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Evaluation of alpha-L-fucosidase for the diagnosis of hepatocellular carcinoma based on meta-analysis

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Abstract

Objectives: The main aim of the present study was to assess the diagnostic value of alpha-L-fucosidase (AFU) for hepatocellular carcinoma (HCC).

Methods: Studies that explored the diagnostic value of AFU in HCC were searched in EMBASE, SCI, and PUBMED. The sensitivity, specificity, and DOR about the accuracy of serum AFU in the diagnosis of HCC were pooled. The methodological quality of each article was evaluated with QUADAS-2 (quality assessment for studies of diagnostic accuracy 2). Receiver operating characteristic curves (ROC) analysis was performed. Statistical analysis was conducted by using Review Manager 5 and Open Meta-analyst.

Results: Eighteen studies were selected in this study. The pooled estimates for AFU vs. α -fetoprotein (AFP) in the diagnosis of HCC in 18 studies were as follows: sensitivity of 0.7352 (0.6827, 0.7818) vs. 0.7501 (0.6725, 0.8144), and specificity of 0.7681 (0.6946, 0.8283) vs. 0.8208 (0.7586, 0.8697), diagnostic odds ratio (DOR) of 7.974 (5.302, 11.993) vs. 13.401 (8.359, 21.483), area under the curve (AUC) of 0.7968 vs. 0.8451, respectively.

Conclusions: AFU is comparable to AFP for the diagnosis of HCC.

Keywords: alpha-L-fucosidase (AFU); diagnosis; hepatocellular carcinoma (HCC); meta-analysis; α -fetoprotein (AFP).

Introduction

In 2018, liver cancer morbidity and mortality ranked seventh and third among global tumors, respectively [1]. The number of new cases of liver cancer was 841,080 and the number of related deaths was 781,631. Hepatocellular carcinoma (HCC) is associated with chronic hepatitis B virus and chronic hepatitis C virus infection [2]. Due to the asymptomatic nature of early HCC and the lack of effective diagnostic and screening strategies, most patients (>80%) present with advanced HCC stages [3]. A lot of cases have shown that early detection of HCC and timely treatment is critical for improving patient survival. Tumor markers are pivotal tools for the early diagnosis of tumors. α -Fetoprotein (AFP) is a major marker widely used in the clinic for the detection of HCC [4]. However, AFP shows poor sensitivity and specificity in the early stages of HCC. Ba et al. (2012) revealed that AFP levels are not elevated in some HCC patients, while they were elevated in other patients with benign liver disease [5]. New biomarkers are urgently needed for specific early diagnosis of HCC patients.

Many groups have proposed alpha-L-fucosidase (AFU) as a tumor marker for the diagnosis of HCC. AFU is a lysosomal enzyme found in all mammalian cells and its activity in HCC patients was reported as significantly higher than that in patients with benign liver disease [6]. However, due to inconsistency among reports, diagnostic value of AFU is still not widely accepted. In this paper, we report a meta-analysis based on the results of published studies. The main aim is to assess the diagnostic value of AFU for HCC compared to AFP.

Methods

Inclusion criteria of the study

(1) No HCC treatment before sample collection, (2) the diagnostic values of AFU and AFP were tested in the same HCC patients, (3) sensitivity and specificity of AFP and AFU are reported or can be obtained by calculation, (4) patients of the control group had only other malignant liver cancer

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or benign liver disease, (5) all patients in the case group were diagnosed as HCC by gold standard assessment.

Exclusion criteria of the study

(1) The sample is from a tissue or another body fluid (i.e., not a serum sample), (2) the patient received HCC treatment before sampling (3) data on specificity and sensitivity are not available in the article, (4) animal study, (5) only had healthy people in control groups, or had no control group in studies.

Search and selection

The keywords selected included “alpha-L-fucosidase”, “AFU”, “hepatocellular carcinoma”, “HCC”, “ α -fetoprotein”, and “AFP”. Keywords search and free words search were adopted. Papers published in English were searched in EMBASE, SCI, and PUBMED, with no limit in year of publication. In addition, the references in the included papers were searched and analyzed.

The two authors (Lei Xi and Chunqing Yang) independently extracted data from the selected articles and solved any discrepancy through discussion. The following data came from each article: first author, publication year, country, AFU test method, number of HCC and control, gender ratio and average age of HCC patients, pathological stage and raw data (true positive(TP), false positive(FP), false negative(FN), and true negative(TN) subjects).

Evaluation of study quality

The quality of each article was evaluated with the new assessment tool, Quality Assessment Diagnostic Accuracy Study 2 (QUADAS-2). QUADAS-2 has two components: risk of bias and applicability concerns. The risk of bias includes four parts: patient selection, index testing, reference standard, flow and timing [7]. The applicability concerns include the first three parts of the risk of bias.

Data analysis

Open Meta-Analyst and Review Manager 5 software were used for statistical analysis [8, 9]. First, a heterogeneity test was conducted. Heterogeneity produced by threshold effects was analyzed by Spearman coefficient and received operational characteristic (ROC) plots [10]. If the

Spearman's value was about 1 and $p < 0.05$, a threshold effect was concluded. The heterogeneity index I^2 produced by the non-threshold effect was examined through the inconsistency test and statistical significance was concluded in case of $I^2 > 0$.

Meta-regression was used to analyze whether heterogeneity is caused by non-threshold effects. p -value < 0.05 was considered statistically significant. The plotted ROC curve was used to calculate the AUC and Q value. Sensitivity, specificity, and DOR were obtained using the diagnostic random effects model and the DerSimonian–Laird method.

Results

Search results

The search retrieved 169 related articles. After removing the doublets, 86 articles remained, of which 23 were eligible for full-text evaluation. The detailed flow chart of the article selection is shown in Figure 1. Five articles were excluded based on not fulfilling inclusion and exclusion criteria and the remaining 18 articles [11–28] were selected in this study. The features of the selected articles are shown in Tables 1 and 2. These articles involved a total of 2,224 patients, of which 1,053 patients with HCC were in the experimental group and 1,171 patients without HCC were in the control group.

Figure 1: Article selection process.

Table 1: Main features of the articles selected.

Author	Year	Country	Test method	HCC/control	Gender (M/F)	Average age, years	Stage
El-houseini	2001	Egypt	PNPF	50/50	37/11	63.7	Child-Pugh
El-houseini	2005	Egypt	PNPF	44/20	34/10	55	TNM
Shao	2009	China	PNPF	30/30	26/4	51.2	N
Zhu, J	2013	China	CNPF	113/102	95/18	55.3	N
Zhang	2015	China	PNPF	116/104	75/41	57.8	N
Wang	2014	China	CNPF	459/210	406/53	51	BCLC
Zhu, JN	2017	China	CNPF	36/36	21/15	50.2	N
Xing	2019	China	PNPF	325/187	157/30	53.7	Child-Pugh
Montaser	2012	Egypt	PNPF	40/40	33/7	54.88	Child-Pugh
El-tayeh	2012	Egypt	PNPF	37/59	29/8	55	TNM
Habachi	2018	Egypt	PNPF	86/89	N	N	Child-Pugh
Mossad	2014	Egypt	PNPF	40/30	14/26	57.3	Child-Pugh
Bukofzer	1989	South Africa	PNPF	72/64	N	N	N
Giardina	1992	Italy	PNPF	21/76	15/6	59.76	N
Hutchinson	1991	England	PNPF	35/35	27/8	46.5	N
Marotta	1991	Japan	PNPF	19/30	N	N	N
Takahashi	1994	Japan	PNPF	67/47	53/14	63.7	N
Tangkijvani	1999	Thailand	PNPF	60/150	50/10	56.5	N

PNPF, PNPF endpoint method; CNPF, CNPF kinetic rate method; N, none; TNM, tumor node metastasis; BCLC, Barcelona clinic liver cancer; M/F, male/female.

Table 2: Subjects' raw data of the articles selected.

Author ^a	TP	FP	FN	TN
El-houseini	35	7	15	43
El-houseini	36	9	8	11
Shao	20	12	10	18
Zhu, J	64	18	49	84
Zhang	80	40	34	69
Wang	124	92	61	182
Zhu, JN	28	13	8	23
Xing	105	100	82	225
Montaser	36	1	4	39
El-tayeh	27	8	10	57
Habachi	61	45	25	44
Mossad	35	4	5	36
Bukofzer	54	19	18	45
Giardina	16	7	5	69
Hutchinson	21	12	14	23
Marotta	16	3	3	27
Takahashi	52	10	15	37
Tangkijvani	49	44	11	106

^aRelated to AFU. TP, true positive; FP, false positive; FN, false negative; TN, true negative.

concentrated on “patient selection” and “index test”. In the “patient selection” part, only four articles used forward-looking design. Twelve articles presented a experiment control design. In the part of “index test,” 10 articles did not use any blinding method, and another article was not clear. On the other hand, the evaluation results of “reference standard” was deemed good, while the evaluation results of “flow and timing” in four articles were bad.

Data analysis

Overall diagnostic accuracy of AFU and AFP for HCC

The DOR forest plot of AFU in HCC diagnosis is shown in Figure 3. Figure 4 is ROC plot of AFU. The sensitivities of AFU and AFP were 0.7352 (0.6827, 0.7818) and 0.7501 (0.6725, 0.8144), respectively, while the specificities of AFU and AFP were 0.7681 (0.6946, 0.8283) and 0.8208 (0.7586, 0.8697) respectively. The DORs of AFU and AFP were 7.974 and 13.401, respectively. The sensitivity, specificity, and DOR of AFU and AFP are presented in Table 3.

AFU heterogeneity test

The purpose of studying heterogeneity is to identify the factors in the statistics that may affect the accuracy of estimations, and to assess whether the different studies combined are appropriate. Threshold effects are the most

Quality of article

The QUADAS-2 evaluation results are shown in Figure 2. It shows that the evaluation results on “risk of bias” are not ideal, while the evaluation results of “applicability concerns” are better. The main bias of these articles is

Figure 2: Summary of risk of bias and applicability concerns.

Figure 3: DOR forest plot for AFU.

Figure 4: ROC diagram of AFU.

important cause of heterogeneity in diagnostic tests. In general, the threshold effect can be seen by the ROC plot. If the planar distribution displays a representative “shoulder arm shape,” there is a threshold effect. In this study, the AFU’s ROC plot did not display a “shoulder arm shape” (see Figure 4), indicating the absence of threshold effect. Also,

Table 3: DOR, sensitivity, and specificity of AFU and AFP.

	AFU	AFP
DOR (95% CI)	7.974(5.302, 11.993)	13.401(8.359, 21.483)
AUC	0.7968	0.8451
Sensitivity (95% CI)	0.7352(0.6827, 0.7818)	0.7501(0.6725, 0.8144)
Specificity (95% CI)	0.7681(0.6946, 0.8283)	0.8208(0.7586, 0.8697)
Tau ² of DOR-Het.	0.538	0.683
Q of DOR-Het.	79.125	76.371
I ² of DOR-Het.	78.515%	77.74%
p of DOR-Het.	<0.001	<0.001
Spearman(p)	−0.396(0.103)	0.406(0.095)
H = (Q/17) ^{0.5}	2.1574	2.1195

DOR, diagnostic odds ratio; AUC, area under the curve; Tau², estimate of between-study variance; Het, heterogeneity; Q, Q statistic; I², inconsistency; H, H statistic.

the Spearman correlation coefficient of −0.396 (p=0.103) confirms that there is no threshold effect.

However, in this analysis, the I^2 of DOR-Het of AFU was 78.515%, indicating that the heterogeneity is caused by non-threshold effects.

Meta-regression for heterogeneity

The heterogeneity was investigated by exploring the characteristics of these samples using meta-regression. Four DORs, which include HCC and control, gender, average age, and year, were examined. The results are that the p-values of the four DORs are all >0.1 , meaning that the four DORs have no heterogeneity.

We also performed a subgroup meta-analysis on the non-continuous data for the subgroup “country” and “test method”. The results of this subgroup meta-analysis are shown in Table 4. The upper part of the table shows that three subgroups can be established, and only subgroup Egypt’s $Q=38.258$, $p<0.001$, and $I^2=86.76\%$ indicate heterogeneity. However, $p>0.05$ means no heterogeneity in the subgroup China and Japan and in these two subgroups, the detection effect of AFU is better.

The two most common detection methods, endpoint method and kinetic rate method, use different substrates to examine AFU activity. In the endpoint method, AFU activity is examined employing *p*-nitrophenyl- α -L-furanoside (PNPF) as a colorimetric substrate [29–31]. The kinetic rate method typically uses 2-chloro-4-nitrophenyl- α -glucopyranoside (CNPF) as a colorimetric substrate [32]. The lower part of Table 4 shows that two subgroups can be established. The subgroup PNPF’s $Q=76.974$, $p<0.001$, and $I^2=81.81\%$ indicate heterogeneity, while $p>0.05$ indicates no heterogeneity in the CNPF’s subgroup.

Discussion

Hepatocellular carcinoma is the leading cause of death in patients with chronic liver disease. HCC is usually

developed in an orderly progression from hepatitis to cirrhosis to early stage of cancer [33,34]. Serum marker detection shows many advantages over histopathological examination and there is a true need for sensitive and specific biomarkers. The serum AFP widely used for clinical detection of early HCC, and AFU has also been suggested as a serum marker for HCC as HCC patients have higher AFU concentrations and patients with benign liver disease have lower AFU levels [35].

This study explored the value of AFU in the diagnosis of HCC. To prevent confounding factors in the comparison we only chosen AFU and AFP in each patient. To improve accuracy, we analyzed only data from patients with chronic liver disease, precluding data from healthy people. We noticed the sensitivity, specificity, DOR, and AUC of AFU was lower than ones of AFP (0.7352 vs. 0.7501, 0.7681 vs. 0.8208, 7.974 vs. 13.401, 0.7968 vs. 0.8451), respectively, but they are approximative, which indicates that the diagnostic value of AFU is comparable to one of AFP [17, 36]. Some papers reported that the combination of AFU and AFP may improve previous diagnostic value [12, 13, 15, 16, 37]. In particular, El-Houseini (2001) reported that the simultaneous determination by AFP and AFU may improve the detection of HCC in cirrhotic patients negative for AFP. In addition, four articles show that AFU determination combined with other tumor markers increases the diagnostic accuracy of HCC [22, 23, 27, 37]. For example, Zhu et al. (2013) reported that the combined determination of GGT-II with AFU or AFP exhibited superior sensitivity and specificity for the diagnosis of HCC. However, many articles included in this analysis do not report a combined determination and the meta-analysis could not be conducted on the combined use of biomarkers for HCC diagnosis. Even though, a paper reported that the AUC of AFU combined with AFP was lower than that of AFP alone [17].

To explore heterogeneity, we excluded sequentially each article one by one and performed the meta-analyses. As none showed any significant change, heterogeneity is

Table 4: Results of subgroup meta-analysis based on DOR.

	Subgroups	Studies	DOR (p)	Q (df)	p of Het	I^2 , %
Country	Subgroup Egypt	6	17.002 (<0.001)	38.258 (5)	<0.001	86.76
	Subgroup China	6	3.839 (<0.001)	5.450 (5)	0.363	8.26
	Subgroup Japan	2	20.120 (<0.001)	1.779 (1)	0.182	43.78
	Subgroup South A	1	7.105 (NA)	NA	NA	NA
	Subgroup Italy	1	31.543 (NA)	NA	NA	NA
	Subgroup England	1	2.875 (NA)	NA	NA	NA
	Subgroup Thailand	1	10.731 (NA)	NA	NA	NA
Method	Subgroup PNPF	15	9.316 (<0.001)	76.974 (14)	<0.001	81.81
	Subgroup CNPF	3	4.659 (<0.001)	1.516 (2)	0.469	0

NA, not applicable; df, degrees of freedom; Het, heterogeneity.

not caused by any individual research. Using a meta-regression analysis, we found that country differences contribute to this heterogeneity. The subgroup analysis based on country demonstrates that only the Egypt subgroup shows heterogeneity except for the samples that could not be grouped, while the Chinese and the Japanese subgroups do not show heterogeneity, indicating better detection effects of AFU in these two subgroups.

Further, because many studies lack information on design, conduct, number of focal lesions in the liver, pathological stage, and focal size of the liver, we could not estimate if these variables cause bias. Wang et al. (2014) reported that preoperative AFU was an independent prognostic factor of overall survival. Patients with a pre-operative AFU >35 nmol/mL/min had a lower recurrence-free survival rate and overall survival rate compared to patients with AFU <35 nmol/mL/min, and they have a higher tendency to form macrovascular invasion. These data need to be confirmed by additional studies [24].

On the other hand, many studies lack detailed data on the pathological stage of HCC, limiting research on the relationship between AFU detection and HCC pathological stage.

Conclusions

AFU is close to AFP for the diagnosis of HCC, and the combined diagnostic value of AFP and AFU is worthy of further study, and the specific performance of AFU depending on a country remains to be clarified.

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