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Association between density of tumor-infiltrating lymphocytes and prognoses of patients with gastric cancer

Peng-Cheng Yu, MD[a], Di Long, MD[a], Cheng-Cheng Liao, MD[b], Sen Zhang, MD[a,*]

Abstract

Introduction: Tumor-infiltrating lymphocytes (TILs) have been shown to be of prognostic significance in patients with gastric cancer. This study aims to investigate the association between density of TILs and prognoses of patients with gastric cancer.

Methods: The relative studies of tumor-infiltrating lymphocytes in tumor tissue from patients with gastric cancer were systematically searched from PubMed and Embase until October 31, 2017. The pooled hazard ratios (HRs) and their 95% confidence intervals (95%CI) for overall survival (OS) were estimated.

Results: Twenty-nine studies involving 4,942 patients were included into analyses. Subset of TILs included $CD8^+$, $CD3^+$, $CD4^+$, and $FOXP3^+$ T cell density. Results from meta-analyses revealed that high density of intratumoral $CD8^+$ T cells (HR = 0.77, 95% CI 0.63–0.95) and $CD3^+$ (HR = 0.62, 95% CI 0.49–0.77) were associated with significantly higher OS than those with low density in patients with gastric cancer. Moreover, a larger number of general TILs density also suggested a favorable prognosis (HR 0.75, 95% CI 0.67–0.84). However, patients with high density of intratumoral $FOXP3^+$ T or $CD4^+$ T cells were not statistically associated with higher or lower OS than those with low density (HR 1.41, 95% CI 0.97–2.05; HR = 0.86, 95% CI 0.47–1.57). Sample size and follow-up period seemed to influence study outcomes.

Conclusion: The present study revealed that high density of intratumoral $CD8^+$ and $CD3^+$ T cells were associated with better OS in patients with gastric cancer.

Abbreviations: CI = confidence interval, HR = hazard ratio, NOS = Newcastle–Ottawa Quality Assessment Scale, OS = overall survival, TILs = tumor-infiltrating lymphocytes.

Keywords: gastric cancer, meta-analysis, overall survival, tumor infiltrating lymphocytes

1. Introduction

Gastric cancer is the most common type of gastrointestinal malignancies in the world.[1] Surgical resection remains the primary curative treatment for gastric cancer. However, less than 30% of patients eligible for curative resection because the majority of gastric cancer cases present in advanced stage due to late onset and nonspecific symptoms.[2] In recent decade, although treatment of gastric cancer has been significantly improved, the prognoses of patients represented by liver metastases is extremely poor, with a 3-year overall survival (OS) lower than 40% even after hepatectomy,[2] or less than 10% after chemotherapy.[3] Therefore, exploring tumor markers to predict patients' prognoses has important clinical value to prolong their survival time.

Previous studies revealed that immune markers are associated with OS for patients with gastric cancer.[4,5] Tumor-infiltrating lymphocytes (TILs) are the major type of infiltrating immune cells.[4,5] The density of TILs is considered a manifestation of the host immune response against tumor cells. Nowadays, the association between TILs and patients' clinical outcomes have been investigated in non-small cell lung cancer,[6] esophageal squamous cell carcinoma,[7] hepatocellular carcinoma,[8] and breast cancers,[9] and so on. Moreover, lots of studies have investigated the prognostic impact of TILs on patients with gastric cancer, but their results were inconsistent. Therefore, this systematic review comprehensively investigated the prognostic effect of TILs for patients with gastric cancer.

2. Materials and methods

Two independent authors performed a systematic review (PCY and DL) according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses statement.[10] The included studies were evaluated as a cohort study performed in accordance with the Newcastle-Ottawa Quality Assessment Scale (NOS) for quality assessment.[11] This tool was chosen because of the unavailability of randomized controlled trials and

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large heterogeneity between studies. The NOS scale includes the following: selection; comparability; and outcome. Full marks according to NOS are represented by 9 points; scores of 0 to 4 indicate low-quality research, and scores of 5 to 9 indicate high-quality research.[11]

3. Literature search

We searched the literature for studies published in Embase, PubMed, Web of Science, and the Cochrane Library through 31 December 2017 reporting the prognostic role of TILs or its subsets among patients with gastric cancer.

Search keywords included "immune cells" OR "tumor infiltrating lymphocytes" AND "gastric cancer" OR "stomach neoplasm" AND "overall survival" OR "prognostic" OR "prognosis." The literature search was restricted to English-language publications. Besides, all the reference lists of identified articles were also reviewed in order to find out potential studies. When the effective data included in the literature were not reported or when data published in different studies overlapped, we contacted the author to confirm the appropriate data. Two authors were responsible for searching the comprehensive database and evaluating availability independently (PCY and DL).

4. Eligibility criteria

Literatures that were eligible for inclusion in this meta-analysis should meet the following criteria: patients were diagnosed with gastric cancer; studies investigated the role of general TILs or T lymphocyte subsets (including $CD3^+$, $CD8^+$, $CD4^+$, and $FoxP3^+$ lymphocytes) on patients with gastric cancer; the TILs measurement must be detected in situ of tumor tissue by application of HE or immunohistochemistry; the lymphocyte infiltration site should be within the tumor tissue, such as tumor parenchyma, tumor stroma; adequate data (hazard ratio [HR] and its associated 95% confidence interval [95% CI]) were provided for further analysis. When more than one studies were published based on the same population, only the latest information and the most complete study were used.

5. Data extraction and quality assessment

Two authors (PCY and DL) independently extracted data from all eligible studies. Uncertainties were resolved through discussion or the third author (SZ). The following information was extracted from all included studies: name of first author, country, recruitment period, sample size, lymph node metastasis, tumor stage, TIL detection method, cut-off for overexpression, TILs subsets and distribution site, follow-up period, and outcome measures. HR and 95% CI for OS were extracted directly from the enrolled studies.

6. Statistical analysis

HR combination analysis was performed under the assumption of clinical homogeneity. The 95% CI represents the statistical effect. The heterogeneity of each study was analyzed by the χ^2 test. With $P < .1$ as the significance level, heterogeneity was expressed as an I^2 value. When heterogeneity was present, a random effects model (DerSimonian–Laird method) was used; otherwise, a fixed effects model (Mantel–Haenszel test) was employed.

An HR >1.0 was considered to indicate a poor OS for patients with low TILs or its subsets infiltration group; an HR < 1.0 in the high TILs or its subsets infiltration group was associated with good OS. A lack of overlap of the HR (CI) with 1 suggested that the results of visual interpretation had statistical value for OS prediction. All *P*-values were calculated with 2-sided tests.

Effect-quantity pooling, heterogeneity testing, sensitivity analysis, and bias testing were analyzed using the meta-package in R (ver.3.2.3; a language and environment for statistical computing; https://www.R-project.org/).

7. Results

7.1. Results of literature search and study characteristics

A total of 656 studies were identified using our search criteria (Fig. 1), of which 445 were rejected and 211 were retained for abstract review. On the basis of the abstract, 145 studies were excluded and 66 retained and read in full. The main reason of exclusion was because these studies were not investigated the role of general TILs or T lymphocyte subsets (including $CD3^+$, $CD8^+$, $CD4^+$, and $FoxP3^+$ lymphocytes) on patients with gastric cancer. In the end, 29 studies involving 3020 patients were included in the systematic review (Table 1).[12–40] The flow diagram for study selection was shown in Fig. 1. Though 2 studies were based on the same population, different variables were investigated. Therefore, all these 2 studies were included into this meta-analysis.[22,33]

Of the 29 studies, 11 came from China,[13–17,22,33–34,38–40] 7 from Japan,[12,23,25–27,35,36] 6 from Korea,[24,28–32] and the other 5 from the United States,[19] Germany,[20] and Italy,[18,37] respectively. Three studies used hematoxylin-eosin staining[14,19,24] while the other 26 studies used immunohistochemistry staining for detection of general or specific TILs subsets of the tumor tissue.[12,13,15–18,20–23,25–40]

The quality of the 29 eligible studies was assessed in strict accordance with the NOS. The scores were 6 points, suggesting that the methodological quality level of each eligible study was sufficiently high (Table 2).

7.2. Pooled analysis

Six studies reported the prognostic value of general TILs,[14,15,18,19,23,24] 9 studies about $CD8^+$ T lymphocytes,[13,16,20,25,27,31–33,36] 14 studies about $FOXP3^+$ T lymphocytes,[17,20–22,26,28,29,31,34–35,37–40] 2 about $CD4^+$ T lymphocytes,[29,33] and 5 about $CD3^+$ T lymphocytes.[12,20,28,30,32] Association between general TILs or its subsets and patients' prognoses in each study was described in Table 3.

Pooled analysis found that high infiltration of general TILs was associated with statistically higher OS than those with low infiltration (HR 0.75, 95% CI 0.67–0.84; Fig. 2). Moreover, high infiltration of $CD8^+$ T lymphocytes (HR = 0.77, 95% CI 0.63–0.95; Fig. 3) and $CD3^+$ T lymphocytes (HR 0.62, 95% CI 0.49–0.77; Fig. 4) were also associated with statistically higher OS than those with low infiltration. However, gastric cancer patients with high infiltration of $FOXP3^+$ T lymphocytes (HR 1.41, 95% CI 0.97–2.05; Fig. 5) or $CD4^+$ T lymphocytes (HR = 0.86, 95% CI 0.47–1.57; Fig. 6) were only associated with slightly higher OS than those with low infiltration.

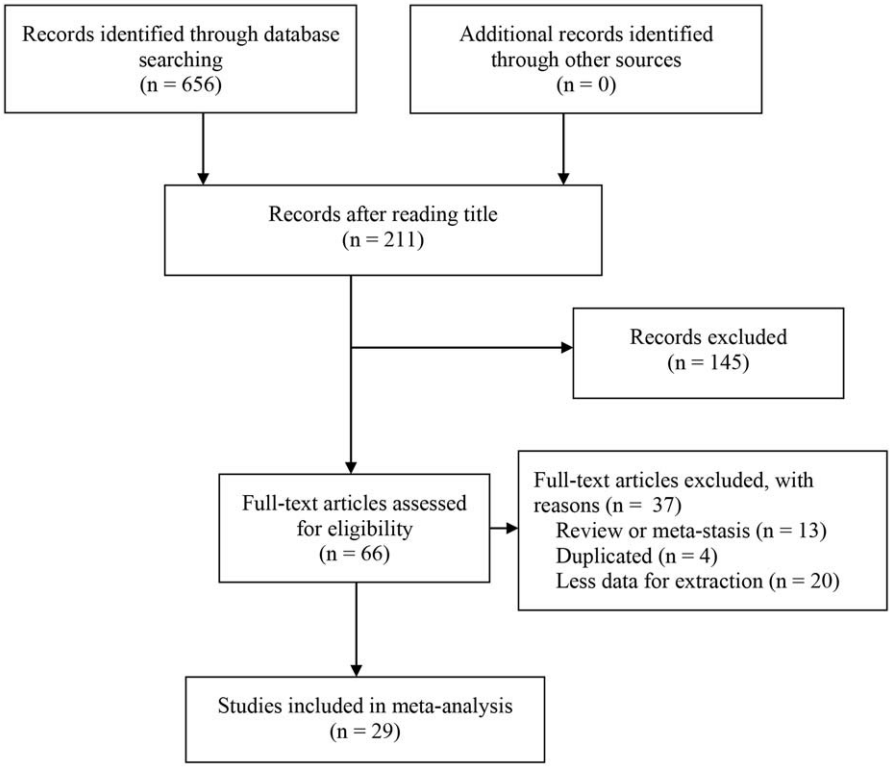

Figure 1. PRISMA flow diagram.

Table 1

Clinicpathological characteristics of the included studies.

Study	Country	Recruitment period	Sample size (M/F)	Lymph node metastasis (P/N)	Tumor stage (I/II/III/IV)	Detected method	Cut-off for overexpression
Chen et al[13]	China	2002–2005	192 (129/63)	51/141	I+II/III+IV: 113/79	IHC	Median
Chen et al[14]	China	1998–2009	152 (117/35)	98/35	10/31/93/18	HE	19/HPF
Dai et al[15]	China	2007–2010	398 (304/94)	–	0-II/III-IV 132/252	IHC	Median
Dong et al[16]	China	2003–2006	100 (72/28)	100	0/0/100/0	IHC	Median
Hu 2 et al[22]	China	2008–2013	102 (69/33)	59/43	I+II/III+IV 26/76	IHC	Median
Geng et al[17]	China	2005–2006	100 (61/39)	68/32	I+II/III+IV: 40/60	IHC	25% field
Li et al[33]	China	2008–2013	192 (138/54)	128/64	I+II/III+IV 48/144	IHC	Median
Liu et al[34]	China	2006–2009	166 (125/41)	118/48	23/41/80/22	IHC	Median
Shen et al[38]	China	1999–2005	135 (89/44)	84/51	40/28/60/7	IHC	Median
Wang et al[39]	China	1998–2004	107 (69/38)	69/38	NA	IHC	Median
Zhou et al[40]	China	2001–2007	133 (89/44)	84/49	I+II/III+IV 50/83	IHC	Median
Arigami et al[12]	Japan	2000–2005	120 (74/46)	66/54	46/16/36/22	IHC	Median
Ishigami et al[23]	Japan	1985–1995	146 (108/38)	74/73	54/26/27/39	IHC	Median
Kawazoe et al[26]	Japan	2002–2010	487 (327/160)	449/38	0/0/358/129	IHC	Median
Kashimura et al[25]	Japan	2000–2004	123 (89/34)	42/81	80/10/13/20	IHC	Median
Kijima et al[27]	Japan	1987–1999	410 (298/122)	247/173	187/134/93/6	IHC	Median
Mizukami et al[35]	Japan	1997–1998	80 (56/24)	41/39	31/28/15/6	IHC	Median
Ohno et al[36]	Japan	1990–1997	84 (57/27)	62/22	I+II/III+IV 31/53	IHC	Median
Kang et al[24]	Korea	2011–2014	120 (96/24)	33/87	74/26/19/1	HE	Median
Kim et al[29]	Korea	2000–2006	180 (126/54)	107/73	0/81/99/0	IHC	Median
Kim et al[31]	Korea	2004–2007	99 (55/44)	57/42	Not clear	IHC	Median
Kim et al[30]	Korea	2003–2004	243 (152/91)	124/119	120/41/82/0	IHC	Median
Kim et al[28]	Korea	2002–2005	396 (270/126)	246/156	165/77/143/11	IHC	Mean
Lee et al[32]	Korea	1995.1–6	220 (156/64)	165/55	67/53/55/45	IHC	75th percentile
Grogg et al[19]	US	1990–1998	110 (72/38)	29/81	15/15/43/37	HE	Median
Haas et al[20]	Germany	1993–2004	52 (40/12)	28/24	20/19/10/3	IHC	Median
Hennequin et al[21]	France	1993–2014	82	–	–	IHC	Median
Giampieri et al[18]	Italy	2007–2013	103 (71/32)	71/32	–	IHC	Median
Perrone et al[37]	Italy	1997–006	110 (53/57)	79/31	NA	IHC	Median

DFS = disease-free survival, F = female, HE, hematoxylin-eosin, HPF = high power field, IHC = immunohistochemistry, M = male, N = negative, NA = not available, P = positive, OS = overall survival, TIL = tumor-infiltrating lymphocyte.

Table 2

Newcastle–Ottawa score quality assessment scale for cohort studies.

	Selection				Comparability		Outcome			
Study	**1**	**2**	**3**	**4**	**1a**	**1b**	**1**	**2**	**3**	**Total star**
Chen et al[13]	a*	a*	b	a*	a*	–†	a*	a*	b	6
Chen et al[14]	a*	a*	b	a*	a*	–†	a*	a*	b	6
Dai et al[15]	a*	a*	b	a*	a*	–†	a*	a*	b	6
Dong et al[16]	a*	a*	b	a*	a*	–†	a*	a*	b	6
Hu et al[22]	a*	a*	b	a*	a*	–†	a*	a*	b	6
Geng et al[17]	a*	a*	b	a*	a*	–†	a*	a*	b	6
Li et al[33]	a*	a*	b	a*	a*	–†	a*	a*	b	6
Liu et al[34]	a*	a*	b	a*	a*	–†	a*	a*	b	6
Shen et al[38]	a*	a*	b	a*	a*	–†	a*	a*	b	6
Wang et al[39]	a*	a*	b	a*	a*	–†	a*	a*	b	6
Zhou et al[40]	a*	a*	b	a*	a*	–†	a*	a*	b	6
Arigami et al[12]	a*	a*	b	a*	a*	–†	a*	a*	b	6
Ishigami et al[23]	a*	a*	b	a*	a*	–†	a*	a*	b	6
Kawazoe et al[26]	a*	a*	b	a*	a*	–†	a*	a*	b	6
Kashimura et al[25]	a*	a*	b	a*	a*	–†	a*	a*	b	6
Kijima et al[27]	a*	a*	b	a*	a*	–†	a*	a*	b	6
Mizukami et al[35]	a*	a*	b	a*	a*	–†	a*	a*	b	6
Ohno et al[36]	a*	a*	b	a*	a*	–†	a*	a*	b	6
Kang et al[24]	a*	a*	b	a*	a*	–†	a*	a*	b	6
Kim et al[29]	a*	a*	b	a*	a*	–†	a*	a*	b	6
Kim et al[31]	a*	a*	b	a*	a*	–†	a*	a*	b	6
Kim et al[30]	a*	a*	b	a*	a*	–†	a*	a*	b	6
Kim et al[28]	a*	a*	b	a*	a*	–†	a*	a*	b	6
Lee et al[32]	a*	a*	b	a*	a*	–†	a*	a*	b	6
Grogg et al[19]	a*	a*	b	a*	a*	–†	a*	a*	b	6
Haas et al[20]	a*	a*	b	a*	a*	–†	a*	a*	b	6
Hennequin et al[21]	a*	a*	b	a*	a*	–†	a*	a*	b	6
Giampieri et al[18]	a*	a*	b	a*	a*	–†	a*	a*	b	6
Perrone et al[37]	a*	a*	b	a*	a*	–†	a*	a*	b	6

†no description or comparability.

Table 3

Associated between tumor-infiltrating lymphocytes and patients' prognoses in each study.

			Outcomes [hazard ratio (95%CI)]	
Study	**TILs or phenotype***	**Follow-up (mons), median (rang)**	**Overall survival**	**Disease-free survival**
Chen et al[13]	$CD8^+$	61 (0.3–81.6)	0.818 (0.529–1.266)	–
Dong et al[16]	$CD8^+$	36.5 (2–88)	0.684 (0.275–1.697)	0.699 (0.286–1.707)
Li et al[33]	$CD8^+$	19 (1–52)	0.764 (0.443–1.318)	–
Kashimura et al[25]	$CD8^+$	–	0.630 (0.390–0.990)	–
Kijima et al[27]	$CD8^+$	30 (1–176)	0.800 (0.330–2.030)	–
Ohno et al[36]	$CD8^+$	38 (3–109)	6.581 (1.959–22.101)	–
Kim et al[31]	$CD8^+$	59 (1–96)	0.485 (0.138–1.701)	–
Lee et al[32]	$CD8^+$	64.4 (1–108)	0.640 (0.408–1.003)	–
Haas et al[20]	$CD8^+$	61	1.260 (0.480–3.320)	–
Hu et al[22]	$FOXP3^+$	19 (1–52)	7.599 (2.155–26.802)	–
Geng et al[17]	$FOXP3^+$	> 60	1.650 (1.120–3.580)	–
Liu et al[34]	$FOXP3^+$	66	5.580 (1.350–23.070)	–
Shen et al[38]	$FOXP3^+$	43 (36–104)	1.158 (0.519–2.584)	–
Wang et al[39]	$FOXP3^+$	62 (2–120)	0.763 (0.442–1.320)	–
Zhou et al[40]	$FOXP3^+$	43 (36–104)	1.906 (1.205–4.238)	–
Kawazoe et al[26]	$FOXP3^+$	–	2.100 (0.630–6.930)	0.640 (0.160–2.510)
Mizukami et al[35]	$FOXP3^+$	87.7	0.850 (0.370–1.970)	–
Kim et al[29]	$FOXP3^+$	45	2.224 (1.206–4.103)	2.253 (1.243–4.085)
Kim et al[31]	$FOXP3^+$	59 (1–96)	0.269 (0.084–0.860)	–
Kim et al[28]	$FOXP3^+$	53.9 (0–84.5)	1.100 (0.600–2.100)	–
Haas et al[20]	$FOXP3^+$	61	0.250 (0.080–0.780)	–
Hennequin et al[21]	$FOXP3^+$	27	–	2.000 (1.000–4.000)
Perrone et al[37]	$FOXP3^+$	–	2.340 (1.270–4.280)	2.000 (1.100–3.650)
Li et al[33]	$CD4^+$	19 (1–52)	1.133 (0.733–1.753)	–
Kim et al[29]	$CD4^+$	45	0.607 (0.338–1.090)	0.708 (0.406–1.236)
Chen et al[14]	TIL	–	0.550 (0.300–0.990)	0.560 (0.320–0.980)
Dai et al[15]	TIL	61.2 (12.2–79.9)	0.788 (0.637–0.975)	–
Ishigami et al[23]	TIL	84	0.600 (0.400–1.700)	–
Kang et al[24]	TIL	22.2 (2.1–50.8)	–	4.839 (0.917–25.525)
Grogg et al[19]	TIL	37 (1–131)	0.820 (0.710–0.960)	–
Giampieri et al[18]	TIL	–	0.390 (0.260–0.580)	0.400 (0.260–0.600)
Arigami et al[12]	$CD3^+$	36 (1–112)	0.650 (0.420–0.970)	–
Kim et al[30]	$CD3^+$	74 (0–123)	0.538 (0.347–0.832)	0.624 (0.399–0.976)
Kim et al[28]	$CD3^+$	53.9 (0–84.5)	0.600 (0.400–1.000)	–
Lee et al[32]	$CD3^+$	64.4 (1–108)	0.670 (0.404–1.123)	–
Haas et al[20]	$CD3^+$	61	0.760 (0.290–1.980)	–

* Variables in multivariate analysis, variables only in univariate analysis were not reported; –, not reported.

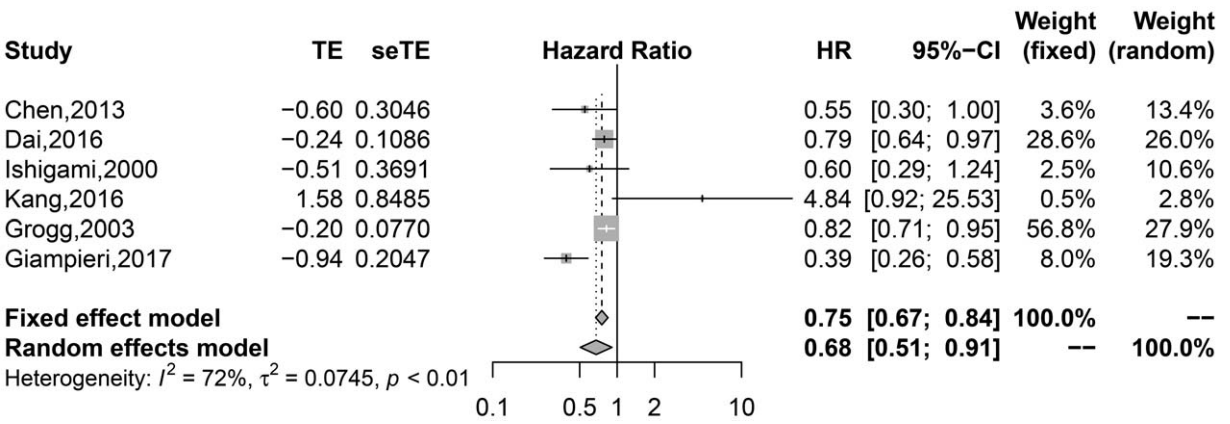

Figure 2. Pooled results for general TILs in patients with gastric cancer.

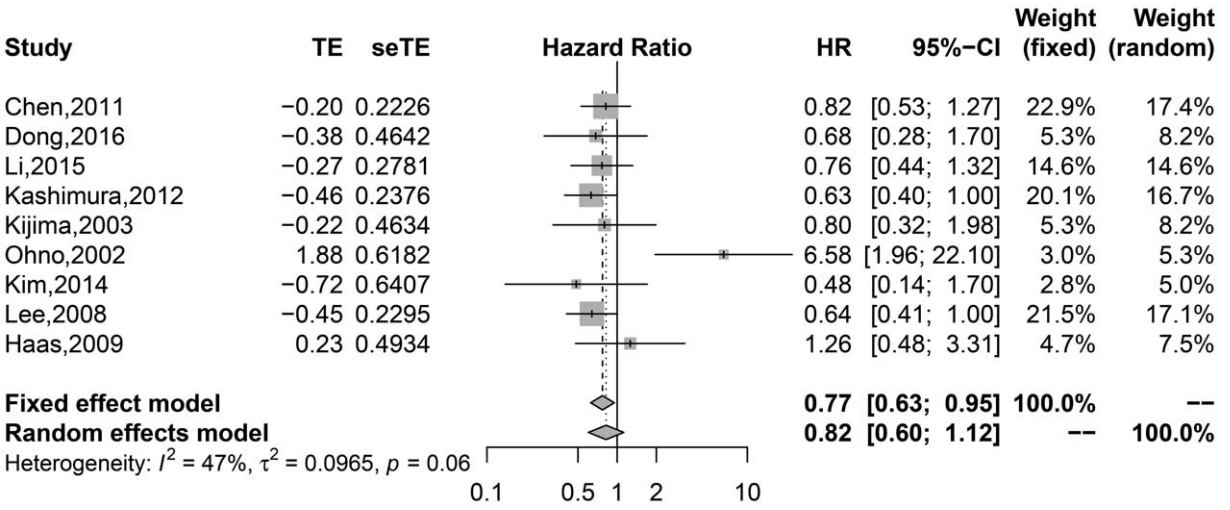

Figure 3. Pooled results for $CD8^+$ TILs in patients with gastric cancer.

8. Discussion

The microenvironment contributes to patients' survival and growth of cancer cells. The gastric tumor microenvironment is frequently filled with a wide range of immune cells, which have been reported to impact on cancer development, progression, and cancer-related immune reactions, emerged as the hotspot of cancer research.

Subgroup analyses based on subset of TILs was performed because of the bidirectional role of TILs in tumor-associated

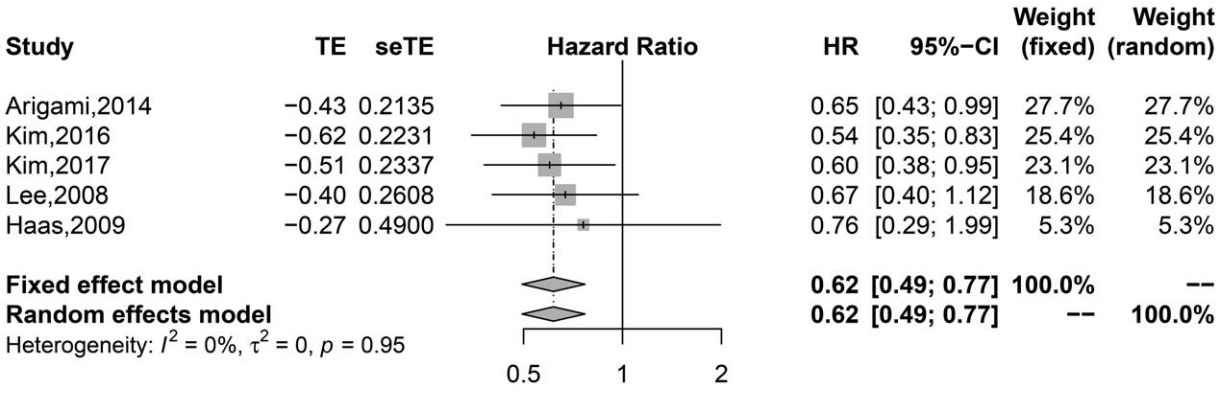

Figure 4. Pooled results for $CD3^+$ TILs in patients with gastric cancer.

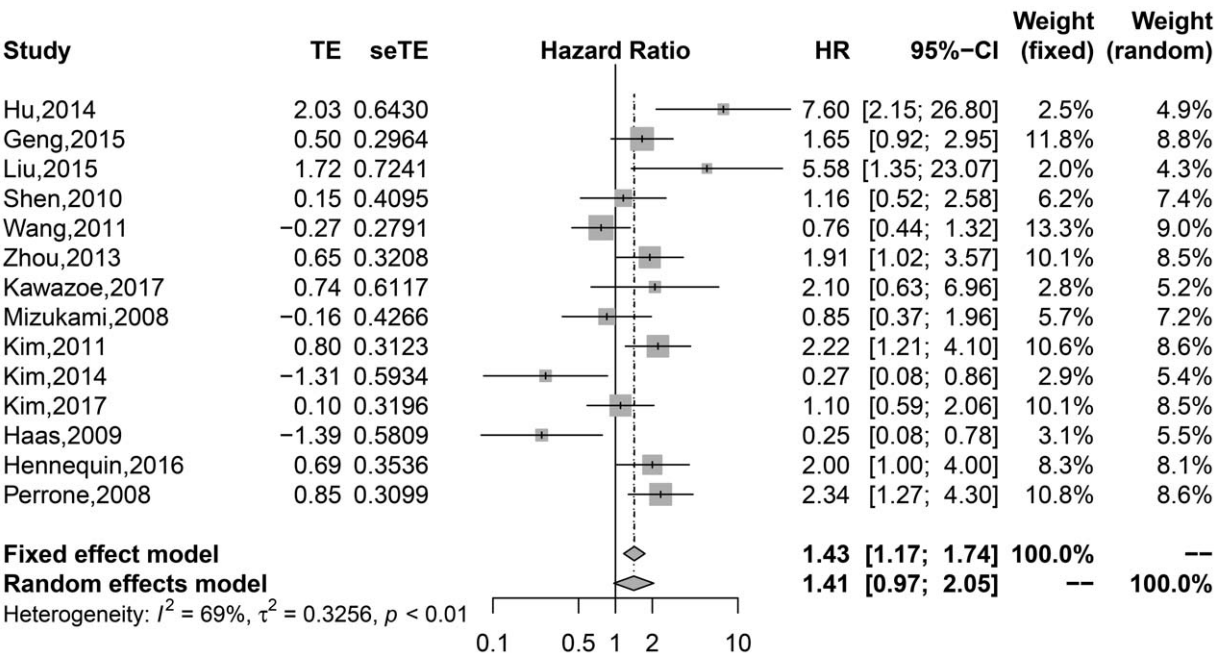

Figure 5. Pooled results for FOXP3$^+$ TILs in patients with gastric cancer.

immune responses. Our study confirmed the importance of intratumoral TILs and its subsets as a prognostic factor, which is in agreement with previous studies.[41–44] Our meta-analysis identified 29 studies that evaluated the prognostic significance of different TIL subsets. It provides evidence that high densities of intratumor general TILs, CD8$^+$, or CD3$^+$ TILs alone are indicative of improved survival, but the presence of FOXP3$^+$ and CD4$^+$ TILs alone are not significantly associated with the prognosis.

Each subset of TILs plays its own roles in the development and progression of gastric cancer. CD8$^+$ TILs is the surface antigens of cytotoxic T lymphocytes. And it is the main effective cells in the anti-tumor immune response. In our study, we found CD8$^+$ TILs were associated with better OS. However, 2 included studies were not in accordance with our drawn conclusions,[20,36] which may be due to sample size bias. However, CD4$^+$ lymphocytes are composed of T helper and regulatory cells. These cells can secret diverse cytokines. Therefore, the roles of CD4$^+$ T cells are complicated by their heterogeneity trait. In the present study, we did not found the significant prognostic value of CD4$^+$ TILs.

CD3 is a common surface antigen of T cells. We found gastric cancer patients with high infiltration of CD3$^+$ TILs had significantly higher OS than those with low infiltration. This finding is consistent with that the infiltration of CD3$^+$ TILs significantly correlated with tumor stage, lymph node metastasis, and depth of tumor invasion.[12,32] Higher infiltration of CD3$^+$ TILs is also significantly correlates with higher OS in other cancers.[45,46] Therefore, as an immunological predictor of tumor stage and disease outcome in cancer patients, the infiltration of CD3$^+$ TILs may decreases during tumor progression.[12] Considering the close relationship between the infiltration of CD3$^+$ TILs and patients' prognoses, immunohistochemical analysis of CD3$^+$ TIL infiltration in endoscopic resected specimens might help to identify tumor stage after endoscopic resection. In addition, measuring CD3$^+$ TIL infiltration in resected specimens may be helpful to identify the induction of adjuvant chemotherapy in patients with gastric cancer.

The prognostic value of intratumoral FOXP3$^+$ TILs infiltration in gastric cancer is still in debate. Some studies showed that FOXP3$^+$ TILs infiltration was associated with decreased overall survival in gastric cancer[17,22,34,40] while other studies failed to uncover such an association.[26,28,35,38,39] A previous meta-analysis found tumor-infiltrating FOXP3$^+$ TILs were a factor for

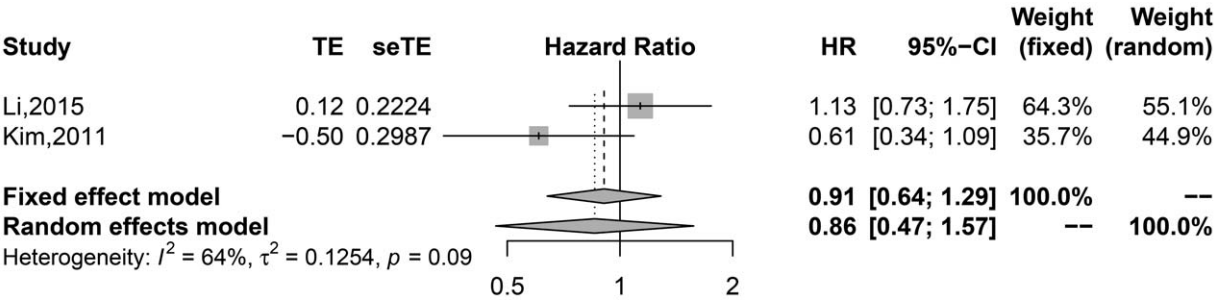

Figure 6. Pooled results for CD4$^+$ TILs in patients with gastric cancer.

a poor prognosis for hepatocellular carcinoma and gastric cancer, but a good prognosis for colorector cancer.[47] Our study included 14 studies investigating the relationship between FOXP3+ T lymphocytes and the prognoses of patients with gastric cancer. We did not found a positive or negative association. These discrepancies may be partly attributed to differences on the method for specimen processing, the difference on the selection of FOXP3+ TILs markers in each study, the ethnics of population, and the histology of gastric cancer patients.

This study has some limitations. First, cut-off for overexpression of TILs is different in some studies. Second, median follow-up in some included studies may be too short to observe long term prognoses. Third, the method of immunohistochemical technique may be discrepency in tissue fixation, antibodies used for T cell detection. For example, 3 studies[14,19,24] with hematoxylin-eosin staining. It may be difficult to distinguish the biomarkers of TIL only via hematoxylin-eosin staining. And fourth, the heterogeneity of some meta-analysis is still remarkable.

9. Conclusion

This meta-analysis suggests that TILs were prognostic markers for OS in gastric cancer patients. In addition, a high density of introtumoral CD8+ and CD3+ lymphocyte indicated good prognosis in gastric cancer, while CD4+ and FOXP3+ lymphocytes demonstrated no obvious effect on survival outcomes. However, due to the limitations of this study, TILs can't fully explain its impact on prognosis. Therefore, further studies should focus on high-quality prospective studies, including a comprehensive clinical pathology, evaluation of information, follow-up strategy, and standardized cut-off values, similar treatment strategies, multivariate analyses of clinicopathological variables of the patients, which will make the study more standardized. Besides, quantitative studies of TILs alone are far from explaining the complex effects of tumor microenvironment, which requiring more rigorous design, greater sample size, more standardized survival analyzes, and longer follow-up studies to produce more credible statistics.

Author contributions

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