

Prognostic value of tumor-infiltrating lymphocytes in hepatocellular carcinoma

A meta-analysis

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Abstract

Background: In patients with hepatocellular carcinoma (HCC), the prognostic role of tumor-infiltrating lymphocytes (TILs) for survival is still controversial. A meta-analysis was performed to investigate the prognostic effect of TILs in HCC.

Methods: We identify studies from PubMed, Embase, and the Cochrane Library to evaluate the prognostic value of TILs in patients with HCC. A meta-analysis was conducted to estimate overall survival and disease-free survival. The hazard ratio (HR) and 95% confidence interval (CI) were calculated employing fixed-effect or random-effect models depending on the heterogeneity of the included trials.

Results: A total of 7905 patients from 46 observational studies were enrolled. For TILs subsets, the density of CD8+, FOXP3+, CD3+, and Granzyme B+ lymphocytes was significantly associated with improved survival ($P < .05$). The density of FOXP3+ TILs in intratumor (IT) was the most significant prognostic marker (pooled HR = 1.894; 95% CI = 1.659–2.164; $P < .001$). Patients with high infiltration of CD8+ TILs in IT (pooled HR = 0.676; 95% CI = 0.540–0.845; $P = .001$) or in margin of tumor (MT) (pooled HR = 0.577; 95% CI = 0.437–0.760; $P < .001$) had better OS. The pooled analysis revealed that high density of Granzyme B+ T-lymphocytes in IT was statistically significant associated with better OS (pooled HR = 0.621; 95% CI = 0.516–0.748; $P < .001$) and DFS (pooled HR = 0.678; 95% CI = 0.563–0.815; $P < .001$). It was interesting that high density of CD3+ in IT foreboded worse OS (pooled HR = 1.008; 95% CI = 1.000–1.015; $P = .037$), but better DFS (pooled HR = 0.596; 95% CI = 0.374–0.948; $P = .029$).

Conclusion: Our findings suggested that some TIL subsets could serve as prognostic biomarkers in HCC. High-quality randomized controlled trials are needed to determine if these TILs could serve as targets for immunotherapy in HCC.

Abbreviations: CI = confidence interval, CIK = cytokine-induced killer, DFS = disease-free survival, FC = flow cytometry, HCC = hepatocellular carcinoma, HR = hazard ratio, HVEM = herpesvirus entry mediator, IHC = immunohistochemistry, IT = intratumor, LI = lymphoplasmacytic infiltration, MT = margin of tumor, NK = natural killer, NOS = Newcastle–Ottawa scale, NR = not report, OS = overall survival, PB = peripheral blood, PCR = polymerase chain reaction, PT = peritumor, ROC curve = receiver operating characteristic curve, SC = survival curve, TILs = tumor-infiltrating lymphocytes, Treg = regulatory T-lymphocytes.

Keywords: CD8, Foxp3, hepatocellular carcinoma, prognosis, tumor-infiltrating lymphocytes

1. Introduction

Hepatocellular carcinoma (HCC) is one of the most common primary malignancies of the liver, representing the 3rd leading

cause of cancer-related death worldwide. Besides early diagnosis, prompt treatment plans identified from the prediction of patients' outcomes also contribute to successful treatment of liver cancer. Therefore, further investigation is needed to discovery precise tumor biomarkers with higher sensitivity and specificity in HCC to determine the optimal treatment programs and predict the prognosis of HCC.

Tumor-infiltrating lymphocytes (TILs), including T-cells, B cells, and natural killer (NK) cells, are one of the representative components of host antitumor immune responses.^[1] There are many specific antigens such as FOXP3, CD3, CD4, CD8, CD16, CD20, CD56 and CD57, CD68, and CD169 in the cell membrane of TILs.^[2] For example, FOXP3, CD3, CD4, and CD8 bind to T-cells; CD16 binds to monocytes; CD20 binds to B cells; CD56 and CD57 binds to natural killer cells; CD68 and CD169 bind to macrophages. With advances in flow cytometry and immunohistochemistry, many researches have shown that specific types of immune cells not only regulate the host defense against cancer,^[3] but also accelerate tumor progression either by selecting an opportune microenvironment to survive for tumor or by creating conditions within the tumor microenvironment that simulate tumor outgrowth.^[4]

The TILs might play as a prognostic biomarker in HCC. TILs have been demonstrated to predict overall survival (OS) and

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disease-free survival (DFS) following resection of both primary and metastatic liver tumors.^[5–7] Hang et al found that high infiltration of Foxp3+ T-cells was associated with poor prognosis,^[5] whereas no correlation between the infiltration of Foxp3+ T-cells and OS was found in Wang et al.^[8] Chew et al revealed that patients with high density of CD8+ T-cell had poor outcome,^[9] but Ramzan et al reported the opposite results.^[10] So, the effect of TILs on HCC prognosis is controversial. Besides, it is not clear whether the associations between the density of TILs and prognosis vary depending on clinical factors such as the pathological stage and anatomic site. Therefore, we performed this meta-analysis to assess the prognostic effect of TILs in HCC.

2. Materials and methods

2.1. Literature search

Relevant articles were identified by 2 reviewers via an electronic search of PubMed, Embase, and Cochrane using the following keywords: (tumour or tumor or cancer or carcinoma), (hepatic or liver), (TILs or tumor-infiltrating lymphocyte or intratumoral lymphocyte or tumor-infiltrating lymphocyte or intratumoral lymphocyte), and (prognostic or prognosis or outcome). And the search time period of the electronic database was from inception to July 7, 2018. Additionally, pertinent studies were searched in reference lists of selected reports and reviews. Unpublished literature was not performed to search. Disagreement on article inclusion between the 2 reviewers was resolved via a 3rd reviewer. A 3rd researcher would determine the final results about the disagreement of the 2 reviewers.

2.2. Inclusion and exclusion criteria

Inclusion criteria for this meta-analysis were as follows: studies researching the prognostic effect of TILs or associated TIL subsets in HCC; report of TILs or associated TIL subsets in tumor surgical specimens; hazard ratio (HR) and 95% confidence interval (CI) could be extracted; when the same author or group reported results obtained from the same cohort of patients in more than one paper, the most recent report or the most informative report was selected.

Exclusion criteria for this meta-analysis were as follows: letters, reviews, case reports, animal trials, abstracts, and expert opinion were excluded; articles in which have no information on overall survival or disease-free survival; patients who only treated with immunotherapy chemotherapy, radiotherapy, interventional therapy, or liver transplantation; nonprimary tumor, such as metastatic tumor or recurrent tumor. Names of authors or journals of the articles were not considered in excluding or including the articles.

2.3. Statistical analysis

The HR and its 95% CI were used to assess the association between TILs and prognosis of HCC. If a direct report of OS and DFS were not available, then extract the survival data from Kaplan–Meier curves by Engauge Digitizer version 4.1 as described previously.^[11] The extraction was performed by 2 independent researchers to decrease error. STATA version 12.0 was performed to merge the results of studies. Statistical heterogeneity between trials was assessed by Chi-squared test and was suggested significant when $I^2 > 50\%$ and/or $P < .05$. The fixed-effect model was used when no heterogeneity was detected

among studies, while the random-effect model was preferred when variance existed. Funnel plot and Egger test were performed to evaluate the publication bias.^[12]

3. Results

3.1. Study selection and characteristics

A total of 1232 articles were initially gathered after duplicate removal. Among them, 58 studies reported the association between TILs and prognosis of HCC. After further screening, 12 researches were excluded (1 studying the same cohort of patients, 10 lacking enough useful data, and 1 treating with immunotherapy). Finally, a total of 46 retrospective studies including 7905 patients from America, China, France, Germany, Japan, The Netherlands, and Singaporean were included in this meta-analysis (Fig. 1).^[8–10,13–55]

The main characteristics of included studies were displayed in Table 1. The prognostic role of TILs in intratumor (IT), margin of tumor (MT), and peritumor (PT) were evaluated in 41, 3, and 9 articles, respectively. Sample sizes of included studies ranged from 36 to 544 patients. The details of tumor stage were offered in 31 articles, and the classifications were diverse. Follow-up time was provided in 27 articles. Specific TIL subsets were detected by methods of flow cytometry (FC), immunohistochemistry (IHC), and polymerase chain reaction (PCR), respectively. All 46 studies were considered to be of adequate quality for the meta-analysis according to NOS assessment (score ≥ 5 points) (Table 1).

3.2. Subgroup analysis

Different TIL subsets have different functions in the process of protumor or antitumor immunoreaction response. Therefore, subgroup analyses based on TIL subsets were carried out. Then, we further conducted subgroup analyses based on the distribution location of TILs. Table 2 shows the various consequences of subgroup analyses.

3.3. Foxp3+ T-lymphocyte subset

A total of 18 articles, including 3015 patients with HCC, researched the association between clinical outcome and the density of Foxp3+ TILs.^[8,16,18–22,24,26,27,29–31,34,42,43,48,49]

3.3.1. Overall survival. Fourteen articles focused on the relationship between OS and the density of Foxp3+ TILs in IT.^[8,16,18,20,24,26,27,29–31,34,42,48,49] Four articles focused on the association between OS and the density of Foxp3+ TILs in PT.^[16,21,22,30] The pooled analysis revealed that low density of infiltration of Foxp3+ TILs in IT was statistically significant associated with better OS (pooled HR = 1.894; 95% CI = 1.659–2.164; $P < .001$). But, there was no statistical significance between the density of Foxp3+ TILs in PT and OS ($P = .054$) (Fig. 2, Table 2).

Subgroup analysis showed that patients with high infiltration of Foxp3+ T-lymphocytes had worse OS in the groups with Asian patients (pooled HR = 1.878, 95% CI = 1.643–2.147; $P < .001$), small sample size (< 100 ; pooled HR = 2.543, 95% CI = 1.639–3.945; $P < .001$), large sample size (≥ 100 ; pooled HR = 1.804, 95% CI = 1.548–2.101; $P < .001$), median cutoff values (pooled HR = 2.098, 95% CI = 1.579–2.787; $P < .001$), and other cutoff values (pooled HR = 1.911, 95% CI = 1.567–2.330; $P < .001$). However, other subgroup showed no statistical significance (Table 3).

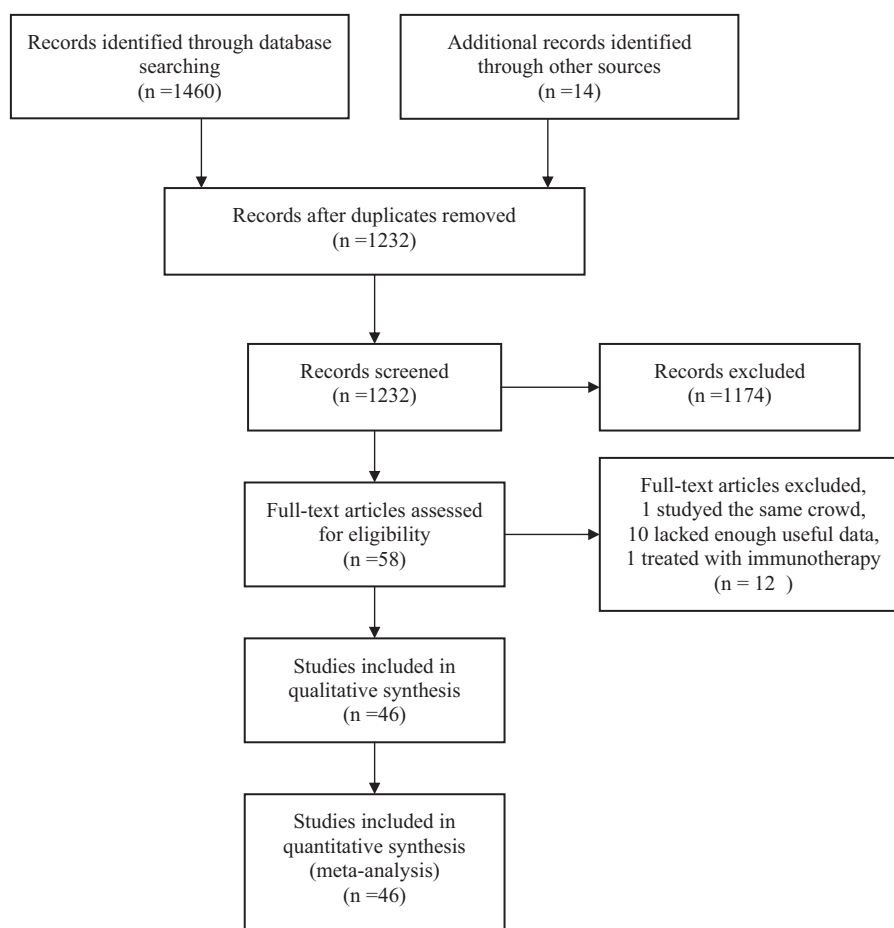


Figure 1. Flow diagram of study selection.

3.3.2. Disease-free survival. Eleven articles focused on the relationship between DFS and the density of Foxp3+ TILs in IT.^[16,18–20,24,27,29–31,34,43] Four articles focused on the association between DFS and the density of Foxp3+ TILs in PT.^[16,21,30] The pooled analysis revealed that low density of infiltration of Foxp3+ TILs in IT was statistically significant associated with better DFS (pooled HR=1.791; 95% CI=1.546–2.076; $P < .001$) and so as Foxp3+ TILs in PT (pooled HR=1.557; 95% CI=1.057–2.293; $P = .025$) (Fig. 3, Table 2).

3.4. CD8+ T-lymphocyte subset

A total of 20 articles, including 3102 patients with HCC, researched the association between clinical outcome and the density of CD8+ TILs.^[9,10,13,14,16,17,23,28,30,34,35,39–41,44,48,50,53–55]

3.4.1. Overall survival. Eighteen articles focused on the relationship between OS and the density of CD8+ TILs in IT.^[9,10,14,16,17,23,28,30,34,35,39–41,44,50,53–55] Two articles focused on the association between OS and the density of CD8+ TILs in MT.^[39,41] Five articles focused on the association between OS and the density of CD8+ TILs in PT.^[16,17,30,39,48] A random model was used because of a significant heterogeneity ($P < .001$, $I^2 = 69.5\%$), and the result demonstrated patients with high infiltration of CD8+ TILs in IT had better OS (pooled HR=

0.676; 95% CI=0.540–0.845; $P = .001$). The pooled analysis revealed that high density of infiltration of CD8+ TILs in MT was statistically significant associated with better OS (pooled HR=0.577; 95% CI=0.437–0.760; $P < .001$). But, there was no statistical significance between the density of CD8+ TILs in PT and OS ($P = .531$) (Fig. 4, Table 2).

Subgroup analysis showed that patients with high infiltration of CD8+ T-lymphocytes had better OS in the groups with Asian patients (pooled HR=0.669, 95% CI=0.536–0.835; $P < .001$), large sample size (≥ 100 ; pooled HR=0.668, 95% CI=0.537–0.831; $P < .001$), median cutoff values (pooled HR=0.701, 95% CI=0.532–0.925; $P = .012$), and other cutoff values (pooled HR=0.656, 95% CI=0.443–0.971; $P = .035$). However, other subgroups showed no statistical significance (Table 3).

3.4.2. Disease-free survival. Ten articles focused on the relationship between DFS and the density of CD8+ TILs in IT.^[13,16,17,28,30,34,41,44,50,53] Only 1 article focused on the association between DFS and the density of CD8+ TILs in the association between DFS and the density of CD8+ TILs in MT.^[41] Three articles focused on the association between DFS and the density of CD8+ TILs in PT.^[16,17,30] The pooled analysis revealed that high density of infiltration of CD8+ TILs in IT was statistically significant associated with better OS (pooled HR=0.712; 95% CI=0.574–0.883; $P = .002$). There was no statistical

Table 1
Main characteristics of all studies included in the meta-analysis.

Study	Country	Subsets	Location	Case number	Tumor stage	Follow-up, mo	Detected method	Cutoff value	HRs provided from	Outcome	Quality score (NOS)
Ikeguchi, 2004	Japan	CD8+	IT	60	5/17/26/12	49 (2–115)	IHC	Median	Report	DFS	7
Ikeguchi, 2005	Japan	CD8+	IT	59	6/16/25/12	57 (2–128)	IHC	Median	Report	OS	5
Fu, 2007	China	CD4+CD25+Foxp3+	PB	75	NR	NR	FC	Mean	SC	OS	5
Gao, 2007	China	CD3+, CD4+, CD8+, Granzyme B+, Foxp3+	IT/PT	302	185/52/65/0	58 (2–109)	IHC	Median	Report	OS/DFS	6
Kobayashi, 2007	Japan	Foxp3+/CD4+, CD8+	IT/PT	147	57/53/37/0	52.8 (0.5–169.1)	IHC	Median	Report	OS/DFS	6
Li, 2008	China	Foxp3+, Foxp3+/CD4+	IT/PB	63	15/46/2/0	NR	IHC	20/mm ²	Report	OS/DFS	7
Sasaki, 2008	Japan	Foxp3+	IT	164	NR	55.5 (2–184)	IHC	Median	SC	DFS	7
Gao, 2009	China	Foxp3+, Granzyme B+, PD-L1, PD-L2	IT	240	106/76/58/0	16 (1.5–68)	IHC	NR	Report	OS/DFS	7
Ju-AJCP, 2009	China	Foxp3+, CD68+	PT	130	I-II66/III-IV64	31.8 ± 1.7	IHC	25%	Report	OS/DFS	7
Ju-CS, 2009	China	Foxp3+	PT	207	98/67/42/0	27.9 (1.5–77)	IHC	25%	SC	OS	7
Pang, 2009	China	CD4+, CD8+	IT	38	3/12/12/11	NR	NR	Median	SC	OS	5
Zhou, 2009	China	Foxp3+	IT	85	NR	NR	IHC	Median	Report	OS/DFS	6
Zhu, 2009	China	CD56+	IT	62	I-II38/III-IV24	NR	IHC	40%	Report	OS/DFS	6
Lin, 2010	China	Foxp3+	IT	102	70/7/23/2	NR	IHC	Mean	Report	OS	7
Chen, 2011	China	Foxp3+	IT	293	I-II202/III-IV91	NR	IHC	Median	Report	OS/DFS	6
Li, 2011	China	CD66b+, CD8+	IT	281	I103/II-III94	29 (1.5–83)	IHC	70%	Report	OS/DFS	6
Shen, 2011	China	Foxp3+	IT	76	I-II51/III-IV25	12 (9–19)	IHC	27/5HPF	SC	OS/DFS	7
Chen, 2012	China	CD3+, CD4+, CD8+, Foxp3+, CD8+/CD3+, Foxp3+/CD4+	IT/PT	141	NR	22.7 (2–70.3)	IHC	Median	Report	OS/DFS	7
Chew, 2012	Singaporean	CD8+, CD56+	IT	46, 36	NR	NR	IHC	Median	Report	OS	6
Huang, 2012	China	Foxp3+, CD8+/Foxp3+	IT	54	I-II23/III-IV31	44 (2–86)	IHC	Median	Report	OS/DFS	7
Mathai, 2012	America	CD8+/Foxp3+	IT	91	40/43/8	56 (25–81)	IHC	Median	Report	OS/DFS	8
Wang, 2012	China	Foxp3+	IT	137	17/71/26/23	27.5 (2–49)	IHC	Median	SC	OS	7
Fu, 2013	China	Granzyme B+	IT	315	NR	55 (1.8–116)	IHC	Median	SC	OS/DFS	6
Lin, 2013	China	CD3+, CD4+, CD8+, CD56+, CD68+, Foxp3+, Foxp3+/CD4+, CD8+/CD3+	IT	132	NR	NR	FC	Minimum P-value	Report	OS/DFS	6
Pan, 2013	China	CD3+, CD8+	IT	163	I100/II-III63	36 (1–87)	IHC	Median	SC	OS	6
Shi-Training, 2013	China	CD20+	MT	120	I-II85/III-IV35	61.5 (2–84.8)	IHC	Median	Report	OS/DFS	6
Shi-Validation, 2013	China	CD20+	MT	200	NR	59.5 (2–73.2)	IHC	Median	Report	OS/DFS	6
Umemoto, 2014	Japan	PD-L1	IT	80	I-II49/III-IV31	80.9	IHC	NR	SC	OS/DFS	6
Zhao, 2014	China	CD57+	IT	163	NR	NR	IHC	Median	SC	OS	6
Brunner, 2015	Germany	CD8+	IT/MT/PT	119	NR	65 (57–71)	IHC	Mean	Report	OS	6
Shi, 2015	China	CD8+	IT	147	I-II90/III-IV57	NR	IHC	Mean	Report	OS	6
Sun, 2015	China	CD3+, CD8+	IT/MT	359	180/27/108/44	NR	IHC	Minimum P-value	Report	OS/DFS	6
Cai, 2016	China	CD39+, Foxp3+, CD39+Foxp3+	IT/PT	324	I-II243/III-IV81	61.03 (2–82.33)	IHC	Minimum P-value	Report	OS	6
Fu-Training, 2016	China	Foxp3+, CD39+Foxp3+	IT	210	I-II159/III-IV51	53.4 (1.5–61.3)	IHC	x-tile analysis	Report	DFS	6
Fu-Validation, 2016	China	Foxp3+, CD39+Foxp3+	IT	138	I-II100/III-IV38	53.4 (1.5–61.3)	IHC	x-tile analysis	Report	DFS	6
Gabrielson, 2016	America	CD3+, CD8+, PD-L1	IT	65	37/13/14/1	39.7 (9–84)	IHC	Median	Report	OS/DFS	5
Hayashi, 2016	Japan	LI	IT	544	NR	52.7 (0.8–168.5)	IHC	NR	Report	OS/DFS	6
Liao, 2016	China	CD16+, CD16+/CD8+	IT	256	I-II192/III-IV64	44 (1.5–84)	IHC	NR	Report	DFS	6
Ramzan, 2016	France	CD8+	IT	63	NR	38.9 (1–130)	IHC	Harrel-C method	SC	OS	6
Tu, 2016	China	Foxp3+/CD4+	IT	57	NR	NR	IHC	ROC curves	SC	OS	6
Wang Q, 2016	America	Foxp3+, CD8+	IT/PT	66	NR	38.2 (35.2–58.2)	PCR	Median	SC	OS	6
Wang Y, 2016	China	Foxp3+	IT	151	NR	NR	IHC	Median	SC	OS/DFS	6
Xie, 2016	China	CD8+, PD-L1	IT	167	I86/II-III81	RN	IHC	Median	Report	OS/DFS	6
Zhang, 2016	China	CD68+, CD169+	IT/PT	354	I217/II-III133	NR	IHC	Median	Report	OS	6
Garnelo, 2017	Singapore	CD3+, CD20+	IT	112	I-II64/III-IV45	RN	IHC	Median	Report	OS	6
Huang-Xu, 2017	China	PD-L1, CD8+	IT	411	231/57/123	32	IHC	Median	Report	OS/DFS	6
Sideras, 2017	The Netherlands	PD-L1, CD8+	IT	153	NR	NR	IHC	Minimum P-value	SC	OS	6
Zhang, 2018	China	CD8+	IT	183	73/60/42	43 (1–80)	IHC	10%	Report	OS	6

CI = confidence interval, DFS = disease-free survival, FC = flow cytometry, HR = hazard ratio, IHC = immunohistochemistry, IT = intratumor, LI = lymphoplasmacytic infiltration, MT = margin of tumor, NOS = Newcastle–Ottawa scale, NR = not report, OS = overall survival, PB = peripheral blood, PCR = polymerase chain reaction, PT = peritumor, ROC curve = receiver operating characteristic curve, SC = survival curve.

Table 2
The pooled associations between TILs subsets and the prognosis of patients with hepatocellular carcinoma.

Subset	Outcome	Location	Study number	Case number	HR (95% CI) model	P-value	Heterogeneity	
							I ² , %	P
Foxp3+	OS	IT	11	1802	1.894 (1.659–2.164) - fixed	<.001	32.9	.112
		PT	4	780	1.673 (0.991–2.822) - random	.054	57.8	.069
	DFS	IT	12	1898	1.791 (1.546–2.076) - fixed	<.001	0	.540
CD8+	OS	PT	3	573	1.557 (1.057–2.293) - fixed	.025	0	.387
		IT	18	2976	0.676 (0.540–0.845) - random	.001	69.5	<.001
		MT	2	478	0.577 (0.437–0.760) - fixed	<.001	0	.947
	DFS	PT	5	775	0.863 (0.544–1.369) - random	.531	58.6	.047
		IT	10	2065	0.712 (0.574–0.883) - random	.002	56.9	.001
CD3+	OS	MT	1	359	0.600 (0.458–0.786)	<.001	–	–
		PT	3	590	0.806 (0.597–1.089) - fixed	.160	0	.525
		IT	7	1274	1.008 (1.000–1.015) - random	.037	81.7	<.001
	DFS	MT	1	359	0.160 (0.080–0.310)	<.001	–	–
		PT	2	443	0.958 (0.643–1.427) - fixed	.833	0	.496
		IT	5	999	0.596 (0.374–0.948) - random	.029	77.0	.002
CD4+	OS	MT	1	359	0.430 (0.260–0.690)	.001	–	–
		PT	2	443	1.232 (0.630–2.409) - random	.543	55.5	.134
		IT	4	613	0.849 (0.666–1.082) - fixed	.185	0	.416
	DFS	PT	2	443	0.965 (0.645–1.444) - fixed	.863	24	.252
		IT	3	575	0.817 (0.634–1.053) - fixed	.118	0	.544
Granzyme B+	OS	PT	2	443	0.969 (0.632–1.485) - fixed	.885	45.1	.177
		IT	3	857	0.621 (0.516–0.748) - fixed	<.001	13.4	.315
	DFS	PT	1	302	1.310 (0.820–2.800)	.389	–	–
		IT	3	857	0.678 (0.563–0.815) - fixed	<.001	42.5	.176
CD56+	OS	IT	3	230	0.920 (0.550–1.550)	.752	–	–
	DFS	IT	2	194	0.518 (0.203–1.323) - random	.169	64.9	.058
CD68+	OS	IT	2	486	0.711 (0.393–1.288) - fixed	.261	0	.608
		PT	2	484	1.088 (0.372–3.181) - random	.878	87.6	.004
	DFS	IT	1	132	2.459 (1.091–5.542) - random	.030	64.9	.092
PD-L1	OS	PT	1	132	0.700 (0.400–1.300)	.236	–	–
		IT	6	1116	2.537 (1.192–5.398)	.016	–	–
	DFS	IT	5	963	1.012 (0.606–1.692) - random	.962	79.9	<.001
Foxp3+/CD4+	OS	IT	5	963	1.209 (0.848–1.724) - random	.294	57.9	.068
		PT	2	288	2.097 (1.176–3.736) - random	.012	65.9	.032
		PB	1	63	0.767 (0.510–1.154) - fixed	.204	0	.836
	DFS	IT	3	420	2.226 (1.030–4.811)	.042	–	–
		PT	2	288	1.497 (0.872–2.570) - random	.143	84.8	.001
CD8+/CD3+	OS	PT	1	63	0.977 (0.924–1.033) - fixed	.417	0	.388
		IT	2	273	1.676 (0.831–3.379)	.149	–	–
	DFS	IT	2	273	0.909 (0.565–1.462) - fixed	.693	33.9	.219
		PT	1	141	1.070 (0.500–2.270)	.861	–	–
CD4+CD25+FoxP3+	OS	PB	1	75	1.000 (0.990–1.010) - fixed	.988	0	.426
	DFS	IT	1	141	0.890 (0.430–1.830)	.752	–	–
CD16+	OS	IT	1	256	2.460 (1.478–4.094)	<.001	–	–
	DFS	IT	1	256	1.457 (1.008–2.106)	.045	–	–
CD16+/CD8+	OS	IT	1	256	1.876 (1.174–2.998)	.009	–	–
	DFS	IT	1	256	1.869 (1.285–2.718)	.001	–	–
CD169+	OS	IT	1	354	0.500 (0.300–0.700)	.008	–	–
	DFS	PT	1	354	1.200 (0.800–1.700)	.378	–	–
CD20+	OS	IT	1	112	1.080 (1.003–1.163)	.041	–	–
	DFS	IM	1 (2 cohort)	340	0.500 (0.369–0.676)	<.001	0	.574
CD39+	OS	IM	1 (2 cohort)	340	0.559 (0.401–0.779)	.001	0	.568
	DFS	IT	1	324	1.625 (1.017–2.596)	.016	–	–
CD39+Foxp3+	OS	PT	1	324	1.548 (1.086–2.206)	.016	–	–
	DFS	IT	1 (2 cohort)	348	1.485 (0.990–2.228)	.056	–	–
CD57+	OS	IT	1	348	1.915 (1.284–2.854) - fixed	.001	0	.783
	DFS	IT	1	163	0.560 (1.236–2.580)	.002	–	–
CD66b+	OS	IT	1	281	1.485 (0.990–2.228)	.056	–	–
	DFS	IT	1	281	2.578 (1.618–4.106)	<.001	–	–
CD8+/Foxp3+	OS	IT	1	281	1.845 (1.169–2.911)	.009	–	–
	DFS	IT	2	145	0.861 (0.762–0.972) - fixed	.016	0	.446
LI	OS	IT	2	145	0.868 (0.776–0.970) - fixed	.012	47.6	.167
	DFS	IT	1	544	1.731 (1.240–2.444)	.001	–	–
	OS	IT	1	544	1.567 (1.242–1.983)	<.001	–	–
	DFS	IT	1	544	1.567 (1.242–1.983)	<.001	–	–

(continued)

Table 2
(continued).

Subset	Outcome	Location	Study number	Case number	HR (95% CI) model	P-value	Heterogeneity	
							I^2 , %	P
PD-L2	OS	IT	1	240	1.100 (0.680–1.790)	.698	–	–
	DFS	IT	1	240	0.960 (0.600–1.530)	.865	–	–

CI = confidence interval, DFS = disease-free survival, HR = hazard ratio, IT = intratumor, LI = lymphoplasmacytic infiltration, MT = margin of tumor, OS = overall survival, PB = peripheral blood, PT = peritumor.

significance between DFS and CD8+ TILs in PT ($P = .160$) (Fig. 5, Table 2).

3.5. CD3+ T-lymphocyte subset

A total of 7 articles, including 1274 patients with HCC, researched the association between clinical outcome and the density of CD3+ TILs.^[16,30,34,35,41,44,52]

3.5.1. Overall survival. Seven articles focused on the relationship between OS and the density of CD3+ TILs in IT.^[16,30,34,35,41,44,52] Only 1 article focused on the association between OS and the density of CD3+ TILs in MT.^[41] Two articles

focused on the association between OS and the density of CD3+ TILs in PT.^[16,30] The pooled analysis revealed high density of CD3+ in IT foreboded worse OS (pooled HR = 1.008; 95% CI = 1.000–1.015; $P = .037$). However, there was no statistical significance between OS and CD3+ TILs in PT ($P = .833$) (Table 2).

3.5.2. Disease-free survival. Five articles focused on the relationship between DFS and the density of CD3+ TILs in IT.^[16,30,34,41,44] Only 1 article focused on the association between DFS and the density of CD3+ TILs in MT.^[41] Two articles focused on the association between DFS and the density of CD3+ TILs in PT.^[16,30] The pooled analysis revealed high

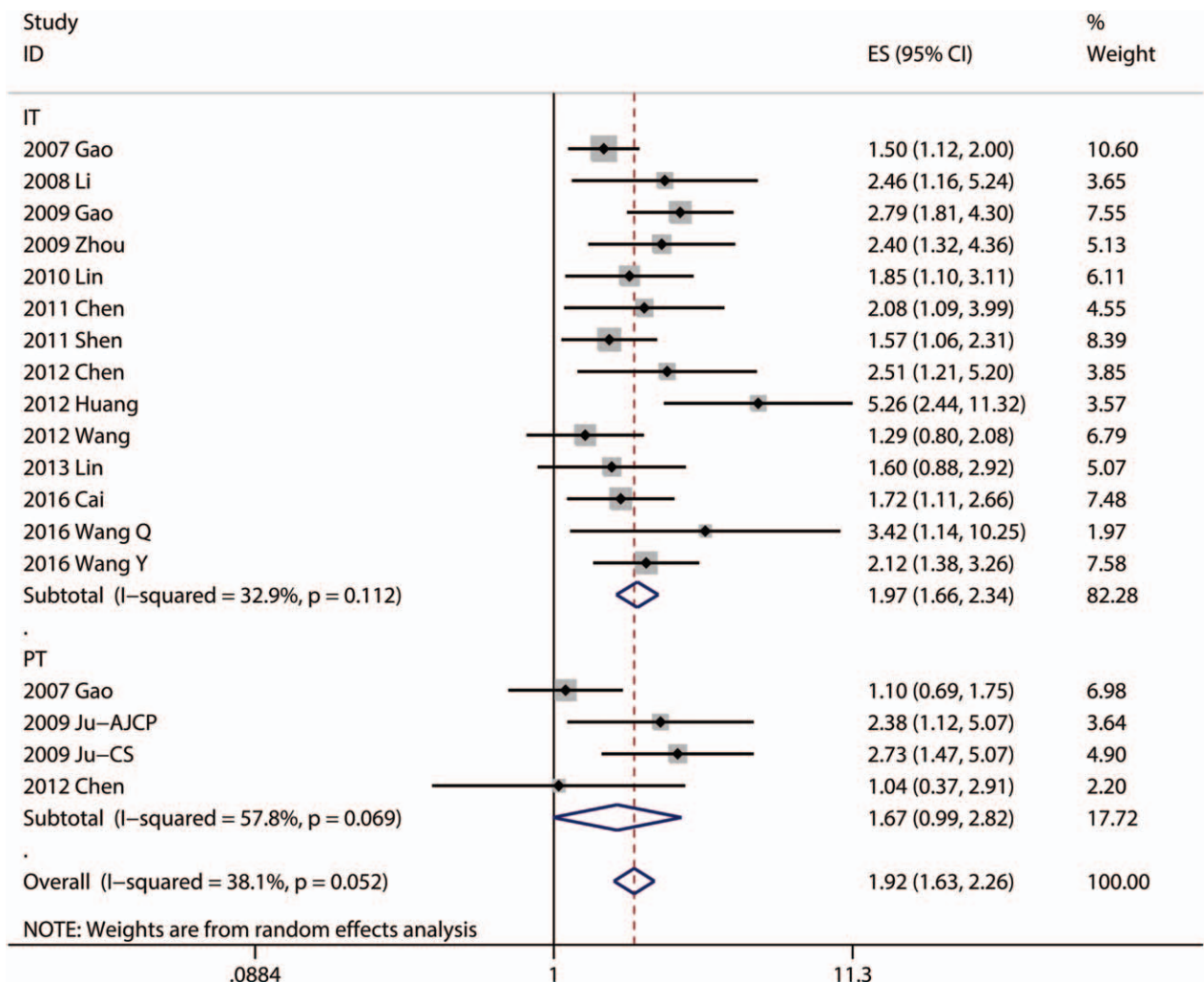


Figure 2. Forest plots of studies evaluating the association between Foxp3+ lymphocytes and overall survival of patients with hepatocellular carcinoma.

Table 3
Pooled hazard ratios for OS according to subgroup analyses.

OS-Intratumor subgroup	Study number	Case number	HR (95% CI) - model	P-value	Heterogeneity	
					I ² (%)	P
Foxp3+						
Ethnicity						
Asian	13	2100	1.878 (1.643–2.147) - fixed	<.001	34.2	.108
Caucasian	1	66	3.421 (1.142–10.253)	.028	–	–
Sample size						
≥100	9	1822	1.804 (1.548–2.101) - fixed	<.001	11.8	.336
<100	5	344	2.543 (1.639–3.945) - random	<.001	53.8	.007
Cutoff value						
Median	8	1229	2.098 (1.579–2.787) - random	<.001	51.5	.079
Others	6	937	1.911 (1.567–2.330) - fixed	<.001	0	.424
CD8+						
Ethnicity						
Asian	14	2576	0.669 (0.536–0.835) - random	<.001	67.8	<.001
Caucasian	4	400	0.770 (0.264–2.248) - random	.632	80.4	.002
Sample size						
≥100	13	2705	0.668 (0.537–0.831) - random	<.001	79.1	.001
<100	5	271	0.728 (0.297–1.788) - random	.489	67.2	<.001
Cutoff value						
Median	10	1539	0.701 (0.532–0.925) - random	.012	64.8	.002
Others	8	1437	0.656 (0.443–0.971) - random	.035	75.2	<.001

CI=confidence interval, HR=hazard ratio, OS=overall survival.

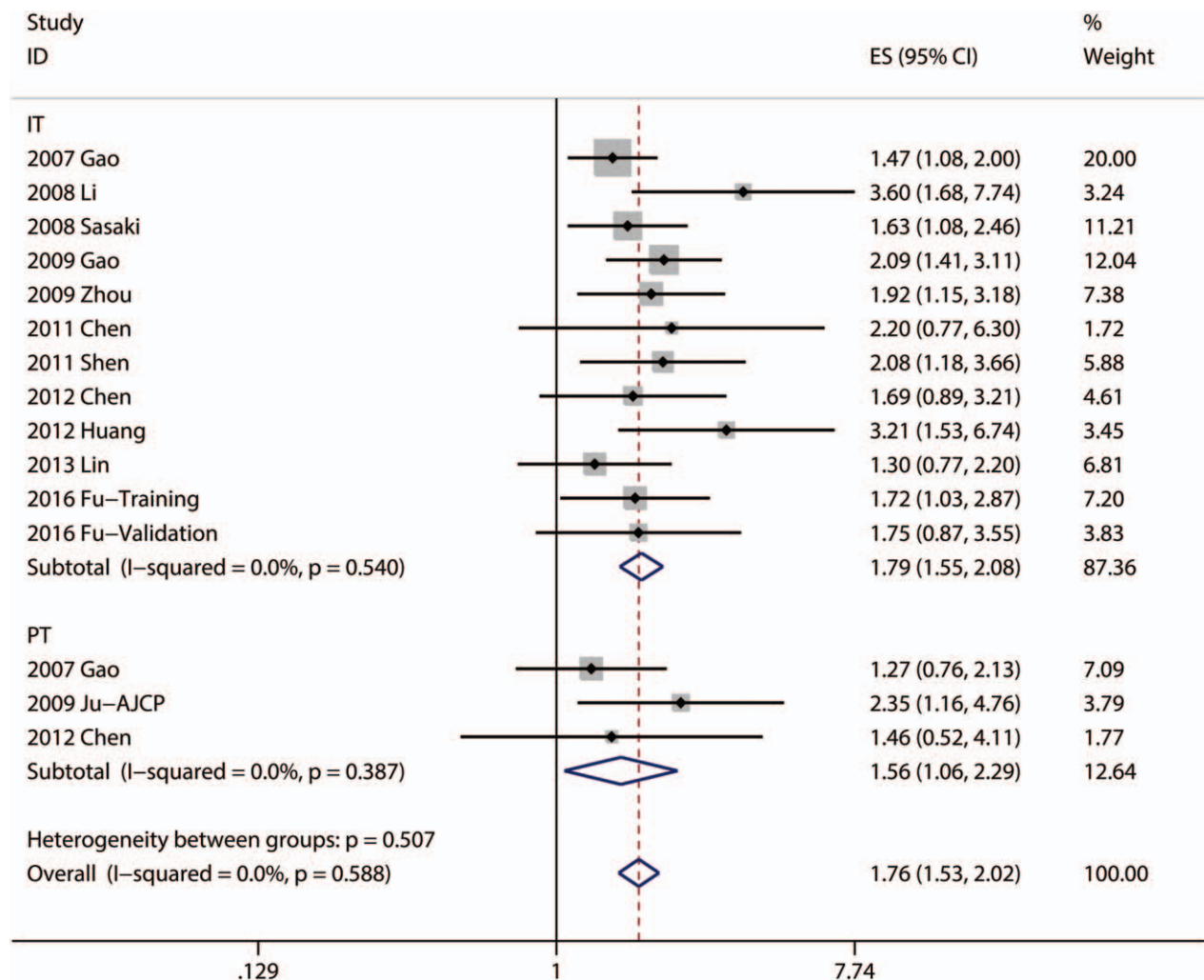


Figure 3. Forest plots of studies evaluating the association between Fxp3+ lymphocytes and disease-free survival of patients with hepatocellular carcinoma.

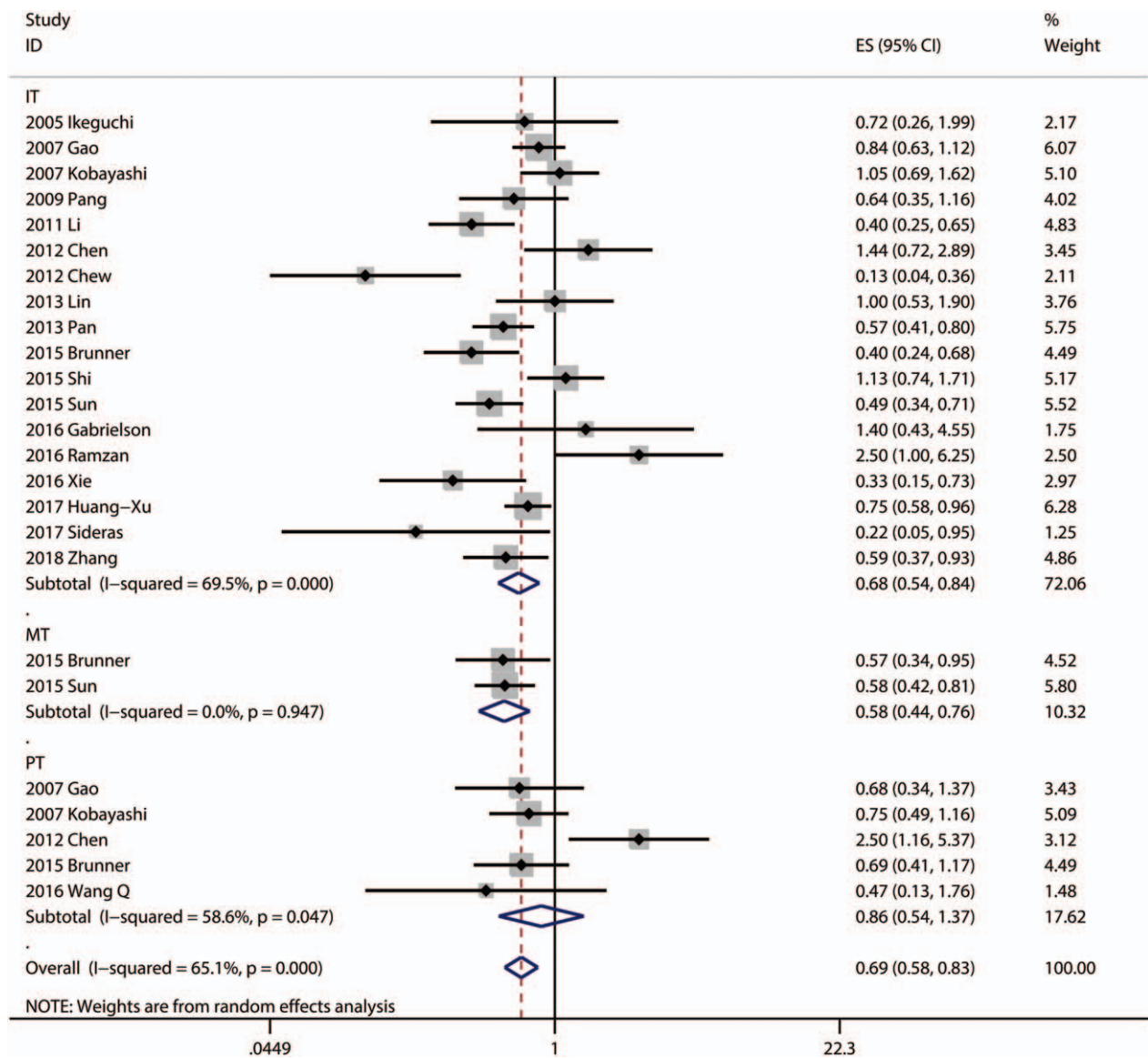


Figure 4. Forest plots of studies evaluating the association between CD8+ lymphocytes and overall survival of patients with hepatocellular carcinoma.

density of CD3+ in IT foreboded better DFS (pooled HR=0.596; 95% CI=0.374–0.948; $P=.029$). However, there was no statistical significance between DFS and CD3+ TILs in PT ($P=.543$) (Table 2).

3.6. CD4+ T-lymphocyte subset

A total of 4 articles, including 613 patients with HCC, researched the association between clinical outcome and the density of CD4+ TILs.^[16,23,30,34]

3.6.1. Overall survival. Four articles focused on the relationship between OS and the density of CD4+ TILs in IT.^[16,23,30,34] Two articles focused on the association between OS and the density of CD4+ TILs in PT.^[16,30] However, there was no statistical significance between OS and CD4+ TILs in IT ($P=.185$) or in PT ($P=.863$) (Table 2).

3.6.2. Disease-free survival. Three articles focused on the relationship between DFS and the density of CD4+ TILs in IT.^[16,30,34] Two articles focused on the association between DFS and the density of CD4+ TILs in PT.^[16,30] However, there was no statistical significance between DFS and CD4+ TILs in IT ($P=.118$) or in PT ($P=.885$) (Table 2).

3.7. Granzyme B+ T-lymphocyte subset

A total of 3 articles, including 857 patients with HCC, researched the clinical outcome of patients with Granzyme B+ T-lymphocytes infiltration.^[16,20,33] The pooled analysis revealed that high density of Granzyme B+ T-lymphocytes in IT was statistically significant associated with better OS (pooled HR=0.621; 95% CI=0.545–0.794; $P<.001$) and DFS (pooled HR=0.678; 95% CI=0.563–0.815; $P<.001$) (Table 2).

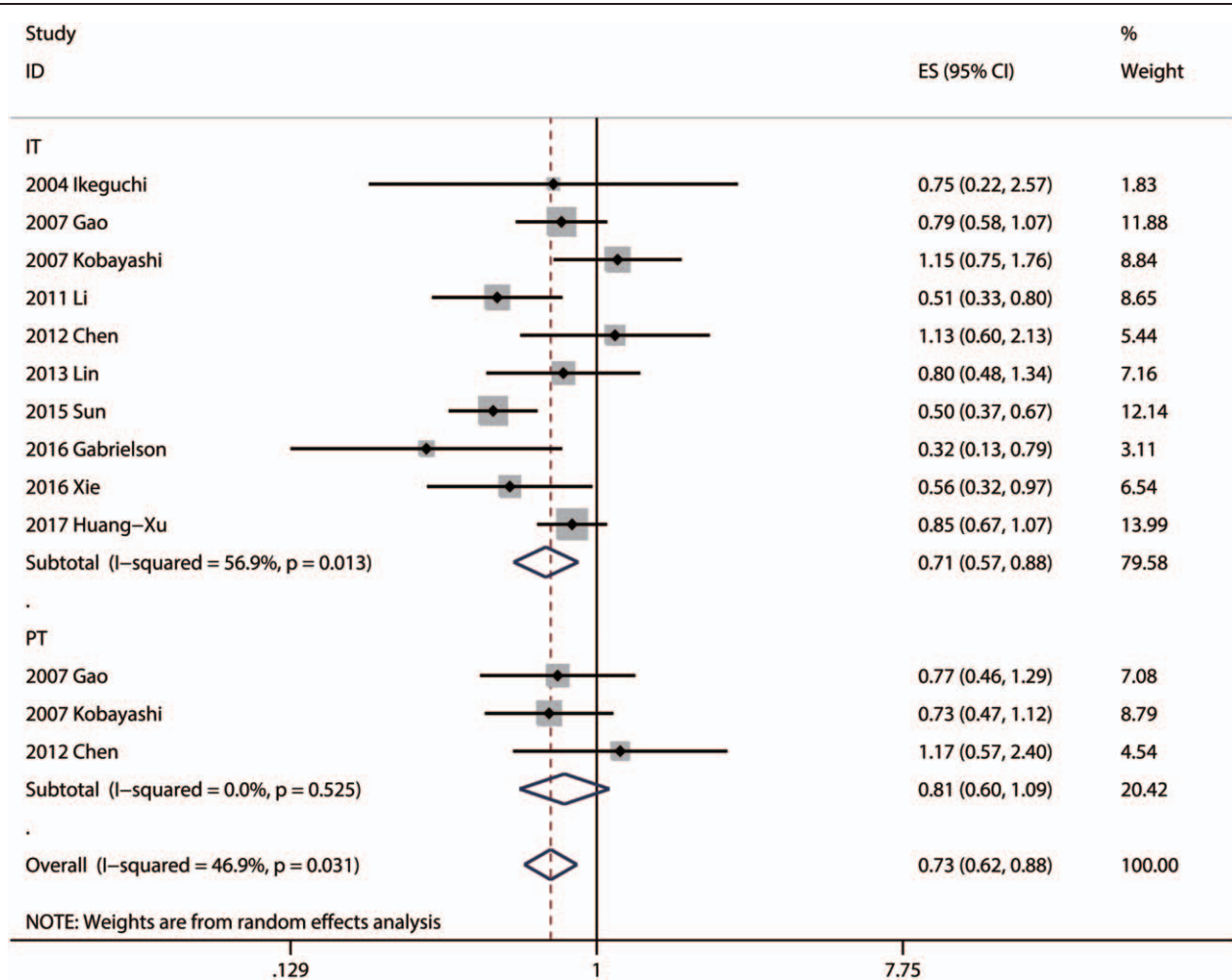


Figure 5. Forest plots of studies evaluating the association between CD8+ lymphocytes and disease-free survival of patients with hepatocellular carcinoma.

3.8. PD-L1 lymphocyte subset

A total of 6 articles, including 1116 patients with HCC, researched the clinical outcome of patients with PD-L1 lymphocyte infiltration.^[20,37,44,50,53,54] A random model was used because of a significant heterogeneity ($P < .001$, $I^2 = 79.9%$), and the result demonstrated that there was no statistical significance between PD-L1 lymphocyte in IT and OS ($P = .962$). A random model was used because of a significant heterogeneity ($P < .068$, $I^2 = 57.9%$), and the result demonstrated that there was no statistical significance between PD-L1 lymphocyte in IT and DFS ($P = .294$).

3.9. CD56+ NK cell subset

A total of 3 articles, including 230 patients with HCC, researched the clinical outcome of patients with CD56+ NK cells infiltration.^[9,25,34] A random model was used because of a significant heterogeneity ($P = .058$, $I^2 = 64.9%$), and the result demonstrated that there was no statistical significance between CD56+ NK cells in IT and OS ($P = .169$), and the same to DFS ($P = .261$) (Table 2).

3.10. CD68+ macrophage subset

A total of 3 articles, including 616 patients with HCC, researched the clinical outcome of patients with CD68+ macrophages

infiltration.^[21,34,51] A random model was used because of a significant heterogeneity ($P = .004$, $I^2 = 87.6%$), and the result demonstrated that there was no statistical significance between CD68+ macrophages in IT and OS ($P = .878$). A random model was used because of a significant heterogeneity ($P = .092$, $I^2 = 64.9%$), and the result demonstrated that high density of CD68+ macrophages in PT was statistically significant associated with better OS (pooled HR = 2.459; 95% CI = 1.091–5.542; $P = .030$) (Table 2).

3.11. FoxP3+/CD4+ T-lymphocytes ratio

A total of 5 articles, including 540 patients with HCC, researched the clinical outcome of patients with FoxP3+/CD4+ T-lymphocytes ratio.^[17,18,30,34,47] A random model was used because of a significant heterogeneity ($P = .032$, $I^2 = 65.9%$), and the result demonstrated that patients with low FoxP3+ /CD4+ T-lymphocytes ratio had better OS in IT and OS (pooled HR = 2.097; 95% CI = 1.176–3.736; $P = .012$) (Table 2).

3.12. Other subsets

The prognostic value of CD4+CD25+FoxP3+ TILs, CD16+ macrophages, CD39+ TILs, CD20+ B cells, CD57+ NK cells, and CD169+ macrophages in patients with HCC have been

investigated in separate articles respectively.^[15,36,38,42,46,51] Subgroup analysis suggested that patients with high density of CD20+ B cells in MT had better OS (pooled HR=0.500; 95% CI=0.369–0.676; $P < .001$) and DFS (pooled HR=0.559; 95% CI=0.401–0.779; $P = .001$) (Table 2).

3.13. Publication bias

We just assessed the publication bias of subgroups which contained more than 10 researches. We used Egger test to evaluate the publication bias. It indicated there was some degree of publication bias in the studies about Foxp3+ in IT with OS ($P = .016$) (Fig. 6A) and with DFS ($P = .048$) (Fig. 6B). And, there was no statistical significance of publication bias in the studies about CD8+ in IT with OS ($P = .653$) (Fig. 6C) or with DFS ($P = .688$) (Fig. 6D).

4. Discussion

The prognostic value of TILs in HCC was quantified in this meta-analysis. Because of the bidirectional role of TILs in immune microenvironment of HCC, subgroup analysis was performed to assess the protumor or antitumor role of different TIL subsets.

Different subsets of TILs have different effect in the development of HCC, which contribute to different prognosis.

FOXP3, CD3, CD4, CD8, CD16, CD20, CD56 and CD57, CD68, and CD169 are mainly located in the surface of lymphocytes, including T-cells, B cells, NK cells, and macrophages. FOXP3 is surface antigen of regulatory T-lymphocytes (Tregs) that are the main effective cells in the protumor immune response.^[56] Correspondingly, Granzyme B and CD8 are surface antigens of cytotoxic T-lymphocytes that play an important role of the antitumor immune response.^[19,20] This is consistent with our research. CD3 is also a common surface antigen of T-lymphocytes. Pan et al and Sun et al considered that high density of CD3+ TILs in IT and MT implied a favorable prognosis, respectively.^[35,41]

Furthermore, there are some other surface antigens, which located in T-lymphocytes, showing different prognostic roles. CD8+ lymphocytes include a group of heterogeneous T-lymphocytes, which can diverse programmed cell death ligand-1 (PD-L1; B7-H1; CD274), galactin-9 and herpesvirus entry mediator (HVEM). Sideras et al showed that low tumor expression of PD-L1 and Galectin-9 are associated with poor survival in patients with resected HCC.^[54] On the contrary, some studies also confirmed overexpression of PD-L1 in HCC was significantly associated with tumor aggressiveness and enhanced risk for postoperative recurrence.^[20,37] These conflicting results, on the association between PD-L1 and prognosis of HCC, were

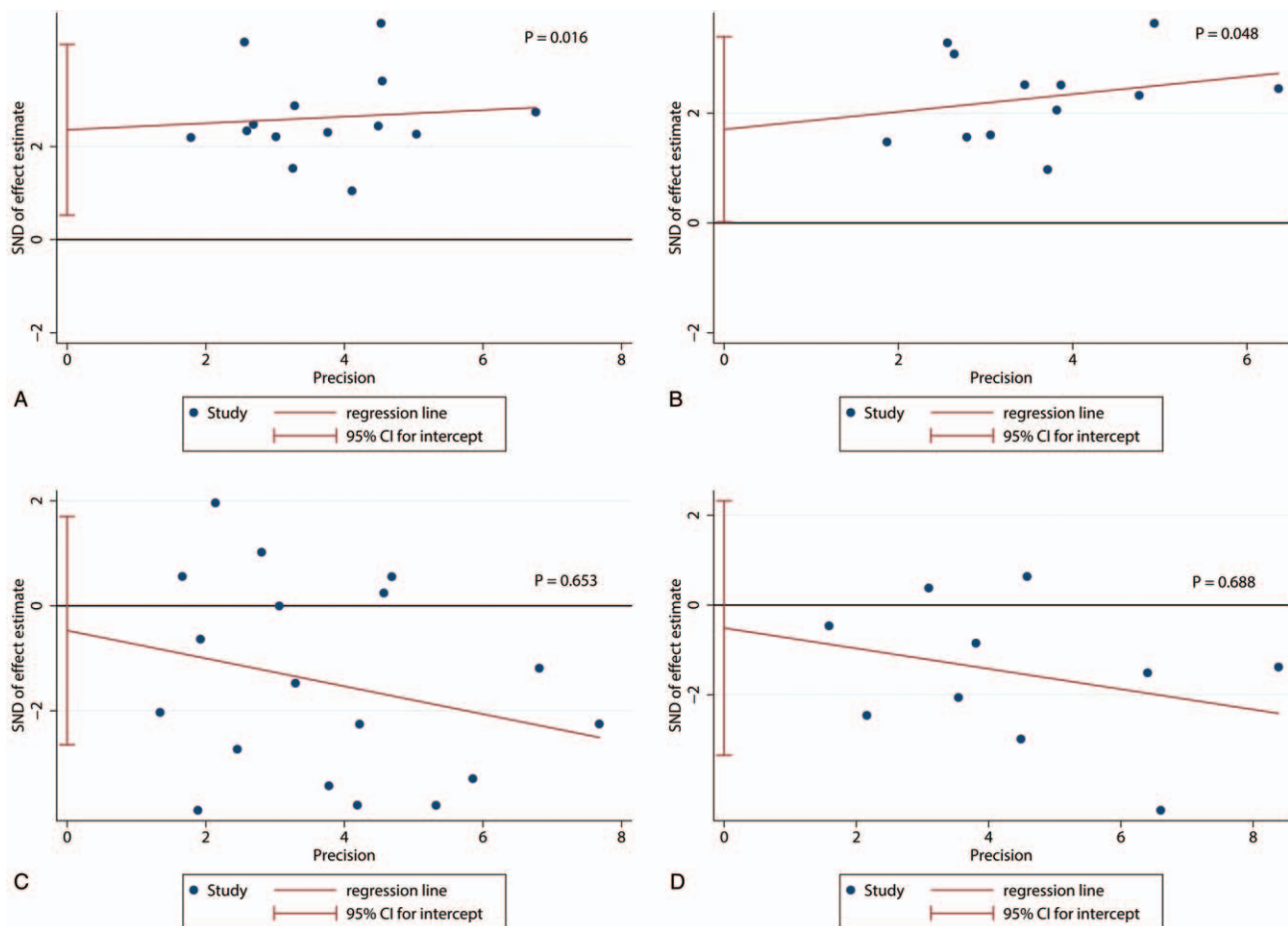


Figure 6. Egger tests of the association between Foxp3+ tumor-infiltrating lymphocytes (TILs) or CD8+ TILs and prognosis. (A) Egger tests of the association between Foxp3+ TILs in intratumor (IT) and overall survival (OS). (B) Egger tests of the association between Foxp3+ TILs in IT and disease-free survival (DFS). (C) Egger tests of the association between CD8+ TILs in IT and OS. (D) Egger tests of the association between CD8+ TILs in IT and DFS.

probably related to the lack of specificity of some anti-PD-L1 antibodies for immunohistochemical staining of PD-L1 in formalin-fixed paraffin-embedded tissues.^[54] Therefore, the role of PD-L1 is very complicated.

The B cells, NK cells, and macrophages also play crucial roles in tumor-associated immune responses. The role of CD20+ (a B-lymphocyte antigen expressed on mature B cells but not on plasma cells) lymphocytes in promoting favorable outcomes in several human cancers has been reported, including breast carcinoma, cutaneous melanoma, and HCC.^[36,57,58] The potential mechanisms included margin-infiltrating B lymphocytes serving as allophycocyanin, stimulating CD8+ T-lymphocytes by releasing proinflammatory cytokines, and exerting a direct killing effect.^[36] CD56 and CD57 are all main markers of NK cells. Zhao et al suggested that IL-37 might mediate antitumor immune responses through recruiting CD57+ NK cells to tumor sites.^[38] CD16, CD68, and CD169 are all main markers of macrophages. CD169 is mainly expressed by cells of the monocyte/macrophage lineage, and CD169+ macrophages enhanced autologous CD8+ T-cell-mediated immunity in vitro and could suppress tumor progression by enhancing CD8+ T-cell activity in human HCC.^[51]

To quantify the immune microenvironment and apply it to a heterogeneous group of patients, a methodology named “Immunoscore” based on the numeration of CD3+ and CD8+ lymphocytes in 2 regions (IT and MT), was provided.^[41,44] The Immunoscore was acquired by summation of the 4 binary score values, the scale being from 0 to 4. For instance, immunoscore 0 (I0) was low densities of both cell types in both regions and immunoscore 4 (I4) was high densities of both cell types in both regions.^[12,59] Gabrielson et al revealed Immunoscore were significantly associated with recurrence and recurrence-free survival independently of other predictive clinicopathologic factors, and patients with a high density of CD3+ and CD8+ cells in 1 or both tumor regions (IT or MT) experienced a significant reduction in the rate of HCC recurrence.^[44]

In addition, we observed that TILs are potential targets for immunotherapy, beside prognostic biomarker for gastric cancer. Recently, cancer immunotherapy has emerged as an appealing field in eliminating the micrometastatic and residual disease of cancer, which contains adoptive cell therapy, monoclonal antibody-based treatment and cancer vaccines, among which cytokine-induced killer (CIK) cells therapy is a promising option. Tumor immunotherapy is currently focusing on the signaling pathway of the programmed cell death protein 1 (PD-1; also known as CD279) binding with PD-L1. PD-1+ TIL was a powerful and specific biomarker in predicting the outcome of CIK immunotherapy in patients with HCC. The correlation between high number of PD-1+ TILs and the high number of both CD4+ and CD8+ TILs suggested that PD-1+ TILs can reflect the existence of endogenous host immune response to tumors, and additional CIK therapy, to some extent, could compensate for immunosuppressive environment and exert a synergistic antitumor effect against HCC.^[60]

As we know, this is the 1st meta-analysis including more than 30 articles to evaluate the prognostic role of TILs in HCC. And there are several limitations which should be taken into consideration in interpreting the conclusions of this study. First, studies included used different cutoff values that could reduce the accuracy of TILs in the process of estimating prognosis. Second, when several HRs could not be directly acquired in the original article, we obtained them by calculating the data extracted from the survival curves. Third, some data comes from univariate

analysis, which may overestimate the effect sizes of some TIL subsets compared to multivariate analysis. Fourth, there are few investigations of some subsets such as CD16+ TILs, CD39+ TILs, CD20+ B cells, CD57+ NK cells, and CD169+ macrophages. This could reduce the credibility of the data in the subgroup analysis. Fifth, there was some degree of publication bias in the studies about Foxp3+ in IT. It could be caused by a greater percentage of Asian articles. Finally, no prospective randomized trials are reported; therefore, future research should be directed at performing prospective randomized trials.

5. Conclusion

In conclusion, our meta-analysis provides strong evidence that TILs would serve as an effective marker for evaluating the prognosis of patients with HCC. Especially, high density of Foxp3+ TILs in the IT was a poor prognostic factor, and high density of CD8+ TILs in IT was a good prognostic factor. More randomized controlled trials were needed to further verify the results of this meta-analysis.

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Author contributions

Ding W and Xu XZ contributed equally to the work as co-first authors; Ding W and Tan YL designed the study and wrote the manuscript; Ding W and Xu XZ designed the research; Qan Y, Xue WB, and Wang YB performed the research and analyzed the data; Du JG and Jin L contributed analytical tools; and Tan YL designed the study and edited the manuscript as the corresponding author.

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