

Prognostic Value of Vascular Endothelial Growth Factor Expression in Patients with Esophageal Cancer: A Systematic Review and Meta-analysis

Meilan Chen, Erhui Cai, Jizheng Huang, Ping Yu, and Ke Li

Abstract

Background: VEGF is a prime mediator of tumorigenesis and metastasis. Various studies assessing the prognostic value of VEGF in patients with esophageal cancer remain controversial. This study aims to comprehensively and quantitatively summarize the evidence on the suitability of VEGF to predict patients' survival.

Methods: Searches were applied to PubMed and EMBASE until December 31, 2011, without language restrictions. Studies were assessed for quality using REMARK (Reporting recommendations for tumor MARKer prognostic studies). Data were collected comparing overall survival in patients with high VEGF level with those with low level. We conducted a systematic review of 31 studies ($n = 2,387$ patients) and completed a meta-analysis of 30 studies ($n = 2,345$ patients) that correlated VEGF levels with overall survival. Data were synthesized with HRs.

Results: The estimated risk of death was 1.82-fold greater in patients with high VEGF expression [95% confidence interval (CI), 1.58–2.08]. The heterogeneity was not significant ($P = 0.130$) between studies. High VEGF expression was associated with worse survival in esophageal squamous cell carcinoma (HR, 1.81; 95% CI, 1.57–2.10) and there was no significance in between-study heterogeneity ($P = 0.185$). Data collected were not sufficient to determine the prognostic value of VEGF in patients with esophageal adenocarcinoma.

Conclusions: In this meta-analysis, elevated VEGF expression was associated with poor survival in patients with esophageal cancer but not esophageal adenocarcinoma.

Impact: These results support further investigation of VEGF expression for predicting poor survival in patients with esophageal carcinoma and may have implications for treatments directed at inhibiting VEGF-mediated angiogenesis. *Cancer Epidemiol Biomarkers Prev*; 21(7); 1126–34. ©2012 AACR.

Introduction

Esophageal cancer, composed of squamous cell carcinoma and adenocarcinoma, is the eighth most common cancer worldwide, with 482,300 new cases annually, and has the sixth highest cancer mortality, with 406,800 deaths registered in 2008 worldwide (1). Despite recent advances in screening and multimodality therapy (2), the outcome for esophageal cancer remains generally poor, emphasizing the need for early detection and prognostic markers. Following the growing knowledge of molecular mechanisms underlying tumor biology, the search for prognostic markers is one of the most active fields in oncology. Currently, the identification of molecular biologic mar-

kers is being pursued to determine the prognosis of patients affected with solid tumors (3). For example, factors related to apoptosis (e.g., p53 and bcl2), growth (e.g., epithelial growth factor, erbB2), or cell cycling [e.g., cyclin, proliferating cell nuclear antigen (PCNA)] have been studied, to correlate markers with survival (4–8).

Expression of VEGF, one of the most potent sources of angiogenesis, has been shown to be responsible for the development and maintenance of a vascular network that promotes tumor growth and metastasis for a wide range of human tumors and human cell lines (9). VEGF is a homodimeric glycoprotein with a molecular weight of approximately 45 kDa. In healthy humans, VEGF promotes angiogenesis in embryonic development and is important in wound healing in adults. VEGF is a key mediator of angiogenesis in cancer, and angiogenesis is essential for cancer development and growth (10). Moreover, a large and growing body of evidence indicates that VEGF expression is associated closely with poor prognosis in patients with cancer (11–13).

At this point, a question arises whether these findings justify the use of VEGF detection, in a routine clinical setting, as a prognostic indicator in patients with

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esophageal cancer. In this study, we conducted a systematic review and meta-analysis to estimate the prognostic importance of elevated VEGF expression for survival among patients with esophageal cancer.

Materials and Methods

Search strategy

To identify all primary research articles that evaluated the level of VEGF expression as a prognostic factor among individuals with esophageal cancer, we searched the PubMed and EMBASE databases up to December 31, 2011, without language restrictions, using a strategy developed with an expert librarian based on terms for esophageal carcinoma, prognostic study (14), and VEGF or "vascular endothelial growth factor." One reviewer (M. Chen) inspected the title and abstract of each citation to identify those studies that were likely to report the prognostic value of VEGF and then obtained the full text. Inclusion criteria for the primary studies were as follows: (i) diagnosis of esophageal cancer in humans was proven, (ii) VEGF evaluation was conducted, and (iii) data reported was related to the prognostic value.

Methodologic and validity assessment

We used published guidelines for reporting tumor marker studies and quality metrics for evaluating studies for inclusion in cancer-related meta-analyses (15, 16). Criteria for eligibility of a study were as follows: (i) a prospective or retrospective cohort design with a well-defined study population and justification for all excluded eligible cases, (ii) assay of the primary esophageal cancer specimens, (iii) a clear description of methods for specimen handling and testing, including selection and preparation of reagents or kits, as well as visualization techniques, (iv) clear statements on the choice of positive/high expression and negative/low expression controls and on assay validation, and (v) a statistical analysis reporting HRs including 95% confidence intervals (CI), or provision of data available for statistical estimation of HRs. Because small cell esophageal carcinoma, esophageal stromal tumors, small adenocarcinomas, and gastrointestinal cancers have different clinical courses, studies that did not distinguish these tumor types from esophageal cancer were excluded.

Quality assessment was conducted in duplicate for each eligible study by independent reviewers (M. Chen and E. Cai) using operationalized prognostic biomarker reporting guidelines (15) and extract details on 18 items (see Table 1), allowing for assessment of study purpose, study population, biomarker measurement, confounder measurement, outcome measurement, and statistical analysis.

Data extraction

Two investigators (M. Chen and J. Huang) reviewed all eligible studies and extracted study characteristics carefully in duplicate, including first author, publication year,

country of origin, histology, disease stage, number of patients, gender, median age, test method, cutoff value, VEGF positivity, and survival data (Table 2). If data from any of the above categories were not reported in the primary article, items were treated as "not reported." We did not contact the author to request the information.

Statistical analysis

For appropriate VEGF evaluation in a single study, the summary HR and their 95% CIs were combined to present the value reported in the study. For some of the trials without reporting HR and 95% CIs directly, mathematical HR approximation was estimated using established methods (17). In 11 studies not quoting the HRs or CIs, HR and CI values were calculated using parameters given by the authors for univariate analysis: the CI for the HR, the observed (O) -expected (E) statistic (the difference between the number of observed and the number of expected events) or its variance, the log-rank statistic or its *P* value were used to allow for an approximate calculation of the HR estimate. When those data were not available, the total number of events, the number of patients at risk in each group, and the log-rank statistics or its *P* value were used to derive an approximation estimate of the HRs. Finally, if the only exploitable data were in the form of graphical representations of the survival distributions, survival rates at some special times were extracted to reconstruct the HR estimate and its 95% CIs.

Heterogeneity of the individual HRs was calculated using the χ^2 test according to Peto's method (18). Between-study heterogeneity was assessed using I^2 and *Q* statistics (19). All eligible studies were categorized by histology, disease stage, and laboratory techniques used. Individual meta-analysis was conducted in each group. When HRs had fine homogeneity, we analyzed the effect of VEGF expression on survival by a fixed-effect model; otherwise Dersimonian-Laird random-effect model was used (20).

The combined HRs were estimated using forest plots graphically. An observed HR of more than 1 implied a worse survival for the VEGF-positive or high VEGF expression group relative to the VEGF-negative or low expression group and was considered statistically significant if the 95% CI did not overlap 1 ($P < 0.05$). Horizontal lines represent 95% CIs. Boxes represent the HR point estimate, and its area is proportional to the weight of the study. The diamond represents the overall summary estimate, with the CI represented by its width. The unbroken vertical line was set at the null value (HR, 1.0).

Assessment of publication bias was conducted using the methods of Song and colleagues (21) and Begg and colleagues (22). Meanwhile, a contour-enhanced funnel plot was conducted to aid in interpreting the funnel plot (23). If studies appeared to be missing in the area of low statistical significance, then it is possible that the asymmetry was due to publication bias. If studies seemed to be missing in the area of high statistical significance, then publication bias was a lesser cause of the funnel

Table 1. Definitions of 18 items of study reporting quality

Study design

1. Objectives or prespecified hypothesis: state the study objectives, prespecified hypothesis or study protocol
2. Sample size: state a statistical sample size or power calculation
3. Follow-up description: state the follow-up period or the median follow-up time
4. Population source: state health care setting from which patients were recruited
5. Population selection criteria: state inclusion or exclusion
6. Population characteristics: state the population characteristics (e.g., age, gender, and disease stage)
7. Number of patients included in each stage of the analysis and reason for dropout: description of number of patients at different stage, including the number of patients who participate in the study, who met the inclusion criteria, and who followed up and reason for dropout

Assay method

1. Sample handling: state the method of storage
2. Assay method: state the type of assay method used to measure VEGF
3. Manufacturer: state the name of company which makes the assay for VEGF
4. Cutoff point determination: state methods used for cutoff point determination

Confounders

1. Conventional risk factors: state the conventional risk factors (e.g., age, gender, depth of tumor, lymph node metastasis)
2. Other biomarkers (e.g., p53, PCNA, and microvessel density): state other biologic marker relating with the disease

Outcome

1. Clinical endpoint: define the clinical endpoint
2. Validation: state the outcome events checked by independent source (e.g., medical records, outpatient visits, by letter, and by telephone)

Analysis

1. Univariate estimate: report the effect of VEGF on outcome
2. Multivariate estimate: adjusted for risk factors or other biomarkers (list above)
3. Missing value: state the number of patients with missing value for VEGF or confounders and how to deal with it

asymmetry. Intercept significance was determined by the *t* test suggested by Egger ($P < 0.05$ was considered representation of statistically significant publication bias). All the statistical analyses were conducted using STATA SE11.0 software (Stata Corporation).

Results

The abstracts and titles of 215 primary studies were identified for initial review using the search strategies as described. After exclusion of articles that were out of the scope of our meta-analysis, we identified 52 potential studies for full-text review. Upon further review, 2 review articles were eliminated (24, 25) and 19 articles were eliminated because of inadequate data for meta-analysis (refs. 26–44; Fig. 1).

These studies followed several different patient cohorts, 26 studies dealt with all types of esophageal squamous cell carcinoma (ESCC; refs. 45–70), one (71) dealt with esophageal adenocarcinoma (EADC), and another 4 dealt with ESCC and EADC (72–75). The studies included were conducted in different countries, 19 of 31 studies were conducted in Japan, 5 in China, 2 in Korea, 2 in Poland, 2 in Brazil, and 1 study being from Sweden. The total number of patients included was 2,387 and ranged from 38 to 149 patients per study (median, 80). Characteristics of the 31 eligible publications are listed in Table 2.

The median or mean age of patients ranged from 55 to 68 years in the 23 studies with age information. The

median proportion of males was 85.4% across the eligible studies. Twenty-five of 31 studies had information on disease stage, and the median proportion of stage I + II was 47.5%. Among 31 eligible studies, 25 used immunohistochemistry (IHC) to assess VEGF expression, 4 used ELISA, and the remaining 2 used reverse transcription PCR (RT-PCR). HRs were reported for every eligible study using available data or the methods described above. Individual studies correlated VEGF levels with survival data. VEGF cutoff points were chosen using different methods in each study. Some studies used a purely binary system (positive or negative) for final analysis, others used a quantitative system. Many quantitative-based studies used the median level as the cutoff value. The proportion of high VEGF expressors in individual studies ranged from 23.9% to 87.0%. Univariate survival analysis alone (log-rank-based comparison of Kaplan–Meier curves) was conducted in 12 of 31 studies ($n = 869$ patients, 36.41%). Multivariate survival analysis (Cox proportional hazards model) was conducted in the remaining 19 studies ($n = 1,518$ patients, 63.59%). Nine of 19 studies based on multivariate survival analysis identified high VEGF expression as an indicator of poor prognosis, and the remaining 10 showed no statistically significant effect of VEGF high expression on survival.

Quality assessment based on REMARK guidelines was conducted on all 31 studies included for systematic review. The mean number of study quality items reported

Table 2. Main characteristics and results of the eligible studies

First author (Year)	Origin country	Histology	Stage I + II (%)	No. of patients	Male (%)	Median/mean age, y	Method	Cutoff	VEGF positive/high (%)	Survival analysis	HR (95%CI)
Kozlowski (2011)	Poland	Esophageal cancer	45.2	73	80.8	64	IHC	10% staining	54.7	Univariate	2.27 (1.03–4.80)
Miroslaw (2010)	Poland	ESCC	31.5	149	91.9	62	ELISA	Median	41.7	Multivariate	2.18 (1.37–3.47)
Tatsuya (2010)	Japan	ESCC	39.6	106	82.1	NR	RT-PCR	Median	50	Multivariate	1.64 (0.97–2.78)
Zhi-Gang Sun (2010)	China	Esophageal cancer	NR	82	78	NR	RT-PCR	NR	51.2	Multivariate	2.51 (1.21–5.19)
Leandro (2009)	Brazil	EADC	36.8	38	78.9	60.6	IHC	30% staining	50	Multivariate	0.37 (0.10–1.44)
Pengfei Liu (2009)	China	ESCC	50.7	73	76.7	61	IHC	30% staining	53.4	Multivariate	2.23 (1.35–5.00)
Akemi (2008)	Japan	ESCC	NR	81	95.1	NR	IHC	10% staining	87.0	Multivariate	1.69 (0.65–4.41)
Ching Tzao (2008)	Taiwan	ESCC	56.5	85	94.1	NR	IHC	10% staining	65.9	Multivariate	1.73 (1.04–2.92)
Hitoshi (2008)	Japan	ESCC	51.3	80	88.6	62.8	ELISA	Median	50	Univariate	1.42 (0.59–3.43)
Reigetsu (2007)	Japan	ESCC	NR	40	77.5	60	IHC	35% staining	32.5	Univariate	3.03 (1.32–6.96)
Takayuki (2007)	Japan	ESCC	35	51	82	68	IHC	Strong staining	31	Multivariate	0.90 (0.42–1.96)
Joon Yong (2006)	Korea	ESCC	54.9	51	92.2	NR	IHC	10% staining	58.8	Multivariate	7.21 (1.71–30.4)
Takuma (2006)	Japan	ESCC	47.5	40	90	65.8	IHC	57% staining	50	Univariate	1.97 (0.73–5.35)
Martin (2005)	Sweden	Esophageal cancer	NR	42	73.8	NR	ELISA	Median	48	Multivariate	1.00 (1.00–1.00)
Hiroya (2004)	Japan	ESCC	59	90	86.7	61	IHC	80% staining	35.6	Univariate	1.68 (0.66–4.31)
Shigeru (2004)	Japan	ESCC	36.6	82	87.8	62.2	IHC	10% staining	62.2	Univariate	1.81 (0.78–4.21)
A.R. Rosa (2003)	Brazil	ESCC	48.9	47	87.2	55	IHC	30% staining	40.4	Multivariate	0.48 (0.18–1.32)
Yasue (2003)	Japan	ESCC	71.4	112	85.7	66	IHC	10% staining	39.3	Univariate	2.15 (1.08–4.28)
Yutaka (2003)	Japan	ESCC	50	92	91.3	60.2	IHC	10% staining	23.9	Multivariate	2.40 (1.10–5.34)
Hiroyuki (2002)	Japan	ESCC	59.4	64	85.9	61.4	IHC	80% staining	37.5	Multivariate	1.03 (0.46–2.34)
HuChong-zhu (2002)	China	ESCC	27.8	72	77.8	59.5	IHC	30% staining	62.5	Univariate	1.50 (0.85–2.65)
Shimada (2002)	Japan	ESCC	NR	52	83	65	IHC	10% staining	44.2	Multivariate	2.43 (1.19–4.94)
Myung-Ju (2001)	Korea	ESCC	43.2	81	93.8	60	IHC	30% staining	51.3	Multivariate	1.27 (0.61–2.63)
Hideaki (2001)	Japan	ESCC	43.9	82	85.4	65	ELISA	Median	36.6	Multivariate	3.83 (1.82–8.08)
Xu Weiguo (2001)	China	ESCC	NR	82	74.4	56	IHC	30% staining	63.4	Univariate	2.86 (1.50–5.44)
Chi-Horng (2000)	Japan	ESCC	NR	117	90.6	NR	IHC	80% staining	30.8	Multivariate	3.35 (1.02–10.98)
Naohiko (1999)	Japan	ESCC	NR	52	75	62.7	IHC	30% staining	57.7	Univariate	1.71 (0.64–4.60)
Uchida (1998)	Japan	Esophageal cancer	56	109	81.7	63.5	IHC	10% staining	59.6	Multivariate	1.80 (0.75–5.01)
Yasuhiko (1998)	Japan	ESCC	56.3	71	90	63.5	IHC	30% staining	69	Univariate	0.94 (0.45–1.95)
Shimada (1998)	Japan	ESCC	33.6	116	84.5	NR	IHC	10% staining	69	Multivariate	1.59 (0.69–4.04)
Kimitoshi (1997)	Japan	ESCC	10.7	75	82.7	60.3	IHC	30% staining	46.7	Univariate	1.78 (0.80–4.21)

NOTE: Esophageal cancer includes ESCC and EADC.

Abbreviation: NR, not reported.

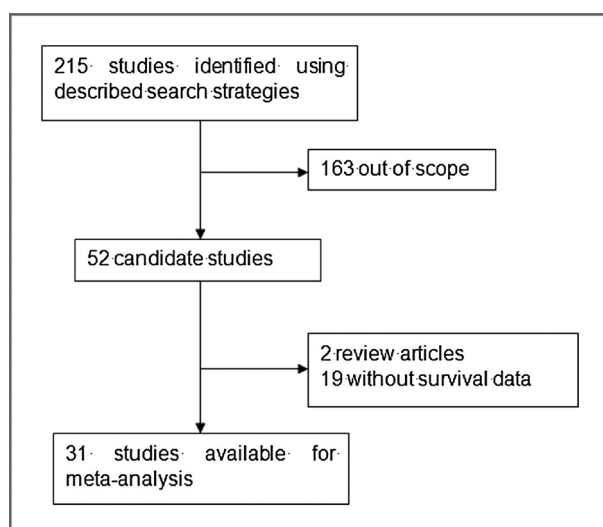


Figure 1. Search strategy flowchart.

was 11 of a possible 18, and there was no statistical difference between studies that assessed the outcome with univariate survival analysis ($n = 12$) or with multivariate survival analysis ($n = 19$), with mean items being 10.7 and 11.2, respectively ($P = 0.297$). All studies reported details of the assay type, manufacturer, cutoff point determination, clinical endpoint, and univariate estimation. More than 80% reported details of population source, sample handling, conventional risk factors, and multivariate estimation. Of note, 17 studies attempted to control for other important prognostic factors that may have confounded the association of high VEGF with survival. Three studies referred to validation of outcome but no studies referred to rational sample size and missing value.

The results of the meta-analysis are reported in Table 3 and Fig. 2. For all studies, with one exception with HR = 1 (95% CI, 1.00–1.00; ref. 73), there did not appear to be any major qualitative evidence for heterogeneity between HRs, as assessed by inspection of the forest plot (Fig. 3). For studies evaluating VEGF levels in ESCCs and esoph-

ageal cancers, the combined HRs were 1.81 (95% CI, 1.57–2.10) and 2.24 (95% CI, 1.41–3.55), respectively, and there was no evidence for heterogeneity within the 2 groups. The pooled HR estimate for survival in the 25 studies using IHC was 1.72 (95% CI, 1.47–2.02), with no evidence for heterogeneity between studies. However, when we limited the analysis to the 14 studies ($n = 1,064$) with a higher proportion of disease stage III + IV (>50%), the combined HR was 1.69 (95% CI, 1.39–2.04; $Q = 21.95$; $I^2 = 40.8\%$; $P = 0.056$). When grouped according to the survival analysis of individual studies, the combined HRs of univariate survival analysis and multivariate survival analysis were 1.85 (95% CI, 1.47–2.31) and 1.80 (95% CI, 1.51–2.14), respectively. There was significant heterogeneity in the multivariate analysis with $I^2 = 43.0\%$ and $P = 0.028$.

Visual assessment of funnel plots provided no evidence of overt publication bias for the studies (Fig. 3). Formal evaluation using Egger's test also failed to reveal evidence for significant publication bias ($P = 0.543$).

Discussion

In this meta-analysis, we found high VEGF expression in esophageal cancers to be associated with an approximate 80% higher risk of death from the disease. Our current finding is in agreement with recent meta-analysis reports on VEGF expression in colorectal cancer, oral carcinoma, and gastric carcinoma (76–78).

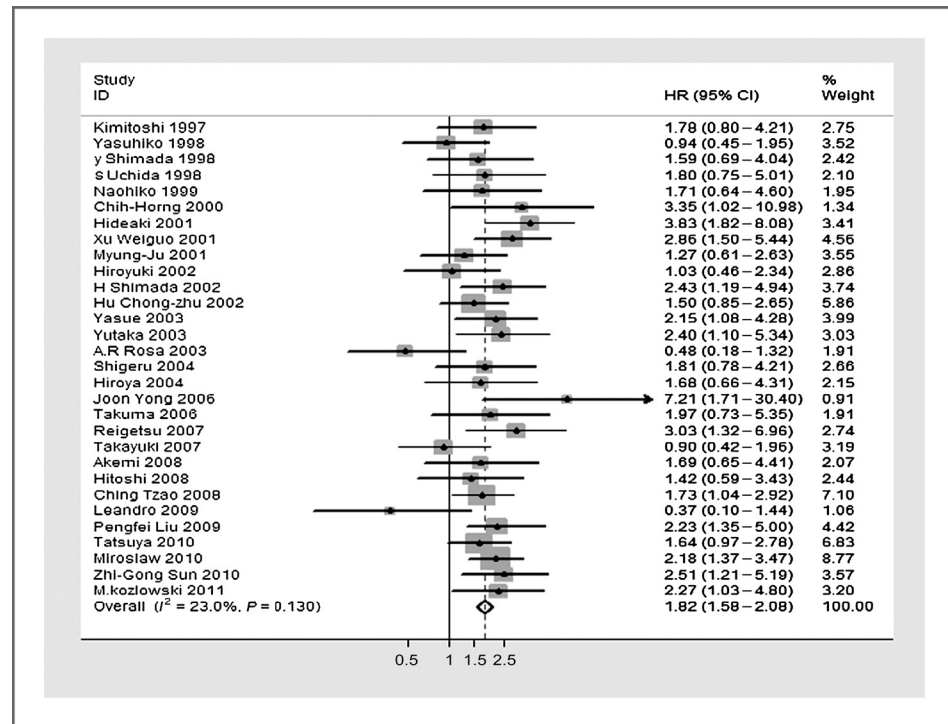
Quality assessment tools are being developed for prognostic studies to help identify study bias and causes of heterogeneity when conducting meta-analysis. We chose to use the REMARK guidelines, which provide a useful start for assessing tumor prognostic markers (15). We operationalized the REMARK guidelines and found that studies reported an average of 11 of 18 quality items. As this is a relatively new tool, there is not much information about what quality constitutes high versus low quality. In our meta-analysis, studies based on multivariate survival analysis tended to be of a slightly higher methodologic

Table 3. Meta-analysis: HR value in esophageal carcinoma subgroups according to histology, methods detecting VEGF, and survival analysis

	No. of studies	Patients	Random-effects HR (95%CI)	Heterogeneity test (Q, I ² , P)
Total	30	2,345	1.82 (1.58–2.08)	37.66, 23.0%, 0.130
VEGF in ESCC	26	2,043	1.81 (1.57–2.10)	31.11, 19.6%, 0.185
VEGF in esophageal cancer	3	264	2.24 (1.41–3.55)	0.30, 0%, 0.861
VEGF by IHC	25	1,846	1.72 (1.47–2.02)	31.57, 24.0%, 0.138
VEGF by ELISA	3	311	2.31 (1.62–3.32)	3.00, 33.4%, 0.223
VEGF by RT-PCR	2	188	1.90 (1.24–2.91)	0.86, 0%, 0.353
Univariate	12	869	1.85 (1.48–2.31)	7.80, 0%, 0.731
Multivariate	18	1,476	1.80 (1.51–2.14)	29.82, 43.0%, 0.028

NOTE: Esophageal carcinoma includes ESCC and EADC.

Figure 2. Meta-analysis (forest plot) of the 30 eligible studies assessing VEGF in esophageal cancer.



quality than studies based on univariate survival analysis, although this is not statistically significant.

In this systematic review with meta-analysis, we combined 30 eligible studies, which included 2,345 patients with esophageal cancer, to yield summary statistics that indicate that high VEGF expression has a significant correlation with poor survival in patients with esophageal cancer. This correlation was observed in both ESCCs and esophageal cancer. When analysis was restricted to studies with more advanced stages (stages III + IV), we found that the combined HR (1.69) was lower than the combined HR for the 30 eligible studies (1.82), suggesting that VEGF expression could be a more important prognostic marker

for early-stage esophageal cancers. When limiting our analysis to studies in esophageal cancers, we found a worse prognostic significance of VEGF. Data were not sufficient to determine the prognostic value of VEGF expression in EADCs. The methods used to detect VEGF also had an impact on significance. We observed that the combined HRs were larger in groups using ELISA (2.31) and RT-PCR (1.90) instead of IHC (1.72). The results were consistent with all methods, with poorer survival in high VEGF expressors, suggesting that VEGF detection techniques are unlikely to be a source of bias. However, it is still important to use standardized, well-defined methods to assess biomarkers. It is important to note that because of the small number of primary studies using ELISA and RT-PCR for analysis, the power to detect potentially important differences is limited. The relationship between serum VEGF and survival should be interpreted cautiously and requires further study. The statistical analysis method chosen to evaluate the survival data also did not have an impact on significance, and results were similar for studies that used either univariate or multivariate survival analysis.

We found no significant heterogeneity among the 30 studies included in our review. When analysis was limited to histologic type and assay method, there was also no heterogeneity detected. However, heterogeneity was observed when analysis was limited to the 18 studies that used multivariate survival analysis. Data for multivariate survival analysis reported in the primary articles were included in the present systematic review with meta-analysis. However, the data we obtained were adjusted for different variables in each study. Adjustment for

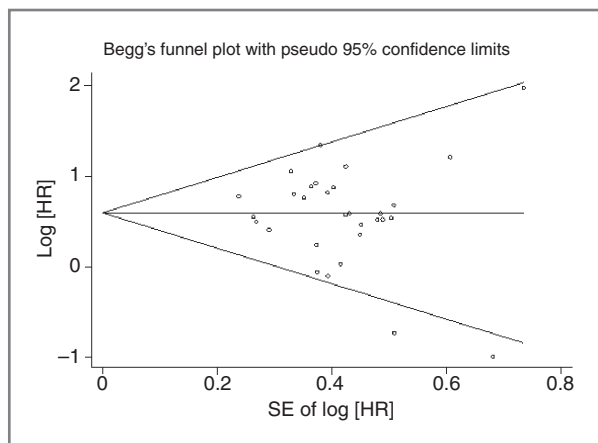


Figure 3. Begg's funnel plot for publication bias test of 30 studies assessing VEGF in esophageal cancer.

potential confounding factors differed across studies, and risk estimates were adjusted for age, gender, depth of tumor penetration, and pathologic stage. The available evidence does not systematically evaluate the independence of the VEGF prognosis association from potential confounders, and the extent of residual confounding is unknown. Thus, this may explain why our collected studies of multivariate survival analysis partly revealed significance in heterogeneity. Publication bias remains a major problem in assessing the validity of clinical research studies. In the present analysis, we did not find evidence that publication bias significantly influenced our results.

Several limitations of this meta-analysis could not be ignored. First of all, although we did not observe significant publication bias between studies, it is uncertain whether the cases are comparably representative in Asia due to 26 of 31 studies conducted. Obviously, it is unavoidable to miss some data because of unpublished studies. Missing information may reflect a negative or more conservative correlation between VEGF and survival, which could lower the significance of VEGF expression as a predictor of mortality (22). Second, studies enrolled in our meta-analysis used IHC to detect VEGF level, which represent potential selection bias. Cutoff values for high VEGF expression differed in the percentage cell staining, varying from 10% to 80%, with 10 studies using 10% and 9 using 30%. Six studies evaluated the association of VEGF with clinical outcome using ELISA or RT-PCR. Although results obtained from different methods are fixed, these findings are consistent with our meta-analysis. Third, the estimated data that we obtained were not adjusted for other variables such as age, gender, histologic grade, and tumor stage. This may cause variability in assessing these

variables between studies. It might be difficult to arrive at a robust conclusion, given the correlation pattern of these prognostic factors. Finally, there still might be a little error when the approximate calculation method was used to estimate the HR values, although 2 investigators calculated them separately.

In conclusion, our results suggest that high VEGF expression may be associated with a poor prognosis in patients with esophageal cancer and provide further support for more definitive investigations into the potential clinical usefulness of measuring VEGF expression in esophageal cancers.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Authors' Contributions

Conception and design: K. Li

Development of methodology: M. Chen, K. Li

Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): M. Chen, E. Cai, Z. Huang, P. Yu, K. Li

Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): M. Chen, E. Cai, Z. Huang, P. Yu, K. Li

Writing, review, and/or revision of the manuscript: M. Chen, K. Li

Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): M. Chen, K. Li

Study supervision: K. Li

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