

ORIGINAL ARTICLE

Prognostic significance of vascular endothelial growth factor expression in esophageal carcinoma: a meta-analysis

Jun Peng¹, Na Shao¹, Hongling Peng¹, Long-Qi Chen²

¹West China Medical School, Sichuan University, Chengdu, Sichuan; ²Department of Thoracic Surgery, West China Hospital of Sichuan University, Chengdu, Sichuan, China

Summary

Purpose: The purpose of this study was to comprehensively and quantitatively review eligible published studies to explore the prognostic significance of vascular endothelial growth factor (VEGF) expression in patients with esophageal carcinoma (EC).

Methods: PubMed and Embase databases were searched until September 11, 2011. A meta-analysis was performed to demonstrate any relationship between VEGF and 5-year overall survival (OS) in EC patients.

Results: The final analysis included 1453 patients from 19 studies. The studies were grouped by patient source, histology, VEGF isoform and cutoff value. The estimated risk of death suggested that VEGF positivity had negative impact on prognosis of patients with EC, esophageal squamous cell carcinoma (ESCC) and Asian patients. The risk ratios (RR) and 95% confidence interval (95% CI) were 1.26 (1.16-1.37) in EC patients, 1.28 (1.16-1.40) in ESCC patients and 1.35

(1.24-1.48) in Asian patients. Furthermore, when the cutoff value was set at 10% in 6 studies, the RR (95% CI) was 1.48 in the VEGF positive group (1.27-1.73). In addition, VEGFC was also correlated with patient poor prognosis with a RR (95% CI) of 1.30 (1.15-1.48). However, EC patients from non-Asian countries and cutoff value at 30% showed no significant correlation with survival. Data were not sufficient to determine the prognostic value of VEGF expression in esophageal adenocarcinoma (EA) patients and VEGFD expression.

Conclusions: VEGF positivity indicated poor prognosis in patients with EC, ESCC and of Asian origin. Cutoff value at 10% may be a more appropriate standard to define VEGF positivity. VEGFC also correlated with poor prognosis in EC patients.

Key words: esophageal carcinoma, meta-analysis, prognostic factor, vascular endothelial growth factor

Introduction

EC is still one of the most lethal malignancies in the gastrointestinal carcinoma family despite improvements in surgical techniques and perioperative management [1]. The prognosis for EC patients is generally poor, with a 5-year OS rate of approximately 10-30%, and it has shown little improvement in recent decades [2,3]. There are two major types of EC: ESCC and adenocarcinoma (AC). In China, ESCC is the dominant type.

The current staging of EC is based on the TNM classification, and the pathological node (pN) status is the most powerful predictor of prognosis in EC [4]. Recently, it has been proposed that

various molecular biological markers such as p53, epidermal growth factor receptor (EGFR), vascular endothelial growth factor (VEGF), cyclin D1, and E-cadherin could be prognostic indicators for EC [5,6]. Angiogenesis, the formation of new tumor-feeding blood vessels from preexisting vasculature, is essential for human tumor growth and metastasis [7]. Furthermore, angiogenesis depends on the production of angiogenic factors by tumor cells and normal cells. Moreover, increased vascularity intensifies the growth of tumor and promotes tumor metastasis. It has also been reported to have prognostic value in some solid tumors, such as lung, colorectal, prostate, and breast cancer [8-11].

VEGF is an angiogenetic factor produced by tumor cells that stimulates growth of endothelial cells [12]. It promotes the proliferation and migration of endothelial cells, enhances the permeability of blood vessels, reduces endothelial cell apoptosis and promotes stromal proteolysis [13]. The VEGF family consists of 4 structurally similar subtypes, including VEGFA, VEGFB, VEGFC, and VEGFD [14,15]. VEGFA is the major mediator of tumor angiogenesis, usually referred to as VEGF [16]. On the contrary, VEGFC and VEGFD are reported to correlate with lymphangiogenesis which is associated with lymph node metastasis [17]. Migration via blood vessels and lymphatics are the most common pathways for EC dissemination, and this contributes to the poor outcome of EC. In addition, VEGF expression was obviously associated with clinical responses to treatment. VEGF induction and vascularization of solid tumors have been shown to contribute to the response to radiation therapy and chemotherapeutic agents [1].

The association between VEGF overexpression and prognosis in patients with EC has been debated over the last years, however, no consensus has been achieved so far. Opposite results have been reported from different authors. Consequently, we conducted a meta-analysis based on eligible published studies to quantitatively review any relation between VEGF overexpression in tumor tissue and prognosis in patients with EC.

Methods

Search strategy and study selection

The electronic databases PubMed and Embase were searched for studies to be included in the present meta-analysis. An upper date limit of September 11, 2011 was applied and no lower date limit was used. An initial search strategy was based on combinations of "VEGF," "vascular endothelial growth factor," "vasculotropin," "esophageal carcinoma," "esophagus carcinoma," "esophageal cancer," "esophagus cancer," and "esophageal neoplasms." The search was performed independently by two investigators (P J and SN).

Our initial selection of all candidate articles relied on careful reading of their abstracts. Articles that could not be classified based on title and abstract were reviewed by their full-text. The primary studies required for meta-analysis were categorized based on full-text review. Authors of eligible studies were not contacted for supplementary or additional, unreported information.

Inclusion criteria for eligible studies were as follows: (1) Study published in English; (2) EC histologically diagnosed; (3) VEGF expression measured in the

primary EC tissue by immunohistochemistry (IHC); (4) Survival information, especially correlation of VEGF with OS; (5) Follow-up time up to 5 years; (6) When studies had the same authors and overlapped study population, only the most recent or the study with the larger patient population was selected to avoid duplication of data. Two reviewers (PJ and SN) independently determined study eligibility. Disagreements were resolved by consensus.

Data extraction and quality assessment

Information was carefully extracted from all included publications using a prespecified data collection form with the following items: the first author's name, publication year, patient origin (Asian and non-Asian), number of patients included in the study, histologic type, stage, VEGF assessment method, antibody and cutoff value of VEGF positivity, number of VEGF positive and negative patients, follow-up time, 5-year OS and number of deaths in VEGF positive and negative group. The data were extracted by two independent reviewers (PJ and PHL), and disagreements were resolved by consensus.

We did not set a predefined minimal number of patients for a study to be included in our meta-analysis, nor weighed each study by a quality score, because Altman [18] has reported that no quality score has received general agreement for meta-analyses of observational studies.

The objective of the meta-analysis

The first endpoint of this meta-analysis was to measure the impact of VEGF expression on survival by estimating the RR and its 95% CI between the positive or negative VEGF groups. The second endpoint was to examine the prognostic value of VEGF expression in Asian and non-Asian people. The third endpoint was to investigate the proper IHC cutoff value of VEGF positivity. The fourth endpoint was to see for any correlation between VEGF subtype expression and OS.

Statistics

For the quantitative aggregation of the survival results, the 5-year OS rate and number of deaths in the VEGF positive and negative groups were extracted from the full-text. When these statistical variables were not given explicitly in an article, they were calculated from available survival curves in the full-text.

The heterogeneity of all included studies was assessed by the statistical value I^2 . If $I^2 \leq 50\%$, the studies with fine homogeneity were considered, and then the fixed-effects model with Mantel-Haenszel method was used for secondary analysis. Otherwise, a random effect model with the DerSimonian and Laird (D-L) method was adopted. By convention, an observed $RR > 1$ implies worse survival for the group with positive VEGF [19]. If the 95% CI did not overlap with 1, we considered the

Table 1. Main characteristics and results of the eligible studies

First author [ref.no]	Year	Patient source	Patients N	Histology	Stage	Antibodies used	Cutoff (%)	No. of VEGF(+)	No. of VEGF(-)
Ahn [20]	2002	Korea	80	ESCC	I+II:35 III+IV:45	Mouse monoclonal IgG antibody (Santa Cruz Biotech, U.S.A.)	30	41	39
Choi [21]	2006	Korea	51	ESCC	I+II:28 III+IV:23	Mouse monoclonal IgG antibody (Santa Cruz Biotechnology, Santa Cruz, CA)	10	21	30
Inoue [22]	1997	Japan	65	ESCC	I+II:7 III+IV:58	Rabbit polyclonal antibody (Pharmacia, Uppsala, Sweden)	30	31	34
Kitadai [23]	1998	Japan	71	ESCC	I+II:40 III+IV:31	Rabbit polyclonal antibody (Santa Cruz Biotechnology, Santa Cruz, CA)	30	49	22
Ogata [24]	2003	Japan	92	ESCC	I+II:46 III+IV:46	Mouse monoclonal IgG antibody (R&D systems, Minneapolis, MN, USA)	10	22	70
Rosa [25]	2003	Brazil	47	ESCC	II:23 III:24	Rabbit polyclonal antibody A-20(Santa Cruz Biotechnology, Santa Cruz, CA, USA)	30	19	28
Uchida [26]	1998	Japan	102	ESCC	I+II:57 III+IV:45	Anti-human VEGF polyclonal antibody-Nippon Medical School, Tokyo, Japan)	10	61	41
Cavazzola [27]	2009	Brazil	38	EA	I+II:14 III +IV:24	A-20 rabbit polyclonal antibody (Santa Cruz Biotechnology, Inc., Santa Cruz, CA, USA).	30	19	19
Kimura [28]	2003	Japan	112	ESCC	I+II:80 III:32	Goat polyclonal anti-VEGF-C antibody (Santa Cruz, CA)	10	44	68
Kozlowski[29]	2011	Poland	73	ESCC:34 EA:39	I+II:32 III:41	Goat monoclonal antibody, 1:7, R&D Systems	10	40	33
Mobius [30]	2007	Germany	54	EA	I:17 II-IV:37	Goat polyclonal antibody (Santa Cruz, CA)	50	24	30
Liu [31]	2009	China	73	ESCC	I+II:37 III+IV:36	Goat polyclonal antibody (Santa Cruz, CA, USA)	30	39	34
Tzao [32]	2008	Taiwan	85	ESCC	I+II:48 III+IV:37	Monoclonal antibody clone 78923.11 against human VEGF-D (1:100; R&D Systems, Minneapolis, MN, USA)	20	56	29

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Noguchi [33]	2002	Japan	71	ESCC	I+II:37 III+IV:34	Anti-VEGF-C polyclonal antibody (1:20, IBL, Gunma, Japan)	NG	38	33
Kato [34]	2002	Japan	64	ESCC	I+II:38 III+IV:26	C-1(Santa Cruz Bio, Inc, Santa Cruz, CA, USA)	80	24	40
Li [35]	2000	Japan	96	ESCC	I+II:50 III+IV:46	Goat anti rabbit IgG (Nippon Medical School, Tokyo, Japan)	10	62	34
Okazawa [36]	2008	Japan	100	ESCC:96 EA:2; UC:1 BC:1	NG	Goat polyclonal antibody (N-19, Santa Cruz Biotechnology, Inc., Santa Cruz, CA)	30	43	57
Nakagawa [37]	2003	Japan	97	ESCC	I+II:44 III+IV:53	Rabbit polyclonal antibody (Santa Cruz Biotechnology, Santa Cruz, CA)	50	47	50
Xu [38]	2004	China	82	ESCC	NG	Anti-VEGF mouse polyclonal antibody (Beijing Zhongshan Biotechnology Co., Ltd., Beijing, China)	30	52	30

ESCC: esophageal squamous cell carcinoma, EA: esophageal adenocarcinoma, UC: undifferentiated carcinoma, BC: basaloid carcinoma, NG: not given

impact of VEGF on OS as statistically significant.

Assessment of publication bias was performed for each of the pooled study groups using the inverted funnel plots, the Egger's test, and the Begg's test. A p -value > 0.05 showed no publication bias in the study.

All statistical analyses were carried out using STATA version 11.0 (Stata Corporation, College Station, TX).

Results

Study selection and characteristics

A total of 563 references were reviewed. After initial exclusion of the articles that were obviously out of the scope of our meta-analysis, we identified 49 potential studies for further detailed evaluation. Upon further review, 19 studies finally met our inclusion criteria for this meta-analysis (Figure 1). Nineteen studies [20-38] published between 1997 and 2011, all reported the prognostic value of VEGF status for survival in patients with EC. The total numbers of patients included was 1453, ranging from 38 to 112 patients per study and the median sample size for all studies was 73 patients. The major characteristics of the 19 included publications are shown in Table 1.

All of the 19 studies used IHC to evaluate VEGF expression in EC, and the subtypes VEGFC and VEGFD were detected in 6 and 1 studies, re-

spectively. Fifteen studies dealt with ESCC, 2 studies dealt with EA and 2 considered all types of EC.

Among the 19 studies, 15 (1241 patients; 85.41%) were performed in Asian patients, and the remaining 4 (212 patients; 14.59%) focused on European or south American population. Eleven of the 19 studies identified VEGF positivity as an indicator of poor prognosis, while the remaining 8 studies showed no statistically significant impact of VEGF positivity on OS. The proportion of patients exhibiting VEGF positivity in each study

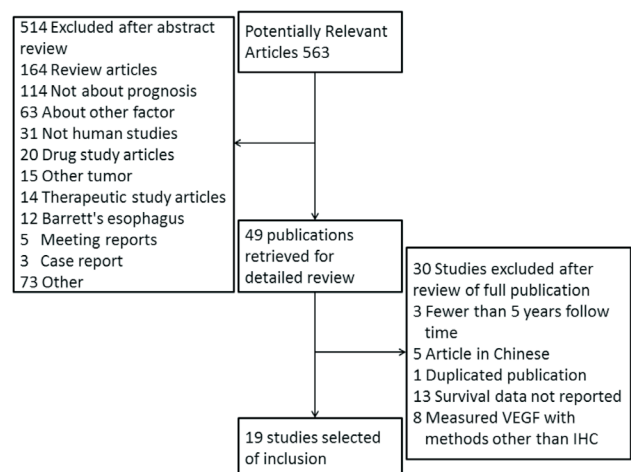


Figure 1. Flow chart of the meta-analysis. Nineteen studies met our inclusion criteria for this meta-analysis.

Table 2. Level of cancer antigens, LDH and D-dimer during chemotherapy

	Studies N	Patients N	RR (95% CI, <i>p</i> -value)	Heterogeneity Test (Q, I ² , P, <i>p</i> -value)
VEGF in EC	19	1453	1.26 (1.16-1.37, 0.000)	36.01, 50.0%, 0.007
Asian	15	1241	1.35 (1.24-1.48, 0.000)	13.80, 13.1%, 0.313
Non- Asian	4	212	0.82 (0.53-1.29, 0.396)	14.03, 78.6%, 0.003
VEGF in ESCC	15	1188	1.28 (1.16-1.40, 0.000)	27.12, 48.4%, 0.019
10% cutoff	6	526	1.48 (1.27-1.73, 0.000)	3.05, 0.0%, 0.692
30% cutoff	8	556	1.12 (0.88-1.43, 0.100)	26.42, 73.5%, 0.000
VEGFC in EC	6	483	1.30 (1.15-1.48, 0.000)	5.62, 11.0%, 0.345

VEGF: vascular endothelial growth factor, VEGFC: vascular endothelial growth factor C, RR: risk ratio, CI: confidence interval, ESCC: esophageal squamous cell carcinoma, EC: esophageal cancer

ranged from 23.91 to 69.01%.

Six studies put the cutoff at 10%, 1 study at 20%, 8 studies at 30%, 2 studies at 50%, 1 study at 80%, and 1 did not mention the cutoff.

Main results

Correlation between VEGF expression and 5-year overall survival

The estimated risk of death in 5 years was 1.26-fold higher in the VEGF positive patients (95% CI: 1.16-1.37, *p*=0.000), indicating that VEGF positivity was an indicator of poor prognosis for EC patients (Table 2; Figure 2).

The prognostic value of VEGF expression in Asian and non-Asian populations

After grouping the studies according to the patient origin (Asian and non-Asian), we observed that in Asian patients' mortality was significantly higher in VEGF positive subjects than in VEGF negative ones (RR=1.35, 95% CI: 1.24-1.48, *p*=0.000). However, no statistically significant effect on OS was observed among non-Asian patients (RR=0.82, 95% CI: 0.53-1.29, *p*=0.396). We found highly significant heterogeneity among the non-Asian studies (Q=14.03, I²=78.6%, *p*=0.003) (Table 2; Figures 3,4).

Correlation between ESCC and VEGF expression

When the 15 studies which dealt with ESCC were combined, the mortality was 1.28-fold higher (95% CI: 1.16-1.40 *p*=0.000) in VEGF positive patients, showing that the prognosis of VEGF positive patients was worse compared to VEGF negative patients with ESCC (Table 2; Figure 5).

Correlation between different cutoffs of VEGF posi-

tivity and 5-year overall survival

In 6 studies using 10% as cutoff, the survival difference was clearer (RR=1.48, 95% CI: 1.27-1.73, *p*=0.000) and larger than the outcome of all 19 studies. However, in 8 studies using 30% as cutoff no statistically survival difference was found (RR=1.12, 95% CI: 0.88-1.43, *p*=0.100). We also found highly significant heterogeneity in these 8 studies (Q=26.42, I²=73.5%, *p*=0.000) (Table 2; Figures 6,7).

Correlation between VEGFC expression and 5-year overall survival in EC patients

When we limited the analysis to the 6 studies that investigated VEGFC expression in patients with EC, the mortality in the VEGFC positive group was 1.30-fold higher (95% CI: 1.15-1.48, *p*=0.000) than in the VEGFC negative group, indicating that VEGFC positivity was an indicator of poor prognosis for EC patients (Table 2; Figure 8).

Assessment of publication bias

Visual assessment of funnel plot provided no evidence of overt publication bias for the all 19 studies included in the meta-analysis (Figure 9). Begg's test (*p*=0.834) and Egger's test (*p*=0.867) also failed to reveal evidence for significant publication bias. Similar results were found for the 15 Asian studies (*p*=0.428 and 0.075, respectively); 4 non-Asian studies (*p*=0.308 and 0.102, respectively); 15 studies of patients with ESCC (*p*=0.621 and 0.572, respectively); 6 studies with cutoff at 10% (*p*=0.26 and 0.102, respectively); 8 studies with cutoff at 30% (*p*=0.386 and 0.308, respectively); 6 studies investigating VEGFC expression in patients with EC (*p*=0.707 and 0.697, respectively). These results suggest that there was no publication bias.

Discussion

Folkman's publication innovated tumor angiogenesis hypothesis in 1971 [39]. From then on scientists have shown great interest in exploring the role of VEGF in this process. Publications of the prognostic value of VEGF expression in EC patients date back to the 1990s. In this meta-analysis, we have combined 19 published studies with 1453 EC patients to demonstrate that VEGF has a significant correlation with poor survival in such patients. The outcome is in agreement with the recent meta-analysis reports on VEGF expression in gastric carcinoma [40], lung cancer [41], hepatocellular carcinoma [42] and colorectal cancer [19]. This correlation was also observed in Asian populations, but in non-Asian populations we found that VEGF did not show any prognostic value, perhaps because only 4 non-Asian publications were eligible. When analysis was limited to ESCC patients, the estimated risk of death (RR=1.28) was similar to all the 19 included studies (RR=1.26), indicating VEGF also had prognostic value in ESCC patients. This outcome is in agreement with the meta-analysis in head and neck squamous cell carcinoma [43]. Data were not sufficient to determine the prognostic value of VEGF expression in EA patients.

When only publications with cutoff at 10% were analyzed, the estimated risk of death (RR=1.48) was higher than all 19 included studies (RR=1.26). However, when the cutoff was 30%, the 95% CI overlapped with 1, and the estimated risk of death (RR=1.12) was lower than all 19 included studies (RR=1.26). These findings suggest that a cutoff of 10% may be more appropriate when we define the VEGF positivity, and it will help avoid failure of diagnosis and predict prognosis.

Subgroup analysis suggested that VEGFC also had a significant impact on prognosis of EC patients. VEGFC is an inducible factor of lymphangiogenesis [17] and correlates with lymph node metastasis. Our results further support the hypothesis that VEGFC positivity is associated with poor prognosis in EC patients [4]. Concerning VEGFD, data were not sufficient to determine its prognostic value.

The present meta-analysis suggests that VEGF positivity is an indicator of poor prognosis in patients with EC. Therefore, inhibiting VEGF-mediated angiogenesis and lymphangiogenesis might be an effective therapy for EC. Recently, several phase II studies [44-46] have shown a little clinical benefit for the tyrosine kinases inhibitor (TKI) sunitinib and VEGF-targeting mono-

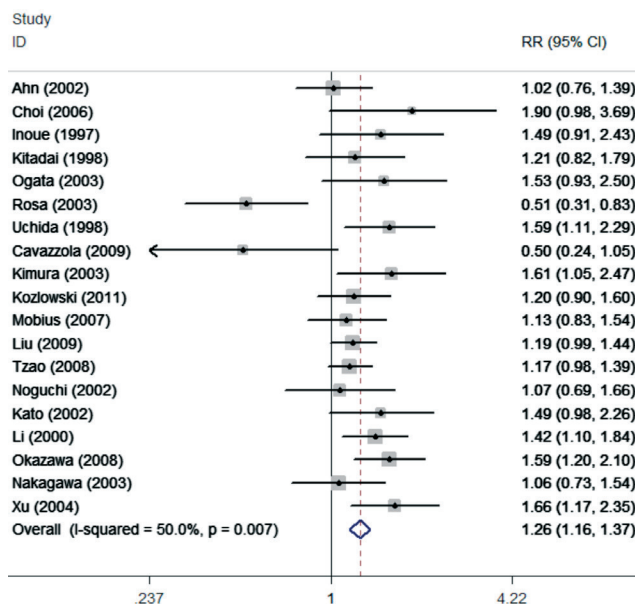


Figure 2. Meta-analysis (Forest plot) on the relation between VEGF expression and 5-year overall survival (OS). Risk ratio (RR) and its 95% CI for OS is 1.26 (1.16-1.37). Each study is shown by lead author/year.

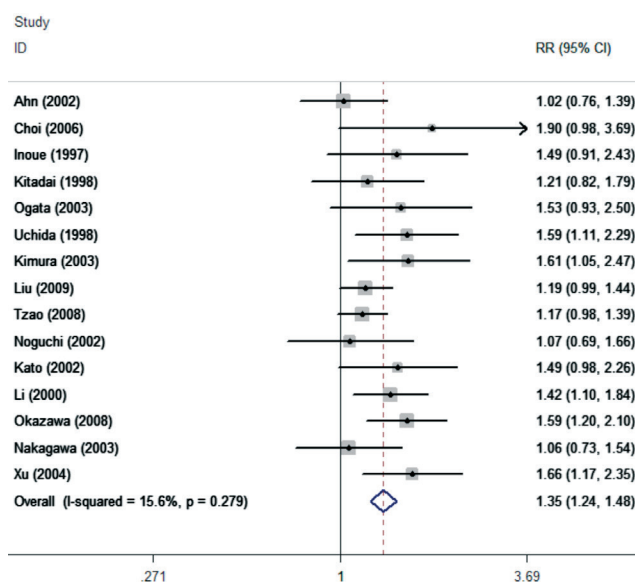


Figure 3. Meta-analysis of VEGF expression in Asian patients. Risk ratio (RR) and its 95% CI for overall survival is 1.35 (1.24-1.48). Each study is shown by lead author/year.

clonal antibody bevacizumab in patients with EC. Phase III studies comparing bevacizumab with supportive care alone are anticipated. Up until now the antiangiogenic agents do not achieve a desired effect and we believe that a more accurate evaluation of the impact of VEGF expression on patient survival is required.

The present meta-analysis is complicated by heterogeneity issues. We found highly significant heterogeneity between 4 non-Asian studies and 8 studies with cutoff at 30%. No heterogeneity was

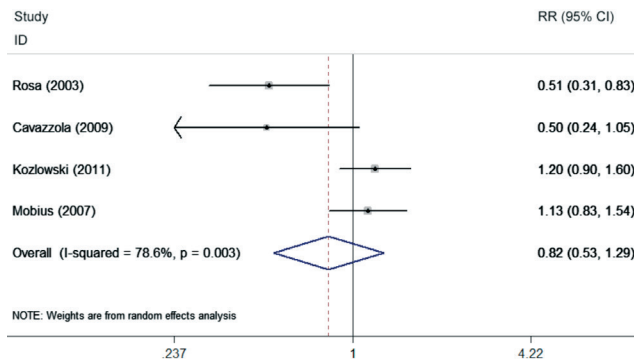


Figure 4. Meta-analysis of VEGF expression in non-Asian patients. Risk ratio (RR) and its 95% CI for overall survival is 0.82 (0.53-1.29). Each study is shown by lead author/year.

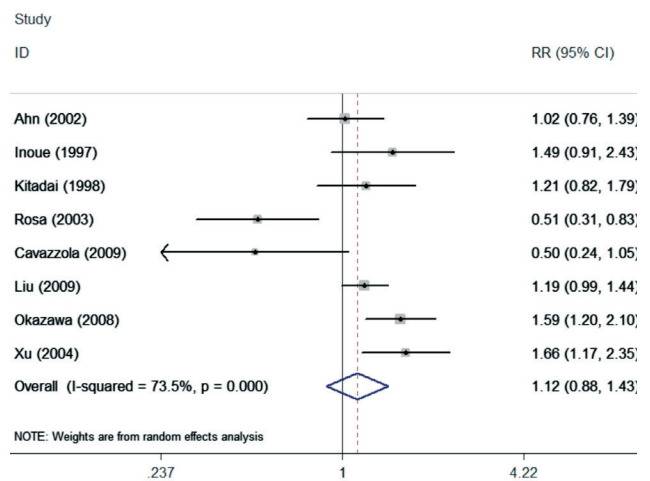


Figure 7. Meta-analysis when cutoff at 30%. Risk ratio (RR) and its 95% CI for overall survival is 1.12 (0.88-1.43). Each study is shown by lead author/year.

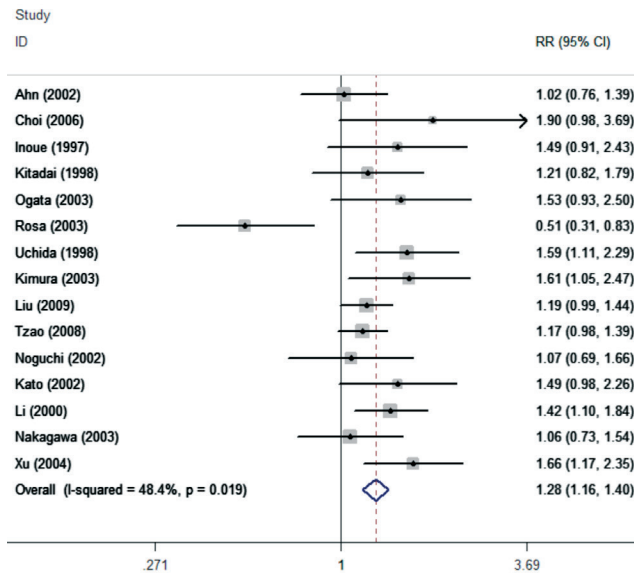


Figure 5. Meta-analysis of VEGF expression in ESCC patients. Risk ratio (RR) and its 95% CI for overall survival is 1.28 (1.16-1.40). Each study is shown by lead author/year.

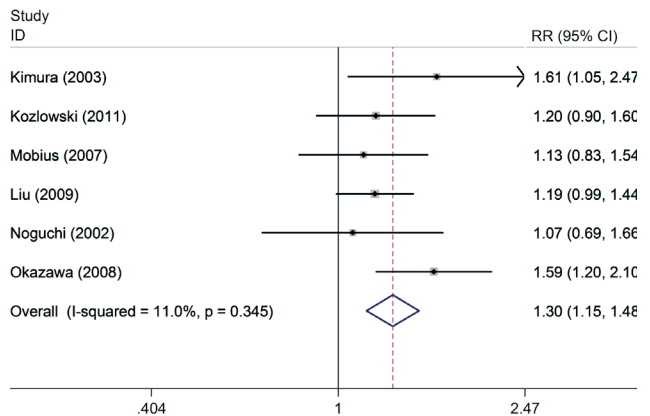


Figure 8. Meta-analysis on the relation between VEGFC expression and OS. Risk ratio (RR) and its 95% CI for overall survival is 1.30 (1.15-1.48). Each study is shown by lead author/year.

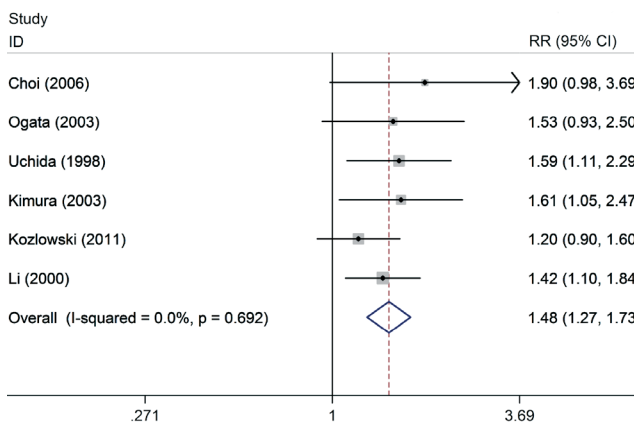


Figure 6. Meta-analysis when cutoff at 10%. Risk ratio (RR) and its 95% CI for overall survival is 1.48 (1.27-1.73). Each study is shown by lead author/year.

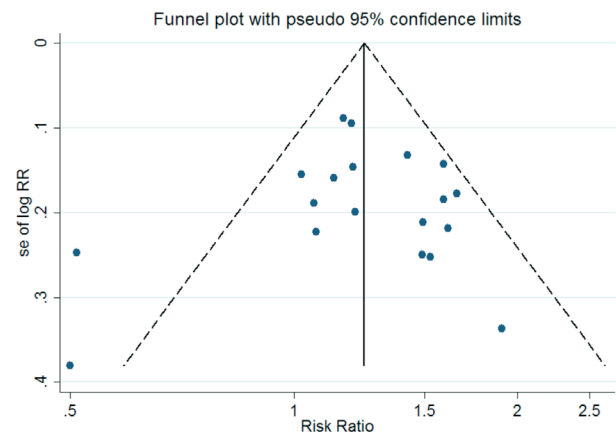


Figure 9. Bias assessment funnel plots for all 19 studies included in the meta-analysis. There is no evidence of overt publication bias for all 19 studies included in the meta-analysis.

detected when the analysis was limited to 15 Asian studies, 15 of which dealt with VEGF in ESCC and 6 used cutoff at 10%. Therefore, the heterogeneity in this meta-analysis could be explained by the different histologic types, geographic isolation, racial differences and many different cutoffs for VEGF positive tissues (10–80%). However, the D-L method we used could improve the heterogeneity factor.

There are several potential sources of bias that should be noted in our meta-analysis. Although we did not detect significant publication bias among the eligible studies, we could not completely exclude the publication bias. Firstly, all of the studies included in our meta-analysis used IHC to assess VEGF expression status, which represented potential selection bias. Secondly, we only collected and analyzed published English language literature, which might introduce bias.

Thirdly, bias may come from our method of data extraction i.e. if the survival data were not directly reported by the authors, we calculated them from the survival curves available in the article.

In conclusion, this meta-analysis shows that VEGF positivity was correlated with poor prognosis in patients with EC and it also suggests that VEGF positive Asian patients and ESCC patients have a worse prognosis than VEGF negative ones. VEGF protein might be a powerful predictor of prognosis, which can help to identify high-risk patients and guide clinical decision and therapy. In addition, it may provide a target for future targeted therapy. Furthermore, cutoff of 10% may be more appropriate when we determine VEGF positivity or negativity. Interestingly, our work suggests that VEGFC is also a predictor of prognosis in EC patients. These results should be investigated and validated by further prospective studies.

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