

Prognostic Significance of Vascular Endothelial Growth Factor Immunohistochemical Expression in Head and Neck Squamous Cell Carcinoma: A Meta-Analysis

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ABSTRACT

Purpose: Vascular endothelial growth factor (VEGF) is considered as a prime mediator of angiogenesis. Various studies examining the relationship between VEGF protein overexpression with the clinical outcome in patients with head and neck squamous cell carcinoma have yielded inconclusive results.

Experimental Design: We conducted a meta-analysis of 12 studies ($n = 1,002$ patients) that evaluated the correlation between VEGF (detected by immunohistochemistry) and 2-year overall survival. The relation between VEGF and lymph node involvement (11 studies, $n = 722$) was also examined. Data were synthesized with random effect and fixed effect risk ratios.

Results: The estimated risk of death in 2 years was 1.88-fold higher in the VEGF-positive patients [95% confidence interval, 1.43–2.45; $P < 0.001$ random effect calculations]. Between-study heterogeneity was nonsignificant ($P = 0.15$) but larger studies tended to provide more conservative estimates ($P = 0.097$). VEGF overexpression was not significantly associated with the presence of lymph node metastasis (risk ratio, 1.20; 95% confidence interval, 0.97–1.49; $P = 0.087$) and there was significant between-study heterogeneity ($P = 0.08$).

Conclusions: Although some modest bias cannot be excluded, VEGF positivity seems to be associated with worse overall survival in patients with head and neck squamous cell carcinoma.

INTRODUCTION

Despite advances in diagnosis and treatment, the prognosis of patients with head and neck squamous cell carcinoma

(HNSCC) has remained largely unchanged (1), and the most reliable prognostic factor is still the presence of lymph node metastasis at the time of diagnosis (2). There is a need for better markers that can identify patients with poor prognosis in early stages of the disease.

Angiogenesis is considered to be a very important biological process in many neoplasms (3, 4). Angiogenesis is crucial both for the growth of a primary tumor and for the development of distant metastasis. Among the factors causing tumor angiogenesis, vascular endothelial growth factor (VEGF) is a leading candidate. VEGF induces the proliferation, differentiation, and migration of vascular endothelial cells (5), increases capillary permeability (5, 6), and enhances endothelial cell survival by preventing apoptosis (7, 8). Recently, targeted cancer therapy has spurred attention to angiogenesis inhibitors (9, 10). More than 150 antiangiogenic compounds (the majority of which target VEGF and/or its receptors) are being developed (9, 11, 12). It is therefore useful to establish whether VEGF expression is a prognostic factor.

Several studies have evaluated whether VEGF protein immunohistochemical expression may be a prognostic factor for survival in patients with HNSCC (13–23). However, the results of various studies are conflicting or inconclusive. It is unknown whether differences in these investigations have been mostly due to their limited sample size or genuine heterogeneity. Thus, we conducted a meta-analysis of all available studies relating VEGF with the clinical outcome in patients with HNSCC.

MATERIALS AND METHODS

Identification and Eligibility of Relevant Studies. Initially, we identified all studies, regardless of publication language, targeting VEGF or any other angiogenesis marker in patients with HNSCC. Sources included MEDLINE and EMBASE (last search update July 2004). The search strategy was based on combinations of “angiogenesis,” “VEGF,” “vasculogenesis,” “growth factor,” “head and neck squamous cell carcinoma,” “oral cancer,” “laryngeal cancer,” and “head and neck cancer.” References of retrieved articles were also screened.

We accepted for the meta-analysis studies measuring VEGF with immunohistochemistry in patients with HNSCC, provided that measurements had been done in the primary tumor. We did not use prespecified quality-related inclusion or exclusion criteria and did not weight each study by a quality score because no such score has received general agreement for meta-analyses of observational studies (24). Whenever reports pertained to overlapping patients, we retained only the largest study to avoid duplication of information. We made an effort to contact by e-mail all primary investigators of studies of VEGF expression in HNSCC to clarify whether they had additional available patient survival data and to standardize their data according to the meta-analysis definitions whenever possible.

Received 9/13/04; revised 11/8/04; accepted 11/16/04.

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We also contacted investigators examining other potential angiogenesis markers in HNSCC in order to obtain potentially unpublished data regarding VEGF expression and survival.

Definitions and Standardizations. We used prespecified rules to standardize as much as possible the definition of VEGF positivity. We defined VEGF positivity as positive cell stain in at least 20% of the tumor cells in continuous scales or at least moderate staining in qualitative scales. The above cutoff was used by the majority of the enrolled studies (15–22). When different definitions were used we contacted the primary investigators, and when data with this cutoff were not possible to retrieve we accepted the cutoff that was closest to this 20% cutoff level.

The main outcome of the meta-analysis was overall survival. Overall all-cause mortality avoids biases that may ensue in death attribution. This was standardized to include 24 months of follow-up in all studies to avoid some studies contributing very long-term follow-up data as compared with others. All studies had at least 24 months of follow-up and censoring was very uncommon before this time point. We also performed sensitivity analysis for VEGF expression and HNSCC-specific deaths including the studies with available information.

The secondary outcome of the meta-analysis was the presence of lymph node metastasis at the time of diagnosis. HNSCC preferentially spreads via the lymphatic vessels, and the presence of lymph node metastasis at the time of diagnosis is one of the key reasons of poor outcome (2). Lymph node metastasis was defined as the involvement of at least one lymph node. We also examined whether VEGF expression was associated with tumor location, clinical stage, and *histologic* differentiation.

Data Extraction. Two investigators extracted data from eligible studies independently and reached consensus to all items. We extracted data on characteristics of studies and patients, measurements, and results. In particular, in each report we recorded the first author, year of publication, country of origin, number of patients analyzed, tumor-node-metastasis (TNM) staging of HNSCC, tumor location, demographics, antibodies used for immunohistochemistry, definition of VEGF positivity, and blinding of VEGF measurements to the study outcomes. The main outcomes were tabulated in 2×2 tables showing the occurrence or not of death during the first 24 months of follow-up and the lymph node status according to VEGF results. We also recorded the number of patients censored alive before 24 months.

Statistical Analyses. Data on the predictive ability of VEGF overexpression for 24-month overall survival were combined across studies using fixed and random effect models for the synthesis of risk ratios (RR; ref. 25). The RR shows the death rate in 2 years in the group with VEGF overexpression divided by the death rate in 2 years in the group without VEGF overexpression. Between-study heterogeneity in the RRs was assessed with the Q statistic (considered significant for $P < 0.10$; ref. 25). In the same way, we estimated the relation between VEGF overexpression and lymph node metastasis and other clinicopathologic correlates.

Fixed effect models (Mantel-Haenszel) assume differences between the results of various studies are due to chance. Random effect models (DerSimonian and Laird) allow that results may differ genuinely between studies. With between-study heterogeneity, random effect models provide wider confidence intervals

(26). In the text we generally present random effect estimates, unless stated otherwise.

Sensitivity analyses examined the effect of limiting the evaluation to studies using the 20% or at least moderate immunohistochemistry cutoff; to studies that clearly stated that VEGF measurements were blinded to outcomes; to HNSCC-specific deaths; to oral cavity tumors; and excluding patients censored alive before 24 months. We examined, using the inverted funnel plots, the Begg-Mazumdar correlation test, and the equivalent regression test, whether the results differed in small versus large studies (claiming significance for $P < 0.10$; ref. 27). We also evaluated whether results were changing gradually over time with the publication of more recent studies (28, 29). Analyses were conducted in SPSS 10.0 (SPSS, Inc., Chicago, IL) and Meta-Analyst (Joseph Lau, Boston, MA); P values are two tailed.

RESULTS

Eligible Studies. The initial search algorithm retrieved a total of 645 references and we evaluated 154 reports in full text. Overall, we identified 28 reports with VEGF measurements in patients with head and neck squamous cell carcinoma. Also, one investigator coauthoring the meta-analysis provided unpublished data for the correlation between VEGF expression and survival and node status. For all the patients, measurements had been done in the primary tumor, and all specimens had been taken before chemotherapy or radiotherapy, with the exception of 4 patients enrolled in the study of Neuchrist et al. (15). Of the published studies, 12 reports were excluded: one study lacked follow-up data per the response of the corresponding author (30), one overlapped with another study (31), one measured VEGF only with methods other than immunohistochemistry (32), and nine lacked informative clinical data (33–41).

Among studies that did not provide eligible data for the meta-analysis, only one had addressed survival (40), but calculations were done for VEGF levels determined by Western blot. Four studies that did not provide eligible data mentioned in brief analyses correlating VEGF with lymph node status (36, 39, 41) or TNM stage (38) but without offering usable data to create 2×2 tables. Two of them (36, 41), with a total of 92 patients, found a statistically significant association between VEGF overexpression and lymph node metastasis, whereas the other two (38, 39), with a total of 87 patients, found no significant association.

Seventeen studies ($n = 1,287$ patients) were eligible for the meta-analysis. Overall, for 12 studies (1,002 patients) data on 2-year survival could be obtained from published (13–16, 18) or corresponded (17, 19–23) information. Five studies ($n = 285$ patients) had data only for lymph node status (42–46). Six of the 12 studies with data on 2-year survival also had data on lymph node status ($n = 437$ patients). Therefore, 11 studies ($n = 722$ patients) were synthesized quantitatively for lymph node status.

Characteristics of the 17 eligible studies are listed in Table 1. Eight reports originated from Japan, seven from Europe, and one from the United States. Unpublished data were included from a Brazilian cohort. The reported median age of patients ranged from 58 to 71 years across the eligible studies. Men accounted for 60.9% of the enrolled patients across the 12 studies with gender

Table 1 Characteristics of the eligible studies

Author (year-country)	N	Median age	Male (%)	Clinical staging I + II (%)	Location	Staining for VEGF positivity	Antibody used	Blinding of VEGF evaluation
Maeda (1998-Japan)	45	63	62	42	45 oral	>5%	A20	Yes
Mineta (2002-Japan)	109	58	72	50	109 tongue	>10%	M293	Yes
Kishimoto (2003-Japan)	62	NR	60	61	62 oral	>20%	R11	Yes
Smith (2000-USA)	56	NR	82	9	32 oral, 24 or-ph	At least moderate	JH121	Yes
Gallo (2002-Italy)	52	62	79	44	18 oral, 12 or-ph, 22 lar	>20%	JH121	Yes
Salven (1997-Finland)	156	60	71	28	44 tongue, 22 mouth floor, 19 lower gum, 12 tonsil, 12 hypo-ph, 47 lar	>0%	M293	Yes
Aebersold (2000-Switzerland)	100	NR	NR	9	40 tonsillar fossa, 29 base of tongue, 21 faucial arch, 6 lateral posterior wall, 4 vallecula	At least moderate	A20	NR
Neuchrist (1999-Austria)	52	52	98	19*	11 supraglottic lar, 41 glottic lar	At least moderate	M293	NR
Uehara (2004-Japan)	63	NR	NR	44	28 tongue, 3 palate, 13 lower alveolar and gingiva, 7 upper alveolar and gingiva, 8 buccal mucosa, 4 mouth floor	>20%	A20	NR
Riedel (2000-Germany)	33	58	79	12	6 oral, 10 or-ph, 8 hypo-ph, 9 lar	>25%	PC37	NR
Moriyama (1997-Japan)	44	NR	NR	NR	23 tongue, 12 gingiva, 4 buccal mucosa, 2 mouth floor, 2 soft palate, 1 lip	At least moderate	A20	NR
Artese (2001-Italy)	52	71	83	NR	25 tongue, 9 gingiva, 8 mouth floor, 3 retromolar, 2 hard palate	>10%	A20	NR
Igarashi (2003-Japan)	58	62	62	NR	58 tongue	>10%	A20	NR
Shintani (2004-Japan)	98	66	NR	45	98 oral	>80%	A20	NR
Tanigaki (2004-Japan)	73	58	79	49	73 tongue	>20%	A20	NR
Kyzas (2004-Greece)	67	65	78	63	18 tongue, 2 mouth floor, 1 palate, 1 tonsil, 9 supraglottic lar, 36 lip	>20%	JH121	Yes
Cunha (2004-Brazil) [†]	167	58	83	29	96 oral, 30 hypo-ph, 41 lar	>20%	A20	Yes

Abbreviations: NR, not reported; or-ph, oropharyngeal; hypo-ph, hypopharyngeal; lar, laryngeal.

*The authors do not have staging data for two patients.

[†]Unpublished data.

information. All of the included studies were observational retrospective studies. The VEGF effect on mortality was apparently adjusted for tumor stage and differentiation in six studies, but only two of them provided the final adjusted RR. Laboratory procedures for VEGF determination were reported in sufficient detail in all studies. For eight reports (619 patients) data on the association of VEGF and clinical stage could be obtained from published information, whereas for 10 studies (694 patients) information for the correlation of VEGF with histologic differentiation could be extracted from the published articles. Eight of the eligible studies (all of them having data on 2-year survival) clearly stated that VEGF determinations were blinded to outcomes (13, 14, 16–18, 21). Cancer was located in the oral cavity in 911 patients (70.8%), pharynx in 196 patients (15.2%), and larynx in 180 patients (14%). Information on the prespecified cutoff (20% or at least moderate staining) could be obtained in 10 studies (15–17, 19–23, 44). Different thresholds in the 0% to 25% range were used by six reports (13, 14, 17, 42, 43, 45) whereas one study (46) with data only for lymph node metastasis applied a cutoff of 80%. The most commonly used VEGF antibody was A 20 (Santa Cruz Biotechnology, Santa Cruz, CA).

Data Synthesis: Survival at 24 Months. VEGF overexpression was associated with a worse prognosis regarding the risk of death within 2 years. Mortality was 1.88-fold higher in VEGF-positive patients ($P < 0.001$; Fig. 1). Between-study heterogeneity was nonsignificant ($P = 0.15$). When patients censored alive before 2 years were excluded from the calculations, the results were similar (RR, 1.91) and there

was again no significant between-study heterogeneity ($P = 0.20$; Table 2). In studies clearly stating that measurements were blinded to outcomes, the survival difference was clearer [RR, 2.22; Table 2] and between-study heterogeneity was still nonsignificant ($P = 0.31$). There was no difference in the summary estimate when the unpublished data were excluded from the analysis (RR, 1.84). Analysis for HNSCC-specific mortality also showed a significant effect (RR, 2.29; $P = 0.016$).

Larger, more precise studies tended to somewhat provide more conservative estimates compared with smaller studies ($P = 0.13$ for the Begg-Mazumdar test, $P = 0.097$ for the equivalent regression test, asymmetrical funnel plot, Fig. 2). However, with one exception, all study estimates suggested that VEGF positivity increased the risk of mortality, and the three largest studies still yielded a significant summary RR of 1.56 ($P < 0.001$). There was no evidence that the observed association had changed significantly over time (RR 1.55 in 1997, 1.98 in 1998, 1.49 in 1999, 1.51 in 2000, 1.78 in 2002, 1.90 in 2003, and 1.88 in 2004).

Data Synthesis: Lymph Node Status. VEGF overexpression tended to be associated with the presence of lymph node metastasis, but the effect was modest and not formally statistically significant (RR, 1.20; $P = 0.087$). There was also statistically significant between-study heterogeneity ($P = 0.08$; Fig. 3). Formal statistical significance was claimed when analyses were limited to studies using the 20% cutoff or those that clearly stated that measurements were blinded to outcomes; however, between-study heterogeneity remained significant (Table 3).

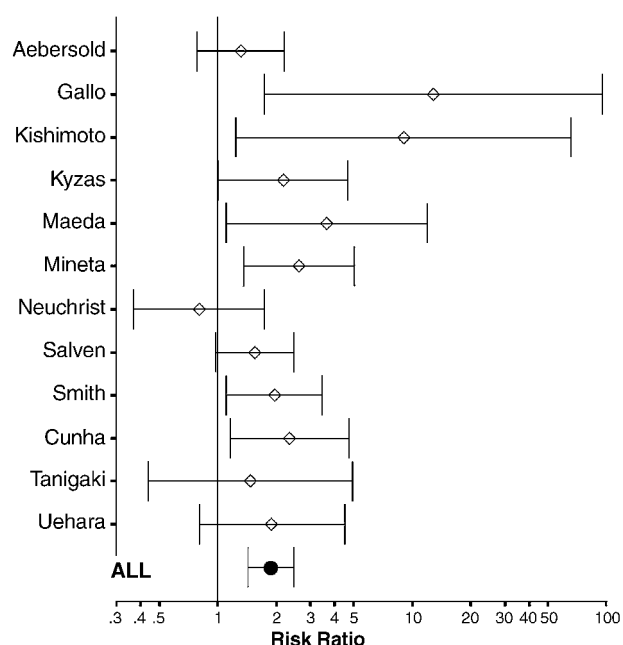


Fig. 1 Meta-analysis of the association between VEGF protein overexpression and the risk of death at 2 years. Each study is shown by the name of the lead author and the RR with 95% CIs. The summary RR and 95% CIs (according to random effect calculations) are also shown (ALL).

VEGF Association with Stage, Differentiation, and Location. We observed a trend towards a correlation of VEGF positivity with higher (III + IV) clinical stage [8 studies ($n = 619$); RR, 1.21; 95% confidence interval (95% CI), 1.00-1.46; $P = 0.05$] and poor histologic differentiation [10 studies ($n = 694$); RR, 1.37; 95% CI, 0.95-1.97; $P = 0.095$]. Tumors located in the oral cavity were not more likely to show VEGF positivity than other tumors [5 studies ($n = 359$); RR, 1.02; 95% CI, 0.88-1.19; $P = 0.77$].

DISCUSSION

This meta-analysis showed that VEGF protein overexpression, as detected with immunohistochemistry, is associated with worse overall survival in patients with HNSCC. All studies, with one exception, had results in the same direction; a prognostic effect on mortality was seen also in the three largest

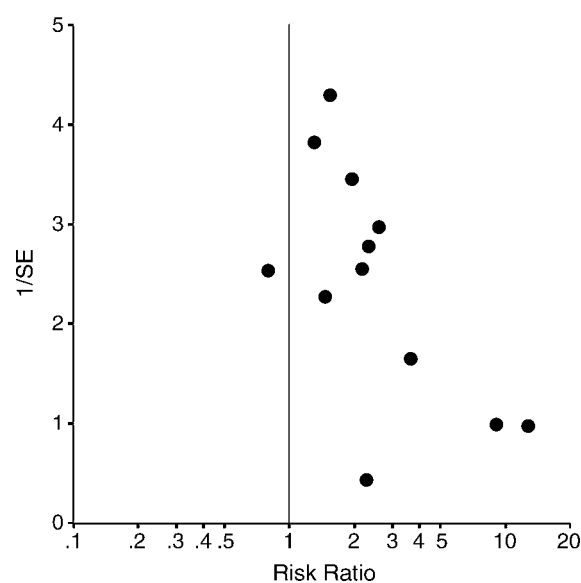


Fig. 2 Inverted funnel plot showing the relation between RR and 1/SE. Larger studies (those with larger 1/SE) show generally smaller RRs.

studies; and VEGF expression had also a significant prognostic effect on HNSCC-specific mortality. However, results should be interpreted very cautiously. VEGF might be a potential prognostic marker in HNSCC, but associations with traditional prognostic factors such as clinical stage or differentiation may explain some of its prognostic effect. Moreover, the strength of the association was significantly smaller in larger studies. This may signify publication bias against the dissemination of “negative” results (47).

Some studies have examined VEGF in HNSCC using methods other than immunohistochemistry (reverse transcription-PCR, ELISA, or Western blot; refs. 32–34, 40). However, only one of these studies (39) had evaluated the correlation between VEGF and survival, and this study used immunohistochemistry measurements as well. Mineta et al. (40) found that high VEGF expression as determined by Western Blot in 60 patients was an independent prognostic factor for 2-year survival. There was also a significant correlation between the levels of VEGF determined by Western Blot and those determined by immunohistochemistry ($P < 0.001$). Although results obtained with different methods are not interchangeable, these findings are consistent with our meta-analysis.

Table 2 RR for association between VEGF and death rate in 24 months

	Studies (n)	RR estimates (95% CI)		
		Random effects	Q	Fixed effects
Total	12 (1,002)	1.88 (1.43-2.45)	15.84*	1.97 (1.59-2.43)
Excluding patients with censoring	12 (942)	1.91 (1.48-2.45)	14.56*	2.03 (1.64-2.50)
Specific 20% cut off	9 (692)	1.81 (1.27-2.58)	12.76*	1.92 (1.48-2.48)
Stated blinding	8 (714)	2.22 (1.66-2.98)	8.27*	2.39 (1.84-3.11)
Excluding unpublished data	11 (935)	1.84 (1.37-2.47)	15.26*	1.92 (1.54-2.39)
Largest studies	3 (312)	1.56 (1.17-2.09)	1.06*	1.54 (1.15-2.07)
HNSCC-specific death	6 (484)	2.29 (1.17-4.48)	12.75†	2.25 (1.54-3.28)
Tumors located in the oral cavity	5 (359)	2.23 (1.61-3.08)	5.60*	2.36 (1.71-3.26)

* $P > 0.1$ for between-study heterogeneity.

† $P < 0.05$ for between-study heterogeneity.

The potential association between VEGF and lymph node status is far from certain for many reasons. The observed effect was modest and not formally significant. Between-study heterogeneity was significant. Moreover, considerable reporting bias probably exists. Also, considerable variability in definitions for VEGF positivity was documented across studies. Other members of the VEGF family such as VEGF-C and VEGF-D, but not VEGF, are considered responsible for tumor lymphangiogenesis and lymph node metastasis (48, 49). However, the capability of VEGF to increase vascular permeability both in blood and lymphatic vessels, and helping cancer cells to enter lymphatics and get established in lymph nodes, may offer a possible explanation for the observed modest association (6).

Some limitations of this meta-analysis should be discussed. First of all, as reported above, potential publication bias is a concern. We tried to identify all relevant data and retrieve additional information that was not available from the published reports, but it is unavoidable that some data could still be missing. Missing information may reflect “negative” or more conservative association of VEGF with survival (27) that could reduce the significance of VEGF expression as a predictor of mortality. Therefore, our results should be interpreted cautiously. Second, in prognostic factors meta-analyses, variability in definitions, outcomes, measurements, and experimental procedures may contribute to between-study heterogeneity (50). In the current meta-analysis, despite the fact that we tried to optimize standardization, some remaining variability in definitions was unavoidable. Although the final estimates of the synthesis of studies using the standardized cutoff did not differ significantly from the overall result in the total study population, conclusions

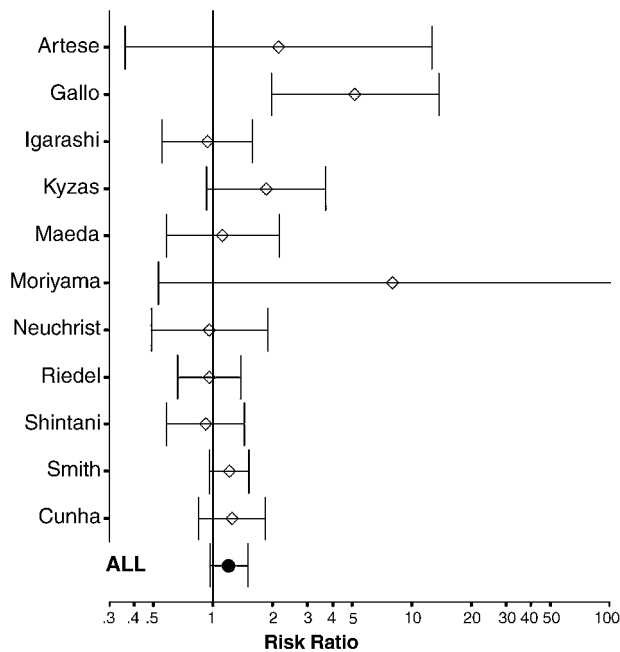


Fig. 3 Meta-analysis of the association between VEGF protein overexpression and the presence of lymph node metastasis at diagnosis. Each study is shown by the name of the lead author and the RR with 95% CIs. The summary RR and 95% CIs (according to random effect calculations) is also shown (ALL).

Table 3 RR for association between VEGF and lymph node status

	Studies (n)	RR estimates (95% CI)		
		Random effects	<i>Q</i>	Fixed effects
Total	11 (722)	1.20 (0.97-1.49)	16.64*	1.30 (1.10-1.53)
Specific 20% cut off	6 (436)	1.50 (1.04-2.17)	11.80†	1.51 (1.22-1.88)
Stated Blinding	5 (387)	1.47 (1.04-2.08)	9.52‡	1.46 (1.19-1.81)
Excluding unpublished data	10 (554)	1.21 (0.94-1.55)	16.52*	1.31 (1.09-1.58)
Largest studies	3 (255)	1.15 (0.97-1.37)	1.32‡	1.17 (0.95-1.44)

*0.05 < *P* < 0.1 for between-study heterogeneity.

†*P* < 0.05 for between-study heterogeneity.

‡*P* > 0.1 for between-study heterogeneity.

need to be drawn cautiously (23, 50). Third, the estimates that we obtained were not adjusted for other variables such as tumor size, histologic differentiation, and clinical staging. However, we found trends for modest correlations of VEGF positivity with higher clinical stage and poor differentiation. Individual-level data would be required to assess the independent prognostic effect of each of these variables (23). Even then, it might be difficult to arrive at robust conclusions, given the correlation pattern of these prognostic factors. Finally, the available data do not allow examining whether VEGF may affect the response to specific therapeutic regimens.

VEGF is the leading candidate but not the only proposed angiogenesis factor. The prognostic value of VEGF in patients with HNSCC should be examined in the context of other proposed molecular markers such as angiogenin, interleukins 10 and 8, platelet-derived endothelial growth factor, fibroblast growth factor, angiopoetins, and thrombospondin (51). Some of the studies included in the meta-analysis had already addressed a significant association of VEGF with other key biomarkers, such as cyclooxygenase-2 (17), *TP53* (14, 18), and the nuclear proliferating marker Ki-67 (18, 21). Also, angiogenesis may not be the only function of this protein. Recent publications show the existence of VEGF receptors in cancer cells in HNSCC (21, 52), in non-small cell lung carcinoma (53), and in cancer cell lines (54), suggesting an autocrine role for VEGF. Considering these caveats about pathophysiology, and despite the limitations reported above, we conclude that VEGF expression might have prognostic significance for patients with HNSCC.

ACKNOWLEDGMENTS

We thank Dr. Gallo (Department of Oto-Neuro-Ophthalmologic Surgery, University of Florence Medical School, Florence, Italy), Dr. Kishimoto (Department of Oral and Maxillofacial Surgery, Biopathological Science, Graduate School of Medicine and Dentistry, Okayama University, Japan), Dr. Aebersold (Department of Radiation Oncology, University of Bern, Inselspital, Bern, Switzerland), Dr. Uehara (Division of Oral and Maxillofacial Surgical Reconstruction and Functional Restoration, Department of Development and Reconstructive Medicine, Graduate School of Biomedical Sciences, Nagasaki University, Sakamoto, Nagasaki, Japan), Dr. Tanigaki (Department of Biology and Function in the Head and Neck, Yokohama City University, Graduate School of Medicine, Kanazawa-ku, Yokohama, Japan), Dr. Soares (Medical and Research Center Hospital do Cancer, SA Paulo, Brazil), and Dr. Sauter (The Wistar Institute, Philadelphia, PA) for providing us with additional data or information from their studies.

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