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Review Article

Tumor-infiltrating FoxP3$^+$ regulatory T-cells links to overall survival and clinicopathological features in colorectal cancer: a meta-analysis

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Abstract: *Background and aim:* The prognostic value of tumor-infiltrating FoxP3$^+$ T-cells (Tregs) for overall survival (OS) and disease-free survival (DFS) in patients with colorectal cancers (CRC) remains controversial. *Methods:* Searching PubMed, EMBASE, Web of Science, and the Cochrane library, up to September 2017, studies on tumor-infiltrating Tregs for CRC were systematically identified. *Results:* Twenty studies, including 5,846 patients, 2,544 of whom had high Tregs infiltration, were included. OS of high Tregs infiltration patients in 1, 3, and 5 years was significantly higher than low Tregs infiltration patients (OR = 1.77, $P < 0.00001$; OR = 1.88, $P < 0.0001$ and OR = 2.09, $P < 0.00001$, respectively). There were no significant differences in 1, 3, and 5-year DFS between high Tregs infiltration patients and low Tregs infiltration patients (OR = 1.18, $P = 0.57$; OR = 1.23, $P = 0.49$ and OR = 1.26, $P = 0.37$, respectively). When studies were stratified further by clinicopathological variables for high Tregs infiltration patients versus low Tregs infiltration patients in CRC, T stage (T3+T4), TNM stage (III+IV), and vascular invasion were significantly lower in high Tregs infiltration patients (OR = 0.57, $P = 0.002$; OR = 0.43, $P < 0.00001$ and OR = 0.71, $P = 0.003$, respectively). However, no differences were found comparing lymph node metastasis, poor histodifferentiation, lymphatic invasion, nerve invasion, and tumor size (> 5 cm) between these two groups (all $P > 0.05$). *Conclusion:* Tumor-infiltrating Tregs, favorite immune cells for avoiding T stage (T3+T4), TNM (III+IV), and vascular invasion, were associated with better OS but not with DFS in CRC.

Keywords: Regulatory T-cells, colorectal cancers, prognostic, meta-analysis

Introduction

Colorectal cancer (CRC) is the third most common cancer, worldwide, accounting for 10% of all new cancer cases. It is the second leading cause of cancer deaths in both male and female patients [1]. At present, the prognosis of patients with CRC primarily depends on the disease stage at diagnosis and other clinicopathological features, including differentiation, depth of invasion, presence of synchronous metastasis, and lymphovascular invasion [2, 3].

Regulatory T-cells (Tregs), essential suppressors of antitumor immune response, are considered a potential prognostic factor, possibly representing a novel therapeutic target [4, 5]. Present evidence has demonstrated that tumor-infiltrating Tregs are the main obstacle for tumor immunotherapy [6-8]. However, inconsistent results about tumor-infiltrating Tregs, in patients with CRC, have been reported involving the prognostic value and clinicopathological features, which may play a positive [9-19], negative [20] or non-predictive [21-28] role in CRC. Results in a meta-analysis in 2014 showed that tumor-infiltrating Tregs are associated with better OS, but not with DFS in CRC patients [29]. A subsequent meta-analysis in 2015 indicated that tumor-infiltrating Tregs were associated with better OS and DFS in CRC patients [30]. In addition to inconsistent results, they also did not clarify the correlation between tumor-Infil-

trating Tregs and clinicopathological features in CRC patients. Recently, many new studies involving the prognostic value and clinicopathological features for tumor-infiltrating Tregs in CRC have been published [16-20, 27, 28].

Considering the important roles of Tregs in CRC and conflicts found in previous reports, reevaluation of the prognostic value of Tregs for outcomes and clinicopathological features in CRC patients is urgently essential. To investigate these apparent differences, this study sought to conduct a meta-analysis to estimate the link between Tregs and CRC patient survival and clinicopathological features, aiming to gain insight into whether Tregs could provide useful guidance concerning biological behavior and treatment in CRC.

Materials and methods

Search strategy

Relevant articles up to September 2017 were identified by two reviewers via an electronic search of PubMed, EMBASE, Web of Science, and Cochrane library using the following search terms: ("colorectal carcinoma" OR "colorectal neoplasms" OR "colorectal tumor" OR "colorectal cancer" OR "large bowel carcinoma" OR "large bowel tumor" OR "large bowel cancer" OR "large intestine carcinoma" OR "large intestine tumor" OR "large intestine cancer" OR "colon cancer" OR "rectal cancer" OR "colon carcinoma" OR "rectal carcinoma" OR "colon tumor" OR "rectal tumor" OR "colon neoplasms" OR "rectal neoplasms" OR "CRC") [Title/Abstract] AND ("Treg" OR "regulatory T cell" OR "Foxp3") [Title/Abstract]. All eligible studies were retrieved. Additionally, possible missing papers were searched in reference lists of selected papers and systematic review. Search for unpublished literature was not performed. Studies were searched by two authors (W Cheng and Z Yang), independently. Disagreements were resolved by consultation with a senior author (M Zhang).

Inclusion and exclusion criteria

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

Exclusion criteria for this study were as follows: (1) Letters, reviews, case reports, conference summary, editorials, and expert advice were excluded; (2) Studies having no information on survival rates or survival curve; (3) Non-surgical treatment studies; (4) Peripheral blood or peritumoral specimens or puncture specimens; and (5) Animal experiments. Names of authors or journals of the articles did not influence the decision in excluding or including articles.

Data extraction

Two investigators independently summarized full texts meeting the inclusion criteria and extracted the following contents: (1) Publication data (first author's name, year of publication); (2) Study characteristics (study design, sample size, scoring protocols to identify $FoxP3^+$ Tregs, number which was defined as high or low Tregs infiltration, follow up times); (3) Number of subjects included in the studies; and (4) 1, 3, and 5-year OS and DFS as well as some associated clinicopathological features including T stage (T3+T4), lymph node metastasis, TNM stage (III+IV), poor histo-differentiation, vascular invasion, lymphatic invasion, nerve invasion, and tumor size (> 5 cm). If a direct report on OS and DFS was not available, then the survival data extracted from Kaplan-Meier curves were gained by Engauge Digitizer version4.1 (http://digitizer.sourceforge.net/), as described previously [32-34]. Authors were contacted by e-mail for additional information if necessary.

Qualitative assessment

Newcastle-Ottawa Scale was used to evaluate the quality of the included 20 studies. This scale estimated three aspects of the studies including selection, comparability, and exposure [31]. A study would be rated with a maximum of one "star" for each high-quality item if including the "selection" and "exposure" categories and a maximum of two "stars" if includ-

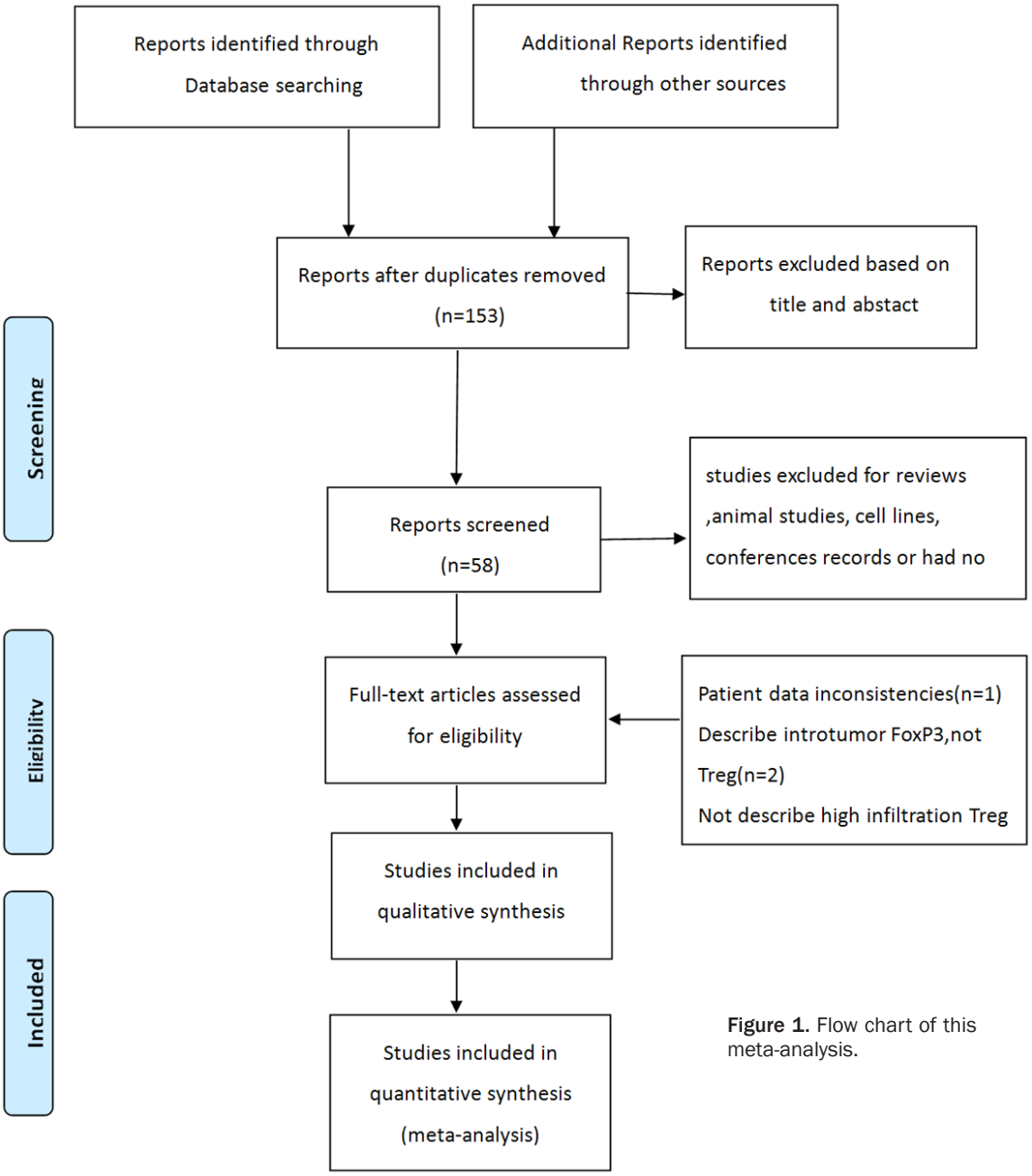


Figure 1. Flow chart of this meta-analysis.

ing the "comparability" category. Quality assessment was conducted by two authors (W Cheng and Z Yang), independently.

Statistical analysis

Primary outcomes for analysis were divided into two sections. The first aim of analysis was to evaluate the prognostic value of tumor-infiltrating Tregs on 1, 3, and 5-year OS and 1, 3, and 5-year DFS in CRC. Odds ratio (OR) and its 95% confidence interval (CI) were used as outcomes evaluation indexes. The second aim was to assess the correlation between tumor-infiltrating Tregs and clinicopathological variables, including T stage (T3+T4), lymph node metastasis, TNM stage (III+IV), poor histo-differentiation, vascular invasion, lymphatic invasion, nerve invasion, and tumor size (> 5 cm). This work was performed by two independent individuals to reduce inaccuracy of extracted survival rates.

Table 1. Main characteristics and results of the 20 studies relating tumor-infiltrating FoxP3$^+$ regulatory T cells on OS and DFS for colorectal cancer

First author-year	Patients source	Number of patients	High/Low	Marker	Method	Antibody	Study quality	Cut-off	Survival
Frey-2009	Switzerland	1252	621/631	FoxP3	IHC	Abcam	7	Other	OS
Sinicrope-2009	American	160	101/59	FoxP3	IHC	Abcam	7	Other	DFS
Lee-2010	Korea	63	23/40	FoxP3	IHC	eBioscience	8	Mean	OS, DFS
Nosho-2010	American	768	384/384	FoxP3	IHC	BioLegend	7	Other	OS
Tosolini-2011	France	125	69/56	FoxP3	IHC	Abcam	6	Mean	DFS
Yoon-2012	American	156	78/78	FoxP3	IHC	Abcam	7	Other	OS
Suzuki-2013	Japan	88	34/54	FoxP3	IHC	Abcam	7	Other	OS, DFS
Xu-2013	China	90	21/69	FoxP3	IHC	Abcam	7	Mean	OS
Kim-2013	Germany	65	27/38	FoxP3	IHC	Abcam	6	Median	OS
Katz-2013	American	188	26/162	FoxP3	IHC	Abcam	8	Other	OS, DFS
Reimers-2014	Netherlands	478	238/240	FoxP3	IHC	Abcam	7	Median	OS, DFS
Zeestraten-2014	Netherlands	244	111/133	FoxP3	IHC	Abcam	7	Median	OS
Ling-2014	Sweden	405	326/79	FoxP3	IHC	Abcam	6	Other	OS
Chen-2014	China	102	47/55	FoxP3	IHC	Abcam	7	Mean	OS
Vlad-2015	Romania	42	21/21	FoxP3	IHC	Abcam	8	Median	OS
Nakagawa-2015	Japan	155	49/106	FoxP3	IHC	Abcam	7	Other	OS, DFS
Hanke-2015	Switzerland	99	34/786	FoxP3	IHC	BioLegend	5	Median	OS
Wang-2015	China	340	181/159	FoxP3	IHC	Abcam	7	Median	OS
Yoshida-2016	Japan	199	99/100	FoxP3/CD3	IHC	Abcam	8	Median	OS
McCoy-2017	Australia	106	53/53	FoxP3	IHC	Abcam	6	Median	OS, DFS

Quality scores were assessed using Newcastle Ottawa Scale; High, high FoxP3$^+$ T cells infiltration; Low, low FoxP3$^+$ T cells infiltration.

All analyses were performed with Review Manager version 5.2 (RevMan, Cochrane Collaboration, Oxford, England), except publication bias which was completed with stata 11.0 (Stata Corporation, College Station, TX). Statistical heterogeneity between trials was evaluated with Chi-square test. Heterogeneity was considered present when $P < 0.05$. In the absence of statistical significance for heterogeneity, the Mantel-Haenszel method for the fixed-effects model was used for meta-analysis. Otherwise, the DerSimonian and Laird method in the random-effects model was selected. 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 OR in the trial. $P < 0.05$ was considered statistically significant.

Results

Study selection and characteristics

A total of 414 trials were retrieved according to the initial search strategy. After screening all titles, abstracts, and full texts, 20 studies [9-28] met the entry criteria and were retrieved for further detailed evaluation (**Figure 1**). All 20 studies were retrospectively analyzed and their characteristics are summarized in **Table 1**. Sample sizes ranged from 42 to 1,252 and the total patient number was 5,846. Of these, 2,544 had high Tregs infiltration. For the 20 included studies, 17 provided data on 1, 3, and 5-year OS, 8 on 1, 3, and 5-year DFS, and 12 on clinical variable. These studies were conducted in 11 countries (Switzerland, United States of America, Korea, France, Japan, China, Germany, Netherlands, Sweden, Romania, and Australia).

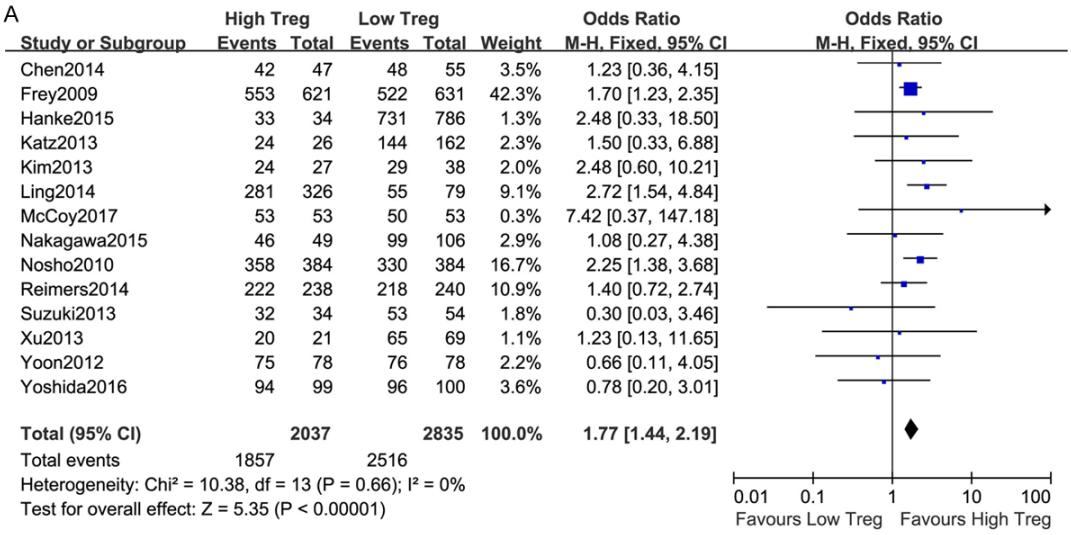
Quality assessment of included studies

Of the included studies, 4 studies [11, 17, 25, 27] were rated as 8 scores, 11 studies [9, 10, 12-14, 16, 18, 21, 23, 26, 35] were rated as 7 scores, 4 studies [15, 22, 24, 28] were rated as 6 scores, and 1 study [19] was rated as a 5 score, according to the Newcastle-Ottawa Scale.

Correlation between Tregs infiltration and OS for CRC

Heterogeneity was significant among the included 17 studies [9-19, 23-28] when comparing

A


High Treg
Low Treg
Odds Ratio
Odds Ratio
Study or Subgroup Events Total Events Total Weight M-H, Fixed, 95% CI
M-H, Fixed, 95% CI
Chen2014 42 47 48 55 3.5% 1.23 [0.36, 4.15]
Frey2009 553 621 522 631 42.3% 1.70 [1.23, 2.35]
Hanke2015 33 34 731 786 1.3% 2.48 [0.33, 18.50]
Katz2013 24 26 144 162 2.3% 1.50 [0.33, 6.88]
Kim2013 24 27 29 38 2.0% 2.48 [0.60, 10.21]
Ling2014 281 326 55 79 9.1% 2.72 [1.54, 4.84]
McCoy2017 53 53 50 53 0.3% 7.42 [0.37, 147.18]
Nakagawa2015 46 49 99 106 2.9% 1.08 [0.27, 4.38]
Nosho2010 358 384 330 384 16.7% 2.25 [1.38, 3.68]
Reimers2014 222 238 218 240 10.9% 1.40 [0.72, 2.74]
Suzuki2013 32 34 53 54 1.8% 0.30 [0.03, 3.46]
Xu2013 20 21 65 69 1.1% 1.23 [0.13, 11.65]
Yoon2012 75 78 76 78 2.2% 0.66 [0.11, 4.05]
Yoshida2016 94 99 96 100 3.6% 0.78 [0.20, 3.01]
Total (95% CI) 2037 2835 100.0% 1.77 [1.44, 2.19]
Total events 1857 2516
Heterogeneity: Chi² = 10.38, df = 13 (P = 0.66); I² = 0%
Test for overall effect: Z = 5.35 (P < 0.00001)
0.01 0.1 1 10 100
Favours Low Treg Favours High Treg

B

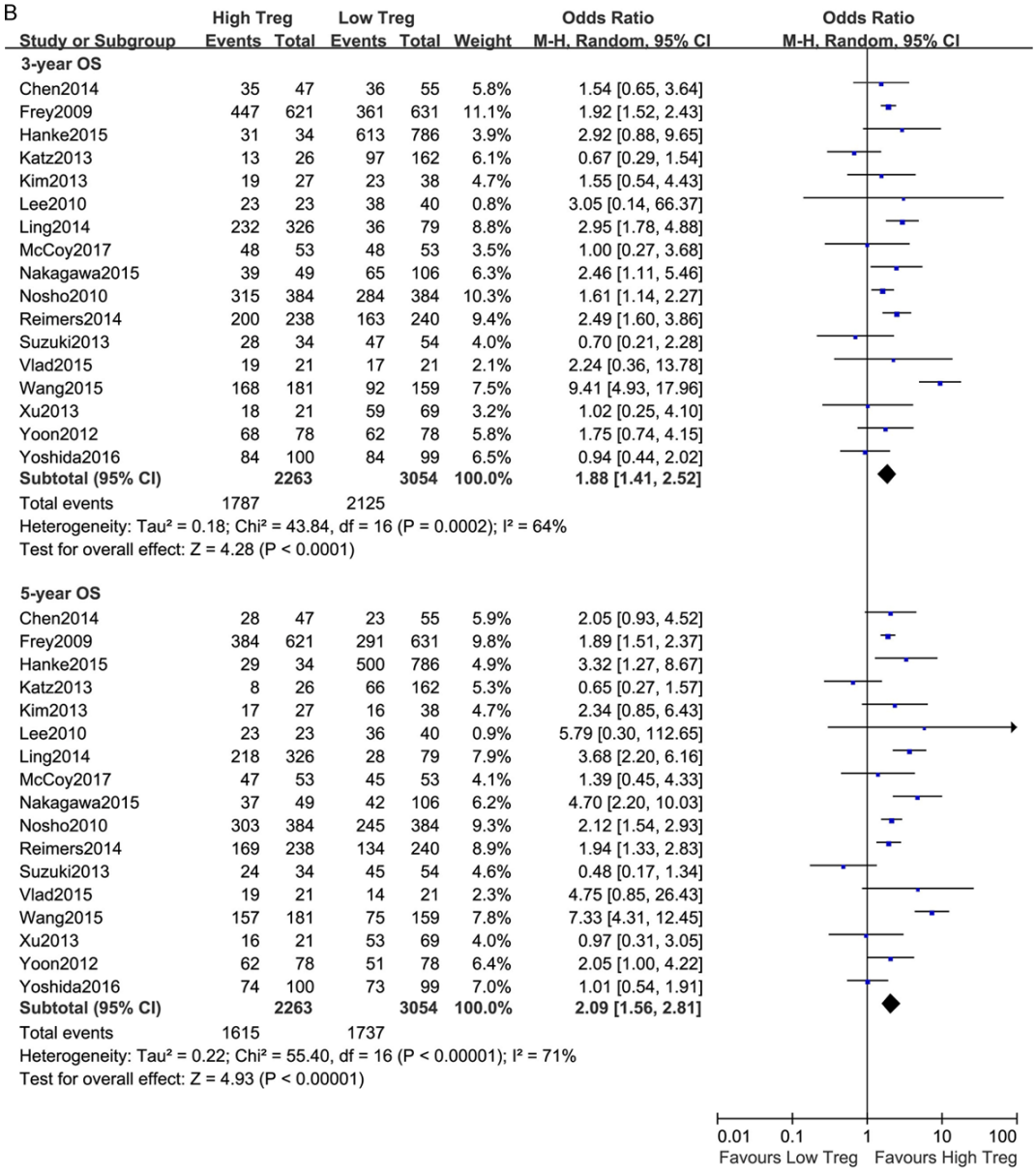

High Treg
Low Treg
Odds Ratio
Odds Ratio
Study or Subgroup Events Total Events Total Weight M-H, Random, 95% CI
M-H, Random, 95% CI
3-year OS
Chen2014 35 47 36 55 5.8% 1.54 [0.65, 3.64]
Frey2009 447 621 361 631 11.1% 1.92 [1.52, 2.43]
Hanke2015 31 34 613 786 3.9% 2.92 [0.88, 9.65]
Katz2013 13 26 97 162 6.1% 0.67 [0.29, 1.54]
Kim2013 19 27 23 38 4.7% 1.55 [0.54, 4.43]
Lee2010 23 23 38 40 0.8% 3.05 [0.14, 66.37]
Ling2014 232 326 36 79 8.8% 2.95 [1.78, 4.88]
McCoy2017 48 53 48 53 3.5% 1.00 [0.27, 3.68]
Nakagawa2015 39 49 65 106 6.3% 2.46 [1.11, 5.46]
Nosho2010 315 384 284 384 10.3% 1.61 [1.14, 2.27]
Reimers2014 200 238 163 240 9.4% 2.49 [1.60, 3.86]
Suzuki2013 28 34 47 54 4.0% 0.70 [0.21, 2.28]
Vlad2015 19 21 17 21 2.1% 2.24 [0.36, 13.78]
Wang2015 168 181 92 159 7.5% 9.41 [4.93, 17.96]
Xu2013 18 21 59 69 3.2% 1.02 [0.25, 4.10]
Yoon2012 68 78 62 78 5.8% 1.75 [0.74, 4.15]
Yoshida2016 84 100 84 99 6.5% 0.94 [0.44, 2.02]
Subtotal (95% CI) 2263 3054 100.0% 1.88 [1.41, 2.52]
Total events 1787 2125
Heterogeneity: Tau² = 0.18; Chi² = 43.84, df = 16 (P = 0.0002); I² = 64%
Test for overall effect: Z = 4.28 (P < 0.0001)
5-year OS
Chen2014 28 47 23 55 5.9% 2.05 [0.93, 4.52]
Frey2009 384 621 291 631 9.8% 1.89 [1.51, 2.37]
Hanke2015 29 34 500 786 4.9% 3.32 [1.27, 8.67]
Katz2013 8 26 66 162 5.3% 0.65 [0.27, 1.57]
Kim2013 17 27 16 38 4.7% 2.34 [0.85, 6.43]
Lee2010 23 23 36 40 0.9% 5.79 [0.30, 112.65]
Ling2014 218 326 28 79 7.9% 3.68 [2.20, 6.16]
McCoy2017 47 53 45 53 4.1% 1.39 [0.45, 4.33]
Nakagawa2015 37 49 42 106 6.2% 4.70 [2.20, 10.03]
Nosho2010 303 384 245 384 9.3% 2.12 [1.54, 2.93]
Reimers2014 169 238 134 240 8.9% 1.94 [1.33, 2.83]
Suzuki2013 24 34 45 54 4.6% 0.48 [0.17, 1.34]
Vlad2015 19 21 14 21 2.3% 4.75 [0.85, 26.43]
Wang2015 157 181 75 159 7.8% 7.33 [4.31, 12.45]
Xu2013 16 21 53 69 4.0% 0.97 [0.31, 3.05]
Yoon2012 62 78 51 78 6.4% 2.05 [1.00, 4.22]
Yoshida2016 74 100 73 99 7.0% 1.01 [0.54, 1.91]
Subtotal (95% CI) 2263 3054 100.0% 2.09 [1.56, 2.81]
Total events 1615 1737
Heterogeneity: Tau² = 0.22; Chi² = 55.40, df = 16 (P < 0.00001); I² = 71%
Test for overall effect: Z = 4.93 (P < 0.00001)
0.01 0.1 1 10 100
Favours Low Treg Favours High Treg

Figure 2. Forest plot for OS of CRC patients. Fixed effects model of OR for survival of follow-up 1-year (A); random effects model of OR for survival of follow-up 3 and 5-year (B) of CRC patients after surgery: high Tregs infiltration patients vs low Tregs infiltration patients.

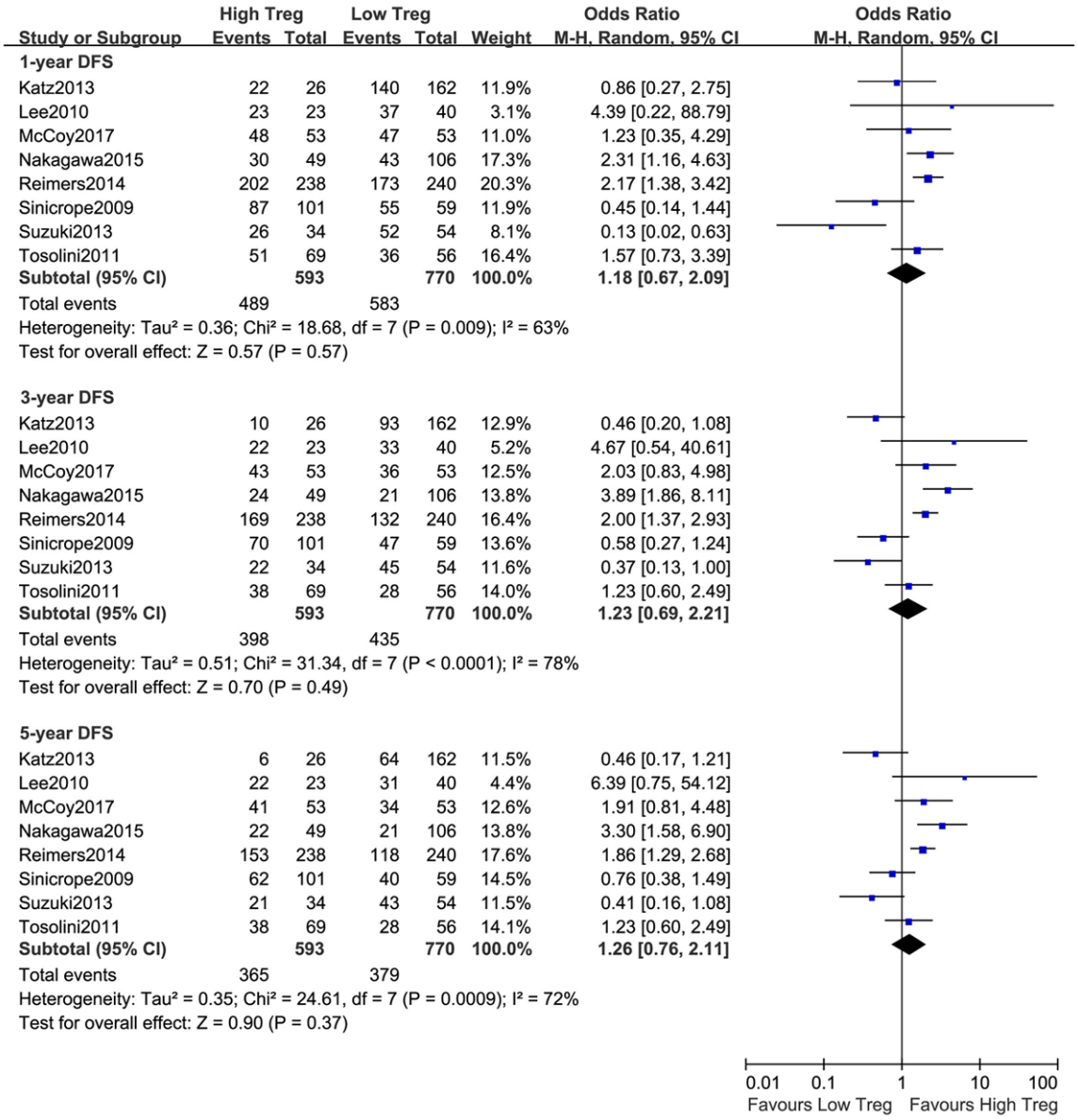


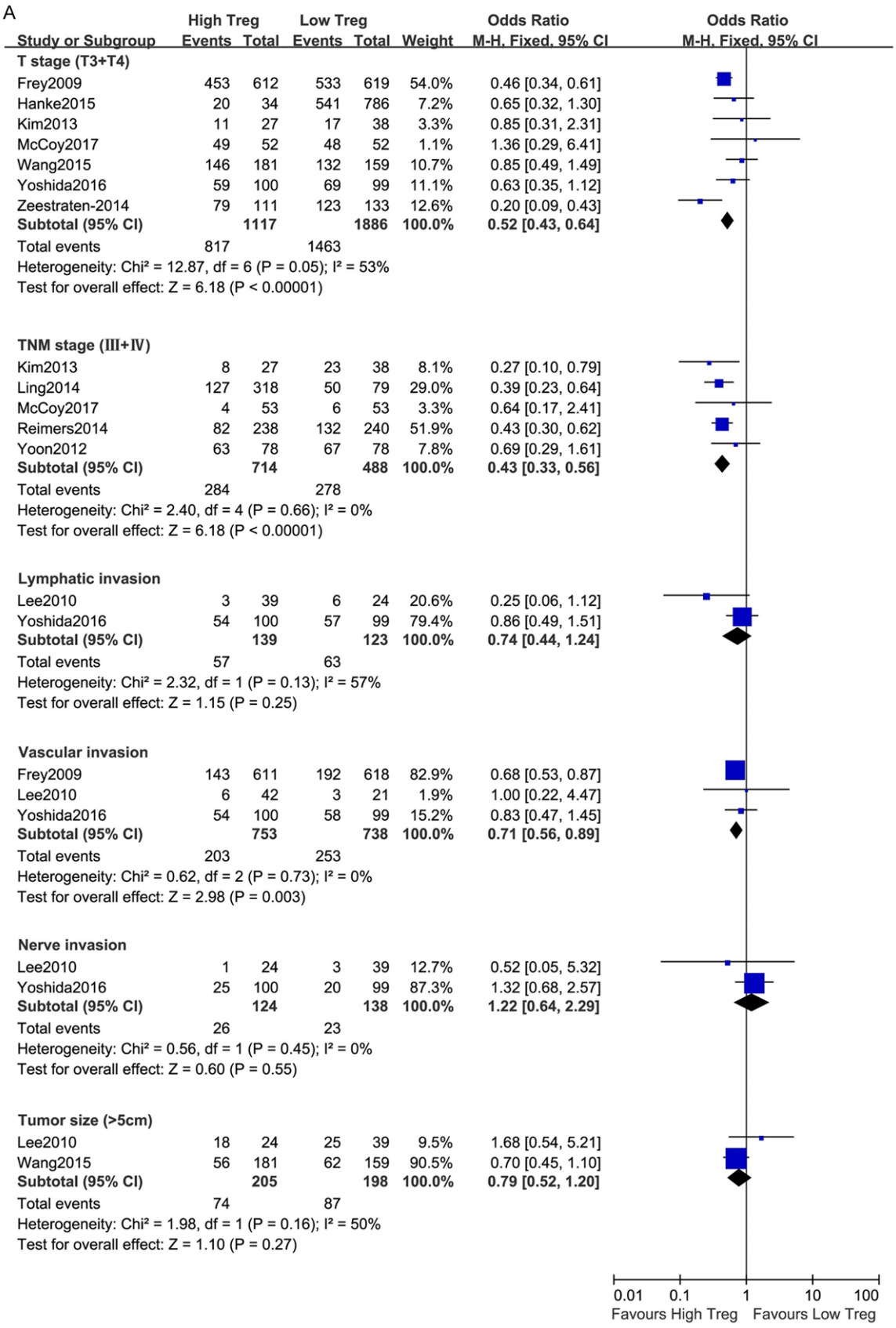
Figure 3. Forest plot for DFS of CRC patients. Random effects model of OR for survival of follow-up 1-year, 3-year, and 5-year of CRC patients after surgery: high Tregs infiltration patients vs low Tregs infiltration patients.

3 and 5-year OS between high Tregs infiltration and low Tregs infiltration for CRC (P = 0.0002, I^2 = 64% and P < 0.00001, I^2 = 71%, respectively). Meta-analysis with the fixed-model for 1-year OS and random-model for 3 and 5-year OS revealed that CRC patients with high Tregs infiltration had significantly high 1, 3, and 5-year OS compared to those with low Tregs infiltration (OR = 1.77, 95% CI = 1.44-2.19, P < 0.00001, **Figure 2A**; OR = 1.88, 95% CI = 1.41-2.52, P < 0.0001 and OR = 2.09, 95% CI = 1.56-2.81, P < 0.00001, respectively, **Figure 2B**).

Correlation between Tregs infiltration and DFS for CRC

Heterogeneity was significant among the included 8 studies [11, 14, 18, 21-23, 25, 28] when comparing 1, 3, and 5-year DFS between high

A


High Treg
Low Treg
Odds Ratio
Odds Ratio
Study or Subgroup Events Total Events Total Weight M-H, Fixed, 95% CI
M-H, Fixed, 95% CI
T stage (T3+T4)
Frey2009 453 612 533 619 54.0% 0.46 [0.34, 0.61]
Hanke2015 20 34 541 786 7.2% 0.65 [0.32, 1.30]
Kim2013 11 27 17 38 3.3% 0.85 [0.31, 2.31]
McCoy2017 49 52 48 52 1.1% 1.36 [0.29, 6.41]
Wang2015 146 181 132 159 10.7% 0.85 [0.49, 1.49]
Yoshida2016 59 100 69 99 11.1% 0.63 [0.35, 1.12]
Zeestraten-2014 79 111 123 133 12.6% 0.20 [0.09, 0.43]
Subtotal (95% CI) 1117 1886 100.0% 0.52 [0.43, 0.64]
Total events 817 1463
Heterogeneity: Chi² = 12.87, df = 6 (P = 0.05); I² = 53%
Test for overall effect: Z = 6.18 (P < 0.00001)
TNM stage (III+IV)
Kim2013 8 27 23 38 8.1% 0.27 [0.10, 0.79]
Ling2014 127 318 50 79 29.0% 0.39 [0.23, 0.64]
McCoy2017 4 53 6 53 3.3% 0.64 [0.17, 2.41]
Reimers2014 82 238 132 240 51.9% 0.43 [0.30, 0.62]
Yoon2012 63 78 67 78 7.8% 0.69 [0.29, 1.61]
Subtotal (95% CI) 714 488 100.0% 0.43 [0.33, 0.56]
Total events 284 278
Heterogeneity: Chi² = 2.40, df = 4 (P = 0.66); I² = 0%
Test for overall effect: Z = 6.18 (P < 0.00001)
Lymphatic invasion
Lee2010 3 39 6 24 20.6% 0.25 [0.06, 1.12]
Yoshida2016 54 100 57 99 79.4% 0.86 [0.49, 1.51]
Subtotal (95% CI) 139 123 100.0% 0.74 [0.44, 1.24]
Total events 57 63
Heterogeneity: Chi² = 2.32, df = 1 (P = 0.13); I² = 57%
Test for overall effect: Z = 1.15 (P = 0.25)
Vascular invasion
Frey2009 143 611 192 618 82.9% 0.68 [0.53, 0.87]
Lee2010 6 42 3 21 1.9% 1.00 [0.22, 4.47]
Yoshida2016 54 100 58 99 15.2% 0.83 [0.47, 1.45]
Subtotal (95% CI) 753 738 100.0% 0.71 [0.56, 0.89]
Total events 203 253
Heterogeneity: Chi² = 0.62, df = 2 (P = 0.73); I² = 0%
Test for overall effect: Z = 2.98 (P = 0.003)
Nerve invasion
Lee2010 1 24 3 39 12.7% 0.52 [0.05, 5.32]
Yoshida2016 25 100 20 99 87.3% 1.32 [0.68, 2.57]
Subtotal (95% CI) 124 138 100.0% 1.22 [0.64, 2.29]
Total events 26 23
Heterogeneity: Chi² = 0.56, df = 1 (P = 0.45); I² = 0%
Test for overall effect: Z = 0.60 (P = 0.55)
Tumor size (>5cm)
Lee2010 18 24 25 39 9.5% 1.68 [0.54, 5.21]
Wang2015 56 181 62 159 90.5% 0.70 [0.45, 1.10]
Subtotal (95% CI) 205 198 100.0% 0.79 [0.52, 1.20]
Total events 74 87
Heterogeneity: Chi² = 1.98, df = 1 (P = 0.16); I² = 50%
Test for overall effect: Z = 1.10 (P = 0.27)
0.01 0.1 1 10 100
Favours High Treg Favours Low Treg

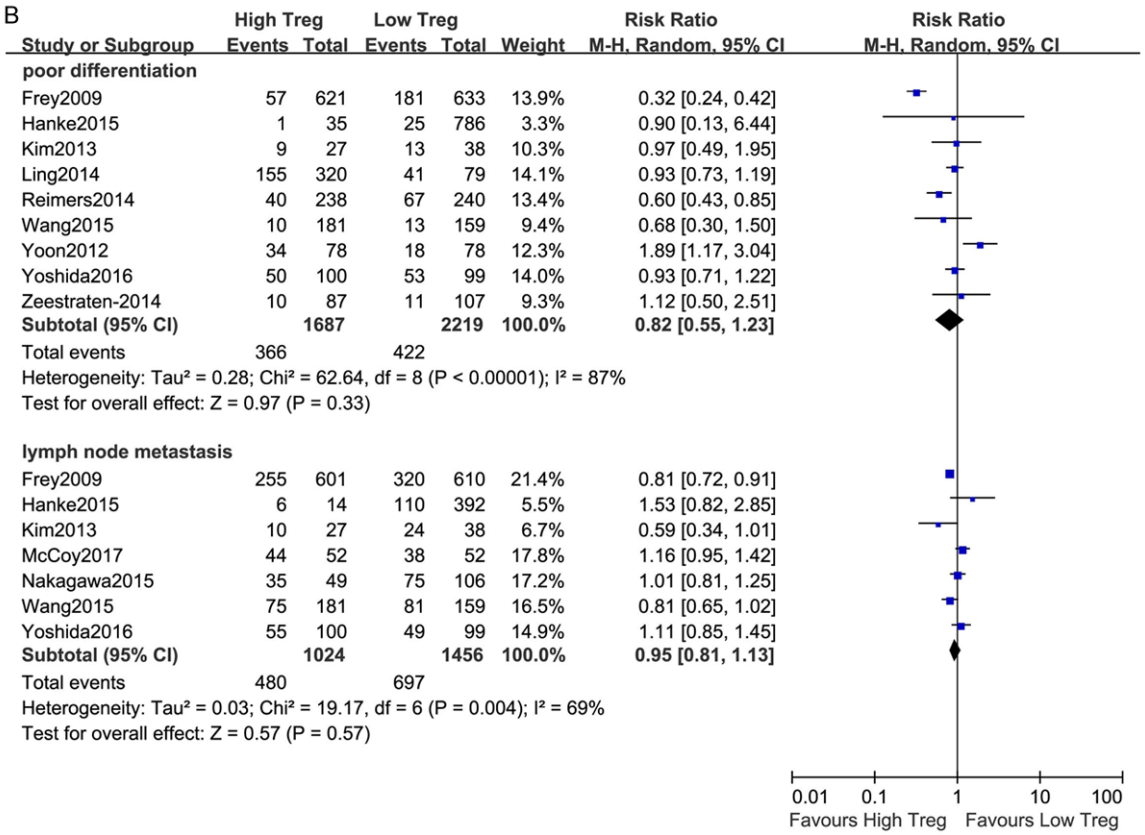


Figure 4. Forest plot for pathological variables in CRC patients after surgery. Correlation between Tregs infiltration and T stage (T3+T4), TNM stage (III+IV), lymphatic invasion, vascular invasion, nerve invasion, and tumor size (> 5 cm) with fixed effects model of OR (A); Correlation between Tregs infiltration and poor histological differentiation and lymph node metastasis with random effects model of OR (B).

Tregs infiltration and low Tregs infiltration for CRC (P = 0.009, I^2 = 63%; P < 0.0001, I^2 = 78%, and P = 0.0009, I^2 = 72%, respectively). Meta-analysis with the random-model showed that there were no significant differences for 1, 3, and 5-year DFS between high Tregs infiltration patients and low Tregs infiltration patients (OR = 1.18, 95% CI = 0.67-2.09, P = 0.57; OR = 1.23, 95% CI = 0.69-2.21, P = 0.49 and OR = 1.26, 95% CI = 0.76-2.11, P = 0.37, respectively, **Figure 3**).

Correlation between Tregs infiltration and clinicopathological variables for CRC

Stratifying for different clinicopathological variables after CRC surgical resection, heterogeneity was significant among included studies when comparing poor histodifferentiation and lymph node metastasis between high Tregs infiltration and low Tregs infiltration for CRC (P < 0.000001, I^2 = 87%; and P = 0.004, I^2 = 69%, respectively, **Figure 4B**). Meta-analysis with the random-model for those two clinicopathological variables and with the fix-model for other clinicopathological variables was performed. Statistical results in 7 studies [9, 16, 19, 24, 27, 28, 33] for T stage (T3+T4), 5 studies [12, 14, 15, 24, 28] for TNM stage (III+IV), and 3 studies [9, 11, 27] for vascular invasion in CRC suggested that T stage (T3+T4), TNM stage (III+IV), and vascular invasion were more likely to occur in low Tregs infiltration (77.6%, 57% and 34.3%) than in high Tregs infiltration (73.1%, 40% and 29%) (OR = 0.52, 95% CI = 0.43-0.64, P < 0.00001; OR = 0.43, 95% CI = 0.33-0.56, P < 0.00001; and OR = 0.71, 95% CI = 0.56-0.89, P = 0.003; respectively, **Figure 4A**). There were no significant differences for lymphatic invasion in 2 studies [11, 27], nerve invasion in 2 studies [11, 27], and tumor size (> 5 cm) in 3 studies [11, 17, 27] in CRC between high Tregs infiltration and low Tregs infiltration (OR = 0.74, 95% CI = 0.44-1.24, P = 0.25; OR = 1.22, 95% CI = 0.64-2.29, P = 0.55 and OR = 0.79, 95% CI = 0.52-1.20, P = 0.27; respective-

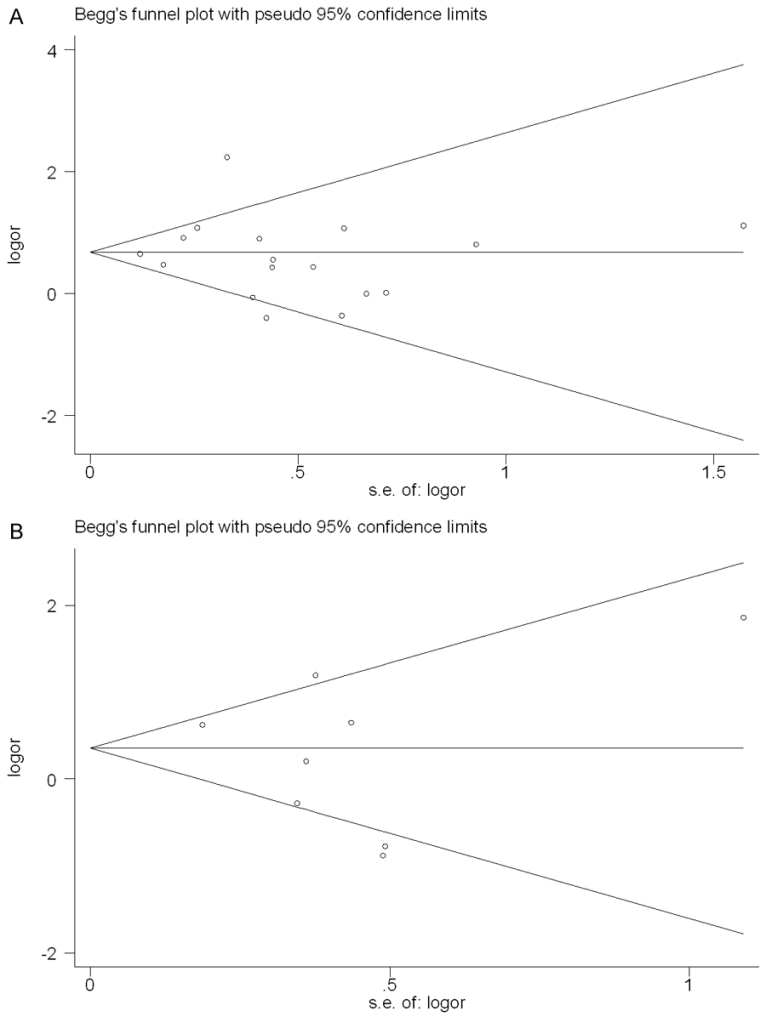


Figure 5. Begg's funnel plot of studies included for OS (A) and DFS (B) in CRC patients. This funnel plot displays in OR against its standard error (s.e.) for each individual study. The horizontal line represents the estimate of the OR, with the dash lines indicate the expected 95% CI for a given standard error.

ly, **Figure 4A**). At the same time, this study explored the relationship between Tregs infiltration and poor differentiation and lymph node metastasis in CRC. It was found that there were no significant differences for poor differentiation in 9 studies [9, 12, 14-16, 19, 24, 27, 35] and lymph node metastasis in 7 studies [9, 16, 19, 24, 27, 28, 35] between high Tregs infiltration and low Tregs infiltration (OR = 0.82, 95% CI = 0.55-1.23, P = 0.33 and OR = 0.95, 95% CI = 0.81-1.13, P = 0.57; respectively, **Figure 4B**).

Publication bias

Begg's funnel plot and Egger's test were used to detect publication bias for OS and DFS for CRC patients in included studies. As shown in **Figure 5A** and **5B**, no obvious asymmetry was revealed in the funnel plot. Begg's test and Egger's test were performed to further detect asymmetry. Statistical evidence suggested that there was no significant publication bias among included studies for this meta-analysis (Begg's test score of P = 0.837 and Egger's test score of P = 0.636 in OS studies and Begg's test score of P = 0.711 and Egger's test score of P = 0.566 in DFS studies).

Discussion

CRC is a common malignant tumor with relatively high morbidity and mortality in China [36]. At present, the main therapy methods for CRC include preoperative concurrent chemoradiotherapy, radical surgery, postoperative concurrent chemoradiotherapy, adjuvant chemotherapy, and molecular target therapy. Selection of the right treatment regimen for CRC is based on clinical pathological variables [37]. Many patients have experienced inadequate treatment or excessive treatment [38, 39]. Choosing better markers, combined with clinical pathological variables, to determine a treatment regimen is a great challenge with CRC.

Tregs are functionally immunosuppressive subsets of CD4+ T-cells found by Sakaguchi in 1995 [40]. They take part in regulating the balance between self-tolerance and self-rejection by secreting IL-4, IL-10, TGF-β, and other cytokines [41]. High Tregs infiltration has been identified as a poor outcome in most common tumors. However, many studies have shown high Tregs infiltration with a better prognosis in CRC [29, 30]. In contrast, there are still some uninterrupted research reports concerning high FoxP3$^+$ T cells infiltration with a poor prognosis [20] or non-predictive role [21-28] in CRC.

Outcomes of the present study indicated that high density of tumor-infiltrating Tregs is associated with better survival for 1, 3, and 5-year OS. The reasons may be that Tregs suppress inflammation and immune responses resulting from bacterial invasion, which may lead to tumorigenesis [42]. Unfortunately, this study also suggests that there were no significant differences for 1, 3, and 5-year DFS between high density tumor-infiltrating Tregs and low density tumor-infiltrating Tregs in CRC. This might indicate that Tregs cannot be used as an indicator of recurrence and metastasis for CRC. Regarding clinicopathological variables after CRC surgical resection, statistical results suggested that there were less tumor-infiltrating Tregs in T stage (T3+T4), TNM stage (III+IV), and vascular invasion for CRC. There were no significant differences for lymphatic invasion, nerve invasion, tumor size (> 5 cm), poor histological differentiation, and lymph node metastasis between high Tregs infiltration and low Tregs infiltration in CRC patients. This implies that low Tregs infiltration is likely to be considered a risk factor for T stage (T3+T4), TNM stage (III+IV), and vascular invasion in CRC patients. Hence, it is possible to extend OS and avoid T stage (T3+T4), TNM stage (III+IV), and vascular invasion by increasing Treg levels on the basis of previous treatment for CRC patients. At the same time, subsequent treatment regimens can be determined according to levels of Tregs infiltration combined with clinicopathological variables for CRC patients after radical surgery. However, well-designed large sample trials still need to be conducted to evaluating roles of Tregs infiltration in CRC.

There are many limitations to this meta-analysis. First, more high-quality studies are needed to confirm these results. Second, patient surgical procedures, surgical quality, postoperative stage, and treatment regimens were inconsistent. Third, antibodies, IHC cell-scoring strategies, and cutoff values were not defined consistently in these studies, therefore, these factors may contribute to potential publication bias.

Publication bias [43] is an important deficiency in meta-analysis, as some studies with negative results have more difficulty being accepted for publication. Thus, researchers are encouraged to publish studies including some negative results. There was, however, no evidence of publication bias of included studies for this meta-analysis.

In conclusion, this meta-analysis demonstrated that high densitiy tumor-infiltrating Tregs were associated with better OS, lower T stage, lower TNM stage, and less vascular invasion in CRC patients. These results might arouse many researchers to pursue therapy strategies increasing Tregs for CRC. Unfortunately, this present meta-analysis also showed no significant differences for DFS in CRC patients between high density tumor-infiltrating Tregs and low density tumor-infiltrating Tregs. Additional high quantity investigations are needed to confirm the exact value of tumor-infiltrating Tregs in CRC patients.

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Disclosure of conflict of interest

None.

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