Original Article

Microvessel density as a prognostic factor in non-small cell lung cancer: a meta-analysis

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Abstract: Purpose: The prognostic role of microvessel density (MVD) in non-small cell lung cancer (NSCLC) remains controversial. The aim of this study was to evaluate the prognostic role of MVD in NSCLC. Methods: Relevant literature was identified using PubMed, EMBASE and Cochrane Library. The patients’ baseline characteristics and survival outcome were extracted. Then meta-analysis was performed to assess the prognostic role of MVD in all patients and different subgroups. Results: A total of 68 eligible studies were included in this meta-analysis. The combined hazard ratio (HR) and 95% confidence interval (95% CI) for overall survival (OS) of 66 studies was 1.12 (1.09-1.16, P<0.001), and the pooled HR and 95% CI for DFS/RFS/PFS of 9 studies was 2.24 (1.53-3.27, P<0.001). The pooled HR and 95% CI for OS in ADC>50% and <50% group were 1.71 [1.42-2.05] and 1.15 [1.10-1.20], respectively. We also did subgroup analyses based on the patients’ area, detection antibody and clinical stage. Conclusions: Our results showed that MVD may be a prognostic factor for DFS/RFS/PFS in patients with NSCLC, but does not seem to have a prognostic role for OS. Standardization of angiogenesis assessment should be established. Furthermore, MVD combined with other markers could be assessed to predict the survival outcome of NSCLC.

Keywords: Microvessel density, non-small cell lung cancer, prognosis, meta-analysis

Introduction

Lung cancer is the leading cause of cancer-related mortality worldwide, which is expected to represent 26% of all female cancer deaths and 29% of all male cancer deaths in 2012 [1]. Non-small cell lung cancer (NSCLC) accounts for approximately 85% of all lung cancer cases [2]. Although diagnostic and therapeutic methods have improved a lot, the overall 5-year survival rate of NSCLC remains poor, lower than 15% [1]. The critical factors that determine the prognosis of NSCLC are tumor size, nodal involvement, and distant metastasis [3]. Prognostic factors are of great help for clinical management and response evaluation of therapy for cancer. Apart from some independent prognostic factors, including age, sex, tumor stage and weight loss [4], many biological factors have been identified for NSCLC, for example, microvessel density (MVD), epidermal growth factor receptor (EGFR), vascular endothelial growth factor (VEGF), p53, cyclooxygenase-2 (COX-2), HER-2/Neu, and Ki67 [5].

Weidner et al. [6] first reported the correlation between tumor metastasis and angiogenesis, which is a process involving the formation of new blood vessels from preexisting vasculature. MVD has been commonly used to measure tumoral angiogenesis. MVD was evaluated by light microscopy after staining [6]. Main antibodies used for microvessel staining are against Von Willebrand Factor (factor VIII), CD31 (platelet/endothelial cell adhesion molecule), CD34 (progenitor cell antigen), and CD105 (Endoglin) [7-13]. It has been demonstrated that the correlation between MVD and prognosis exists in patients with breast cancer [14], colorectal cancer [15], pituitary adenomas [16], and head and neck cancer [17].
Many researchers have investigated the prognostic role of MVD in NSCLC patients. But the results remain controversial. Some studies concluded that MVD is related to poorer survival in NSCLC [18-20], but some studies did not reach this conclusion [10, 11, 21, 22]. The aim of this meta-analysis was to determine whether MVD can be a prognostic factor in NSCLC. We conducted a meta-analysis of published studies relating MVD and NSCLC, and quantitatively summarized the available evidence.

**Materials and methods**

**Search strategy**

We followed the developed guidelines for the systematic reviews of evaluations of prognostic variables [23]. PubMed, EMBASE and Cochrane Library were searched to identify potentially relevant published literature (last search on Feb 1st, 2015). The search items included ('microvessel density' OR 'microvascular density' OR 'MVD'), AND ('outcome' OR 'survival' OR 'prognosis') AND ('lung cancer'). Reference lists of existing reviews were also checked for any potentially relevant additional studies.

**Inclusion and exclusion criteria**

Studies were considered eligible if they met all of the following criteria:

1. The study population consisted of patients with NSCLC; 2. The study measured the MVD of the tumor; 3. The study investigated the correlation between the MVD of the tumor and the survival outcome; 4. Sufficient survival data were reported to estimate the log-hazard ratio (logHR) and variance with methods developed by Parmar, Williamson and Tierney [24-26].

Studies were excluded based on any of the following criteria:

1. Studies were review articles, laboratory articles or letters; 2. The study investigated other types of cancer or other markers; 3. The study lacked key information for calculation.

The criteria were assessed by two investigators (XL Ma and J Zhang) independently. Any disagreement was resolved by consensus.

**Data extraction**

Two independent reviewers (XL Ma and J Zhang) extracted the data from the selected studies, with any discrepancies being discussed. The primary data were survival outcomes including multivariate or univariate Cox hazard regression analysis, or Kaplan-Meier survival curves of survival outcomes and log-rank $P$ value. Additional data included first author, year of publication, country, number of patients, mean age, tumor stage, histological types, antibody, attitude and other clinical characteristics.

**Statistical analysis**

The logHR and variance were used for aggregation of the survival results, but they were not given directly in most studies. Based on the methods developed by Parmar, Williamson and Tierney [24-26], these values were either calculated from the HR and 95% confidence interval (CI) where quoted, or from the Kaplan-Meier survival curves and the log-rank $P$ value. The software used for these indirect calculations was designed by Matthew Sydes and Jayne Tierney of the Medical Research Council Clinical Trials Unit, London, UK [26]. Despite the overall analysis, we also did subgroup analysis on the basis of different clinical characteristics, for
Table 1. Main characteristics of the included studies

<table>
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<tr>
<th>First author</th>
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The logHR and variance for individual studies were entered into RevMan 5.1 (Cochrane collaboration, Oxford, UK) and pooled together. The overall prognostic role of MVD was recorded as an HR and 95% CI (i.e. an HR>1 reflected adverse survival associated with higher MVD, and would be considered statistically significant if the 95% CI did not overlap 1). And forest plots were used to estimate the role of MVD on the survival outcome in NSCLC patients. Heterogeneity was assessed with a P-value of <0.10 or I²>50% taken to reflect the presence of significant heterogeneity. In this case, a random effect model was used. A P-value of <0.05 was taken to reflect significance for the analyses. Publication bias was assessed by inspection of the funnel plot with Begg’s rank correlation, and P>0.05 was considered that there was no potential publication bias. Publication biases were evaluated using the Begg and Egger’s funnel plot by STATA 11.0 (STATA Corporation, College Station, TX).

**Results**

**Studies selection and characteristics**

Our initial search using PubMed and EMBASE retrieved 382 potentially relevant references. After screening the titles and abstracts of identified articles, 222 articles were excluded for the following reasons: laboratory studies (n=23), review articles (n=4), articles on other cancers (n=44), duplicate (n=4), or not related to the current study (n=147). Then, we evaluated the 160 potential studies in full text and 92 articles were further excluded because of lacking data to be calculated for analysis. Finally, 68 studies [7-13, 18-22, 27-82] were included in this study. The study selection process was shown in Figure 1.

The main characteristics of the included studies were summarized in Table 1. The studies were from 18 different countries and published between 1995 and 2013. The total number of patients encompassed in the 68 eligible studies was 10358 with the mean number of 152 (range 17-515). The patients were all diagnosed with NSCLC including squamous cell carcinoma (SCC), adenocarcinoma (ADC), large cell carcinoma (LCC), epidermoid carcinoma (EC), carcinosarcoma (CS), adenosquamous carcinoma (ADSC), adenosquamous carcinoma (ADC), large cell carcinoma (LCC), epidermoid carcinomas (EC), adenosquamous carcinoma (ADSC), carcinosarcoma (CS) and bronchioloalveolar carcinoma (BAC). As to the survival data, among the 68 included studies, 38 reported HR and 95% CI directly, 26 reported Kaplan-Meier survival curves with log-rank p value and the rest 4 reported both of them. The antibodies used to assess the MVD included anti-CD31 antibody, anti-CD34 antibody, anti-CD105 antibody and anti-factor VIII (FVIII) antibody. The survival outcome included overall survival (OS), disease-free survival (DFS), progression-free survival (PFS), recurrence-free survival (RFS), and other.
Prognostic role of MVD in NSCLC

Figure 2. Estimated hazard ratio (HR) summary for overall survival (OS) in all patients.
free survival (DFS), recurrence-free survival (RFS) and progression-free survival (PFS).

**Correlation between MVD level and survival outcome (OS and DFS/RFS/PFS)**

**Overall analysis:** Since the test of between-study heterogeneity was significant for OS ($I^2$=82.4%, P<0.001) and DFS/RFS/PFS ($I^2$=92.5%, P<0.001), random-effects models were used to calculate the pooled HR. For the overall population from 66 included studies, worse OS (HR1.12, 95% CI 1.09-1.16) was observed among patients with higher MVD level (Figure 2). And from the 9 studies that reported the DFS/RFS/PFS, the pooled HR (95% CI) was 2.24 [1.53-3.27] (Figure 3).

**Subgroup analysis**

**Asian and non-Asian countries:** Among the 68 included articles, 34 were from China and Japan (Asian) and the other half were from Europe, the USA and Brazil (non-Asian). When grouping the meta-analysis by patients' nation, we found that the combined HRs and 95% CIs for OS in patients in Asian and non-Asian were 1.13 [1.08-1.19] and 1.22 [1.15-1.29], respectively.

**Detection antibodies:** Four antibodies were used in the included studies: anti-CD31 antibody, anti-CD34 antibody, anti-CD105 antibody and anti-FVIII antibody. Since the different detection antibodies may influence the prognostic value, we made subgroup analysis to further analyze the prognostic role of MVD. The pooled HR and 95% CI for OS was 1.20 [1.10-1.30] in CD31 group, 1.10 [1.06-1.14] in CD34 group, 1.94 [1.43-2.62] in CD105 group and 1.49 [1.30-1.70] in FVIII group.

**Clinical stage:** Patients were in different stages (I, II, III or IV by TNM grading) of NSCLC. According to the percentage of patients in stage I and II, we divided the studies into stage I-II>50% group and stage I-II<50% group. The pooled HR and 95% CI for OS in stage I-II>50% and <50% group were 1.18 [1.14-1.23] and 1.13 [1.04-1.22], respectively.
Table 2. Meta-analyses of MVD to predict the survival outcome

<table>
<thead>
<tr>
<th>Survival outcome</th>
<th>Data sets (number)</th>
<th>Model</th>
<th>HR (95% CI)</th>
<th>Log-rank P</th>
<th>Heterogeneity (P, I²)</th>
<th>Publication bias (Egger's)</th>
<th>Conclusion</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total OS</td>
<td>91</td>
<td>Random</td>
<td>1.12 [1.09-1.16]</td>
<td>&lt;0.001</td>
<td>&lt;0.001, 82.4%</td>
<td>&lt;0.001</td>
<td>Positive</td>
</tr>
<tr>
<td>Total DFS/RFS/PFS</td>
<td>13</td>
<td>Random</td>
<td>2.24 [1.53-3.27]</td>
<td>&lt;0.001</td>
<td>&lt;0.001, 91.6%</td>
<td>0.038 (Begg's)</td>
<td>Positive</td>
</tr>
<tr>
<td>Asia OS</td>
<td>44</td>
<td>Random</td>
<td>1.13 [1.08-1.19]</td>
<td>&lt;0.001</td>
<td>&lt;0.001, 80.9%</td>
<td>&lt;0.001</td>
<td>Positive</td>
</tr>
<tr>
<td>Non-Asia OS</td>
<td>47</td>
<td>Random</td>
<td>1.22 [1.15-1.29]</td>
<td>&lt;0.001</td>
<td>&lt;0.001, 82.2%</td>
<td>&lt;0.001</td>
<td>Positive</td>
</tr>
<tr>
<td>Detection antibodies (anti-CD31) OS</td>
<td>26</td>
<td>Random</td>
<td>1.20 [1.11-1.31]</td>
<td>&lt;0.001</td>
<td>&lt;0.001, 74.6%</td>
<td>&lt;0.001</td>
<td>Positive</td>
</tr>
<tr>
<td>Detection antibodies (anti-CD34) OS</td>
<td>41</td>
<td>Random</td>
<td>1.10 [1.06-1.14]</td>
<td>&lt;0.001</td>
<td>&lt;0.001, 83.5%</td>
<td>&lt;0.001</td>
<td>Positive</td>
</tr>
<tr>
<td>Detection antibodies (anti-FVIII) OS</td>
<td>5</td>
<td>Fixed</td>
<td>1.93 [1.45-2.55]</td>
<td>&lt;0.001</td>
<td>0.341, 11.4%</td>
<td>0.624 (Begg's)</td>
<td>Positive</td>
</tr>
<tr>
<td>Clinical Stage (I-II/total&gt;50%) OS</td>
<td>19</td>
<td>Random</td>
<td>1.49 [1.30-1.70]</td>
<td>&lt;0.001</td>
<td>&lt;0.001, 87.5%</td>
<td>&lt;0.001</td>
<td>Positive</td>
</tr>
<tr>
<td>Clinical Stage (I-II/total&lt;50%) OS</td>
<td>72</td>
<td>Random</td>
<td>1.18 [1.14-1.23]</td>
<td>&lt;0.001</td>
<td>&lt;0.001, 81.3%</td>
<td>&lt;0.001</td>
<td>Positive</td>
</tr>
<tr>
<td>Clinical Stage (Illa/total&gt;10%) OS</td>
<td>17</td>
<td>Random</td>
<td>1.13 [1.04-1.22]</td>
<td>0.003</td>
<td>&lt;0.001, 83.7%</td>
<td>0.001</td>
<td>Positive</td>
</tr>
<tr>
<td>Clinical Stage (Illa/total&lt;10%) OS</td>
<td>27</td>
<td>Random</td>
<td>1.49 [1.21-1.84]</td>
<td>&lt;0.001</td>
<td>&lt;0.001, 73.7%</td>
<td>0.649</td>
<td>Positive</td>
</tr>
<tr>
<td>Histology Type (ADC/total&gt;50%) OS</td>
<td>31</td>
<td>Random</td>
<td>1.71 [1.42-2.05]</td>
<td>&lt;0.001</td>
<td>&lt;0.001, 74.6%</td>
<td>&lt;0.001</td>
<td>Positive</td>
</tr>
<tr>
<td>Histology Type (ADC/total&lt;50%) OS</td>
<td>52</td>
<td>Random</td>
<td>1.15 [1.10-1.20]</td>
<td>&lt;0.001</td>
<td>&lt;0.001, 84.6%</td>
<td>&lt;0.001</td>
<td>Positive</td>
</tr>
</tbody>
</table>

MVD, microvessel density; FVIII, factor VIII; ADC, adenocarcinoma; OS, overall survival; DFS, disease-free survival; RFS, recurrence-free survival; PFS, progression-free survival; HR, hazard ratio; CI, confidence interval.
We also divided the studies into stage IIIa>10% group and stage IIIa<10% group according to the percentage of patients in stage IIIa. The pooled HR and 95% CI for OS in stage IIIa>10% and <10% group were 1.49 [1.21-1.84] and 1.08 [1.04-1.13], respectively.

**Histological type:** Patients were in different subtypes including SCC, ADC, LCC, EC, ADSC and BAC. According to the percentage of patients with ADC, we divided the studies into ADC>50% group and ADC<50% group. The pooled HR and 95%CI for OS in ADC>50% and <50% group were 1.71 [1.42-2.05] and 1.15 [1.10-1.20], respectively.

The results of meta-analyses for overall and subgroup analysis were shown in Table 2.

**Assessment of publication bias**

Egger and Begg's tests were used to examine publication bias. Significant publication biases were found in the results of meta-analyses except in the subgroup of anti-CD105 antibody for OS (P=0.624) and the subgroup of stage IIla<10% for OS (P=0.649). The plots of publication bias for OS and DFS/RFS/PFS in all patients were shown in Figure 4.

**Discussion**

Previous meta-analyses of studies investigating the prognostic value of MVD, a marker of angiogenesis, have been published for different malignancies. Uzzan et al. [14] investigated the prognostic role of MVD on breast cancer. Des Guetz et al. [15] investigated on colorectal cancer and Zhang et al. [83] investigated on renal cell carcinoma. They demonstrated that MVD predicted poor survival in breast cancer and colorectal cancer, but not in renal cell carcinoma. Our meta-analysis of all published studies from which statistical data could be obtained or calculated examined the prognostic role of MVD on the patients with NSCLC. In our study, the pooled HR (95% CI) for OS was only 1.12 [1.09-1.16] and the pooled HR (95% CI) for DFS/RFS/PFS was 2.24 [1.53-3.27]. Moreover, the pooled HRs were all below 2 in each subgroup. As a rule of thumb, prognostic factors with a HR<2 is of limited practical use [84]. Thus, our results show that MVD may be a prognostic factor for DFS/RFS/PFS in patients with NSCLC, but does not seem to be a prognostic factor for OS.

Our findings are in agreement with the meta-analysis by Trivella et al. [85] in 2007, but are inconsistent with the results in the meta-analysis by Meert et al. [86] in 2002. The meta-analysis in 2007 included 17 centers with 3200 patients. They reported the pooled HR of 1.05 (95% CI 1.01-1.09, P=0.03) for MVD counts obtained by the Chalkley method and 1.03 (0.97-1.09, P=0.3) by the all vessels method. And they concluded that MVD did not seem to be a prognostic factor in patients with non-metastatic surgically treated NSCLC. The meta-analysis in 2002 included 14 studies on FVIII (1866 patients), 10 on CD34 (1440 patients) and 8 on CD31 (1093 patients). They found that a high microvessel count in the primitive lung tumor was a statistically significant poor prognostic factor for survival in NSCLC whatev-
er it was assessed by FVIII (HR: 1.81; 95% CI: 1.16-2.84), CD34 (HR: 1.99; 95% CI: 1.53-2.58) or CD31 (HR: 1.80; 95% CI: 1.10-2.96). And they concluded that microvessel count appears to be a poor prognostic factor for survival in surgically treated NSCLC. Compared to the two previous meta-analyses, we did a more thorough literature research and included far more articles and much more patients (68 articles with 10358 patients) using the methods developed by Parmar, Williamson and Tierney [24-26], with 36 articles before 2002 and 47 before 2007. Furthermore, we covered some important clinical features which may influence prognosis of NSCLC patients, for example, nation, detection antibodies, clinical stage and histology type.

Jusufovic et al. [7] found that higher MVD predicted poor outcome in Serbia (HR: 6.27 [2.90-13.54]). In China, Wu et al. [82] also reached this conclusion. However, Kreuter et al. [10] in Germany found that increased MVD is a prognostic indicator for better survival (0.52 [0.28-0.97]). So we analyzed the prognostic role of MVD in patients from different nations. The pooled HRs for OS in Asian (1.13) and non-Asian (1.22) groups were all very low and did not differ sharply. Thus we conclude that the prognostic value is almost the same in either Asian or non-Asian patients.

Anti-CD31, anti-CD34, anti-CD105 and anti-FVIII antibodies are commonly used antibodies for evaluation of angiogenesis quantification in solid human tumors [87]. Researchers using different antibodies draw different conclusions regarding to which antibody was superior [34, 38, 55, 58]. The above four studies were before 2002. Therefore, in 2002, the international consensus statement [87] on the methods and criteria for assessment of angiogenesis suggests, but crucially does not prove that CD34 should be the antigen of choice. But after 2002, all the four antigens were still used for staining in angiogenesis assessment and different researchers had different voices. When we divided the studies by detection antibody, we found that the anti-CD105 group had the highest pooled HR (1.93) for OS. Our results indicated that CD105 may be the best antigen for prognostic use. So we suggest that standardization of angiogenesis assessment is warranted.

Moreover, researchers have been studying the prognostic role of MVD on NSCLC on different stages and histological subtypes. Tanaka et al. [67] focused on patients in stage IIIa. Giatromanolaki et al. [29], Zhu et al. [69] and Medetoglu et al. [13] focused on early stages (stage I-II). While, many other researchers [39, 55, 77] examined patients in all stages. When analysis was strictly restricted to studies mainly on different clinical stages, we found that there was no remarkable difference between the stage I-II>50% and <50% group. However, the pooled HR in stage IIIa>10% group is higher than that in stage IIIa<10% group. As we know, treatment for patients with stage IIIA NSCLC has been a controversial issue. Our study suggested that MVD had a relatively higher predictive value in patients with stage IIIA NSCLC.

Kaira et al. [74] and Minami et al. [62] examined patients with ADC. Eerola et al. [46] focused on LCC and other researchers [9, 12, 58] studied patients in different histology subtypes. Therefore, we suggest that study on MVD should be investigated on different stages and every histological subtype of NSCLC to clarify the prognostic role of MVD on NSCLS patients in different situations. Adenocarcinoma (ADC) has become the most frequent type of lung cancer encountered [74]. We divided the studies into ADC>50% and <50% groups. The pooled HR in the ADC>50% group is much higher than that in the ADC<50% group, indicating that MVD may provide more information about the survival in ADC patients.

In addition, now that MVD cannot be an independent prognostic marker in NSCLC, we suggest other markers that can be applied to all stages and histological subtypes of NSCLC should be found. Moreover, the combination of MVD with other markers could also potentially predict the survival in patients with NSCLC.

The publication bias was a major concern for all meta-analysis. Begg’s test and funnel plot indicated that significant publication bias was found (Table 2). But no publication bias was found in the anti-CD105 (P=0.102) and IIIa>10% (P=0.649) subgroup. We attempted to minimize publication bias by making our literature search as complete as possible. However, we could not take into account the few studies published not in English or Chinese. Another
source of publication bias may be that non-significant results might not be reported [88-90].

Significant heterogeneity was observed when analyzing the logHR estimates for OS of the prognostic value of MVD from the included studies ($I^2=82.4\%$, $P<0.001$). To exclude technique biases, methodological and clinico-pathological characteristics of each study were analyzed. We analyzed them in subgroups by nation, detection antibodies, clinical stage and histology type. The heterogeneity could not be eliminated by these attempts. However, when we grouped the analysis into IIIa $>10\%$ and $<10\%$ groups, the heterogeneity for OS could shrink slightly to $73.7\%$ and $77.2\%$. The shrink of heterogeneity could also be found in other subgroups. Thus, potential sources of heterogeneity may be from the study methodology, clinico-pathological characteristics of patients, as well as the cut-off value of the vessel count.

Besides, limitations still exist in this meta-analysis. Firstly, we could not include the studies that could not be calculated using the methods developed by Parmar, Williamson and Tierney, which may affect the result of the survival outcome. Secondly, the data extraction based on the survival curve may not be accurate with the original study, which leads to another source of bias. Moreover, except for the multivariate analysis of logHR and SE extracted directly from the studies, the logHR and SE calculated from the Kaplan-Meier survival curves and log-rank p value are univariate analysis. To ensure data integrity, we combined the univariate analysis and multivariate analysis, which could affect the analysis. In addition, almost all the studies did not evaluate the prognostic role of MVD in different clinical stages or histology types. We grouped the studies by the proportion of patients in different clinical stages or histology types, which may lower the suggestive power of the subgroup analysis.

In conclusion, this meta-analysis suggested that higher MVD was related to poorer DFS/RFS/PFS in NSCLC, but not associated with poorer OS. Standardization of angiogenesis assessment should be established. Future studies revealing the prognostic role of MVD should investigate more on each stage and every histological subtype of NSCLC separately. Furthermore, MVD combined with other markers may predict the survival outcome of NSCLC.

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Disclosure of conflict of interest

None.

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