Original Paper

Prognostic Role of Tumor-Infiltrating Lymphocytes in Lung Cancer: a Meta-Analysis

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Key Words

Tumor-infiltrating lymphocytes • Lung Cancer • Prognosis • Meta-analysis

Abstract

Background/Aims: The role of Tumor-infiltrating lymphocytes (TILs) in the prognosis of patients with lung cancer is still controversial. We performed a meta-analysis to evaluate the prognostic role of TILs in lung cancer. **Methods:** Studies were recruited by searching PubMed, Embase and the Cochrane Library and assessed by further quality evaluation. The pooled hazard ratios (HRs) with 95% confidence intervals (CIs) were calculated to investigate the association between TIL subsets and lung cancer patients' outcome. **Results:** A total of 29 articles including 8,600 patients were enrolled into the meta-analysis. Our results indicated that high level of CD8⁺ cells infiltration in tumor stroma (TS) or tumor nest (TN) was associated with better OS in lung cancer patients (HR = 0.76, 95% CI 0.62-0.93, *P* = 0.006; HR = 0.80, 95% CI 0.67-0.96; *P* = 0.018, respectively). Similar results could be also observed in CD3⁺ T cells infiltration. High CD4⁺ T lymphocytes infiltration in TS was explicitly accompanied by better OS (HR = 0.65, 95% CI 0.46-0.91; *P* = 0.013), rather than in TN. In contrast, high density of FOXP3⁺ T cells infiltration in TS showed a poor PFS (HR = 2.67, 95% CI, 1.74-4.08, *P* < 0.001). **Conclusion:** This meta-analysis clarified that high level of CD8⁺ and CD3⁺ T cells infiltration in TS or TN, and high CD4⁺ T lymphocytes infiltration in TS showed better OS in lung cancer patients, whereas high density of FOXP3⁺ T cells infiltration in TS could be recognized as a negative prognostic factor.

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Introduction

As a malignant tumor with the highest morbidity and mortality, lung cancer has become a major public health problem worldwide [1]. With the development of the comprehensive treatment strategies, the therapeutic effect of lung cancer has been improved recently. However, the long-term survival of patients is still poor, with a 5-year survival rate of around 18% [2]. Traditionally, the prognosis of lung cancer was evaluated according to TNM staging based on anatomy and histopathology, which has been proved not accurately enough by growing evidence nowadays. Therefore, a new biological marker is needed for more accurate prognosis and effective therapies as a complement to the TNM system.

Tumor-infiltrating lymphocytes (TILs) might be one of potential biomarkers for cancer prognosis. Early in 1922, McCarfy et al. [3] brought up the concept of TILs, and considered the infiltration of lymphocytes into tumor tissue as an antitumor activity of immune system. Unexpectedly, in the late 1990s, the infiltration of neoplastic tissues by immune inflammatory cells was reported to promote tumor progression. Until recently, a bidirectional role of TILs within the tumor microenvironment in tumor progression has been confirmed: It can not only suppress tumor growth by destroying cancer cells or inhibiting their outgrowth, but also promote tumor progression either by selecting for tumor cells that are more fit to survive in an immunocompetent host or by establishing conditions within the tumor microenvironment that facilitate tumor outgrowth [4]. TILs are a heterogeneous population comprising mainly T lymphocytes, and to a lesser degree, B lymphocytes and natural killer cells (NK) [5]. It is important to discuss TILs subsets separately due to their different physiological and pathological effects in tumor microenvironment. With the development of immunohistochemistry, more and more subtypes of TILs have been discovered. CD3, a biomarker of T lymphocytes, is expressed in almost all T lymphocytes. According to the cell surface markers, T lymphocytes mainly include the following subtypes: CD8⁺ cytotoxic T lymphocytes (CTL), CD4⁺ T helper lymphocytes (Th), CD45RO⁺ memory T cells (Tm), and FOXP3⁺ regulatory cells (Tregs), etc.

The correlation between TILs and clinical outcome have been investigated in many cancers, such as lung cancer [6], colorectal cancer [7, 8], breast cancer [9], melanoma [10], ovarian cancer [11], pancreatic cancer [12] and so on. In some studies, TILs were found to be a better predictor of patients' survival than TNM staging [6-9]. However, the prognostic value of TILs in lung cancer is still controversial, varying with the distribution site and cell types. It was reported that high density of CD8⁺ T cells in tumor stroma (TS) was associated with a longer overall survival (OS) [6], whereas Kawai et al. considered that only CD8⁺ T cells in tumor nest (TN) were associated with patients' survival [13]. However, Goc et al. reported that the level of CD8⁺ T cells in both TN and TS had prognostic value for lung cancer patients [14]. These controversial results were also reported in CD3⁺ T cells [15, 16]. Compared to CD8⁺ and CD3⁺ T cells, regulatory T cells (Foxp3⁺) showed the opposite effect on prognosis in most studies [17-19]. All the published literatures we have searched so far did not come to a conclusion whether TILs can be used as a potent biomarker for prognosis. Therefore, a systematical and comprehensive meta-analysis to investigate the relationship between TILs and the survival of patients with lung cancer is urgently required. To our knowledge, this meta-analysis is the first to evaluate the prognostic value of TILs in lung cancer patients.

Materials and Methods

We carried out this meta-analysis following the guidelines of the Systematic Reviews and Meta-Analyses (PRISMA) [20] and the Observational Studies in Epidemiology (MOOSE) guidelines [21].

Search strategy

Literatures were searched through PubMed, Embase and the Cochrane Library (last update by Aug 20, 2015). Keywords used in the search strategy were "tumor-infiltrating lymphocyte OR TILs OR tumor

infiltrating lymphocyte OR intratumoural lymphocyte OR intratumoral lymphocyte OR intra-tumoral lymphocyte" (all fields) AND "lung" (all fields) AND "tumor OR tumour OR neoplasm OR cancer OR carcinoma" (all fields) AND "prognosis OR prognostic OR survival OR outcome" (all fields). There were no other limitations in the database searching process. Besides title, abstract and full text, all the reference lists of identified articles were also reviewed in order to find out potential studies. Two authors were responsible for the comprehensive database search and availability evaluation independently (Y. Geng and Y. Shao).

Inclusion and exclusion criteria

Literatures that were eligible for inclusion in this meta-analysis met the following criteria: (1) Studies in lung cancer reporting the prognostic impact of TILs or associated TILs subsets (including CD3⁺, CD8⁺, CD4⁺, and FoxP3⁺ lymphocytes); (2) Studies provided sufficient data to estimate the hazard ratio (HR) and 95% confidence intervals (CI) were included in the meta-analyses; (3) The sample size of studies was specific restricted: only studies with sample size ≥ 50 were enrolled in this meta-analyses. The exclusion criteria were as follows: (1) Studies with sample size < 50 were excluded, because too small sample size would induce publication bias, whereas if the lower limit was too large, there would be few qualified studies left; (2) If more than one study were targeted at the same patient cohort, only the most recent or complete study would be selected; (3) Some styles of literature such as case reports, letters, reviews, conference abstracts and animal trials were excluded.

Data extraction and quality assessment

Parameters of all eligible studies were collected, including first author's surname, publication year, origin of population, sample size, tumor stage, lymph node metastasis, follow-up period, TILs subsets and distribution site, the cut-off definition, HRs of TILs and each TILs subset for OS, disease-free survival (DFS) and relapse-free survival (PFS) as well as corresponding 95% CIs. If univariate and multivariate analyses were both involved in a study, only the latter was selected due to its high precision.

The quality of each study was assessed independently by two researchers according to the Newcastle-Ottawa Quality Assessment Scale (NOS) [22]. Scores ranged from 0 to 9 for quality assessment, and studies with scores ≥ 6 were rated as high quality.

Statistical analysis

The death risk of lung cancer patients with different level of TILs was evaluated by HRs and 95% CIs: an observed HR > 1 indicated worse prognosis in patients with high-density TILs and a HR < 1 suggested better prognosis. If the statistical variables were not reported in the article directly, we calculated them from available numerical data in the articles according to the methods described by Tierney [23]. The data from Kaplan-Meier survival curves were read by three independent researchers using Engauge Digitizer version 4.1 to reduce reading variability, and the specific method was described in our previous study [24]. We obtained additional information and original data needed for meta-analysis by sending e-mail to the corresponding authors of eligible articles. Statistical heterogeneity was assessed by visual inspection of forest plots, by performing the Chi-square test (assessing the *P* value), and by calculating the *I*² statistic [25, 26]. If the *P* value was less than 0.05 and/or *I*² exceeded 50%, indicating the presence of heterogeneity, a random-effects model (the DerSimonian-Laird method) would be applied. Otherwise, the fixed-effects model (the Mante-Haenszel method) was used. Subgroup analysis and meta-regression were further performed to explore the source of identified heterogeneity. Publication bias was estimated by visually assessing the asymmetry of an inverted funnel plot, and was quantified by Begg's and Egger's tests. For all analyses, STATA version 12.0 (Stata Corporation, College Station, TX, USA) was used, with significance defined as a *P*-value less than 0.05.

Results

Study characteristics

Using the described searching strategy, 962 references were initially retrieved. After screening the titles, abstracts, publication types and full text of each publication, 40 articles investigated the correlation between TILs and patients' outcome in lung cancer. Among these,

11 articles were excluded (seven without some important data, one investigating the same patient cohorts with others, and three with too small sample size). Finally, 29 articles were enrolled into the meta-analysis (Fig. 1) [6, 13-19, 27-47]. The total number of patients from Japan, America, France, Norway, Korea, Greece, Denmark, China, Germany, Austria, Italy, Finland and England was 8,600, ranging from 56 to 1290 patients per study. Six studies included fewer than 100 patients and 15 studies enrolled over 200 patients.

Fig. 1. Flow diagram of the study selection process.

Among these, only four articles reported the prognostic value of generalized TILs [27, 31, 38, 45], and others focused on specific TILs subsets. HE (hematoxylin-eosin) staining and immunohistochemistry (IHC) staining were applied for the detection of generalized TILs and specific TILs subsets respectively. The cut-off values contained median level ($n=11$), mean level ($n=2$), and some semiquantitative methods. The other major details of these eligible studies, such as sex, tumor stage, follow-up time, etc, are showed in Table 1.

Quality assessment was performed for each study included in our meta-analyses according to the NOS, with scores ranging from 4 to 8 (mean = 6.7). A higher value indicated a better methodology. Therefore, all these 29 studies were enrolled in the subsequent analyses.

Subgroup analysis

The prognosis of lung cancer patients is significantly associated with the cell type and distribution site of TILs. Thus, we performed subgroup analyses bases on TILs subsets, and then based on distribution site of each subset. The main results of subgroup analyses are summarized in Table 2.

Generalized TILs

Three articles researched the relation between the density of generalized TILs in both TN and TS and patients' survival [27, 31, 38, 45]. Three of the articles evaluated OS [27, 38, 45] and two of them evaluated PFS [27, 38]. Our results showed that high density of generalized TILs was associated with a favorable PFS in lung cancer (the pooled HR = 0.42, 95% CI 0.28-0.61, $P < 0.001$), rather than OS (HR = 0.95, 95% CI 0.77-1.18, $P = 0.66$).

CD8⁺ T lymphocyte subset

A total of 18 articles, encompassing 6,543 tumor patients, focused on the association between the infiltration of CD8⁺ T lymphocytes and the survival of lung cancer patients [6, 13, 14, 28-30, 32-34, 38-40, 42-47]. Stratified by the distribution of TILs, there were 11 of TN, 6 of TS and 6 of both TN and TS, respectively.

Tumor nest

Ten studies [13, 14, 29, 30, 32, 33, 39, 40, 42, 44] which assessed the infiltration of CD8⁺ T lymphocytes in TN were involved in OS analysis, with a significant heterogeneity among them ($P = 0.002$, $I^2 = 66.4\%$). Hence, a random model was applied to overall data integration, and the result suggested that patients with high CD8⁺ T lymphocytes infiltration had better

Table 1. Main characteristics of all studies included in the meta-analysis. TS tumor stroma, TN tumor nest, NR not report, IHC immunohistochemistry, HE haematoxylin-eosin, HPV high-power fields, OS overall survival, PFS recurrence-free survival, DFS disease-free survival, DSS disease-special survival, QIF quantitative immunofluorescence

Study	Subset	Location	Origin of population	Sample number (male/female)	Tumor stage (I / II / III / IV)	Follow-up (months)	Detected method	Cut-off for overexpression	Outcome
Goc 2014 [14]	CD8 ⁺	TS	France	376(302/74)	167/101/104/2	≥90	IHC	383 per mm ²	OS
	CD8 ⁺	TN	France	376(302/74)	167/101/104/2	≥90	IHC	114 per mm ²	OS
Kinoshita 2013 [17]	FOXP3 ⁺	TS	Japan	200(93/107)	200/0/0/0	3-87	IHC	mean (6/HPF)	OS, PFS
Liu 2012 [28]	CD8 ⁺	both	China	259(118/41)	0/0/96/63	median 20.8(2-44)	IHC	median	OS
	FOXP3 ⁺	both	China	259(118/41)	0/0/96/63	median 20.8(2-44)	IHC	median	OS
Kayser 2012 [15]	CD3 ⁺	TS	Germany	232(167/65)	91/59/77/3	median 32(1-210)	IHC	median 74.6 per mm ²	OS
	CD3 ⁺	TN	Germany	232(167/65)	91/59/77/3	median 32(1-210)	IHC	median 19.8 per mm ²	OS
Ilie 2012 [29]	CD8 ⁺	TN	France	632(467/165)	274/185/173/0	median 30(0-112)	IHC	median 110 per mm ²	OS
Sterlacci 2012 [30]	CD4 ⁺	TN	Austria	383(280/103)	219/67/79/18	over 60	IHC	mean 16.84/0.28 mm ²	OS
	CD8 ⁺	TN	Austria	383(280/103)	219/67/79/18	over 60	IHC	mean 17.92/0.28 mm ²	OS
	FOXP3 ⁺	TN	Austria	383(280/103)	219/67/79/18	over 60	IHC	mean 1.17/0.28 mm ²	OS
Tao 2012 [18]	FOXP3 ⁺	TS	Japan	87(56/31)	NR	NR	IHC	25/HPF	OS, PFS
Horne 2011 [27]	TIL	both	American	273(130/143)	273/0/0/0	NR	HE	moderate lymphocytes	OS, PFS
Al-shibli 2010 [16]	CD3 ⁺	TS	Norway	335(253/82)	212/91/32/0	median 86(48-216)	IHC	50%	OS
	CD3 ⁺	TN	Norway	253(82/335)	212/91/32/0	median 86(48-216)	IHC	1%	OS
Kilic 2011 [31]	TIL	both	American	219(NR/NR)	219/0/0/0	over 24	HE	none to mild infiltrate	PFS
Ruffini 2009 [32]	CD8 ⁺	TN	Italy	1290(1084/206)	714/265/214/0	NR	IHC	staining in 20% of cells	OS
Al-shibli 2008 [6]	CD4 ⁺	TS	Norway	335(253/82)	212/91/32/0	median 96(10-179)	IHC	25%	DSS
	CD4 ⁺	TN	Norway	335(253/82)	212/91/32/0	median 96(10-179)	IHC	5%	DSS
	CD8 ⁺	TN	Norway	335(253/82)	212/91/32/0	median 96(10-179)	IHC	5%	DSS
Kawai 2008 [13]	CD8 ⁺	TN	Japan	199(139/60)	0/0/0/199	NR	IHC	median 12/HPV	OS
	CD8 ⁺	TS	Japan	199(139/60)	0/0/0/199	NR	IHC	median 13/HPV	OS
Wakabayashi 2003 [33]	CD4 ⁺	TS	Japan	144(97/47)	188/II-III/56	over 36	IHC	median 210/HPV	OS
	CD8 ⁺	TN	Japan	178(118/60)	1107/II-III/71	over 36	IHC	median 35/HPV	OS
Hiraoka 2006 [34]	CD4 ⁺	TS	Japan	109(71/38)	167/II-III/42	over 60	IHC	median 168/HPV	OS
	CD8 ⁺	TS	Japan	109(71/38)	167/II-III/42	over 60	IHC	median 151/HPV	OS
Shimizu 2010 [35]	FOXP3 ⁺	TS	Japan	100(60/40)	68/14/18/0	over 24	IHC	median 3/HPV	PFS
Petersen 2006 [36]	CD3 ⁺	both	American	64(34/30)	64/0/0/0	over 30	IHC	50%	PFS
Suzuki 2013 [19]	Foxp3 ⁺	TS	American	478(176/302)	478/0/0/0	over 24	IHC	1.67 normal	PFS
	Foxp3 ⁺	TS	American	478(186/292)	478/0/0/0	over 24	IHC	1.67 normal	PFS
Dieu-Nosjean 2008 [37]	CD3 ⁺	both	France	74(60/14)	62/12/0/0	over 48	IHC	1.5 mean score/tumor IPF	OS
Ikedo 2006 [38]	CD8 ⁺	both	Japan	83(65/18)	140/II-III/43	over 60	IHC	5/HPV	OS
	TIL	both	Japan	83(65/18)	140/II-III/43	over 60	HE	50/HPV	OS
Yoshida 2006 [39]	CD4 ⁺	TN	Japan	125(86/39)	71/26/25/2	median 21.7	IHC	median 220/HPV	OS
Eerola 2000 [40]	CD8 ⁺	TN	Finland	56(51/5)	I-II/37/III-IV/19	over 60	IHC	median 10/HPV	OS
Johnson 2000 [41]	CD3 ⁺	TN	England	95(59/36)	54/17/20/0	NR	IHC	10/HPV	OS
Kikuchi 2007 [42]	CD8 ⁺	TN	Japan	161(110/51)	195/II-III-IV/66	median 111.7	IHC	median	OS
	CD8 ⁺	TS	Japan	161(110/51)	195/II-III-IV/66	median 111.7	IHC	median	OS
Donnem 2015 [43]	CD8 ⁺	TS	Norway Denmark	797(510/287)	407/275/115/0	median 65.5	IHC	50%	OS, DFS, DSS
Alifano 2014 [44]	CD8 ⁺	TN	France	303(244/59)	114/85/97/7	NR	IHC	96 per mm ²	OS
Schalper 2015 [45]	TIL	both	American	188(91/97)	I-II/119/III-IV/55	NR	HE	the top tertile	OS
	TIL	both	Greece	341(300/41)	I-II/200/III-IV/135	NR	HE	60%	OS
	CD3 ⁺	both	American	188(91/97)	I-II/119/III-IV/55	NR	QIF	60%	OS
	CD3 ⁺	both	Greece	341(300/41)	I-II/200/III-IV/135	NR	QIF	the top tertile	OS
	CD8 ⁺	both	American	188(91/97)	I-II/119/III-IV/55	NR	QIF	the top tertile	OS
	CD8 ⁺	both	Greece	341(300/41)	I-II/200/III-IV/135	NR	QIF	the top tertile	OS
Kim 2015 [46]	CD8 ⁺	both	Korea	331(317/14)	131/118/79/0	NR	IHC	median 251.5 per mm ²	OS, DFS
Koh 2015 [47]	CD8 ⁺	both	Korea	497(231/266)	342/46/109/0	NR	IHC	100 per mm ²	DFS

OS (HR=0.80, 95% CI 0.67-0.96; $P = 0.018$) (Fig. 2A). In order to explore the potential factors responsible for the heterogeneity, we conducted subgroup analyses, sensitivity analysis and meta-regression. The subgroups were defined according to the main features of pooled studies, including cut-off values, follow-up time, publication year, patients' ethnicity, and sample size. We found that high level of CD8⁺ T lymphocytes was associated with improved OS in groups with Caucasian patients (HR = 0.83, 95% CI 0.75-0.92; $P < 0.001$), special cut-off values (non-median; HR = 0.75, 95% CI 0.58-0.96; $P = 0.024$), long time follow-up (≥60 months; HR = 0.76, 95% CI 0.64-0.90; $P = 0.002$), more recent publication (after 2009; HR = 0.84, 95% CI 0.75-0.93; $P = 0.001$) and large sample size (≥300; HR = 0.84, 95% CI 0.75-0.93; $P = 0.001$); However, statistical significance was not shown in other subgroups (Table 3). Sensitivity analysis was performed by sequential omission of individual studies using the fixed-effects model, and the result pattern was not obviously impacted by any single study. The meta-regression showed that the cut-off values ($P = 0.646$), follow-up time ($P = 0.390$), publication year ($P = 0.803$), patients' ethnicity ($P = 0.572$) and sample size ($P = 0.803$) may contributed to the heterogeneity more or less, but not definitively.

Table 2. The pooled associations between TILs subsets and the prognosis of patients with lung cancer. TS tumor stroma, TN tumor nest, OS overall survival, PFS recurrence-free survival, DSS disease-special survival, HR hazard ratio, CI confidence intervals

Subset/distribution	Outcome	No. of studies	No. of patients	HR (95%CI) - model	P value	heterogeneity I^2 (%)	P	Publication bias Begg's P	Egger's P
Generalized TILs									
both	OS	4	885	0.95 (0.77-1.18) - fixed	0.66	0	0.521	0.734	0.818
	PFS	2	492	0.42 (0.28-0.61) - fixed	<0.001*	0	0.409	-	-
CD8*									
TN	OS	10	3703	0.80 (0.67-0.96) - random	0.018*	66.4	0.002	0.371	0.366
	DSS	1	335	0.67 (0.46-0.97)	0.023*	-	-	-	-
TS	OS	5	1642	0.76 (0.62-0.93) - random	0.006*	63.4	0.027	0.462	0.856
	DFS	1	797	0.72 (0.62-0.85)	<0.001*	-	-	-	-
	DSS	1	797	0.49 (0.30-0.82)	<0.001*	-	-	-	-
both	OS	5	1102	0.74 (0.63-0.88) - fixed	<0.001*	0	0.406	0.806	0.261
	DFS	2	828	0.68 (0.55-0.85) - fixed	0.001*	0	0.523	-	-
CD3*									
TN	OS	3	662	0.66 (0.45-0.97) - random	0.036*	60.1	0.082	1.000	0.636
TS	OS	2	567	0.65 (0.50-0.84) - fixed	0.001*	0	0.487	-	-
both	OS	3	603	0.72 (0.51-1.03) - random	0.068	53.4	0.093	1	0.327
	PFS	2	138	0.50 (0.10-2.42) - random	0.392	84.4	0.011	-	-
CD4*									
TN	OS	2	508	0.43 (0.07-2.61) - random	0.359	92.9	0.001	-	-
	DSS	1	335	0.94 (0.64-1.37)	0.632	-	-	-	-
TS	OS	2	253	0.65 (0.46-0.91) - fixed	0.013*	0	0.395	-	-
	DSS	1	335	0.42 (0.20-0.59)	<0.001*	-	-	-	-
FOXP3*									
TS	OS	2	287	2.67 (1.74-4.08) - fixed	<0.001*	0	0.829	-	-
	PFS	5	1343	2.14 (1.68-2.72) - fixed	<0.001*	0	0.898	1.000	0.879
TN	OS	1	383	0.92 (0.39-2.17)	0.849	-	-	-	-
both	OS	1	159	1.22 (0.91-1.64)	0.186	-	-	-	-

Table 3. Subgroup analyses of the relationship between CD8* T lymphocyte subsets and OS. OS overall survival, HR hazard ratio, CI confidence intervals

Outcome subgroup	No. of patients	No. of studies	HR (95%CI)	P value	Heterogeneity I^2	P
Overall effect	3703	10	0.80 (0.67-0.96) - random	0.018*	66.4	0.002
Patients' ethnicity						
Asian	663	4	0.91 (0.58-1.43) - random	0.677	83.9	<0.001
Caucasian	3040	6	0.83 (0.75-0.92) - fixed	<0.001*	37.6	0.156
cut-off values						
median	1351	6	0.85 (0.64-1.13) - random	0.261	75.7	0.001
others	2352	4	0.75 (0.58-0.96) - random	0.024*	50.7	0.108
Follow-up months						
≥60	1279	5	0.76 (0.64-0.90) - fixed	0.002*	38.0	0.168
<60	2424	5	0.89 (0.69-1.15) - random	0.357	78.6	0.001
Publication year						
Before 2009	719	5	0.83 (0.55-1.27) - random	0.398	80.5	<0.001
After 2009	2984	5	0.84 (0.75-0.93) - fixed	0.001*	36.3	0.179
Sample size						
≥300	2984	5	0.84 (0.75-0.93) - fixed	0.001*	36.3	0.425
<300	719	5	0.83 (0.55-1.27) - random	0.398	80.5	<0.001

Tumor stroma

As regards the 5 studies providing OS [13, 14, 34, 42, 43], a random model was used to calculate the pooled HR and its 95% CI due to the high heterogeneity between these studies ($P = 0.027$, $I^2 = 63.4\%$). The pooled HR indicated that high level of CD8* T lymphocytes in TS significantly predicted better OS (HR=0.76, 95% CI 0.62-0.93; $P=0.006$) (Fig. 2A).

Both tumor nest and stroma

Five studies researched the CD8* T lymphocytes in both TN and TS [28, 38, 45-47]. Because these studies did not display obvious heterogeneity ($P=0.406$, $I^2=0\%$), a fixed model was used for calculating the pooled HR. Our result showed that high level of CD8* T lymphocytes in both TN and TS is a favorable predictor for OS (HR=0.74, 95% CI 0.63-0.88; $P<0.001$) and DFS (HR=0.68, 95% CI 0.55-0.85; $P<0.001$) (Fig. 2A).

Fig. 2. Forest plots of studies evaluating hazard ratios of high level of TILs subsets in lung cancer by the distribution. (A) Forest plots of the prognostic effect of CD8⁺ T cells. (B) Forest plots of prognostic effect of CD3⁺ T cells. (C) Forest plots of prognostic effect of CD4⁺ T cells. (D) Forest plots of prognostic effect of FOXP3⁺ T cells.

Fig. 3. Funnel plots of CD8⁺ T cell infiltration in tumor nest.

CD3⁺ T lymphocyte subset
Six articles provided data regarding the association between CD3⁺ T cells infiltration and survival outcomes [15, 16, 36, 37, 41, 45]. Three of them [15, 16, 41] investigated CD3⁺ T cells infiltration in TN, and the pooled HR for OS was

0.66 (95% CI 0.45-0.97; $P=0.036$), with a significant evidence of heterogeneity ($P=0.082$, $I^2=60.1\%$) (Fig. 2B). Two studies which focused on CD3⁺ T cells infiltration in TS [15, 16] confirmed a positive predictive effect for OS (HR=0.65, 95% CI 0.50-0.84; $P=0.001$) without heterogeneity (Fig. 2B). However, high CD3⁺ T cells infiltration in both TN and TS did not show a positive outcome for both OS and PFS (Fig. 2B).

CD4⁺ T lymphocyte subset

Five eligible articles [6, 30, 33, 34, 39] provided the HR and 95% CI regarding the correlation between CD4⁺ T cells and lung cancer patients' survival. We found that high CD4⁺ T lymphocytes infiltration in TS was explicitly accompanied by better OS (HR=0.65, 95% CI 0.46-0.91; *P*=0.013), rather than in TN (HR=0.43, 95% CI 0.07-2.61; *P*=0.359) (Fig. 2C).

FOXP3⁺ Treg subset

Six articles researched the prognostic value of FOXP3⁺ Tregs in lung cancer patients [17-19, 28, 30, 35]. Five studies investigated FOXP3⁺ Tregs in TS [17-19, 35], and the pooled HRs for OS and PFS were 2.67 (95% CI, 1.74-4.08) and 2.14 (95% CI, 1.68-2.72), respectively, without any heterogeneity (Fig. 2D). Thus, it is reliable that high level of FOXP3⁺ Tregs in TS is associated with poor outcome in lung cancer. There was no prognostic effect of FOXP3⁺ Tregs in TN [30] both TN and TS [28], with only one study in each group.

Publication Bias

The publication bias of all enrolled studies was evaluated using Egger's and Begg's tests. In subgroup analyses, the *P* values of Egger's and Begg's tests were all greater than 0.05 (Table 2). Additionally, a funnel plot was applied to detect publication bias for the subgroups with the largest number of studies. The funnel plot of the CD8⁺ T cells infiltration in TN was substantially symmetric (Fig. 3). Therefore, significant publication bias was not observed in our meta-analyses.

Discussion

The TNM staging system has been used for over 80 years, but provides incomplete prognostic information. Clinical outcome may vary widely among patients within the same histological tumor stage [48]. Recently, a combination of the density and location of TILs subsets has defined the Immunoscore as a complement to the TNM system for the classification of malignancy [49-51]. Although derived from the immune contexture [52, 53], Immunoscore is more convenient to evaluated than the immune contexture. In colorectal cancer, the Immunoscore is a simple and powerful prognostic biomarker: it has been confirmed significant prognostic value even in Cox multivariate including TNM classification [7, 8]. However, this concept is not well explored in lung cancer. It's timely to definitively clarity which subset or location of TILs is appropriate for lung cancer Immunoscore. We conducted a meta-analysis combining 29 studies and 8,600 patients to provide clinical evidence for Immunoscore in lung cancer. The statistical results confirmed that high density of generalized TILs was associated with favorable PFS, rather than OS. Subgroup analysis was performed according to TILs subsets including CD8⁺, CD3⁺, CD4⁺ and FOXP3⁺ T cells, and we found better OS in patients with high level of CD8⁺ T cells infiltration in TS, TN, and in both TS and TN. Compared with CD8⁺ T cells in TN, the prognostic effect of CD8⁺ T cells in TS appeared more significant. However, the positive prognostic value of CD3⁺ T cells was only found in TS and TN, but not in both TS and TN. High density of CD4⁺ T cells infiltration in TS, rather than in TN, was associated with better prognosis in lung cancer. However, high density of FOXP3⁺ T cells infiltration in TS could be recognized as a negative prognostic factor. These results were interpreted to indicate that: firstly, various TILs subsets and distribution sites were generally associated with outcome of patients, but the pooled HRs didn't show dramatic differences in survival. Combining different TILs subsets, especially those have opposite effects on survival, would be helpful for prediction of prognosis, such as CD3⁺ and FOXP3⁺, CD8⁺ and FOXP3⁺, CD4⁺ and FOXP3⁺ T cells. Secondly, it is essential to stratify each subset of T lymphocytes according to the infiltration location, and the prognostic effect of TILs in TS seemed to be superior to those in TN. This conclusion is based on some physiological theory. Tumor cells use the deregulation of the Fas/Fas ligand (FasL) signaling pathway to escape the immune reactions directed against them. By downregulation of Fas and upregulation of FasL lung cancer cells may escape from cytotoxic T-cell effects and induced apoptosis [54].

As FasL concentrations will be highest in between tumor cell and thus in the “intraepithelial” compartment, changes of Fas/FasL ratios can lead to inactivation of T-cells, especially the intraepithelial CD4⁺ and CD8⁺ lymphocytes [15]. The native TILs in both TN and TS express high level of PD-1, connected with PD-L1 which causes anergy of TILs [55]. Cancer associated fibroblasts (CAF) is reported to induce the extravasation of active T cells with PD-1 low expression from the circulatory system via upregulating transforming growth factor (TGF)- β and vascular endothelial growth factor (VEGF), which lead to more comprehensive immune response in TS [17, 56, 57]. Additionally, the density of CD8⁺ cell infiltrating into cancer nests was decreased in tumor with negative expression of human leukocyte antigen (HLA) class I, but the density of CD8⁺ cells infiltrating in stroma was unaffected by expression of HLA class I. Down-regulation of HLA class I expression was a poor prognostic factor in many tumor including NSCLC [42]. Thus, it is reasoned that the anti-tumor immune response mainly depends on the TILs in TS, and the number and function of TILs in TS may particularly influence patients’ prognosis. This gives an explanation of our results showing TILs in TS to be of statistically significant prognostic value compared with TILs in TN. In view of the standpoints aforementioned, we propounded the Immunoscore in lung cancer based on the combination of different TILs (CD3⁺ and FOXP3⁺ T cells, CD8⁺ and FOXP3⁺, CD4⁺ and FOXP3⁺) in TS, which is helpful to predict prognosis more accurately. Recently, a research of Suzuki et al. showed that the combination of stromal CD3⁺ and FOXP3⁺ T cells acted as an independent prognostic factor in lung cancer [19]. However, further prospective studies with large sample size are needed to find the most promising combination of TILs for the establishment of Immunoscore in lung cancer.

Dialectically, these results should be carefully understood for following reasons. First, different cut-off points were used in those studies, and the deficiency of a unified cut-off value may affect the precision of TILs as a predictive biomarker in cancer prognosis. Second, several HRs were calculated based on the data extracted from the survival curves, which inevitably leads to small statistical errors. Third, TILs in tumor nest and stroma were discussed in this study. However, because many of the included studies did not address the location and several scores were based on a mix of cores located in the central of the tumor and in the invasive margin, we didn’t carry out specific discussion about the detailed localization: central tumor versus invasive margin. Fourth, other subsets of TILs such as B lymphocyte were not included in this research owing to few investigations. Finally, although we performed subgroup analyses pertaining to prognostic association of subsets and distribution of TILs, there was still significant heterogeneity displayed in our meta-analysis, which was most likely due to variation in patient origin, publication year, tumor pathological type, tumor stage, sample size, follow-up time, and cut-off values among others.

Conclusion

In conclusion, we found that high level of CD8⁺ and CD3⁺ T cells infiltration in TS or TN show better OS in lung cancer patients, whereas high density of FOXP3⁺ T cells infiltration in TS could be recognized as a negative prognostic factor. Considering the limitations of present analysis, the conclusions should be regarded cautiously. Further prospective multi-center studies with larger sample size are needed to test the most promising combination of TILs for the establishment of Immunoscore in lung cancer.

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Disclosure Statement

The authors declare that they have no conflict of interest.

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