Increased vascular endothelial growth factor expression predicts a worse prognosis for laryngeal cancer patients: a meta-analysis

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Abstract

Objective: This study assessed the relationship between vascular endothelial growth factor expression and the laryngeal cancer prognosis.

Methods: Systematic computerised searches of PubMed were performed up to 31 January 2015. Prognostic endpoints were overall survival and disease-free survival. The pooled hazard ratios for overall survival and disease-free survival were also calculated.

Results: Seven studies containing 975 patients were included. The pooled hazard ratio was 1.703 (95 per cent confidence interval, 1.373 to 2.112; z score = 4.85, p = 0.000) for overall survival and was 1.918 (95 per cent confidence interval, 1.410 to 2.609; z score = 4.15, p = 0.000) for disease-free survival. No significant publication bias was found. A sensitivity analysis showed that the results were robust. Power analyses also showed there was enough power to detect the calculated hazard ratios.

Conclusion: The study found that vascular endothelial growth factor overexpression predicted a worse prognosis for laryngeal cancer patients. This supports a strategy of targeted therapy by blocking the vascular endothelial growth factor receptor.

Key words: Laryngeal Neoplasms; Vascular Endothelial Growth Factors; Disease-Free Survival; Survival; Biological Markers

Introduction

According to the latest cancer statistics, there were 13 560 cases of laryngeal cancer and 3640 laryngeal cancer deaths in the USA in 2015.¹ Globally, the age-standardised incidence of laryngeal cancer was 5.1 per 100 000 and the age-standardised mortality rate was 2.2 per 100 000 in more developed areas; these figures were 3.5 and 2.0 per 100 000, respectively, in less developed areas.² In recent years, improvements in chemotherapy, radiotherapy and conservative surgery have led to improved preservation of organs and their functions, leading to a better quality of life for laryngeal cancer patients.³ However, patient prognosis has not improved: the 5-year relative survival rates for 1975–1977, 1987–1989 and 2004–2010 were 66 per cent, 66 per cent and 63 per cent, respectively.¹ It is therefore important to identify prognosis factors for this disease and for developing personalised treatment.

Vascular endothelial growth factor controls tumour angiogenesis by promoting endothelial cell growth and migration. Previous studies found that vascular

endothelial growth factor overexpression was associated with poor prognosis for many human cancers, including colorectal cancer, hepatocellular cancer, gastric cancer, cervical cancer and osteosarcoma.^{4–8} A meta-analysis found that vascular endothelial growth factor positivity was associated with worse overall survival for head and neck squamous cell carcinoma patients, with an estimated risk of death within 2 years of 1.88 (95 per cent confidence interval (CI), 1.43 to 2.45; p < 0.001).⁹ Although the larynx is, strictly speaking, part of the head and neck region, it has several clinical and molecular peculiarities. A comparative genomic study identified differences in chromosomal patterns and cancer progression between laryngeal cancer and other head and neck cancers.¹⁰ The American Cancer Society classifies the larynx as part of the respiratory system, that is, as separate from the oral cavity and pharynx. The relationship between vascular endothelial growth factor expression and laryngeal cancer prognosis is unknown.

A meta-analysis was performed to summarise the scientific evidence for an association between vascular

endothelial growth factor expression and the laryngeal cancer prognosis.

Materials and methods

Database and literature search

Systematic computerised searches of the Medline and Web of Science databases up to 31 January 2015 were performed. The following search terms were used: 'laryngeal carcinoma', 'laryngeal cancer', 'vascular endothelial growth factor', 'VEGF', 'prognosis', 'disease-free survival' and 'overall survival'. The search strategy for Medline was 'laryngeal neoplasms' [MeSH Terms] AND (vascular endothelial growth factor* [Title/Abstract] OR VEGF* [Title/Abstract]) AND (Survi* [Title/Abstract] OR prognosis [Title/Abstract]). For Web of Science, the search strategy was (Ts = Laryn*) and (Ts = vascular endothelial growth factor* or Ts = VEGF*) and (Ts = Survi* or Ts = prognosis). In addition, the references of all relevant studies were manually reviewed to supplement the search results.

Study selection

Relevant studies were manually selected based on the following criteria: (1) vascular endothelial growth factor expression was measured immunohistochemically or by enzyme-linked immunosorbent assay or other detection methods; (2) overall survival and disease-free survival were compared for different levels of vascular endothelial growth factor expression in laryngeal cancer; (3) hazard ratios and 95 per cent CIs for overall survival or disease-free survival stratified by the level of vascular endothelial growth factor expression were obtained by multivariate Cox proportional hazards regression or univariate Kaplan–Meier log-rank tests, or from the reported data or survival curves.

Data extraction

The following data types were extracted into standardised forms: (1) basic information such as the first author, publication year, country, study design and number of patients enrolled per year; (2) patient characteristics such as sex, age, clinical stage and treatment; (3) the vascular endothelial growth factor detection method; and (4) outcome, such as overall survival and disease-free survival.

Study selection and data extraction were carried out independently by two reviewers. Disagreements were resolved by discussion between the two.

Statistical analysis

Methods used to combine time-to-event outcomes were summarised using the log hazard ratio and its variance.^{11,12} If individual studies provided a hazard ratio but not a 95 per cent CI, the latter was calculated using the formulas: $b = \ln(\text{hazard ratio})$; standard error of the mean (SEM) = $b \div \text{inverse_normal_}$

distribution ($p \div 2$); and 95 per cent CI = $\exp(b \pm 1.96 \times \text{SEM})$. If individual studies provided neither hazard ratios nor 95 per cent CIs, data were extracted from Kaplan–Meier survival curves using a previously reported method and hazard ratio calculations spreadsheet (in additional file 1 of the paper at <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC1920534/?tool=pubmed#S1>).¹³ Survival curves were read using Engauge Digitizer version 4.1 (<http://digitizer.sourceforge.net/>). Statistical heterogeneity was investigated using inconsistency (I^2) statistics: an I^2 value of 50 per cent or more represented substantial heterogeneity. Studies without substantial heterogeneity were pooled using a fixed-effect model; studies with heterogeneous data were pooled using a random-effects model. Potential publication bias was estimated using a funnel graph, Egger's regression tests and Begg's adjusted rank correlation tests. A sensitivity analysis was conducted using only pooled adjusted hazard ratios. Meta-analyses were performed using Stata 12.0 (StataCorp, College Station, Texas, USA). As a limited number of studies was included in this meta-analysis, we also carried out power analyses. Statistical power was calculated using the power and sample size calculation in PS: Power and Sample Size Calculation version 3.0 (<http://biostat.mc.vanderbilt.edu/wiki/Main/PowerSampleSize>).

Results

Description of eligible studies

In all, seven studies met our inclusion criteria for meta-analysis.^{14–20} The detailed steps in the literature search are shown in Figure 1. Data from 975 patients were used in pooled analyses; study sample sizes ranged from 59 to 289. Two studies each were from USA, Spain and China, and the other was conducted in Greece. In six studies, patient treatments included surgery; in the other study, patients underwent primary radiotherapy only. Vascular endothelial growth factor expression was detected immunohistochemically in six studies and by enzyme-linked immunosorbent assay in the other. Overall follow up ranged from 60 months to 200 months. Six studies used univariate and multivariate analysis to evaluate prognostic outcomes; for these, adjusted hazard ratios were used in this meta-analysis. One study used only univariate analysis to evaluate prognostic outcomes; for this study, crude hazard ratios were used in the meta-analysis. Table I shows the main characteristics of the seven studies included in this meta-analysis.

Overall survival

Six studies reported overall survival as a prognostic outcome^{14–18,20}; there was no heterogeneity between these studies ($I^2 = 0.0$ per cent, $p = 0.512$). A pooled hazard ratio of 1.703 (95 per cent CI, 1.373 to 2.112; z score = 4.85, $p = 0.000$) was obtained using a fixed-effect model (Figure. 2). The symmetrical

FIG. 1

Flow diagram showing the study selection process.

funnel plot (Figure 3) and the Begg's test (z score = 0.38, $p = 0.707$) and Egger's test ($t = 0.87$, $p = 0.432$) suggested no significant publication bias. A sensitivity analysis using only adjusted hazard ratios found the pooled hazard ratio to be 1.688 (95 per cent CI, 1.35 to 2.104; z score = 4.66, $p = 0.000$), indicating that the result was robust. A power analysis found that this sample had sufficient power (51.3 per cent) to detect a hazard ratio of 1.703.

These results show that vascular endothelial growth factor overexpression was associated with worse overall survival for laryngeal cancer patients.

Disease-free survival

Five studies reported disease-free survival as a prognostic outcome;^{14,15,17–19} there was no heterogeneity between these studies ($I^2 = 0.0$ per cent, $p = 0.512$). A pooled hazard ratio of 1.918 (95 per cent CI, 1.410 to 2.609; z score = 4.15, $p = 0.000$) was obtained using a fixed-effect model (Figure 4). The symmetrical funnel plot (Figure 5), and the Begg's test (z score = 0.24, $p = 0.806$) and Egger's test ($t = 0.55$, $p = 0.619$) suggested no significant publication bias. A sensitivity analysis of pooled adjusted hazard ratios showed that the pooled hazard ratio was 1.938 (95 per cent CI, 1.405 to 2.673; z score = 4.03, $p = 0.000$), indicating that the result is robust. A power analysis showed this sample had sufficient power (43.6 per cent) to detect a hazard ratio of 1.918.

These results show that vascular endothelial growth factor overexpression was associated with a worse disease-free survival rate in laryngeal cancer patients.

Discussion

Vascular endothelial growth factor was first identified in 1983, and first purified and cloned in 1989. It functions as an endothelial cell-specific mitogen in vitro and as an angiogenic inducer in a variety of in vivo models.²¹ This study showed that vascular endothelial growth factor overexpression predicts a worse prognosis for laryngeal cancer patients: the pooled hazard ratio was 1.703 (95 per cent CI, 1.373 to 2.112) for overall survival and 1.918 (95 per cent CI, 1.410 to 2.609) for disease-free survival. Several mechanisms could mediate the association between vascular endothelial growth factor overexpression and poor laryngeal cancer prognosis. Angiogenesis is important for the growth and progression of solid tumours, and vascular endothelial growth factor both induces mitosis and regulates the endothelial cell permeability.²² Vascular endothelial growth factor is also important for inducing immune tolerance in the tumour microenvironment.²³ Deezagi *et al.* reported that small interfering RNA-mediated vascular endothelial growth factor inhibition induces apoptosis in cancer cells.²⁴ Therefore, promotion of angiogenesis and immune tolerance by vascular endothelial growth factor overexpression may worsen laryngeal cancer prognosis.

Other risk factors are also reported to affect laryngeal cancer prognosis: these include the tumour–node–metastasis (TNM) tumour category (as defined by the Union for International Cancer Control), nodal involvement (reported for both radiotherapeutic or surgical series), different locations (glottic tumours have a more favourable prognosis than supraglottic or subglottic tumours), alcohol consumption, and the

TABLE I
CHARACTERISTICS OF STUDIES INCLUDED IN THE META-ANALYSIS

Study	Year	Country	Design	Enrollment period	Clinical stage	Pts (n)	Sex (M/F)	Age (y)	Treatment	VEGF detection method	FU (mon)	Outcomes	Statistical analysis methods
Liu <i>et al.</i> ¹⁴	2013	China	Prospective	Jan 2000 – Oct 2005	I–IV	176	170/6	<58.85, >58.91	Partial or total laryngectomy	IHC	~60	DFS & OS	U & M
Pentheroudakis <i>et al.</i> ¹⁵	2012	Greece	Retrospective	May 1985 – Jun 2008	I–III	289	277/12	63 (36–82)	Potentially curative surgery and/or radical external beam irradiation	IHC	~200	DFS & OS	U & M
Liu <i>et al.</i> ¹⁶	2011	China	Prospective	Jan 2003 – Sep 2005	I–IV	69	66/3	<58.29, >58.40	Laryngectomy	IHC	~60	OS	U & M
Rueda <i>et al.</i> ¹⁷	2011	Spain	Retrospective	1993–2003	III–IV	59	NR	39–73 (median 58)	Surgery, chemotherapy & radical radiotherapy	IHC	~200	DFS & OS	U only*
Parikh <i>et al.</i> ¹⁸	2007	USA	Retrospective	1975–2000	T _{1–2} N ₀	123	106/17	Median 64.06 ± 10.9, range 38–90	Primary radiation therapy	IHC	Median 9.9 years	DFS & OS	U & M
Hinojar-Gutiérrez <i>et al.</i>	2007	Spain	Retrospective	NR	T _{1–4} N ₀	76	74/2	median 64, range 41–87	Surgery	IHC	Median 71 (range 40–128)	DFS	U & M
Teknos <i>et al.</i> ²⁰	2002	USA	Prospective	NR	III–IV	183	179/4	NR	Surgery, chemotherapy & radical radiotherapy	ELISA	~80	OS	U & M

Pts = patients; M = male; F = female; y = years; VEGF = vascular endothelial growth factor; mon = months; FU = follow-up; IHC = immunohistochemistry; ELISA = enzyme-linked immunosorbent assay; DFS = disease-free survival; OS = overall survival; U = univariate multivariate analysis; M = multivariate analysis; T = tumour–node–metastasis tumour grade; N = tumour–node–metastasis node grade; NR = not reported. *Crude hazard ratio used for meta-analysis.

FIG. 2

Meta-analysis of overall survival in laryngeal squamous cell carcinoma patients with different levels of vascular endothelial growth factor expression. HR = hazard ratio; CI = confidence interval

expression of genes such as *BCL2*, *p53* and *BAX*.²⁵ These prognostic factors are confounders in the assessment of the relationship between vascular endothelial growth factor expression and laryngeal cancer prognosis. This study included seven studies, of which six reported multivariate analyses and hazard ratios adjusted by sex, age, smoking history, alcohol intake, histological grade, primary tumour site, TNM tumour classification, clinical stage, the presence of metastasis and the expression of some tumour markers. A

sensitivity analysis of pooled adjusted hazard ratios showed that the results were robust.

The results of this study support a therapeutic strategy of blocking the vascular endothelial growth factor receptor. Several clinical trials have confirmed the effectiveness of this type of therapy. In a prospective trial, Yoo *et al.* treated locally advanced head and neck cancer with bevacizumab, erlotinib and concurrent chemoradiation.²⁶ After a median follow up of 46 months, they reported 3-year locoregional control

FIG. 3

Funnel plot showing an analysis of publication bias in the pooled hazard ratios for overall survival. SEM = standard error of the mean; HR = hazard ratio

FIG. 4

Meta-analysis of progression-free survival in laryngeal squamous cell carcinoma patients with different levels of vascular endothelial growth factor expression. HR = hazard ratio; CI = confidence interval

and distant metastasis-free survival rates of 85 per cent and 93 per cent, respectively, and 3-year estimated progression-free survival, disease-specific survival and overall survival rates of 82 per cent, 89 per cent and 86 per cent, respectively. In a phase II trial by Hainsworth *et al.*, combined modality treatment comprising chemotherapy, radiotherapy, bevacizumab and erlotinib in patients with locally advanced squamous carcinoma of the head and neck resulted in estimated three-year progression-free and overall survival rates of 71 per cent and 82 per cent, respectively.²⁷ The

clinical data showed acceptable toxicity levels. This meta-analysis had some limitations. Firstly, some of the studies included failed to provide sufficient data on time-to-event outcomes for direct inclusion in the meta-analysis. Further, data extracted from survival curves was used instead of direct patient data, which may have led to inaccuracies. Secondly, the studies included different clinical tumour stages, different treatments and different methods of detecting vascular endothelial growth factor. Therefore, a subgroup analysis would have been preferable. However, this was

FIG. 5

Funnel plot showing an analysis of publication bias in the pooled hazard ratios for progression-free survival. SEM = standard error of the mean; HR = hazard ratio

impossible owing to the limited number of studies included in the meta-analysis. Fortunately, there was no heterogeneity between studies.

- **Vascular endothelial growth factor is an angiogenic factor that promotes endothelial cell growth and migration**
- **Outcomes derived from the pooled data of 7 studies involving 975 laryngeal cancer patients were included in a meta-analysis**
- **Vascular endothelial growth factor overexpression predicted a worse prognosis**
- **Pooled hazard ratios for overall survival and disease-free survival were 1.703 (95 per cent CI, 1.373 to 2.112) and 1.918 (95 per cent CI, 1.410 to 2.609), respectively**

Conclusion

This meta-analysis found that vascular endothelial growth factor overexpression predicts a worse prognosis for laryngeal cancer patients. This evidence supports a strategy of targeted therapy by blocking the vascular endothelial growth factor receptor.

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