Serum angiopoietin 2 acts as a diagnostic biomarker for hepatocellular carcinoma: a meta-analysis of diagnostic test accuracy studies.

Anas Elgenidy (anas.elgenidy@gmail.com)
Cairo University Kasr Alainy Faculty of Medicine https://orcid.org/0000-0002-4532-8574
Ahmed K Awad
Ain Shams University Faculty of Medicine
Ahmed M Afifi
Baylor College of Medicine
Prasun K Jalal
Baylor College of Medicine

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Abstract

Background & Aims: HCC is characteristically a hypervascular tumor where angiogenesis is directly linked to its progression. Angiopoietin-2 (Ang-2) promotes vascular remodeling as the Ang-2-Tie2 pathway suppresses interactions between endothelial cells and vascular mural cells: pericytes and vascular smooth muscle cells. However, the importance of Ang-2 has a controversial outcome between the studies, thus we aimed to systematically analyze the diagnostic utility of serum Ang-2 in patients with HCC in comparison with cirrhotic liver diseases.

Methods: We searched PubMed, Scopus, Embase, and Web of Science databases until Jan 2022, to identify studies assessing serum levels of Ang-2 in patients with HCC. Studies that measured Serum Angiopoietin 2 levels of hepatocellular carcinoma patients, articles that were published in peer-reviewed international journals and had enough data for qualitative and quantitative analysis were included with no language restriction.

We conducted our double-arm meta-analysis using the “meta” package in R version 4.1.0. Random-effects meta-analysis models were employed to estimate the pooled serum level on angiopoietin 2 level. The data was continuous, we used the mean difference with a 95% confidence interval to assess the estimated effect measure. A leave-one-out meta-analysis was performed to show how each individual study affects the overall estimate by removing one study alternately from the meta-analysis.

Results: Ten studies with 3175 patients met our criteria and were included in our meta-analysis. The serum level of Ang-2 was higher in the HCC group when compared to healthy participants, Chronic liver disease patients or patients with liver cirrhosis.

Ang-2 as a marker was compared between HCC and healthy participants with significant favor for HCC (p=0.00001). The cumulative mean difference was significant at 2.88 (95% CI of 1.87, 3.89). Furthermore, Ang-2 as a marker was compared between HCC and cirrhotic liver patients with significant favor for HCC (p=0.003) with significant mean difference(MD) 2.52 (95% CI of 0.85, 4.18), while when compared between HCC and Chronic liver disease patients revealed a significant favor for HCC (p=0.0002) with MD 1.93 (95% CI of 0.92, 2.93).The pooled sensitivity, specificity, positive likelihood ratio, negative likelihood ratio were 0.79, 0.89, 9.86, and 0.08, respectively. The summary ROC plot showed an area under the curve (AUC) of 0.9816 and Q*=0.9403.

Conclusion: Having both high pooled sensitivity and specificity, serum Ang-2 shows the potential to have a vital role as an independent diagnostic marker in HCC over decades of other biomarkers used.

Introduction

Hepatocellular carcinoma is the most common form of liver cancer and accounts for nearly 90% of cases with an estimate that, by 2025, > 1 million individuals will be diagnosed by liver cancer annually (1). Corresponding to 20,000 new cases per year in the USA, HCC has been reported in previous studies as an incidence of 16 cases per 100,000 population (2). Mortality rates are quite comparable to the incidence rates from different parts of the world, both of which have been increasing in recent years (3). Accounting for more than 50% of the cases, Hepatitis B virus (HBV) infection is the most prominent risk factor for HCC development, yet hepatitis C virus (HCV) infection has substantially decreased due to patients achieving sustained virological response (SVR) with antiviral drugs (4). Nevertheless, patients with cirrhosis are still considered to be at high risk for HCC incidence even after HCV clearance. In most cases, HCC arises in the setting of chronic liver disease and has been a leading cause of death among patients with cirrhosis (3). The prognosis of HCC mostly depends on its stage and the severity of liver disease at the time of diagnosis; advanced-stage HCC has a very poor prognosis, with a median survival time of 9.0 (20) months. Overall, HCC patients showed 1-, 2-, 3-, and 5-year survival rates equal to 49.3%, 35.3%, 26.6%, and19.5% respectively. The median survival time of HCC patients first hospitalized from 2009 to 2015 was higher than those in 2002 to 2008 (5).

HCC is characteristically a hypervascular tumor where angiogenesis is directly linked to its progression (6). As one of the hallmarks of malignancy, tumor angiogenesisallows delivery of oxygen and nutrition to tumors, thus not only contribute to tumors’ growth but also its metastasize and dissemination(7). Tumor dormancy is a confined small mass solid tumor within 1–2 mm in diameter which cannot grow beyond 3 mm if being avascular, yet once being vascularized, they can grow and metastasize remotely rapidly with missed diagnosis (8). Although the diagnosis of HCC is usually based on non-invasive criteria, molecular classification of the tumor using tissue biopsies is growing as a crucial need in clinical practice (9). Alpha-fetoprotein (AFP), Lens culinaris agglutinin A-reactive fraction of alpha-fetoprotein (AFP-L3), and des-gamma-carboxy prothrombin (DCP) have been established as HCC-specific tumor markers, yet with their limited success and increasing false positives (10), the usage of angiogenic factors as markers have been proposed in the literature (23–25).

Being regulated by the balance between pro- and anti-angiogenic factors, angiogenesis constitutes of several angiogenic signals, including angiopoietin/Tyrosine kinase with Ig and EGF homology domains 2 (TIE2) signaling, vascular endothelial growth factor (VEGF)/VEGF receptor (VEGFR) signaling, and platelet-derived growth factor (PDGF)/PDGF receptor (PDGFR) signaling. However, angiogenesis suppressors include angiostatin, endostatin, and thrombospondin-1 (11). Shifting from tumor dormancy to tumor progression results from the imbalance between pro and anti-angiogenic factors toward the pro-angiogenic outcome. Ligands of the tyrosine kinase with Ig and EGF homology domain tie 1 and 2, The angiopoietin (Ang) family consists of Ang1, Ang-2, and Ang3/4, in which vascular smooth-muscle cells produce Ang1 is mainly - an agonist for Tie2-; however, tumor cells predominantly secret Ang-2- an antagonist for Tie2 (12)(13).

Ang-1 mediates the stabilization and maturation of developing vessels, whereas Ang-2 disrupts the stabilizing effect of Ang-1. Furthermore, Ang-2 promotes vascular remodeling as the Ang-2-Tie2 pathway suppresses interactions between endothelial cells and vascular mural cells: pericytes and vascular smooth muscle cells (14). Several studies (23–25) have recommended Ang-2 as diagnostic marker for HCC, while other recommend it as marker for only liver cirrhosis. However, others studies have showed great results for serum neuropilin-1 (32) and micro-RNA (37), thus he importance of Ang-2 has been discussed

in the literature to an extent with always controversial outcomes between the studies, thus we aimed to systematically analyze the prevalence of serum Ang-2 among liver diseases with its diagnostic accuracy and prediction of HCC.

Methods

The Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guideline was fulfilled in this systematic review and meta-analysis (15).

Search strategy:

We searched PubMed, Scopus, Embase, and Web of Science for studies that measured Serum Angiopoietin 2 levels of Hepatocellular carcinoma patients up to January 27, 2022.

The following search terms were used: ("angiopoietin 2" OR "Ang 2" OR "angiopoietin 2") AND ("hepatocellular carcinoma" OR "Liver Neoplasm" OR "carcinoma of liver" OR "Liver Cell Carcinoma" OR "Cancer of Liver" OR "Liver Cancer" OR "Hepatic Cancer" OR "Hepatocellular Cancer"). Moreover, we reviewed the reference lists of retrieved articles to complement the broad search.

Eligibility criteria:

Studies that measured Serum Angiopoietin 2 levels for diagnosis of hepatocellular carcinoma patients, articles that were published in peer-reviewed international journals and had enough data for qualitative and quantitative analysis were included with no language restriction. We excluded conference papers, unpublished articles, letters to the editor, posters, and animal studies.

Selection process:

Three independent authors screened the articles and decided whether one met the inclusion criteria of the review. While in case of disagreement, the senior was opt in to take the decision to whether include the article or not.

Data Extraction:

Two independent authors extracted the following data from the included studies as baseline characteristics: name of the First author, publication year, country, study design, gender, mean age, total sample size, stage of hepatocellular cancer, serum level on angiopoietin 2 of different groups, the received medical or surgical treatment, method of measurement of the serum marker level, the cut-off value, sensitivity, specificity of Ang-2**,** We extracted data for both cancer and non-cancer patients (for available studies) separately.

Quality assessment:

We used the National Institute of Health Study Quality Assessment Tool (16) for Observational Cohort and Cross-Sectional Studies to assess the internal validity of the included observational cohort studies, Newcastle-Ottawa Scale (NOS)(17) to assess the case-control studies and QUADAS-2 tool to evaluate bias risk and applicability of diagnostic accuracy studies (18).

Two independent reviewers (AE and AA) screened the methodological quality of included studies and in case of discrepancies were resolved by discussion.

The NIH tool consists of fourteen domains, each of the domains was given yes, no or not applicable. The NOS tool judges the studies on three broad perspectives: the selection of the study groups, the comparability of the groups, and the ascertainment of either the exposure or outcome. QUADAS-2 consists of four key domains: patient selection, index test, reference standard, and flow and timing.

Data analysis:

We conducted Our double-arm meta-analysis using the “meta” package in R version 4.1.0(19)(20). Random-effects meta-analysis models were employed to estimate the pooled serum level on angiopoietin 2 level. Heterogeneity was evaluated using the inconsistency (I^2) and Chi-squared (X2) test. I2 > 50% was considered substantial heterogeneity in the studies, and a P value less than 0.05 was considered statistically significant. The data was continuous, we used the standardized mean difference with a 95% confidence interval to assess the estimated effect measure. A leave-one-out meta-analysis was performed to show how each individual study affects the overall estimate by removing one study alternately from the meta-analysis.

We calculated true positive (TP), true negative (TN), false positive (FP), and false-negative (FN) using the extracted sensitivity and specificity by RevMan calculator (21). The diagnostic accuracy meta-analysis was conducted using Meta-DiSc software, we measured the Pooled estimates of sensitivity, specificity, positive likelihood ratio (PLR), negative likelihood ratio (NLR), and diagnostic odds ratio (DOR) and plotted with a 95% confidence interval (CI).

The performance of a diagnostic test was represented by plotting the summary receiver operating characteristic (SROC) curve which represents the results for sensitivity and specificity by looking at the curve, the x-axis represents the specificity, the y-axis represents the sensitivity and the diagonal represents the value of sensitivity and specificity of the index test. The closer curves to the top-left corner the better the performance of the test.

Heterogeneity was evaluated using Spearman’s correlation coefficient, inconsistency (I^2), Chi-square test, and Cochran’s Q test. The pooled effect was calculated using a random-effects model of DerSimonian–Laird was employed to estimate the pooled effect when heterogeneity was present (I^2 > 50%, P < 0.05), and a fixed-effects model of Mantel–Haenszel was utilized when no heterogeneity was found.

According to Wan's method (22), studies that reported serum Ang 2 levels as median and range or median and interquartile range (IQR) were converted to mean and standard deviation (SD). studies that reported serum Ang 2 levels in pg/ml or ug/L were converted to nanogram/milliliter. However, few studies presented the plots without sufficient data, we obtained it by using GetData Graph Digitizer software.

Results

Search results

Our search strategy resulted in a total number of 291 studies. After the title and abstract screening and removing the duplicates, 139 articles were eliminated, and 31 full-text articles were evaluated for eligibility. Following the full-text screening, 10 papers (23–32) met our criteria and were included in our meta-analysis **(Figure 1).** Seven studies were Case-control studies, two were retrospective cohort and one was a prospective cohort.

Summary of the included studies

With a mean age of 62.5 (6.8), Our study included 3175 Participants of which 1887 patients were male. Moreover, 1579 patients had hepatocellular carcinoma, 344 had liver cirrhosis, and 294 had chronic liver disease. Various methods of measurements were used, such as Enzyme linked immunosorbent assay kit (ELISA), sandwich ELISA, multiplex immunoassay, and Ang-2 ELISA kit. The baseline characteristics are illustrated in (Table 1)

Quality assessment:

Following the NIH Study Quality Asseappssment Tool guidelines and after the interrater consensus, one study was considered of good quality and two were of Fair quality mostly due to no justification for the sample size or power description, and no blinding to the outcome

For case-control studies, judged by following NOS guidelines, four were of good quality and three were of fair quality mostly due to non-matching of the cases and controls regarding the confounders, selection of controls with no description.

while for diagnostic accuracy studies, one study has a high risk of bias regarding the selection of patients and insufficient exclusion criteria. All the studies did not provide sufficient data regarding blinding of the test interpreter to results of the reference standard as shown in Appendix Tables 1,2,3.

Data- analysis

The first analysis of nine studies including 2379 patients, Ang-2 as a marker was compared between HCC and healthy participants with significant favor for HCC (p=0.00001). The cumulative mean difference was significant at 2.88 (95% CI of 1.87, 3.89). The second analysis of seven studies including 1753 patients, Ang-2 as a marker was compared between HCC and cirrhotic liver patients with significant favor for HCC (p=0.003). The cumulative mean difference was significant at 2.52 (95% CI of 0.85, 4.18). Third analysis of four studies including 983 patients Ang-2 as a marker was compared between HCC and Chronic liver disease patients with significant favor for HCC (p=0.0002). The cumulative mean difference was significant at 1.93 (95% CI of 0.92, 2.93). Heterogeneity analysis demonstrated high heterogeneity for the analyses. Further details were illustrated **in** Figure 2.

Publication bias

Visual inspection of the funnel plot of our first analysis between HCC and healthy patients revealed some asymmetry. However, the Egger test showed no evidence of substantial publication bias (t ¼ 1.86, P ¼ 0.078) according to our significance level.

meta-analysis of Diagnostic test accuracy studies

Out of 10 included studies, seven studies used Ang-2 as the index test for differentiating patients with HCC from healthy controls. Its sensitivity varied widely from 51% to 100% with pooled sensitivity of 0.79 (95% CI 0.76, 0.81), the specificity from 68% to 100% with pooled specificity of 0.85 (95% CI 0.82, 0.88), pooled PLR of 5.71 (CI 95% 2.94, 11.09), and pooled NLR of 0.12 (95% CI 0.03, 0.41). We also measured the diagnostic odds ratio, OR 68.79 (95% CI 12.08 to 391.71). **Figure 3** shows the summary ROC plot and an overview of the six serological tests with their summary sensitivity and specificity with the area under the curve (AUC) of 0.9548 and Q*=0.8970.

For heterogeneity, the Spearman correlation coefficient was: -0.607 p-value= 0.148 that indicates no significant correlation, and the threshold effect played no role in the heterogeneity of estimates.

Sensitivity Analysis and leave-one-out:

A leave-one-out analysis revealed that no single study effects on the overall effect size **(Figure 4).** When the outlying studies (Scholz 2007, Wenli 2015) were removed, the heterogeneity did not change considerably and remained high.

Discussion

Our meta-analysis is the first to discuss the serum levels of Ang-2 in hepatocellular carcinoma patients as a potential biomarker, and the first diagnostic accuracy meta-analysis performed to discuss such a topic. Our meta-analysis showed that when comparing healthy individuals to HCC patients, Ang-2 presented a statistically significant favor for HCC with MD 2.88 (95% CI of 1.87, 3.89), which was inferior when Ang-2 was compared between HCC and cirrhotic liver patients with significant favor for HCC with MD 2.52 (95% CI of 0.85, 4.18). The sensitivity varied widely between our studies from 0 to 100%, yet the pooled sensitivity of Ang-2 is 0.79 (95% CI 0.76, 0.82); furthermore, the specificity varied from 13–100% with pooled specificity of 0.89 (95% CI 0.85, 0.91).

These two representative parameters indicated an overall decent diagnostic value of Ang-2 as a promising noninvasive marker for HCC diagnosis. Moreover, the pooled positive likelihood ratio (PLR) and negative likelihood ratio (NLR) are 9.86 (CI 95% 4.42, 22.02), and 0.08 (95% CI 0.01, 0.44), respectively indicating high accuracy with diagnostic OR of 68.79 (95% CI 12.08 to 391.71). Nevertheless, considering the thresholds of PLR almost 10 and NLR < 0.1, the values for PLR (9.86) and NLR (0.08) in the present meta-analysis suggest caution regarding the diagnostic power of Ang-2 for HCC screening alone.

Tie-2 is an important cell-specific growth factor receptor, whose specific ligands are angiopoietins 1 and 2. Although Ang-1 is an agonistic ligand inducing stabilization and maturation of newly developed blood vessels, Ang-2 is considered a biological antagonist disrupting the stabilization caused by Ang- 1(36). Therefore, one of the proposed HCC tumor biomarkers is Ang-2. However, some varying findings were presented in literature from many quantitative analyses, such as Pestana et al. (24) who reported improvement in Ang-1 over Ang-2, Kuboki et al. (28) who reported increase in VEGF levels in patients with HCC, and Nouh et al. (25) who reported that AFP may show superiority over Ang-2 and combination of both significantly increase the sensitivity and specificity of detecting HCC, thus these variations suggesting the necessity of performing a systematic review and meta-analysis to evaluate the diagnostic accuracy of Ang-2 in the diagnosis of HCC. The present meta-analysis showed that when comparing healthy individuals to HCC patients, serum level of Ang-2 presented a statistically significant favor for HCC, which was significant yet inferior when Ang-2 was compared between HCC and cirrhotic liver patients favoring HCC.

As a potential diagnostic biomarker for HCC, Ang-2 has many exceptional advantages as compared with histopathological examination or Alpha Feto-protein (AFP): Ang-2 is obtained through a minimally invasive procedure, Ang-2 has an early presentation with the adequate amount in both HCC and cirrhotic patients, Ang-2 is significantly associated with long-term survival of patients with HCC proposing it as an independent prognostic factor (30). Kuboki et al. declared that not only high serum levels of Ang-2 have been associated with low survival rates in contrast with low levels which correlate to higher survival rates but also Ang-2 plays a vital role as a prognostic factor (28); however, only Sharma et al. didn't find any correlation of Ang-2 with survival (27). AFP is the most commonly used serum biomarker for HCC; nevertheless, AFP is characterized not only with 30–40% of HCC patients having serum AFP level within normal range but also with a high threshold for screening (400ng/ml) which indicate normal levels with small HCC lesions (< 3cm) leading to misdiagnosis in early tumor stages(37). However, the serum level of Ang-2 not only has increased in a direct proportionate of tumor size but also the administration of Ang-2 inhibitor has been associated with tumor size reduction by 62% over a period of 26 days in preclinical models (24). On the other hand, Scholz et al. mentioned that serum level of Ang-2 varies indirect relationship with tumor size, yet without statistical significance (P = 0.067 for the comparison of tumors > 80 mm with tumors < 30 mm and P = 0.073 for the comparison of tumors of 31–80 mm with tumors < 30 mm) (23).

Compared to other biomarkers such as mRNA, Midkine, or AFP, Ang-2 proved to have more diagnostic power. A systematic review and meta-analysis of the diagnostic effect of mRNA (38) between HCC patients and chronic liver disease patients were performed showing a pooled sensitivity and specificity were 85.2% (73.3–88.4%) and 79.2% (68.4–87.0%), respectively. Not only the pooled specificity was lower compared to our diagnostic accuracy but also the Area under the curve (AUC) -0.89 (95% CI: 0.85–0.91); furthermore, a moderate to significant heterogeneity existed without declaration. Another proposed tumor biomarker is Midkine (MDK) -a heparin-binding growth factor- was compared to AFP as a diagnostic biomarker for HCC in a systematic review and meta-analysis. The diagnostic accuracy of this meta-analysis (39) showed a pooled sensitivity of MDK (85%) and AFP (52%) and a pooled specificity of MDK (82%) and AFP (94%), in which sensitivity of both still lower than our Ang-2 pooled sensitivity, yet with higher specificity for AFP over Ang-2. Also, a separate meta-analysis (40) of MDK alone showed nearly the same results. The study of OA et.al 2021(31) has compared the double markers of AFP and DCP with Ang-2, and the double markers showed higher sensitivity and specificity than Ang-2 but still lower than our pooled sensitivity of Ang-2.

Taken together, serum Ang-2 may be a novel biomarker in HCC as a whole, also increasing the diagnostic accuracy of early-stage HCC. Significant heterogeneity was discovered in this meta-analysis (I^2 = 98), yet threshold analysis was done to explore the source of heterogeneity if because of different cut-off values used, and in our case, Spearman's correlation coefficient was 0.607 (p = 0.148). An absence of publication bias was also revealed by the funnel plot. The main limitation was presence of high heterogeneity rates among studies, probably caused by some differences in the methods, patient characteristics, and samples used. we also tried to perform a subgroup analysis. furthermore, we performed sensitivity analyses to avoid the systematic errors. Sensitivity analysis, with Leave-one-out analysis, revealed no single study showed to be affecting the high heterogeneity, the heterogeneity unchanged significantly with the removal of outlier studies (Scholz 2007, Wenli 2015). However, we have overcome it by using a random effects model meta-analyses which take the level of heterogeneity in consideration. Since clinical and methodological diversity always occur in a meta-analysis, statistical heterogeneity is inevitable (Cochrane book, 9.5.2). For heterogeneity of diagnostic accuracy meta-analysis, the Spearman test was performed to assess whether the different cut-off points from different studies have a direct effect on the heterogeneity and it indicated that the threshold effect plays no role in the heterogeneity. Based on the highest sensitivity, we suggest Chen 2017 et al cut-off point – 433.6 ng/ml – is the most reliable cut-off value.

Conclusion

Having both high pooled sensitivity and specificity, serum Ang-2 shows the potential to have a vital role as an independent diagnostic marker in HCC over other biomarkers used: AFP, MicroRNA. Therefore, further studies are needed to elaborate the potentiality of having a new efficient biomarker in an attempt to have a change of health care policy to start considering Ang-2 as one of the HCC biomarkers in clinical practice.

Declarations

Ethics approval and consent to participate: Not applicable

Consent for publication: Not applicable

Availability of data and materials: The data is available from the corresponding author, upon request.

Competing interests: None

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Table

Table 1: Characteristics of the included studies.

Author, Year	country	Study design	Gender (male/female)				age				Total n	Stage	Received treatment	device
			HCC	healthy	chronic	cirrhosis	HCC	healthy	chronic	cirrhosis				
KUBOKI ,2007	Japan	Case control	17/4	-	-	-	65 ≤(n=11) >65 (10)	-	-	-	31	Stage II + III: 10 . Iva: 11	NO	Quantikine immunoassay (R & D systems, Minneapolis, .MN, USA)
Scholz 2007	Germany	Retrospective cohort	72/21	108/72			65 (44–82)	57 (30–79)	-	-	314	grade 1 : 6 grade 2 : 35 stage 3: 16	-	Ang-2 ELISA kits were purchased from R&D systems (Wiesbaden, ,Germany)
Sharma 2013	India	Case control	-	-	-	-	-	-	-	-	200	-	No	Ang-2 ELISA kits from R and D Systems, Minneapolis, USA
Nouh 2015	Egypt	Case control	51/19 For HCC and healthy		-	-	55.13 ±8.16 for HCC and healthy groups		-	-	70	-	-	ELISA kit
Wenli 2015	China	Case control	117/24	12/15	22/6	15/20	57.5 ± 11.6	40.5 ±12.4	41.1±12.7	54.5 ±12.9	249	-	No	double antibody sandwich reverse competition ELISA method, the kit was purchased from ADL Biotech Dev Co., USA
Chen 2017	China	Case control	137/36	237/66	128/40		54.13 ±10,65	52.11 ±9,86	53.29 ±9,34		544	I–TNM II: 114 III–IV: 59	radiofrequency ablation (RFA), transcatheter arterial chemoembolization (TACE) and surgical resection	Enzyme-linked immunosorbent assay (ELISA) (R&D Systems, Inc. MN, USA)
Feillaci 2018	Italy	prospective cohort	16/5	-	-	88/46	58.8 ± 9	-	-	62.7 ±10.1	183	Edmondson-Steiner grade (1/2/3) (n): 1/12/8	Direct-acting antivirals (DAAs)	enzyme-linked immunosorbent assay kit (R&D Systems, Minneapolis, MN)
Pestana 2018	USA	case control	567/200	-	-	-	60 (n = ≤ 327) >60 (n = 440)	-	-	-	1042	TNM staging* Stage I-II: 253 Stage IIIA-IIIB: 225 Stage IIIC-IVB: 266	Surgery or transplant, Local therapy, Systemic therapy, AND Best supportive care	multiplex immunoassay (Myriad, Human Discovery MAP, v3.3, Austin, TX, USA)
Abdel gafar 2021	Egypt	Retrospective cohort	27/23	27/23	23/26	-	59.2 ± 6.7	57.5 ±7.1	-	58.8 ±6.3	149	BCLC staging : stage 0 : 4 patients stage A : 13 stage B : 12 stage C: 21 .patients	No	quantitative sandwich (R&D Systems, Minneapolis, USA)
AO 2021	Japan	Case control	198/77	14/6	62/36	-	71 (40-92)	60 (28-84)	67 (35-84)	-	393	UICC stage (I/II/III/IV): 128/68/59/2	No	sandwich ELISA (R&D Systems, Inc., MN, USA)
											TOAL =3175			

Figures

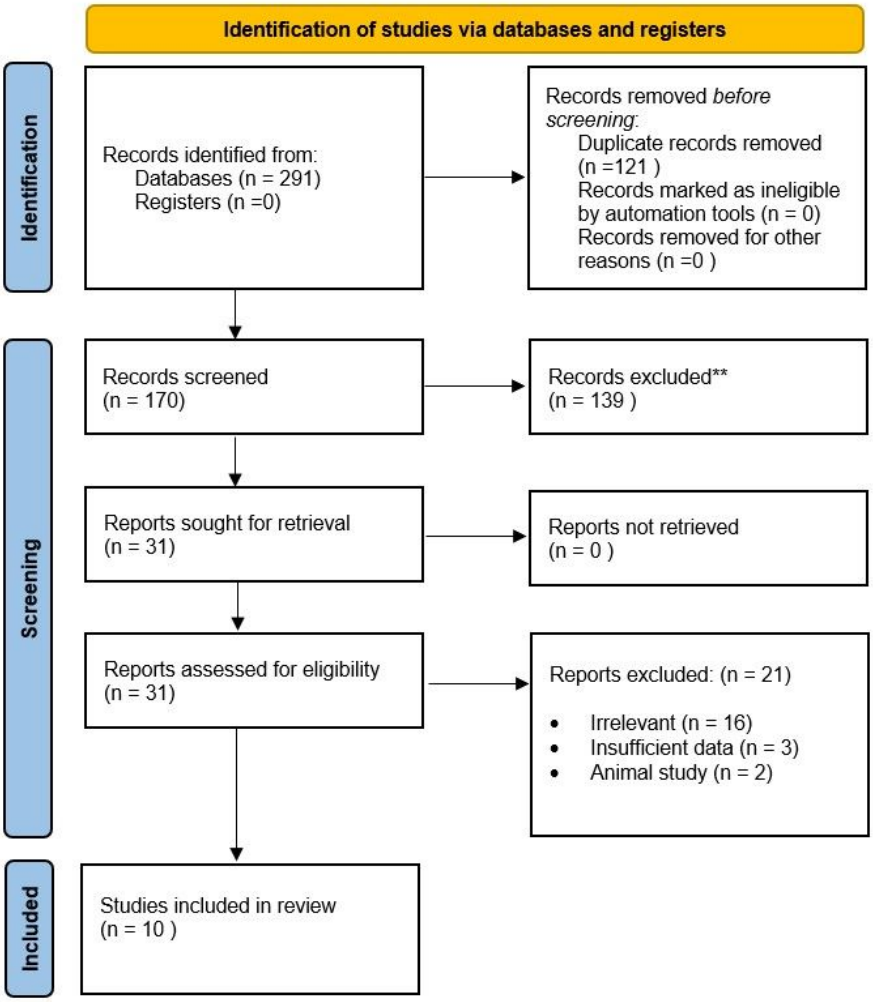

Figure 1

Prisma flow diagram

From: Page MJ, McKenzie JE, Bossuyt PM, Boutron I, Hoffmann TC, Mulrow CD, et al. The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. BMJ 2021;372:n71. doi: 10.1136/bmj.n71

For more information, visit: http://www.prisma-statement.org/

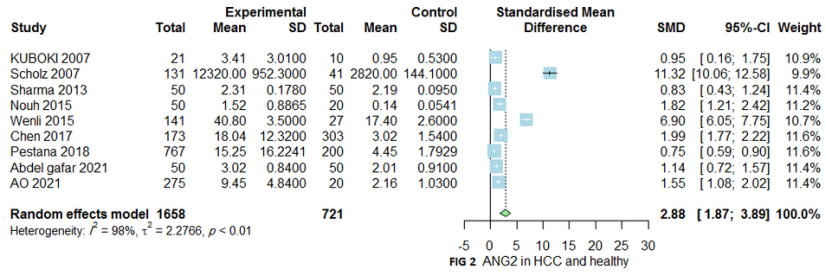

FIG 2 ANG2 in HCC and healthy

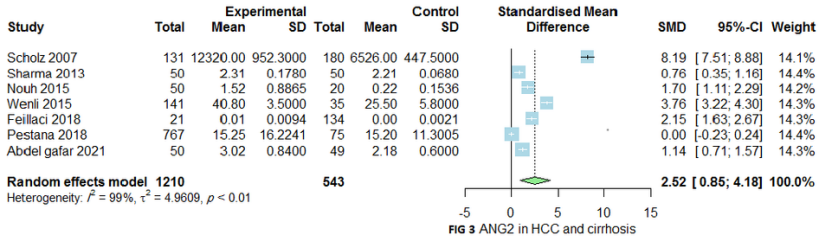

FIG 3 ANG2 in HCC and cirrhosis

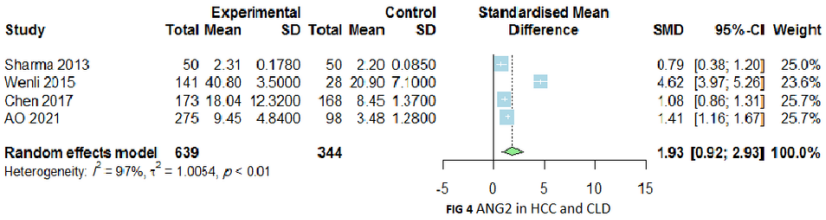

FIG 4 ANG2 in HCC and CLD

Figure 2

Forrest plots show the mean difference in Ang-2 between the HCC group, Cirrhotic group, CLD group, and healthy control group.

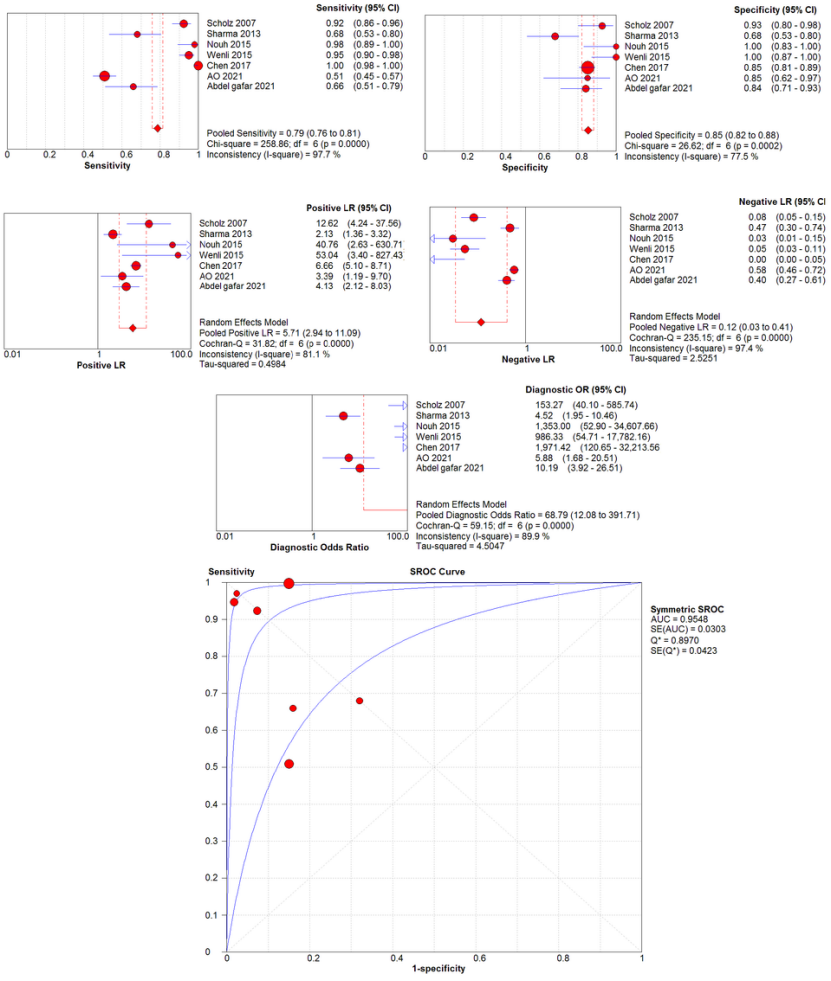

Figure 3

DTA for the Ang-2 for diagnosis HCC.

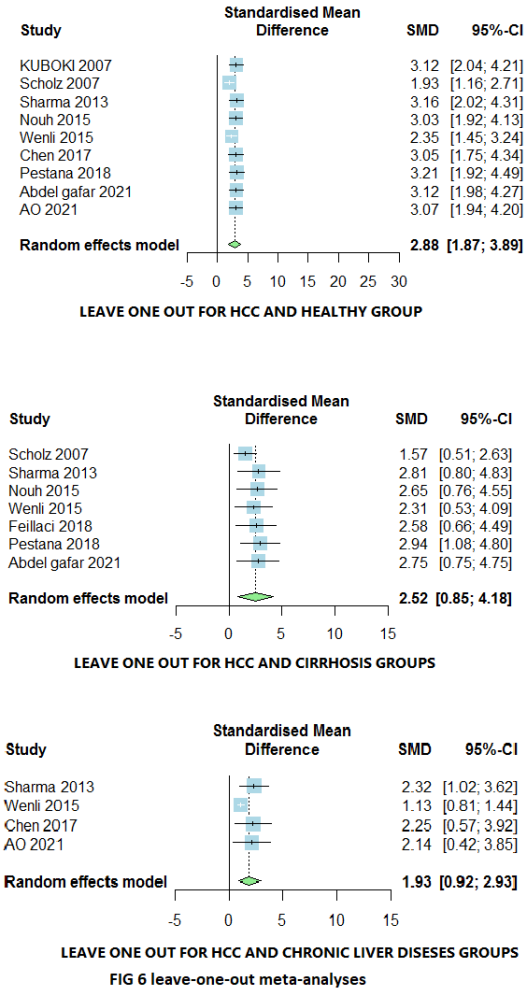

Figure 4

Legend not included with this version

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- riskofbiasTables.docx