Prognostic value of tumor-infiltrating lymphocytes in esophageal cancer: an updated meta-analysis of 30 studies with 5,122 patients

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Background: The prognostic role of tumor-infiltrating lymphocytes (TILs) in esophageal cancer (EC) patients is controversial; therefore, we performed a meta-analysis to obtain a consensus.

Methods: The PubMed, PubMed Central, Embase, Cochrane Library, and Web of Science databases were searched. The pooled hazard ratios (HRs) with 95% confidence intervals (CIs) were calculated using fixed effect or random effect models depending on the heterogeneity.

Results: A total of 30 articles comprising 5,122 patients were included in this meta-analysis. High levels of generalized TIL infiltration were associated with better overall survival (OS) (HR =0.67, 95% CI: 0.47–0.95, P=0.02) in EC patients. High CD8+ T-cell infiltration and high CD4+ T-cell infiltration were associated with better OS (HR =0.68, 95% CI: 0.60–0.78, P<0.001; HR =0.70, 95% CI: 0.57–0.85, P<0.001, respectively). However, the pooled results showed that neither CD3+ nor FOXP3+ T-cell infiltration were associated with patient survival (P>0.05). Moreover, for esophageal squamous cell carcinoma (ESCC), high CD8+ T lymphocyte infiltration in the TN (Tumor nest) or TS (Tumor stroma) significantly predicted better OS (pooled HR =0.70, 95% CI: 0.57–0.85; P=0.001; pooled HR =0.77, 95% CI: 0.65–0.91; P=0.003).

Conclusions: High levels of generalized TILs, high CD8+ T-cell infiltration and high CD4+ T-cell infiltration have the potential to serve as prognostic markers in EC patients. Moreover, high CD8+ TIL in TNs or TS can predict better OS in ESCC patients.

Keywords: Tumor-infiltrating lymphocytes; esophageal cancer; prognosis; tumor immunity; meta-analysis

Submitted Jan 01, 2020. Accepted for publication Jun 04, 2020.
doi: 10.21037/atm-20-151
View this article at: http://dx.doi.org/10.21037/atm-20-151

Introduction

Esophageal cancer (EC) is the eighth most common cancer and the sixth most common cause of cancer-related death worldwide, and its incidence continues to increase (1). EC mainly has two different pathological types: esophageal squamous cell carcinoma (ESCC) and esophageal adenocarcinoma (EAC) (2). ESCC accounts for approximately 90% of ECs worldwide and is the predominant subtype of EC in Asia, Africa, and South America, while EAC remains the predominant subtype

in North America and Europe (2). ESCC arises from epithelial cells, whereas EAC arises from metaplastic Barrett's esophagus (3). These two subtypes differ with regard to etiology, ethnic distribution, pathogenesis, precursor lesions, and location in the esophagus (4). Despite significant advances in screening, diagnosis and treatment modalities, the long-term outcomes of EC patients remain poor. At present, the prognosis of patients is mainly predicted by the histopathology-based TNM classification system, which does not provide sufficiently detailed information to delineate definitive clinical outcomes in EC patients. Therefore, a new biological marker that can more precisely stratify patients with regard to long-term prognosis is needed.

Emerging evidence suggests that tumor-infiltrating lymphocytes (TILs) can be a potential prognostic biomarker. The correlation between TILs and prognosis has been studied in several types of solid tumors, such as lung cancer (5), breast cancer (6), colorectal cancer (7), melanoma (8), hepatocellular carcinoma (9) and ovarian cancer (10). The heterogeneous population of TILs mainly includes T cells as well as smaller populations of B cells and natural killer cells (11,12). After decades of research, scientists have reached a consensus that TILs play a bidirectional role in the tumor microenvironment (13). On the one hand, TILs can suppress tumor growth by directly destroying tumor cells; on the other hand, TILs can select tumor cells that are suitable for growth in immunocompetent hosts (12). The distribution of TILs can also influence the prognosis to some extent. TILs divide into two groups based on their locations: (I) tumor nest (TN), which contains cells infiltrating within the epithelium of the invasive tumor cell nests; and (II) tumor stroma (TS), which contains cells infiltrating either the tumor stroma adjacent to the cancer epithelia or the stroma along the invasive margin of the cancer epithelia. Overall, although the influence of TILs on tumor progression is highly complex, their pivotal localization in the tumor microenvironment and their influence on a patient's clinical outcome cannot be denied.

Before 2000, researchers had been studying the association between TILs and the prognosis of EC patients, and efforts in this direction have not ceased, with continued improvements in techniques and more in-depth insights relating to the field of tumor immunology. Many studies have verified TILs for use in prognostic prediction in addition to their crucial role in tumor-associated immune responses in EC (14-19). However, the prognostic role of TILs in EC remains controversial, varying with the distribution site and cell types. Zhang *et al.* found that high infiltration of CD8+ T cells in TS was associated with better clinical outcome for ESCC patients (19); however, the infiltration of CD8+ lymphocytes showed no prognostic value in Chen KY's study (16). Svensson *et al.* found that high infiltration of CD8+ T cells was associated with better clinical outcome for EAC patients (20), whereas no correlation between the infiltration of CD8+ T cells and clinical outcome was found in either Rauser's study or Stein's study (15,21). Chen *et al.* revealed that patients with a high density of CD4+ T cells had a better outcome (16), whereas no correlation between the infiltration of CD4+ T cells and clinical outcome was reported in Zhu's study (17). Zhang *et al.* stated that high infiltration of FOX3+ T cells was associated with worse outcomes (18), but Stein *et al.* revealed contrasting results (15). Thus, a meta-analysis that can systematically and comprehensively gather and analyze all available data is urgently needed. The present work aimed to evaluate the prognostic role of TILs in EC.

We present the following article in accordance with the PRISMA 2009 reporting checklist (available at http://dx.doi.org/10.21037/atm-20-151).

Methods

Search strategy

This meta-analysis was performed according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines and the Meta-analysis of Observational Studies in Epidemiology (MOOSE) guidelines (22,23).

The PubMed, Embase, Cochrane Library, and Web of Science databases (last update on March 20, 2020) were searched for relevant articles. The following keywords and combinations were used in the search strategy: "(((((Prognosis) OR Prognostic) OR Survival) OR Outcome)) AND ((((((((((((Esophageal Neoplasms) OR Esophageal Neoplasm) OR Neoplasm, Esophageal) OR Esophagus Neoplasm) OR Esophagus Neoplasms) OR Neoplasm, Esophagus) OR Neoplasms, Esophagus) OR Neoplasms, Esophageal) OR Cancer of Esophagus) OR Cancer of the Esophagus) OR Esophagus Cancer) OR Cancer, Esophagus) OR Cancers, Esophagus)) AND ((((((((((((TILS) OR Lymphocytes, Tumor-Infiltrating) OR Lymphocytes, Tumor Infiltrating) OR Tumor-Infiltrating Lymphocytes) OR Lymphocyte, Tumor-Infiltrating) OR Tumor Infiltrating Lymphocytes) OR Tumor-Infiltrating

Lymphocyte) OR Tumor-Derived Activated Cells) OR Activated Cell, Tumor-Derived) OR Activated Cells, Tumor-Derived) OR Tumor Derived Activated Cells) OR Tumor-Derived Activated Cell))." In addition to the title, abstract, and full text, the reference lists of identified articles were perused in order to ascertain other potential studies. Eligible reports were identified by 2 reviewers (Yidong Zhang and Xiao Geng), and disagreements were resolved by a third reviewer (Wei Guo).

Inclusion and exclusion criteria

Articles extracted from the databases were selected in accordance with the following criteria: (I) data pertaining to the prognostic role of either TILs or specific TIL subsets in EC were reported; (II) HRs and 95% CIs could be extracted directly or indirectly; and (III) sample size was greater than 50. The exclusion criteria were as follows: (I) small sample size (<50) that might cause publication bias; or (II) certain types of studies, including encompassing reviews, letters, case reports, animal trials, and conference abstracts. Additionally, if a particular patient cohort was included in more than 1 study, only the most recent or complete study was considered.

Data extraction

Two researchers independently collected data, including author information, publication year, tumor histology, TIL subsets and distribution sites, population origin, tumor stage, cutoff values, sample size, follow-up period, detection methods, HRs, and 95% CIs. Overall survival (OS), disease-free survival (DFS), and cancer-specific survival (CSS) (where possible) were selected as indexes in our study because these data were available in most of the included studies. If multivariate anal1ysis was performed, it was preferred over univariate analysis because it offers some control over other potential confounding factors. Kaplan-Meier curves were used to extract HR with 95% CI if it could not be obtained directly from the article.

The quality of each included study was evaluated independently by 2 researchers according to the Newcastle-Ottawa Quality Assessment Scale (NOS) (24). Scores ranged from 0 to 9 for quality assessment, and studies with scores ≥6 were considered to be of high quality.

Statistical analysis

The prognostic roles of different TIL subsets at different sites within the tumor microenvironment in EC patients were assessed by HRs and 95% CIs. An HR >1 was considered to indicate a worse prognosis among patients with high TIL infiltration, whereas an HR <1 was considered to indicate a better prognosis. If HRs and 95% CIs were reported, they were retrieved directly. Otherwise, the HR was calculated with data extracted from Kaplan–Meier survival curves using Engauge Digitizer version 4.1 (M. Mitchell, Engauge Digitizer, http://digitizer.sourceforge.net) (25). Important supplementary information was obtained by sending emails to the corresponding authors. The I^2 statistic and chi-square test (P value) were performed, and forest plots were visually inspected to assess statistical heterogeneity (26,27). When I^2 was below 50% and/or the P-value was greater than 0.05, heterogeneity was suggested, and a random effects model (the DerSimonian-Laird method) was used; otherwise, a fixed effects model was used. Subgroup analysis was performed to further analyze integrated data derived from a sufficient number of studies, which showed clinical significance. Publication bias was assessed using Egger's linear regression test and Begg's funnel plot, and P<0.05 was thought to have statistical significance. All analyses were performed using STATA (Stata Corporation, College Station, TX, USA), and significance was defined as a P value < 0.05.

Results

Study characteristics

Using the search strategy described above, 13,894 articles were initially retrieved. After the titles, abstracts, publication types, and full text of the publications were screened, we identified 49 articles that investigated the association between TILs and outcomes in EC patients. Among these articles, 19 were excluded after further in-depth screening (12 for the lack of extractable important data, 5 for small sample size, and 2 for cohort duplication). Finally, 30 articles were included in our meta-analysis (*Figure 1*) (28). The total number of patients in our meta-analysis was 5,122 (range from 70 to 514 patients per study) and originated from Japan, China, Australia, Germany, France, Switzerland, Sweden and England.

Among the selected articles, only 8 reported the prognostic value of generalized TILs, while the remaining studies focused on specific TIL subsets (*Table 1*). Generalized TILs were detected with hematoxylin-eosin (HE) staining, while specific TIL subsets were identified with

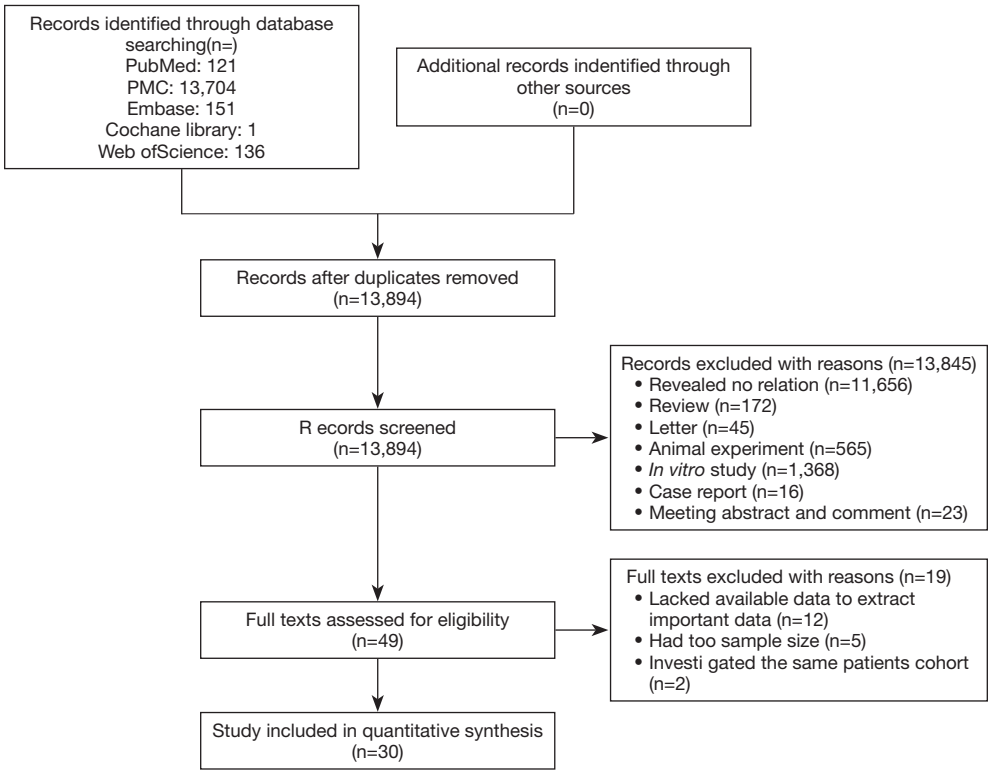

Figure 1 Flow diagram of the study selection process.

immunohistochemistry (IHC) staining and HE staining. The details of tumor stage were provided in 23 articles, although various categorization methods were applied. Follow-up time was mentioned in 19 articles. The cutoff values involved mean values (n=6), median values (n=11), and values from some semiquantitative methods. Of the 30 studies, only 10 provided Kaplan–Meier survival curves when reporting the survival as it related to a subset of TILs.

Quality assessment was performed for each study included in our meta-analysis according to the NOS, with scores ranging from 7 to 8 (mean, 7.87). A higher value indicates a better methodology. Therefore, all 30 studies were included in subsequent analyses.

Generalized TILs

Eight articles assessed the association between the density of generalized TILs and patient survival (29-36). Seven articles comprising 1,458 patients evaluated OS, and the pooled results showed that patients with a high level of generalized TILs had better OS (pooled HR =0.67; 95% CI: 0.47–0.95; P=0.02) (*Figure 2*, *Table 2*) than did patients with a lower level of generalized TILs. Of these 8 articles, 4 (involving 982 patients) evaluated DFS, and the pooled results showed that a high level of generalized TILs was not correlated with DFS (pooled HR =1.13; 95% CI: 0.79–1.61; P=0.52) (*Figure 2*, *Table 2*).

CD8+ T-cell subset

Twenty articles assessed the relationship between the density of the CD8+ T-cell subset and patient survival (14,15,17,19-21,29,37-49). 17 articles (12,14-17,19-21,29,39,40,43,44,46,47,49,50) comprising 2,529 patients evaluated OS, and the pooled results showed that patients with a high level of CD8+ TILs had better OS (pooled HR =0.68; 95% CI: 0.60–0.78; P<0.001) (*Figure 3*, *Table 2*) than did patients with a lower level of CD8+ TILs. Six articles (16,17,20,21,29,46) comprising 1,180 patients evaluated DFS, and the pooled results showed that a high level of CD8+ TILs was not correlated with DFS (pooled HR =0.82; 95% CI: 0.67–1.01; P=0.06) (*Figure 3*, *Table 2*). Finally, three articles (38,41,45) comprising 400 patients evaluated CSS, and the pooled results showed that patients

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

Study	Subset	Histologic type	Origin of population	Sample number (M/F)	Tumor stage (I/II/III/IV)	Follow-up (months)	Cut-off for overexpression	Outcome	Quality assessment
Chen, 2011	CD3+	ESCC	China	112 (80/32)	13/63/20/16	NR	≥Moderate	OS	8
	CD3+	ESCC	China	112 (80/32)	13/63/20/16	NR	≥Moderate	OS	
	CD8+	ESCC	China	112 (80/32)	13/63/20/16	NR	≥Moderate	OS	
	CD8+	ESCC	China	112 (80/32)	13/63/20/16	NR	≥Moderate	OS	
	FOXP3+	ESCC	China	112 (80/32)	13/63/20/16	NR	≥Moderate	OS	
	FOXP3+	ESCC	China	112 (80/32)	13/63/20/16	NR	≥Moderate	OS	
Chen, 2017	CD3+	ESCC	China	514 (444/70)	58/188/262/6	Median 32.8	Median	OS, DFS	8
	CD3+	ESCC	China	514 (444/70)	58/188/262/6	Median 32.8	Median	OS, DFS	
	CD4+	ESCC	China	514 (444/70)	58/188/262/6	Median 32.8	Median	OS, DFS	
	CD4+	ESCC	China	514 (444/70)	58/188/262/6	Median 32.8	Median	OS, DFS	
	CD8+	ESCC	China	514 (444/70)	58/188/262/6	Median 32.8	Median	OS, DFS	
	CD8+	ESCC	China	514 (444/70)	58/188/262/6	Median 32.8	Median	OS, DFS	
Dutta, 2012	CD8+	EAC	England	98 (83/15)	I22/II44/III55	Median 45	The top tertile	CSS	8
Jesinghaus 2017	CD3+	ESCC	Germany	125 (95/30)	NR	Nr	Top 1/3	OS	8
Jiang, D. X 2017	TILs	ESCC	China	235 (197/38)	I–II 136/III–IVa 99	Median 36	20%	OS, DFS	8
	TILs	ESCC	China	235 (197/38)	I–II 136/III–IVa 99	Median 36	10%	OS, DFS	
	CD4+	ESCC	China	235 (197/38)	I–II 136/III–IVa 99	Median 36	10%	OS, DFS	
	CD8+	ESCC	China	235 (197/38)	I–II 136/III–IVa 99	Median 36	10%	OS, DFS	
	CD8+	ESCC	China	235 (197/38)	I–II 136/III–IVa 99	Median 36	10%	OS, DFS	
	FOXP3+	ESCC	China	235 (197/38)	I–II 136/III–IVa 99	Median 36	10%	OS, DFS	
	FOXP3+	ESCC	China	235 (197/38)	I–II 136/III–IVa 99	Median 36	10%	OS, DFS	
Jiang, Y. B 2017	TILs	ESCC	China	428 (335/93)	0–II 143/175/40	Median 34.4 (0.3–147.1)	Moderate	OS, DFS	8
Li, 2016	TILs	ESCC	China	121 (96/25)	51/46/24/0	Median 34 [3–64]	Percentage-quartile	OS, DFS	8
	TILs	ESCC	China	121 (96/25)	51/46/24/0	Median 34 [3–64]	Percentage-quartile	OS, DFS	
Lv, 2011	CD8+	ESCC	China	181 (141/40)	I–II 117/III–IV 65	Median 44 [1–87]	Median	OS	8
Liu, 2017	TILs	ESCC	China	198 (147/51)	18/77/103/0	Median 54 [6–152]	10%	DFS	8
Ma, 1999	TILs	ESCC	China	377 (268/109)	1/150/198/28	Over 60	The top tertile	OS	8

Table 1 *(continued)*

Table 1 *(continued)*

Study	Subset	Histologic type	Origin of population	Sample number (M/F)	Tumor stage (I/II/III/IV)	Follow-up (months)	Cut-off for overexpression	Outcome	Quality assessment
Morita, 2001	TILs	ESCC	Japan	122 (110/12)	I–II 30/III–IV 92	over 60	3 normal	OS	8
Nakajima,2009	CD4	EC	Japan	125 (108/17)	37/40/28/20	NR	Mean 68.2/HPV	OS	8
	CD8+	EC	Japan	125 (108/17)	37/40/28/20	NR	Mean 27.8/HPV	OS	
Nishimura, 2019	CD8+	ESCC	Japan	80 (64/16)	14/9/21/36	Median 59	Median	OS	8
Noble, 2016	CD3+	EAC	England	128 (112/16)	NR	Median 42	Median	CSS	8
	FOXP3+	EAC	England	128 (112/16)	NR	Median 42	Median	CSS	
	CD8+	EAC	England	128 (112/16)	NR	Median 42	Median	CSS	
	CD4+	EAC	England	128 (112/16)	NR	Median 42	Median	CSS	
Rauser, 2009	CD3+	EAC	Germany	118 (109/9)	47/34/28/8	Median 33 (0.8–164)	0.9%	OS, DFS	8
	CD8+	EAC	Germany	118 (109/9)	47/34/28/9	Median 33 (0.8–164)	0.5%	OS, DFS	
Schumacher, 2001	CD8+	EC	Germany	70	8/17/29/16	Median 17 [6–85]	Mean 150/3 HPV	OS, DFS	8
Stein, 2017	CD3+	EAC	Switzerland	111 (96/15)	NR	NR	Median 61/0.849 mm^2	OS	7
	CD8+	EAC	Switzerland	111 (96/15)	NR	NR	Median 22/0.849 mm^2	OS	
	FOXP3+	EAC	Switzerland	111 (96/15)	NR	NR	Median 3/0.849 mm^2	OS	
Sudo, 2017	TILs	ESCC	Japan	223 (203/20)	39/49/135/0	NR	50%	OS, DFS	8
Sugimura, 2015	CD8+	EC	Japan	210 (186/24)	NR	35.1 median	Median 15/HPV	OS	8
Svensson, 2017	CD3+	EAC	Sweden	98	NR	NR	Mean	OS, DFS	7
	CD8+	EAC	Sweden	98	NR	NR	Mean	OS, DFS	
	FOXP3+	EAC	Sweden	98	NR	NR	Mean	OS, DFS	
Tsuchikawa, 2011	CD4+	ESCC	Japan	98 (84/14)	25/31/23/19	over 60	Mean 1.4/HPV	OS	8
	CD8+	ESCC	Japan	98 (84/14)	25/31/23/19	over 60	Mean 0.8/HPV	OS	
Vacchelli, 2015	CD8+	EC	France	174	NR	NR	The top tertile	CSS	8
	FOXP3+	EC	France	196 (177/19)	NR	NR	The top tertile	CSS	
Wang, 2000	TILs	ESCC	China	97 (75/22)	I–IIa 41/IIb–III 42/IV 14	median 44.3 [7–108]	The top tertile	OS	8
Wang, 2014	CD8	ESCC	China	90 (72/18)	I–II 31/III–IV 59	NR	Median 19/HPV	OS, DFS	8
Yasunaga, 2000	TILs	ESCC	Japan	78 (70/8)	NR	NR	≥Moderate	OS	8

Table 1 *(continued)*

Table 1 *(continued)*

Study	Subset	Histologic type	Origin of population	Sample number (M/F)	Tumor stage (I/II/III/IV)	Follow-up (months)	Cut-off for overexpression	Outcome	Quality assessment
Yoshioka, 2008	CD4	ESCC	Japan	122 (105/17)	I–II 76/III–IV 46	NR	Median	OS	7
	CD8	ESCC	Japan	122 (105/17)	I–II 76/III–IV 46	NR	Median	OS	
	FOXP3	ESCC	Japan	122 (105/17)	I–II 76/III–IV 46	NR	Median	OS	
Zhang, 2011	CD8+	ESCC	China	135 (100/35)	I–II 74/III–IV 61	Median 49 [7–78]	Mean 10/0.0625 mm^2	OS	7
	CD8+	ESCC	China	135 (100/35)	I–II 74/III–IV 62	Median 49 [7–78]	Mean 20/0.0625 mm^2	OS	
Zhu, Li, Bo 2017	CD8+	ESCC	China	220 (117/103)	II	Median 53.25	16.90%	OS, DFS	8
	CD4+	ESCC	China	220 (117/103)	II	Median 53.25	5.00%	OS, DFS	
Zhu, Li, Mu 2017	CD8+	ESCC	China	133 (75/58)	II	Median 42.6	Median	OS, DFS	8
	FOXP3+	ESCC	China	133 (75/58)	II	Median 42.6	Median	OS, DFS	
Zingg, 2010	CD3	EAC	Australia	105 (90/15)	I–IIA 33/IIB–III 72	NR	Median 563/HPV	OS	8
	CD4	EAC	Australia	105 (90/15)	I–IIA 33/IIB–III 72	NR	Median 33/HPV	OS	
	CD8	EAC	Australia	105 (90/15)	I–IIA 33/IIB–III 72	NR	Median 225/HPV	OS	
	FOXP3	EAC	Australia	105 (90/15)	I–IIA 33/IIB–III 72	NR	Median 167/HPV	OS	

EC, esophageal cancer; ESCC, esophageal squamous cell carcinoma; EAC, esophageal adenocarcinoma; NR, not report; OS, overall survival; DFS, disease-free survival; CSS, cancer-special survival; HPV, high-power fields.

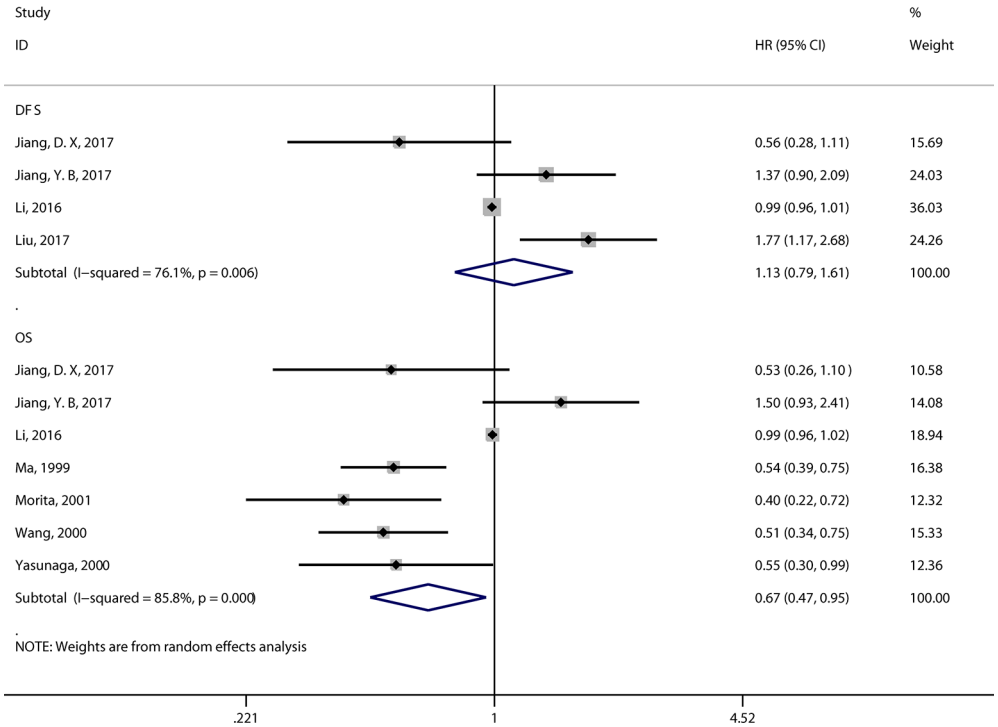

Figure 2 Forest plot for the prognostic effect of generalized TILs.

Table 2 The pooled associations between TILs subsets and the prognosis of patients with esophageal cancer

Subset	Outcome	Study number	Case number	HR (95% CI)	Model	P value	Heterogeneity	
							I^2 (%)	P value
TILS	OS	7	1,458	0.67 (0.47–0.95)	Random	0.02	86	<0.00001
TILS	DFS	4	982	1.13 (0.79–1.61)	Random	0.52	76	0.006
CD3	OS	7	1,164	1.02 (0.70–1.48)	Random	0.92	66	0.007
CD3	DFS	3	711	1.07 (0.57–2.02)	Random	0.83	78	0.01
CD3	CSS	1	128	1.03 (1.00–1.07)	–	0.07	–	–
CD4	OS	5	964	0.70 (0.57–0.85)	Fixed	0.0004	0	0.91
CD4	DFS	1	514	0.66 (0.39–1.11)	–	0.12	–	–
CD4	CSS	1	128	0.93 (0.88–0.98)	–	0.01	–	–
CD8	OS	16	2,449	0.69 (0.61–0.78)	Fixed	<0.00001	0	0.50
CD8	DFS	6	1,180	0.82 (0.67–1.01)	Fixed	0.06	19	0.29
CD8	CSS	3	400	0.85 (0.76–0.94)	Fixed	0.001	25	0.26
FOXP3	OS	7	916	0.69 (0.43–1.10)	Random	0.12	70	0.003
FOXP3	DFS	3	466	0.81 (0.40–1.65)	Random	0.57	76	0.02
FOXP3	CSS	2	323	1.18 (0.62–2.25)	Random	0.62	87	0.005

TILs, tumor-infiltrating lymphocytes; OS, overall survival; DFS, disease-free survival; CSS, cancer-special survival; HR, hazard ratio; CI, confidence intervals.

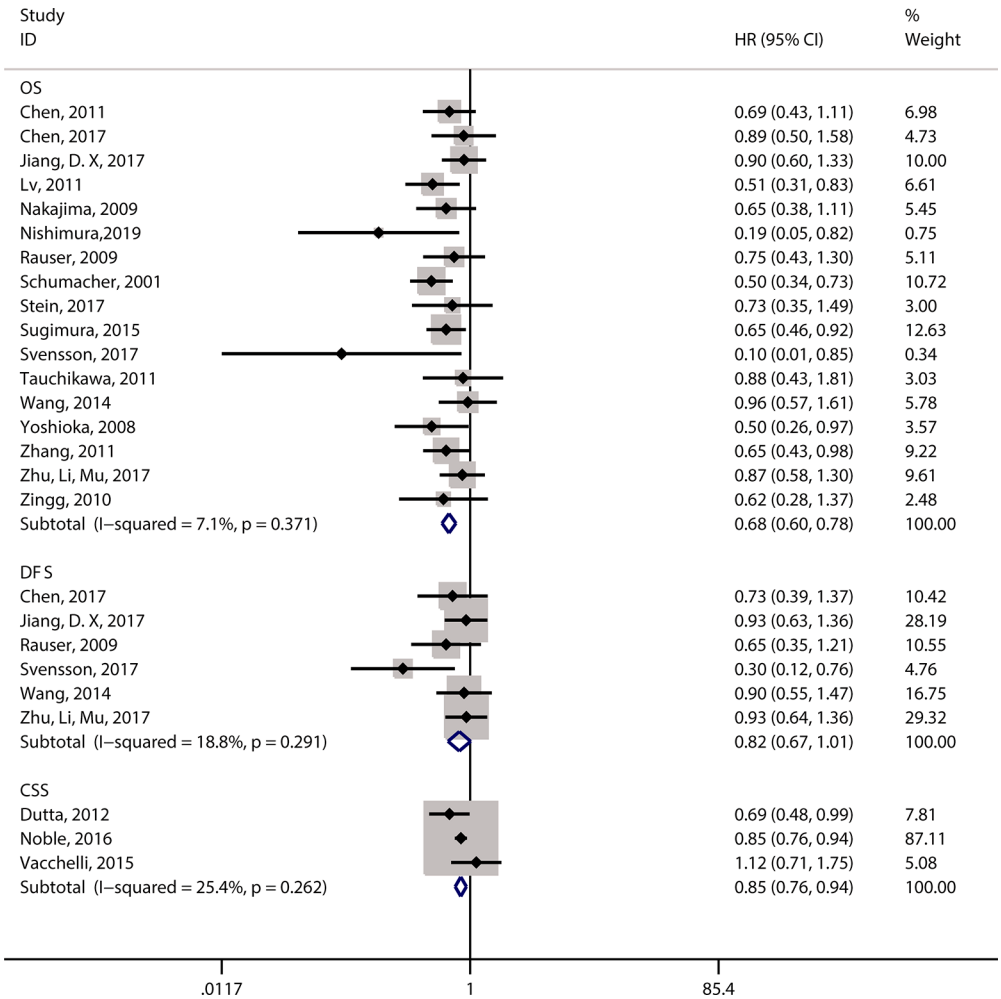

Figure 3 Forest plot for the prognostic effect of CD8+ T cells.

with a high level of CD8+ TILs had better CSS (pooled HR =0.85; 95% CI: 0.76–0.94; P=0.001) (*Figure 3*, *Table 2*) than did patients with a lower level of CD8+ TILs.

The subgroups were defined according to the patients' ethnicity, histology, sample size, cutoff values, publication year, and patients' country. The results showed that a high level of CD8+ TILs was associated with improved OS in patients with ESCC (pooled HR =0.74, 95% CI: 0.63–0.87; P=0.0005), patients with EAC (pooled HR =0.67, 95% CI: 0.46–0.98; P=0.04), studies with a large sample size (≥100; pooled HR =0.70, 95% CI: 0.61–0.81; P<0.001), and studies published after 2010 (pooled HR =0.72, 95% CI: 0.63–0.84; P=0.02) (*Table 3*). High levels of CD8+ TILs showed a better OS in both Asian patients (pooled HR =0.72, 95% CI: 0.62–0.83; P<0.001) and Caucasian patients (pooled HR =0.58, 95% CI: 0.44–0.76; P<0.001). In addition, high levels of CD8+ TILs indicated a better OS in patients from China (pooled HR =0.76, 95% CI: 0.64–0.91; P=0.002), Japan (pooled HR =0.67, 95% CI: 0.53–0.84; P<0.001), and Germany (pooled HR =0.57, 95% CI: 0.42–0.78; P<0.001) (*Table 3*).

Moreover, for ESCC, we also considered the distribution site of CD8+ TILs. Six studies that assessed the infiltration of CD8+ T lymphocytes in TNs also conducted an OS analysis (14,16,17,19,29,50), the results of which suggested that patients with high CD8+ T lymphocyte infiltration in the TN had better OS (pooled HR =0.70, 95% CI: 0.57–0.85; P=0.001) (*Figure 4*). In addition, five studies that assessed the infiltration of CD8+ T lymphocytes in TS performed OS analysis (14,16,19,29,39) and showed that high CD8+ T lymphocyte infiltration in TS significantly predicted better OS (pooled HR =0.77, 95% CI: 0.65–0.91; P=0.003) (*Figure 4*). Four studies researched CD8+ T lymphocyte

Table 3 Subgroup analyses of the relationship between CD8+ T lymphocyte subsets and OS

Outcome subgroup	Study number	Case number	HR (95% CI)	Model	P value	Heterogeneity	
						I^2 (%)	P value
Ethnicity							
Asian	12	2,035	0.72 (0.62–0.83)	Fixed	<0.00001	0	0.45
Caucasian	5	494	0.58 (0.44–0.76)	Fixed	<0.0001	9	0.36
Histology							
ESCC	10	1,700	0.74 (0.63–0.87)	Fixed	0.0005	14	0.32
EAC	4	424	0.67 (0.46–0.98)	Fixed	0.04	8	0.35
Both	3	405	0.59 (0.47–0.74)	Fixed	<0.00001	0	0.57
Sample size							
<100	5	436	0.57 (0.33–0.98)	Random	0.11	60	0.04
>100	12	2,093	0.70 (0.61–0.81)	Fixed	<0.00001	0	0.85
Cut-off values							
Median	9	1546	0.70 (0.58–0.83)	Fixed	0.0002	12	0.34
Others	8	983	0.67 (0.57–0.80)	Fixed	<0.0001	14	0.32
Publication year							
Before 2010	4	427	0.58 (0.46–0.74)	Fixed	<0.00001	0	0.62
After 2010	13	2,102	0.72 (0.63–0.84)	Fixed	0.02	8	0.36
Country							
China	7	1,400	0.76 (0.64–0.91)	Fixed	0.002	0	0.49
Japan	5	635	0.67 (0.53–0.84)	Fixed	0.0008	0	0.83
Australia	1	105	0.62 (0.28–1.37)	–	0.24	–	–
Germany	2	180	0.57 (0.42–0.78)	Fixed	0.0005	28	0.24
Sweden	1	98	0.10 (0.01–0.85)	–	0.04	–	–
Switzerland	1	111	0.73 (0.35–1.49)	–	0.38	–	–

ESCC, esophageal squamous cell carcinoma; EAC, esophageal adenocarcinoma; OS, overall survival; HR, hazard ratio; CI, confidence intervals.

infiltration in both TNs and TS (44,46-48). However, the results showed that high levels of CD8+ T lymphocytes in both TNs and TS were not associated with OS (pooled HR =0.82, 95% CI: 0.63–1.07; P=0.14) (*Figure 4*).

CD3+ T-cell subset

Eight studies investigated the prognostic value of CD3+ TILs in patients with EC. Seven studies (14-16,20,21,49,51) comprising 1,164 patients evaluated OS, and the pooled results showed that high level of CD3+ TILs were not associated with OS (pooled HR =1.02; 95% CI: 0.70–1.48; P=0.92) (*Figure 5*, *Table 2*). Three studies (16,20,21) comprising 711 patients evaluated DFS, and the pooled results showed that high level of CD3+ TILs were also not associated with DFS (pooled HR =1.07; 95% CI: 0.57–2.02; P=0.83) (*Figure 5*, *Table 2*).

CD4+ T-cell subset

Six studies investigated the prognostic value of CD4+ TILs in patients with EC. Five studies (16,44,47,49) comprising 964 patients evaluated OS, and the pooled results showed that high levels of CD4+ TILs were associated with better OS (pooled HR =0.70; 95% CI: 0.57–0.85; P<0.001) (*Figure 6*, *Table 2*) compared to lower levels of C4+ TILs.

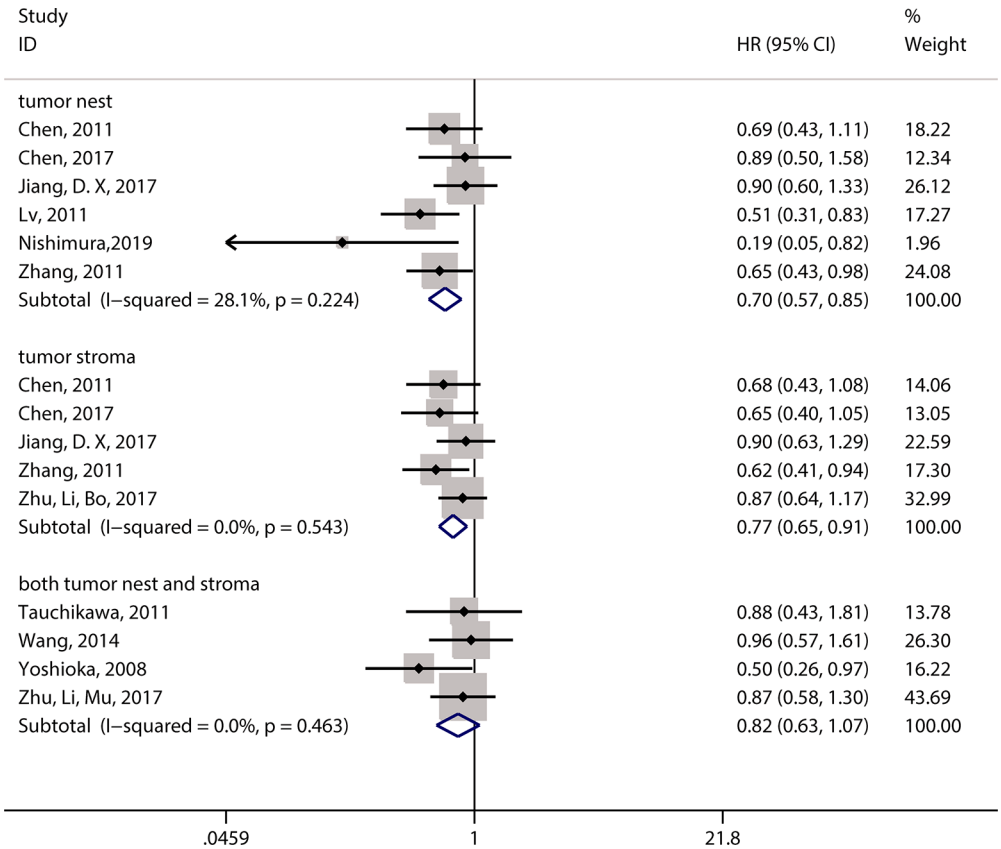

Figure 4 Subgroup analysis of the prognostic effect of CD8+ T cells in esophageal squamous cell carcinoma.

FOXP3+ T-cell subset

Nine studies investigated the prognostic value of FOXP3+ TILs in patients with EC. Seven studies (14,15,17,20,29,47,49) comprising 916 patients evaluated OS, and the pooled results showed that FOXP3+ TILs were not associated with OS (pooled HR =0.69; 95% CI: 0.43–1.10; P=0.12) (*Figure 7*, *Table 2*). Three studies (17,20,29) comprising 466 patients evaluated DFS, and the pooled results showed that FOXP3+ TILs were not associated with DFS (pooled HR =0.81; 95% CI: 0.40–1.65; P=0.57) (*Figure 7*, *Table 2*).

Publication bias

Because the subgroup analyzing the relationship between CD8+ TILs and OS contained 17 studies, it is necessary to evaluate the publication bias of this subgroup. The P values of the Egger's (P=0.112) and Begg's tests (P=0.303) were both greater than 0.05 (*Figure 8A*,*B*), indicating that no significant publication bias was observed.

Discussion

Many studies published in recent years have demonstrated that the various TIL subsets possess different prognostic predictive values in quite a few types of cancers, including gastric, breast, colorectal, and lung cancers (5-7,52-57). However, to the best of our knowledge, the prognostic roles of TILs in EC remain controversial. It has been repeatedly mentioned in previous studies that different subsets of lymphocytes may have different and even opposing prognostic effects, which makes sense in terms of the different functions of the corresponding subsets of lymphocytes in the tumor microenvironment. A meta-analysis published by Zheng *et al.* demonstrated that some TIL subsets could serve as prognostic biomarkers for EC patients (58). The two main differences between our meta-analysis and the previous one is as follows: (I) In our meta-analysis, 30 observational studies (comprising 5,122 patients) were summarized; however, the previous meta-analysis included only 22 studies and 2,909 patients. (II) In Zheng's meta-analysis, the researchers combined ESCC and EAC,

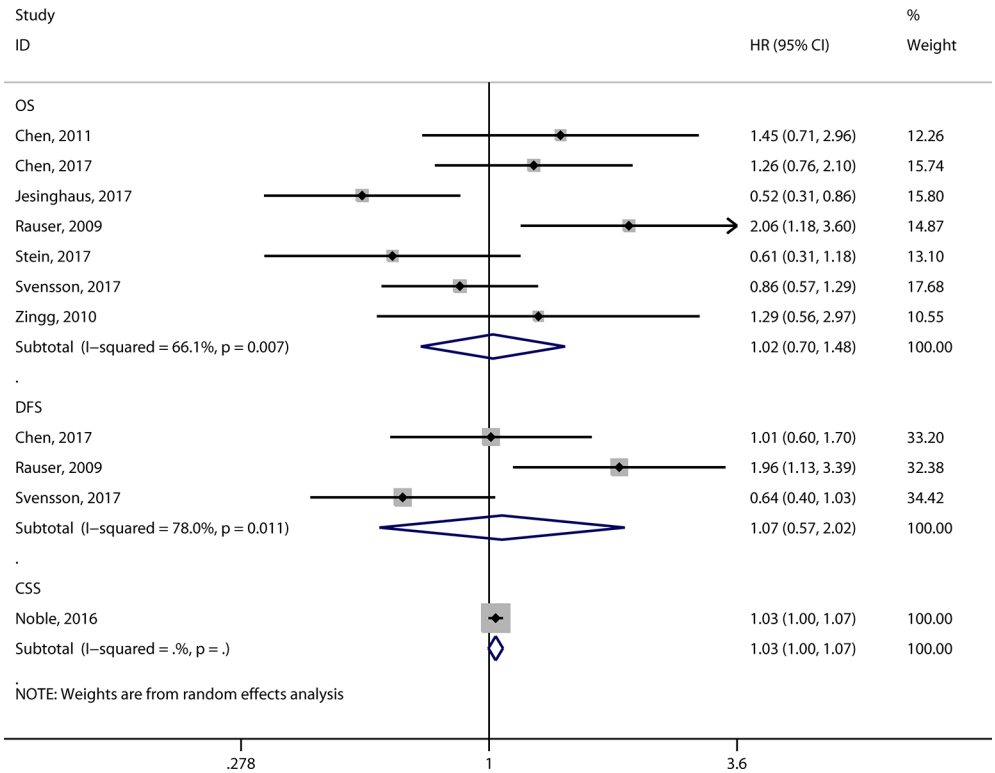

Figure 5 Forest plot for the prognostic effect of CD3+ T cells.

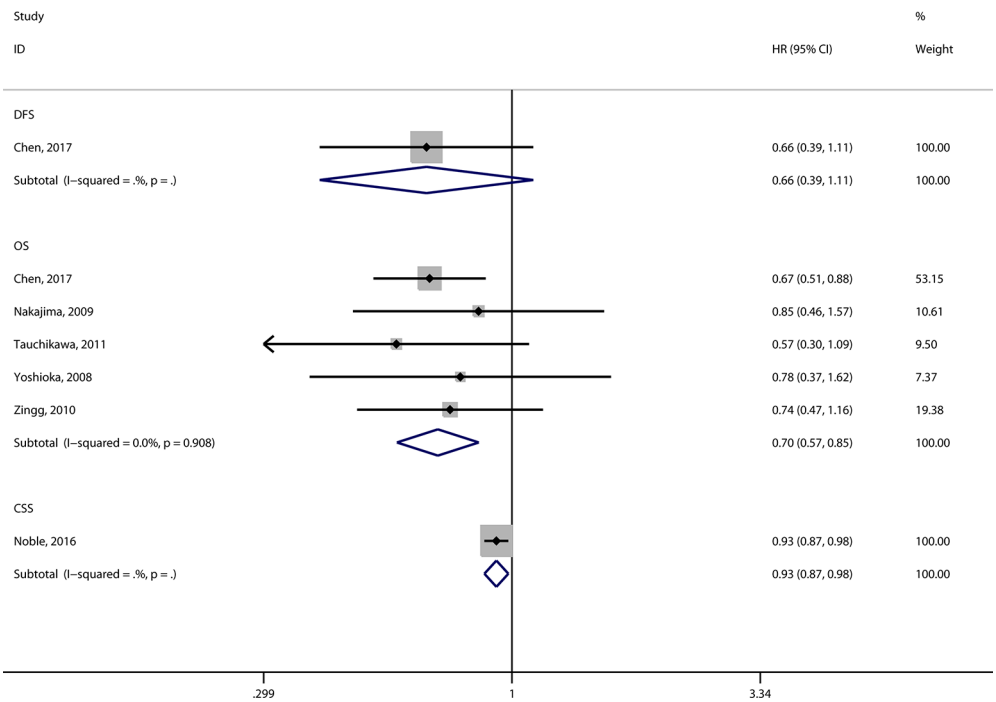

Figure 6 Forest plot for the prognostic effect of CD4+ T cells.

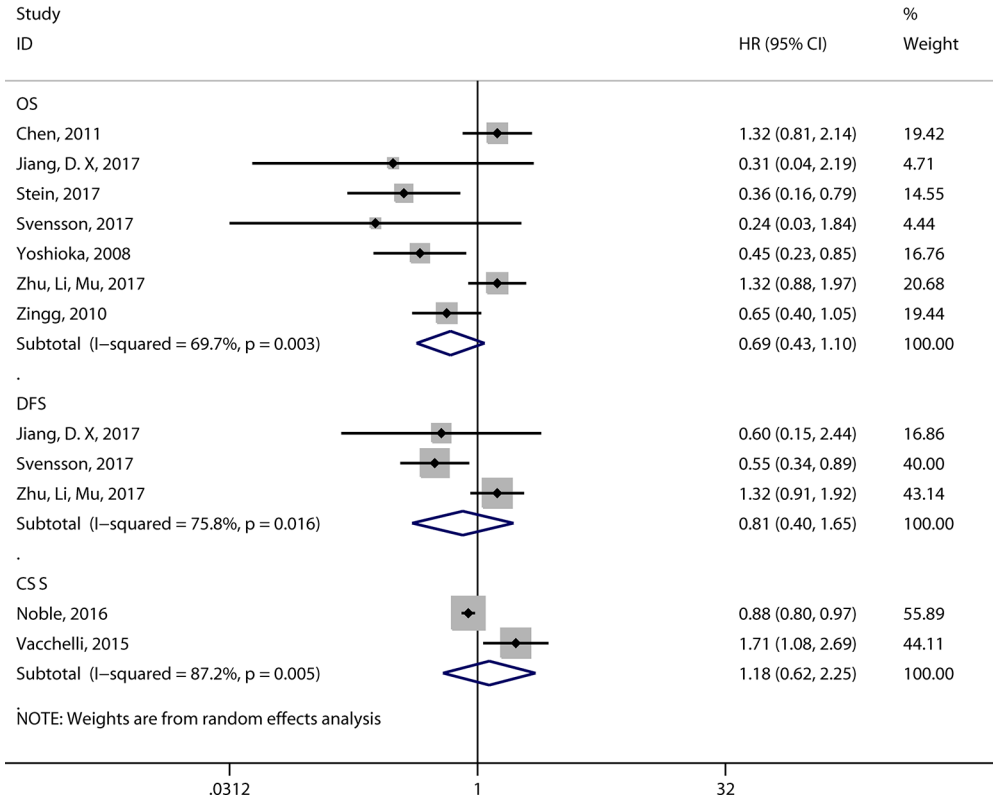

Figure 7 Forest plot for the prognostic effect of FOXP3+ T cells.

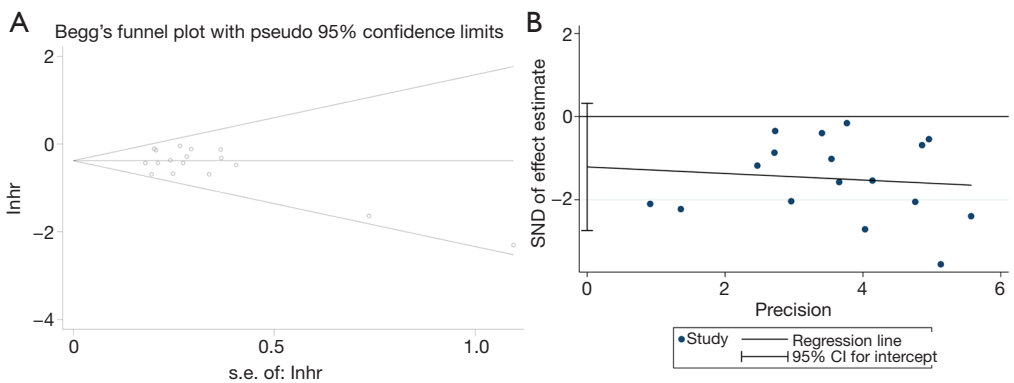

Figure 8 Begg's funnel plot and Egger's test for the assessment of potential publication bias in studies investigating the association between CD8+ TILs and overall survival of patients with esophageal cancer. No evidence of publication bias is observed. (A) Begg's P=0.303. (B) Egger's P=0.112.

and they did not mention the prognostic effect of TILs in different locations (in ESCC). Molecular analysis has shown that ESCC are more reminiscent of other SCCs than of EAC, which itself bears a striking resemblance to chromosomal instability (CIN) gastric cancer (59). In our meta-analysis, we performed subgroup analyses to investigate the prognostic value of CD8+ TILs for both ESCC and EAC patients. Moreover, we also investigated the prognostic effect of CD8+ TILs in both the TNs and TS in ESCC patients. Taking these results and insights into account, our meta-analysis was more comprehensive and stratified TILs according to their subsets and locations within the tumor

microenvironment in both ESCC and EAC.

In our studies, generalized TILs indicate overall TILs regardless of the subtypes and location. Generalized TILs were reported in eight studies, and the evaluation of TILs was conducted on HE-stained tumor sections without further distinguishing subgroups by IHC. According to our results, a high density of generalized TILs was associated with favorable clinical outcomes. This result was in accordance with many previous studies in which the prognostic role of generalized TILs was evaluated (5-7,52,60). In our study, a high level of CD8+ T-cell infiltration could predict a better OS for EC. The results are in agreement with the findings of previous studies that showed an association between CD8+ T cells and better prognosis in other types of cancers (5,7,52,55,60). In the subgroup analysis, high CD8+ T-cell infiltration was associated with favorable clinical outcomes in almost every subgroup analyzed, such as patients with ESCC, patients with EAC, Asian patients, Caucasian patients, and patients from China, Japan, or Germany. Moreover, in ESCC, a high number of CD8+ T cells in the TN or TS could predict a better OS for ESCC patients. These results indicate that CD8+ T-cell infiltration plays a definite anticancer role in EC. In our study, high CD4+ TIL infiltration was also a favorable prognostic biomarker. Although the prognostic value of CD4+ TIL infiltration was assessed by several studies, the role of CD4+ lymphocytes in the tumor microenvironment still remains questionable. CD4+ lymphocytes can exert different functions, ranging from the cytotoxic cell response of stimulated Th1 cells to the immunosuppressive response of regulatory T (Treg) cells (61,62). FOXP3 is the most specific marker on Treg cells, which are commonly considered immunosuppressive. A study reported the negative prognostic role of FOXP3+ cells (63), while other studies found that a high level of FOXP3+ lymphocyte infiltration is a positive prognostic marker (37,64). A meta-analysis by Shang *et al.* concluded that FOXP3+ Treg cells were associated with improved survival in several types of cancers, including EC. Furthermore, the molecular subtype and tumor stage significantly influenced the prognostic value of FOXP3+ TILs (65). According to our meta-analysis, high levels of FOXP3+ lymphocyte infiltration were not correlated with a favorable prognosis in EC patients. Finally, we assessed the prognostic value of CD3+ TILs. CD3 is a general surface antigen of T cells, which may represent the entire tumor-infiltrating T cell population (66). Due to the complex functions of different kinds of T cells, CD3+ TILs may not correlate with patients' clinical outcome, which is also consistent with our meta-analysis.

At present, the mainstream approach for predicting the clinical outcome in cancer patients is still the traditional TNM classification based on histopathological examination of surgically resected tumor tissues. However, it has been recognized for a long time that the TNM staging system is not precise enough, as survival outcomes can vary significantly among patients within the same stage (67). For many years, researchers have been exploring ways to complement TNM staging with immunologically relevant biomarkers; this can be referred to as "immunoscore". In the last few years, achievements have been made to include the prognostic value of immunological biomarkers to TNM staging in various types of cancers (9,68-71). To date, many attempts have been made to explore the prognostic value of various TIL subsets for EC patients. To the best of our knowledge, this is the most comprehensive meta-analysis to systematically combine data regarding the prognostic roles of various subsets of TILs in EC. Moreover, the results of our study should be interpreted with caution for the following reasons. First, the main limitation of this study is the heterogeneity within the tumor subgroups, cut-off values and detecting methods included in our meta-analysis. Molecular analysis has shown that ESCC are more reminiscent of other SCCs than of EAC. So, the prognostic value of TILs may be different between these two different tumor subgroups. Moreover, because now there is no consensus on cut-offs, several studies were unclear about their data-dependent cut-offs, and this may lead to different results. In addition, Because the detection methods of TILs may also be different, and this also affect the results. we need to conduct multi-center, prospective researches which use homogeneous cohorts to determine the appropriate T-cell makers, cut-off values and detection methods. Second, as HRs were not provided directly by some of the included studies, the required data had to be extracted from survival curves, which would undoubtedly introduce measurement error. Third, some studies only applied univariate Cox regression analysis, which tended to overestimate the prognostic effects of TILs, as other influencing factors were not adequately controlled. Fourth, in our meta-analysis, some studies included patients who received neoadjuvant treatment, but the separate HRs of these patients were not provided. Other studies only included patients who did not receive neoadjuvant treatment, which undoubtedly caused a bias. Finally, for some TIL subsets, data could only be obtained from a single study; thus, data integration was

not feasible. Overall, for most TIL subsets, corresponding studies that assessed their prognostic values did not have a sufficient number of patients within their cohorts. However, this problem is inevitable to some extent, as studies focusing on the prognostic roles of TILs in EC patients are far from abundant.

In summary, we found that high levels of generalized TILs, high CD8+ T-cell infiltration and high CD4+ T-cell infiltration were associated with better OS in EC patients. Moreover, high numbers of CD8+ TILs in the TN or TS can predict better OS for ESCC patients. Additional randomized controlled trials with larger sample sizes are needed to determine the most promising combination of TILs for the establishment of an immunoscore for patients with EC.

Acknowledgments

We thank Ms. Meihua Xiong, Fang Zhou and all the staff in Dr. Jie He's Laboratory, Department of Thoracic Surgery, for their support during the study.

Funding: This study was supported by the National Key R&D Program of China (2018YFC1313100, 2016YFC1303200), the National Natural Science Foundation of China (81972316), the Beijing Nova Program (Z181100006218032), the CAMS Innovation Fund for Medical Sciences (CIFMS) (2017-I2M-1-005, 2017-I2M-2-003), and the Institutional Fundamental Research Funds (2018PT32012, PY2018B02, 2019PT320022).

Footnote

Reporting Checklist: The authors have completed the PRISMA 2009 reporting checklist. Available at http://dx.doi.org/10.21037/atm-20-151

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at http://dx.doi.org/10.21037/atm-20-151). The authors have no conflicts of interest to declare.

Ethics Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. All analyses in this meta-analysis were based on previous published studies which have been performed in accordance with the Declaration of Helsinki (as revised in 2013) and approved by an appropriate ethics committee.

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Cite this article as: Gao Y, Guo W, Geng X, Zhang Y, Zhang G, Qiu B, Tan F, Xue Q, Gao S, He J. Prognostic value of tumor-infiltrating lymphocytes in esophageal cancer: an updated meta-analysis of 30 studies with 5,122 patients. Ann Transl Med 2020;8(13):822. doi: 10.21037/atm-20-151

