Systematic Review and Meta-Analysis

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Microvessel density as a prognostic factor in esophageal squamous cell cancer patients

A meta-analysis

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Abstract

Background: To date, literature has emerged that shows contradictory results about the prognostic role of microvessel density (MVD) in esophageal squamous cell cancer (ESCC). The aim of the study set out to evaluate the correlation between MVD and the prognosis of ESCC.

Methods: Identified publications from various databases were obtained and reviewed. A meta-analysis was performed to evaluate the prognostic role of MVD among ESCC patients.

Results: A total of 11 eligible studies containing 891 ESCC cases were included in the meta-analysis. The pooled hazard ratio for overall survival was 2.39 (95% confidence interval 1.92–2.96, $P < .001$). Heterogeneity among the studies was not significant, and publication bias was not found. Subgroup analyses were also performed on different issues, such as districts, antibodies, and median age.

Conclusion: High MVD is a prognostic factor among ESCC that indicated worse prognosis in these patients. More studies are needed, and through abundant evidence, the topic could be re-evaluated by then.

Abbreviations: CI = confidence interval, ESCC = esophageal squamous cell cancer, HR = hazard ratio, MVD = microvessel density, NOS = Newcastle–Ottawa Scale, OS = overall survival, PCNA = proliferating cell nuclear antigen, VEGF = vascular endothelial growth factor, vWF = von Willebrand Factor.

Keywords: esophageal squamous cell cancer, meta-analysis, microvessel density, prognostic factor

1. Introduction

Esophageal cancer is the eighth most frequent cancer worldwide. It is also the sixth most common cause of cancer death, accounting for over 5.4% of all cancer deaths.[1] The occurrence of the disease varies from geographic regions. The incidence is 4.5 per 100,000 individuals in USA, while some of the highest incidences are found in Asia, with approximately 100 per 100,000 individuals affected in the Linxian district of China.[1,2] It remains one of the most lethal cancers of all malignancies, with a 5-year survival rate of 17% once diagnosed.[3] Esophageal squamous cell cancer (ESCC) comprises the majority cases of esophageal malignancies, followed by adenocarcinomas.[4] Apart from independent prognostic factors such as histological type, tumor size, lymph node metastases,[5,6] several biological factors have been recognized to affect the outcomes of the disease as well. These biomarkers include vascular endothelial growth factor (VEGF), p53, proliferating cell nuclear antigen (PCNA), Her-2, and microvascular density (MVD).[7–10] The correlation between tumor metastasis and angiogenesis was first reported by Weidner et al.[11] Angiogenesis as an intratumoral process to form new blood vessels was later proved to be related with the outcomes of various malignancies, such as lung cancer,[12] colorectal cancer,[13] breast cancer,[14] etc. MVD is the most common pathological approach to assess angiogenesis, involving microscopic estimation and microvessel staining.[11] Currently, routine antibodies for staining endothelial cells of microvessel include those against pan-endothelial marker CD34,[15] homodimer trans-membrane protein CD105,[16] platelet/endothelial cell adhesion molecule CD31,[17] and von Willebrand Factor (vWF).[18] The prognostic role of MVD in ESCC was reported in various studies, and many suggested MVD as a crucial prognostic factor in ESCC and led to adverse outcomes,[17–20] whereas some did not reach to any conclusive result indicating that MVD is associated with the prognosis of ESCC.[21,22]

Due to those inconsistent results above, we herein aimed to perform a systematic review and meta-analysis with summarized evidence to determine the prognostic role of MVD among ESCC patients.

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2. Methods

2.1. Literature search

The current study is a meta-analysis; hence, ethical approval was not necessary. Two reviewers (GM and JZ) independently searched PubMed, Embase, Cochrane Library, China National Knowledge Infrastructure (CNKI), and Wanfang Database for eligible studies up till March 25, 2017. The search keywords were as follows: "Microvascular Density" or "Microvessel Density" and "Esophageal Neoplasms" or "Esophageal Cancer" or "Esophageal Carcinoma" and "Survival" or "Prognosis" or "Outcome."

2.2. Inclusion criteria

Eligible studies should met all the criteria as follows: In studies on esophageal cancer, all included patients should be confirmed with squamous cell carcinoma; MVD was assessed and its association with ESCC prognosis was reported; Data provided within the literatures were feasible for log hazard ratio (log HR) calculation, according to methods by Parmar et al,[23] Williamson et al,[24] and Tierney et al[25]; Eligible study categories include cohort study, case–control study, and randomized controlled trials (RCTs), if any.

2.3. Exclusion criteria

Literatures should be excluded if any of the following was matched: review or systematic review; case reports; studies on animals, in vitro studies, or any other types of laboratory studies; and studies that lack credible or extractable data.

2.4. Data extraction

Basic information was extracted as follows: names of first author, publication year, country, median age, number of patients involved and gender, clinical stage, tumor stages, antibodies applied for immunohistochemical staining, and evaluation of high MVD.

The primary data for calculation were multivariate/univariate Cox hazard regression analysis, the Kaplan–Meier survival curves with *P* values, or HR with 95% confidence interval (95% CI) for overall survival (OS). The literature selection and data extraction were performed by 2 reviewers (GM and JZ) independently, with any discrepancies being discussed and reassessed.

2.5. Methodological assessment

Quality of each study was assessed according to Newcastle–-Ottawa Scale (NOS) criteria.[26] Three aspects of each study were evaluated as follows: subject selection: 0 to 4; comparability of subject: 0 to 2; and clinical outcome: 0 to 3. The total score ranged from 0 to 9; study that scored 6 or more was eligible for data-pooling and any literature that scored 7 or more was considered of good quality. The whole evaluation process was conducted by 2 reviewers independently.

2.6. Statistical analysis

The STATA (version 11; Stata Corporation, College Station, TX) was applied for data analysis. LogHRs and variances were extracted for pooling the survival results. If not directly given among the literatures, the HR with 95% CI or Kaplan–Meier curves with *P* values were applied for calculation. Multivariate analyses were prior used if univariate and multivariate survival analyses were both provided. Adjusted HR was first applied if adjusted and unadjusted HRs all existed. Heterogeneity assumption of pooled HRs was assessed by I^2 statistic test and Chi-square based Q-test.[27] The fixed-effect model (the Mantel–-Haenszel method)[28] was applied if the heterogeneity between studies was not statistically significant ($P > .10$ or $I^2 < 50\%$). If else, to reduce the impact of heterogeneity, HR should be evaluated by the random-effect model. Publication bias was assessed through methods of Begg and Mazumdar[29]; if *P* value was no more than .05, then publication bias was considered statistically significant.

3. Results

3.1. Study selection

A total of 248 studies were retrieved from initial search for eligible studies. Abstracts were carefully screened of each identified literatures. Studies were excluded for reasons as follows: duplicate literatures (n = 25), laboratory studies (n = 113), reviews (n = 48), and case reports (n = 34). Full texts of 28 potential studies were retrieved, and then 16 studies were further excluded: 7 studies aimed on irrelevant topics, 5 focused on biological technics such as immunostaining, 3 studies lack available data for quantitative synthesis, 1 study[30] scored no more than 5 according to quality assessment, and 1 literature[31] reported the association between MVD and survival of esophageal adenocarcinoma. In all, 11 studies eventually met our criteria of inclusion for the final analyses.

The process to obtain eligible publication is displayed in Fig. 1.

3.2. Study characteristics

Among the 11 eligible studies, 10 were from Asia, including 8 from Japan[17–19,32–36] and 2 from Korea.[20,21] The study from Turkey[37] was the only one conducted on Caucasian. Altogether, 891 patients were included, with mast majority of male patients. All cases included were ESCC, and tumor stages varied from 0 to IV. Antibodies applied for immunohistochemical staining were against CD34, CD31, Factor VIII, or vWF. HRs were directly given in 6 studies,[17,19–21,32,33] and the rest were extracted from survival curves.[18,34–37] All eligible studies scored no less than 6. High MVDs were assessed quantitatively or defined through intensity levels of staining.

To conclude, basic information for all included studies is summarized in Table 1.

3.3. Meta-analysis results

The prognostic role of high MVD was valued by survival time OS. All 11 studies were eligible to examine OS, and the pooled HR was 2.39 (95% CI 1.92–2.96, $P < .001$), indicating that high intratumoral MVD was associated with inferior outcomes on OS (Fig. 2). The heterogeneity was statistically insignificant ($I^2 = 0\%$, $P = .625$); therefore, fixed-effect model was applied for calculation.

3.4. Subgroup analysis

In accordance with basic information and extracted data from all eligible literatures, subgroups were sorted due to varied districts (Asian/Japanese), antibodies for staining (CD34), median age

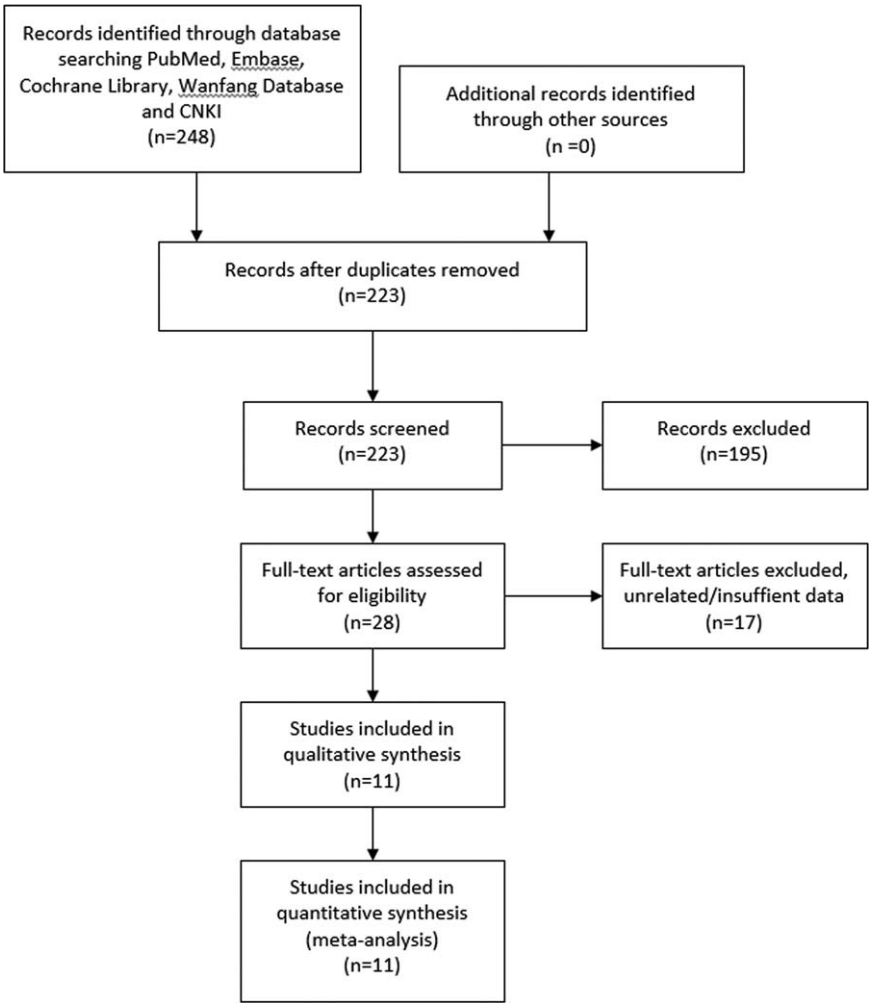

Figure 1. The selection process for eligible studies.

(>60 years), and specific definition of high MVD (>60/mm^2). Disease-free survival (DFS) was reported in 2 studies,[20,21] thus the data were also combined for a pooled result.

3.4.1. Asian/Japanese. Altogether, among 10 Asian studies, 8 were from Japan. The combined HR for OS in Asian was 2.26 (95% CI 1.80–2.84, $P < .001$), heterogeneity was not significant ($I^2 = 0\%$, $P = .747$), and fixed-effect model was applied (Fig. 2). With regard to Japanese patients, heterogeneity was not found and the pooled HR for OS was 2.31 (95% CI 1.81–2.95, $P < .001$, $I^2 = 0\%$).

3.4.2. Antibodies for immunohistochemical staining. Antibodies against CD34 were used within 7 of the included studies for vasculature staining. The combined HR was 2.26 (95% CI 1.74–2.94, $P < .001$). Heterogeneity was not detected and fixed-

Table 1

Characteristics of the included literatures.

Author	Year	Country	Median age	N (F/M)	Clinical stage	Antibody	HR estimation	Evaluation of high MVD	MVD results (high/low)	Quality score
Ha et al[21]	2014	Korea	—	115	I–IV	CD34	HR+CI	>60/mm^2	70/45	8
Faried et al[19]	2007	Japan	62	130 (16/114)	0–IV	CD34	HR+CI	I.L.	64/66	6
Zhang et al[17]	2006	Japan	61.7	51 (8/43)	II–III	CD31	HR+CI	I.L.	20/31	7
Choi et al[20]	2006	Korea	63	51 (4/47)	0–IV	CD34	HR+CI	>60/mm^2	8/43	8
Kato et al[33]	2002	Japan	61.4	64 (9/55)	I–IV	CD34	HR+CI	I.L.	30/34	7
Hironaka et al[32]	2002	Japan	62	73 (13/60)	I–III	CD31	HR+CI	I.L.	36/37	6
Nakagawa[36]	2001	Japan	60.7	95	0–IV	FVIII	Survival curves	I.L.	48/47	7
Elpek et al[37]	2001	Turkey	—	53 (23/30)	I–IV	CD34	Survival curves	>92/mm^2	30/23	6
Shih et al[18]	2000	Japan	61.5	95	I–III	vWF	Survival curves	>60/mm^2	28/67	7
Kitadai et al[35]	1998	Japan	63.5	71	I–IV	CD34	Survival curves	>43/mm^2	35/36	6
Igarashi et al[34]	1998	Japan	64.3	93 (9/84)	0–IV	CD34	Survival curves	>116/mm^2	48/45	7

CI = confidence interval, F = female, HR = hazard ratio, I.L. = intensity level, M = male, MVD = microvessel density, N = number of patients.

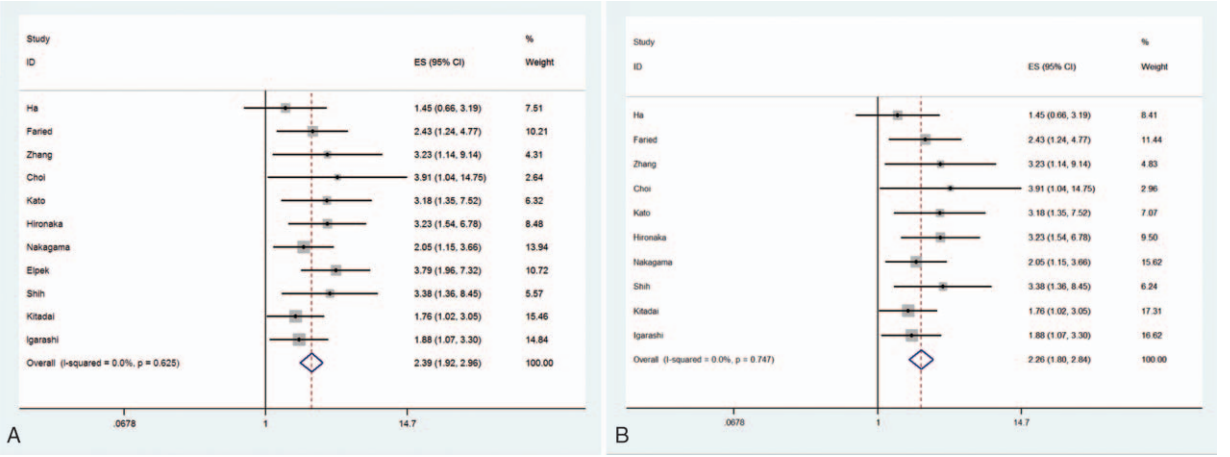

Figure 2. The pooled hazard ratio (HR) for OS in ESCC patients (A) and Asian patients (B) with high intratumoral MVD.

effect model was used to perform the analysis ($P=.414$, $I^2=1.4\%$).

3.4.3. Definition of high MVD. The quantitative measurement to define high MVD varied between studies, whereas 3 studies were coherent that vessel counts over 60/mm^2 be considered as high MVD. The pooled result for OS was also indicative. The HR was 2.31 (95% CI 1.34–3.99, $P=.003$), and heterogeneity was statistically insignificant ($P=.27$, $I^2=23.5\%$).

3.4.4. DFS. The pooled HR for DFS was 2.37 (95% CI: 0.66–8.56, $P=.189$). Heterogeneity was significant ($P=.052$, $I^2=73.6\%$) and random-effect model was used.

3.4.5. Age. Median age was provided in 9 studies that were all over 60 years old. Combined HR for OS in this case was 2.35 (95% CI 1.85–2.99, $P<.001$). Heterogeneity was not significant, thus fixed-effect model was applied ($P=.799$, $I^2=0\%$).

All summarized results are listed in Table 2.

3.5. Publication bias

Publication bias was not found in this meta-analysis, with reference to the plots of publication in Fig. 3 ($P=.213$).

4. Discussion

The present study set out to determine the prognostic role that MVD might have among ESCC patients. Data were pooled and a meta-analysis was performed. As a result, high MVD was a prognostic factor, which indicated poorer outcomes among ESCC patients. Accordingly, the correlation between MVD and Asian/Japanese patients who suffered ESCC was also identified; high MVDs have an adverse impact on these cases. When precisely defined (>60/mm^2), the prognostic role of high MVD in ESCC resulted the same. As to ESCC patients whose median ages were above 60 years and intratumoral vessels stained by CD34, high MVD was also a poor prognostic factor among ESCC patients, respectively. As to DFS, the number of included studies is very limited, and heterogeneity was also significant. Therefore, no conclusion could be drawn on the topic of correlation between MVD and DFS of ESCC.

In accordance with the results above, high MVD is related with poorer outcomes among ESCC patients. Such is the case in squamous cell cancer, but when it comes to other histological types of esophageal cancer, little was reported and the correlation remains unclarified. In a cohort study involving 98 adenocarcinomas, no significant association between MVD and survival was found according to Dutta et al.[31] In ESCC, the occurrence of lymph node metastasis is also an independent poor prognostic factor.[38] MVD with lymph node (LMVD) was also reported in several studies. Seemingly, LMVD that indicated lymphatic metastasis should have a negative impact on ESCC survival; interestingly, no correlation between LMVD and OS was detected among any of these studies.[39–41] As to other malignances such as lung adenocarcinoma, LMVD was reported to cause worse prognosis,[42,43] so was the same with colorectal cancer.[44,45] To conclude, although the role of MVD in ESCC has been identified in this study, the prognostic role of MVD in other pathological types and the role of LMVD remains unclear,

Table 2

Meta-analyses of high MVD and survival of ESCC patients.

	N of studies	Model	HR (95% CI)	Log-rank *P*	Heterogeneity (*P*, I^2)
Total OS	11	Fixed	2.39 (1.92–2.96)	<.001	.625, 0%
Asian OS	10	Fixed	2.26 (1.80–2.84)	<.001	.747, 0%
Japanese OS	8	Fixed	2.31 (1.81–2.95)	<.001	.777, 0%
Anti-CD34 OS	7	Fixed	2.26 (1.74–2.94)	<.001	.414, 1.4%
>60/mm^2 OS	3	Fixed	2.31 (1.34–3.99)	.003	.270, 23.5%
Total DFS	2	Random	2.37 (0.66–8.56)	.189	.052, 73.6%
Median age >60 OS	9	Fixed	2.35 (1.85–2.99)	<.001	.799, 0%

CI = confidence interval, DFS = disease-free survival, HR = hazard ratio, N = number, OS = overall survival.

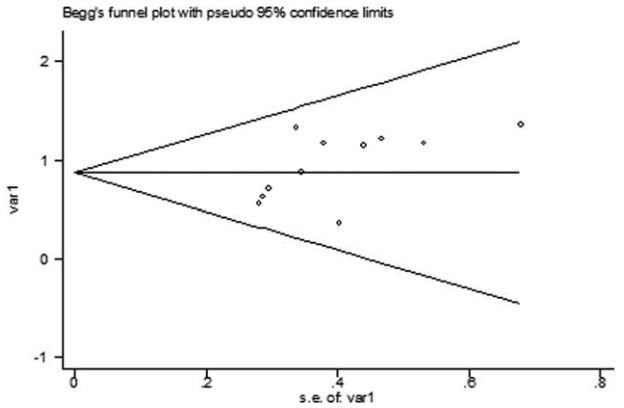

Figure 3. The Begg publication bias plots of the studies that reported the correlation between MVD and ESCC. The publication bias was insignificant ($P = .213$).

and they should be revalued when abundant clinical evidence has emerged by then.

Similar to other malignances, ESCC growth is closely associated with vascularization. Folkman[46] firstly revealed the correlation between tumor growth and angiogenesis. Tumor angiogenesis is a complicated process mediated by various angiogenetic factors that were either released from cancer cells or synthesized by host cells.[47] Among these factors, VEGF was considered to be the key factor of most specificity.[48,49] Various prior studies were conducted on the topic to recognize the correlation between VEGF and MVD when ESCC was diagnosed; however, the results were incoherent. Some studies reported a positive relation between VEGF expression and MVD.[35,50] On the contrary, however, no significant result was found on the question of whether VEGF level correlates with MVD.[33,51] Therefore, more studies are needed to further explore the question and MVD results should be referred together with VEGF level to assess the angiogenesis condition of ESCC cases.

With regards to MVD, several issues should be considered. Although MVD is closely related with tumor behavior such as invasion and metastasis, the parameter itself has restrictions. First of all, evaluation of MVD value was mostly based on subjective judgments, such as hot-spot selection and vessel-counting.[52] Although software, such as CIAS (computer-aided image analysis system), was designed to mellow these bias, yet its accuracy needs to be further tested.[17,53] Second, the MVD was derived from a tissue section, which means that MVD could not indicate the whole in vivo condition or the dynamic tumoral status. Lastly, to date, debate continues on which antibody was most suitable for immunohistochemical staining in MVD assessment. CD34 was a frequently used marker, but it failed to differentiate normal vessels and newly formed vessels.[15] Some believed that CD105 has superior specificity with newly generated endothelial cells,[53,54] yet few studies measured ESCC MVD through CD105, and evidence remained insufficient to draw a conclusion. Despite the flaws mentioned above, to date, MVD is still the most widely used method, and is considered as the golden standard to assess angiogenesis quantitatively.[11]

To our knowledge, this is the first meta-analysis conducted to demonstrate the prognostic role of MVD among ESCC patients. Yet, there are several limitations in our study. First of all, currently, existing literatures are limited. All the included studies were either cohort study or retrospective study, with no RCTs been found. Second, the basic information of included cases was incoherent. The stages of ESCC ranged from 0 to IV, and the definitions of high MVD were also inconsistent. Despite the subgroups performed on some study characters, we failed to cover them all. For instance, all included patients staged between 0 and IV in each study, respectively; therefore, subgroups could not be performed on tumor stages and our topic. Furthermore, the antibodies applied for microvessel-counting varied between studies. As mentioned earlier, to our knowledge, we cannot define which is the most reliable. However, with detailed protocol, carefully pooled data, neither publication bias nor heterogeneity was found, and the results of the study are guaranteed reliable.

To conclude, high MVD is a prognostic factor among ESCC, and would lead to worse outcomes in these patients. Antibody for histological staining is a crucial issue, and needs to be further compared for liability. More studies are in need to examine the correlation between MVD and clinical outcome of ESCC patients, and through abundant evidence, we may re-evaluate the topic by then.

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