ORIGINAL RESEARCH

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The prognostic value of vascular endothelial growth factor in patients with renal cell carcinoma: a systematic review of the literature and meta-analysis

Abstract

Objective: Vascular endothelial growth factor (VEGF) serum level or tumor expression may be prognostic in renal cell carcinoma (RCC). The purpose of this meta-analysis was to examine the prognostic value of serum VEGF level and tumor expression in patients with RCC.

Methods: PubMed and EMBASE databases were searched until September 26, 2016. Prospective and retrospective studies of RCC patients that had VEGF levels measured were included. Outcome measures were overall survival (OS), disease-specific survival (DSS), and progression-free survival (PFS).

Results: A total of 14 studies were included in the meta-analysis. In patients with RCC, elevated serum VEGF level was not associated with OS (pooled hazard ratio [HR] = 1.16; 95% confidence interval [CI]: 0.52 to 2.60; $p = 0.716$), but was associated with poor DSS (pooled HR = 4.22; 95% CI: 2.02 to 8.79; $p < 0.001$) and PFS (pooled HR = 1.50; 95% CI: 1.22 to 1.85; $p < 0.001$). Removal of one study, however, resulted in elevated serum level being associated with poorer OS. Tumor VEGF expression was not associated with OS (pooled HR = 1.48; 95% CI: 0.74 to 2.95; $p = 0.263$), but was associated with worse DSS (pooled HR = 1.83; 95% CI: 1.24 to 2.71; $p = 0.003$).

Conclusion: In patients with RCC, elevated serum VEGF level is associated with worse OS, DSS, and PFS, while tumor expression is only associated with worse DSS. The number of studies, however, was limited and the results should be interpreted with caution.

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Renal cell carcinoma (RCC) accounts for approximately 2% of malignant diseases worldwide [1]. It is the urological malignancy with the worst survival, with an estimated 5-year survival of between 60% and 70% [2]. While the 5-year survival of patients with stage I disease is over 90%, the 5-year survival of patients with stage IV disease is only around 20% [3]. Almost one-third of patients will have distant metastasis at diagnosis, and one-third who have traditional surgery (nephrectomy) will develop a recurrence [4]. There are three types of renal cell carcinoma; clear cell renal cell carcinoma (ccRCC), papillary renal cell carcinoma (pRCC), and chromophobe renal cell carcinoma (cRCC), with ccRCC accounting for 80% to 90% of cases [5]. Although a number of risk factors for RCC have been identified, including smoking, obesity, and hypertension, the etiology of the disease has yet to be clearly determined [6].

Vascular endothelial growth factor (VEGF) is an endothelial cell mitogen with a number of isoforms that plays an important role in angiogenesis (primarily VEGF-A), and is associated with pathological angiogenesis in a number of malignancies [7,8]. VEGF is expressed in RCC tumor cells and not in normal renal tissue, and appears to promote the development of RCC [9,10]. Study has shown that VEGF expression is correlated with tumor grade, size, and disease stage, lymph node involvement, vascular invasion, and poor survival in patients with RCC [9,10]. Recent study has shown that a high serum VEGF level is associated with an increased risk of recurrence and cancer-specific death in patients with RCC [11]. On the other hand, earlier studies did not find an association with VEGF level and RCC prognosis [12,13]. There have also been some inconsistencies with respect to the prognostic value of VEGF tumor expression in RCC [14].

Thus, the purpose of this study was to perform a systematic review of the literature and meta-analysis examining the prognostic value of serum VEGF level and VEGF tumor expression in patients with RCC.

Materials and Methods

Literature search strategy and study selection

This systematic review and meta-analysis was conducted in accordance with PRISMA guidelines [15]. PubMed and EMBASE databases were searched until November 2015 using combinations of the following keywords: vascular endothelial growth factor, VEGF, renal cell carcinoma, RCC, prognosis, and prognostic factors. References lists of relevant studies were also hand-searched for studies of potential interest. Searches

were conducted by two reviewers, and a third was consulted for resolution of any disagreements.

Inclusion criteria for the meta-analysis were: 1) prospective or retrospective cohort studies; 2) patients with renal cell carcinoma; 3) VEGF serum levels or VEGF tumor expression measured at baseline or at some time point in the trial; and 4) quantitative outcomes reported. Letters, comments, editorials, case report, proceedings, and personal communications were excluded, as were studies that did not report a quantitative outcome.

After identifying articles based on the inclusion and exclusion criteria, the articles were screened by the information in the title and abstract. If the title and the abstract did not indicate the article provided data regarding VEGF level or expression, or prognosis or any of the outcomes of interest, the article was excluded. The full texts of the remaining articles were then reviewed to determine study eligibility.

Data extraction

Information and data extracted from eligible studies included: name of the first author, year of publication, number of patients, mean patient age, percentage males/females, study design, initial tumor stage, metastasis status and location, follow-up time, comorbidities, surgery or other treatment (radical nephrectomy, partial nephrectomy, or targeted ablative therapy, e.g., percutaneous ablative therapies), VEGF level/expression and time obtained, medical therapy administered, if any, and dosage and frequency, pathological type (ccRCC, pRCC, cRCC). Data were extracted by two reviewers, and a third was consulted for resolution of any disagreements.

Quality assessment

The Quality in Prognostic Studies (QUIPS) tool [16] was used to assess the quality of the included studies. The tool is valid for evaluating prognostic studies with regard to six areas of potential study bias: study participation, study attrition, measurement of prognostic factors, measurement of and controlling for confounding variables, measurement of outcomes, and analysis approaches. Quality assessment was also performed by the two independent reviewers, and a third reviewer was consulted for any uncertainties.

Outcome measures and data analysis

The primary outcome was overall survival (OS), and secondary outcomes were progression-free survival (PFS) and disease-specific survival (DSS). Outcomes were determined for

serum VEGF level, and tumor VEGF expression. Hazard ratios (HRs) with 95% confidence intervals (CIs) for survival outcomes were extracted for each individual study. If the available data were presented as a Kaplan-Meier curve, survival rates at specified times were extracted in order to reconstruct the HR estimate and its variance, with the assumption that the rate of patients censored was constant during the study follow-up [17]. A HR > 1 implied worse survival for the group with a high VEGF level.

A χ^2 -based test of homogeneity was performed, and the inconsistency index (I²) and Q statistics were determined. The I² statistic indicates the percentage of the observed between-study variability due to heterogeneity rather than chance, and a value > 50% indicates moderate to large heterogeneity. If heterogeneity existed between studies (Q statistic $p < 0.1$ or I² statistic > 50%), a random-effects model

(DerSimonian-Laird method) of analysis was used; otherwise, a fixed-effect model was used (Mantel-Haenszel method). Combined effects were calculated, and a two-sided p value < 0.05 was considered to indicate statistical significance.

Sensitivity analysis was carried out using the leave one-out approach. Publication bias was only assessed if there were more than 10 studies, because more than 10 are necessary to detect funnel plot asymmetry [18]. All analyses were performed using Comprehensive Meta-Analysis statistical software, version 2.0 (Biostat, Englewood, NJ, USA).

Results

A flow diagram of study selection is shown in Figure 1. A total of 655 studies were identified in the database searches, and of these 613 were excluded based on inclusion/exclusion criteria after review of the titles and abstracts. The full texts of the

FIGURE 1. Flow diagram of study inclusion.

FIGURE 2. Meta-analysis for (A) overall survival, (B) disease-specific survival, and (C) progression-free survival.

FIGURE 3. Quality assessment for (A) risk of bias graph for the included studies, (B) risk of bias for individual study.

remaining 42 articles were reviewed, and of these 23 were excluded, the reasons for which are shown in Figure 1. Thus, 19 studies were included in the quantitative synthesis [10,19-36]. Five of these studies [20, 22, 25, 33, 36] did not provide data (HR or Kaplan-Meier curve) to calculate the HR for OS, PFS, or DSS, and therefore could not be included in the meta-analysis.

The characteristics of the 19 studies are summarized in Table 1. The 19 studies included a total of 1,875 patients, the average age of the patients ranged from 54 to 65 years, and the majority was male. The most common cancer type was ccRCC, and in most studies the majority of patients had stage I disease at diagnosis. Approximately half of the studies reported data regarding metastasis, and between 10% and approximately 50% of patients were found to have metastasis at diagnosis.

Serum VEGF level

Only three studies [11,30,32] provided HR data, or data from which the HR could be calculated, and were included in the analysis of OS. Relatively high heterogeneity was observed among the three studies; therefore, a random-effects model of analysis was used (Q statistic = 11.249, I^2 = 82.22%, p = 0.004). The overall analysis revealed that serum VEGF level was not associated with OS in patients with RCC (pooled HR = 1.16; 95% CI: 0.52 to 2.60; p = 0.716; Figure 2A).

Only two studies [11,23] provided HR data or data from which the HR could be calculated, and were included in the analysis of DFS. No heterogeneity was found among the studies, therefore, a fixed-effect model was used (Q statistic = 0.014, I^2 = 0%, p = 0.905). The overall analysis revealed that a

high serum VEGF level was associated with poor DSS (pooled HR = 4.22; 95% CI: 2.02 to 8.79; $p < 0.001$; Figure 2B).

Two studies provided enough information for estimating HRs for PFS, and were included in the meta-analysis [21,30]. There was no heterogeneity among the studies, therefore, a random-effects model was used (Q statistic = 0.380, $I^2 = 0\%$, $p = 0.538$). The overall analysis revealed high serum VEGF level was associated with poor PFS (pooled HR = 1.50; 95% CI: 1.22 to 1.85; $p < 0.001$; Figure 2C).

VEGF tumor expression

Five studies [19,24,26,28,34] either provided HR data or data from which the HR could be calculated, and were included in the analysis of OS. Relatively high heterogeneity was observed among the five studies, therefore, a random-effects model of analysis was used (Q statistic = 15.993, $I^2 = 74.99\%$, $p = 0.003$). The overall analysis revealed that tumor VEGF expression was not associated with OS in patients with RCC (pooled HR = 1.48; 95% CI: 0.74 to 2.95; $p = 0.263$; Figure 2A).

Analysis of the four studies that provided tumor VEGF expression data [27,29,31,35] indicated that high VEGF expression was associated with worse DSS (pooled HR = 1.83; 95% CI: 1.24 to 2.71; $p = 0.003$, Figure 2B).

An analysis of PFS and tumor VEGF expression could not be performed because not enough studies provided these data.

Sensitivity analysis

Sensitivity analysis of VEGF tumor expression and DSS was performed using the leave-one-out approach (Table 2). The direction and magnitude of combined estimates of DSS did not vary markedly with the removal of the studies, indicating that the meta-analysis had good reliability and the data was not overly influenced by each study. However, the results indicated the pooled estimates of OS might be affected by the study by Minardi *et al.* [19]. After removal of the Minardi *et al.* study, the overall analysis indicated that high level of VEGF tumor expression was a prognostic factor for poor OS (HR = 3.63; 95% CI: 1.92 to 6.85; $p < 0.001$).

Quality assessment

Results of the quality assessment of the studies are summarized in Figure 3. The majority of the studies were of low risk with respect to study attrition, prognostic factor measurement, outcome measurement and analysis. However, seventeen of nineteen studies had high risk in study participation, and eight studies did not account confounding factors for analysis.

Discussion

VEGF is a key mediator of angiogenesis, and is crucial for the development and metastasis of tumors, including RCC. The main findings of this meta-analysis are that while a high serum level of VEGF is not associated with OS, it is associated with poorer PFS and DSS. Similarly, a high level of VEGF tumor expression was not associated with worse OS, it was associated with poorer DSS. However, when the study by Minardi *et al.* [19] was removed from the analysis of OS, the result indicated that high tumor expression was associated with poorer OS. However, it is important to note that the number of studies provided data with respect to the different outcomes was limited.

While the prognosis of patients with stage I RCC is good, with a 5-year survival of around 90%, one-third of patients will have distant metastasis at diagnosis and the outcomes for these patients is much poorer [2-4]. Many markers have been examined for use as prognostic factors including VEGF and VEGF receptors (VEGFR), VEGF vascular density, CD31, CD34, carbonic anhydrase IX, and the von Hippel-Lindau pathway [12,13,20,22,25-27,37-39]. Of these, VEGF and its receptors are among the most studied.

VEGF is a regulator of angiogenesis, and has been found to be associated with abnormal angiogenesis and tumor growth in a number of different malignancies [7,8]. Briefly, there are five VEGF ligands in humans which bind to VEGFRs and induce a biological response [7,8]. Abnormal VEGF signaling results in abnormal tissue growth in various malignancies, and anti-angiogenic therapies have been found to inhibit the generation of new blood vessels and the growth and metastasis of tumor cells.

Studies have provided somewhat inconsistent results with respect to the prognostic value of VEGF and RCC. For example, a recent study that was included in the current meta-analysis examined 124 patients with RCC that was surgically treated, and found that tumor stage and a high VEGF level were predictors of recurrence and cancer-specific death, and that serum VEGF level may be useful for determining RCC subtype prior to surgery [11]. On the other hand, earlier studies have shown that VEGF levels were not associated with cause-specific survival [12], only provided prognostic information for patients with a good performance status (2 or 3) [13], or only identified patients with aggressive disease [40]. Another more recent study indicated that high CD31 expression was associated with significantly better survival, and that VEGFR3 had no association with survival [37].

Five studies that were included in the qualitative synthesis did not provide sufficient data for calculating a HR, and thus were not included in the meta-analysis. Three earlier studies showed that a normal tumor necrosis factor (TNF)- α level, but not VEGF, was prognostic for patients with RCC [36], VEGF expression in RCC tumor tissue was not prognostic [33], and in pRCC MIB-1, VEGF, CD31, and c-met oncogenic protein were not prognostic, while Fuhrman grade was an independent predictor of survival [25]. A more recent study examined CD147 and VEGF expression in paraffin-embedded specimens from 53 patients with advanced RCC and 12 healthy controls, and reported that patients who were CD147-/VEGF- had the best outcomes, while patients who were CD147+/VEGF+ had the worst [22]. Multivariate analysis indicated that CD147+/VEGF+ and CD147-/VEGF- co-expression were independent prognostic indicators for RCC [22]. A study performed in 2010 by Kim *et al.* [20] examined RCC tissue for carbonic anhydrase IX, cyclooxygenase-2 (COX-2), and VEGF expression in tissue from 62 patients with RCC, and found that high carbonic anhydrase and COX-2 staining were associated with a better response to cytokine therapy, whereas VEGF expression did not provide prognostic information.

VEGF gene polymorphisms may alter VEGF concentrations and thus affect angiogenesis, and may be the reason for discordant results of the association between VEGF and RCC prognosis. In 2013 Zhang *et al.* [41] performed a systematic review of the literature and meta-analysis to examine the associations VEGF gene polymorphisms and RCC. They found five studies published between 2000 and 2012 that examined eight polymorphisms, and reported that only the -2578C/A gene polymorphism may be associated with an increased risk of RCC.

Therapies targeting the VEGF pathway have been successful in the treatment of a number of malignancies, and have shown value in the treatment of RCC [42]. Two recent meta-analyses have focused on VEGF pathway-targeted therapy in advanced RCC. In 2011, Liu *et al.* [42] pooled the data from seven RCTs that included 3,451 patients and reported that compared with interferon therapy or placebo, VEGF pathway-targeted therapies (sorafenib, sunitinib, and bevacizumab) improved PFS and OS in patients with metastatic RCC. A more current meta-analysis suggested that second-line use of mammalian target of rapamycin inhibitors (mTORi) was associated with prolonged survival as compared with second-line use of VEGF tyrosine kinase inhibitors (TKI) in patients with metastatic RCC [3]. More recently, RNA interference-mediated VEGF gene silencing has shown

promise with respect to tumor suppression in patients with metastatic RCC [43,44].

There are limitations to the current analysis that need to be considered. Only three of the included studies in the meta-analysis were prospective; the others were retrospective and associated with potential biases. Furthermore, the numbers of studies examining the different outcome measures with respect to serum VEGF level and Tumor expression were limited. The treatment of metastatic RCC is not standardized, and differences in treatments may have affected survival rates. Although tumor stages were reported in the majority of the studies, only approximately half of the studies reported metastasis data, and outcomes by disease stage were not examined. We did not examine outcomes based on subtype of RCC, and study has shown that VEGF may be associated with different outcomes with different RCC subtypes. Lastly, there are a number of VEGF isoforms (e.g., VEGF-A, VEGF-C), and data were not sufficient to perform the analysis based on any specific isoforms.

Conclusions

In summary, the results of this meta-analysis suggest that while a high serum level of VEGF is not associated with OS, it is associated with poorer PFS and DSS. Similarly, a high level of VEGF tumor expression was not associated with worse OS, but was associated with poorer DSS. However, when one study was removed from the analysis of OS, the result indicated that high tumor expression was associated with poorer OS. However, it is important to note that the number of studies provided data with respect to the different outcomes was limited. More study is necessary to determine the exact role of VEGF with respect to the prognosis of patients with RCC.

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