
Prognostic Value of Tissue Vascular Endothelial Growth Factor Expression in Bladder Cancer: a Meta-analysis

Yu-Jing Huang, Wei-Xiang Qi, Ai-Na He, Yuan-Jue Sun, Zan Shen, Yang Yao*

Abstract

Objective: The prognostic role of vascular endothelial growth factor (VEGF) in bladder cancer remains controversial. This meta-analysis aimed to explore any association between overexpression and survival outcomes. Methods: We systematically searched for studies investigating the relationships between VEGF expression and outcome of bladder cancer patients. Study quality was assessed using the Newcastle-Ottawa Scale. After careful review, survival data were extracted from eligible studies. A meta-analysis was performed to generate combined hazard ratios (HRs) for overall survival (OS), disease-free survival (DFS) and disease-specific survival (DSS). Results: A total of 1,285 patients from 11 studies were included in the analysis. Our results showed that tissue VEGF overexpression in patients with bladder cancer was associated with poor prognosis in terms of OS (HR, 1.843; 95% CI, 1.231-2.759; P = 0.003), DFS (HR, 1.498; 95% CI, 1.255-1.787; P = 0.000) and DSS (HR, 1.562; 95% CI, 0.996-1.00; P = 0.052), though the difference for DSS was not statistically significant. In addition, there was no evidence of publication bias as suggested by Begg’s and Egger’s tests except for DFS (Begg’s test, P = 0.221; Egger’s test, P = 0.018). Conclusion: The present meta-analysis indicated elevated VEGF expression to be associated with a poor prognosis in patients with bladder cancer.

Keywords: Vascular endothelial growth factor - prognosis - bladder cancer - meta-analysis

Introduction

Bladder cancer is the second most common malignancy of the urinary tract after prostate cancer, with approximately 390,000 new cases annually, and has the sixth highest cancer mortality (Jemal et al., 2011). Despite recent advances in screening and multimodality therapy, the outcome for bladder cancer remains generally poor, emphasizing the need for early detection and prognostic markers. Currently, the most widely studied prognostic factors are related to pathological characteristics of the neoplasm, including tumor size, grade, stage, and vascular invasion (Thieblemont et al., 1996; Kanda et al., 2006; Youssef et al., 2011; van Rhijn, 2012). However, a variety of other potential prognostic markers remain to be further characterized (Kanda et al., 2006).

Angiogenesis, defined as the formation of new blood vessels from existing vasculature, plays an important role in tumor growth and metastasis by providing oxygen, nutrients and growth factors to the cancer cells (Folkman, 1995). Vascular endothelial growth factor (VEGF), a homodimeric glycoprotein with a molecular weight of approximately 45 kDa, is considered to be one of the most important regulators in tumor angiogenesis (Ferrara, 2004). Furthermore, the invasiveness of some tumors have recently been linked to high levels of VEGF, leading several authors to conclude that an important relationship between VEGF and prognosis exists for bladder cancer (Crew et al., 1997; Inoue et al., 2000; Bernardini et al., 2001; Theodoropoulos et al., 2004; Yang et al., 2004; Zu et al., 2006; Pignot et al., 2009; Shariat et al., 2010; Li et al., 2011). However, conflicting results were showed in other studies regarding the ability of VEGF to predict prognosis in bladder cancer (Suzuki et al., 2005; Nadaoka et al., 2008; Szarvas et al., 2008; Szarvas et al., 2009; Ma et al., 2010; Zaravinos et al., 2012).

Therefore, in this study, we sought to conduct a meta-analysis to estimate the prognostic importance of elevated VEGF expression for survival among patients with bladder cancer.

Materials and Methods

Search strategy

We searched Medline, PubMed, Embase, and the Web of Science using the search terms: ('VEGF' or vascular endothelial growth factor) and (cancer or carcinoma) and ('bladder' and 'prognosis'). The last search was updated in November 2012. To expand our search, references of the retrieved articles were also screened for additional studies.

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period used to determine study inclusion. Only studies
There was no pre-specified sample size or follow-up
free survival (DFS) or disease-specific survival (DSS).

correlation of VEGF with overall survival (OS), disease-
(2) VEGF evaluation using tissue-based methods, and (3)
follows: (1) proven diagnosis of bladder cancer in humans,
exclusion criteria criteria for primary studies were as
identification and included. Study inclusion/exclusion criteria criteria for primary studies were as follows: (1) proven diagnosis of bladder cancer in humans, (2) VEGF evaluation using tissue-based methods, and (3) correlation of VEGF with overall survival (OS), disease-free survival (DFS) or disease-specific survival (DSS). There was no pre-specified sample size or follow-up period used to determine study inclusion. Only studies written in English were included. Studies not directly reporting hazard ratios (HRs) were allowed if data were available for statistical estimation. When more than one of the same patient populations was included in several publications, only the most recent or complete study was used to avoid duplication of information.

Quality assessment
Quality assessment was performed in each of the acceptable studies by two reviewers independently using the Newcastle–Ottawa Quality Assessment Scale for cohort studies (Table 1) (Wells et al., 2003). This scale is an eight-item instrument that allows for assessment of patient population and selection, study comparability, follow-up, and outcome of interest. A star system of the NOS (range, 0–9 stars) has been developed for the evaluation. The highest value for quality assessment was 9 stars. Any discrepancies were resolved by a consensus reviewer.

Data extraction
Two investigators extracted data from eligible studies independently, discussed discrepancies and reached consensus for all items. The following information was extracted from each article: (1) basic information from papers, such as first author’s name, year of publication; (2) information of study designation, such as study design; (3) demographic data such as inclusion criteria, patient age, sex, and treatment during follow-up; (4) tumor data such as VEGF expression in the primary site, stage, grade, vascular invasion, and metastases; (5) survival data such as OS, DFS and DSS; (6) variables such as number of patients analyzed, method of tissue VEGF measurement, cut-off values for VEGF levels, and geographical district of the patients. The primary data were the HRs and 95% confidence intervals (CIs) for survival outcomes, including OS, DFS and DSS.

Statistical analysis
The primary outcome for analysis was survival in patients with high VEGF values as compared to those with low VEGF values. HRs with 95% CIs were reported for individual studies with HR>1 and 95% CI for the aggregated HR not crossing 1 designates a prognostic role of high VEGF. When HRs were not reported in an article, they were calculated to use established methods reported by Parmar et al. (1998).

Forrest plots were undertaken to evaluate the heterogeneity of combined HRs. Statistical assessment was performed using a χ²-based test of homogeneity and evaluation of the inconsistency index (I²) statistic. Heterogeneity was defined as p<0.10 or I²>50% (Higgins et al., 2003). When heterogeneity was judged between primary studies, a fixed effect model was used for pooled analyses. If not, a random effect model was used (DerSimonian et al., 1986). Egger’s test was performed to test for publication bias (Egger et al., 1997).

Statistically significant test was determined by a P-value of less than 0.05 for a summary HR and publication bias. All analyses were carried out using STATA.
Table 2. Main Characteristics and Results of the Included Studies

| Article & publication year | Study design | Country | Treatment | Age(y) | Number of patients (M/F) | Tumor grade | Study quality points | Subtype of patients | VEGF detection method | Survival analysis | Hazard ratio | Cut-off level | Number with high VEGF | Conclusion |
|---------------------------|--------------|---------|-----------|--------|-------------------------|-------------|---------------------|---------------------|---------------------|-------------------|-------------|--------------|------------------|-------------|------------|
| Zaravinos et al. 2012     | C            | Greece  | S         | 77(68/9) | 72.12                   | NR          | 6                   | 9                   | VEGF-A              | mRNA, OS, DFS     | Estimated    | Median       | 40               | Negative    |
| Li et al. 2011            | R            | China   | S         | 93(82/11) | 67                     | NR          | 7                   | 9                   | VEGF-C              | Antibody, OS, DFS | Report       | mRNA value of 3 | 55            | Positive    |
| Pignot et al. 2009        | R            | France  | S         | 84(67/17) | 68                     | 0/84        | 6                   | 9                   | VEGF-A              | Antibody, OS, DFS | Reported     | Median       | 50%             | Positive    |
| Herrmann et al. 2007      | R            | Germany | S         | 262(NR)  | 62                      | NR          | 5                   | 9                   | VEGF-D              | Antibody, OS, DFS | Estimated    | 25%           | 98            | Indeterminate |
| Yang et al. 2004          | R            | China   | Multi-treatment | 161(NR) | 58                     | 63/98       | 5                   | 9                   | VEGF-C              | Antibody, OS      | Estimated    | 10%           | 88            | Positive    |
| Theodoropoulos et al. 2004| R            | Greece  | Multi-treatment | 93(71/22) | 68                     | 75/18       | 7                   | 9                   | VEGF-C              | Antibody, OS, DFS | Estimated    | Median       | 46            | Positive    |
| Zu et al. 2006            | R            | China   | Multi-treatment | 45(NR)  | 58                     | 29/16       | 5                   | 9                   | VEGF-C              | Antibody, DFS     | Estimated    | Median       | 50%           | Positive    |
| Nadaoka et al. 2008       | R            | Japan   | NR        | 72(NR)   | 66.46                  | 122/97      | 4                   | 9                   | VEGF-D              | Antibody, DSS, DFS | Reported     | 10%           | 44            | Negative    |
| Szarvas et al. 2008       | R            | Hungary | S         | 107(NR)  | 71.6                   | 57/56       | 5                   | 9                   | VEGF-A              | mRNA, DSS         | Reported     | Median       | 54            | Negative    |
| Suzuki et al. 2005        | R            | Japan   | Multi-treatment | 87(75/12)| 66.66                  | 33/54       | 5                   | 9                   | VEGF-C              | Antibody, DSS, DFS| Reported     | 10%           | 36           | Positive    |
| Sharriott et al. 2010     | R            | Canada  | NR        | 204(NR)  | 60                     | NR          | 6                   | NR                  | VEGF-A              | Antibody, OS, DFS | Estimated    | Median       | 175           | Positive    |
| Herrmann et al. 2004      | R            | Germany | S         | 262(NR)  | 62                      | NR          | 5                   | 9                   | VEGF-D              | Antibody, OS, DFS | Estimated    | 25%           | 98            | Indeterminate |

Summary table of studies included in meta-analysis. Study design is described as case-controlled (C) or retrospective (R). Treatment describes whether the patients received curative surgical resection (S). Tumour grade was most often described using the WHO classification, but occasionally other systems were utilized. Study quality is listed using the results of the Newcastle–Ottawa questionnaire (Table 1); NR, not reported.

Results

Study identification and eligibility

An electronic search yielded 118 articles, of which 71 were excluded on the basis of their abstracts. We then screened the remaining 47 articles in full text. Upon further review, 17 articles was eliminated on the basis of without survival data, 11 articles were excluded because there is no special result of VEGF, and 6 articles were eliminated due to inadequate data for calculation. We also excluded a previous study with data overlap and a study investigating the association of serum VEGF level with survival. The selection process and reasons for exclusion have been summarized in Figure 1. From the 11 studies that were included (Bernardini et al., 2001; Theodoropoulos et al., 2004; Yang et al., 2004; Suzuki et al., 2005; Zu et al., 2006; Herrmann et al., 2007; Nadaoka et al., 2008; Szarvas et al., 2008; Herrmann et al., 2007; Li et al., 2011; Zaravinos et al., 2012), a total of 1285 patients were analyzed. The characteristics of the selected studies are presented in Table 2.

Quality assessment

Quality assessment using the Newcastle–Ottawa Scale was performed on all 11 studies included for meta-analysis. Of note, there was no study attempting to control for important prognostic factors that may have confounded the association of high VEGF with survival. The NOS scores of 1-3, 4-6 and 7-9 were defined as low, intermediate and high-quality studies, respectively. Our NOS results showed that the median overall score was 5

Figure 1. Flow Chart of the Meta-analysis

Figure 2. Random-effects Model of Hazard Ratio (95% confidence interval) of OS Associated with High VEGF Levels Versus Low Levels

Figure 3. Fixed-effects Model of Hazard Ratio (95% confidence interval) of DFS Associated with High VEGF Levels Versus Low Levels

(range 4 to 7), which indicated that the quality of included trials was acceptable.

Overall survival

The pooled hazard ratio for OS showed that high VEGF level was significantly associated with OS (HR, 1.843; 95% CI, 1.231-2.759; P = 0.003; Figure 2). There was significant heterogeneity (P = 0.008, I² = 68.2%; χ² = 15.71), and the pooled HR for OS was performed by using the random-effects model.

Disease-free survival

The pooled hazard ratio for PFS showed that high VEGF level was significantly associated with DFS (HR, 1.498; 95% CI, 1.255-1.787; P = 0.000; Figure 3). No significant heterogeneity was found (P = 0.459, I² = 0.0%, χ² = 3.63), and the pooled HR for DFS was performed by using the fixed-effects model.
Yu-Jing Huang et al


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However, several other studies demonstrated that the level
of VEGF expression did not predict outcomes for patients
with bladder cancer (Suzuki et al., 2005; Nadaoka et al.,
2009; Pignot et al., 2009; Shariat et al., 2010; Li et al., 2011).

As a result, the role of VEGF expression in bladder cancer is
undetermined. Yang et al. (2004) both announce the
association between VEGF over expression and poor
outcome of survival in several cancers, such as lung
cancer (Zhan et al., 2009), hepatocellular carcinoma
(Schoenleber et al., 2009), gastric cancer (Chen et al.,
2011), ovarian cancer (Yu et al., 2012), and osteosarcoma
(Qu et al., 2012). However, the prognostic value of VEGF
in bladder cancer was undetermined. Yang et al. (2004)
and Theodoropoulos et al. (2004) both announce the
association between VEGF over expression and poor
outcome of patients with bladder cancer. Then several
following studies supported their results (Zu et al., 2006;
Pignot et al., 2009; Shariat et al., 2010; Li et al., 2011).

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Meta-analysis is useful to integrate results from
independent studies for a specified outcome. Pooled
results from the combining relevant studies are statistical
powerful, and make it possible to detecting effects that
may be missed by individual studies. This meta-analysis
presents combined results from 11 studies of 1285
patients and reveals that tissue VEGF over expression
are associated with prognosis in terms of OS (HR, 1.843;
95% CI, 1.231-2.759; P = 0.003), DFS (HR, 1.498; 95%
CI, 1.255-1.787; P = 0.000) and DSS (HR, 1.562; 95%
CI, 0.996-1.00; P = 0.052), though the difference in DSS
was not statistically significant. Additionally, significant
heterogeneity was found in OS (P = 0.008, I² = 68.2%,
$\chi^2 = 15.71$) and DSS (P = 0.032, I² = 59.2, $\chi^2 = 12.24$), but not
for DFS (P = 0.459, I² = 0.0%, $\chi^2 = 3.63$). Thus, a random
effect model was used in combining OS and DSS.

There were several potential sources of heterogeneity
among the studies. First, Studies might differ in the
characteristics of included patients (age, histological
type, tumor grade, stage, tumor size, treatment received,
and the duration of follow-up). Furthermore, in some
studies, patients were excluded because of insufficient
tissue source, insufficient clinical data or insufficient
survival data. All of these could potentially lead to
selection bias or recruitment bias. Second, language also
induces a bias, as positive results tend to be published
in English in international journals. Although our search
was not restricted, all the studies included were written
in English. Third, the differences of methodology and
cut-off values among included studies also were sources
of heterogeneity and caused selection biases potentially.
The variability of IHC techniques, which we could not
avoided, may prevent tissue VEGF measurements from
standardization. Although four studies chose the median
VEGF level as the cut-off value, values varied among
studies obviously. Additionally, the heterogeneity in
tissue samples cannot be ignored. Fourth, observers in
some studies were not blinded to the outcome data, which
contributed to information bias.

Discussion

Inducing angiogenesis is one of the hallmarks of
cancer (Hanahan et al., 2011), and VEGF, as one of the
most important regulators in tumor angiogenesis (Ferrara,
2004), has been thought to be valuable of predicting poor
outcome of survival in several cancers, such as lung
cancer (Zhan et al., 2009), hepatocellular carcinoma
(Schoenleber et al., 2009), gastric cancer (Chen et al.,
2011), ovarian cancer (Yu et al., 2012), and osteosarcoma
(Qu et al., 2012). However, the prognostic value of VEGF
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Meta-analysis is useful to integrate results from
independent studies for a specified outcome. Pooled
results from the combining relevant studies are statistical
powerful, and make it possible to detecting effects that
may be missed by individual studies. This meta-analysis

![Figure 4. Random-effects Model of Hazard Ratio (95% confidence interval) of DSS Associated with High VEGF Levels Versus Low Levels](image-url)
case-controlled study, providing a lower level of evidence. And furthermore, publication bias was detected even we expanded our search to the best of our ability. Finally, the small number of included patients negated the possibility of exploring possible sub-group analyses and explored heterogeneity among study populations.

In the conclusion, this meta-analysis for the first time demonstrated that high levels of VEGF are associated with a poor prognosis in patients with bladder cancer. However, one should be cautious when interrupting these results due to the limitations of our studies. Further high-quality studies are still needed to confirm these results.

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