Systematic Review and Meta-Analysis

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Prognostic role of tumor-infiltrating lymphocytes in gastric cancer

A systematic review and meta-analysis

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Abstract

Background: The potential prognostic value of tumor-infiltrating lymphocytes (TILs) in gastric cancer remains controversial. This meta-analysis examines the association between TILs and survival outcomes in gastric cancer.

Methods: Twenty-two eligible studies were identified using the PubMed and Google Scholar databases. The combined sample size of the 22 studies was 2941, and the median sample size of the individual studies was 122 patients (52–220). The main clinical outcomes examined were overall cancer survival (OCS) and overall cancer relapse-free survival (OCRFS).

Results: Tumor tissue CD3(+) TILs, indicative of pan-T-cell expression, had a positive effect on survival with a hazard ratio (HR) of 0.64 (95% confidence interval [CI] 0.52–0.78) for OCS, as did the non-FOXP3(+) T-cell subgroup with an HR of 0.66 (95% CI 0.57–0.75), particularly in CD8(+) lymphocytes (HR = 0.63, 95% CI 0.48–0.83). On the contrary, high FOXP3(+) T-cell expression was correlated with reduced OCS, with an HR of 1.75 (95% CI 1.26–2.42). Analysis of the seven studies evaluating OCRFS revealed improved OCRFS with infiltration of non-FOXP3(+) TILs with an HR of 0.59 (95% CI 0.42–0.81) but not FOXP3(+) T lymphocytes with an HR of 1.82 (95% CI 1.30–2.53).

Conclusion: The results from this meta-analysis suggest that high expression of TILs, mainly by CD8 lymphocytes, may be a potential prognostic biomarker in patients with gastric cancer.

Abbreviations: CI = confidence interval, CSS = cancer-specific survival, DFS = disease-free survival, EBV = Epstein–Barr virus, HR = hazard ratio, OCRFS = overall cancer relapse-free survival, OCS = overall cancer survival, OS = overall survival, RFS = relapse-free survival, TIL = tumor-infiltrating lymphocyte.

Keywords: gastric cancer, meta-analysis, prognosis, TIL subtype, tumor-infiltrating lymphocytes

1. Introduction

Gastric cancer is the third leading cause of cancer-related mortality.[1] Despite remarkable advances in gastric cancer treatment, including targeting agents, such as trastuzumab and ramucirumab,[2,3] the prognosis of patients with advanced gastric cancer remains poor. A growing body of evidence suggests that immunotherapy may be suitable for treating cancers with a high tumor mutation burden, such as gastric cancer.[4] Thus, a more in-depth understanding of tumor immunity based on immunity-specified research outcomes for the successful treatment of tumor immunotherapy is needed.

Based on the presence or absence of immune cell infiltrations, tumors can be classified into 2 groups: T-cell-inflamed tumors and non-inflamed tumors.[5] T-cell-inflamed tumors, where tumor cells are surrounded by different infiltrating inflammatory cells (eg, T cells, B cells, myeloid lineage leukocytes, natural killer cells, macrophages, and dendritic cells) that contribute to either pro- or anti-tumor activities, are considered immunologically responsive.[6] Of the infiltrating inflammatory cells, tumor-infiltrating lymphocytes (TILs) act as major determinants of the host immune response to tumor cells. The degree of TIL infiltration is thought to be associated with controlling the growth, progression, and metastasis of cancer, as well as with predicting the response of cytotoxic treatments, such as chemotherapy and radiotherapy. However, the results of studies examining TILs and clinical outcomes of breast, oesophagus, and lung malignancies have conflicted.[7–9]

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JHH and YHK contributed equally to this work.

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Due to small sample sizes and the use of varying T-cell subsets, the prognostic role of TILs in gastric cancer has not been clearly elucidated. An understanding of the clinical role of TILs would allow the classification of patients by prognosis and the identification of high-risk cases requiring aggressive approaches. Therefore, we performed a literature-based meta-analysis of eligible studies to obtain evidence-based results on the prognostic role of TILs in gastric cancer.

2. Materials and methods

2.1. Search strategy and selection criteria

PubMed and Google Scholar were used to identify studies published up to December 2016 containing one or more of the following keywords: "stomach neoplasm," "stomach cancer," "gastric cancer," "tumor-infiltrating lymphocytes," "CD3," "CD4," "CD8," "CD25," "CD45RO," "FOXP3," "HELIOS," "T-bet," "RORr T cell," "PD1," or "IL17." The reference lists of relevant studies were also searched. Only studies meeting the following criteria were included: clinical study on gastric cancer patients; assessment of overall survival (OS), cancer-specific survival (CSS), disease-free survival (DFS), or relapse-free survival (RFS) using multivariate hazard ratios (HRs); and evaluation of TIL expression in primary tumor tissue (not blood or lymph nodes). Exclusion criteria were as follows: review articles, letters, or abstracts; evaluations of neoadjuvant chemotherapy; lack of appropriate data; and non-English or unpublished articles. Statistical data were reviewed prior to inclusion in the final sample.

2.2. Data extraction, quality assessment, and statistical methods

For the meta-analysis, the effect size was evaluated using multivariate HRs with 95% confidence intervals (CIs) comparing high TIL expression to cancer survival. Survival outcomes were measured from the time at which the baseline tissue sample was obtained to the date of the event or date of last follow-up. The definitions of the event for OS (or CSS) and DFS (or RFS) were death from any cause or cancer recurrence, respectively. OS (or CSS) was defined as overall cancer survival (OCS), and DFS (or RFS) was defined as overall cancer relapse-free survival (OCRFS). The quality of selected studies was systematically evaluated by 2 reviewers using the Newcastle–Ottawa scale. Data were recorded on a predefined form. The meta-analysis statistics were obtained using RevMan (version 5.3.5). The heterogeneity of the combined HRs was assessed using Cochran Q test and Higgins I^2 statistic. A P-value of $<.1$ was considered statistically significant. If heterogeneity was observed among the studies ($P<.1$), a random effect model (DerSimonian and Laird method) was applied; if no heterogeneity was observed ($P>.1$), the fixed effects model was used. Publication bias was evaluated using the funnel plot with Egger bias indicator test.

3. Results

3.1. Study selection

The literature search identified 1943 unique studies. In total, 1809 studies were removed based on the following criteria: unpublished, non-English, letters or abstracts, withdrawn articles, review articles, non-human studies, and articles that could not be accessed. Of the remaining 134 studies, 81 were excluded because they were not relevant to the current analysis. Of the remaining 53 studies, 5 did not have OS data, 8 did not calculate multivariate HRs for survival, and 18 did not evaluate TIL expression in tumor tissue. In total, 22 eligible studies were selected for the final analysis; 21 of these were included in the meta-analysis of OCS (OS or CSS), and 7 were included in the meta-analysis of OCRFS (DFS or RFS). Six studies evaluated both OS (or CSS) and DFS (or RFS). A flow chart of the article selection process is shown in Figure 1.

3.2. Study characteristics

The main features of the 22 eligible studies are summarized in Table 1. Briefly, the studies were published between 2000 and 2016 and had sample sizes ranging between 52 and 220 patients (median: 122); the combined sample size was 2941. All studies were non-randomized and retrospective.

3.3. Quality assessment and meta-analysis

Study quality was systematically assessed using the criteria of the Newcastle–Ottawa scale. Studies were evaluated based on: selection of study groups, comparability of the groups, and ascertainment of exposure and outcome. The criteria were assessed using a star scoring system, with higher scores given to higher quality studies. A summary of the quality assessment is provided in Table 2.

3.4. Effect of TIL expression in gastric cancer tissues on OCS

The OCS analysis included 21 studies evaluating expression of all TIL subtypes in gastric tissue samples. Pooled analyses of CD3(+) cells or TILs in tumor tissue, indicative of pan-T-cell expression, were positively correlated with OCS (pooled HR = 0.64, 95% CI 0.52–0.78, Fig. 2) with low heterogeneity ($P=.71$, $I^2=0\%$). Pooled analyses of each non-FOXP(+) subgroup revealed significant correlations between expression of CD4(+) and CD8(+) in tumor tissues and OCS (CD4: pooled HR = 0.70, 95% CI 0.55–0.90, Fig. 3A; CD8: pooled HR = 0.63, 95% CI 0.48–0.83, Fig. 3B). A fixed-effects model was used given the low heterogeneity among the studies (CD4: $P=.13$, $I^2=43\%$; CD8: $P=.88$, $I^2=0\%$). The pooled HR of 0.66 (95% CI 0.57–0.75) observed for the non-FOXP(+)-cell subgroups reflects a significant association between high non-FOXP3(+) TIL expression in cancer tissues and OCS (Supplementary Fig. 1, http://links.lww.com/MD/C377). In contrast, a pooled analysis of the FOXP3(+) regulatory T-cell (Treg) subgroup revealed that high FOXP3(+) expression was correlated with reduced OCS (pooled HR = 1.75, 95% CI 1.26–2.42, Fig. 3C) with high heterogeneity ($P=.003$, $I^2=63\%$).

3.5. Effect of TIL expression in gastric cancer tissues on OCRFS

The OCRFS was assessed in 7 studies evaluating expression of all TIL subtypes in tissue samples. Subgroup analyses of OCRFS were conducted by non-FOXP3(+) or FOXP3(+) T-cell population. Pooled analyses of non-FOXP3(+) subgroups such as TILs, CD4(+) or CD8(+) expression was positively correlated with OCRFS (pooled HR = 0.59, 95% CI 0.42–0.81) with low

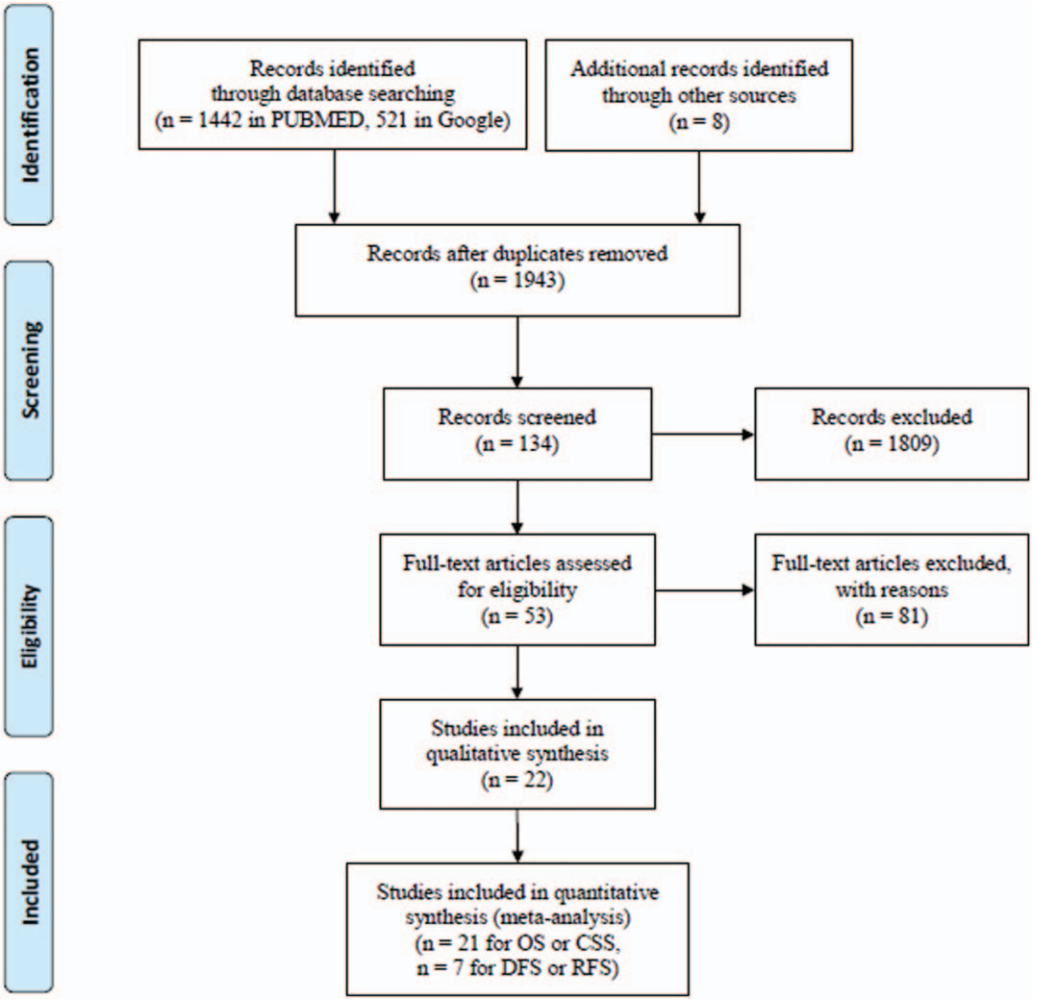

Figure 1. Flow chart of the selection process of eligible articles. CSS = cancer-specific survival, DFS = disease-free survival, OS = overall survival, RFS = relapse-free survival.

heterogeneity among the studies ($P = .58$, $I^2 = 0\%$; Fig. 4A). The FOXP3(+) Treg subgroup was negatively correlated with OCRFS (pooled HR = 1.82, 95% CI 1.30–2.53) with low heterogeneity among the studies ($P = .92$, $I^2 = 0\%$; Fig. 4B).

3.6. Analysis of clinical outcomes based on the location of TILs in gastric cancer tissues

The biologic functions of TILs differ based on their location in the tumor.[6,32] Due to the small number of studies evaluating peritumoral TILs, only intratumoral TILs were assessed. The analysis of the studies of intratumoral TILs showed more robust HRs for OCS than for all TILs. A pooled analysis of the pan-T-cell TILs, such as CD3/TIL, CD4, and CD8, in intratumoral gastric cancer tissues revealed a correlation between tissue expression and OCS (CD3/TIL: pooled HR = 0.64, 95% CI 0.52–0.78; CD4: pooled HR = 0.70, 95% CI 0.55–0.90; CD8: pooled HR = 0.64, 95% CI 0.48–0.85; Supplementary Fig. 2a–c, http://links.lww.com/MD/C377). In contrast, a pooled analysis of the intratumoral FOXP3(+) Treg subgroup showed that high FOXP3 (+) expression was significantly correlated with reduced OCS (pooled HR = 1.89, 95% CI 1.54–2.31, Supplementary Fig. 2d, http://links.lww.com/MD/C377).

3.7. Effect of gastric cancer tissue TILs on OCS in Asian and Western populations

Recently, molecular analyses of gastric cancer have suggested that different immune signature between Asian and Western populations affect the outcome[32]; therefore, the prognostic role of TILs in studies of each Asian and Western gastric cancer patients were evaluated, respectively. Subgroup analyses for each TIL subtype in Asian studies indicated that CD3/TIL, CD4, and CD8 expression levels were positively correlated with OCS (CD3/TIL: pooled HR = 0.65, 95% CI 0.53–0.80; CD4: pooled HR = 0.70, 95% CI 0.55–0.90; CD8: pooled HR = 0.63, 95% CI 0.46–0.85; Supplementary Fig. 3a–c, http://links.lww.com/MD/C377) with low heterogeneity among the studies ($P = .71$, $I^2 = 0\%$; $P = .13$, $I^2 = 43\%$; and $P = .85$, $I^2 = 0\%$, for CD3, CD4, and CD8, respectively). FOXP3 expression was correlated with reduced OCS (pooled HR = 1.66, 95% CI 1.13–2.44) with high heterogeneity ($P = .0001$, $I^2 = 69\%$, Supplementary Fig. 3d, http://links.lww.com/MD/C377). Western studies showed similar patterns in the prognostic role of TILs; however, due to a smaller number of studies, a lower statistical significance was observed (CD3/TIL: pooled HR = 0.45, 95% CI 0.19–1.03; CD8: pooled HR = 0.64, 95% CI 0.34–1.19; FOXP3: pooled

Table 1

Characteristics of the 22 eligible studies evaluating TIL expression in gastric tissue samples and patient survival.

Author	Year published	Country	Sample no	Location of TILs	Outcome	Subtype of TILs	High (%)	Stage	FUD	Criteria for cutoff value of high vs low expression	Quantification method
Kang[10],*	2016	Korea	120	Str	DFS	str-TIL	61	I–IV	22.2 mo†	Median (≥25 or <25) (stir-TIL score, ×40 LPF)	NS‡
Liu[11]	2015	China	166	IT/Str	OS	Foxp3+	50	I–IV	65.88 mo†	Median (counts, ×400 HPF)	Blinded‡
Li[12]	2015	China	192	IT	OS	CD4+/CD8+	48/54	I–IV	NS	Scores ≤1+ (low) Scores ≥2 (high) (HPF, ×400)§	Blinded‡
Geng[13]	2015	China	100	IT	OS	Foxp3+	76	I–IV	>5 y	>25% (rate of positive cell staining, ×400 HPF)	NS‡
Ma[14]	2014	China	197	IT/Str	OS	Foxp3+	12	I–IV	102 mo¶	>25 (high), <5 (low) (count, ×400 HPF)	Blinded‡
Kim[15],‖	2014	Korea	99	IT/Str	OS	CD8+/Foxp3+	40/49	I–IV	59 mo¶	CD8+(60th percentile) FoxP3+ TIL (median) (counts)	NS
Arigami[16]	2014	Japan	120	IT	OS	CD3+	50	I–IV	36 mo†	Median (>60) (cell counts, ×200 HPF)	Blinded‡
Zhou[17]	2013	China	133	IT	OS	Foxp3+	65	T1–T4	43 mo†	NS	Blinded‡
Wakatsuki[18]	2013	Japan	74	IT	OS	CD45RO+	50	I–IV	NS	Mean (cell counts, ×400 HPF)	NS
Tuncel[19]	2013	Turkey	52	IT	OS	CD8+/FoxP3+	54/50	I–IV	NS	>10 (cell counts, ×400 HPF)	Blinded**
Dong[20]	2013	China	100	IT	OS/DFS	CD8+	38	I–IV	36.5 mo†	Median (cell counts, ×400 HPF)	Blinded‡
Chen[21]	2013	China	152	IT	OS/DFS	T-bet+	NS	I–IV	NS	>19.05 (cell counts, ×200 HPF)	Blinded‡
Kashimura[22]	2012	Japan	123	IT	OS/DFS	Foxp3+	50	I–IV	NS	Median (cell counts, ×400 HPF)	Blinded††
Wang[23]	2011	China	107	IT/Str	OS	Foxp3+	50	I–IV	62 mo†	Median (cell counts, ×400 HPF)	Blinded‡
Kim[24]	2011	Korea	180	IT	OS/RFS	CD4+	50	I–III	45 mo†	Median (cell counts, ×NS)	NS
Du[25]	2011	China	179	IT	OS/DFS	Foxp3+	49	I–IV	21 mo†	Median	Blinded‡‡
Shen[26]	2010	China	133	IT	OS	Foxp3+	50	I–IV	43 mo†	Median (cell counts, ×400 HPF)	Blinded‡
Perrone[27]	2008	Italy	110	IT	OS/RFS	Foxp3+	53	II–III	3 y§§	≥6 (cell counts, ×NS)	Blinded‡
Lee[28]	2008	Korea	220	IT	OS	CD3+/CD8+/CD45RO+	35/25/30	I–IV	64.4 mo¶	Mean	NS
Chiaravalli[29]	2006	Italy	96	IT	OS	CD3+/CD8+	50/56	I–IV	64.3 mo¶	>14.9 (CD3+) >9.5 (CD8+) (cell counts, ×400 HPF)	NS
Ishigami[30]	2002	Japan	185	IT	OS	CD3ζ	65	I–IV	NS	>66% (CD3ζ/CD3ε ratio, ×400 HPF)	NS
Ishigami[31]	2000	Japan	66	IT	CSS	TIL	33	I–IV	7 y 3 mo¶	>150¶¶ (cell counts, ×400 HPF)	NS

CSS = cancer-specific survival, DFS = disease-free survival, FUD = follow-up duration, HPF = high power field, IT = intratumoral, LPF = lower power field, mo = months, NS = no statement, OS = overall survival, RFS = relapse-free survival, Str = stromal, TIL = tumor-infiltrating lymphocyte, xNS = no statement about power field, y = years.

* Epstein–Barr virus (EBV)-associated gastric cancer.

† Median.

‡ Counted by 2 observers.

§ Assessed in terms of staining intensity and percentage of positive cells as follows: 0 (negative, ≤5% of cells staining positive), 1+ (weak staining, 6–25% of cells staining positive), 2+ (moderate staining, 26–50% of cells staining positive), and 3+ (strong staining, > 50% of cells staining positive). The final score for each slide was presented as the average of 3 representative high-power fields.

¶ Mean.

‖ MSI+ gastric cancer with microsatellite instability (MSI).

** Counted by 1 observer.

†† No statement about the total number of observers to count cells.

‡‡ Counted by 3 observers.

§§ Minimum.

¶¶ Those with >25 natural killer cells found in 25 intratumoral field and >150 lymphocytes per HPF were designated as the high infiltrating lymphocyte (IL) group.

HR = 2.24, 95% CI 1.38–3.65; Supplementary Fig. 4a–c, http://links.lww.com/MD/C377).

4. Discussion

Recent advances in tumor immunology have shown dynamic and complex interactions between immune and tumor cells, and these interactions are crucial for tumor progression.[33] Thus, it is important to identify the prognostic role of TILs in gastric cancer. The present meta-analysis of 2941 cases, which provides a quantitative assessment of the prognostic value of TILs in gastric cancer patients, revealed a significant association between high pan-T-cell marker (+) TIL levels in tumor tissue and improved survival. TIL subtypes, such as CD8(+), CD4(+), and FOXP3(+) lymphocytes, play different roles in predicting the clinical outcome of gastric cancer patients. These results indicate that the immune cell subtypes in tumors are important for predicting the clinical outcomes of gastric cancer patients.

A growing body of evidence has shown that high densities of TILs are associated with a favorable prognosis in some immunogenic tumors, and gastric cancer is thought to be an immunogenic tumor.[34] High pan-T-cell expression or TILs in tumor tissue was significantly correlated with favorable OCS. This suggests that the adaptive immunity mediated by T lymphocytes acts as an active antitumor response by eradicating cancer cells and avoiding tumor growth.[35] In a study of 200 gastric cancer patients, the high-density groups for CD3, CD8, and CD45RO had significantly longer survival times than the corresponding low-density groups.[28] The association between TILs and good prognosis is well known for breast cancer[36]; however, this relationship in gastric cancer patients is unclear. A recent in vitro study reported that adaptive immune responses can be initiated in the inflammatory microenvironment of gastric tumors, and TILs can induce apoptosis in gastric cancer models.[37] Releasing tumor antigens into the tumor microenvironment using cytotoxic chemotherapy or radiotherapy induces cell-mediated apoptosis via activation of cytotoxic T-cell lymphocytes. Interestingly, an increase in the number of TILs in patients with microsatellite instability or Epstein–Barr virus (EBV)-associated gastric cancer was associated with a

Table 2

Quality assessment of 21 nonrandomized studies included in the meta-analysis using the Newcastle–Ottawa scale criteria.

		Selection				Comparability	Outcome			
Author	Year published	S1	S2	S3	S4	C1	O1	O2	O3	Total stars
Kang[10]	2016	B (1*)	A (1*)	A (1*)	A (1*)	AB (1*)	B (1*)	B (0)	B (1*)	8
Liu[11]	2015	B (1*)	A (1*)	A (1*)	A (1*)	AB (1*)	A (1*)	A (1*)	B (1*)	9
Li[12]	2015	B (1*)	A (1*)	A (1*)	A (1*)	AB (1*)	A (1*)	B (0)	D (0)	7
Geng[13]	2015	B (1*)	A (1*)	A (1*)	A (1*)	AB (1*)	B (1*)	A (1*)	D (0)	8
Ma[14]	2014	B (1*)	A (1*)	A (1*)	A (1*)	AB (1*)	B (1*)	A (1*)	D (0)	8
Kim[15]	2014	B (1*)	A (1*)	A (1*)	A (1*)	AB (1*)	B (1*)	A (1*)	D (0)	8
Arigami[16]	2014	B (1*)	A (1*)	A (1*)	A (1*)	AB (1*)	A (1*)	B (0)	A (1*)	8
Zhou[17]	2013	B (1*)	A (1*)	A (1*)	A (1*)	AB (1*)	A (1*)	A (1*)	A (1*)	9
Wakatsuki[18]	2013	B (1*)	A (1*)	A (1*)	B (0)	AB (1*)	B (1*)	A (1*)	D (0)	6
Tuncel[19]	2013	B (1*)	A (1*)	A (1*)	B (0)	AB (1*)	A (1*)	A (1*)	D (0)	6
Dong[20]	2013	B (1*)	A (1*)	A (1*)	A (1*)	AB (1*)	B (1*)	A (1*)	B (1*)	9
Chen[21]	2013	B (1*)	A (1*)	A (1*)	A (1*)	AB (1*)	A (1*)	A (1*)	D (0)	7
Kashimura[22]	2012	B (1*)	A (1*)	A (1*)	B (0)	AB (1*)	B (1*)	A (1*)	D (0)	7
Wang[23]	2011	B (1*)	A (1*)	A (1*)	A (1*)	AB (1*)	A (1*)	A (1*)	A (1*)	9
Kim[24]	2011	B (1*)	A (1*)	A (1*)	A (1*)	AB (1*)	A (1*)	A (1*)	D (0)	8
Du[25]	2011	B (1*)	A (1*)	A (1*)	A (1*)	AB (1*)	B (1*)	A (1*)	A (1*)	9
Shen[26]	2010	B (1*)	A (1*)	A (1*)	A (1*)	AB (1*)	A (1*)	A (1*)	A (1*)	9
Perrone[27]	2008	B (1*)	A (1*)	A (1*)	A (1*)	AB (1*)	A (1*)	A (1*)	B (1*)	9
Lee[28]	2008	B (1*)	A (1*)	A (1*)	B (0)	AB (1*)	B (1*)	A (1*)	B (1*)	8
Chiaravalli[29]	2006	B (1*)	A (1*)	A (1*)	B (0)	AB (1*)	B (1*)	A (1*)	A (1*)	8
Ishigami (2002)[30]	2002	B (1*)	A (1*)	A (1*)	B (0)	AB (1*)	B (1*)	A (1*)	D (0)	7
Ishigami (2000)[31]	2000	B (1*)	A (1*)	A (1*)	B (0)	AB (1*)	B (1*)	A (1*)	B (1*)	8

more pronounced response to treatment as well as longer OS.[10,38,39]

Furthermore, in this meta-analysis, high expression of CD8(+) lymphocytes was the strongest predictor of improved survival. Cytotoxic T cells have a crucial role in determining the clinical outcomes of patients. Thus, it could be speculated that a large fraction of the tumor-reactive CD8(+) T cells among TILs recognize specific mutant neoantigens formed in the individual patient's tumor.[40] Immune checkpoint blockade using monoclonal antibodies against CTLA-4 and PD-1/PD-L1 has shown a long-lasting clinical response in many solid tumors, such as gastric cancer.[4] Currently, it is recognized that neoantigens and major histocompatibility complex induce CD8(+) T-cell activation, resulting in the elimination of cancer cells. Therefore, mutation burdens in the tumor genome and related cytotoxic TILs are reliable predictors of clinical outcomes.

The transcription factor forkhead box P3 (FOXP3), characterized by the $CD4^+CD25^+FOXP3^+$ phenotype, is a key intracellular molecule for the development and functioning of Treg, and it is considered to be one of the most specific Treg markers.[41] Tregs play an essential role in modulating host responses to tumors and infections, and they are known to be the mediators of self-immune tolerance in the tumor microenvironment via suppression of antitumor cytotoxic T cells. Thus, a high infiltration of Tregs in tumor tissues is expected to be associated with an unfavorable outcome via immune escape of the tumor.[42] In this analysis, high expression of FOXP3(+) cells was significantly negatively correlated with clinical outcomes. In a previous study of 100 gastric cancer specimens, high FOXP3 expression in gastric cancer tissue was associated with lymph node metastasis and poorer survival.[13] Thus, targeting Treg cell is one of the most clinically relevant and therapeutic strategies.[43]

The different microlocalization of TILs in various tumor tissues could have distinct clinical roles in determining their relationship to disease prognosis.[6,36,44,45] The migration factors for TILs were

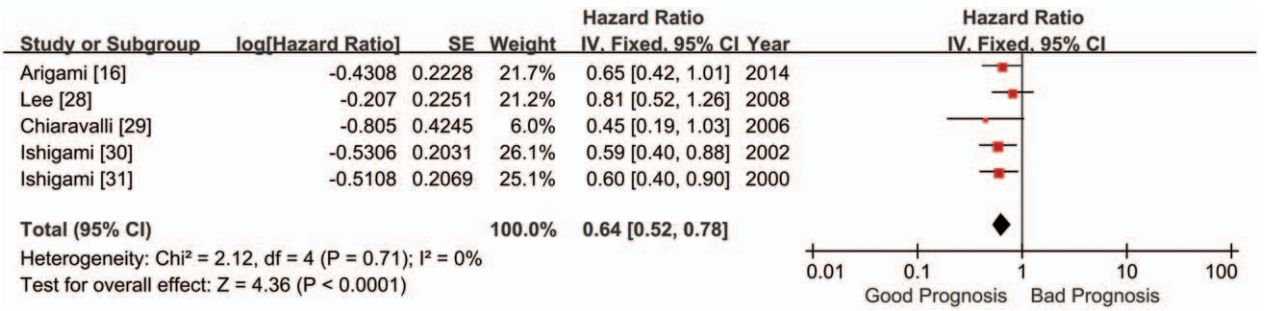

Figure 2. Forest plot of hazard ratios for the prediction of overall cancer survival (OCS) by CD3(+) cells or tumor-infiltrating lymphocytes (TILs) in gastric cancer tissues. High expression of CD3(+) cells or TILs were favorably correlated with OCS (pooled hazard ratio = 0.64, 95% confidence interval [CI] 0.52–0.78) with low heterogeneity ($P = .71$, $I^2 = 0\%$).

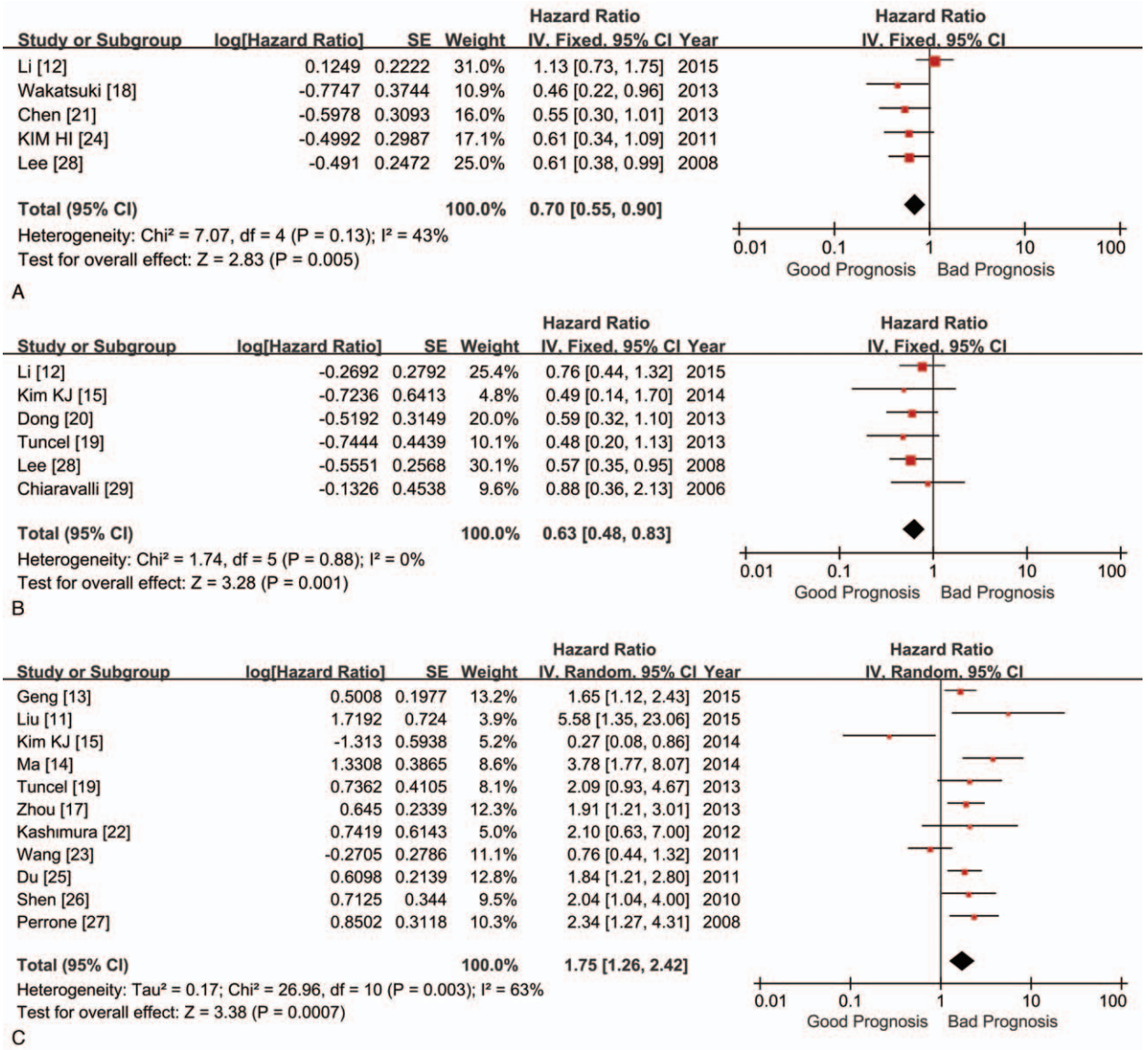

Figure 3. Forest plot of hazard ratios for the prediction of overall cancer survival (OCS) by (A) CD4(+), (B) CD8(+), and (C) FOXP3(+) tumor-infiltrating lymphocytes in gastric cancer tissues. High expression of (A) CD4(+) and (B) CD8(+) were significantly correlated with increased OCS (CD4: pooled hazard ratio [HR] = 0.70, 95% confidence interval [CI] 0.55–0.90; CD8: pooled HR = 0.63, 95% CI 0.48–0.83), while high (C) FOXP3(+) expression was correlated with reduced OCS (pooled HR = 1.75, 95% CI 1.26–2.42) with high heterogeneity ($P = .003$, $I^2 = 63\%$).

mainly produced in the peritumoral region as a result of the tumor-stromal reaction.[46] In breast cancer, because the evaluation of the stromal compartment has been proven to be more reproducible between studies, the evaluation of stromal or peritumoral lymphocytes is recommended as the principal parameter by the current international guideline.[6,36] In this meta-analysis, the prognostic pattern of TILs in the intratumoral lymphocytes subgroup was similar to those in the overall population. Recently, Kang et al, in a study of EBV-associated gastric cancer, showed that the stromal TIL positivity was significantly associated with better clinical outcomes than intratumoral TIL positivity tumors.[10] On the contrary, an analysis of the localization pattern of FOXP3(+) cells in 80 gastric cancer patients by immunohistochemistry showed that the survival of patients with a diffuse pattern of FOXP3(+) cells was significantly poorer than that of those with a peritumoral pattern.[44] The biologic difference between the peritumoral and intratumoral localizations of TILs needs to be further investigated.

There is a lack of evidence about the biologic differences among gastric cancers from different geographic regions, and recent studies have reported different immune signature between East Asian and Western patients.[32] However, little has been known about the prognostic role of TILs in different ethnicities. We analyzed the prognostic role of TILs in Eastern and Western studies. The prognostic roles of TILs in Eastern and Western patients, which involved a positive correlation between OCS and the high expression of CD4(+) or CD8(+) T cells and a negative correlation between OCS and FOXP3(+) T cells, were similar. However, the statistical significance was reduced in the Western studies. It should be noted that international phase III randomized trials have reported different treatment outcomes as a function of patient ethnicity. In the AVAGAST trial,

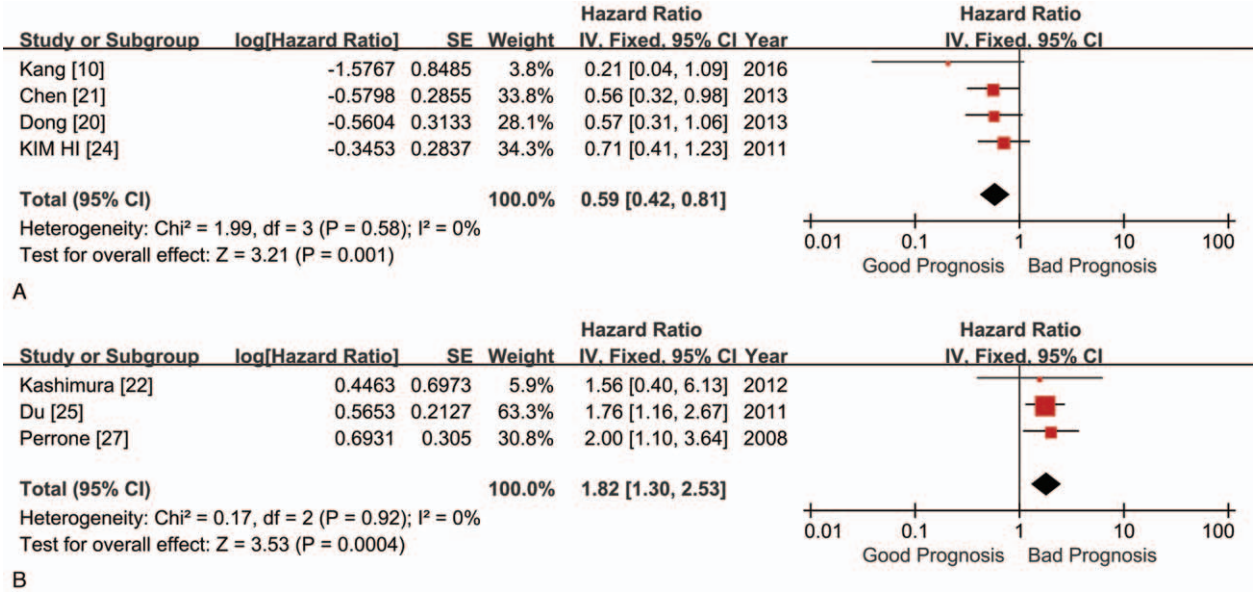

Figure 4. Forest plot of hazard ratios for the prediction of overall cancer relapse-free survival (OCRFS) by (A) non-FOXP3(+) and (B) FOXP3(+) tumor-infiltrating lymphocyte subtypes in gastric cancer tissues. High expression of (A) non-FOXP3(+) subgroups was positively correlated with OCRFS (pooled hazard ratio [HR] = 0.59, 95% confidence interval [CI] 0.42–0.81) with low heterogeneity among the studies ($P = .58$, $I^2 = 0\%$), while high expression of (B) FOXP3(+) Treg subgroup was negatively correlated with OCRFS (pooled HR = 1.82, 95% CI 1.30–2.53) with low heterogeneity among the studies ($P = .92$, $I^2 = 0\%$).

bevacizumab showed a survival benefit in non-Asians but not in Asians.[47] In contrast, in the LOGiC trial, benefit from lapatinib was observed in Asians but not in non-Asians.[48] Further studies must be conducted to clarify the clinical outcome of the immune checkpoint blockade using monoclonal antibodies against PD-1/PD-L1 in gastric cancer according to geographic regions.

There are further challenges to overcome before utilizing TILs as prognostic biomarkers in clinical treatment. There is no current consensus for interpreting TILs in gastric cancer, and the appropriate cutoff values need to be standardized. Studies included in our meta-analysis were characterized by differences in sample size, baseline patient characteristics (eg, age, tumor stage, and treatment type), follow-up duration, and detection methods. These results should be interpreted cautiously. To reduce the heterogeneity of the studies, however, we selected only high-quality studies using a quality assessment protocol based on the Newcastle–Ottawa scale, and heterogeneity was found only in the FOXP3(+) TILs subset analysis.

In conclusion, our meta-analysis strongly suggests that high TILs, mainly by CD8 lymphocytes, are potential biomarkers and accurate predictors of good prognosis in patients with gastric cancer. The clinical benefit of high TILs expression in gastric cancer tissue should contribute to future research regarding both conventional and novel therapies targeting immune cells.

Author contributions

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