence of HCC was found in AFP-L3-positive cirrhosis patients than in AFP-positive cirrhosis patients. AFP-L3 was also highly related to biological malignancies of HCC, such as portal vein invasion and intrahepatic metastasis,[46] which may explain the usefulness of AFP-L3 in monitoring treatment response and the recurrence of HCC.[47–49]

There are some limitations in the present study: the data were extracted for subgroups of early HCC in 5 of 6 articles, which may lead to bias in the patient samples. Additionally, all 6 articles were case-control studies, which may present better results than the real world. The cutoff values of AFP-L3% were different among the 6 studies, which may affect the merged diagnostic indicator of AFP-L3% for the diagnosis of early liver cancer. The gold standard diagnostic method used in each study was different, which may produce bias. The analysis of the source of heterogeneity was not sufficient, and only a subgroup analysis of detection methods was performed due to the limited data.

In conclusion, the meta-analysis revealed that AFP-L3% exhibits high specificity and low sensitivity for diagnose early HCC, suggesting that AFP-L3% alone is not ideal for the screening of early HCC but valuable for the discrimination between malignant and benign diseases in patients with elevated total AFP. Among the current methods for AFP-L3 detection, the µTAS method is the best for improving the sensitivity of detection and new highly sensitive methods may provide more favorable results for diagnose HCC. The combination of AFP-L3% with other conventional serum biomarkers could improve its diagnostic performance and enable monitoring applications for AFP-L3% in early HCC. More attention should be paid to the advantage of the high specificity of AFP-L3% for diagnose early HCC, and this is a major direction for the future development of the clinical applications of AFP-L3%.

Author contributions

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Formal analysis: Jian-Ming Zhou.
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Writing – review & editing: Ting Wang, Kun-He Zhang.

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