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Diagnosis accuracy of serum Glypican-3 level in patients with hepatocellular carcinoma and liver cirrhosis: a meta-analysis

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Abstract. – OBJECTIVE: The diagnostic value of serum GPC3 levels in patients with hepatocellular carcinoma (HCC) and liver cirrhosis remains controversial. Therefore, we performed a meta-analysis to assess the diagnostic accuracy of serum GPC3 for HCC and liver cirrhosis (LC).

MATERIALS AND METHODS: A systematic search was performed for the relevant studies. Sensitivity, specificity and other measures regarding the accuracy of serum GPC3 in the diagnosis of HCC were performed by random-effects models. Summary receiver operating characteristic curve (sROC) analysis was taken to summarize GPC3's performance.

RESULTS: 17 studies were included in our meta-analysis. The pooled sensitivity and 95 % confidence intervals (95% CIs) for GPC3 were 56% (53%-59%) and 89% (87%-90%) in specificity. The pooled positive LR and 95% confidence intervals (95% CIs) for GPC3 were 7.82 (3.86-15.85) and 0.48 (0.39-0.59) respectively in negative LR. The summary diagnostic odds ratio (DOR) and 95% CIs for GPC3 were 26.73 (10.31-69.26), and the area under sROC (AUC) and 95% CIs for GPC3 were 0.8827 (0.8324-0.9330).

CONCLUSIONS: GPC3 is acceptable as a serum marker for the diagnosis of HCC, which can elevate the accuracy of diagnosis.

Key Words:
Glypican3, Hepatocellular carcinoma (HCC), Liver cirrhosis, Diagnosis accuracy.

Introduction

HCC is the sixth most common cancer and the third most common causes of cancer deaths across the world[1]. Patients diagnosed of advanced liver cancer are often associated with poor prognosis[2]. Early detection of hepatocellular carcinoma can improve the survival rate of patients, which become more and more important[3]. European Research Association of liver (EASL) recommended that patients with liver disease had liver ultrasound examination and serum alpha-fetoprotein (AFP) levels every six months[4]. However, ultrasound is an indirect diagnostic method with good accuracy, depending on its skills and having the ability to distinguish liver cancer from non-neoplastic nodules[5]. In addition, the level of serum alpha-fetoproteinis is not an accurate sensitivity biomarker with the range of sensitivity only from 40 to 65%[6]. Therefore, several promising biomarker discovered brought hope to improve the accuracy of diagnosis of liver cancer.

Glypican-3 (GPC-3) belongs to a glypican family of heparan sulfate proteoglycans[7]. GPC-3 is normally expressed in fetal liver and placenta, and has negligible normal expression in adult liver tissue[8]. At present, many studies found in hepatocellular carcinoma, GPC-3 increased its expression in hepatocytes, despite its loss of expression of GPC3 expression in healthy people with hepatitis[9]. In addition, patients with liver cancer had higher levels of serum GPC3 than healthy people with hepatitis. Therefore, it has been suggesting that serum GPC3 is a specific marker of liver cancer[10,11]. But other studies have reported conflicting results. In this meta-analysis, we collect relevant researches in recent years about diagnostic accuracy of GPC3, aiming to explore the diagnostic value of GPC3 in HCC and liver cirrhosis.

Methods

Literature Search Strategy

In a comprehensive electronic searching of PubMed, EMBASE, Web of Science and the Cochrane Database, two investigators independently carried many articles up to date June 1,

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2015. Keywords used in the search process is as follows: (1) HCC: HCC, primary liver cancer, hepatocellular carcinoma, liver cancer; (2) Serum, blood; (3) GPC3: GPC3, phosphatidylinositol 3 proteoglycan. Published research design and publication status is not set limited, but publishing language is English. In addition, we pay the relevant articles to determine related information is not to be missed and we manually search for a reference list of articles selected to determine the more relevant publications.

Criteria for Inclusion and Exclusion of Published Studies

Inclusion criteria was as follows: (1) research was about discussion of diagnostic accuracy of serum GPC3 in HCC; (2) data samples were patients with liver cancer in study group and control group was patients with liver cirrhosis or hepatitis; (3) true positive (TP), true negative (TN), false positives (FP), and false negatives (FN) values were showed or could be computed; (4) the article was written in English. Exclusion criteria was as follows: (1) research on animals; (2) research was about evaluation of gene expression or polymorphisms in GPC3 or did not provide the sensitivity or specificity using GPC3 as a liver cancer marker; (3) letters, editorials, expert comments, and clinical researches without the original data; (4) lack of a control group in reports and studies; (5) repeated studies.

Data Extraction

Data extracted by two independent investigators from the article encountered was in accordance with the inclusion and exclusion criteria. The following data extracted from the study included as follows: first author's name, year of publication, research and design, the number of patients, the cut-off values. The sensitivity and specificity of assessment data was extracted (results of true-positive and false-negative, true negative and false positive). Any differences on data extraction were solved by a third independent investigators.

Methodological Quality Assessment

We assessed study quality using Quality Assessment of Studies of Diagnostic Accuracy (QUADAS)[12] recommended from Cochrane Collaboration, which contains 11 projects specifically developed to assess the quality of a preliminary study of the diagnostic test. Each item score was labeled as "Yes", "no" or "unclear."

Statistics

Statistical analysis was used by the Stata 12.0 and meta-disc 1.4 software. Cochran Q test and I^2 heterogeneity was used to estimate the value of including research. Heterogeneity can be interpreted by threshold effect. Meta regression analysis was used to explain the observed heterogeneity. Characteristic of HCC and methods may be the source of heterogeneity. Pooled sensitivity, specificity, diagnostic odds ratio (DOR) and positive (PLR) and negative likelihood ratio (NLR) are obtained from the random effects Model. Overall diagnosis of forest plots were used to depict the 95% sensitivity, specificity. The SROC AUC used to describe the overall diagnostic performance of each marker.

Results

Study Selection Process

A total of 471 potentially relevant articles was determined by searching PubMed and EMBASE and other databases. After reviewing their titles and abstracts, 444 articles were excluded, including repeated studies, case reports, expert commentary and observational studies. After reviewing the full text, some studies or meta-analysis not related to our study design were excluded, but also some studies lack of sufficient data to estimate the sensitivity or specificity and not meeting the study group and the control group inclusion criteria. In searching for all the references in the study included, we found no articles met our inclusion criteria. Finally, the 17 studies were included in our meta-analysis (Figure 1). Characteristics contained the study as shown in Table I 13-29, including 2572 patients (1201 HCC and 1371 controls). In all the literature, HCC patients with or without hepatitis B virus (HBV) or hepatitis C virus (HCV) and liver cirrhosis patients with or without in HBV or HCV in control group were included.

Quality of the Studies

QUADAS quality assessment of the included studies was shown in Figure 2. The quality of include literatures was not satisfactory. For example, in the domain of "acceptable reference standard", ten studies did not provide clear reference standard of GPC3. Although all studies reported the diagnostic standard for HCC, however, no studies stated whether the index results were blindly or not. In the domain of "relevant clinical

information," seven studies did not provide enough relevant clinical information of HCC.

Diagnostic value of GPC3 for HCC

The sensitivity and specificity of each study were displayed on Figures 3-4. The pooled sensitivity and 95% confidence intervals (95% CIs) for GPC3 were 56% (53%-59%) and 89% (87%-90%) in specificity. The sensitivity and specificity of each study were displayed on Figures 5-6. The pooled positive LR and 95% confidence intervals (95% CIs) for GPC3 were 7.82 (3.86-15.85) and 0.48 (0.39-0.59) respectively in negative LR. Regarding pooled sensitivity, specificity, pooled positive LR and negative LR, significant heterogeneity was also found across different studies and the random effects model was used to present overall summary of all above. The summary diagnostic odds ratio (DOR) and 95% CIs for GPC3 were 26.73 (10.31-69.26), and the area under sROC (AUC) and 95% CIs for GPC3 were 0.8827 (0.8324-0.9330), as shown in Figures 7-8.

Investigation for Heterogeneity

For sensitivity and specificity, I^2 was 90.7% and 94.3%, respectively. These results indicated that significant heterogeneity in this study. Heterogeneity can be interpreted by threshold effect. Threshold effect may be caused by difference cut-off value, objective method, population or others. At this meta-analysis, Spearman correlation coefficient was 0.020, *p* value was 0.940, indicating that heterogeneity was not caused by threshold effect in this study. Meta regression was used to analyzing the characteristics of the study to explain the heterogeneity. The results are shown in Table II. However, we didn't find any differences in cut-off value assay type, characteristics of HCC and controls which had a statistically significant effect on diagnostic accuracy.

Publication Bias

Deeks' funnel plot asymmetry test was used to evaluate publication bias among 17 studies. The slope coefficient of the regression line had a p value of 0.28, which indicated that the data did not have a likelihood of publication bias (Figure 9).

Discussion

Compared to the tissue pathology and imaging studies, serum markers have advantages of more convenient and lower costs in HCC patients. GPC3 is a candidate serum marker for HCC patients. GPC3 protein significantly inhibits embryonic development, but in most normal adult tissues, several studies showed, GPC3 was highly expressed in HCC tissue, promoting cell growth by stimulating Wnt signaling[30,31].

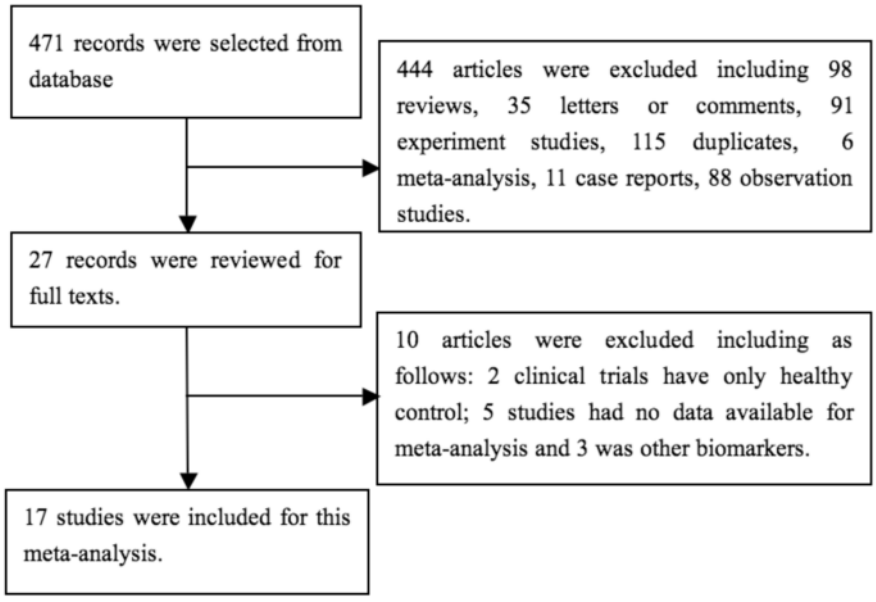

Figure 1. Flow diagram of study selection for our meta-analysis.

Table I. 1 Characteristics of studies included in the present meta-analysis.

Study	Study/ Control	Characteristics of HCC	Characteristics of controls	Assay type	Cut-off value	HCC value(ng/ml)	Controls value (ng/mL)
Yu, et al[13], 2015	192/101	NA	LC or hepatitis patients;	Chemiluminescent immunoassay	30 ng/mL	108.67 ±230.04	3.99 ±7.68
Lee, et al[14], 2014	120/40	62.5% HBV	HCV patients; 50% had LC	ELISA	73 ng/mL	75.8 ±117.5	66.4 ±33.2
Badr, et al[15], 2014	30/30	NA	LC (HCV) patients	ELISA	240 ng/mL	551.47 ±185.25	98.23 ±73.54
Chen, et al[16], 2013	155/440	NA	LC or hepatitis patients;	ELISA	25.25 ng/mL	99.94 ±267.2	19.44 ±50.88
Abdelgawad , et al[17], 2013	40/20	67.5% HCV 17.5% HBV	LC patients;	ELISA	4.9 ng/mL	7.7	3.24
Gomaa, et al[18], 2012	31/30	12.9% HBV 87.1% HCV	LC patients with HCV/HBV	ELISA	5.41 ng/mL	8.13 ±3.25	3.14 ±1.16
Wang, et al[19], 2012	78/97	HBV associated	LC patients with HBV	ELISA	NA	NA	NA
Qiao, et al[20], 2011	101/88	76.2% HBV 7.9% HCV	LC (HBV/HCV) or hepatitis patients;	ELISA	26.8 ng/mL	29.29 ±17.34	12.09 ±9.69
Abd El Moety, et al[21], 2011	10/40	HCV	LC or hepatitis patients;	ELISA	2.0 ng/mL	34.63 ±23.8	NA
Zhang, et al[22], 2010	36/93	NA	LC or hepatitis patients with HCV/HBV;	Immunoassay	3.10 ng/mL	34.63 ±23.8	24.60 ±24.01
Youssef, et al[23], 2010	40/40	HCV and HBV associated	LC patients with HBV/HCV;	ELISA	4.6 ng/mL	NA	NA
Liu, et al[24], 2010,	75/32	84% HBV 16% HCV	LC patients with HBV/HCV	ELISA	300 ng/L	NA	NA
Tangkijvanich, et al[25], 2010	100/100	59% HBV - 11% HCV	LC or hepatitis patients with HBV/HCV	ELISA	NA	46.3 (0–7826.6)	0 (0–43.6)
Beale, et al[26], 2008	50/41	60% ALD - 40% NAFLD	LC patients	ELISA	NA	161.41 ±422.33	125.41 ±281.05
Yoshitaka Hippo, et al[27], 2004	69/38	NA	LC patients	ELISA	2.0 ng/mL	4.84 ±8.91	1.09 ±0.74
Nakatsura , et al[28], 2003	40/50	12.1% HBV 40.9% HCV	LC patients with HBV/HCV	ELISA	10U/mL	NA	NA
Capurro, et al[29], 2003	34/91	44.1% HBV 27.2% HCV 14.7% ALD	LC or hepatitis patients with HBV/HCV	ELISA	117 ng/mL	NA	NA

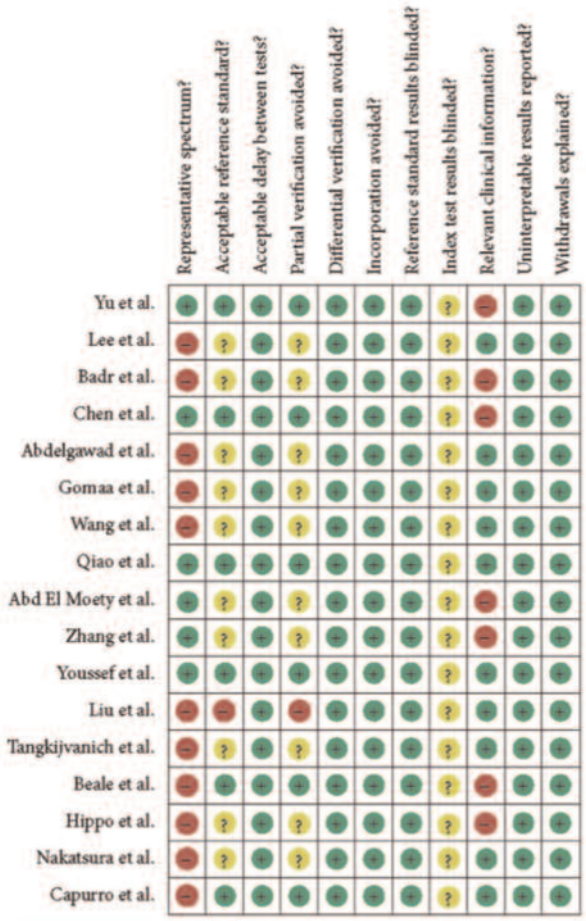

Figure 2. Assesment of included studies by QUADAS tool on 11 aspects according to guidance recommended by Cochrane Collaboration.

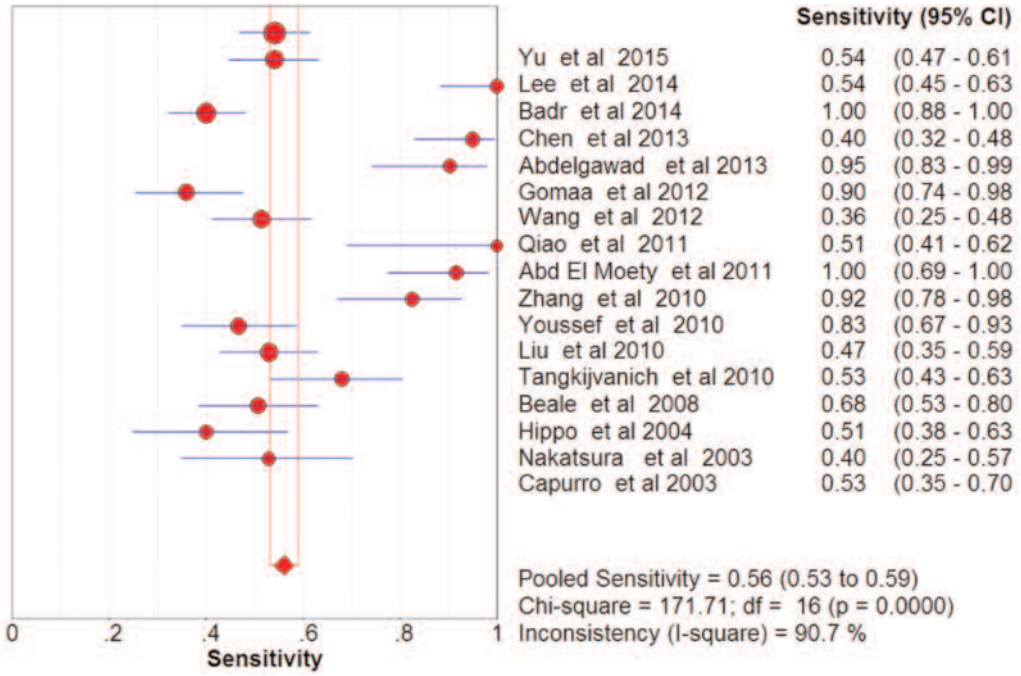

Figure 3. Forest plots of sensitivity in using GPC3 as a diagnostic marker for HCC in the 17 studies included for meta-analysis.

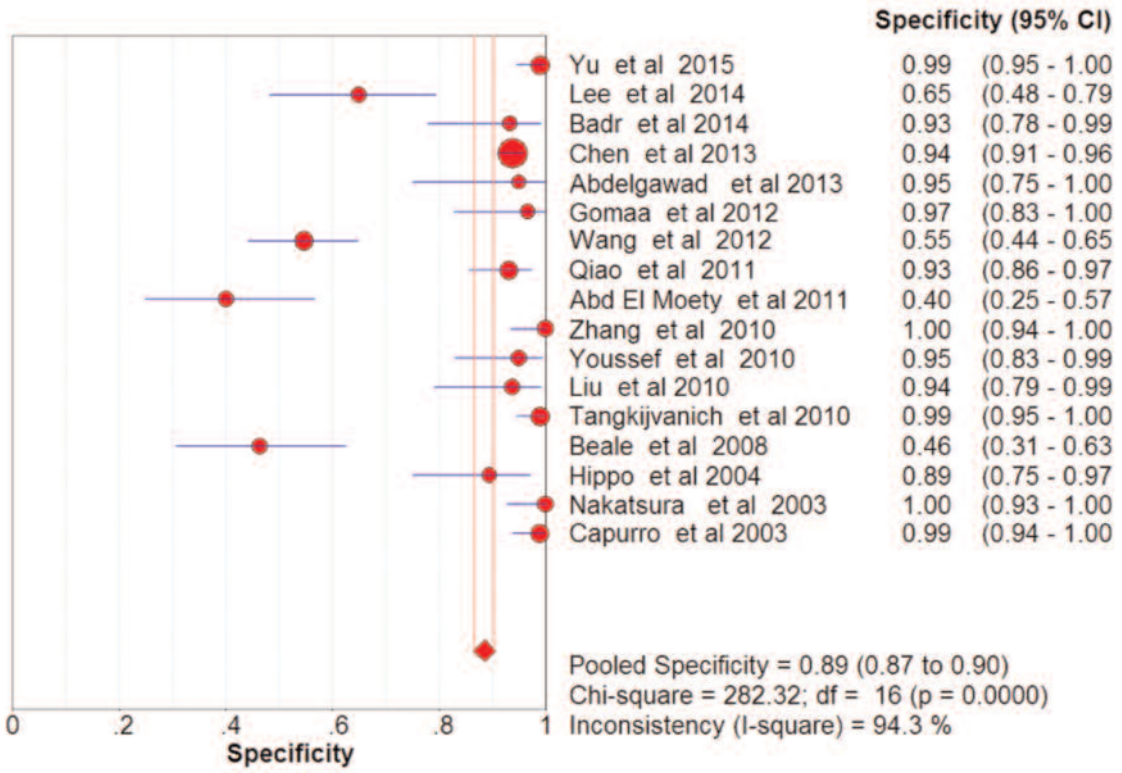

Figure 4. Forest plots of specificity in using GPC3 as a diagnostic marker for HCC in the 17 studies included for meta-analysis

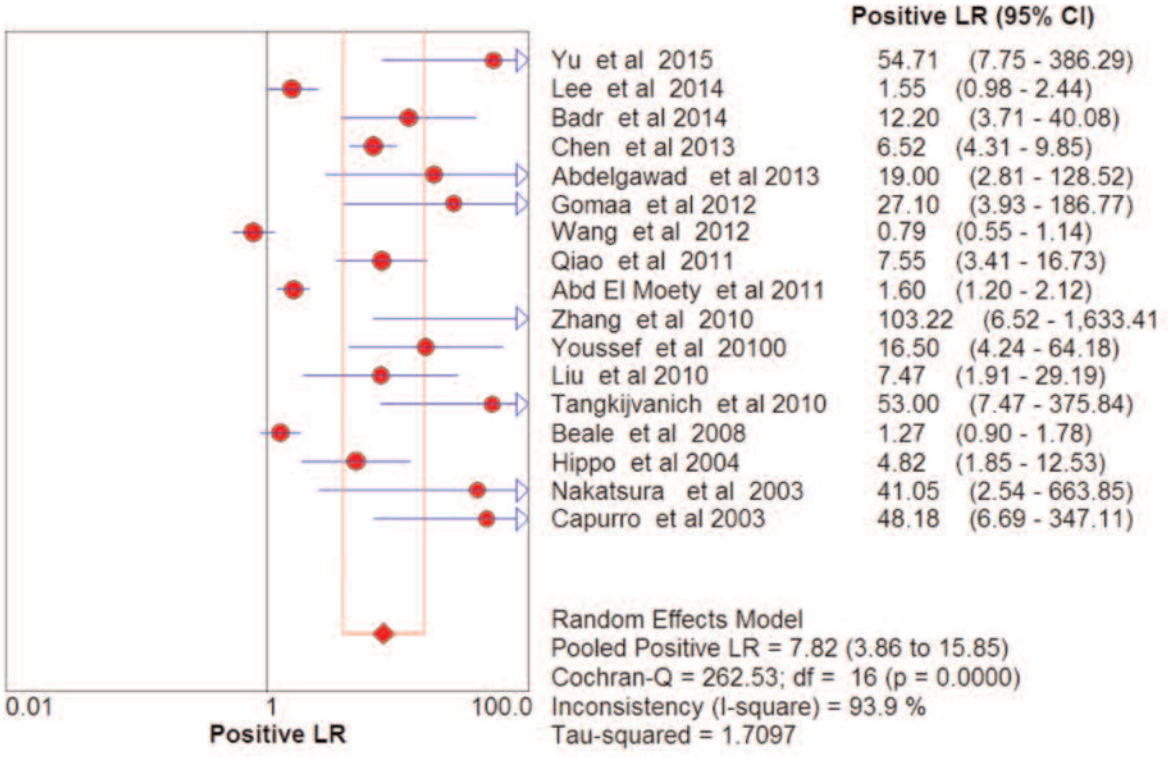

Figure 5. Forest plots of positive LR in using GPC3 as a diagnostic marker for HCC in the 17 studies included for meta-analysis

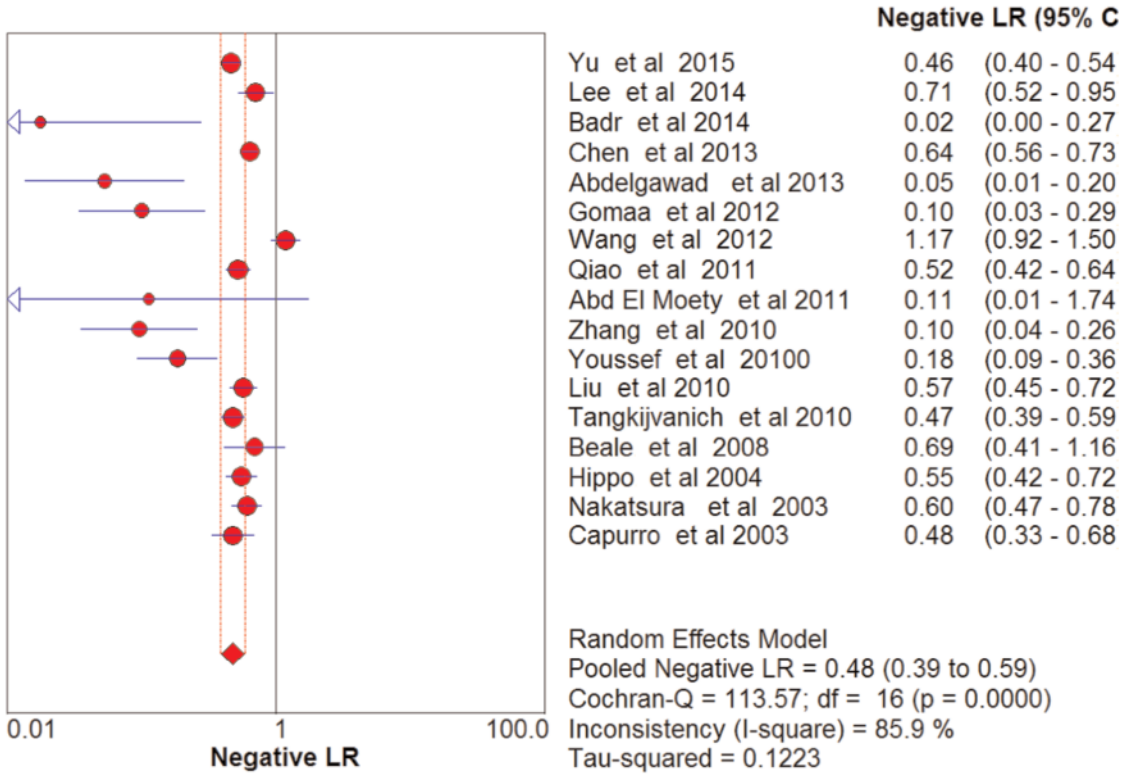

Figure 6. Forest plots of negative LR in using GPC3 as a diagnostic marker for HCC in the 17 studies included for meta-analysis

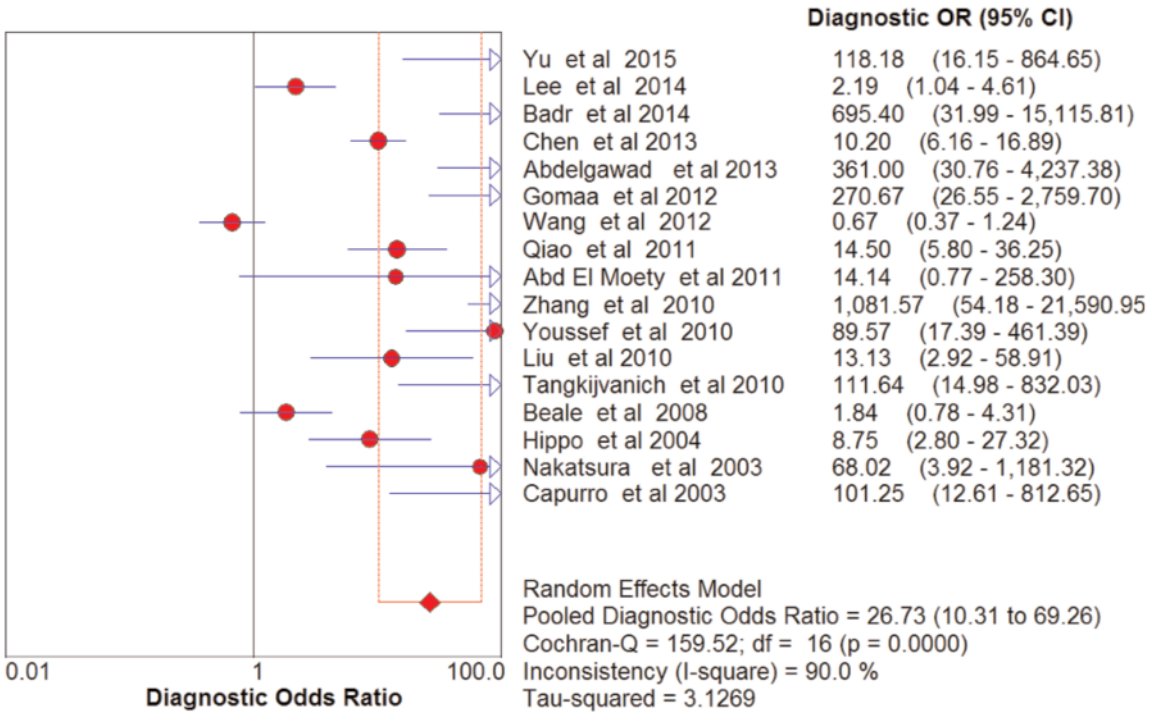

Figure 7. Forest plots of diagnostic OR (DOR) in using GPC3 as a diagnostic marker for HCC in the 17 studies included for meta-analysis.

In this meta-analysis, we identified 17 researches of serum GPC3 diagnostic accuracy study in HCC. Thirteen studies found that GPC3 is an ideal diagnostic marker for HCC, with an acceptable sensitivity or specificity. Specifically in these 13 studies, elevated serum GPC3 were showed in HCC patients compared with hepatitis and cirrhosis patients. But the other four works found lower serum levels or lower sensitivity in patients with liver cirrhosis and HCC. In careful reviewing all the investigations and their references, we found that hepatitis B virus or HCV infection and GPC3 antibody had no interaction in testing. Instead, the possible reason is that GPC3 is not able to differentially diagnose HCC and liver cirrhosis, and thus there were conflicting results in it. Our aim of the report is to determine whether serum GPC3 level detected had diagnostic value in HCC and liver cirrhosis.

Our meta-analysis firstly determined the inclusion criteria, made a clear provision in HCC and controls. Studies only with healthy control people could not be included in our studies, and liver cirrhosis or hepatitis patients could be included as control group. Ultimately, this meta-analysis included 17 selected literatures. We found that, GPC3 have higher diagnostic value in HCC and liver cirrhosis patients, with sensitivity of 56%, specificity of 89%, DOR of 26.73 and the area under the SROC curve 0.88. When the area under the SROC curve was at 0.7-0.9, it indicated that the diagnostic method had a good diagnostic value.

17 studies were from different countries, with different detection reagents and detection methods and varied equipment greatly, and cut-off value reported was quite different. Due to large differences in cut-off value, it might cause threshold effect and a large heterogeneity in this study. Our findings also confirmed that a large heterogeneity in our meta-analysis in sensitivity, specificity, positive likelihood ratio and negative likelihood ratio etc. So we first determined whether there was a threshold effect by Spearman correlation analysis. Our findings showed that this study did not exist a threshold effect. Thus, heterogeneity might come from the role of other factors. Therefore, we conducted a meta-regression analysis and found that cut-off values, characteristics of HCC and control, detection reagents were not the source where heterogeneity existed. Furthermore, publication bias analysis in our meta analysis found no evidence of publication bias in this meta.

In addition, we also found that the quality of the included reports generally could not be entirely satisfactory. Therefore, we believe that the following improvements could enhance the quality of literature and reduce the occurrence of heterogeneity: (1) double-blind studies should be designed to avoid bias; (2) the cohorts of hepatitis and liver cirrhosis patients might be set as subgroups; (3) studies could use two or more different GPC3 antibodies to measure GPC3 level; (4) it was Improper to examine the stability of GPC3 during long-term storage. The diagnostic performance of serum GPC3 maybe greatly affected, if the stability of serum GPC3 in long-term storage.

Conclusions

Based on currently available literature, our meta-analysis showed a high diagnostic efficacy in GPC3 detection in diagnosis of liver cancer and cirrhosis, with a higher area under the SROC curve. Thus, prior to the discovery of new liver cancer specific markers, clinical GPC3 detection methods can be taken to improve the early diagnosis of liver cancer.

Conflict of Interest

The Authors declare that there are no conflicts of interest.

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