R E V I E W

Prognostic value of plasma fibrinogen in hepatocellular carcinoma: a meta-analysis

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Background: Elevated plasma fibrinogen levels have been associated with tumor progression in several malignancies. Our study aims to characterize the clinical significance of elevated plasma fibrinogen levels in patients with hepatocellular carcinoma (HCC).

Materials and methods: Relevant published articles were systematically searched in electronic databases including PubMed, Embase, and Web of Science. The pooled differences in plasma fibrinogen levels among HCC, cirrhotic, and control groups were expressed as weighted mean differences (WMDs) and their corresponding 95% CIs. The associations between elevated fibrinogen and overall survival (OS) and disease-free survival (DFS)/recurrence-free survival (RFS) were expressed as HRs and their 95% CIs, whereas the associations between elevated fibrinogen and various types of clinical characteristic of patients with HCC were expressed as ORs and their corresponding 95% CIs.

Results: Results showed that the plasma fibrinogen levels in patients with HCC were not significantly different than that in healthy controls (WMD = 0.50, 95% CI = [–0.82, 1.82], $P = 0.457$) or patients with cirrhosis (WMD = −0.62, 95% CI = [–1.56, 0.33], $P = 0.200$). However, our results showed that compared to those with normal levels, patients with HCC and elevated plasma fibrinogen levels showed poorer OS (HR = 2.08, 95% CI = [1.67, 2.59], $P < 0.0001$) and DFS/RFS (HR = 1.90, 95% CI = [1.52, 2.37], $P < 0.0001$). Results of trial sequential analysis of the OS indicated that currently available studies were sufficient to validate the negative prognostic value of elevated plasma fibrinogen in patients with HCC. Clinicopathological analyses showed that high plasma fibrinogen levels were associated with tumor progression as indicated by advanced tumor stage, larger tumor size, increased tumor number, and the presence of vascular invasion.

Conclusion: Elevated plasma fibrinogen levels are associated with poor prognosis and advanced tumor progression. Plasma fibrinogen may serve as a negative prognostic biomarker in patients with HCC.

Keywords: hepatocellular carcinoma, fibrinogen, prognosis, tumor progression, meta-analysis

Introduction

Hepatocellular carcinoma (HCC) is a primary malignancy of the liver and is now the second leading cause of cancer deaths worldwide, following lung cancer.[1] The global incidence of HCC is still on the rise with the highest prevalence in East Asia,[1] where the mortality rate is 33.5 per 100,000 people.[2] The majority of HCC cases occur with underlying chronic hepatitis B or C viral infection and cirrhosis.[3,4] The other main risk factors for HCC are alcohol intoxication and aflatoxin.[3,5] Current treatment modalities include radical therapies (eg, surgical resection, liver transplantation, radiofrequency ablation) and adjuvant therapies (eg, transarterial chemoembolization [TACE], systemic

chemotherapy, sorafenib). Although these methods have significantly improved the long-term survival of patients with HCC, the overall prognosis is largely limited by the extent of liver dysfunction.[6] Although various tumor staging systems are available to predict prognosis, they too have major limitations. For instance, the Milan criteria are solely based on the preoperative imaging without consideration of molecular signatures[7] and biopsy has the risk of introducing tumor cell seeding.[8] Therefore, it would be useful to identify effective preoperative prognostic biomarkers that may facilitate outcome prediction and indicate the requirement for aggressive therapy in high-risk patients with HCC.

To date, the most common serum biomarker used by clinicians to diagnose HCC is alpha-fetoprotein (AFP). However, the reliability of AFP is questionable due to its lack of sensitivity and specificity.[9] In addition, AFP marker is less valuable in the early stages of HCC when the tumor size is less than 3 cm.[10] Therefore, new and more effective prognostic serum biomarkers are needed to improve clinical outcomes.

Fibrinogen is a 340-kDa soluble plasma glycoprotein synthesized in the liver by hepatocytes. It is an important coagulation factor. In the coagulation cascade, fibrinogen is enzymatically converted to fibrin by activated thrombin and subsequently forms a clot. In situations such as tissue injury, infection, and inflammation, fibrinogen concentrations are rapidly elevated.[11,12] Recently, emerging evidence has associated the elevation of fibrinogen level with tumor progression in several malignancies, such as breast cancer,[13] gallbladder cancer,[14] gastric cancer,[15] colorectal cancer,[16] and lung cancer.[17] Few studies have also reported that patients with HCC and elevated pretreatment plasma fibrinogen levels have poor outcomes.[18,19] However, these studies were limited in size, varied in cutoff concentrations, or administered heterogenous treatments, and the correlations between elevated fibrinogen and clinicopathological parameters of HCC were unclear. In addition, there are conflicting findings on the levels of plasma fibrinogen in HCC patients compared to that in control or cirrhotic groups. Several studies reported that HCC patients showed higher levels of pretreatment plasma fibrinogen compared to healthy controls or cirrhotic patients.[20–22] However, Basili et al[23] and Liu et al[24] found no significant difference in the plasma fibrinogen levels between HCC and control or cirrhotic groups, and Hessien et al[25] indicated lower levels of fibrinogen in HCC patients compared to that in control and cirrhotic patients. Therefore, our present study aims to summarize the currently available studies to examine 1) whether the pretreatment plasma fibrinogen levels were significantly different among HCC, cirrhotic, and healthy subjects; 2) the

association between elevated plasma fibrinogen and prognosis in patients with HCC; and 3) the association between elevated fibrinogen levels and tumor progression in patients with HCC. To the best of our knowledge, no meta-analysis has been performed to assess elevated plasma fibrinogen risk in patients with HCC.

Materials and methods

This meta-analysis was conducted in accordance with the PRISMA guidelines. The studies included in this meta-analysis are previously published, and therefore, ethical approval and informed consent are not required.

Literature search strategy

Relevant published literatures were systematically searched in electronic databases including PubMed, Embase, and Web of Science. The last update was September 2017. The keywords used included "Fibrinogen", "liver neoplasm", and "hepatocellular carcinoma". Specifically, the keywords used in PubMed were (Fibrinogen) AND (neoplasm, liver [MeSH Terms] OR hepatocellular carcinoma). In Embase, the keywords used were Quick search: Fibrinogen AND (liver neoplasm OR hepatocellular carcinoma). In Web of Science, the keywords used were Fibrinogen AND (liver neoplasm OR hepatocellular carcinoma) [Topic]. There were no restrictions to the search process in terms of language, date, research design, or publication status.

Study selection criteria

Studies were included when they met the following inclusion criteria:

1. diagnosis of HCC was proven by pathological methods;
2. pretreatment plasma fibrinogen was measured in all participants;
3. mean and SD of pretreatment plasma fibrinogen levels were reported in patients with HCC, cirrhosis, and/or healthy controls;
4. complete clinicopathological characteristics were evaluated prior to treatment; and
5. outcomes included overall survival (OS), disease-free survival (DFS), and/or recurrence-free survival (RFS).

Studies were excluded when they met the following exclusion criteria:

1. experiments were performed in vitro or in vivo;
2. study designs were case reports and reviews;
3. fibrinogen peptide chains, mRNA or protein expression, or fibrinogen degradation products were measured;

4. conference proceedings that contained only summaries;
5. articles were not in English or Chinese; and
6. literature with data that could not be extracted.

Outcomes of interest

The primary outcomes of interest were OS, DFS, and RFS. Another outcome of interest was the association between fibrinogen levels and clinicopathological parameters. Other outcomes of interest were pretreatment plasma fibrinogen levels of HCC, cirrhotic, and/or healthy subjects.

Data extraction and quality assessment

Two independent researchers performed literature selection, evaluation, and extraction. Data on the following characteristics were retrieved: first author; year of publication; country in which the study was conducted; number of HCC, cirrhotic, and control subjects enrolled; age; sex; pretreatment plasma fibrinogen level measurements and methods; plasma fibrinogen cutoff values; methods used to determine cutoff values; treatments received; and follow-up period. Discrepancies were resolved by discussion to consensus. If both univariate and multivariate outcomes were provided in an article, we chose the latter. Efforts were made to contact the corresponding authors for any information not reported in the article.

The Newcastle–Ottawa Scale (NOS) was used to assess the quality of the studies included. NOS employs a "star" rating system that evaluates eight items, which are categorized into three domains: selection (four items: maximum one star each), comparability (one item: maximum two stars), and outcome (three items: maximum one star each). The NOS assessment, which ranges from 0 (worst) to 9 (best) stars, provides a semiquantitative appraisal for the overall quality of each study. Studies with scores of 6 or higher were regarded as high quality.

Statistical analyses

All statistical analyses were performed using Stata 13.1 (StataCorp LP, College Station, TX, USA). The pooled differences in plasma fibrinogen levels among HCC, cirrhotic, and control groups were expressed as weighted mean differences (WMDs) and their corresponding 95% CIs. The associations between elevated fibrinogen and OS or DFS/RFS were expressed as the HRs and their corresponding 95% CIs, and the associations between elevated fibrinogen and various types of clinical characteristics of patients with HCC were expressed as ORs and their corresponding 95% CIs. Heterogeneity among studies was assessed using Cochran's

Q test and Higgins I^2 statistic.[26] If no significant heterogeneity was indicated among studies ($P > 0.1$, $I^2 < 50\%$), a fixed effect model was applied for analysis.[27] Otherwise, the random effect model was adopted.[28] Sensitivity analysis was performed using sequential omission of individual studies to evaluate the influence of an individual study on the overall pooled estimates. If the pooled estimates were affected after removal, it indicated that the results were inconsistent. Egger's linear regression tests were used to assess for publication bias, and any indication of publication bias was further tested with trim and fill analysis. $P < 0.05$ indicated significant bias.

Trial sequential analysis (TSA) was conducted to minimize type I error, evaluate required information size, and assess whether current available studies were sufficient for a firm conclusion. The required information size refers to the required number of participants to produce statistically significant result in meta-analysis. The diversity-adjusted required information size of 784 patients was calculated on the basis of type I error of 5%, type II error of 20%, the control event proportion of 40%, and a relative risk reduction of 20% in outcomes. When the cumulative Z-curve crossed the trial sequential monitoring boundary for benefit, harm or futility, or the required information size, this result suggests conclusive evidence within this meta-analysis. Otherwise, more clinical studies are warranted to reach a consistent conclusion.

Results
Study selection process

Figure 1 depicts the fully detailed process of article search and selection. The electronic database searches yielded a total of 1,128 potential articles (PubMed = 410, Embase = 565, and Web of Science = 153). The titles and abstracts were screened, and 1,094 articles were excluded because they covered irrelevant research topics (n = 1,019), lacked full text (n = 20), or were duplicates (n = 55). Thirty-four full-text articles were retrieved for detailed evaluation. Eighteen articles were eliminated due to the following reasons: in vivo/in vitro study (n = 2); case report (n = 2); study that measured fibrinogen peptide chains, mRNA or protein expression, or fibrinogen degradation product (n = 12); cases that included other primary liver tumor besides HCC (n = 1); and data not presented as mean ± SD (n = 1). A total of 16 articles were included in this study: eight articles measured and compared the plasma fibrinogen levels among HCC, cirrhotic, and healthy subjects, and eight articles studied the clinicopathological and prognostic value of plasma fibrinogen in HCC.
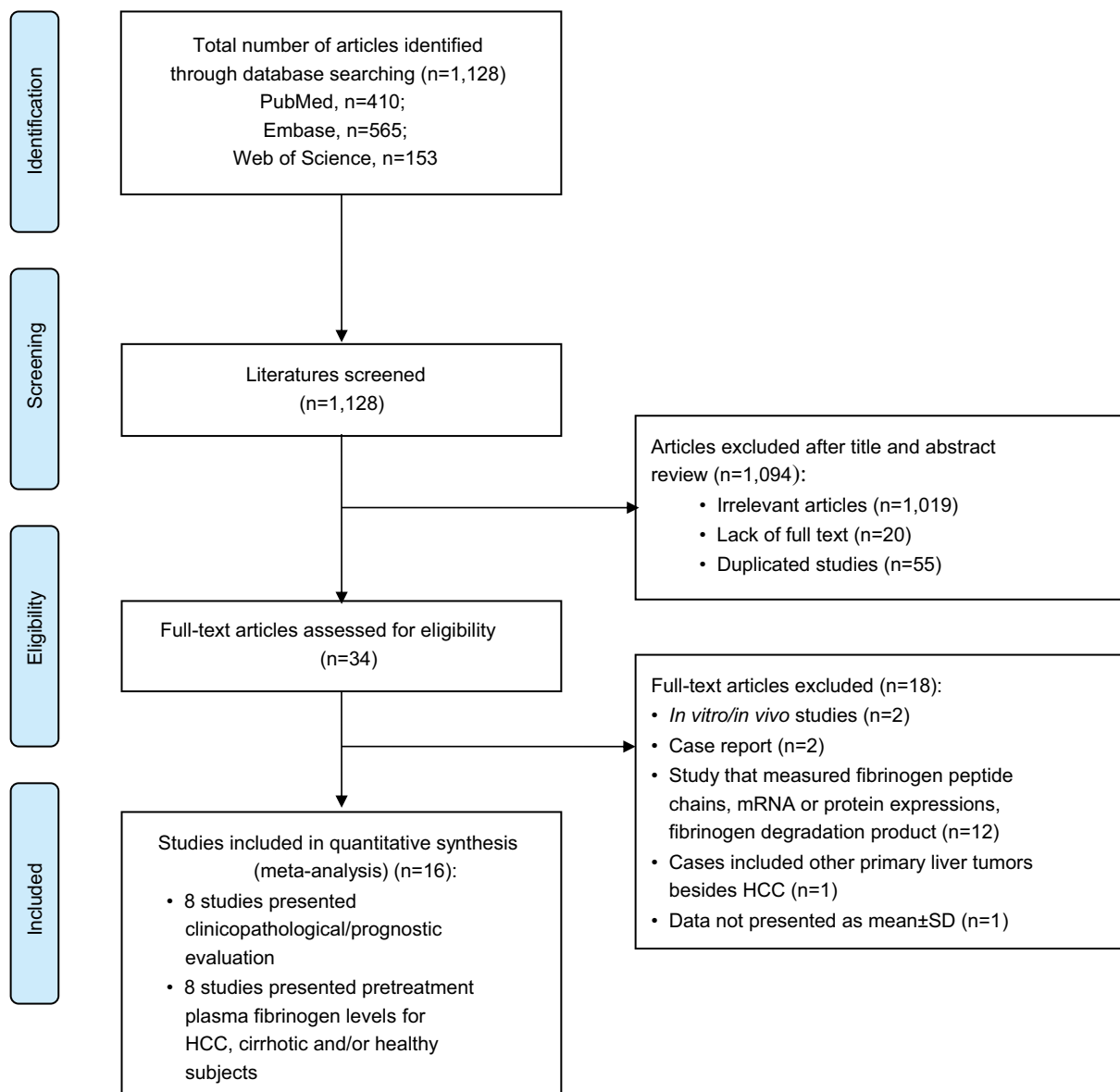
Figure 1 Flow chart of article search and selection.
Note: PLoS Medicine (OPEN ACCESS) Moher D, Liberati A, Tetzlaff J, Altman DG, The PRISMA Group (2009). Preferred Reporting Items for Systematic Reviews and Meta-Analyses: The PRISMA Statement. *PLoS Med* 6(7): e1000097. doi:10.1371/journal.pmed1000097.[55]
Abbreviation: HCC, hepatocellular carcinoma.

Study characteristics and quality assessment

Eight studies that reported OS, DFS, or RFS were included in the meta-analysis to assess the prognostic value of plasma fibrinogen in patients with HCC. The main characteristics of the included studies are presented in Table 1. All studies were conducted in Asia, involving a total of 1,961 patients with HCC. Pretreatment plasma fibrinogen levels were measured using Clauss assay or serum biochemistry method. The cutoff values for plasma fibrinogen level were determined by different methods, including receiver operating characteristics (ROC), clinical reference range, or median. The cutoff values

divided patients with HCC into an elevated plasma fibrinogen group and a normal plasma fibrinogen group. All patients with HCC also received either curative treatment or palliative treatment. In addition, eight studies were included to examine the pretreatment plasma fibrinogen levels in HCC, cirrhotic, and/or healthy subjects. Six studies presented plasma fibrinogen levels in patients with HCC and healthy controls, three studies presented data for patients with cirrhosis and controls, and six studies presented data for patients with HCC and patients with cirrhosis. The details of the studies are provided in Table 2.

We also used the NOS to evaluate the quality of the eight studies that reported OS, DFS, and RFS. All of the studies

Table 1 Study characteristics of the included studies for prognostic analysis

Author	Year	Country	No. of HCC patients	Age (range in years)	Sex (M/F)	Cutoff value	Cutoff level method	Fibrinogen (range)	Fibrinogen measurement	Treatment received	Follow-up (range in months)	Outcome
Wang et al[32]	2016	China	41	Mean = 49 ± 10.7 (27–72)	38/3	2.675 g/L	ROC	Mean = 2.68 ± 1 g/L (0.84–5.06 g/L)	Clauss method	Liver transplantation	Mean = 43 months–>5 years	OS, DFS 1, 3, 5 years
Fu et al[33]	2017	China	130	Median = 49.5 (13–72)	121/9	2.345 g/L	ROC	–	–	Liver transplantation	>5 years	OS, DFS 1, 3, 5 years
Ruan et al[35]	2016	China	352	Mean = 50.6 ± 11.7	315/37	3.43 g/L	ROC	Mean = 3.4 ± 2.0 g/L	Serum biochemistry	Curative resection	Median = 42 months	OS 3.5 years
Kinoshita et al[18]	2013	Japan	113	Median = 72 (45–87)	76/37	315 mg/dL	ROC	Median = 279 mg/dL (91–720 mg/dL)	Clauss method	Curative treatment (surgical resection, RFA/PEI) or palliative treatment (TACE, TAI, systemic chemotherapy, sorafenib, BSC)	Median = 18 months (1–88)	OS 1, 3, 5 years
Liu et al[24]	2017	China	192	Mean = 46 ± 7.8	164/28	4 g/L	Clinical reference range	–	–	Operation	3 years	OS, RFS 3 years
Wang et al[34]	2017	China	539	Median = 53 (19–88)	480/59	2.83 g/L	Median	Median = 2.83 g/L	Serum biochemistry	All patients received treatment	5 years	OS, DFS 5 years
Zhang and Long[19]	2017	China	308	Mean = 51.2 ± 12.9	220/88	4.0 g/L	Clinical reference range	Mean = 3.4 ± 0.9	Clauss method	RFA, resection, TACE, sorafenib, or others	>5 years	OS, DFS 1, 3, 5 years
Li et al[36]	2015	China	286	Mean = 47.6 ± 11.6 (12–73)	261/25	3.24 g/L	ROC	Median = 2.98 g/L	–	Liver resection (n = 184) or transplantation (n = 102)	Median = 23 months (1–132)	RFS 1, 3 years

Abbreviations: BSC, best supportive care; DFS, disease-free survival; HCC, hepatocellular carcinoma; OS, overall survival; PEI, percutaneous ethanol injection; RFA, radiofrequency ablation; RFS, recurrence-free survival; ROC, receiver operating characteristics; TACE, transcatheter arterial chemoembolization; TAI, transcatheter arterial infusion.

Table 2 Baseline plasma fibrinogen levels of HCC, cirrhotic, and healthy subjects

Study	Year	Country	No. of controls	No. of HCC subjects	No. of cirrhotic subjects	Plasma fibrinogen level (g/L)			Method
						Controls	HCC subjects	Cirrhotic subjects	
Zhu et al[22]	2009	China	30	114	–	2.90 ± 0.46	3.75 ± 1.41	–	COULTER ACL-200 automated coagulant analyzer
Alkim et al[29]	2012	Turkey	10	11	13	2.6 ± 0.4	2.3 ± 0.5	2.4 ± 0.6	Conventional method
Hessien et al[25]	2015	Egypt	10	10	10	2.74 ± 0.44	0.99 ± 0.28	1.91 ± 0.43	Clauss method using commercially available kit
Doğan et al[21]	1997	Turkey	–	20	44	–	3.51 ± 1.30	2.36 ± 1.08	Standard method
Basili et al[23]	1997	Italy	–	18	18	–	2.30 ± 1.45	1.81 ± 0.72	Clauss method employing the ortho quantitative fibrinogen assay
Casaril et al[20]	1989	Italy	–	49	110	–	4.25 ± 1.80	2.64 ± 1.05	Routine laboratory screening
Al Ghumlas et al[30]	2005	Saudi Arabia	40	13	67	3.07 ± 1.17 g/L	4.58 ± 2.06	2.83 ± 1.44	Turbidimetric method of Ellis and Stransky
van der Walt et al[31]	1977	South Africa	19	19	–	3.15 ± 0.76	5.83 ± 2.78	–	Ellis and Stransky

Note: Data are presented as mean ± SD.
Abbreviation: HCC, hepatocellular carcinoma.

scored between 8 and 9, indicating that the quality of the included studies was high (Table 3).

Plasma fibrinogen levels among HCC, cirrhotic, and healthy subjects

We examined the plasma fibrinogen levels in HCC, cirrhotic, and healthy subjects. A random effect model showed no significant difference in the plasma fibrinogen levels among patients with HCC and healthy controls (WMD = 0.50, 95% CI = [−0.82, 1.82], $P = 0.457$; Figure 2A). However, compared to healthy individuals, patients with cirrhosis had significantly lower plasma fibrinogen levels (WMD = −0.44, 95% CI = [−0.86, −0.01], $P = 0.043$; Figure 2B). The plasma fibrinogen levels were not significantly different between patients with HCC and patients with cirrhosis (WMD = −0.62, 95% CI, [−1.56, 0.33], $P = 0.200$; Figure 2C).

Association between plasma fibrinogen and HCC prognosis
Correlation between elevated plasma fibrinogen and OS

Meta-analysis of the six OS-related studies showed little heterogeneity among studies ($I^2 = 25.7\%$, $P = 0.242$). A fixed effect model showed that patients with HCC and high pretreatment plasma fibrinogen levels had significantly poorer OS compared to those with normal fibrinogen levels (HR = 2.08, 95% CI = [1.67, 2.59], $P < 0.0001$; Figure 3A). Sensitivity analysis showed that the pooled result was relatively stable

(Figure 3B). Egger's test ($P = 0.008$) showed the presence of publication bias. Further, trim and fill analysis showed that after adjusting for three studies, the recalculated pooled HR for OS was 1.85 (95% CI = [1.51, 2.27]; Figure 3C), which was not significantly changed from the initial estimate (HR = 2.08, 95% CI = [1.67, 2.59]). Our data suggest that the presence of publication bias has no significant effect on the overall finding.

We also performed TSA meta-analysis on the OS results. Although the number of participants had not reached the required information size, our TSA showed that the cumulative Z-curve crossed the trial sequential monitoring boundary for harm, confirming the poorer survival rate among patients with HCC and high levels of plasma fibrinogen. Our results suggest that currently available studies are sufficient to conclude the negative prognostic value of elevated plasma fibrinogen in patients with HCC (Figure 4).

Correlation between elevated plasma fibrinogen and DFS/RFS

Meta-analysis of the 5 DFS-/RFS-related studies showed low heterogeneity among studies ($I^2 = 36.3\%$, $P = 0.179$). A fixed effect model showed that patients with HCC and high pretreatment plasma fibrinogen levels had significantly poorer outcomes compared to those with normal fibrinogen levels (HR = 1.90, 95% CI = [1.52, 2.37], $P < 0.0001$; Figure 5A). Sensitivity analysis showed that the pooled result was relatively stable (Figure 5B). Egger's test ($P = 0.095$) showed no publication bias.

Table 3 Quality assessment of the included studies for prognostic evaluation

	Selection				Comparability	Exposure			Score
	Representativeness of the exposed cohort	Selection of the nonexposed cohort	Ascertainment of exposure	Demonstration that outcome of interest was not present at start of study	Comparability of cohorts on the basis of the design or analysis	Assessment of outcome	Was follow-up long enough for outcomes to occur	Adequacy of follow-up of cohorts	
Wang et al (2016)[32]		*	*	*	**	*	*	*	8
Fu et al (2017)[33]		*	*	*	**	*	*	*	8
Ruan et al (2016)[35]		*	*	*	**	*	*	*	8
Kinoshita et al (2013)[18]	*	*	*	*	**	*	*	*	9
Liu et al (2017)[24]	*	*	*	*	**	*	*	*	9
Wang et al (2017)[34]	*	*	*	*	**	*	*	*	9
Zhang and Friedman (2012)[4]	*	*	*	*	**	*	*	*	9
Li et al (2015)[36]		*	*	*	**	*	*	*	8

Notes: The Newcastle–Ottawa Scale (NOS) was used to assess the quality of the studies included. NOS employs a "star" rating system that evaluates eight items, which are categorized into three domains: selection (four items: maximum one star each), comparability (one item: maximum two stars), and outcome (three items: maximum one star each). The NOS assessment, which ranges from 0 (worst) to 9 (best) stars, provides a semiquantitative appraisal for the overall quality of each study. Studies with scores of 6 or higher were regarded as high quality.

Association between plasma fibrinogen and clinicopathological parameters of HCC

Clinicopathological analyses showed that elevated plasma fibrinogen levels were significantly associated with advanced tumor stage (stage III–IV vs stage I–II, OR = 2.37, 95% CI = [1.83, 3.07], $P < 0.00001$), larger tumor size (≥ 5 vs <5 cm, OR = 3.04, 95% CI = [2.22, 4.15], $P < 0.00001$), increased tumor number (multiple vs single, OR = 1.54, 95% CI = [1.17, 2.05], $P = 0.002$; >3 vs ≤ 3 tumor nodules, OR = 1.99, 95% CI = [1.02, 3.87], $P = 0.04$), and vascular invasion (OR = 3.81, 95% CI = [2.25, 6.44], $P < 0.00001$). Plasma fibrinogen levels were significantly higher in patients with HCC and mild cirrhosis (Child A) compared to those with moderate or advanced cirrhosis (Child B/C) (Child A vs Child B/C, OR = 1.53, 95% CI = [1.01, 2.33], $P = 0.04$). Higher plasma fibrinogen was also marginally associated with increased serum AFP level (OR = 1.37, 95% CI = [1.00, 1.86], $P = 0.05$). No significant association was found when evaluating age, sex, hepatitis B surface antigen (HBsAg), and alcohol use. The correlations between plasma fibrinogen and the clinical features of HCC are depicted in Figure 6 and summarized in Table 4.

Discussion

In this meta-analysis, we identified eight studies[20–23,25,29–31] that examined the pretreatment plasma fibrinogen levels in HCC, cirrhotic, and/or healthy subjects. We observed that patients with cirrhosis had significantly lower plasma fibrinogen levels compared to healthy controls (WMD = -0.44, 95% CI = [-0.86, -0.01], $P = 0.043$). Fibrinogen is a product of hepatocytes. Therefore, patients with cirrhosis and compromised liver function may generally have less plasma fibrinogen levels than healthy counterparts.[25,29] However, our WMD analyses showed that the pretreatment plasma fibrinogen levels in HCC cases were not significantly elevated when compared with that in healthy subjects (WMD = 0.50, 95% CI = [-0.82, 1.82], $P = 0.457$) or patients with cirrhosis (WMD = -0.62, 95% CI = [-1.56, 0.33], $P = 0.200$). Therefore, plasma fibrinogen level is not usable as a diagnostic marker for HCC detection.

On the other hand, our prognostic value analyses, which included eight studies[18,19,24,32–36] with a total number of 1,961 patients with HCC, demonstrated that those patients with HCC and elevated fibrinogen levels are more likely to experience poorer OS (HR = 2.08, 95% CI = [1.67, 2.59], $P < 0.0001$) and recurrence (HR = 1.90, 95% CI = [1.52, 2.37], $P < 0.0001$). Furthermore, TSA indicated that currently available studies are sufficient to validate the negative
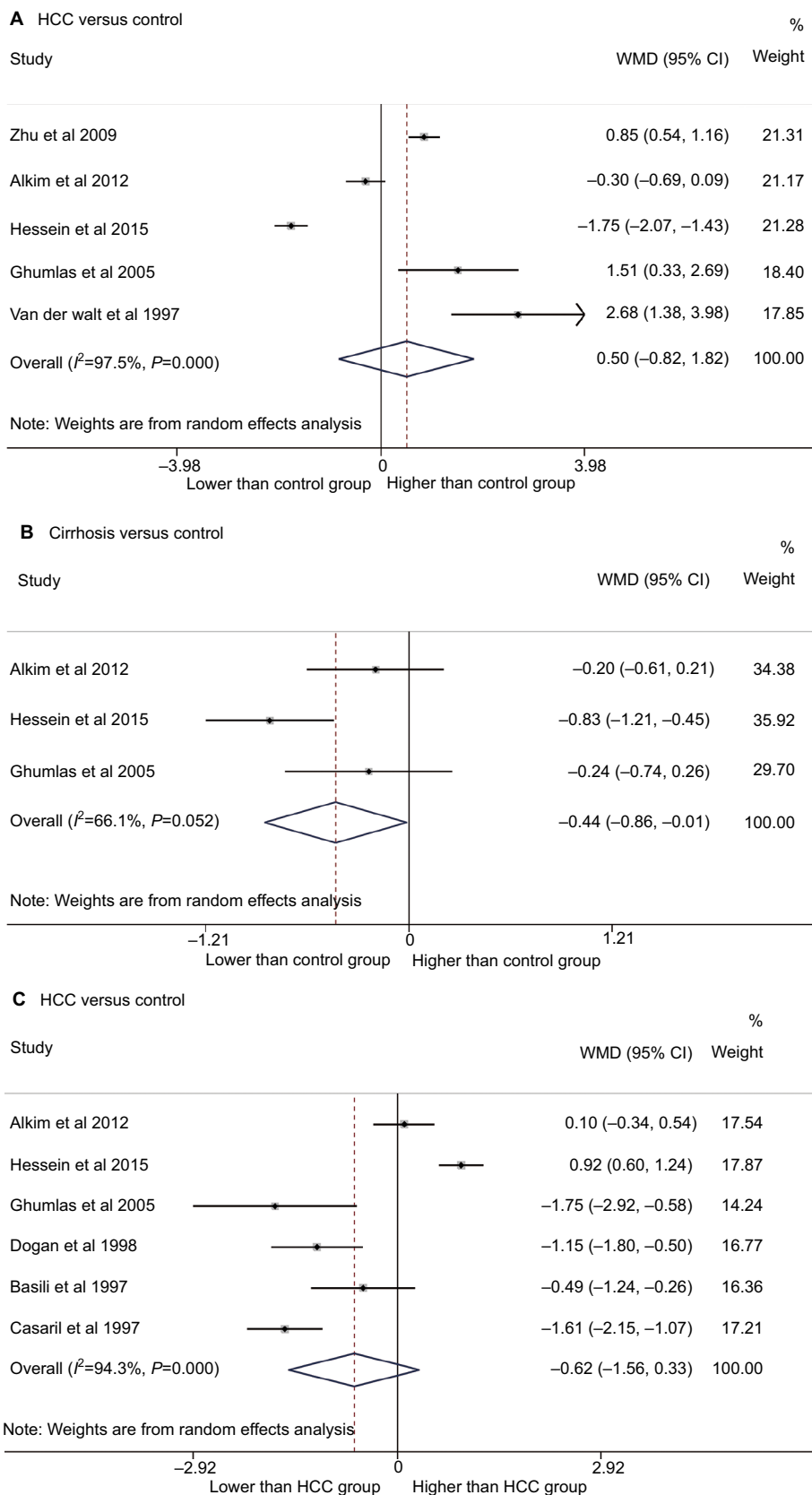
A HCC versus control

%

Study   WMD (95% CI)   Weight

Zhu et al 2009   0.85 (0.54, 1.16)   21.31

Alkim et al 2012   −0.30 (−0.69, 0.09)   21.17

Hessein et al 2015   −1.75 (−2.07, −1.43) 21.28

Ghumlas et al 2005   1.51 (0.33, 2.69)   18.40

Van der walt et al 1997   2.68 (1.38, 3.98)   17.85

Overall (I^2=97.5%, P=0.000)   0.50 (−0.82, 1.82)   100.00

Note: Weights are from random effects analysis

| −3.98 | 0 | 3.98 |
Lower than control group   Higher than control group

B Cirrhosis versus control

%

Study   WMD (95% CI)   Weight

Alkim et al 2012   −0.20 (−0.61, 0.21)   34.38

Hessein et al 2015   −0.83 (−1.21, −0.45) 35.92

Ghumlas et al 2005   −0.24 (−0.74, 0.26)   29.70

Overall (I^2=66.1%, P=0.052)   −0.44 (−0.86, −0.01) 100.00

Note: Weights are from random effects analysis

| −1.21 | 0 | 1.21 |
Lower than control group   Higher than control group

C HCC versus control

%

Study   WMD (95% CI)   Weight

Alkim et al 2012   0.10 (−0.34, 0.54)   17.54

Hessein et al 2015   0.92 (0.60, 1.24)   17.87

Ghumlas et al 2005   −1.75 (−2.92, −0.58) 14.24

Dogan et al 1998   −1.15 (−1.80, −0.50) 16.77

Basili et al 1997   −0.49 (−1.24, −0.26) 16.36

Casaril et al 1997   −1.61 (−2.15, −1.07) 17.21

Overall (I^2=94.3%, P=0.000)   −0.62 (−1.56, 0.33)   100.00

Note: Weights are from random effects analysis

| −2.92 | 0 | 2.92 |
Lower than HCC group   Higher than HCC group

Figure 2 Forest plot for plasma fibrinogen levels in (**A**) patients with HCC compared to that in healthy controls, (**B**) cirrhotic patients compared to healthy controls, and (**C**) patients with HCC compared to patients with cirrhosis.
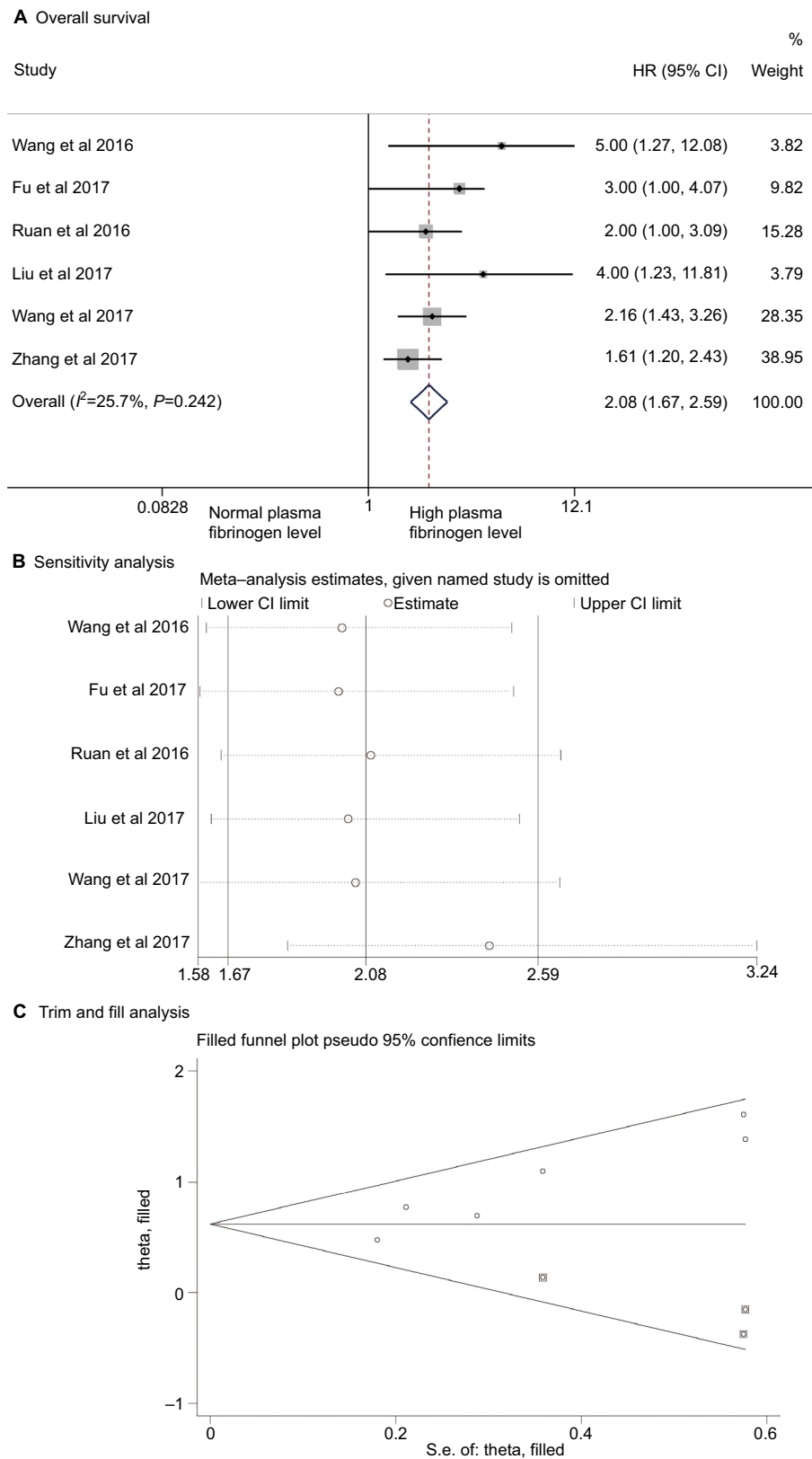**Abbreviations:** HCC, hepatocellular carcinoma; WMD, weighted mean difference.

A Overall survival

%

Study | HR (95% CI) | Weight

Wang et al 2016 — 5.00 (1.27, 12.08) — 3.82
Fu et al 2017 — 3.00 (1.00, 4.07) — 9.82
Ruan et al 2016 — 2.00 (1.00, 3.09) — 15.28
Liu et al 2017 — 4.00 (1.23, 11.81) — 3.79
Wang et al 2017 — 2.16 (1.43, 3.26) — 28.35
Zhang et al 2017 — 1.61 (1.20, 2.43) — 38.95
Overall (I^2=25.7%, P=0.242) — 2.08 (1.67, 2.59) — 100.00

0.0828 — Normal plasma fibrinogen level — 1 — High plasma fibrinogen level — 12.1

B Sensitivity analysis

Meta–analysis estimates, given named study is omitted

| Lower CI limit   ○Estimate | Upper CI limit

Wang et al 2016
Fu et al 2017
Ruan et al 2016
Liu et al 2017
Wang et al 2017
Zhang et al 2017

1.58 1.67 — 2.08 — 2.59 — 3.24

C Trim and fill analysis

Filled funnel plot pseudo 95% confience limits

theta, filled

S.e. of: theta, filled

Figure 3 Association of elevated plasma fibrinogen level with overall survival in patients with hepatocellular carcinoma.
Notes: (**A**) Forest plot of HRs for overall survival. (**B**) Sensitivity analysis of overall survival. (**C**) Trim and fill analysis for the overall survival. The squares represent the adjusted studies.
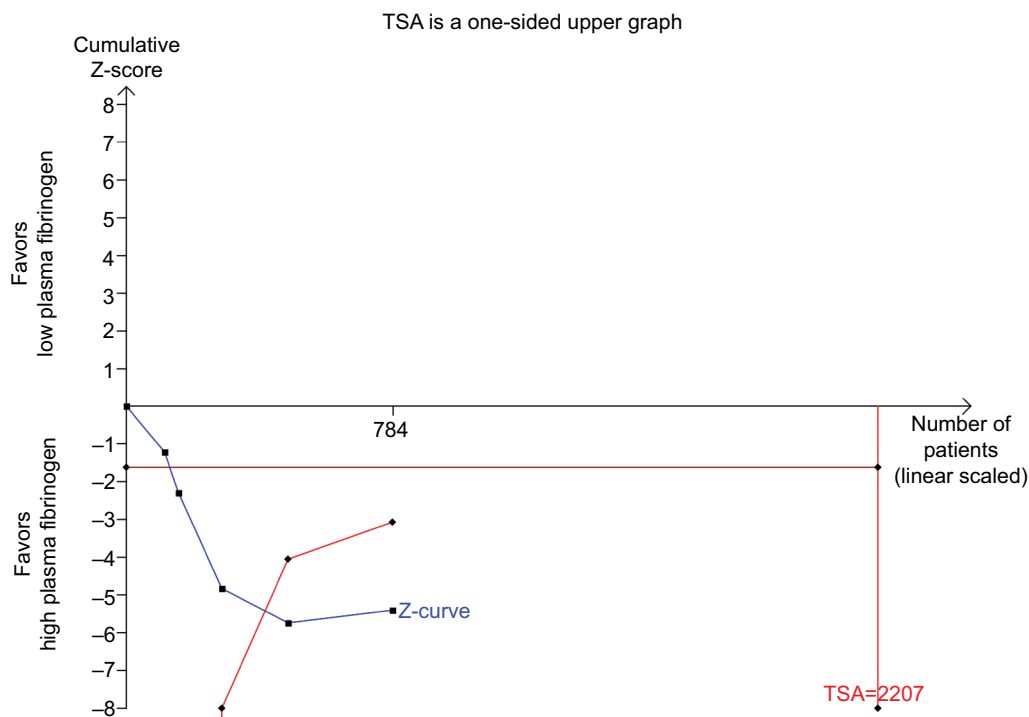
Figure 4 TSA of the association between elevated plasma fibrinogen levels and overall survival in patients with hepatocellular carcinoma.
Notes: The diversity-adjusted required information size of 784 patients was calculated on the basis of type I error of 5%, type II error of 20%, the control event proportion of 40%, and a relative risk reduction of 20% in outcomes. The cumulative Z-curve crossed the trial sequential monitoring boundary for harm, confirming the poorer survival rate in patients with hepatocellular carcinoma and high plasma fibrinogen.
Abbreviation: TSA, trial sequential analysis.

prognostic value of elevated plasma fibrinogen levels in patients with HCC. Our clinicopathological analyses also indicated that patients with HCC and elevated plasma fibrinogen levels are likely to be associated with a higher pathological stage including advanced tumor stage, larger tumor size, increased tumor number, and the presence of vascular invasion, implicating that plasma fibrinogen level is closely related to tumor progression.

In addition to our analysis, recent studies also indicated a positive correlation between elevated plasma fibrinogen levels and poorer prognosis in other solid tumors including esophageal carcinoma,[37–39] small cell lung carcinoma,[40] non-small cell lung carcinoma,[41] upper urinary tract urothelial carcinoma,[42,43] epithelial ovarian cancer,[44] colorectal cancer,[45] renal cell carcinoma,[46,47] and gastric cancer.[48,49] There are several theories that may explain this association: 1) tumor progression is usually coupled with elevated inflammatory responses and leukocyte infiltration into tumor stroma. Infiltrated leukocytes may convert fibrinogen into fibrin matrix, which encapsulates the tumor cells and promotes tumor angiogenesis;[50] 2) fibrinogen may serve as an extracellular matrix protein that regulates the growth of cancer cells by

binding to growth factors (eg, vascular endothelial growth factor, fibroblast growth factor-2, platelet-derived growth factor) and promote cellular adhesion, proliferation, and metastasis; and[51–53] 3) fibrinogen promotes the adhesion of tumor cells to platelets, which leads to platelet aggregation and thrombin formation around tumor cells. The tumor-associated platelets and thrombin protect tumor cells against the attack of natural killer cells.[54] Moreover, in vitro studies also suggested that high levels of fibrinogen induced epithelial–mesenchymal transition, which is a hallmark for cancer invasion and metastasis.[14]

As a plasma biomarker, fibrinogen has its advantages: blood sampling is a minimally invasive method that patients tolerate well, and it allows replicated sampling for long-term surveillance. Plasma fibrinogen levels can be determined easily by using routine coagulation parameters tested in the laboratory, which is rapid and cost-effective compared to other measurements, such as serum AFP level. Similar to serum AFP level, which has been identified as a significant prognostic marker in HCC, fibrinogen may also achieve similar accuracy for predicting clinical outcomes. In fact, Fu et al[33] found that subgroups with negative AFP levels or

A Disease-free/recurrence-free survival
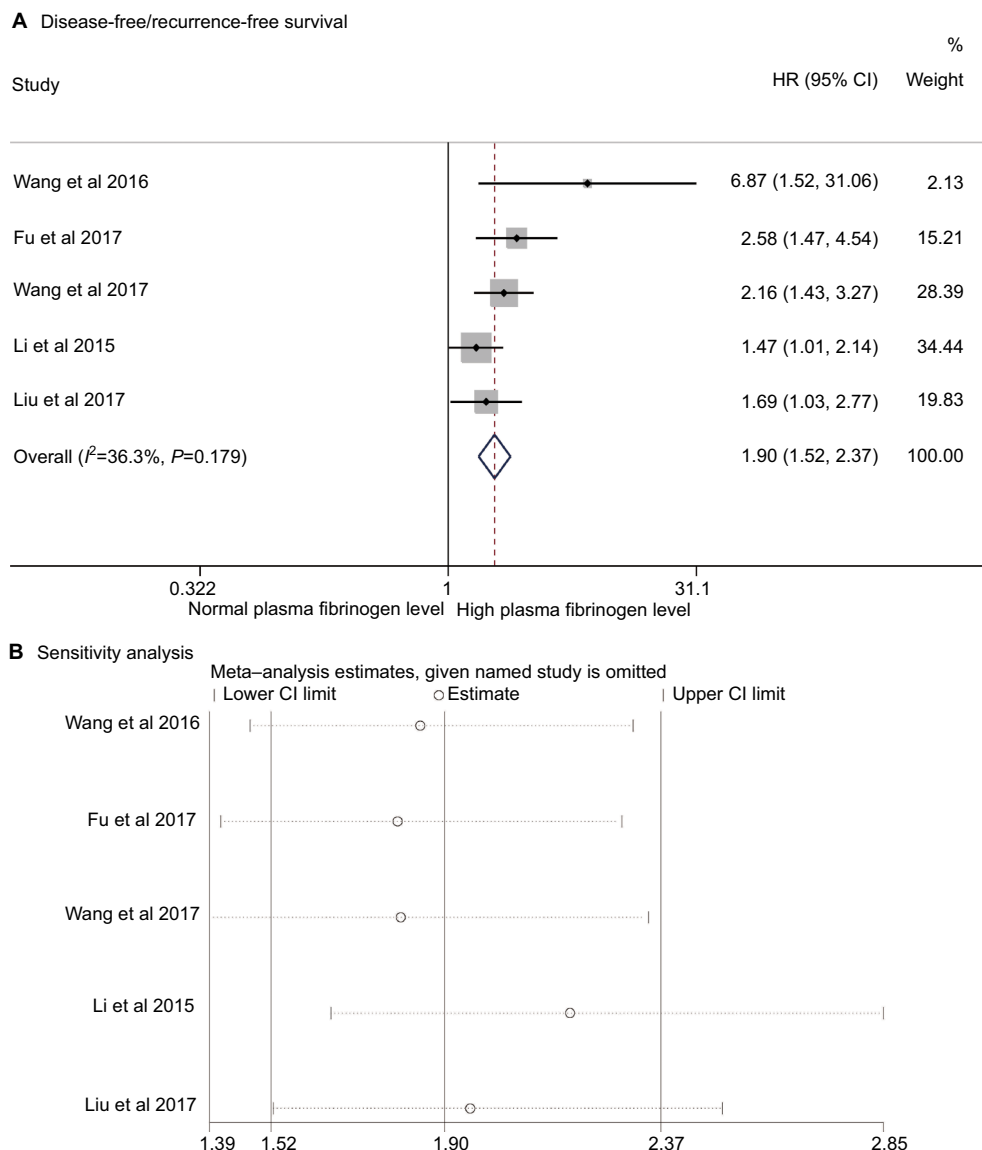

B Sensitivity analysis

Figure 5 Association of elevated plasma fibrinogen level in patients with hepatocellular carcinoma and disease-free/recurrence-free survival.
Notes: (**A**) Forest plot of HR for disease-free/recurrence-free survival. (**B**) Sensitivity analysis of disease-free/recurrence-free survival.

less aggressive clinicopathology but with elevated plasma fibrinogen levels still demonstrated poorer survival outcomes, suggesting that plasma fibrinogen could be a better prognostic biomarker compared to AFP, although further studies are warranted.

Most importantly, the examination of pretreatment plasma fibrinogen levels may help clinicians identify and provide more aggressive treatments to the appropriate group of patients to improve clinical outcomes. By comparing the normal- and high-fibrinogen groups, Zhang and Long[19] found that patients with HCC and elevated plasma fibrinogen levels had poorer responses to TACE, a recommended adjuvant therapy for intermediate and advanced HCC. The poor response to TACE treatment in high-fibrinogen groups

requires further examination due to its small sample size, and it is not one of our primary outcomes due to the limited number of studies available.

Aside from the limited studies available, there are several other limitations in our analyses. The association between the elevated plasma fibrinogen levels and poor survival outcomes could potentially be overestimated because all included studies are published studies. Positive results are always favored for publication when compared to negative results; thus exaggerating the effect of the group tested. There are variations in the plasma fibrinogen measurement methods, the cutoff values, tumor stages, and treatments received among all included studies; however, analysis suggested little heterogeneity among studies. Moreover, all patients included in the
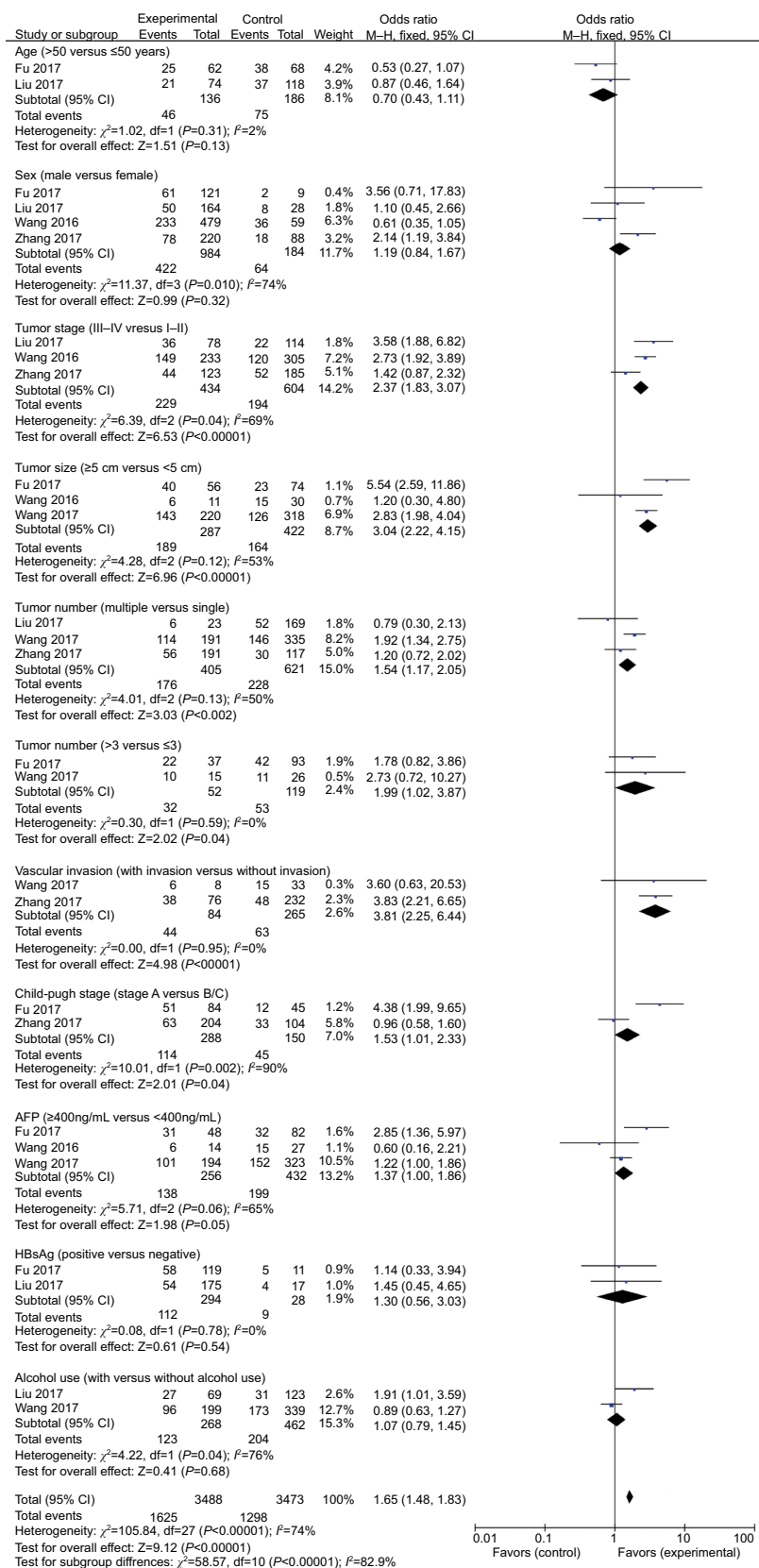
Figure 6 Association between elevated plasma fibrinogen levels and clinicopathological parameters including age (>50 vs ≤50 years), sex (male vs female), tumor stage (III–IV vs I–II), tumor size (≥5 vs <5 cm), tumor number (multiple vs single; >3 vs ≤3), vascular invasion (with vs without invasion), Child-Pugh stage (A vs B/C), serum AFP level (≥400 vs <400 ng/mL), HBsAg (positive vs negative), and alcohol use (with vs without alcohol use).
Abbreviations: AFP, alpha-fetoprotein; HBsAg, hepatitis B surface antigen.

Table 4 Overall analysis of the association between elevated plasma fibrinogen levels and clinical features of patients with hepatocellular carcinoma

Variables	No. of study	OR (95% CI)	P-value	Heterogeneity	
				I² (%)	P-value
Age (>50 vs ≤50 years)	2	0.70 (0.43, 1.11)	0.13	2	0.31
Sex (male vs female)	4	1.19 (0.84, 1.67)	0.32	74	0.010
Tumor stage (III–IV vs I–II)	3	2.37 (1.83, 3.07)	<0.00001	69	0.04
Tumor size (≥5 vs <5 cm)	3	3.04 (2.22, 4.15)	<0.00001	53	0.12
Tumor number (multiple vs single)	3	1.54 (1.17, 2.05)	0.002	50	0.13
Tumor number (>3 vs ≤3)	2	1.99 (1.02, 3.87)	0.04	0	0.59
Vascular invasion (with invasion vs without invasion)	2	3.81 (2.25, 6.44)	<0.00001	0	0.95
Child-Pugh (stage A vs B/C)	2	1.53 (1.01, 2.33)	0.04	90	0.002
AFP (≥400 vs <400 ng/mL)	3	1.37 (1.00, 1.86)	0.05	65	0.06
HBsAg (positive vs negative)	2	1.30 (0.56, 3.03)	0.54	0	0.78
Alcohol use (with vs without alcohol use)	2	1.07 (0.79, 1.45)	0.68	76	0.04

Abbreviations: AFP, alpha-fetoprotein; HBsAg, hepatitis B surface antigen.

prognostic evaluation were Asians, which may not reflect as general phenomena in other ethnic populations. All of these factors could have influenced the outcome of our evaluation.

Conclusion

From our analyses of eight high-quality studies, we conclude that patients with HCC and elevated plasma fibrinogen levels have poor survival outcomes. High plasma fibrinogen levels are associated with heavier tumor burden and advanced tumor progression. Therefore, plasma fibrinogen level may serve as a prognostic biomarker in HCC.

Acknowledgments

Author contributions

GH, HJ, ZJ, and XZ conceived and designed the study. GH, HJ, and YL searched the database and checked them according to the eligible criteria and exclusion criteria. YW and WC analyzed the data. BS and YL designed the methodology. ZJ and XZ supervised the planning and execution of the research activity. GH and HJ wrote the paper. All authors contributed toward data analysis, drafting and critically revising the paper and agree to be accountable for all aspects of the work. All authors read and approved the final manuscript.

Disclosure

The authors report no conflicts of interest in this work.

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