Research Paper

Prognostic value of tumor-infiltrating Foxp3+ regulatory T cells in patients with breast cancer: a meta-analysis

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Abstract

Purpose: The prognostic value of tumor-infiltrating Foxp3+ regulatory T cells (Tregs) in breast cancer remains controversial. Therefore, we performed this meta-analysis to determine the impact of Foxp3+ Tregs infiltration on survival outcomes.

Methods: Relevant literature was retrieved from Pubmed, Web of science and Cocohrane until May 30, 2016. Meta-analysis was performed using hazard ratios (HRs), odds ratio (OR) and 95 % confidence intervals (CI) as effect measures.

Results: Fourteen studies (10,259 patients) were included. Meta-analysis showed that high Foxp3+ Tregs infiltration was correlated with high histological grade (OR= 2.96, 95%CI [2.03-4.31]), estrogen receptor (ER) negativity (OR= 0.38, 95%CI [0.23-0.60]), human epidermal growth factor receptor type 2 (HER2) positivity (OR=2.43, 95%CI [1.69-3.51]). The detection of FOXP3+ Tregs was significantly associated with recurrence-free survival (RFS) of patients (HR = 1.58, 95 % CI [1.03–2.44]).

Conclusion: Our meta-analysis suggests that high Foxp3+ Tregs infiltration is associated with poor RFS in breast cancer patients and predicts histological grade, estrogen receptor and HER-2 status.

Key words: Foxp3; Regulatory T cells; Breast cancer; Prognosis; Meta-analysis.

Introduction

Breast cancer is the most commonly diagnosed cancer in women and the second leading cause of cancer-related mortality among women worldwide [1, 2]. Despite recent progress in breast cancer diagnosis and therapy, the prognosis of breast cancer patients is still poor. Local tumor microenvironment, including tumor cells, extracellular substrates, cytokines and tumor-infiltrating immune cells play an essential role in tumor formation, growth, invasion, and metastasis [3-5]. Regulatory T cells (Tregs) are a subgroup of CD4 + T helper cells that suppress T cell immunity. Forkhead box protein P3 (FoxP3) is a transcription factor imperative and sufficient for the induction of immunosuppressive function of Tregs and could be the most specific marker for Tregs in tumors [6].

Due to the ability to inhibit anti-tumor immunity, high level of tumor-infiltrating Foxp3+ Tregs is expected to be associated with poor prognosis. However, recent studies have challenged this idea and shown that Foxp3+Tregs could improve survival in some tumors [7-9]. To resolve the contradiction, in this study we performed a meta-analysis to investigate the correlation of Foxp3+Tregs and the survival of breast cancer patients.

Methods

Search strategy

Pubmed, Web of science, Cocohrane (last search updated in May 30, 2016) were searched by using the following keywords (Foxp3 OR Forkhead transcription Factors), (Breast cancer OR breast neoplasm OR breast tumor), (Survival OR prognosis OR outcome OR mortality). The identified studies were selected based on following criteria:

Inclusion criteria

(1) Histological type of cancer was breast cancer. (2) Samples were collected by core-needle biopsy or surgery. (3) FOXP3 expression was detected by immunohistochemistry (IHC). (4) Hazard ratio (HR), odds ratio (OR) and their 95 % confidence interval (CI) were reported; if not, outcomes RFS and OS were reported. (5) Published in English. (6) When duplicate data were reported, only the most recent data were included.

Exclusion criteria

(1) Reviews, meta-analysis and conference abstracts were excluded. (2) Animal or cell line studies. (3) Peripheral blood samples or Foxp3 expressed on tumor cells.

Data extraction

Two investigators independently extracted the data from each study, including the first author's surname, geographical location, sample size, publication date, the source of the subjects, protein detection method, median follow-up time and survival data, the discrepancy was resolved by discussion.

Methodological assessment

To evaluate the study methodology, two investigators scored each study independently using Newcastle-Ottawa Scale (NOS) criteria [10]: (1) subject selection: 0-4; (2) comparability of subject: 0-2; (3) clinical outcome: 0-3. NOS scores ranged from 0 to 9; and a score≥7 indicated good quality.

Statistical analysis

The heterogeneity was assessed by X^2 test and the I^2 statistic of inconsistency [11, 12]. Statistically significant heterogeneity was defined as X^2 P < 0.1 or I^2 > 50%. We conducted subgroup analysis by stratifying Tregs location and geographical location. For the pooled analysis of the correlation between FoxP3+ Tregs infiltration and clinicopathological features (Tumor size, histological grade, Lymphnodes metastasis, ER and HER-2 status), odds ratios (ORs) and their 95% CIs were combined to estimate the effect. We performed sensitivity analysis to evaluate the influence of single study on the overall outcome. In addition, we performed funnel plots and Begg's linear regression test to examine publication bias [13]. All analysis was performed using STATA version 12.0.

Results

Study selection and characteristic

As shown in Figure 1, we identified 778 articles. 235 duplicated articles were excluded. We reviewed the titles and abstracts of 543 articles and excluded 503 irrelevant articles. After we reviewed the full texts 26 articles were further excluded. After the selection, 14 articles were finally enrolled for meta-analysis of the prognostic value of tumor-infiltrating Foxp3+ T cells in breast cancer [14-27].

We extracted RFS data from 8 of the 14 articles and OS data from 11 of the 14 articles. These studies evaluated patients from East Asia, Europe, Middle East and North America, including 3 from China, 2 from Japan, 2 from Korea, 2 from Netherlands, 2 from UK, 1 from Turkey and 2 from Canada. All the included studies evaluated FOXP3+ Tregs with immunohistochemical method. The 14 studies included 10,259 patients, the sample sizes ranged from 86 to 3,277. Six of these studies enrolled less than 200 patients and eight studies included more than 200 patients. The NOS scores of these studies ranged from 5 to 8, thus the quality of all included studies was good. Table 1 listed clinical features of 14 included studies.

Main results of meta-analysis

Correlation of FOXP3+Tregs with clinicopathological parameters

Our meta-analysis showed statistically significant association between Foxp3+Tregs and histological grade (III vs. I, Π OR=2.96, 95%CI 2.03-4.31, Random effect). In addition, the presence of FOXP3+Tregs showed significant difference between ER positive and ER negative groups (OR=0.38, 95%CI 0.23-0.60, Random effect). There was also a significant difference in the incidence of FOXP3+Tregs detection between HER2 positive group and HER2 negative group (OR=2.43, 95%CI 1.69-3.51, Random effect). Other factors such as tumor size (>2 cm vs. ≤2 cm OR=1.08, 95%CI 0.86-1.35, Random effect) and lymph node metastasis (Yes vs. No OR=1.08, 95% CI 0.85-1.39, Random effect) had no significant association with Foxp3+ Tregs infiltration (Table 2).
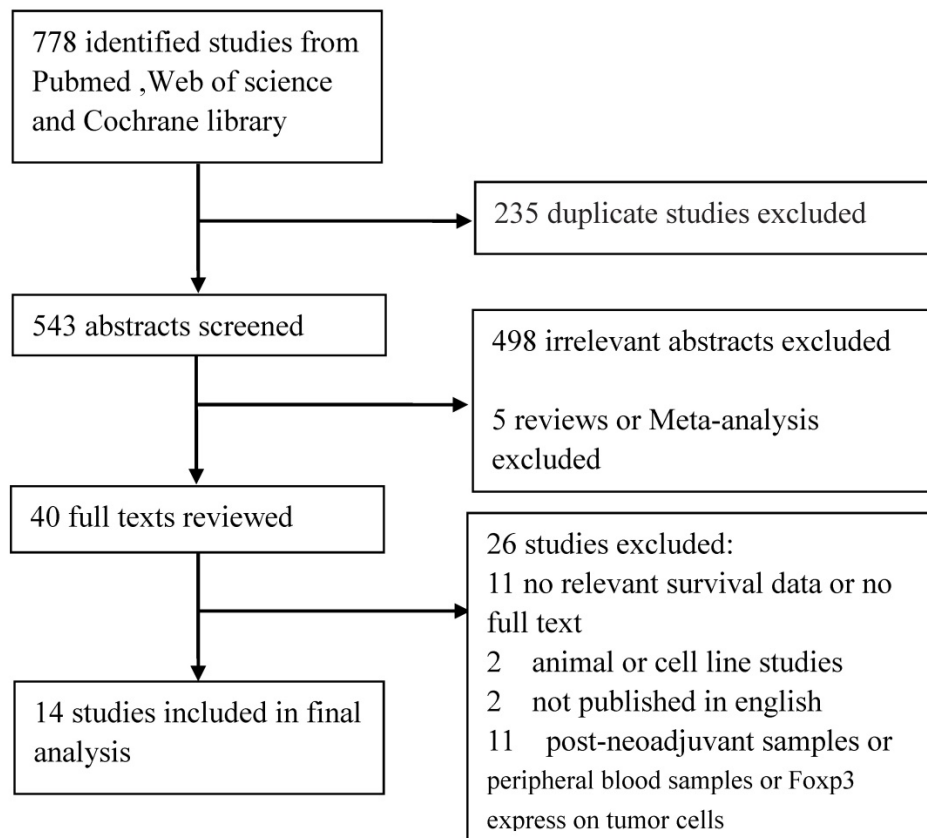
Figure 1. Flow chart shows study selection procedure

Table 1. Main features and methodological assessment of the included studies

Study	Publication year	Patients source	No. of patients	NOS score	Tregs Location	Methods	Outcome	Follow-up (M)
Engels	2015	Netherland	2426	7	Total	IHC	OS/RFS	70.8
Kim	2014	Korea	143	5	peritumoral	IHC	OS/RFS	69
Sun	2014	China	218	8	Total	IHC	OS/RFS	72
Demir	2013	Turkey	101	6	Intratumoral	IHC	OS	27,7
Lee	2013	Korea	86	6	peritumoral	IHC	OS	73.5
Miki	2013	Japan	100	6	Total	IHC	OS	NR
Liu	2011	China	1270	7	Peritumoral Intratumoral	IHC	OS	66
Bates	2006	UK	237	6	Total	IHC	OS/RFS	87.6
Mahmoud	2011	UK	1448	6	Peritumoral Intratumoral	IHC	OS	128
Liu	2012	China	132	6	Peritumoral Intratumoral	IHC	OS/RFS	62
De krui	2010	Netherland	556	7	Intratumoral	IHC	RFS	228
West	2013	Canada	175	6	Total	IHC	RFS	83
Maeda	2014	Japan	90	6	Total	IHC	RFS	67
Liu S	2014	Canada	3277	6	Intratumoral	IHC	OS	151

IHC: Immunohistochemistry; OS: Overall survival; NR: Not reported; M: Months

Table 2. Meta-analysis of high Tregs infiltration and clinicopathological features of breast cancer patients

| Stratification of breast cancer (Foxp3+ High vs Low)	No. of studies	No. of patients	Pooled OR (95% CI)		Heterogeneity	
			Fixed	Random	I^2	P-value
Tumor size (>2cm vs ≤2cm)	7	5,748	0.99(0.89-1.11)	1.08(0.86-1.35)	63.5%	0.012
LN metastasis (Yes vs No)	9	5,842	1.02(0.92-1.13)	1.08(0.85-1.39)	73.3%	<0.0001
Histological grade (III vs I, II)	9	6,078	2.33(2.09-2.60)	2.96(2.03-4.31)	85.6%	<0.0001
ER status (Positive vs Negative)	9	6,247	0.32(0.28-0.38)	0.38(0.23-0.60)	87.8%	<0.0001
HER-2 status (Positive vs Negative)	9	3,789	2.26(1.88-2.71)	2.43(169-3.51)	54.1%	0.026
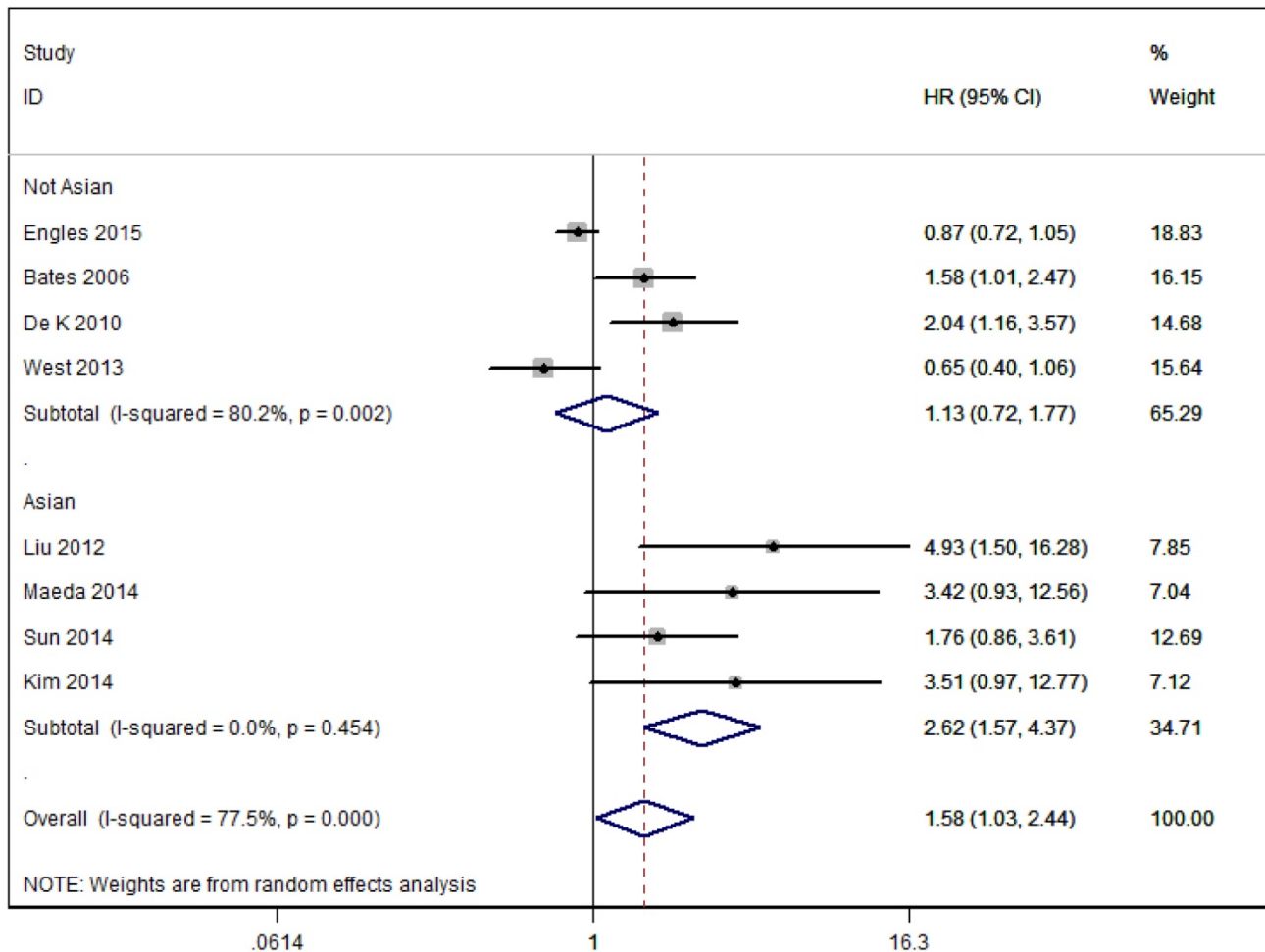
Figure 2. Meta-analysis of the effects of Foxp3+ Tregs infiltration on RFS of patients with breast cancer.

Impact of FOXP3+ Tregs on survival outcomes

As shown in Figure 2, the pooled results of eight studies that assessed RFS showed that patients with high overall Foxp3+ T cells infiltration had significantly decreased RFS compared to patients with low/negative Foxp3+ Tregs infiltration in the random effects model (combined HR 1.58, 95% CI 1.03–2.44) and presented a significant degree of heterogeneity (I^2=77.5%, p<0.0001). Subgroup analysis based on geographical location showed similar results to overall analysis in Asian group patients (Asia: HR = 2.62, 95 % CI [1.57, 4.37], I^2=0.0%), but not in No-Asian group patients (Europe and North America: HR = 1.13, 95 % CI [0.72, 1.77], I^2=80.2%).

As shown in Figure 3, eleven studies assessed OS with the pooled random HR of 1.15 (95%CI 0.89-1.48) and there was evidence for heterogeneity (I^2=73.0%, P<0.0001). Subgroup analysis based on Tregs site showed poor OS in breast cancer patients wit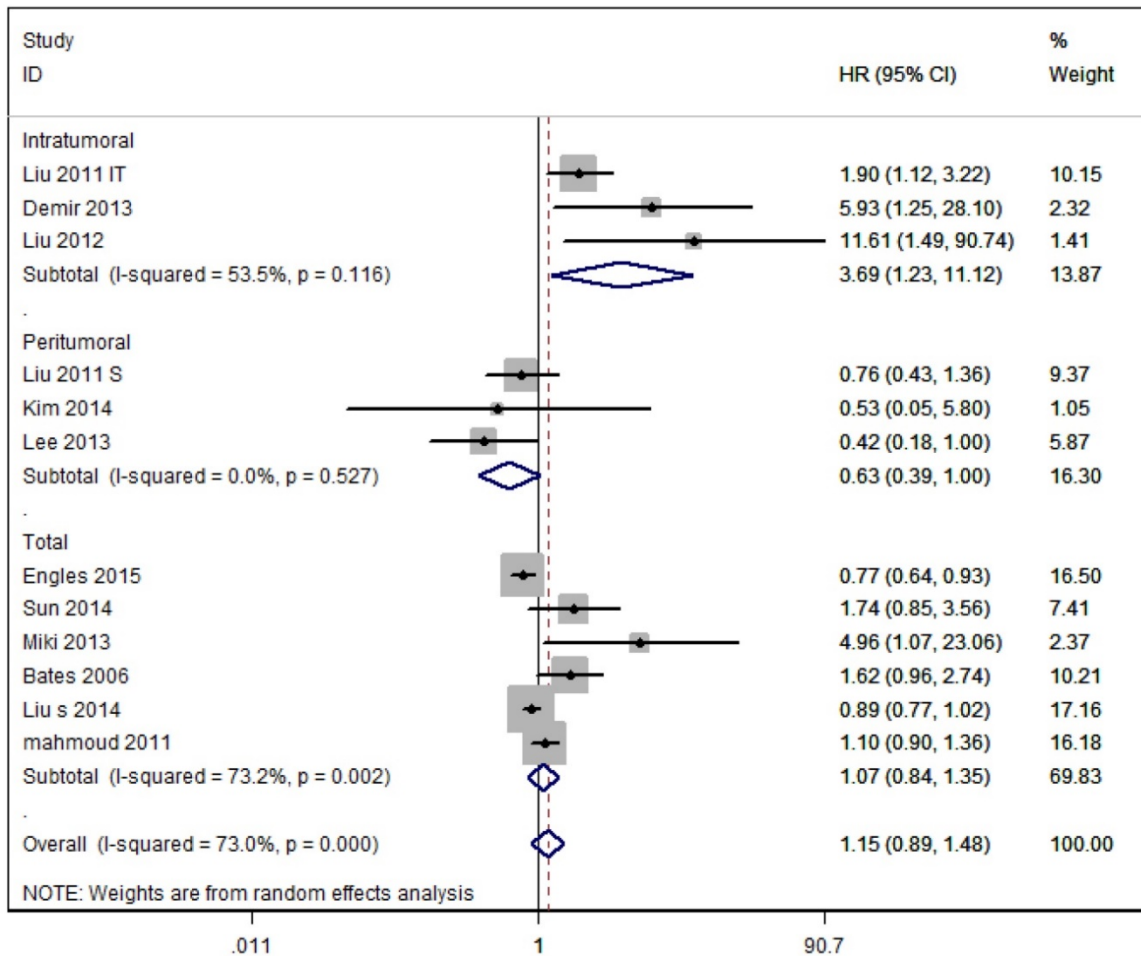h intratumoural detection of FOXP3+ Tregs, but not in those with peritumoral or Total detection of FOXP3+ Tregs (Intratumoural: HR = 3.69, 95 % CI [1.23-11.12], I^2= 53.5 %; Peritumoral: HR = 0.63, 95% CI [0.39-1.004], I^2= 0.0 %; Total: HR = 1.07, 95% CI [0.84-1.35], I^2 = 73.2 %).
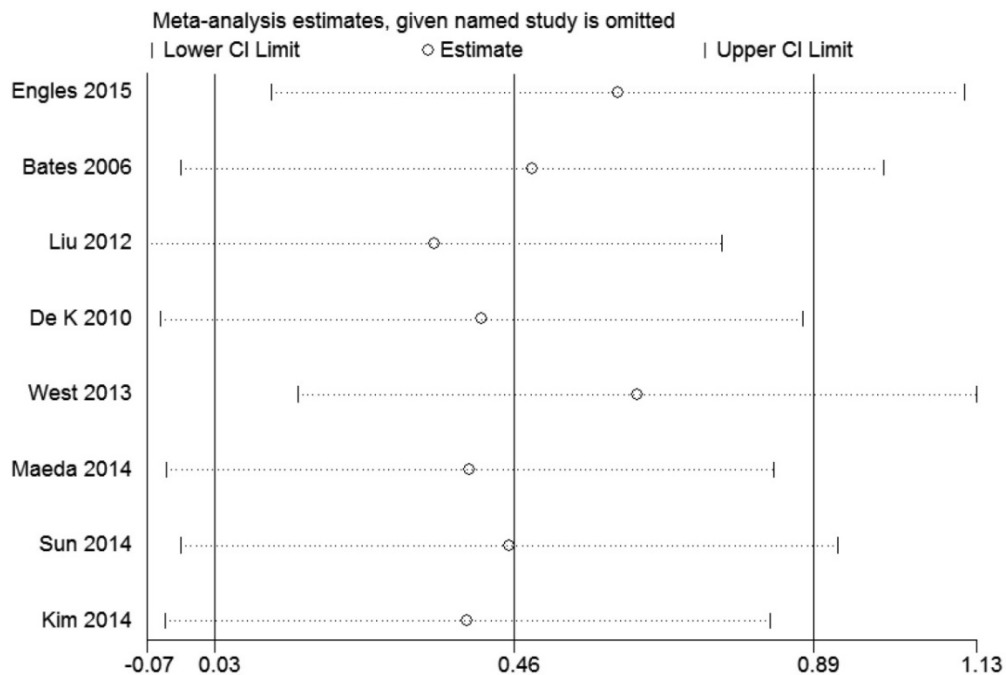
Sensitivity analysis

By sensitivity analysis we found similar HRs and 95% CIs if we omitted any single study, indicating that the results of RFS and OS were relatively stable (Figure 4 and 5).

Publication bias

For RFS, the evaluation of publication bias by Bgger tests (p=0.386, Figure 6) showed no publication bias for all included studies. Egger's linear regression did not reveal obvious publication bias (P=0.057). For OS, Bgger's funnel plot did not show obvious asymmetry (P = 0.119, Figure 7), and Egger's linear regression did not reveal obvious publication bias (P = 0.069).

Study ID		HR (95% CI)	% Weight
Intratumoral			
Liu 2011 IT		1.90 (1.12, 3.22)	10.15
Demir 2013		5.93 (1.25, 28.10)	2.32
Liu 2012		11.61 (1.49, 90.74)	1.41
Subtotal (I-squared = 53.5%, p = 0.116)		3.69 (1.23, 11.12)	13.87
.			
Peritumoral			
Liu 2011 S		0.76 (0.43, 1.36)	9.37
Kim 2014		0.53 (0.05, 5.80)	1.05
Lee 2013		0.42 (0.18, 1.00)	5.87
Subtotal (I-squared = 0.0%, p = 0.527)		0.63 (0.39, 1.00)	16.30
.			
Total			
Engles 2015		0.77 (0.64, 0.93)	16.50
Sun 2014		1.74 (0.85, 3.56)	7.41
Miki 2013		4.96 (1.07, 23.06)	2.37
Bates 2006		1.62 (0.96, 2.74)	10.21
Liu s 2014		0.89 (0.77, 1.02)	17.16
mahmoud 2011		1.10 (0.90, 1.36)	16.18
Subtotal (I-squared = 73.2%, p = 0.002)		1.07 (0.84, 1.35)	69.83
.			
Overall (I-squared = 73.0%, p = 0.000)		1.15 (0.89, 1.48)	100.00
NOTE: Weights are from random effects analysis			

.011   1   90.7

Figure 3. Meta-analysis of the effects of Foxp3+ Tregs infiltration on OS of patients with breast cancer. As Liu et al. 2012 reported two HRs based on the location of Foxp3+ Tregs, we used their data separately.

Meta-analysis estimates, given named study is omitted

| Lower CI Limit   ○ Estimate | Upper CI Limit

Engles 2015

Bates 2006

Liu 2012

De K 2010

West 2013

Maeda 2014

Sun 2014

Kim 2014

-0.07   0.03   0.46   0.89   1.13

Figure 4. Sensitivity analysis of the pooled hazard ratios coefficients on the relationships between Foxp3+ Tregs and RFS of patients with breast cancer.
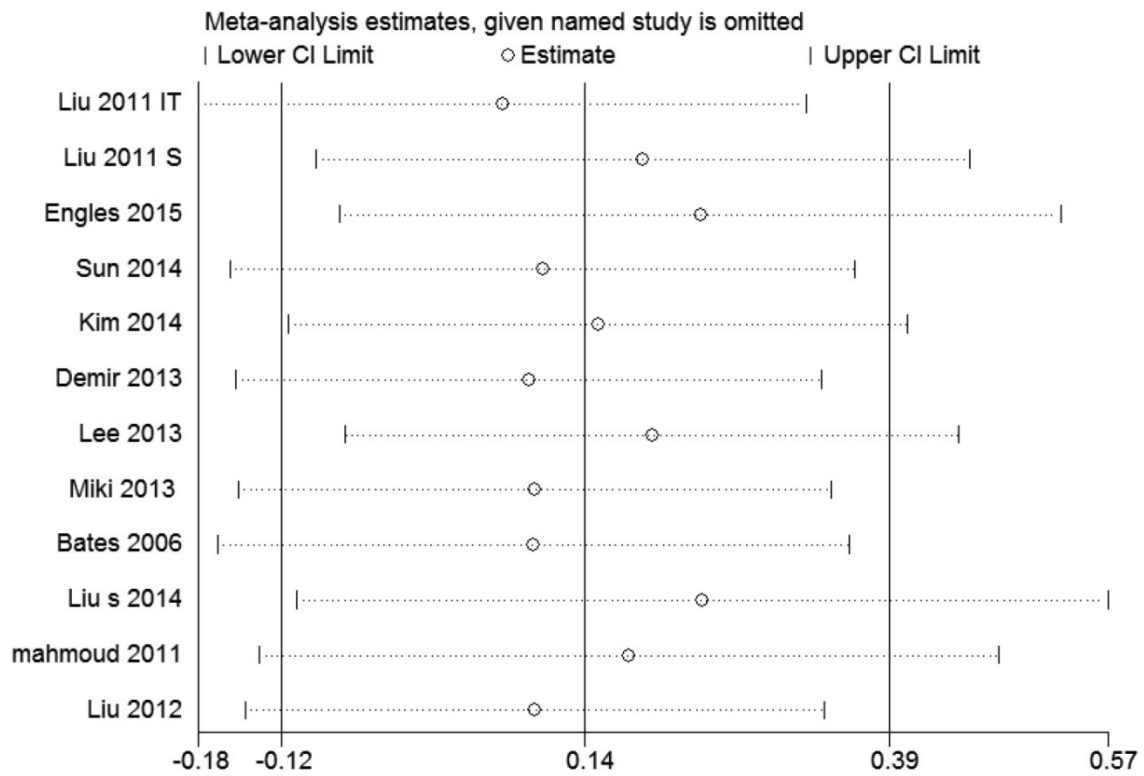
Figure 5. Sensitivity analysis of the pooled hazard ratios coefficients on the relationships between Foxp3+ Tregs and OS of patients with breast cancer.
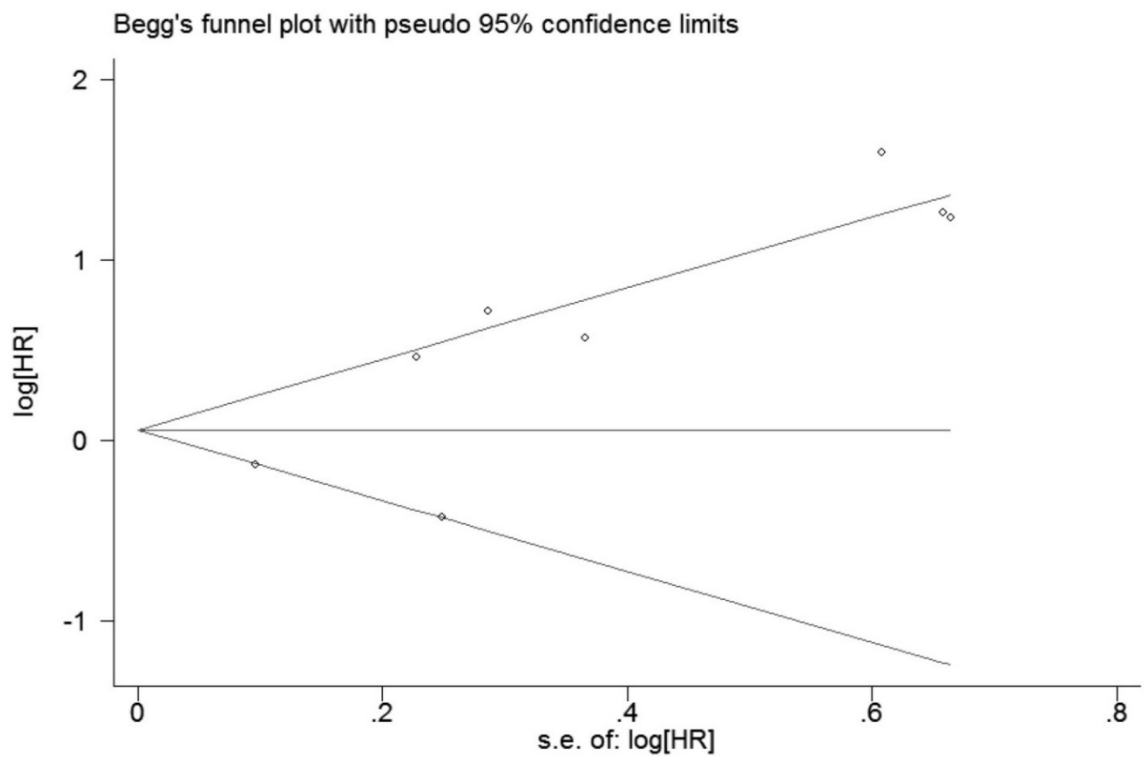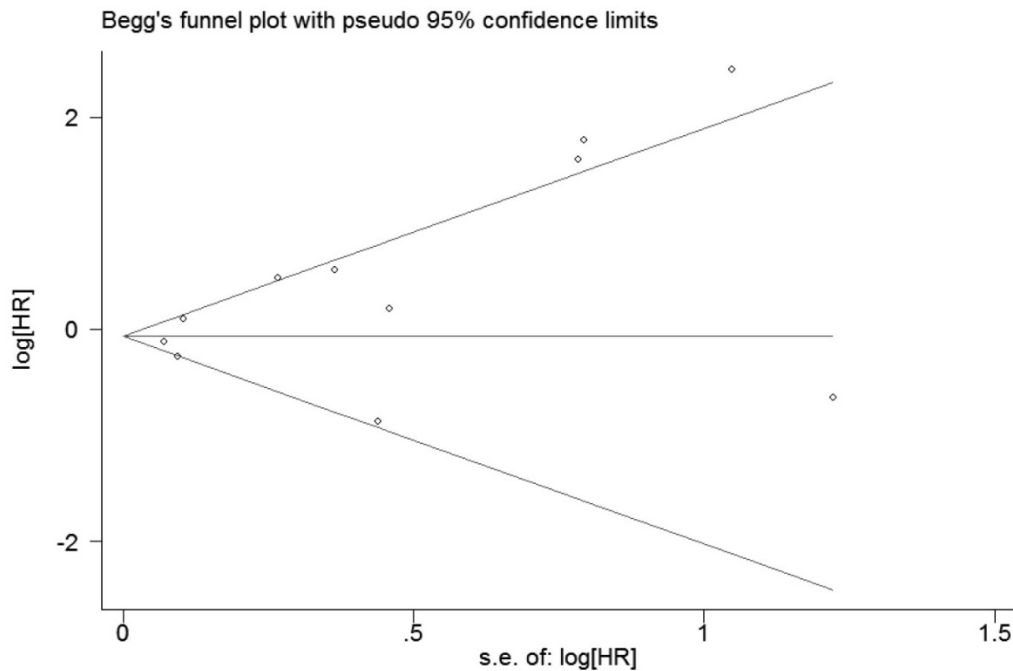

Figure 6. Funnel plot of publication biases on the relationships between Foxp3+ Tregs infiltration and RFS of patients with breast cancer.

Figure 7. Funnel plot of publication biases on the relationships between Foxp3+ Tregs infiltration and OS of patients with breast cancer.

Discussion

Tumor immunity has big impact on clinical outcome of cancer patients [28]. Tregs play an important role of immunosuppression by regulating the balance between tolerance and rejection of self and altered self and secreting cytokines such as TGF-b, IL-4 and IL-10 [29]. Tregs markers such as GITR, OX-4, CTLA-4, CD127 and transcription factor FoxP3 have been reported, and FoxP3 is regarded as the most specific marker [30, 31]. Emerging evidence suggests that Tregs play an essential role in immune evasion mechanisms of cancer [32-35]. However, prognostic significance of FOXP3+ Tregs in breast cancer patients is still unclear. A study showed that Foxp3+Tregs were associated with good outcome of breast cancer patients [12]. Nevertheless, other studies suggested that Foxp3+Tregs were correlated with poor prognosis of breast cancer [17, 20]. Insignificant association between Foxp3+Tregs and prognosis of breast cancer patients was also reported [15, 16]. The inconsistency may be caused by different genetic backgrounds, chemotherapy agents and surgical procedures. A recent review suggested that Tregs are equipped with cytotoxic potential which may directly kill effector T cell [36]. This may explain the relationship between infiltration of Foxp3+ Tregs and poor RFS of breast cancer patients. In addition, our data showed that high infiltrated Foxp3+ Tregs was associated with a worse OS. However, the results were not statistically significant. As we know, many kinds of factors may affect overall survival rate of

patients. Thus Foxp3+ Treg alone may be not enough to predict OS of breast cancer patients.

In this meta-analysis, we investigated the association between Foxp3+Tregs and clinical outcome of breast cancer patients. Combining the data of 10,259 patients from 14 studies, our analysis revealed that high overall Foxp3+Tregs infiltration was positively correlated with high histological grades, ER negativity and Her-2 overexpression, and short RFS. However, FOXP3+Tregs infiltration was not associated with OS of breast cancer patients. Subsequently, sensitivity analysis confirmed that our results were stable. Funnel plot analysis revealed no publication bias. The relationship between Foxp3+Tregs and poor RFS may be explained by the immunosuppressive function of Foxp3+Tregs in breast cancer. On the other hand, the relationship between Foxp3+Tregs and OS may be explained by that Foxp3+Tregs infiltration alone is not enough to predict the OS of breast cancer patients. The ratio between effector T cells and Tregs may serve as a better independent prognostic factor for OS of breast cancer patients [37].

We acknowledge that our meta-analysis has several limitations. First, we only selected the studies published in English. Second, the test for heterogeneity of included studies was significant ($I^2 = 77.5\%$, $p<0.0001$ and $I^2=73\%$, $p<0.001$). We employed subgroup analysis and sensitivity analysis to find out the source, but the results were negative. Despite these limitations, the selection process of the eligible articles was strict and all included studies had high

quality. Moreover, 10,259 patients were included in our meta-analysis, which made our results convincible.

In conclusion, our meta-analysis indicates that the presence of high levels of FOXP3+ Tregs is associated with poor RFS for breast cancer patients and predicts histological grade, estrogen receptor and HER-2 status.

Acknowledgements

Competing Interests

The authors have declared that no competing interest exists.

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