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Prognostic and clinicopathological significance of hypoxia-inducible factors 1α and 2α in hepatocellular carcinoma: a systematic review with meta-analysis

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Abstract

Background: Hepatocellular carcinoma (HCC) is a highly recurrent tumor after resection and has been closely related to hypoxia. Hypoxia-inducible factors 1α and 2α (HIF-1α and HIF-2α) have been shown to contribute to tumor progression and therapy resistance in HCC. We evaluated the prognostic and clinicopathological significance of HIF-1α and HIF-2α in HCC patients.

Methods: We systematically searched Embase, Cochrane, PubMed, Scopus and Web of Science (WOS) from inception to 1 June 2020 for studies evaluating HIF-1α and/or HIF-2α expression in HCC. Selected articles evaluate at least one factor by immunohistochemistry (IHC) in HCC patients who underwent surgical resection, and its relationship with prognosis and/or clinicopathological features. Study protocol was registered in the International Prospective Register of Systematic Reviews (PROSPERO; CDR42020191977). We meta-analyzed the data extracted or estimated according to the Parmar method employing STATA software. We evaluated the overall effect size for the hazard ratio (HR) and odds ratio (OR) with 95% confidence interval (CI), as well as heterogeneity across studies with the I^2 statistic and chi-square-based Q test. Moreover, we conducted subgroup analysis when heterogeneity was substantial. Publication bias was assessed by funnel plot asymmetry and Egger's test.

Results: HIF-1α overexpression was correlated with overall survival (OS), disease-free survival (DFS)/recurrence-free survival (RFS) and clinicopathological features including Barcelona Clinic Liver Cancer (BCLC), capsule infiltration, intrahepatic metastasis, lymph node metastasis, tumor–node–metastasis (TNM), tumor differentiation, tumor number, tumor size (3 cm), vascular invasion and vasculogenic mimicry. We also detected a possible correlation of HIF-1α with alpha-fetoprotein (AFP), cirrhosis, histological grade, tumor size (5 cm) and albumin after subgroup analysis. Initially, only DFS/RFS appeared to be associated with HIF-2α overexpression. Subgroup analysis denoted that HIF-2α overexpression was related to OS and capsule infiltration.

Conclusions: HIF-1α and HIF-2α overexpression is related to poor OS, DFS/RFS and some clinicopathological features of HCC patients, suggesting that both factors could be useful HCC biomarkers.

Keywords: clinicopathological features, hepatocellular carcinoma, hypoxia-inducible factor 1α, hypoxia-inducible factor 2α, prognosis

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Introduction

Hepatocarcinoma or hepatocellular carcinoma (HCC) is the main type of primary liver tumor, which currently represents the sixth most common cancer and the fourth cause of cancer-related death worldwide.[1–3] HCC is frequently diagnosed in advanced stages, resulting in a high mortality rate. Moreover, despite early diagnosis allowing

curative resection, the recurrence rate in these patients usually reaches up to 60%.[4] Typical biomarkers such as alpha-fetoprotein (AFP) have been shown not to have enough value to predict HCC prognosis and metastatic recurrence.[4] Recent research has focused on discovering further useful biomarkers which include serum metabolites or enzymes.[5,6] Therefore, determining new effective biomarkers is necessary to predict the clinical prognosis and treatment response of HCC individuals accurately.

Hypoxia is a shared phenomenon among solid tumors, such as HCC, that plays a critical role in tumor development and progression, and is also associated with resistance to both radiation and sorafenib treatment in HCC.[7,8] The cellular response to low oxygen tension is mainly mediated by the hypoxia-inducible factors (HIFs), heterodimeric transcription factors comprising a constitutively expressed subunit (HIF-β) and an oxygen-regulated subunit (HIF-1α, HIF-2α and HIF-3α). Although both factors are frequently overexpressed in HCC, HIF-1α mediates acute hypoxia whereas HIF-2α likely drives the chronic hypoxia response.[8,9]

HIF-1α has been reported to be overexpressed in several tumors,[10–17] denoting a correlation between HIF-1α high expression and tumorigenesis, cancer progression and worse prognosis. Even though various research supports the role of HIF-1α overexpression in prompting invasion[18,19] and HCC patients' survival shortening,[19] the relationship of HIF-1α with every clinicopathological feature and prognosis in HCC still remains inconclusive. HIF-2α upregulation has also been linked to poor prognosis in diverse malignancies[11,14,20–24] while, in HCC, HIF-2α has been shown to promote invasion and metastasis.[25,26] However, there is a lack of documentation on the association between HIF-2α overexpression and a poorer outcome in HCC, given that existing results are controversial and inconsistent.[25,26]

In the present study, we conducted a systematic review with meta-analysis of the available evidence on the relationship between HIF-1α or HIF-2α expression and prognosis and clinicopathological features in HCC. Our aim was to assess the strength of this association to understand better the development and progression of HCC as well as to make better clinical decisions and to improve HCC patients' survival further.

Methods

This analysis was conducted following the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines (Supplemental Table 1).[27] The study protocol was previously registered in the International Prospective Register of Systematic Reviews (PROSPERO, registration number CRD42020191977).

Study objectives

We firstly aimed at evaluating the prognostic value of HIF-1α or HIF-2α expression in HCC patients who underwent surgical resection, concerning overall survival (OS) and disease-free survival (DFS)/recurrence-free survival (RFS). The second purpose was to explore the association of HIF-1α or HIF-2α expression with tumor and patient characteristics.

Search strategy

A comprehensive literature search of Embase, Cochrane, PubMed, Scopus and Web of Science (WOS) databases was performed prior to 1 June 2020. Studies eligible for this analysis were identified using the following search strategy: ("HCC" OR "hepatocarcinoma" OR "hepatocellular carcinoma") AND ("HIF" OR "hypoxia-inducible factor") (Supplemental Table 2).

Criteria for inclusion and exclusion

Studies meeting the following criteria were selected: (1) patients with distinctive HCC diagnosis by pathology; (2) HIF-1α or HIF-2α protein expression determined using immunohistochemistry (IHC); (3) samples obtained via surgical resection; (4) relationship between the HIF-1α or HIF-2α expression in HCC and clinicopathological features or survival information was examined; (5) appropriate statistical methodology was used; (6) articles in English.

We excluded studies complying with the following: (1) studies conducted only on cell lines or animals; (2) reviews, case reports, letters, book chapters or meeting communications; (3) tumor samples without intratumoral tissues, or just involving the paracarcinoma tissues; (4) the detection method was not IHC; (5) studies in which the required data were not provided or could not be calculated/estimated; (6) articles without English full text.

Table 1. Baseline characteristics of included articles.

Study (reference)	Year	Sample size	Sample size (M/F)	Intervention	Age range	Mean/ median age	Study quality	HIF-1α measurement	Survival analysis	Hazard ratios	High HIF-1α definition	Number of patients with 'high' HIF-1α
Huang *et al.*[29]	2005	36	32/4	Resection	19–77	45.90	6/9	IHC	OS	Estimated	Positive staining[a]	24
Wada *et al.*[30]	2006	60	45/15	Resection	44–79	63	6/9	IHC	DFS	Reported	>1% nuclear staining and/or strong cytoplasmic staining	7
Xie *et al.*[31]	2008	72	59/13	Surgical resection	23–71	50.57	7/9	IHC	OS/DFS	Reported	III and IV[b]	37
Dai *et al.*[32]	2009	110	95/15	Hepatectomy	28–75	52.40	7/9	IHC/RT-qPCR	OS/DFS	Reported	III and IV[b]	39
Liu *et al.*[33]	2010	200	169/31	Radical resection	NR	NR	7/9	IHC	OS/TTR	Reported	>50% with nuclear staining	126
Xiang *et al.*[34]	2011	309	262/47	Curative resection	NR	NR	7/9	IHC	NR	NR	⩾10% nuclear staining	85
Li *et al.*[35]	2012	35	30/5	Curative hepatectomy	34–68	50 ± 9.19	5/9	IHC	NR	NR	Positive staining[c]	28
Xia *et al.*[36]	2012	406	331/75	Curative resection	NR	51.10	7/9	IHC	OS/TTR	Estimated	4–5 (+) or 6–7 (++)[d]	212
Xiang *et al.*[37]	2012	69	61/8	Curative resection	NR	NR	7/9	IHC	OS/RFS	Reported	⩾10% nuclear staining	30
Cui *et al.*[38]	2013	55	34/21	Surgery	20–73	41	7/9	IHC	OS	NEP	3 (moderate staining) and 4–6 (strong staining)[e]	30
Ma *et al.*[39]	2013	207	156/51	Resection	23–80	57	6/9	IHC	NR	NR	⩾4[f]	147
Wang *et al.*[40]	2014	45	34/11	Hepatectomy	36–78	NR	6/9	IHC/RT-qPCR	OS	NEP	⩾3[g]	32
Yang *et al.*[41]	2014	126	110/16	Surgical resection	19–66	48.80	7/9	IHC/WB/RT-qPCR	CS/DFS	Estimated	III and IV[b]	72
Huang *et al.*[42]	2015	47	35/12	Surgery	33–74	53	6/9	IHC	NR	NR	⩾10% cyplasmic staining	19
Li *et al.*[43]	2015	102	87/15	Hepatectomy	NR	NR	6/9	IHC	OS/DFS	Estimated	2–4[h]	64
Srivastava *et al.*[44]	2015	179	142/37	Curative hepatectomy	NR	57.50	5/9	IHC	OS/RFS	Reported	III and IV[b]	108
Zhao *et al.*[45]	2015	97	NR	Surgical resection	NR	NR	5/9	IHC	CS	Estimated	3–6[i]	63
Tang *et al.*[46]	2016	143	130/13	Curative resection	21–70	49.47	6/9	IHC	CS/TTR	Estimated	Scores determined by software based on the percentage of positively stained cells and the staining intensity	72
Wang *et al.*[47]	2017	201	169/32	Hepatectomy	NR	NR	6/9	IHC	CS	Estimated	⩾4[j]	94
Dai *et al.*[48]	2018	90	84/6	Curative hepatectomy	13–81	54	6/9	IHC	OS/DFS	Reported	2 and 3[b]	39
Tian *et al.*[49]	2018	65	38/27	Surgery	25–77	46.50 ± 2.80	6/9	IHC/WB	NR	NR	>3[k]	30
Wang *et al.*[50]	2018	419	313/106	Surgery	NR	NR	7/9	IHC	OS	Reported	⩾30%	223 (6 missing)
Zou *et al.*[51]	2018	138	116/22	Surgery	NR	NR	6/9	IHC/RT-qPCR/WB	CS/TTR	Estimated	Optimal cut-off point of the relative integrated optical densities based on patients' outcome	73

(Continued)

Table 1. (Continued)

Study (reference)	Year	Sample size	Sample size (M/F)	Intervention	Age range	Mean/ median age	Study quality	HIF-1α measurement	Survival analysis	Hazard ratios	High HIF-1α definition	Number of patients with 'high' HIF-1α
Gong *et al.*[52]	2019	137	115/22	Primary surgical resection	NR	NR	7/9	IHC	OS/TTR	Estimated	⩾6[l]	68
Wu *et al.*[53]	2019	119	NR	Curative resection	NR	NR	5/9	IHC	OS	Estimated	⩾6[l]	67
Zhou *et al.*[54]	2020	90	74/16	Surgery	NR	NR	2/9	IHC	OS	NEP	NR	NR
Qian *et al.*[55]	2020	111	NR	Surgery	NR	NR	5/9	IHC	OS	Estimated	>2[f]	57 (2 missing)
Study (Reference)	**Year**	**Sample size**	**Sample size (M/F)**	**Intervention**	**Age range**	**Mean/ median age**	**Study quality**	**HIF-2α measurement**	**Survival analysis**	**Hazard ratios**	**High HIF-2α definition**	**Number of patients with "high" HIF-2α**
Bangoura *et al.*[56]	2004	97	76/21	Resection	34–78	61.40 ± 8.90	6/9	IHC	NR	NR	++ (dark brown)	31
Bangoura *et al.*[57]	2007	315	260/55	Curative surgical resection	46–79	60.80	7/9	IHC	CS	Estimated	Positive staining[m]	219
Sun *et al.*[58]	2013	246	198/48	Curative resection	NR	NR	6/9	IHC/WB/RT-qPCR	OS	Reported	>50%	118
Yang *et al.*[41]	2014	126	110/16	Surgical resection	19–66	48.80	7/9	IHC/WB/RT-qPCR	CS/DFS	Estimated	III and IV[b]	17
Yang *et al.*[59]	2016	206	177/29	Radical resection	31–84	57.20	7/9	IHC/WB	OS/RFS	Reported	>50%	67
Jiang *et al.*[60]	2018	84	70/14	Curative surgery	NR	NR	7/9	IHC/WB/RT-qPCR	NR	NR	⩾4[n]	34
Chen *et al.*[61]	2019	139	116/23	Hepatectomy	NR	NR	7/9	IHC/WB/RT-qPCR	OS/DFS	Estimated	Median value of final scores (product of the percentage of stained cells by the staining strength) as a cut-off	67
Cao *et al.*[62]	2020	328	NR	Surgery	NR	NR	5/9	IHC	OS	NEP	3–9[j]	NR

CS, cumulative survival; DFS, disease-free survival; F, female; HIF, hypoxia-inducible factor; ICH, immunohistochemistry; M, male; NEP, no estimation possible; NR, not reported; OS, overall survival; RFS, recurrence-free survival; RT-qPCR, reverse transcription-quantitative polymerase chain reaction; TTR, time to recurrence; WB, western blot.

[a]The sum of sections with weak and strong staining.

[b]Nuclear staining in 10–50% of cells and/or distinct/moderate cytoplasmic staining (III or 2), and nuclear staining in >50% of cells and/or strong cytoplasmic staining (IV or 3).

[c]The sum of cases with weak (10–25%), moderate (26–50%) and strong staining (>51%).

[d]Final scores were assessed by the sum of the intensity (0, negative; 1, weak; and 2, strong), and the staining extent based on the percentage of positive tumor cells (0, negative; 1, 1–25%; 2, 26–50%; 3, 51–75%; and 4, 76–100%).

[e]Final scores were assessed by the sum of the intensity (0, negative; 1, weak; 2, intermediate; 3, strong), and the extent of immunoreaction (0, 0%; 1, <5%; 2, 5–50%; and 3, >50%).

[f]Final scores were assessed by multiplying the result of the percentage of positive cells (⩽5%, 0; –25%, 1; –50%, 2; –75%, 3; >75%, 4) by the staining intensity (colorless, 0; pale yellow, 1; deep yellow and brownish red, 2; sepia, 3).

[g]Final scores were assessed by the sum of the cytoplasmic staining degree (0, no or negligible staining; 1, pale yellow staining; 2, brown-yellow staining; 3, brown staining) and the punctuation obtained based on the percentage of positively stained cells (0, <5%; 1, 5–25%; 2, >25–50%; and 3, >50%).

[h]Final scores were assessed by determining the percentage of immunoreactive tumor cells (0,0%; 1, 1–25%; 2, 26–50%; 3, 51–75%; 4, 76–100%).

[i]Final scores were assessed by the sum of the positivity extent (0, <10%; 1, <25%; 2, <50%; 3, >50%) and the staining intensity (0, no appreciable; 1, barely detectable; 2, readily visible; 3, dark brown staining).

[j]Final scores were assessed by multiplying the positivity extent (0, <10%; 1, <25%; 2, <50%; 3, >50%) by the staining intensity (0, no appreciable; 1, barely detectable; 2, readily visible; 3, dark brown staining).

[k]Final scores were assessed by multiplying the degree of staining (negative control, 0; light yellow, 1; tan, 2; sepia, 3) by the scoring of the positive cells proportion (⩽10%, 1; 11–50%, 2; 51–75%, 3; >75%, 4).

[l]Final scores were assessed by multiplying the stained area (1, 1–25%; 2, 26–50%; 3, 51–75%; 4, 76–100%) by the staining intensity (0, 1, 2, or 3)

[m]More than 65% of cells were stained intensely (+++) or moderately (++) or weakly (+).

[n]Final scores were assessed by multiplying the results of the percentage (0, <10%; 1, 10–30%; 2, 31–60%; 3, >61%) and the intensity (0, lack of any immunoreactivity; 1, light yellow; 2, yellow-brown; and 3, brown) of immune-staining cells.

Data extraction and quality assessment

Three authors, CMB, PFP and FF independently screened the full text of selected studies to confirm eligibility, assess quality, and extract data. Discrepancies were resolved by discussion and consensus.

The baseline characteristics of each included study were extracted and are shown in Table 1.[29–62] The Newcastle–Ottawa scale (NOS) score was used for assessing the quality of selected articles, which ranged from 0 to 9.[28] Studies with scores ≥ 5 were regarded as high-quality studies; while low-quality studies were not included in the quantitative synthesis. Furthermore, we collected in Supplemental Table 3 the IHC antibodies and the staining procedure employed in the included articles.

Statistical analysis

STATA software version 16 (Stata Corporation, College Station, TX, USA) was employed to assess the correlation between HIF-1α or HIF-2α expression and prognosis and clinicopathological features in HCC.

We measured the effect of HIF expression on HCC in two steps. Firstly, we pooled the OS, DFS, RFS and time to recurrence (TTR) by hazard ratio (HR) and 95% confidence interval (CI) to calculate the effective value to assess the correlation between each HIF and HCC prognosis. OS was measured from the intervention date until either the day of death or the last follow-up visit. DFS, RFS and TTR were defined as the period from the intervention date to the date of last follow-up or recurrence. HR and the corresponding 95% CI were combined across studies. The Parmar method[63] was used to extract data when no direct information could be obtained from the primary study. Secondly, the strength of association between HIF-1α or HIF-2α overexpression and tumor clinicopathological features was evaluated by estimating the odds ratio (OR) with 95% CI. Combined HR >1 and OR >1 suggested a higher risk of poor survival and a higher incidence of the analyzed feature, respectively, related to HIF overexpression. These relationships were significant when $p<0.05$.

Heterogeneity was tested using the chi-square-based Q test, showing significant levels when the p-value was <0.1. The I^2 statistic, a quantitative measure of inconsistency across studies, was also calculated. The I^2 varies from 0% (no observed heterogeneity) to 100% (maximal heterogeneity). $I^2 \geq 50\%$ was considered to represent substantial heterogeneity. The restricted maximum likelihood (REML) method as the random-effect model was employed when heterogeneity was confirmed by at least one statistical method. Otherwise, the fixed-effects model with inverse variance (IV) method was used. To explore the heterogeneity sources, we conducted subgroup analyses based on sample size, NOS score, follow-up and median age.

We assessed the possibility of publication bias by evaluating funnel plot asymmetry and Egger's test. When Egger's p-value was <0.05 and the funnel plot was asymmetric, significant publication bias existed. In this case, the trim-and-fill method was used to estimate a corrected effect size after adjustment, which helped to determine whether the publication bias substantially affected the robustness of the pooled results.

Results

Study characteristics

A total of 3888 applicable studies were identified after the database search, 2172 studies were duplicates, and after scanning titles and abstracts another 1386 articles were omitted for the following reasons: animal or cells studies, non-HCC or HIF articles, reviews or similar. The full text of 330 articles was assessed for eligibility, finding 24 with full text in Chinese, 264 without HIF IHC or analysis about survival or clinicopathological features, seven did not employ surgical resection and one was about HIF-3α. Thus, these 296 papers were also excluded from our study. After screening, 34 studies[29–62] were assessed for quality and data extraction. Cao *et al.*[62] and Zhou *et al.*[54] did not provide enough data to calculate HR and its 95% CI. Moreover, Zhou *et al.*[54] did not reach the quality threshold (Table 1). Eventually, 32 publications were eligible for quantitative meta-analysis: 25 on HIF-1α, six on HIF-2α and one about both factors (Figure 1).

The baseline of included articles and results of quality assessment are summarized in Table 1. These studies were published from 2004 to 2020 and a total of 3578 (eight were 'missing' in HIF-1α expression analysis) and 1213 HCC patients consisted of HIF-1α and HIF-2α, respectively. All included patients came from Asia, mostly from China. For HIF-1α, patients' number across studies ranged

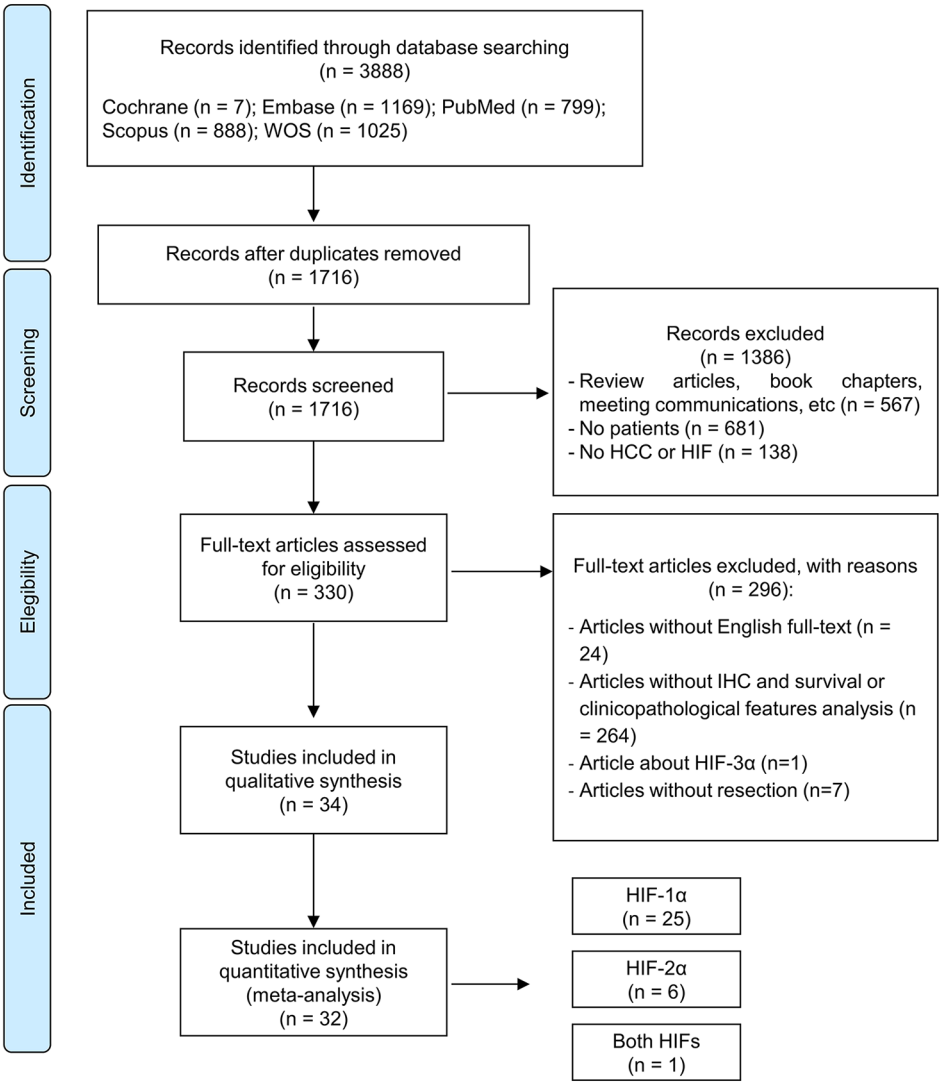

Figure 1. PRISMA flowchart of study selection.
HCC, hepatocellular carcinoma; HIF, hypoxia-inducible factor; IHC, immunohistochemistry; PRISMA, preferred reporting items for systematics reviews and meta-analysis; WOS, Web of Science.

from 35 to 419, and 1846 (51.7%) had HIF-1α overexpression. From 84 to 315 patients by study were enrolled to HIF-2α analysis, 553 (45.6%) showed HIF-2α overexpression. Among the 25 HIF-1α articles, 18 provided data on OS, eight on DFS/RFS and 23 on clinicopathological features; while for HIF-2α, five of seven articles supplied OS, three DFS/RFS and all of them clinicopathological features. Five HIF-1α studies evaluated TTR; nonetheless, only one article reported the HR and estimation was not possible according to the Parmar method. Hence, TTR analysis was not included.

Patients in all studies underwent surgical resection. In Wada *et al.*[30] and Dai *et al.*[32] some of the enrolled patients received preoperative antitumor therapy. Moreover, patients undergoing external beam radiotherapy had postoperative adjuvant treatment in Xiang *et al.*[37] The rest of the included studies had no intervention prior to surgery. Regarding etiology, within the 32 papers included 21 evaluated patients with hepatitis B,[29–31,33–39,41,43,44,46,50–52,57–59,61] nine assessed patients with hepatitis C,[30,34,36,41,43,44,57–59] and only one alcoholic patient was assessed;[44] it should be noted that all the studies were performed in the Asiatic population, where hepatitis B is the key etiology factor. Likewise, 20 articles evaluated HCC patients derived from cirrhosis.[29–34,36,39,41,43,44,46,48,50–52,56–59] In summary, we extracted these data and among the patients included in the present meta-analysis, 78.2% had hepatitis B, 6.6% had hepatitis C and 66.1% had cirrhosis.

Association of HIF protein expression with prognosis

Based on the meta-analysis, we evaluated the prognostic value of HIF-1α and found that high expression correlated with OS (HR 1.73; 95% CI 1.54–1.94; $p=0.00$) and with DFS/RFS (HR 1.64; 95% CI 1.36–1.99; $p=0.00$), not finding significant heterogeneity (Figure 2A).

We also assessed the correlation between HIF-2α protein levels and prognosis. The results suggest that there is no significant association between HIF-2α high expression and OS in HCC patients (HR 1.25; 95% CI 0.68–2.32; $p=0.48$), assuming heterogeneity among studies. However, HIF-2α overexpression appears to be associated with DFS/RFS (HR 1.37; 95% CI 1.05–1.79; $p=0.02$), and no heterogeneity was shown (Figure 2B).

Association of HIF protein expression with clinicopathological features

Otherwise, we evaluated the possible correlation between HIF expression and different clinicopathological features of HCC patients.

High HIF-1α protein levels were positively associated with Barcelona Clinic Liver Cancer (BCLC) staging (OR 2.49; 95% CI 1.56-3.98; $p=0.00$), capsule infiltration (OR 2.48; 95% CI 1.29–4.77; $p=0.01$), intrahepatic metastasis (OR 2.90; 95% CI 1.62–5.20; $p=0.00$), lymph node metastasis (OR 3.74; 95% CI 1.73–8.07; $p=0.00$), tumor–node–metastasis (TNM) classification (I, II–III) (OR 1.59; 95% CI 1.21–2.09; $p=0.00$), TNM (I-II, III) (OR 2.62; 95% CI 1.69–4.08; $p=0.00$), TNM (I–II, III–IV) (OR 2.23; 95% CI 1.37–3.64; $p=0.00$), tumor differentiation (OR 1.78; 95% CI 1.07–2.96; $p=0.03$), tumor number (OR 1.50; 95% CI 1.15–1.96; $p=0.00$), tumor size (3 cm) (OR 3.70; 95% CI 1.29–10.63; $p=0.02$), vascular invasion (OR 2.61; 95% CI 1.82–3.75; $p=0.00$) and vasculogenic mimicry (OR 2.61; 95% CI 1.67–4.09; $p=0.00$) (Figure 3). We also found that there is no statistical significance with other tumor features, including AFP levels (20 ng/ml) (OR 1.39; 95% CI 0.92–2.09; $p=0.11$), AFP (400 ng/ml) (OR 1.49; 95% CI 0.67–3.33; $p=0.33$), age (50 years) (OR 0.86; 95% CI 0.57–1.31; $p=0.49$), age (60 years) (OR 1.03; 95% CI 0.68–1.55; $p=0.90$), albumin (35 U/L) (OR 0.60; 95% CI 0.26–1.38; $p=0.23$), alanine aminotransferase (ALT) (40 U/L) (OR 0.86; 95% CI 0.60–1.24; $p=0.42$), ALT (80 U/L) (OR 1.04; 95% CI

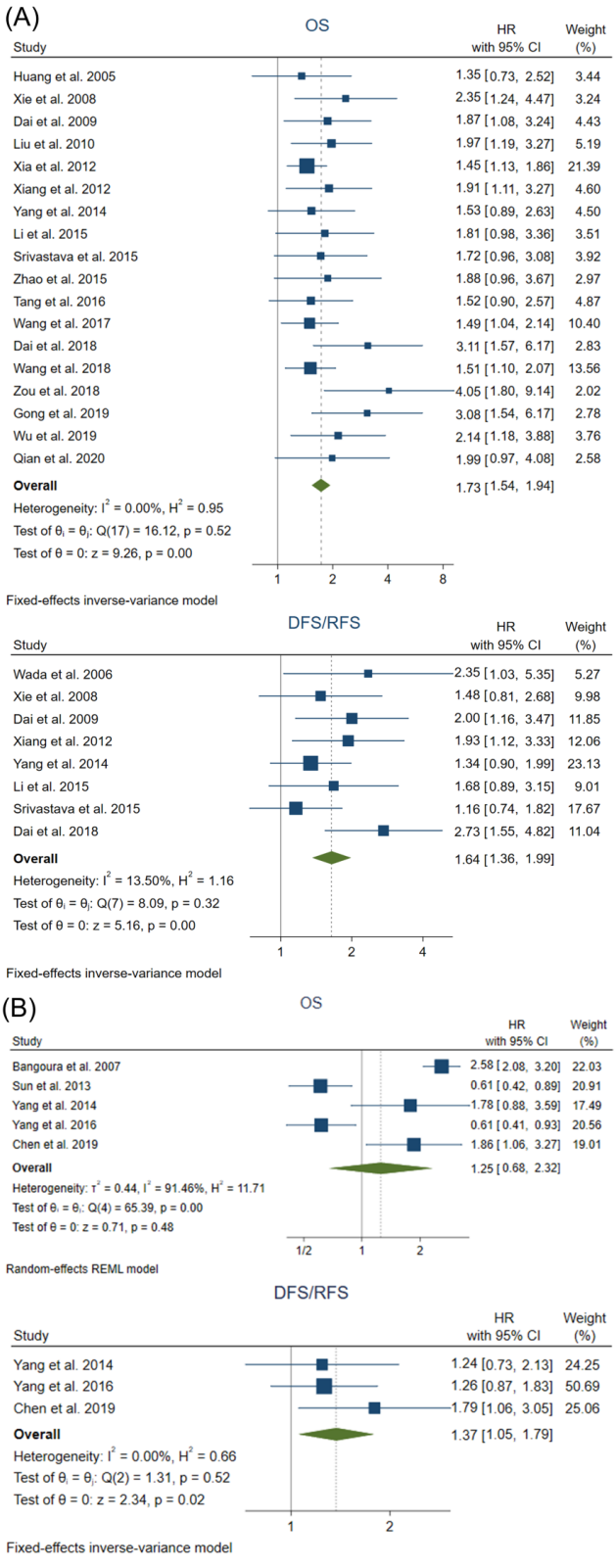

Figure 2. Meta-analysis of the prognostic value of HIF-1α and HIF-2α in HCC patients. Forest plot of OS and DFS/RFS for (A) HIF-1α and (B) HIF-2α. CI, confidence interval; DFS, disease-free survival; HR, hazard ratio; HCC, hepatocellular carcinoma; HIF, hypoxia-inducible-factor; OS, overall survival; REML, restricted maximum likelihood; RFS, recurrence-free survival.

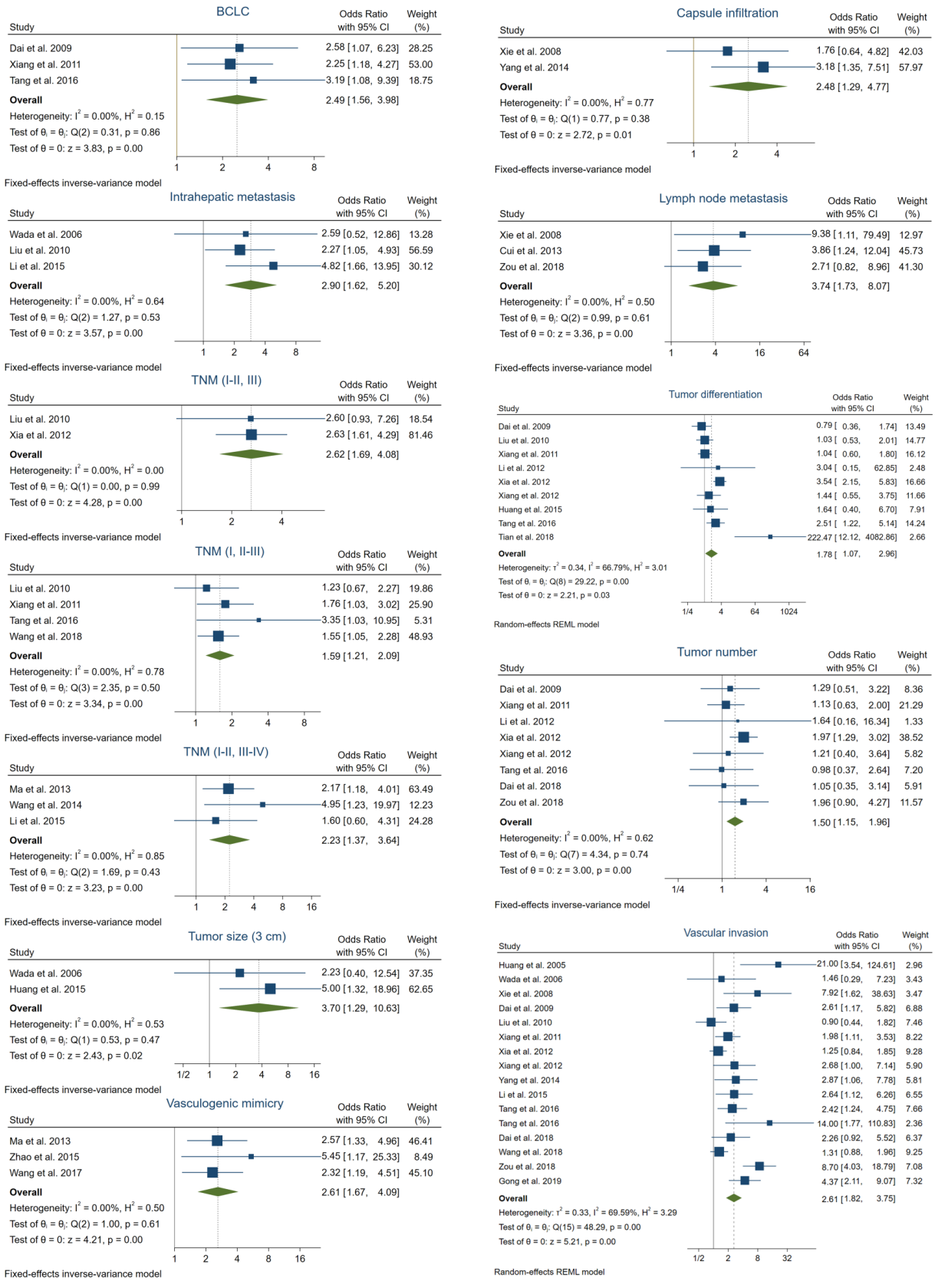

Figure 3. Forest plot of the clinicopathological features significantly associated with HIF-1α overexpression in HCC patients.
BCLC, Barcelona Clinic Liver Cancer; CI, confidence interval; HCC, hepatocellular carcinoma; HIF, hypoxia-inducible-factor; REML, restricted maximum likelihood; TNM, tumor–node–metastasis.

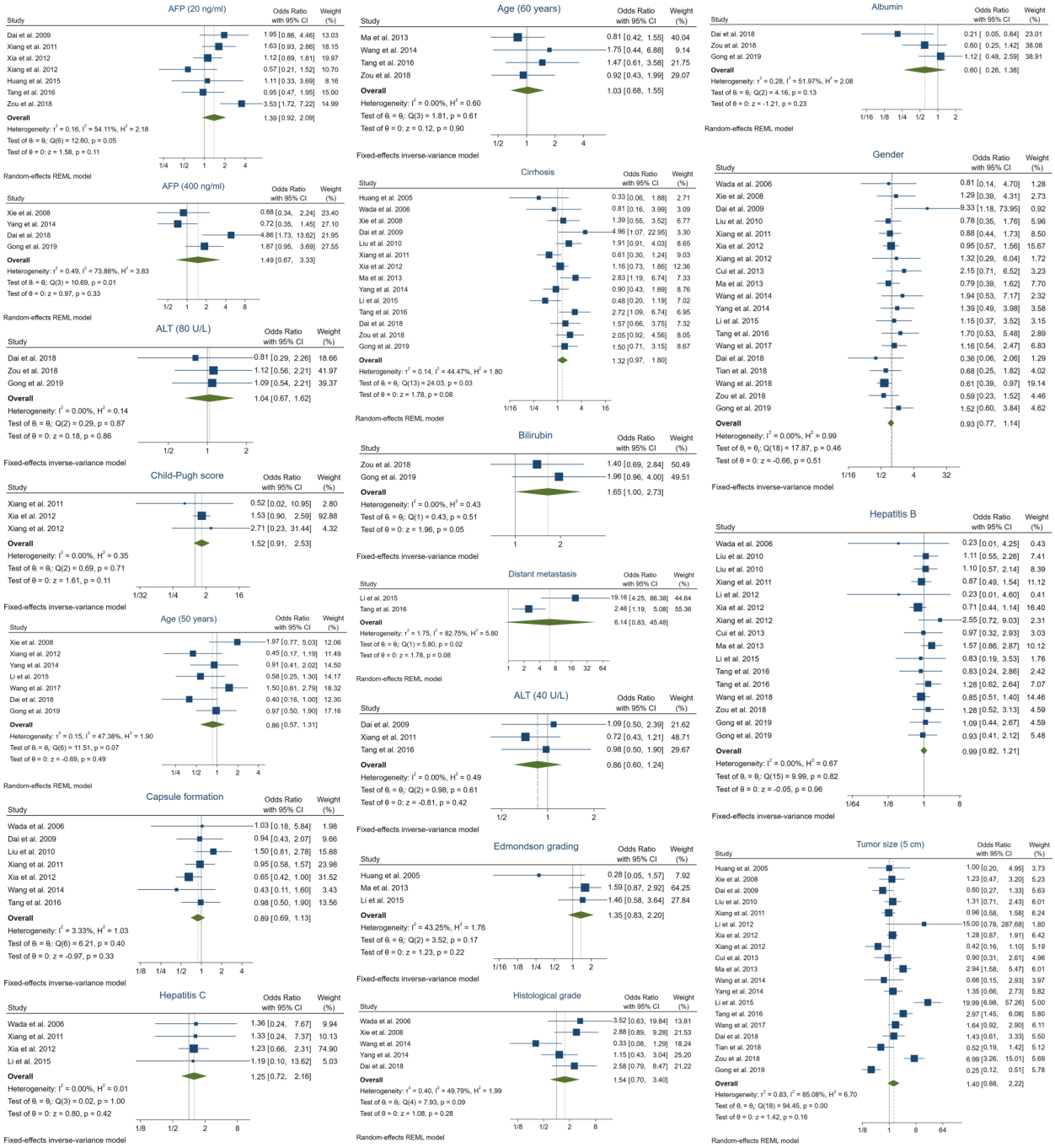

Figure 4. Forest plot of the clinicopathological features not significantly associated with HIF-1α overexpression in HCC patients.
AFP, alpha-fetoprotein; ALT, alanine aminotransferase; CI, confidence interval; HCC, hepatocellular Carcinoma; HIF, hypoxia-inducible-factor; REML, restricted maximum likelihood.

0.67–1.62; $p = 0.86$), bilirubin (1 μmol/L) (OR 1.65; 95% CI 1.00–2.73; $p = 0.0501$), capsule formation (OR 0.89; 95% CI 0.69–1.13; $p = 0.33$), Child–Pugh score (OR 1.52; 95% CI 0.91–2.53; $p = 0.11$), cirrhosis (OR 1.32; 95% CI 0.97–1.80; $p = 0.08$), distant metastasis (OR 6.14; 95% CI 0.83–45.48; $p = 0.08$), Edmondson grading (OR 1.35; 95% CI 0.83–2.20; $p = 0.22$), gender (OR 0.93; 95% CI 0.77–1.14; $p = 0.51$), hepatitis B (OR 0.99; 95% CI 0.82–1.21; $p = 0.96$), hepatitis C (OR 1.25; 95% CI 0.72–2.16; $p = 0.42$), histological grade (OR 1.54; 95% CI 0.70–3.40; $p = 0.28$) and tumor size (5 cm) (OR 1.40; 95% CI 0.88–2.22; $p = 0.16$) (Figure 4).

All HIF-2α studies were included in the analysis of clinical and pathological features. Positive HIF-2α expression was not significantly associated with any feature analyzed: AFP levels (400 ng/ml) (OR 0.88; 95% CI 0.60–1.30; $p = 0.52$), age (50 years) (OR 1.17; 95% CI 0.79–1.73; $p = 0.44$), capsule formation (OR 1.31; 95% CI 0.93–1.83; $p = 0.12$), capsule infiltration (OR 1.82; 95% CI 0.54–6.13; $p = 0.33$), cirrhosis (OR 1.22; 95% CI 0.91–1.64; $p = 0.19$), Edmondson grading (OR 11.05; 95% CI 0.02–6167.72; $p = 0.46$), gender (OR 0.95; 95% CI 0.68–1.35; $p = 0.79$), hepatitis B (OR 1.03; 95% CI 0.76–1.39; $p = 0.86$), histological grade (OR 0.93; 95% CI 0.43–1.99; $p = 0.85$), necrosis (OR 1.32; 95% CI 0.25–6.98; $p = 0.74$), TNM (I–II, III–IV) (OR 1.12; 95% CI 0.40–3.10; $p = 0.83$) tumor number (OR 1.44; 95% CI 0.92–2.27; $p = 0.11$), tumor size (5 cm) (OR 1.20; 95% CI 0.36–3.99; $p = 0.77$) and vascular invasion (OR 1.16; 95% CI 0.67–2.00; $p = 0.60$) (Figure 5).

Subgroup analysis

To explore the potential heterogeneity sources, subgroup analysis for heterogeneity parameters was performed.

When subgroups for HIF-1α were based on sample size, HIF-1α expression was related to AFP levels (20 ng/ml) ($n \geq 100$: OR 1.59; 95% CI 1.03–2.46; $p = 0.04$), cirrhosis ($n < 300$: OR 1.48; 95% CI 1.06–2.07; $p = 0.02$), tumor size (5 cm) ($n \geq 200$: OR 1.46; 95% CI 1.04–2.06; $p = 0.03$) and vascular invasion ($n \geq 100$: OR 2.39; 95% CI 1.58–3.61; $p = 0.00$) ($n < 100$: OR 3.22; 95% CI 1.87–5.55; $p = 0.00$) ($n \geq 200$: OR 1.32; 95% CI 1.04–1.68; $p = 0.02$) ($n < 200$: OR 3.54; 95% CI 2.69–4.66; $p = 0.00$) ($n \geq 300$: OR 1.39; 95% CI 1.08–1.79; $p = 0.01$) ($n < 300$: OR 3.22; 95% CI 2.13–4.88; $p = 0.00$) ($n < 400$: OR 3.04; 95% CI 2.08–4.45; $p = 0.00$); nonetheless, heterogeneity continued to be substantial in some cases. Sample size subgroups provided assumable heterogeneity for AFP levels (20 ng/ml) ($n < 100$, $n \geq 200/300$), age (50 years) ($n \geq 100$, $n < 200$), albumin ($n \geq 100$), cirrhosis ($n < 100$), tumor size (5 cm) ($n < 100$, n≥300) and vascular invasion ($n < 100$, $n \geq 200$, $n < 200$, $n \geq 300$, $n \geq 400$). NOS score subgroups displayed an association between HIF-1α overexpression and albumin ($n < 7$: OR 0.45; 95% CI 0.21–0.93; $p = 0.03$), tumor differentiation ($n \geq 6$: OR 1.76; 95% CI 1.04–2.97; $p = 0.04$), tumor size (5 cm) ($n = 6$: OR 2.27; 95% CI 1.10–4.70; $p = 0.03$) ($n < 7$: OR 2.45; 95% CI 1.20–4.99; $p = 0.01$) and vascular invasion ($n \geq 7$: OR 1.99; 95% CI 1.36–2.90; $p = 0.00$) ($n < 7$: OR 4.00; 95% CI 2.14–7.46; $p = 0.00$), where only albumin subgroup presented low heterogeneity ($n < 7$). In addition, NOS classification resolved heterogeneity for AFP (20 ng/ml) ($n \geq 7$), AFP (400 ng/ml) ($n \geq 7$), age (50 years) ($n \geq 7$), cirrhosis ($n \geq 7$) and histological grade ($n \geq 7$), but not showing association with protein expression. Curiously, the single elimination of Zou *et al.*[51] in AFP (20 ng/ml), and Xia *et al.*[36] and Tian *et al.*[49] in tumor differentiation led to an assumable heterogeneity, although there was no correlation with HIF-1α overexpression. However, the deletion of Xiang *et al.*[34] and Ma *et al.*[39] for cirrhosis, and Wang *et al.*[40] for histological grade, achieved low heterogeneity and a significant association with HIF-1α (Table 2).

For HIF-2α, when subgroups were based on sample size, HIF-2α expression was linked to OS ($n < 200$: HR 1.83; 95% CI 1.18–2.84; $p = 0.01$), where heterogeneity was solved. Moreover, heterogeneity was eliminated from vascular invasion ($n < 100$, $n < 200$, $n < 300$), but no association was found. The follow-up subgroup showed a relationship between OS and HIF-2α overexpression (follow-up ≥72: HR 2.47; 95% CI 2.02–3.03; $p = 0.00$) with reduced heterogeneity. Capsule infiltration was analyzed according to median age, displaying correlation to HIF-2α (years ≥ 50: OR 2.71; 95% CI 1.55–4.73; $p = 0.00$) and low heterogeneity. Subgroup classification by NOS did not exhibit any change. Thus, tumor size heterogeneity could not be resolved (Table 2).

Subgroups with only one study were not considered. The results of subgroup analysis revealed that sample size, NOS score, median age and follow-up time likely triggered heterogeneity.

Publication bias

There was pronounced asymmetry denoting publication bias on the OS parameter for HIF-1α that was confirmed by Egger's test ($p = 0.0027$). Hence, the trim-and-fill method was used, in which seven studies were imputed and the global effect size was corrected (HR 1.559; 95% CI 1.405–1.731). Instead, there was no asymmetry detected for DFS/RFS ($p = 0.0631$) (Table 3; Figure 6A). Concerning the clinicopathological features of HIF-1α, asymmetry was only identified in gender ($p = 0.0255$), tumor differentiation ($p = 0.0428$) and vascular invasion ($p = 0.0016$).

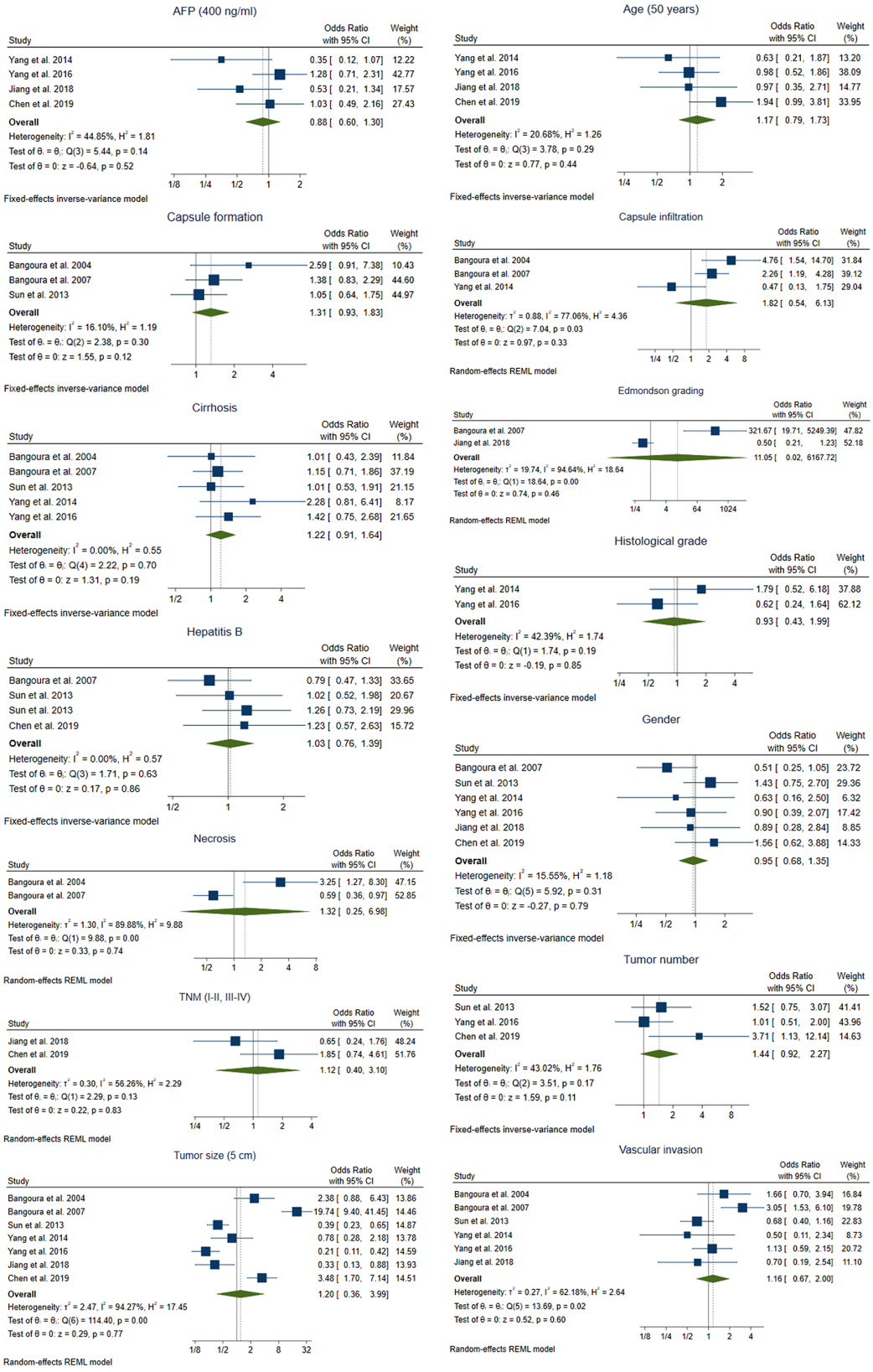

Figure 5. Forest plot of the association between HIF-2α overexpression and clinicopathological features in HCC patients.

AFP, alpha-fetoprotein; CI, confidence interval; HCC, hepatocellular carcinoma; HIF, hypoxia-inducible-factor; REML, restricted maximum likelihood; TNM, tumor–node–metastasis.

Table 2. Subgroup analysis of prognostic and clinicopathological features of HIF-1α and HIF-2α.

HIF-1α										
Subgroups	**Number of studies (*n*)**	**Number of cases (*n*)**	**HIF-1α$^+$ (*n*)**	**HIF-1α$^+$ (%)**	**Pooled data**			**Test for heterogeneity**		**Model used**
					OR	**95% CI**	***p*-value**	***p*-value**	**I^2 (%)**	
AFP (20 ng/ml)										
Sample size (*n*)										
⩾100	5	1106	481	43.49	1.59	1.03–2.46	0.04	0.06	55.89	REM
<100	2	116	49	42.24	0.74	0.35–1.59	0.45	0.40	0.00	FEM
⩾200/300	2	715	297	41.54	1.31	0.91–1.89	0.15	0.32	0.00	FEM
<200/300	5	507	233	45.96	1.39	0.73–2.64	0.31	0.02	64.06	REM
⩾400	1	406	212	52.22	1.12	0.69–1.81	0.65	–	–	REM
<400	6	816	318	38.97	1.45	0.88–2.40	0.14	0.04	57.84	REM
NOS score										
⩾7	4	894	366	40.94	1.27	0.93–1.75	0.13	0.20	35.04	FEM
<7	3	328	164	50.00	1.61	0.67–3.88	0.29	0.03	68.87	REM
Without Zou *et al.*[51]	6	1084	457	42.16	1.21	0.91–1.60	0.19	0.40	3.17	FEM
AFP (400 ng/ml)										
Sample size (*n*)										
⩾100	2	263	140	53.23	1.16	0.45–2.98	0.75	0.06	72.69	REM
<100	2	162	76	46.91	2.03	0.38–10.90	0.41	0.02	82.78	REM
NOS score										
⩾7	3	335	177	52.84	1.11	0.72–1.71	0.65	0.14	49.57	FEM
<7	1	90	39	43.33	4.86	1.73–13.62	0.00	–	–	REM
Age (50 years)										
Sample size (*n*)										
⩾100	4	566	298	52.65	1.00	0.70–1.43	0.99	0.32	13.66	FEM
<100	3	231	106	45.89	0.70	0.26–1.94	0.50	0.03	71.05	REM
⩾200	1	201	94	46.77	1.50	0.81–2.79	0.20	–	–	REM
<200	6	596	310	52.01	0.77	0.55–1.09	0.14	0.15	38.69	FEM
NOS score										
⩾7	4	404	207	51.24	0.95	0.63–1.43	0.82	0.20	35.07	FEM
<7	3	393	197	50.13	0.74	0.33–1.66	0.46	0.03	68.99	REM

(Continued)

Table 2. (Continued)

HIF-1α										
Subgroups	**Number of studies (*n*)**	**Number of cases (*n*)**	**HIF-1α⁺ (*n*)**	**HIF-1α⁺ (%)**	**Pooled data**			**Test for heterogeneity**		**Model used**
					OR	**95% CI**	***p*-value**	***p*-value**	***I*² (%)**	
Albumin										
Sample size (n)										
⩾100	2	275	141	51.27	0.82	0.45–1.51	0.53	0.31	2.23	FEM
<100	1	90	39	43.33	0.21	0.05–0.84	0.03	–	–	REM
NOS score										
⩾7	1	137	68	49.64	1.12	0.48–2.59	0.80	–	–	REM
<7	2	228	112	49.12	0.45	0.21–0.93	0.03	0.21	36.71	FEM
Cirrhosis										
Sample size (*n*)										
⩾100	10	1878	958	51.01	1.40	0.95–2.07	0.09	0.01	59.07	REM
<100	4	258	107	41.47	1.18	0.67–2.06	0.56	0.42	0.00	FEM
⩾200	4	1122	570	71.36	1.35	0.73–2.48	0.34	0.03	68.62	REM
<200	10	1014	495	48.82	1.32	0.90–1.95	0.16	0.08	36.74	REM
⩾300	2	715	297	41.54	0.89	0.48–1.66	0.72	0.14	54.49	REM
<300	12	1421	768	54.05	1.48	1.06–2.07	0.02	0.07	35.53	REM
⩾400	1	406	212	52.22	1.16	0.73–1.86	0.53	–	–	REM
<400	13	1730	853	49.31	1.35	0.95–1.92	0.10	0.02	48.04	REM
NOS score										
⩾7	7	1360	639	46.99	1.20	0.91–1.58	0.19	0.16	35.56	FEM
<7	7	776	426	54.90	1.37	0.76–2.45	0.30	0.03	57.23	REM
Without Xiang *et al.*[34] and Ma *et al.*[39]	12	1620	833	51.42	1.33	1.05–1.69	0.02	0.12	33.52	FEM
Histological grade										
Sample size (*n*)										
⩾100	1	126	72	57.14	1.15	0.43–3.04	0.78	–	–	REM
<100	4	267	115	43.07	1.70	0.58–4.95	0.33	0.06	60.50	REM
NOS score										
⩾7	2	198	109	55.05	1.67	0.79–3.54	0.18	0.24	28.40	FEM
<7	3	195	78	40.00	1.40	0.32–6.02	0.65	0.04	68.67	REM

(Continued)

Table 2. (Continued)

HIF-1α										
Subgroups	**Number of studies (*n*)**	**Number of cases (*n*)**	**HIF-1α$^+$ (*n*)**	**HIF-1α$^+$ (%)**	**Pooled data**			**Test for heterogeneity**		**Model used**
					OR	**95% CI**	***p*-value**	***p*-value**	**I^2 (%)**	
Without Wang *et al.* 2014[41]	4	348	155	44.54	2.04	1.12-3.69	0.02	0.53	0.00	FEM
Tumor differentiation										
Sample size (n)										
≥100	5	1168	534	45.72	1.53	0.86-2.74	0.15	0.00	76.26	REM
<100	4	216	107	49.54	4.82	0.59–39.55	0.14	0.01	81.86	REM
⩾200	3	915	423	46.23	1.59	0.70–3.60	0.27	0.00	83.89	REM
<200	6	469	218	46.48	2.36	0.85–6.51	0.10	0.01	75.68	REM
⩾300	2	715	297	41.54	1.93	0.58–6.40	0.28	0.00	90.39	REM
<300	7	669	344	51.42	1.68	0.92–3.07	0.09	0.01	53.66	REM
⩾400	1	406	212	52.22	3.54	2.15–5.83	0.00	–	–	REM
<400	8	978	429	43.87	1.42	0.93–2.17	0.10	0.01	37.68	REM
NOS score										
5	1	35	28	80.00	3.04	0.15–62.85	0.47	–	–	REM
6	3	255	121	47.45	7.25	0.47–111.92	0.16	0.01	90.53	REM
7	5	1094	492	44.97	1.38	0.79–2.43	0.26	0.00	72.22	REM
NOS (threshold 6)										
⩾6	8	1349	613	45.44	1.76	1.04–2.97	0.04	0.00	70.54	REM
<6	1	35	28	80.00	3.04	0.15–62.85	0.47	–	–	REM
NOS (threshold 7)										
⩾7	5	1094	492	44.97	1.38	0.79–2.43	0.26	0.00	72.22	REM
<7	4	290	149	51.38	5.47	0.79–37.76	0.08	0.03	80.72	REM
Without Tian *et al.*[49]	7	913	399	43.70	1.25	0.92–1.69	0.16	0.40	3.09	FEM
Tumor size (5 cm)										
Sample size (*n*)										
⩾100	11	2079	1052	50.60	1.75	0.90–3.39	0.10	0.00	91.66	REM
<100	8	467	250	53.53	0.88	0.59–1.31	0.53	0.27	20.05	FEM
⩾200	5	1323	664	50.19	1.46	1.04–2.06	0.03	0.08	51.84	REM
<200	14	1223	638	52.17	1.37	0.71–2.65	0.35	0.00	84.81	REM
⩾300	2	715	297	41.54	1.15	0.84–1.57	0.38	0.37	0.00	FEM
<300	17	1831	1005	54.89	1.45	0.85–2.46	0.17	0.00	84.36	REM
⩾400	1	406	212	52.22	1.28	0.87–1.91	0.21	–	–	REM
<400	18	2140	1090	50.93	1.41	0.86–2.31	0.18	0.00	84.50	REM

(Continued)

Table 2. (Continued)

HIF-1α										
Subgroups	**Number of studies (*n*)**	**Number of cases (*n*)**	**HIF-1α$^+$ (*n*)**	**HIF-1α$^+$ (%)**	**Pooled data**			**Test for heterogeneity**		**Model used**
					OR	**95% CI**	***p*-value**	***p*-value**	**I^2 (%)**	
NOS score										
5	1	35	28	80	15.00	0.78–287.68	0.07	–	–	REM
6	9	1027	575	55.99	2.27	1.10–4.70	0.03	0.00	84.53	REM
7	9	1484	699	47.10	0.84	0.57–1.24	0.38	0.01	65.64	REM
NOS (threshold 6)										
⩾6	18	2511	1274	50.74	1.34	0.84–2.13	0.22	0.00	85.49	REM
<6	1	35	28	80	15.00	0.78–287.68	0.07	–	–	REM
NOS (threshold 7)										
⩾7	9	1484	699	47.10	0.84	0.57–1.24	0.38	0.01	65.64	REM
<7	10	1062	603	56.78	2.45	1.20–4.99	0.01	0.00	83.02	REM
Vascular invasion										
Sample size (*n*)										
⩾100	11	2233	1106	49.53	2.39	1.58–3.61	0.00	0.00	75.38	REM
<100	5	327	137	41.90	3.22	1.87–5.55	0.00	0.13	44.29	FEM
⩾200	4	1334	646	48.43	1.32	1.04–1.68	0.02	0.38	2.54	FEM
<200	12	1226	597	48.69	3.54	2.69–4.66	0.00	0.11	35.11	FEM
⩾300	3	1134	520	45.86	1.39	1.08–1.79	0.01	0.41	0.00	FEM
<300	13	1426	723	50.70	3.22	2.13–4.88	0.00	0.00	57.60	REM
⩾400	2	825	435	52.73	1.28	0.97–1.70	0.08	0.87	0.00	FEM
<400	14	1735	808	46.57	3.04	2.08–4.45	0.00	0.00	57.00	REM
NOS score										
⩾7	9	1848	892	48.27	1.99	1.36–2.90	0.00	0.01	63.60	REM
<7	7	712	351	49.30	4.00	2.14–7.46	0.00	0.02	58.51	REM
HIF-2α										
Subgroups	**Number of studies (*n*)**	**Number of cases (*n*)**	**HIF-2α$^+$ (*n*)**	**HIF-2α$^+$ (%)**	**Pooled data**			**Test for heterogeneity**		**Model used**
					HR	**95% CI**	***p*-value**	***p*-value**	**I^2 (%)**	
Overall Survival										
Sample size (*n*)										
⩾200	3	767	404	53.67	1.00	0.39–2.60	1.00	0.00	96.06	REM
<200	2	265	84	31.70	1.83	1.18–2.84	0.01	0.93	0.00	FEM

(Continued)

Table 2. (Continued)

HIF-2α										
Subgroups	**Number of studies (*n*)**	**Number of cases (*n*)**	**HIF-2α$^+$ (*n*)**	**HIF-2α$^+$ (%)**	**Pooled data**			**Test for heterogeneity**		**Model used**
					HR	**95% CI**	***p*-value**	***p*-value**	**I^2 (%)**	
⩾300	1	315	219	69.52	2.58	2.08-3.20	0.00	–	–	REM
<300	4	717	269	37.52	1.01	0.55-1.87	0.97	0.00	84.09	REM
NOS score										
⩾7	3	660	353	53.48	1.44	0.61–3.42	0.41	0.00	93.51	REM
<7	2	372	135	36.29	1.00	0.35–2.84	1.00	0.01	85.67	REM
Follow-up (months)										
>72	2	454	286	63.00	2.47	2.02–3.03	0.00	0.29	11.74	FEM
⩽72	3	578	202	34.95	0.82	0.44–1.54	0.54	0.02	81.14	REM
Subgroups	**Number of studies (*n*)**	**Number of cases (*n*)**	**HIF-2α$^+$ (*n*)**	**HIF-2α$^+$ (%)**	**Pooled data**			**Test for heterogeneity**		**Model used**
					OR	**95% CI**	***p*-value**	***p*-value**	**I^2 (%)**	
Capsule infiltration										
Sample size (*n*)										
⩾100	2	441	236	53.51	1.15	0.25–5.27	0.85	0.04	77.39	REM
<100	1	97	31	31.96	4.76	1.54–14.70	0.01	–	–	REM
⩾200	1	315	219	69.52	2.26	1.19–4.28	0.01	–	–	REM
<200	2	223	48	21.52	1.54	0.16–14.79	0.71	0.01	85.41	REM
NOS score										
⩾7	1	315	219	69.52	2.26	1.19–4.28	0.01	–	–	REM
<7	2	223	48	21.52	1.54	0.16–14.79	0.71	0.01	85.41	REM
Median age (years)										
⩾50	2	412	250	60.68	2.71	1.55–4.73	0.00	0.26	21.15	FEM
<50	1	126	17	13.49	0.47	0.13–1.75	0.26	–	–	REM
Tumor size (5 cm)										
Sample size (*n*)										
⩾100	5	1032	488	47.29	1.34	0.27–6.71	0.72	0.00	96.16	REM
<100	2	181	65	35.91	0.89	0.13–6.10	0.90	0.01	87.06	REM
⩾200	3	767	404	52.67	1.17	0.07–18.75	0.91	0.00	98.22	REM
<200	4	446	149	33.41	1.25	0.43–3.60	0.69	0.00	81.42	REM
⩾300	1	315	219	69.52	19.74	9.40–41.45	0.00	–	–	REM
<300	6	898	334	37.19	0.74	0.30–1.85	0.52	0.00	88.12	REM

(Continued)

Table 2. (Continued)

Subgroups	Number of studies (*n*)	Number of cases (*n*)	HIF-2α$^+$ (*n*)	HIF-2α$^+$ (%)	Pooled data			Test for heterogeneity		Model used
					OR	95% CI	*p*-value	*p*-value	I^2 (%)	
NOS score										
⩾7	5	870	404	46.44	1.32	0.26-6.80	0.74	0.00	95.19	REM
<7	2	343	149	43.44	0.91	0.15–5.39	0.92	0.00	90.05	REM
Vascular invasion										
Sample size (n)										
⩾100	4	893	421	47.14	1.13	0.53–2.42	0.75	0.01	75.80	REM
<100	2	181	65	35.91	1.27	0.62–2.60	0.51	0.27	16.26	FEM
⩾200	3	767	404	52.67	1.30	0.55–3.07	0.54	0.00	82.57	REM
<200	3	307	82	26.71	1.08	0.56–2.06	0.82	0.31	15.06	FEM
⩾300	1	315	219	69.52	3.05	1.53–6.10	0.00	–	–	REM
<300	5	759	267	35.18	0.90	0.64–1.27	0.55	0.38	3.84	FEM
NOS score										
⩾7	4	731	337	46.10	1.24	0.57–2.70	0.59	0.04	63.40	REM
<7	2	343	149	43.44	1.00	0.42–2.35	0.99	0.09	66.09	REM

AFP, alpha-fetoprotein; CI, confidence interval; FEM, fixed-effects model; HIF, hypoxia-inducible factor; HR, hazard ratio; NOS, Newcastle–Ottawa scale; OR, odds ratio; REM, random-effects model.

In gender, trim-and-fill estimated the new global effect (OR 0.827; 95% CI 0.687–0.997) and imputed five studies. Likewise, six 'missing' studies were included in the vascular invasion funnel plot, adjusting effect size (OR 1.749; 95% CI 1.121–2.729). Conversely, trim-and-fill analysis did not report any 'missing' study for tumor differentiation. All imputed studies for HIF-1α parameters were on the left side of the funnel plot (Table 3; Figure 7).

Conversely, there was no asymmetry evidence on OS ($p=0.9273$) and DFS/RFS ($p=0.5480$) for HIF-2α (Table 3; Figure 6B). Concerning the clinicopathological features, only AFP levels ($p=0.0213$) presented asymmetry, where the trim-and-fill method imputed one study on the right side of the funnel plot and corrected the global effect (OR 1.001; 95% CI 0.694–1.444) (Table 3; Figure 8).

Discussion

Hypoxia is a common microenvironment characteristic of solid tumors, such as HCC, which arises as consequence of defective vascularization and intense metabolic activity.[8] Despite HCC being characterized by being one of the most hyper-vascularized tumors, hypoxic regions are frequently present in HCC due to rapid proliferation of tumor cells and the formation of aberrant blood vessels.[19,26] Although a reduction in oxygen supply is initially harmful for cell survival, some tumor cells adapt to the hypoxic microenvironment by decreasing energy consumption and enhancing anaerobic metabolism.[25] This adaptive response is mainly accomplished by HIFs, which entails a set of pro-survival changes implicated in aggressive tumor progression, therapy resistance, selection of more invasive clones and poor clinical outcomes.[8]

Due to difficult detection and the high recurrence rate of early HCC,[4] discerning the risk of recurrence and mortality in HCC patients is key to guide surveillance and determine possible adjuvant therapies. Thus, in this study we evaluated the main hypoxia response mediators, HIF-1α and HIF-2α, as potential clinical biomarkers for predicting HCC prognosis. To investigate the relationship between HIF protein expression and HCC, the present meta-analysis aimed to

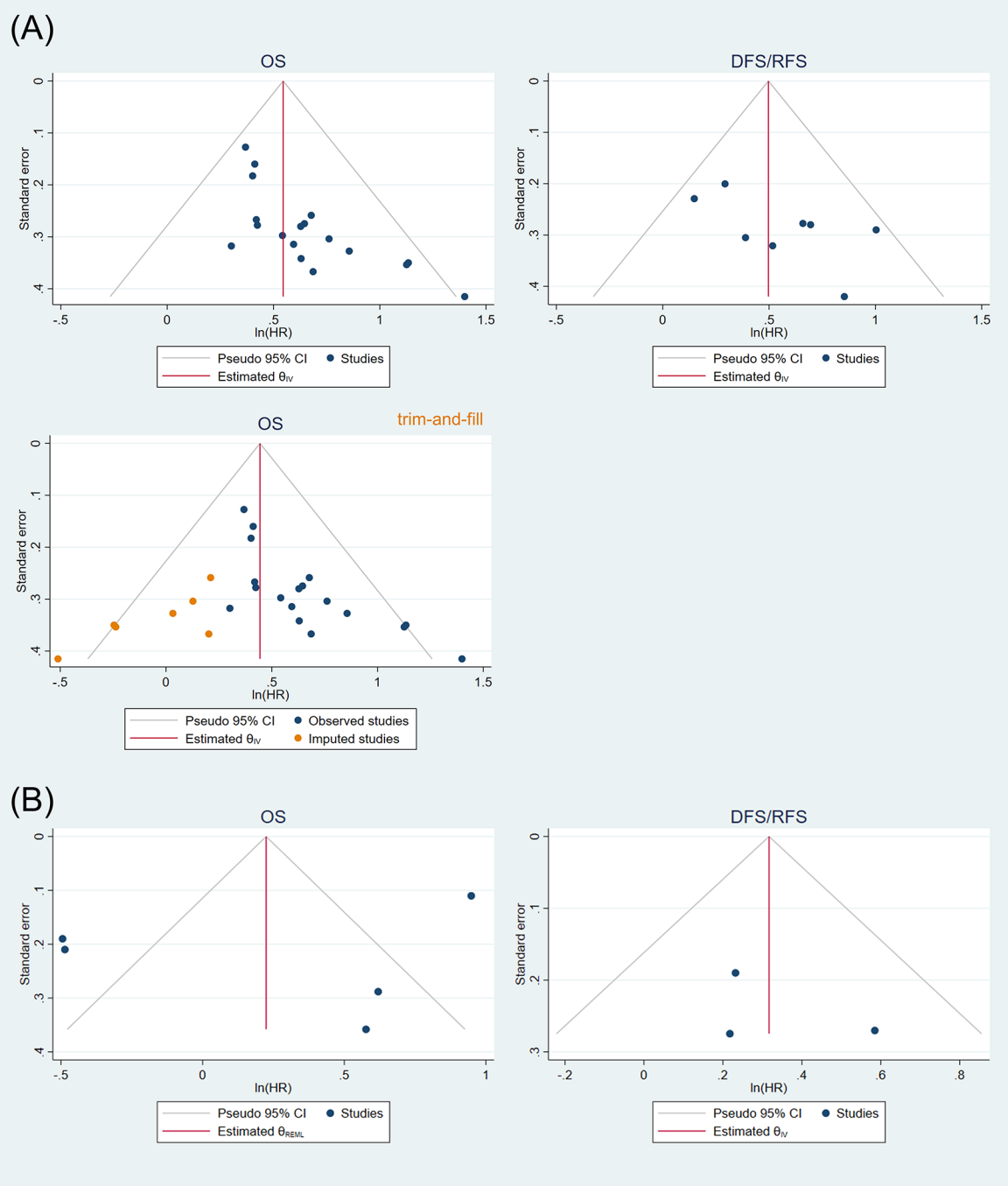

Figure 6. Publication bias analysis of the prognostic value of HIF-1α and HIF-2α. Funnel plot of OS and DFS/RFS for (A) HIF-1α with trim-and-fill funnel plot for OS, and (B) HIF-2α.
CI, confidence interval; DFS, disease-free survival; HIF, hypoxia-inducible-factor; HR, hazard ratio; IV, inverse variance; OS, overall survival; REML, restricted maximum likelihood; RFS, recurrence-free survival.

examine the association between HIF-1α or HIF-2α overexpression and the prognosis and clinicopathological features of HCC patients.

A total of 26 high-quality studies (3570 patients) were included for HIF-1α analysis. Pooled results showed that overexpression of HIF-1α leads to poor OS and DFS/RFS in HCC. Previous studies conducted by Zheng *et al.*[19] and Cao *et al.*,[18] which enrolled seven and eight articles, respectively, evaluated HIF-1α expression in HCC. According to our results, both studies showed association between high HIF-1α levels and DFS, and Zheng *et al.*[19] also reported correlation with OS.

Table 3. Assessment of publication bias on prognostic and clinicopathological features of HIF-1α and HIF-2α.

HIF-1α					
Survival	**Number of studies**	**Egger's test (*p*-value)**	**Model used**	**Trim-and-fill HR (95% CI)**	**Imputed studies**
OS	18	0.00*	FEM	1.56 (1.41–1.73)	7
DFS/RFS	8	0.06	FEM	–	–
Clinicopathological feature	**Number of studies**	**Egger's test (*p*-value)**	**Model used**	**Trim-and-fill OR (95% CI)**	**Imputed studies**
AFP (20 ng/ml)	7	0.59	REM	–	–
AFP (400 ng/ml)	4	0.41	REM	–	–
Age (50 years)	7	0.18	REM	–	–
Age (60 years)	4	0.24	FEM	–	–
Albumin	3	0.07	REM	–	–
ALT (40 U/L)	3	0.33	FEM	–	–
ALT (80 U/L)	3	0.59	FEM	–	–
BCLC	3	0.58	FEM	–	–
Bilirubin	2	0.51	FEM	–	–
Capsule formation	7	0.93	FEM	–	–
Capsule infiltration	2	0.38	FEM	–	–
Child–Pugh score	3	0.85	FEM	–	–
Cirrhosis	14	0.97	REM	–	–
Distant metastasis	2	†	REM	–	–
Edmondson grading	3	0.08	FEM	–	–
Gender	19	0.03*	FEM	0.83 (0.69–1.00)	5
Hepatitis B	16	0.91	FEM	–	–
Hepatitis C	4	0.94	FEM	–	–
Histological grade	5	0.82	REM	–	–
Intrahepatic metastasis	3	0.70	FEM	–	–
Lymph node metastasis	3	0.39	FEM	–	–
TNM (I, II–III)	4	0.35	FEM	–	–
TNM (I–II, III)	2	0.99	FEM	–	–
TNM (I–II, III–IV)	3	0.50	FEM	–	–
Tumor differentiation	9	0.04*	REM	1.78 (1.07–2.96)	0
Tumor number	8	0.32	FEM	–	–

(Continued)

Table 3. (Continued)

HIF-1α					
Clinicopathological feature	**Number of studies**	**Egger's test (*p*-value)**	**Model used**	**Trim-and-fill OR (95% CI)**	**Imputed studies**
Tumor size (3 cm)	2	0.47	FEM	–	–
Tumor size (5 cm)	19	0.47	REM	–	–
Vascular invasion	16	0.00*	REM	1.75 (1.12–2.73)	6
Vasculogenic mimicry	3	0.33	FEM	–	–
HIF-2α					
Survival	**Number of studies**	**Egger's test (*p*-value)**	**Model used**	**Trim-and-fill HR (95% CI)**	**Imputed studies**
OS	5	0.93	REM	–	–
DFS/RFS	3	0.55	FEM	–	–
Clinicopathological feature	**Number of studies**	**Egger's test (*p*-value)**	**Model used**	**Trim-and-fill OR (95% CI)**	**Imputed studies**
AFP (400 ng/ml)	4	0.02*	FEM	1.00 (0.69-1.44)	1
Age (50 years)	4	0.27	FEM	–	–
Capsule formation	3	0.17	FEM	–	–
Capsule infiltration	3	0.63	REM	–	–
Cirrhosis	5	0.46	FEM	–	–
Edmondson grading	2	†	REM	–	–
Gender	6	0.67	FEM	–	–
Hepatitis B	4	0.54	FEM	–	–
Histological grade	2	0.19	FEM	–	–
Necrosis	2	†	REM	–	–
TNM (I–II, III–IV)	2	†	REM	–	–
Tumor number	3	0.08	FEM	–	–
Tumor size (5 cm)	7	0.89	REM	–	–
Vascular invasion	6	0.46	REM	–	–

AFP, alpha-fetoprotein; ALT, alanine aminotransferase; BCLC, Barcelona Clinic Liver Cancer; CI, confidence interval; DFS, disease-free survival; FEM, fixed-effects model; HIF, hypoxia-inducible factor; HR, hazard ratio; OR, odds ratio; OS, overall survival; REM, random-effects model; RFS, recurrence-free survival; TNM, tumor–node–metastasis.
*p-value <0.05.
†Convergence not achieved during tau2 estimation.

Various research performed in lung cancer,[10] renal cell carcinoma,[11] pancreatic cancer,[64] esophageal cancer,[12] gastric tumors,[13] colorectal cancer,[14] head and neck cancer,[15] oral squamous cell carcinoma,[16] bone tumors,[17] breast cancer,[65] endometrial cancer,[66] and epithelial ovarian cancer,[67] also revealed a significant correlation between HIF-1α overexpression and poor prognosis. Moreover, HIF-1α expression has been associated with worse prognosis in advanced

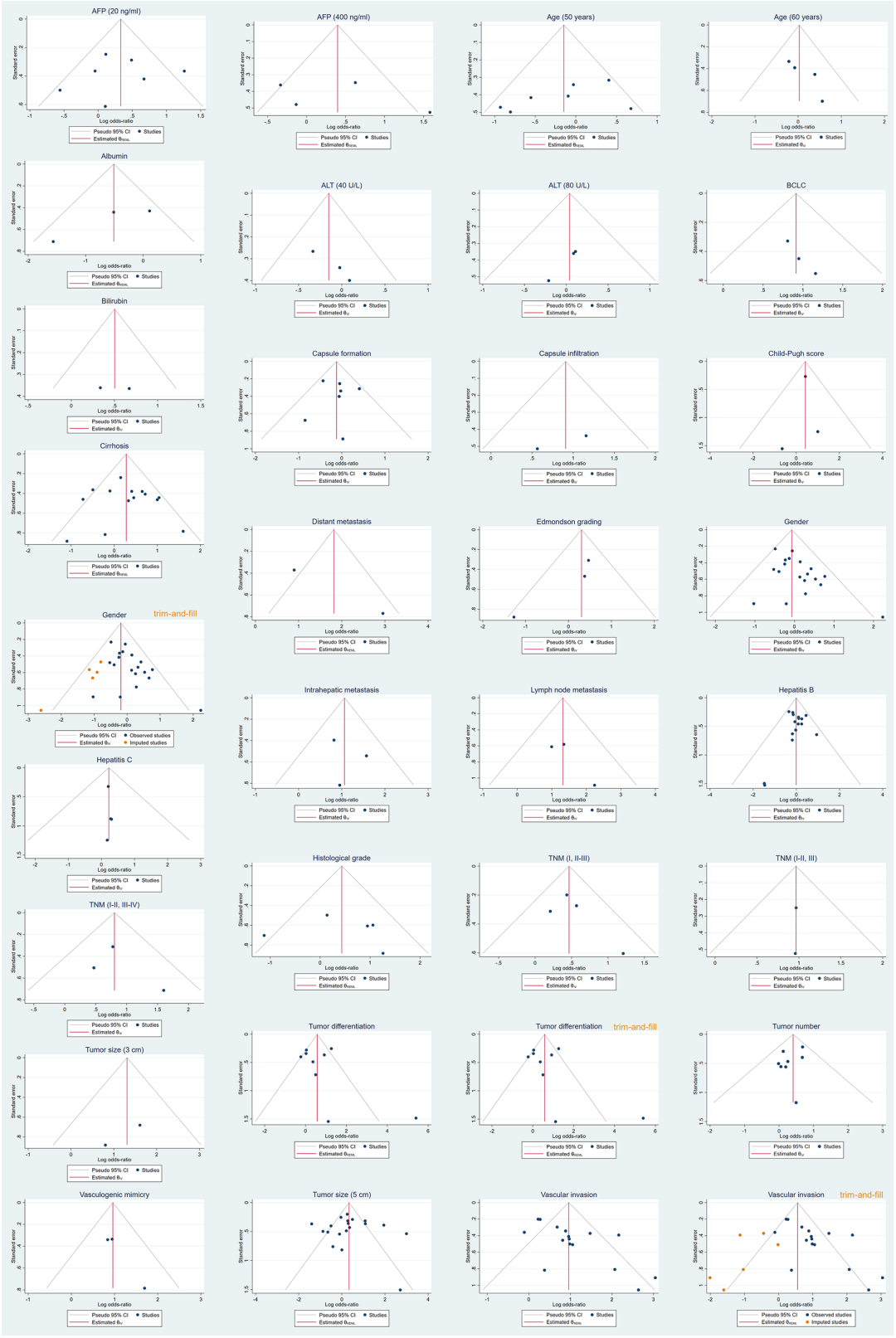

Figure 7. Publication bias analysis of the association between HIF-1α overexpression and clinicopathological features by funnel plot asymmetry. For gender, tumor differentiation and vascular invasion the trim-and-fill funnel plot is also represented.
AFP, alpha-fetoprotein; ALT, alanine aminotransferase; BCLC, Barcelona Clinic Liver Cancer; CI, confidence interval; IV, inverse variance; HIF, hypoxia-inducible-factor; REML, restricted maximum likelihood; TNM, tumor–node–metastasis.

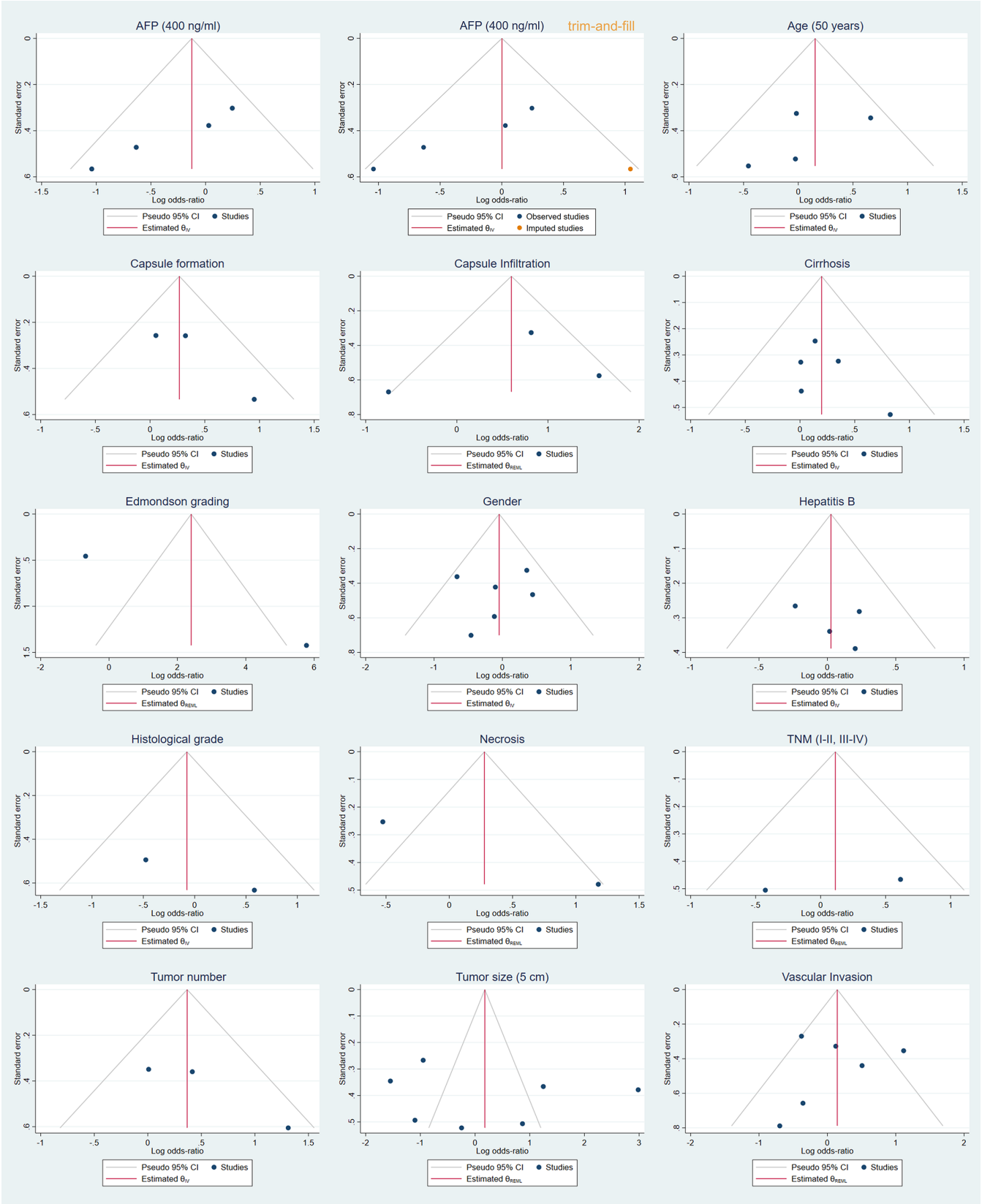

Figure 8. Publication bias analysis of the association between HIF-2α overexpression and clinicopathological features by funnel plot asymmetry. For AFP (400 ng/ml) the trim-and-fill funnel plot is also represented.
AFP, alpha-fetoprotein; CI, confidence interval; IV, inverse variance; HIF, hypoxia-inducible-factor; REML, restricted maximum likelihood; TNM, tumor–node–metastasis.

cancer patients treated with chemotherapy and/or radiotherapy; thus, in addition to a useful biomarker, targeting HIF-1α could be an interesting therapeutic approach to improve survival in advanced cancer patients.[68]

Here, significant results were observed in some clinicopathological features, including BCLC staging, capsule infiltration, intrahepatic metastasis, lymph node metastasis, TNM classification, tumor differentiation, tumor number, tumor size (contrasting greater or less than 3 cm), vascular invasion and vasculogenic mimicry. After subgroup analysis according to sample size and NOS score, we also observed a possible HIF-1α correlation with AFP levels, cirrhosis, tumor size (5 cm) and albumin, while histological grade showed association after removing one study.

The two previous meta-analyses[18,19] found a correlation with vascular invasion, and Cao *et al.*[18] reported no significant association with capsule formation, cirrhosis, tumor differentiation or tumor size. Nevertheless, differences could be explained by the lower number of studies included in such meta-analyses and the absence of subgroup analyses.[18,19]

Meanwhile, seven high-quality articles (1213 patients) were employed to analyze HIF-2α. Initially, only DFS/RFS appeared to be associated with HIF-2α expression; nonetheless, subgroup analysis denoted that HIF-2α overexpression is also markedly related to OS when grouped by sample size and follow-up time.

A meta-analysis by Yao *et al.*[26] and another by Luo *et al.*[25] evaluated HIF-2α in HCC and multiple types of cancer, respectively. Luo *et al.*[25] found a relation between OS and HIF-2α with multivariate but not with univariate analysis. Likewise, this meta-analysis reported that HIF-2α overexpression results in poor OS in additional tumors including lung or colorectal cancers, among others.[25] In contrast, Yao *et al.*[26] did not observe significant correlation among OS and this transcription factor, which can be explained based on the fact that our work included most recent articles and excluded those with full text in Chinese.[26] No previous study has evaluated the impact on DFS or RFS.

Other research has observed the association between HIF-2α overexpression and a worse prognosis in further tumors such as lung cancer,[21] renal cell carcinoma,[11] gastric cancer,[23] colorectal cancer,[14,24] oral squamous cell carcinoma[22] or head and neck cancer.[20]

In addition, concerning the clinicopathological parameters, only capsule infiltration was related to HIF-2α high levels when the median age is ⩾50 years. This result agrees with Yao *et al.*,[26] but they also found an association with vein invasion and histological grade. Besides, both that meta-analysis and ours observed no correlation of HIF-2α with cirrhosis, necrosis and tumor size.[26]

Hypoxia is the principal physiological stimulus inducing angiogenesis in HCC through the upregulation of angiogenic factors. Vascular endothelial growth factor (VEGF) is crucial for blood vessel formation by promoting the growth and migration of endothelial cells, and it is transcriptionally regulated by both HIFs.[69] Some studies included in this meta-analysis showed that high VEGF levels were associated with angiogenesis, microvessel density, vasculogenic mimicry and poor prognosis; having a positive correlation between VEGF and HIF-1α.[29,30,34,44,45] Other angiogenic factors have also been linked to HIF regulation and have been described to be involved in angiogenesis in HCC, such as angiopoietin-2,[30] HIF-1α targets bone morphogenetic protein 4 (BMP4), while HIF-2α targets stem cell factor (SCF) and plasminogen activator inhibitor-1 (PAI-1), and both HIFs target erythropoietin and platelet-derived growth factor (PDGF) expression.[69] Consequently, hypoxia, and more specifically HIFs, contribute to angiogenesis in HCC and could be related to the results obtained in this meta-analysis associating high HIF-1α expression with increased vascular invasion and vasculogenic mimicry.

Furthermore, epithelial to mesenchymal transition and metastasis can be induced under hypoxia in HCC cells.[69] Several studies have described that the increase in invasion and worse prognosis in HCC patients could be related to the expression of invasion-related proteins such as metalloproteinases, interleukin-8 (IL-8) or E-cadherin.[32,34,40,43,44] In preclinical studies similar results were also found, associating high invasion and metastasis with the HIF-1α targets SNAIL-1, granulocyte chemotactic protein-2 (CXCL6), IL-8 and Rab11-family interacting protein 4 (Rab11-FIP4); and the HIF-2α targets SERPINB3, CUB domain-containing protein 1 (CDCP1) and SCF.[69–71] These findings can explain the relationship between both HIFs and the clinicopathological factors related to metastasis, such as intrahepatic or lymph node metastasis and capsule infiltration.

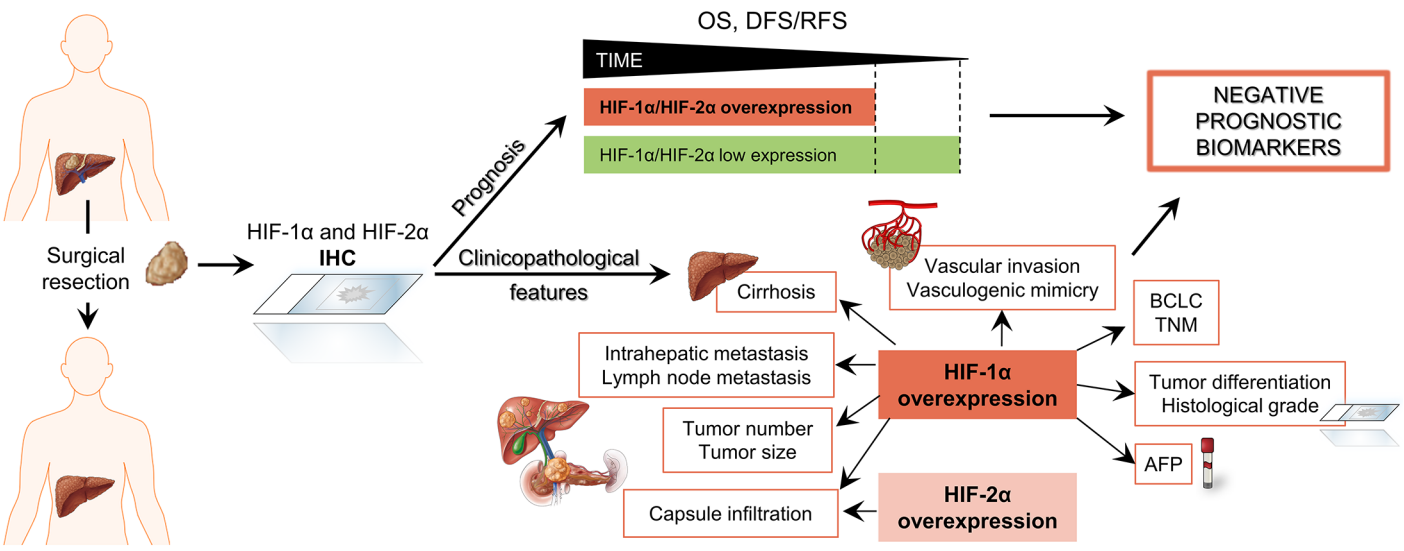

Figure 9. Graphical abstract.
AFP, alpha-fetoprotein; BCLC, Barcelona Clinic Liver Cancer; DFS, disease-free survival; HIF, hypoxia-inducible factor; IHC, immunohistochemistry; OS, overall survival; RFS, recurrence-free survival; TNM, tumor–node–metastasis.

This meta-analysis is a highly comprehensive study performing a detailed quantitative analysis of clinical evidence on HIF-1α and HIF-2α correlation with prognostic variables such as OS and DFS/RFS and with clinicopathological features in HCC patients who underwent surgical resection. Moreover, this work includes an assessment of heterogeneity, subgroup analysis and publication bias. Hence, this is the first meta-analysis analyzing both main hypoxia-inducible factors. Previous meta-analyses evaluating the relationship between HIF-1α or HIF-2α expression and tumor outcome, which were included in the discussion of our results, were performed prior to 2015 and, thus, comprised a lower number of studies and evaluated fewer parameters. Moreover, some studies did not assess publication bias and/or subgroup analysis.

Even though in the present meta-analysis we exhaustively evaluated the association between both HIFs and tumor outcome, some limitations should be acknowledged. Despite full-text articles being obtained, those written in Chinese were excluded and, therefore, likely relevant data. Data extraction was not always possible because of the absence of required variables for the estimation, such as patient follow-up or patient numbers in each group. Furthermore, studies included employed diverse or unspecified antibodies for HIF detection, with miscellaneous or insufficient staining procedure description and different cut-off values for IHC scores of HIFs, which could lead to higher heterogeneity. All the articles included were performed with an Asiatic population, mainly from China, where hepatitis B is the key etiology factor.[1] In accordance with this, most of the articles evaluated the number of patients with hepatitis B or C; however, there are no available studies enrolling populations from other origins, such as western countries where etiological factors such as obesity or non-alcoholic fatty liver disease (NAFLD) prevail.[1] The research volume in HIF-2α analysis was low due to the low number of studies found in the literature; then, more high-quality articles would be needed. Besides, some variables were not collected uniformly between studies, hindering the assessment of established aims and the potential sources of heterogeneity. Finally, publication bias was denoted in some parameters.

In summary, HIF overexpression is linked to a more aggressive behavior of HCC. In this meta-analysis, 51.7% and 45.6% of patients displayed HIF-1α and HIF-2α overexpression, respectively. HIF-1α and HIF-2α seem to act as negative prognosis markers, being linked to poor OS, DFS, RFS and some clinicopathological features of HCC patients. This evidence suggests that both HIFs are useful biomarkers for predicting HCC prognosis that may improve clinical decisions, especially when combined with other prognostic-related markers. These results have been represented graphically in Figure 9.

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Author contributions

All authors were responsible for study conception and design, interpretation of the data and drafting of the manuscript. Systematic literature review, data extraction and data analysis were performed by CMB, PFP and FF. Moreover, JGG and JLM carried out the study supervision. The final version of the manuscript was approved by all authors.

Conflict of interest statement

The authors declare that there is no conflict of interest.

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Supplemental material

Supplemental material for this article is available online.

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