The prognostic significance of high/positive expression of tissue VEGF in ovarian cancer

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ABSTRACT

Background & aim: At present, numerous reports have shown that high/positive expression of tissue vascular endothelial growth factor (VEGF) may be associated with the prognosis of patients with ovarian cancer. However, their results still remained controversy. Thus, this meta-analysis was designed to analyze and assess the prognostic value of tissue VEGF expression in patients with ovarian cancer.

Method: We searched PubMed, Embase, Cochrane Library and Web of Science to October, 2017. Hazard Ratio (HR) with its 95% confidence intervals (CIs) was used to evaluate the association between high/positive expression of tissue VEGF and the prognosis of ovarian cancer patients. All statistical analyses were performed using standard statistical procedures provided in RevMan 5.2.

Result: A total of 18 studies (including 1145 patients) were included for this meta-analysis. The positive/high expression of tissue VEGF had an obvious association with overall survival (OS) (HR 2.24, 95% CI 1.36–3.70; \(P=0.002\)), progression-free survival (PFS) (HR 1.60, 95% CI 1.11–2.31; \(P=0.01\)) and disease-free survival (DFS) (HR 3.49, 95% CI 1.27–9.56; \(P=0.02\)) of patients with ovarian cancer respectively.

Conclusion: The present meta-analysis indicated that positive/high expression of tissue VEGF may have a close association with survival of ovarian cancer.

INTRODUCTION

Cancer, especially ovarian cancer in women, is a major public health problem worldwide. Ovarian cancer is currently the fifth leading cause of cancer deaths in women, associated with an estimated 14,180 deaths in the United States in 2015 [1]. According to the report, the estimated new cases of ovarian cancer is 21,290 and locates in the third place in genital system cancer, which only rank next to the incidence of uterine cervix and corpus cancer. Despite surgical resection is the optimal option, patients with ovarian cancer usually experience poor prognosis, possibly due to a lack of diagnosis in early stage. The majority of patients are diagnosed with advanced disease, and at least nearly half of patients dead within 5 years after their diagnosis [1]. Thus, appropriate prognostic markers are needed to predict patients’ post-treatment prognosis and survival of patients at high risk of recurrence or metastasis.

At present, many advances have been made in the treatment approaches, imaging techniques and staging procedures. However, there is no effective method indicating the prognosis of patients before operation or treatment, for the Federation International of Gynecology and Obstetrics (FIGO) stage which is usually accepted to indicate the prognosis of gynecological tumors must be made after surgery according to a precise pathologic result. For decades, many studies have shown that various growth factors could stimulate angiogenesis in physiological and pathological conditions. Among these, vascular endothelial growth factor (VEGF) has been shown to play a major role in the proliferation and migration of endothelial
cells, providing nourishment to the growing tumors and allowing the tumor cells to establish continuity with the host vasculature [2]. Recent studies have demonstrated a significant correlation between VEGF expression and microvessel density (MVD) in malignant tumors arising from several organs [3]. In addition, several studies have demonstrated that serum VEGF has a significant association with the prognosis of ovarian cancer [4–7]. In recent years, numerous reports have shown that high/positive expression of tissue VEGF may be associated with the prognosis of patients with ovarian cancer [8–25]. However, their results still remain controversy. Thus, this meta-analysis is designed to analyze and assess the prognostic value of tissue VEGF expression in patients with ovarian cancer.

**RESULTS**

**Included studies and characteristics**

After five studies are excluded (two studies for lack of available data, two studies are about cervical cancer, one study for just review), eventually a total of 18 studies (including 1145 patients) are included in this meta-analysis, of which 12 studies are included to analyze the association between positive/high expression of tissue VEGF and OS of patients with ovarian cancer. Nine and five studies are included to analyze the association between positive/high expression of tissue VEGF and PFS and DFS of patients with ovarian cancer respectively. Of the included studies, six studies detect the expression of tissue VEGF with polymerase chain reaction (PCR), 11 studies detect the expression of tissue VEGF with immunohistochemistry (IHC). Seven studies were conducted in Japan, three in Italy, three in USA, one in Germany, one in Austria, one in China, one in UK and one in Egypt. The sample sizes ranged from 18 to 97 patients (Table 1).

The detail search process and summary of studies were showed in study flow diagram (Figure 1). The other study characteristics were showed in Table 1.

**The association between high/positive expression of tissue VEGF and OS of ovarian cancer patients**

A total of 12 studies [12, 14–17, 20–25] were included to analyze the relationship of positive/high tissue VEGF expression with overall survival (OS) in patients with ovarian cancer. Pooled result of meta-analysis showed significant correlation between positive/high expression of tissue VEGF and overall survival, with the pooled HR being 2.24 (95% CI 1.36–3.70; \( P=0.002 \)). As much significant heterogeneity between studies (\( I^2=71\% \)) and \( P=0.0001 \), a random-effect model was used (Figure 2).

The association between high/positive expression of tissue VEGF and PFS of ovarian cancer patients

Nine studies reported the association between positive/high expression of tissue VEGF and progression-free survival (PFS) [9, 11, 13, 14, 17, 20, 22–24], We also used random-effect model to estimate the pooled result. The combined result indicated that the expression of tissue VEGF had significant association with PFS of patients with ovarian cancer (HR 1.60, 95% CI 1.11–2.31; \( P=0.01 \)) (Figure 3).

The association between high/positive expression of tissue VEGF and DFS of ovarian cancer patients

Considering significant heterogeneity between studies (\( I^2=87\% \) and \( P=0.00001 \)), a random-effect model was used to estimate the association between tissue VEGF expression and DFS of patients with ovarian cancer. The pooled HR indicated that positive/high expression of tissue VEGF had an obvious association with DFS in patients with ovarian cancer (HR 3.49, 95% CI 1.27–9.56; \( P=0.02 \)) (Figure 4).

Subgroup and publication bias

In order to analyze the prognostic effects of positive/high tissue VEGF expression on OS, we conducted subgroup analysis grouping according to detection method of tissue VEGF expression (IHC or PCR) and quality score (15, 16–18, 19) of included studies. All pooled results were estimated with fixed-effect model because of good homogeneity among the studies, except subgroup of IHC. Statistically significant effect of tissue VEGF expression on OS was observed in all subgroups except quality score of 16–18. For the subgroups according to detection method, significant results were found in subgroups of both IHC (HR 3.70; 95% CI 1.44, 9.52; \( P=0.007 \)) and PCR (HR 1.73; 95% CI 1.05, 2.86; \( P=0.03 \)). For the subgroups according to quality score, significant results were also found in subgroups of both score=15 (HR 2.35; 95% CI 1.22, 4.53; \( P=0.01 \)) and score=19 (HR 2.17; 95% CI 1.18, 3.98; \( P=0.01 \)) (Table 2).

Funnel plots were conducted for assessing the publication bias of included literatures and we could roughly assess the publication bias by seeing whether their shapes were of any obvious asymmetry. The funnel plots showed no obvious evidence of publication bias with regard to the effects on OS (Figure 5).

**DISCUSSION**

Recently, it was given increasing interest that high/positive expression of tissue VEGF may be associated...
with the prognosis of patients with ovarian cancer. VEGF is a heparin binding dimeric glycoprotein, which is related with angiogenic, mitotic, and microvascular permeability-inducing activities, leading to the extravasation of plasmaproteins and to proangiogenic stromal changes [26]. It has been earlier implicated that various paracrine effects of VEGF are always mediated by binding with high affinity to the tyrosine kinase receptors flt-1 and KDR/flk-1 [27]. Previous animal experimental study revealed the relevance of VEGF in tumor tissue by blockage of

| Study                          | Country   | N  | Median age (year) | Cut-off value | FIGO stage | Study design | Detection method | Follow-up time (month) | Outcome | n  | Quality score |
|-------------------------------|-----------|----|-------------------|---------------|------------|--------------|-------------------|------------------------|----------|----|----------------|
| Brustmann H. 2004             | Austria   | 41 | NR                | 10%           | I-III      | NR           | IHC               | 22                     | DFS      | 21 | 19             |
| Gadducci A et al. 2003        | Italy     | 45 | NR                | ≥ 4+          | NR         | Retrospective | IHC               | NR                     | OS, PFS  | 14 | 15             |
| Garzetti GG et al. 1999       | Italy     | 32 | 58.5 (34-69)      | NR            | I-III      | Retrospective | IHC               | 37 (12-96)             | DFS      | 15 | 17             |
| Goodheart MJ et al. 2005      | USA       | 77 | 50 (21-85)        | ≥ 4+          | I          | Retrospective | IHC               | 73 (24-176)            | PFS      | 5  | 18             |
| Hartenbach EM et al. 1997     | USA       | 18 | 66.5 (29-83)      | 25 cycles     | III/IV     | NR           | RT-PCR            | 49.5 (15-78)           | OS       | 12 | 16             |
| Hata K et al. 2004             | Japan     | 85 | 54 (19-81)        | 0.14          | I-IV       | Retrospective | PCR               | NR                     | PFS      | 43 | 19             |
| Ino K et al. 2006              | Japan     | 67 | NR                | 50%           | I-IV       | Retrospective | IHC               | 60 (1-121)             | OS, PFS  | 49 | 19             |
| Li L et al. 2009               | China     | 78 | (34-72)           | 10%           | I-IV       | NR           | IHC               | NR                     | OS, DFS  | 46 | 15             |
| Nakayama K et al. 2002        | Japan     | 60 | 53 (22-81)        | Positive      | I-IV       | Retrospective | PCR               | 40.6 (6-128)           | OS, PFS  | 30 | 15             |
| Nishida N et al. 2004          | Japan     | 80 | 54.4 (23-79)      | 10%           | I-IV       | Retrospective | IHC               | 33.2 (2.75-89.75)      | DFS      | 58 | 17             |
| Raspollini MR et al. 2004      | Italy     | 83 | 60 (33-79)        | 30%           | III        | Retrospective | IHC               | 44.8 (3-204)           | OS, DFS  | 50 | 19             |
| Secord AA et al. 2007          | USA       | 67 | NR                | >1.2 fold to actin | NR | Retrospective | Immunoblot | NR                     | OS, PFS  | 33 | 17             |
| Shen GH et al. 2000            | Japan     | 64 | 54 (21-88)        | 25%           | I-IV       | Retrospective | IHC               | 31 (3-120)             | OS       | 31 | 19             |
| Shimogai R et al. 2008         | Japan     | 66 | 55.4 (21-78)      | 4%            | I-IV       | Prospective  | RT-PCR            | 154 (92.9-398.5)       | OS, PFS  | 26 | 19             |
| Sinn BV et al. 2009            | Germany   | 97 | 58 (34-80)        | mRNA: 30.52   | I-IV       | NR           | RT-PCR            | 38 (2-83)              | OS, PFS  | NR | 15             |
| Sundar SS et al. 2006          | UK        | 88 | 63 (25-88)        | NR            | I-IV       | Retrospective | IHC               | 28.5 (0.49-165.3)      | OS, PFS  | NR | 19             |
| Ueda M et al. 2005             | Japan     | 73 | NR                | 50%           | I-IV       | NR           | IHC               | NR                     | OS       | 36 | 15             |
| Kassim SK et al. 2004          | Egypt     | 24 | 43.5 (21-65)      | 120pg/mg      | I-IV       | Retrospective | RT-PCR            | 36                     | OS       | NR | 17             |

Abbreviations: N, total number of eligible patients; n, number of patients with high/positive tissue VEGF; OS, overall survival; DFS, disease-free survival; PFS, progression-free survival; NR, not report; RT-PCR, reverse-transcriptase polymerase chain reaction; IHC, immunohistochemistry.
Figure 1: Flow diagram following the PRISMA template of the search strategy for studies included in this meta-analysis.

Figure 2: Forest plot for the association between high/positive expression of tissue VEGF and OS of ovarian cancer patients.
VEGF-induced stimulation endothelial cells in vitro, which prevented VEGF dependent tumor vascularization, growth, and metastases of mouse ovarian tumor cells in syngeneic mice [28]. Previously, several studies investigated VEGF expression in serous carcinomas in relation to proliferation [27, 29, 30]. In addition, several studies have demonstrated that both serum and tissue VEGF have a significant relevance with the prognosis of ovarian cancer [4, 6, 12, 13, 18]. To our best knowledge, Brustmann H initially reported a reciprocal relation of VEGF in serous ovarian carcinomas in 2004 [8]. It was found that the expression of VEGF seemed to be relevant to outcome, tumor stage, and grade, which may be helpful in understanding growth and spread of serous ovarian

Table 2: The pooled results of subgroups for the association between high/positive expression of tissue VEGF and OS

| Subgroups     | Pooled results | P value | Analytical effect model |
|---------------|----------------|---------|-------------------------|
|               | HR             | 95% CI  |                         |
| Detection method |                |         |                         |
| IHC           | 3.70           | 1.44, 9.52 | 0.007 | Random-effect model |
| PCR           | 1.73           | 1.05, 2.86 | 0.03  | Fixed-effect model |
| Quality score |                |         |                         |
| 15            | 2.35           | 1.22, 4.53 | 0.01  | Fixed-effect model |
| 16-18         | 1.11           | 0.67, 1.84 | 0.68  | Fixed-effect model |
| 19            | 2.17           | 1.18, 3.98 | 0.01  | Fixed-effect model |

Abbreviations: HR, hazard ratio; CI, confidence intervals.

Figure 3: Forest plot for the association between high/positive expression of tissue VEGF and PFS of ovarian cancer patients.

Figure 4: Forest plot for the association between high/positive expression of tissue VEGF and DFS of ovarian cancer patients.
Garzetti GG et al. in 1999 suggested that VEGF expression contributed to neoplastic invasiveness probably related to 72-kilodalton metalloproteinase [10]. They found that in cystoadenocarcinomas, expression of VEGF is related to the MMP2 index, suggesting that VEGF has a role in neoplastic invasiveness. It has been suggested that VEGF family members (VEGF-A, VEGF-B, VEGF-C, VEGF-D), basic fibroblast growth factor, and thymidine phosphorylase are expressed in a variety of human tumors in different ways [31–35]. VEGF-A, also known as vascular permeability factor, is considered to play an important role in tumor angiogenesis [35]. It has been shown that VEGF-C expression is associated with hyperplasia of lymphatic vessels [34]. It is conceivable that VEGF-C might play a crucial role in lymphatic proliferation and also in spread of solid tumors. Ueda M et al. reported that VEGF-C may play a critical role for the prevalent progression of the tumor by inducing tumor invasion, lymph node metastasis, and vascularity and subsequently inhibiting apoptosis in ovarian carcinomas [25]. In the present study, we included all of VEGF family members expressing in tumor tissue.

Though many studies reported the significant association between high or positive expression of VEGF and prognosis of ovarian cancer, their results still remained controversy. The purpose of this study was to investigate and analyze the high/positive expression of VEGF as a prognostic biomarker in ovarian cancer. We perform this meta-analysis with 18 studies including 1145 patients. Our analysis results statistically support the conclusions that high/positive expression of VEGF had a significant association with the prognosis of ovarian cancer, with the pooled HRs were 2.24 (95% CI 1.36–3.70; \( P = 0.002 \)) in overall survival, 1.60 (95% CI 1.11–2.31; \( P = 0.01 \)) in progression-free survival and 3.49 (95% CI 1.27–9.56; \( P = 0.02 \)) in disease-free survival. In addition, we also conducted subgroup analysis to estimate the prognostic value of VEGF in different groups. Our analysis results showed that high/positive expression of VEGF had a significant association estimating with fixed-effect model in all subgroups except the subgroup of quality score 16-18.

Several limitations, however, exists in this meta-analysis. One of the main limitations is the inconsistency of the cut-off values of VEGF (definition of positive/high expression of VEGF), which may directly affect the results of studies and result in between-study heterogeneity. The positive criteria of VEGF was various in these studies, as shown in Table 1. In the study of Kassim SK et al. 2004, they determined the cut-off for VEGF that maximizes the sum of sensitivity (5/6, 83.3%) and specificity (15/18, 83.3%) in discriminating good from poor outcome of the disease, with cut-off value of VEGF equal to 120 pg/mg protein. At this cutoff, there was a significant correlation between VEGF positivity and the poor survival of the patients (\( X^2 = 9.0, P < 0.01 \)). Western blot analysis showed

Figure 5: Funnel plots for detecting publication bias of the included studies.

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that the subtype VEGF₁₆₅ is the only VEGF subtype that has been detected in all the ovarian tumors included in the study [15]. The second limitation is the different clinical stages of ovarian patients. As is well known, the survival of tumor patients is significant associated with their clinical stages. In this study, the majority of included studies involved ovarian cancer patients with FIGO stage of I-IV and failed to discuss the prognostic significance of VEGF with different stages. More important, the clinical stage of patients seems related with the level of VEGF in tissue. In the study of Kassim SK et al, all stages of ovarian cancer patients showed significantly increased mean rank of VEGF over that in the benign group (mean ranks are 6.6, 15.5, 20.33, 25, and 30 in benign, stages I, II, III, and IV, respectively, \( X^2 = 22.8, P < 0.001 \)).

Thirdly, all the studies we included were observational retrospective studies, expect studies that did not report their study design type. There is no prospective study in the analysis, which may affect the strength of evidence of analysis results. Finally, the prognosis of patients may also be influenced by other multiple factors, such as age of patients, therapy methods they received, histological type and VEGF detection methods, which should also be taken into consideration. Thus, further prospective researches need to be designed to clear the association between positive/high expression of tissue VEGF and prognosis of ovarian cancer patients with different clinical stages.

In conclusion, positive/high expression of tissue VEGF may have a close association with worse survival in patients with ovarian cancer considering of the evident statistical significance. Elevated tissue VEGF expression may be used as a prognostic biomarker for early identification of poor prognosis.

**MATERIALS AND METHODS**

**Including and excluding criteria of this meta-analysis**

Including criteria: (1) All of randomized, controlled trials (RCTs), observational prospective or retrospective studies were included; (2) included people with a pathological diagnosis of ovarian cancer; (3) tumor tissue VEGF was detected by Immumohistochemical staining or polymerase chain reaction; (4) definition of positive/high expression of tissue VEGF was reported; (5) the outcomes including overall survival, disease-free survival, progression-free survival were reported; (6) sufficient data were provided.

Excluding criteria: (1) Trials on animals; (2) abstracts, letters, editorials, expert opinions, reviews, case reports; (3) patients having other primary tumors; (4) studies without sufficient data; (5) the definition of positive/high expression of tissue VEGF (cut-off value) did not give or meet our including criteria.

**Search strategy**

We searched PubMed, EMBASE, Cochrane Library and Web of Science to October, 2017. Our searching terms and procedures were as follows: (1) “VEGF”; (2) “ovarian cancer” OR “ovarian carcinoma” OR “ovarian tumor”; (3) “prognosis” OR “survival” OR “outcome”. Search strategy was (1) AND (2) AND (3). Other related terms, including references of some literatures we read, were also searched in English. Two assessors independently screened the titles and abstracts of each study. Once relevant studies became certain, the full texts were obtained for further evaluation.

**Quality assessment**

Two reviewers assessed the quality of all the included studies using REPorting recommendations for tumor MARKer prognostic studies (REMARK) [36] independently, and the total scores of each study were displayed in the characteristics table (Table 1). The scores were judged according to relevant information about the study design, pre-planned hypotheses, patient and specimen characteristics, assay methods, and statistical analysis methods as well helpful presentations of data and important elements to include in discussions [36].

**Data extraction**

Data for the analysis were extracted independently by two reviewers, and disagreement was resolved by their discussion. In addition, the extracted contents including total number of eligible patients, published years, country, number of patients with high/positive tissue VEGF, Median age, follow-up time, definition of positive/high expression or cut-off value, FIGO stage, study design, detection method, outcome, and quality score were extracted using a standardized form.

Data collected were input into RevMan 5.2 software for analysis [37].

**Statistical analysis**

In this meta-analysis, the prognostic value of tissue VEGF expression in patients with ovarian cancer was measured by estimating the HR between high/positive expression of tissue VEGF groups and low/negative expression of tissue VEGF groups. The associated 95% CI were also measured. The heterogeneity between studies was evaluated with \( P \) value and \( I^2 \). \( I^2 \leq 50\% \) and \( P \geq 0.10 \) or \( P \leq 0.10 \) was deemed to existing significant heterogeneity [38, 39], and pooled HR was estimated using a random-effect model. On the contrary, if statistical study heterogeneity was not observed (\( I^2 \leq 50\% \) and \( P \geq 0.10 \)), a fixed effects model was used. For the pooled HR estimating OS, we performed subgroup analysis by detection method of tissue VEGF expression (IHC or PCR) and quality score (15, 16–18, 19)
of included studies. Additionally, publication bias was assessed by Begg’s and Egger’s test. If the shape of funnel plots revealed no obvious evidence of asymmetry, we considered that there was no obvious publication bias. All statistical analyses were performed using standard statistical procedures provided in RevMan 5.2 [37].

CONCLUSIONS

In conclusion, positive/high expression of tissue VEGF may have a close association with worse survival in patients with ovarian cancer considering of the evident statistical significance. Elevated tissue VEGF expression may be used as a prognostic biomarker for early identification of poor prognosis.

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CONFLICTS OF INTEREST

The author declared no conflicts of interest.

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