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Review Article

Relationships between tumor-infiltrating FoxP3+ regulatory T cells and overall survival and lymph node metastasis in gastric cancer: a meta-analysis

Wei Cheng[1*], Zongguo Yang[2*], Juanjuan Yuan[3], Dongwei Xing[1], Minguang Zhang[1]

[1]Shanghai Municipal Hospital of Traditional Chinese Medicine, Shanghai University of Traditional Chinese Medicine, Shanghai 200071, China; [2]Shanghai Public Health Clinical Center, Fudan University, Shanghai 201508, China; [3]Hefei Hospital Affiliated to Anhui Medical University/The Second People's Hospital of Hefei, Hefei 230011, Anhui, China. [*]Equal contributors.

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Abstract: Objective: Relationships between tumor-Infiltrating FoxP3+ T cells (Tregs) and overall survival (OS) and disease-free survival (DFS) in patients with gastric cancer (GC) remains controversial. Thus, we conducted a meta-analysis to estimate the prognostic value of tumor infiltrating Tregs for OS and DFS in patients with GC. Methods: PubMed, Embase, Web of science and Cochrane library up to July 2017 were used to search relevant literatures. All studies involved with prognostic value of tumor infiltrating Tregs in patients with GC were systematically identified. Results: 22 studies including 2521 patients, 1311 of whom had high Tregs infiltration in GC were included. The OS of high Tregs infiltration patients in 1, 3 and 5-year were significantly lower than low Tregs infiltration patients (OR = 0.61, 95% CI = 0.38-0.97, *P* = 0.04; OR = 0.54, 95% CI = 0.35-0.81, *P* = 0.003 and OR = 0.56, 95% CI = 0.35-0.90, *P* = 0.02, respectively). There is no significant difference in 1, 3 and 5-year DFS between high Tregs infiltration patients and low Tregs infiltration patients (OR = 0.57, 95% CI = 0.27-1.18, *P* = 0.13; OR = 0.62, 95% CI = 0.33-1.16, *P* = 0.13 and OR = 0.73, 95% CI = 0.34-1.57, *P* = 0.41, respectively). When the studies were stratified further by the pathological variables for high Tregs infiltration patients versus low Tregs infiltration patients in GC, finding high Tregs infiltration is prone to lymph node metastasis (OR = 1.73, 95% CI = 1.08-2.77, *P* = 0.02) and no relationship with T stage (T3+T4), poor histodifferentiation, TNM stage (III+IV), vascular invasion and lymphatic invasion. They also provided some other important clinical prognostic information. Conclusion: Tumor infiltrating Treg, a risk factor of lymph node metastasis, was associated with poor OS, but not with poor DFS, for GC.

Keywords: Regulatory T cells, gastric cancer, prognostic, meta-analysis

Introduction

Despite the dramatic decline in gastric cancer (GC) incidence over the past 50 years, it remains the world's third leading cause of cancer mortality reported in 2012 to be only preceded by lung cancer and liver cancer [1]. More than 70% of GC occur in developing countries [2], and the vast majority of them are adenocarcinomas, which can be further subdivided into intestinal and diffuse types [3]. Even diagnosed at a localized stage, the prognosis of gastric cancer remains poor [4]. So that the identification of new clinical, biological and molecular features affecting prognosis is an important thing in clinical trial design and evaluation of new treatments for GC.

The transcription factor forkhead box P3 (FoxP3) is a key intracellular molecule for regulatory T cells (Tregs) development and function [5], which is considered to be the most specific Tregs marker so far. Under normal conditions, FoxP3+ Tregs are essential suppressors of antitumor immune responses and thus maintain immunological tolerance to host tissues [6]. A large number of experiments have demonstrated high Tregs infiltration were expected to be associated with an unfavorable outcome in GC [7, 8]. Tus, Tregs are considered as a potential prognostic factor and they may also represent a novel therapeutic target. However, this idea has been challenged by recent studies showing that high Tregs infiltration are not always associated with poor prognosis. On the contrary, it can

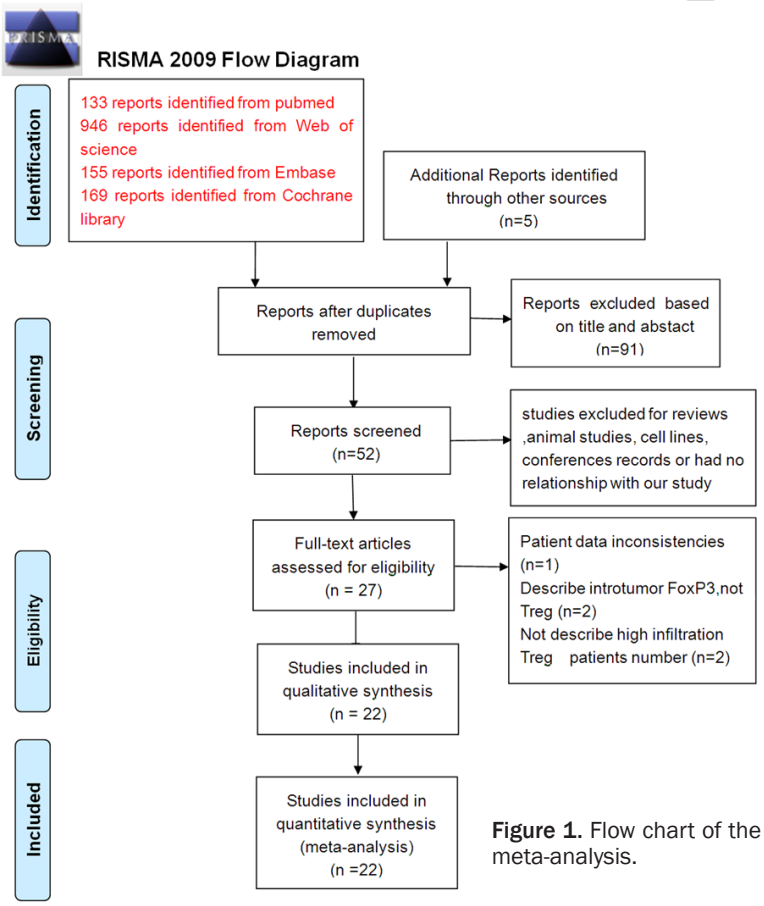


Figure 1. Flow chart of the meta-analysis.

improve survival in GC [9, 10]. So far, the results are still conflicting and whether tumor infiltration Tregs were beneficial or not to the prognosis of GC were not known.

To investigate this apparent differences, we sought to conduct a meta-analysis to estimate the prognostic value of tumor infiltrating Tregs for OS and DFS in GC, as well as the clinicopathological features.

Materials and methods

Search strategy

Relevant articles up to July 2017 were identified by two reviewers via an electronic search of PubMed, Embase, Web of science and Cochrane library using the following medical subject headings: (FoxP3 or regulatory T cells or Tregs) and (gastric cancer or gastric carcinoma or gastric tumor or gastric neoplasm or stomach cancer or stomach tumor or stomach carcinoma or stomach neoplasm). All eligible studies were retrieved. Additionally, possible missing papers were searched in reference lists of selected papers and systematic review. A search for unpublished literature was not performed. Literature was searched by two authors (Wei Cheng and Zongguo Yang) independently. Disagreements were resolved by consultation with senior author (Minguang Zhang).

Inclusion and exclusion criteria

The inclusion criteria for this study were as follows: (1) GC patients were diagnosed clearly by histopathologic examinations; (2) The data were collected from cohorts or medical centers; (3) Report of Tregs in tumor surgical specimens; (4) The detection method for Tregs in GC was immunohistochemical (IHC) or flow cytometry; (5) Association of high and low Tregs infiltration patients with OS and/or DFS and contained survival curves; (6) When the same author or group reported several results which contained same patient population in more than one article, the most recent report or the most complete report was included.

The exclusion criteria for this study were as follows: (1) Letters, reviews, case reports, conference summary, editorials, and expert advice were excluded; (2) Non-surgical treatment study; (3) Non-primary GC, such as metastatic tumor or recurrent tumor; (4) GC patients who had chemotherapy or radiotherapy before gastric cancer operation.

Name of authors or journals of the articles did not influence our decision in excluding or including the articles.

Qualitative assessment

The Newcastle-Ottawa Scale was used to evaluate the quality of the included 22 studies, which estimated three aspects of the studies including selection, comparability, and exposure [11]. A study will be rated as a maximum of one “star” for each high-quality item if including the “selection” and “exposure” categories and

Table 1. Main characteristics and results of the 22 studies relating tumor-Infiltrating FoxP3+ regulatory T cells on OS and DFS for gastric cancer

First author-year	Study location	Ethnicity	Study design	Number of patients	High/low	Marker	Method	Antibody	Study quality	Cut-off	Survival
Mizukami-2008	Japan	Asian	R	80	40/40	FoxP3	IHC	eBioscience	7	Median	OS
Perrone-2008	Italy	Caucasian	R	110	58/52	FoxP3	IHC	eBioscience	7	Median	OS, DFS
Haas-2009	Germany	Caucasian	R	52	26/26	FoxP3	IHC	Abcam	6	Mean	OS
ShinYoungPark-2010	Korea	Asian	R	173	132/41	FoxP3	IHC	eBioscience	8	Mean	OS, DFS
Shen-2010	China	Asian	R	133	66/67	FoxP3	IHC	Biolegend	7	Median	OS
Du-2011	China	Asian	R	179	92/87	FoxP3	IHC	Abcam	7	Median	OS, DFS
Wang-2011	China	Asian	R	107	53/54	FoxP3	IHC	Abcam	7	Mean	OS
Lu-2011	China	Asian	R	60	30/30	FoxP3	IHC	eBioscience	7	Median	OS
Kim-2011	Korea	Asian	R	180	90/90	FoxP3/CD4	IHC	Abcam	6	Median	OS, DFS
Ishigami-2012	Japan	Asian	R	141	76/65	FoxP3	IHC	DAKO	8	Median	OS
Kashimura-2012	Japan	Asian	R	123	62/61	FoxP3	IHC	Abcam	7	Median	OS, DFS
Tuncel-2013	Turkey	Caucasian	R	52	26/26	FoxP3	IHC	Abcam	6	Median	OS
Deng-2013	China	Asian	R	99	48/51	FoxP3	IHC	Abcam	7	Mean	OS
Zhou-2013	China	Asian	R	133	87/46	FoxP3	IHC	Biolegend	8	Mean	OS
Geng-2014	China	Asian	R	100	76/24	FoxP3	IHC	Novus	5	25%	OS
Kim-2014	Korea	Asian	R	99	49/50	FoxP3	IHC	Abcam	7	Median	OS
Hou-2014	China	Asian	R	111	77/34	FoxP3	IHC	Sigma	7	10%	OS
Ma-2014	China	Asian	R	197	24/173	FoxP3	IHC	Abcam	8	25%	OS
Suh-2015	Korea	Asian	R	116	72/44	FoxP3	IHC	eBioscience	6	20%	OS, DFS
Liu-2015	China	Asian	R	166	83/83	FoxP3	IHC	eBioscience	7	Median	OS
Choi-2016	Korea	Asian	R	28	19/9	FoxP3/CD4	FCM	eBioscience	5	Median	OS, DFS
Hennequin-2016	France	Caucasian	R	82	41/41	FoxP3	IHC	eBioscience	7	Mean	DFS

Quality score was assessed using Newcastle Ottawa Scale; High, high Treg; Low, low Treg. Study design is described as prospective (P) or retrospective (R).

a maximum of two "star" if including the "comparability" category. The quality assessment was conducted by two authors (Wei Cheng and Zongguo Yang) independently.

Statistical analysis

The primary outcomes for analysis were divided into two sections. The first aim of the analysis was to evaluate prognostic value of tumor infiltrating Tregs on 1, 3, 5-year OS and 1, 3, 5-year DFS in GC, Odds ratio (OR) and its 95% confidence interval (CI) were used as outcomes evaluation indexes. When the prognosis data were plotted as a Kaplan-Meier curve, the Engauge Digitizer version 4.1 (http://digitizer.sourceforge.net/) as described before [12-14] was used to extract the data. The second aim was to assess the relationship between tumor infiltrating Tregs and the clinical variables including T stage (T3+T4), lymph node metastasis, TNM stage (III+IV), poor histo-differentiation, vascular invasion and lymphatic invasion. This work was performed by two independent individuals to reduce the inaccuracy of extracted survival rate.

Review Manager version5.2 (RevMan, Cochrane Collaboration, Oxford, England) was used for meta-analysis, and publication bias and Sensitivity analysis was conducted by Stata 11.0 (Stata Corporation, College Station, TX). Statistical heterogeneity between trials was evaluated with chi-squared based Q-test and I^2 metric. We considered heterogeneity present when $P<0.05$. In the absence of statistically significant for heterogeneity, the Mantel-Haenszel method in the fixed-effect model was used for the Meta analysis. Otherwise, the DerSimonian and Laird method in the random-effect model was selected. The odds ratio (OR) with 95% CI was used to assess treatment efficacy and clinicopathological value. The combined result was an average OR and 95% CI weighted according to the standard error of the OR in the trial. Publication bias was assessed by Begg's funnel plot and Egger's test. $P<0.05$ was considered statistically significant.

Results

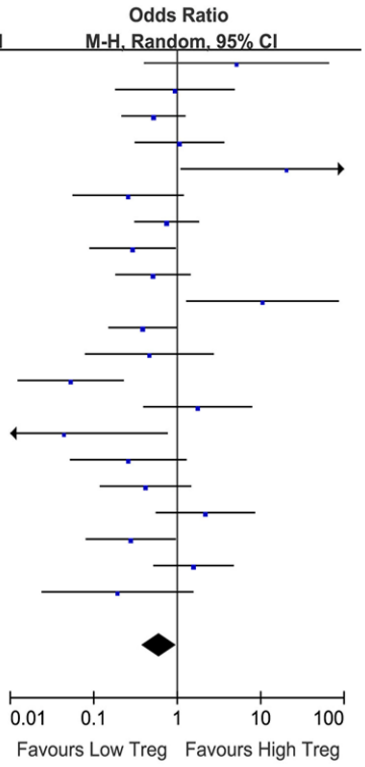
Study selection and characteristics

We retrieved 1403 trials according to initial search strategy. After screening all titles, abstracts, and full texts, 22 studies [9, 10, 15-34] met our entry criteria and were retrieved

A 1-year OS

Study or Subgroup	High Treg Events	High Treg Total	low Treg Events	low Treg Total	Weight	Odds Ratio M-H, Random, 95% CI
Choi-2016	18	19	7	9	2.5%	5.14 [0.40, 66.15]
Deng-2013	45	48	48	51	4.3%	0.94 [0.18, 4.89]
Du-2011	72	87	83	92	6.9%	0.52 [0.21, 1.26]
Geng-2014	64	76	20	24	5.6%	1.07 [0.31, 3.68]
Haas-2009	26	26	19	26	2.0%	20.38 [1.10, 378.65]
Hou-2014	62	77	32	34	4.6%	0.26 [0.06, 1.20]
Ishigami-2012	53	65	65	76	6.9%	0.75 [0.31, 1.83]
Kashimura-2012	50	62	57	61	5.7%	0.29 [0.09, 0.96]
Kim-2011	79	90	84	90	6.3%	0.51 [0.18, 1.45]
Kim-2014	48	49	41	50	3.2%	10.54 [1.28, 86.70]
Liu-2015	67	83	76	83	6.7%	0.39 [0.15, 0.99]
Lu-2011	26	30	28	30	3.9%	0.46 [0.08, 2.75]
Ma-2014	18	24	170	173	4.8%	0.05 [0.01, 0.23]
Mizukami-2008	37	40	35	40	4.7%	1.76 [0.39, 7.93]
Perrone-2008	48	58	52	52	2.1%	0.04 [0.00, 0.77]
Shen-2010	59	66	65	67	4.4%	0.26 [0.05, 1.30]
ShinYoungPark-2010	111	132	38	41	5.5%	0.42 [0.12, 1.48]
Suh-2015	68	72	39	44	5.1%	2.18 [0.55, 8.60]
Tuncel-2013	14	26	21	26	5.6%	0.28 [0.08, 0.96]
Wang-2011	47	53	45	54	6.0%	1.57 [0.52, 4.76]
Zhou-2013	78	87	45	46	3.2%	0.19 [0.02, 1.57]
Total (95% CI)		**1270**		**1169**	**100.0%**	**0.61 [0.38, 0.97]**
Total events	1090		1070			

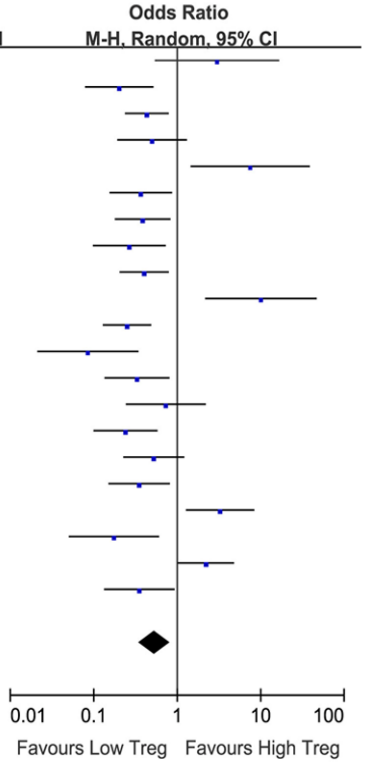
Heterogeneity: Tau² = 0.62; Chi² = 46.38, df = 20 (P = 0.0007); I² = 57%
Test for overall effect: Z = 2.10 (P = 0.04)



B 3-year OS

Study or Subgroup	High Treg Events	High Treg Total	Low Treg Events	Low Treg Total	Weight	Odds Ratio M-H, Random, 95% CI
Choi-2016	15	19	5	9	3.1%	3.00 [0.54, 16.69]
Deng-2013	25	48	43	51	4.9%	0.20 [0.08, 0.52]
Du-2011	40	87	61	92	5.8%	0.43 [0.24, 0.79]
Geng-2014	38	76	16	24	4.9%	0.50 [0.19, 1.31]
Haas-2009	24	26	16	26	3.3%	7.50 [1.45, 38.85]
Hou-2014	36	77	24	34	5.1%	0.37 [0.15, 0.87]
Ishigami-2012	41	65	62	76	5.4%	0.39 [0.18, 0.83]
Kashimura-2012	44	62	55	61	4.8%	0.27 [0.10, 0.73]
Kim-2011	57	90	73	90	5.6%	0.40 [0.20, 0.79]
Kim-2014	47	49	35	50	3.5%	10.07 [2.16, 46.93]
Liu-2015	38	83	64	83	5.6%	0.25 [0.13, 0.49]
Lu-2011	13	30	27	30	3.8%	0.08 [0.02, 0.34]
Ma-2014	14	24	140	173	5.0%	0.33 [0.13, 0.81]
Mizukami-2008	31	40	33	40	4.5%	0.73 [0.24, 2.20]
Perrone-2008	31	58	43	52	5.1%	0.24 [0.10, 0.58]
Shen-2010	48	66	56	67	5.2%	0.52 [0.23, 1.22]
ShinYoungPark-2010	78	132	33	41	5.2%	0.35 [0.15, 0.82]
Suh-2015	63	72	30	44	4.9%	3.27 [1.27, 8.39]
Tuncel-2013	5	26	15	26	4.1%	0.17 [0.05, 0.61]
Wang-2011	32	53	22	54	5.4%	2.22 [1.02, 4.80]
Zhou-2013	61	87	40	46	4.8%	0.35 [0.13, 0.93]
Total (95% CI)		**1270**		**1169**	**100.0%**	**0.54 [0.35, 0.81]**
Total events	781		893			

Heterogeneity: Tau² = 0.69; Chi² = 83.64, df = 20 (P < 0.00001); I² = 76%
Test for overall effect: Z = 2.92 (P = 0.003)



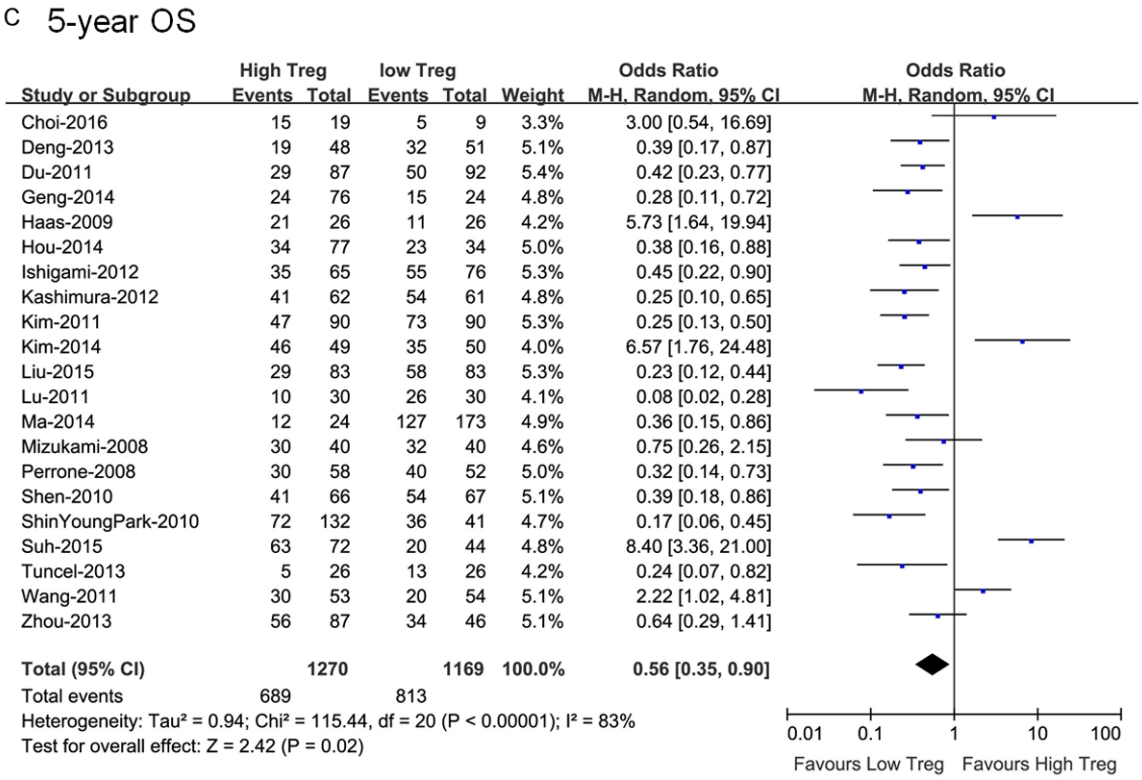


Figure 2. Forest plot for OS of gastric cancer patients. Random effect model of OR for survival of follow-up 1-year (A), 3-year (B) and 5-year (C) of gastric cancer patients after surgery: high FoxP3+ T cells infiltration patients vs low FoxP3+ T cells infiltration patients.

for further detailed evaluation (**Figure 1**). All 22 studies were retrospectively analyzed, and their characteristics are summarized in **Table 1**. Sample sizes ranged from 28 to 197, and the total patients number was 2521, 1311 of whom had high Tregs infiltration. For 22 included studies, 21 provided data on 1, 3, 5-year OS; 8 on 1, 3, 5-year DFS; 16 on clinical pathological variable. The studies were conducted in 7 countries (China, Germany, France, Italy, Japan, South Korea and Turkey).

Quality assessment of included studies

For our 22 studies, there are 4 studies [18, 25, 28, 32] rated as 8 scores, 12 studies [15, 16, 19-22, 24, 27, 30, 31, 33, 34] rated as 7 scores, 4 studies [9, 17, 23, 26] rated as 6 scores, and 2 studies [10, 29] rated as 5 scores according to Newcastle-Ottawa Scale.

Correlation between tregs infiltration and OS for GC

Heterogeneity was significant among the included studies when comparing 1-year OS, 3-year OS and 5-year OS between high Tregs infiltration and low Tregs infiltration for GC (P = 0.0007, I^2 = 57%; P<0.00001, I^2 = 76%, and P<0.00001, I^2 = 83%, respectively). Meta-analysis with random-model revealed that GC patients with high Tregs infiltration had significantly lower 1, 3, 5-year OS compared to those with low Tregs infiltration (OR = 0.61, 95% CI = 0.38-0.97, P = 0.04, **Figure 2A**; OR = 0.54, 95% CI = 0.35-0.81, P = 0.003, **Figure 2B** and OR = 0.56, 95% CI = 0.35-0.90, P = 0.02, **Figure 2C**, respectively). So, high Tregs infiltration was indicated to be associated with poor OS for GC.

Correlation between tregs infiltration and DFS for GC

Heterogeneity was significant among the included studies when comparing 1-year DFS, 3-year DFS and 5-year DFS between high Tregs infiltration and low Tregs infiltration for GC (P = 0.0004, I^2 = 73%; P = 0.0001, I^2 = 76%, and P<0.00001, I^2 = 85%, respectively). Meta-analysis with random-model showed that there was no significant difference for 1, 3, 5-year DFS between high Tregs infiltration patients

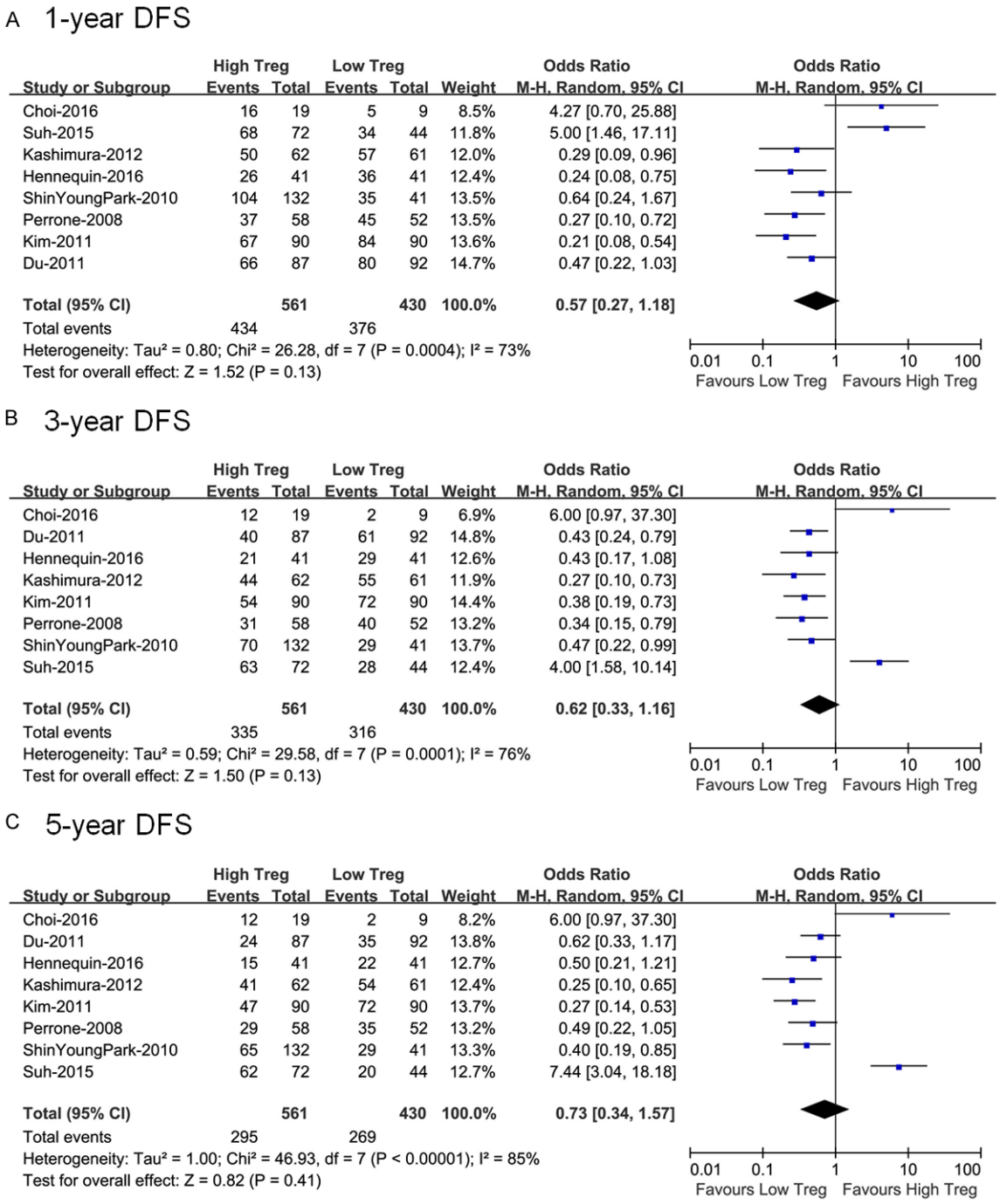


Figure 3. Forest plot for DFS of gastric cancer patients. Random effect model of OR for survival of follow-up 1-year (A), 3-year (B) and 5-year (C) of gastric cancer patients after surgery: high FoxP3+ T cells infiltration patients vs low FoxP3+ T cells infiltration patients.

and low Tregs infiltration patients (OR = 0.57, 95% CI = 0.27-1.18, *P* = 0.13, **Figure 3A**; OR = 0.62, 95% CI = 0.33-1.16, *P* = 0.13, **Figure 3B** and OR = 0.73, 95% CI = 0.34-1.57, *P* = 0.41, **Figure 3C**, respectively). So, high Tregs infiltration was not alway considered to be involved with poor DFS for GC.

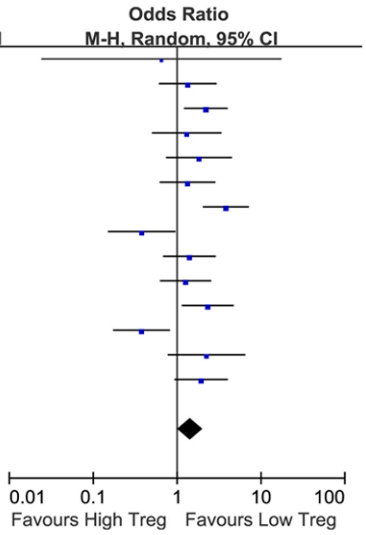
Correlation between tregs infiltration and clinical variable for GC

Stratifying for the different clinical variables after GC surgical resection, heterogeneity was significant among the included studies when comparing T stage (T3+T4), lymph node metas-

A T stage (T3+T4)

Study or Subgroup	High Treg Events	Total	Low Treg Events	Total	Weight	Odds Ratio M-H, Random, 95% CI
Choi-2016	18	19	9	9	1.0%	0.65 [0.02, 17.51]
Deng-2013	27	48	25	51	7.6%	1.34 [0.61, 2.95]
Du-2011	48	87	33	92	9.0%	2.20 [1.21, 4.01]
Geng-2014	52	76	15	24	6.4%	1.30 [0.50, 3.39]
Hou-2014	61	77	23	34	6.8%	1.82 [0.74, 4.51]
Ishigami-2012	18	65	17	76	7.8%	1.33 [0.62, 2.86]
Kim-2011	67	90	39	90	8.8%	3.81 [2.03, 7.16]
Kim-2014	31	49	41	50	6.6%	0.38 [0.15, 0.95]
Liu-2015	66	83	61	83	8.1%	1.40 [0.68, 2.88]
Shen-2010	43	66	40	67	8.2%	1.26 [0.62, 2.55]
ShinYoungPark-2010	82	132	17	41	8.2%	2.32 [1.13, 4.73]
Suh-2015	21	72	23	44	7.7%	0.38 [0.17, 0.82]
Wang-2011	47	53	42	54	5.8%	2.24 [0.77, 6.49]
Zhou-2013	59	87	24	46	8.0%	1.93 [0.93, 4.02]
Total (95% CI)		**1004**		**761**	**100.0%**	**1.43 [1.01, 2.04]**
Total events	640		409			

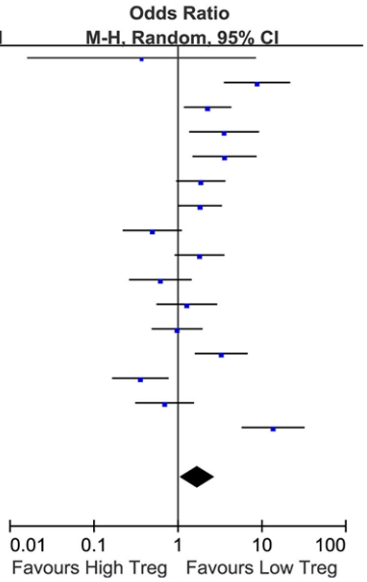
Heterogeneity: Tau² = 0.26; Chi² = 33.91, df = 13 (P = 0.001); I² = 62%
Test for overall effect: Z = 2.00 (P = 0.05)



B lymph node metastasis

Study or Subgroup	High Treg Events	Total	Low Treg Events	Total	Weight	Odds Ratio M-H, Random, 95% CI
Choi-2016	17	19	9	9	1.8%	0.37 [0.02, 8.49]
Deng-2013	35	48	12	51	6.2%	8.75 [3.53, 21.69]
Du-2011	67	87	55	92	6.9%	2.25 [1.18, 4.32]
Geng-2014	57	76	11	24	6.0%	3.55 [1.36, 9.22]
Hou-2014	61	77	17	33	6.3%	3.59 [1.49, 8.62]
Ishigami-2012	33	65	27	76	6.9%	1.87 [0.95, 3.68]
Kim-2011	60	90	47	90	7.1%	1.83 [1.00, 3.34]
Kim-2014	24	49	33	50	6.5%	0.49 [0.22, 1.11]
Liu-2015	64	83	54	83	6.8%	1.81 [0.91, 3.58]
Ma-2014	11	24	100	173	6.3%	0.62 [0.26, 1.46]
Perrone-2008	43	58	36	52	6.4%	1.27 [0.55, 2.93]
Shen-2010	40	66	41	67	6.8%	0.98 [0.49, 1.96]
ShinYoungPark-2010	95	132	18	41	6.7%	3.28 [1.59, 6.77]
Suh-2015	23	72	25	44	6.6%	0.36 [0.16, 0.77]
Wang-2011	33	53	38	54	6.5%	0.69 [0.31, 1.56]
Zhou-2013	72	87	12	46	6.3%	13.60 [5.75, 32.19]
Total (95% CI)		**1086**		**985**	**100.0%**	**1.73 [1.08, 2.77]**
Total events	735		535			

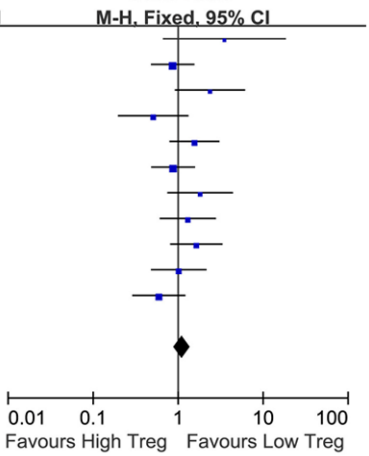
Heterogeneity: Tau² = 0.73; Chi² = 82.40, df = 15 (P < 0.00001); I² = 82%
Test for overall effect: Z = 2.27 (P = 0.02)



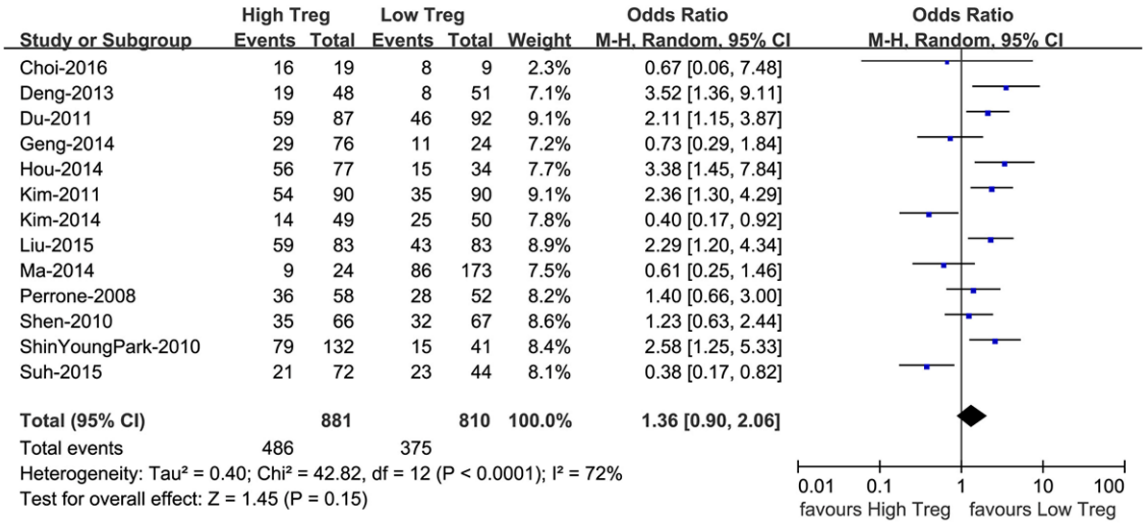
C Poor histological differentiation

Study or Subgroup	High Treg Events	Total	Low Treg Events	Total	Weight	Odds Ratio M-H, Fixed, 95% CI
Choi-2016	14	19	4	9	1.0%	3.50 [0.66, 18.49]
Du-2011	45	87	51	92	16.7%	0.86 [0.48, 1.55]
Geng-2014	56	76	13	24	3.6%	2.37 [0.91, 6.14]
Hou-2014	51	77	27	34	8.8%	0.51 [0.20, 1.32]
Ishigami-2012	30	65	27	76	9.3%	1.56 [0.79, 3.06]
Kim-2011	51	90	54	90	16.3%	0.87 [0.48, 1.58]
Liu-2015	74	83	68	83	5.1%	1.81 [0.75, 4.41]
Perrone-2008	26	58	20	52	8.1%	1.30 [0.61, 2.78]
Shen-2010	45	66	38	67	8.4%	1.64 [0.81, 3.32]
Suh-2015	33	72	20	44	9.4%	1.02 [0.48, 2.16]
Zhou-2013	36	87	25	46	13.3%	0.59 [0.29, 1.22]
Total (95% CI)		**780**		**617**	**100.0%**	**1.11 [0.88, 1.38]**
Total events	461		347			

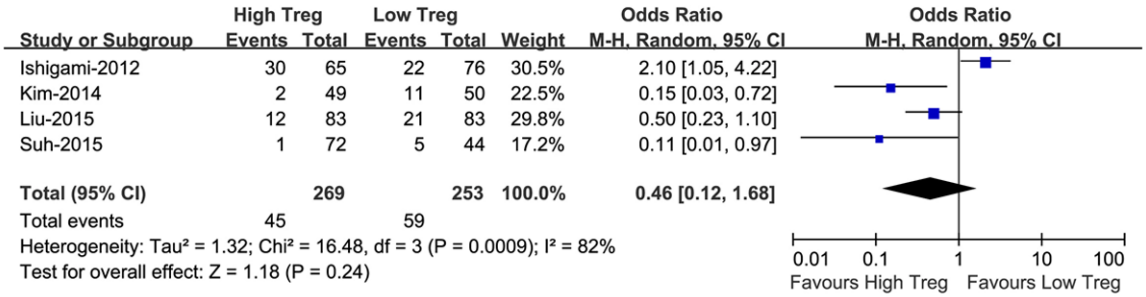
Heterogeneity: Chi² = 14.59, df = 10 (P = 0.15); I² = 31%
Test for overall effect: Z = 0.87 (P = 0.38)



D TNM stage (III+IV)



E vascular invasion



F lymphatic invasion

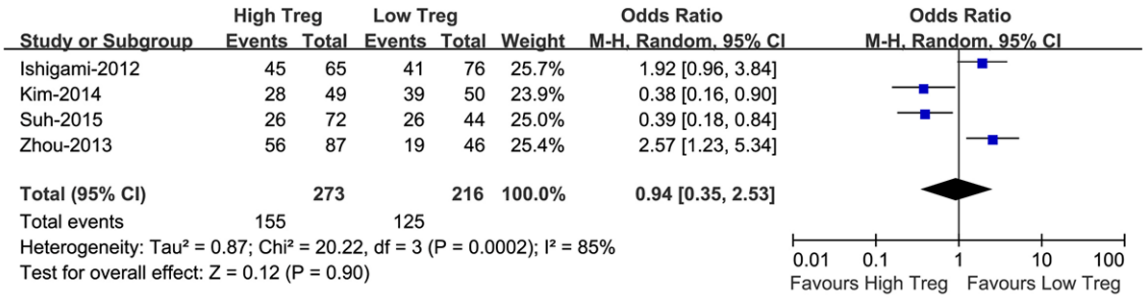


Figure 4. Forest plot for pathological variables in gastric cancer patients after surgery. A: Relationship between FoxP3+ T cells infiltration and T stage (T3+T4) with Random effect model of OR; B: Relationship between FoxP3+ T cells infiltration and lymph node metastasis with random effect model of OR; C: Relationship between FoxP3+ T cells infiltration and poor histological differentiation with fixed effect model of OR; D: Relationship between FoxP3+ T cells infiltration and TNM stage (III+IV) with random effect model of OR; E: Relationship between FoxP3+ T cells infiltration and vascular invasion with random effect model of OR; F: Relationship between FoxP3+ T cells infiltration and lymphatic invasion with random effect model of OR.

tasis, TNM stage (III+IV), vascular invasion and lymphatic invasion between high Tregs infiltration and low Tregs infiltration for GC ($P = 0.001$, $I^2 = 62\%$; $P<0.00001$, $I^2 = 82\%$; $P<0.0001$, $I^2 = 72\%$; $P = 0.0009$, $I^2 = 82\%$ and $P<0.0007$, $I^2 = 82\%$, respectively). Meta-analysis with random-model was used for those clinical variables. Statistical results in 14 studies [9, 10, 18-21, 23, 25, 27-31, 33] suggested that there was no significant difference for T stage (T3+T4)

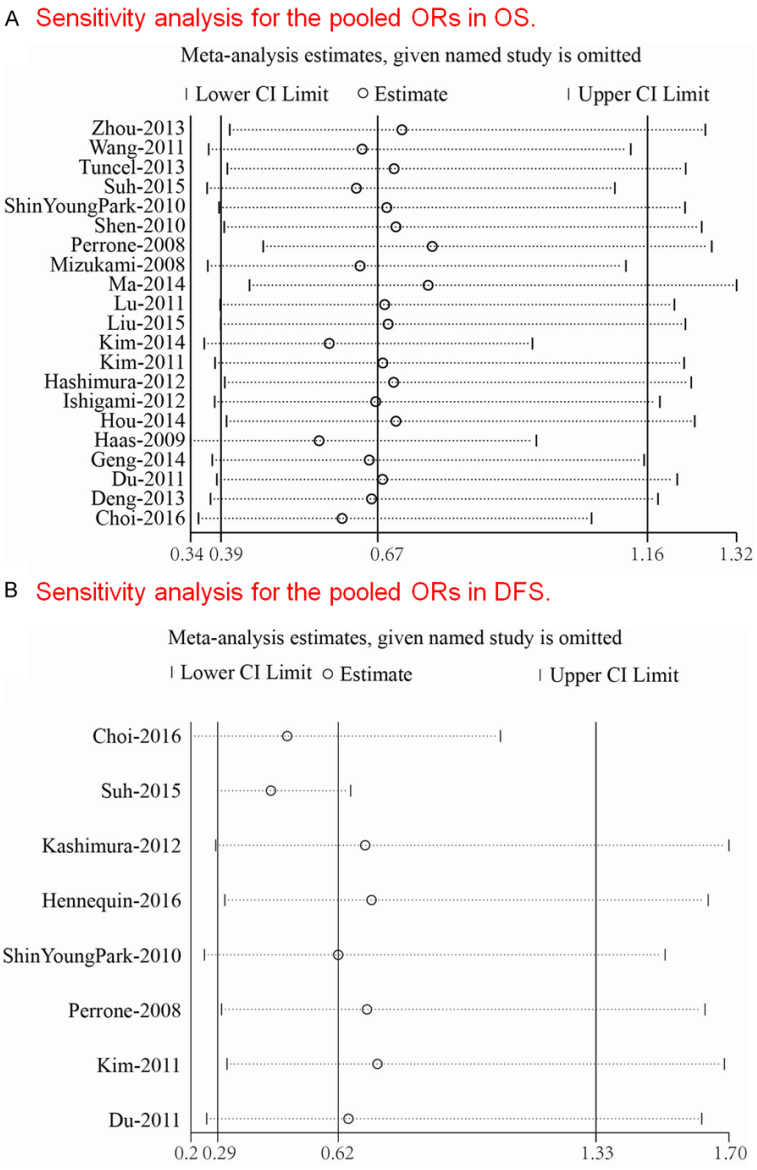


Figure 5. Sensitivity analysis for the pooled ORs in OS (A) and DFS (B) in GC. The sensitivity analysis was conducted by estimating the average OR in the absence of each study.

between high Tregs infiltration and low Tregs infiltration (OR = 1.43, 95% CI = 1.01-2.04, *P* = 0.05. **Figure 4A**). When the different variables were further stratified based on lymph node status in 16 studies [9, 10, 15, 18-21, 23, 25, 27-33], lymph node metastasis was significantly higher in high Tregs infiltration than low Tregs infiltration (OR = 1.73, 95% CI = 1.08-2.77, *P* = 0.02. **Figure 4B**). When the different variables were further stratified in view of histological differentiation status in 11 studies [9, 10, 15, 19, 20, 23, 25, 28, 29, 31, 33], TNM stage (III+IV) in 13 studies [9, 10, 15, 18-20, 23, 27, 29-33], vascular invasion in 4 studies [9, 25, 30, 33], lymphatic invasion in 4 studies [9, 25, 28, 30] in GC, there were no significant difference between high Tregs infiltration and low Tregs infiltration (OR = 1.11, 95% CI = 0.88-1.38, *P* = 0.38, **Figure 4C**; OR = 1.36, 95% CI = 0.90-2.06, *P* = 0.15, **Figure 4D**; OR = 0.46, 95% CI = 0.12-1.68, *P* = 0.24, **Figure 4E** and OR = 0.94, 95% CI = 0.35-2.53, *P* = 0.9, **Figure 4F**, respectively). So, high Tregs infiltration was considered to be a risk factor for lymph node metastasis, and no relationship with T stage (T3+T4), poor histodifferentiation, TNM stage (III+IV), vascular invasion and lymphatic invasion in GC.

Sensitivity analyses

In order to estimate the influence of single study on the pooled ORs, we carried out sensitivity analysis by evaluating the average OR in the absence of each study. The results showed that our meta-analysis was statistically reliable (**Figure 5**).

Publication bias

Begg's funnel plot and Egger's test were used to detect the publication bias for OS and DFS in GC patients in inclued sdudies. As shown in **Figure 6**, no obvious asymmetry was revealed in the funnel plot. We proceeded to perform Begg's test and Egger's test to further detected the asymmetry. Statistical evidence suggested that there was no significant publication bias among included studies for the meta-analysis (Begg's test score of *P* = 0.651, Egger's test score of *P* = 0.495 in OS studies and Begg's test score of *P* = 0.174, Egger's test score of *P* = 0.181 in DFS studies).

Discussion

GC is a common malignant tumor with high morbidity and mortality in China [35]. At pres-

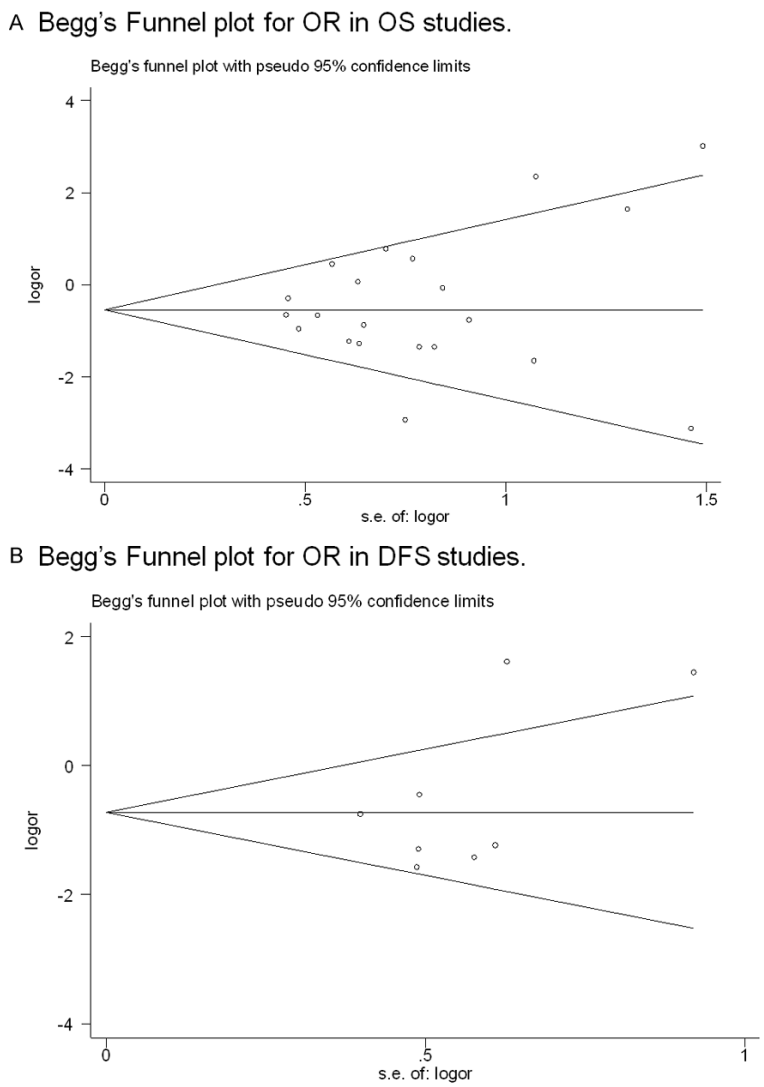


Figure 6. Begg's funnel plot of studies included for OS (A) and DFS (B) in GC patients. The funnel plot displays in OR against its standard error (s.e.) for each individual study. The horizontal line represents the estimate of the OR, with the dash lines indicate the expected 95% CI for a given standard error.

ent, the main therapy methods of GC remain radical surgery, radiotherapy and chemotherapy. Adjuvant therapy after surgery is still based on the pathological stage and the corresponding pathological risk factors [36]. So many patients experienced with inadequate treatment or excessive treatment. How to choose a better biomarker to determine the need for subsequent treatment will be a great challenge in the precise treatment of GC. Tregs are functionally immunosuppressive subsets of CD4+ T found by Sakaguchi in 1995 [37]. They control the balance between self tolerance and self rejection by secreting IL-4, IL-10, TGF-β and other cytokines [38]. At presently, FoxP3 is considered as the most specific marker for Tregs, because it is critical for the development and function of Tregs [39]. Large number of studies have demonstrated high Tregs infiltration with a poor prognosis in GC [7, 8]. They believe that Tregs promote GC cells to evade immune surveillance by suppressing immune responses, leading to tumor progression. But there are still some uninterrupted research reports on high Tregs infiltration with a better prognosis in GC [9, 10, 17, 21, 30]. Their studies have suggested that chronic inflammation caused by Helicobacter pylori is thought to lead to atrophy, intestinal metaplasia, dysplasia and ultimately GC. Tregs suppress the immune reactions of cytotoxic T cells caused by Helicobacter pylori rather than by cancer cells. Subsequent carcinogenesis driven by Helicobacter pylori-associated inflammation could be prevented by Tregs [17, 30]. Another study demonstrated that Tregs also have cytotoxic effects on cancer cells by secreting cytotoxic molecules, such as granzyme B or perforin [40]. So far, prognostic values of Tregs infiltration in GC are still controversial. Thus, this meta-analysis aimed to provide an evidence-based conclusion.

Our outcomes demonstrated that high density of tumor-infiltrating Tregs have been associated with poor OS in GC, consistent with current mainstream views [7, 8]. The reasons for this may be that Tregs inhibit immune function and promote tumor immune escape. But, our studies also suggested that there was no significant difference for DFS in GC, which was inconsistent with published views [7, 8]. This may mean that Tregs cannot be used as an indicator of recurrence and metastasis in GC. For clinical

variables after GC surgical resection, statistical results suggested that there was no significant difference for T stage (T3+T4), poor histological differentiation, TNM stage (III+IV), vascular invasion and lymphatic invasion between high Tregs infiltration and low Tregs infiltration. But, we found that there was significant difference for lymph node metastasis between high Tregs infiltration and low Tregs infiltration in GC patiens. The GC patients with high Tregs infiltration are more likely suffering from lymph node metastasis. So, it is possible for us to prolong OS and prevent lymph node metastasis for patients with GC by reducing Tregs number in future. We can also consider to determine treatment strategies according to level of tumor infiltration Tregs. But, well-designed with large samples trials still need to be conducted in future.

There are still so many limitation in our study. First of all, prognosis data extracted from survival curves might be less reliable than reported directly in studies. Second, only English publications were searched. Third, the antibody, IHC cell-scoring strategy and the cutoff value were not defined similarly in some studies. These factors may contribute to potential publication bias. However, there still existed high heterogeneity in some meta-analysis, our sensitivity analyses indicated that the results were stable and the heterogeneity did not influenced the analysis results.

Publication bias [41] is an important limitation in meta-analysis, because some studies with negative results are more difficult to be accepted for publication. Thus we should encourage some researchers to publish their studies including some negative results. There is no evidence of publication bias in our included studies.

In conclusion, our meta-analysis demonstrated that high density of tumor-infiltrating Tregs, a risk factor of lymph node metastasis, were associated with poor OS in GC patients. In view of immunosuppressive effects of Tregs for adaptive immune responses, that arouse many researchers to pursue therapy strategies to deplete Tregs from GC patients to enhance antitumor immunity respond [42-44]. But, our meta-analysis also showed that there was no significant difference for DFS in GC patients between high density of tumor-infiltrating Tregs and low density of tumor-infiltrating Tregs. So, additional high quantity prospective investigations are still needed to confirm the exact value of tumor-infiltrating Tregs in GC patients.

Acknowledgements

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Disclosure of conflict of interest

None.

Abbreviations

GC, gastric cancer; OS, overall survival; DFS, disease-free survival; Tregs, regulatory T cells; CI, confidence interval; OR, odds ratio.

Address correspondence to: Minguang Zhang and Dongwei Xing, Shanghai Municipal Hospital of Traditional Chinese Medicine, Shanghai University of Traditional Chinese Medicine, 274 Zhijiang Road, Jing'an District, Shanghai 200071, China. Tel: 021-56639828; E-mail: mgzhang09@163.com (MGZ); xdw021@163.com (DWX)

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