ECRONICON OPEN ACCESS

EC PHARMACOLOGY AND TOXICOLOGY
Research Article

The Effect of Age and Gender on Free VEGF-A Levels in Patients with Colorectal Cancer Treated with Bevacizumab-Based Treatment

Apostolos Papachristos[1]*, Haralabos Kalofonos[2] and Gregory Sivolapenko[1]

[1]Laboratory of Pharmacokinetics, Department of Pharmacy, School of Health Sciences, University of Patras, Rion-Patras, Greece

[2]Division of Medical Oncology, University Hospital of Patras, Rio-Patras, Greece

***Corresponding Author**: Apostolos Papachristos, Laboratory of Pharmacokinetics, Department of Pharmacy, School of Health Sciences, University of Patras, Rion-Patras, Greece.

Received: May 15, 2019; **Published:** May 28, 2019

Abstract

VEGF-A is the most significant circulating factor that regulates angiogenesis. Several studies have demonstrated the predictive and prognostic role of VEGF-A levels in various cancer types treated with anti-angiogenic agents. However, there are still no available predictors of clinical response in these patients and the effect of factors not related to treatment or disease on VEGF-A is not clarified in cancer patients treated with anti-angiogenic agents. Studies in healthy volunteers have shown that age and gender do not affect VEGF-A levels. The aim of the present study was to investigate the effect of age and gender on the levels of VEGF-A in cancer patients receiving bevacizumab-based treatment for mCRC in combination with chemotherapy. 27 patients treated with bevacizumab-based chemotherapy were included in this study. Pre- and post-dose concentrations of free VEGF-A were measured in serum during several cycles of treatment. A commercially available ELISA kit was used for measurement of the levels. Statistical analysis was performed with Spearman rank order. 67% of the patients were males and 33% females; the mean age was 67 years (31 - 84). In total 76 samples were analyzed. Mean free VEGF-A levels pre-dose were 302.5 ng/L (63.3 - 822.6) and mean free VEGF-A levels post-dose were 92.2 ng/L (4.3 - 217.3). Age and gender were not correlated significantly with neither pre-dose ($p = 0.62$ and $p = 0.48$, respectively) nor post-dose ($p = 0.99$ and $p = 0.58$, respectively) levels. Therefore, our results indicate that the further evaluation of VEGF-A as biomarkers inpatients with colorectal cancer receiving bevacizumab is warranted as it is related with demographic factors.

Keywords: *Biomarkers; Cancer; Age; Gender; VEGF-A*

Abbreviations

VEGF: Vascular Endothelial Growth Factor; FGF: Fibroblast Growth Factor; VEGFR: Vascular Endothelial Growth Factor Receptor; VEGF-A: Vascular Endothelial Growth Factor A; VEGF-B: Vascular Endothelial Growth Factor B; VEGF-C: Vascular Endothelial Growth Factor C; VEGF-D: Vascular Endothelial Growth Factor D; VEGF-E: Vascular Endothelial Growth Factor E; PLGF/PIGF: Placental growth factor; PDGF: Platelet-Derived Growth Factor; CRC: Colorectal Cancer; CEA: Carcinoembryonic Antigen; mCRC: Metastatic Colorectal Cancer; PFS: Progression Free Survival; OS: Overall Survival; ELISA: Enzyme-linked Immunosorbent Assay; OD: Optical Density; VCAM: Vascular Cell Adhesion Molecules; ICAM: Intracellular Adhesion Molecule; bFGF: Basic Fibroblast Growth Factor; NSCLC: Non-Small-Cell Lung Carcinoma; BC: Breast Cancer

Introduction

In 1971 Judah Folkman described first the process of angiogenesis and its contribution to tumor growth [1]. Since then angiogenesis pathway has been extensively studied. The dominant factor controlling angiogenesis is a glycoprotein called vascular endothelial growth

factor (VEGF) [2-4]. The VEGF family includes 5 sub-types: VEGF-A, VEGF-B, VEGF-C, VEGF-D and placental growth factor (PIGF). VEGFs bind with high affinity to receptors (VEGFR-1, VEGFR-2, and VEGFR-3) and promote angiogenic signals to vascular endothelium [3]. These receptors are primarily expressed on endothelial cells and consist of seven immunoglobulin-like domains in their extracellular region, one transmembrane domain and one intracellular tyrosine kinase domain [5]. VEGF-A is considered the major activator of angiogenesis. It acts selectively on vascular endothelial cells, stimulating both normal and abnormal angiogenesis. More specifically, it directly induces endothelial mitogenesis, promotes endothelial survival, increases vascular permeability [6] and the expression of tissue plasminogen activator, urokinase plasminogen activator, collagenases and matrix metalloproteinases [7,8]. VEGF-A binds to VEGFR-1 and VEGFR-2, though the VEGF-A/VEGFR-2 pathway is considered the major activator of angiogenesis [9,10], whereas binding to VEGFR-1 results in the sequestration of VEGF-A [11]. As far as the other VEGF family members are concerned, PIGF is thought to promote angiogenesis dominantly in pathologic conditions [11,12].

Several studies have also investigated and detected the prognostic and predictive significance of VEGF-A and VEGFR in colorectal [13,14], lung [15,16], gastric [17] and pancreatic cancers [18]. Thus, angiogenesis and especially VEGF-A have become attractive pharmacologic targets.

The fist-approved agent against VEGF-A is bevacizumab; a recombinant humanized (93% human, 7% murine) IgG1 monoclonal antibody. It has been approved as monotherapy or in combination with chemotherapy and in various dosage regimens for the treatment of metastatic colorectal cancer (mCRC), metastatic breast cancer (mBC), unresectable advanced, metastatic or recurrent non-small cell lung cancer (NSCLC), advanced or metastatic renal cell cancer, advanced ovarian and cervical cancers and as a single for advanced, recurrent glioblastoma [19]. The mechanism of action of bevacizumab includes binding to circulating VEGF-A and the blocking of VEGF-A binding to its receptors (VEGFR-1 and VEGFR-2) on the surface of endothelial cells, which results in the inhibition of tumor angiogenesis, growth, and metastases [20].

It is known that there is no significant effect of age or gender on levels of VEGF-A in healthy volunteers [21]. However, the effect of age and gender is not clear in cancer patients and especially in patients receiving anti-VEGF treatment. Given the prognostic and predictive significance of VEGF-A levels in cancer the aim of the present study was to investigate the effect of age and gender on the levels of VEGF-A in cancer patients receiving bevacizumab-based treatment for mCRC in combination with fluoropyrimidines-containing chemotherapy.

Materials and Methods

Patients

27 patients with mCRC, who were treated at University Hospital of Patras, Greece in 2016 were enrolled in the study. Patients were 18 years of age or older, had histopathologically confirmed mCRC and received bevacizumab-based treatment in combination with chemotherapy. Bevacizumab (Avastin®, Roche Registration Ltd.) was administered as an intravenous infusion at a dose of 5 mg/kg once every 2 weeks or at a dose of 7.5 mg/kg once every 3 weeks. The study was conducted in accordance with the Declaration of Helsinki and the International Conference on Harmonisation (ICH) Good Clinical Practice. Approval was obtained by the Hospital's Ethics Committee. Prior to study enrolment, all patients provided signed informed consent.

Samples

Pre- and post-dose (after the end of infusion) concentrations of free VEGF-A (unbound to bevacizumab) were measured in serum during several cycles of treatment after cycle 1. Two blood samples (one pre-dose and one post-dose) were drawn. Blood samples were collected in serum separator tubes and were allowed to clot for 30 minutes. After centrifugation at 1000×*g* for 20 minutes, the serum was removed and stored in aliquots at ≤-20°C until analysis.

Measurement of free VEGF-A

A commercially available ELISA kit (Quantikine® human VEGF, R&D Systems® Europe) was used for measurement of the levels. The detection limit of the assay was 9 ng/L, and the precision was 6.7% (CV%) [22]. Optical density was measured at 450 nm with a correction at 550 nm using an ELISA plate reader (ELx800™, BioTek Instruments). All standards' and samples' readings were performed in duplicate.

Finally, a standard curve was generated with VEGF-A concentrations ranging from 31.2 to 2000 ng/L. The best fit line was determined by regression analysis using OriginPro 8.0 software (OriginLab® Corporation).

Statistics

Statistical analysis was performed with Spearman rank order on SPSS version 24 (IBM® SPSS® Statistics).

Results and Discussion

Of the 27 patients enrolled 67% were males and 33% females, the mean age of the study population was 67 years (31 - 84). In total 76 samples (38 pre-dose and 38-post-dose) were collected and analyzed. For the study population mean free VEGF-A levels pre-dose were 302.5 ng/L (63.3 - 822.6) and mean free VEGF-A levels post-dose were 92.2 ng/L (4.3 - 217.3) (Table 1).

	Frequency (%)
Sex	
Female	33%
Male	67%
Age (years)	
< 55	31%
55 - 65	19%
≥ 65	50%

Table 1: *Baseline patient characteristics.*

Gender did not appear to influence significant free VEGF-A levels before or after the administration of bevacizumab. More specifically, mean free VEGF-A levels pre-dose in male patients were 295.9 ng/L (63.3 - 524.8) and in female patients 267.8 ng/L (95.8 - 822.6). Although levels in female patients pre-dose were approximately 10% lower this difference was not statistically significant (p = 0.6272). Post-dose free VEGF-A levels were very similar for male and female patients 106.1 ng/L (4.3 - 207.4) and 106 ng/L (7.7 - 217.3) respectively (p = 0.9994) (Table 2).

	Pre-dose VEGF-A levels (ng/L)	**Post-dose VEGF-A levels (ng/L)**
Males	295.9 (63.3 - 524.8)	106.1 (4.3 - 207.4)
Females	267.8 (95.8 - 822.6)	106 (7.7 - 217.3)

Table 2: *Free VEGF-A levels per gender.*

In order to study the effect of age on free VEGF-A levels in our population, patient were divided in two groups based on their age (< 65 vs. ≥ 65 years). Pre-dose levels were higher in younger patient (<65 years) 330.7 ng/L (120.2 - 767.2) compared to older patients (≥ 65 years) 277.2 ng/L (63.3 - 822.6). However, this difference was not considered as statistically significant (p = 0.4753). No significant difference was noted in post-dose levels of free VEGF-A (p = 0.5818). Levels were quite similar between the two groups 103.5 ng/L (4.3 - 217.3) and 114.4 ng/L (18.5 - 176.1) in younger and older patients respectively (Table 3).

	Pre-dose VEGF-A levels (ng/L)	**Post-dose VEGF-A levels (ng/L)**
Age < 65 years	330.7 (102.2 - 767.2)	103.5 (4.3 - 217.3)
Age ≥ 65 years	277.2 (63.3 - 822.6)	114.4 (18.5 - 176.1)

Table 3: *Free VEGF-A levels per age group.*

Our results indicate that age and gender are not significant factors that may affect the levels of free VEGF-A in patients with cancer, who are receiving active anti-angiogenic treatment. Larsson A., *et al.* have reported similar results in 80 healthy blood donors [21]. Our

findings are even more important in the era of personalized medicine, as it is indicated that any prognostic or predictive significance of VEGF-A levels is not biased by other factors such as age and gender.

Indeed, research for biomarkers of angiogenesis and anti-angiogenesis and their successful use in the optimization of angiogenesis inhibition therapy is an ongoing challenge. Several studies have revealed the significant role of VEGF levels in various cancer types.

In 2008 Burstein HJ., *et al.* sought to determine the efficacy and safety of bevacizumab and vinorelbine in 56 women with mBC and to explore the role of baseline plasma VEGF as a predictor of treatment outcome. Median VEGF levels were 32.6 pg/ml with broad distribution (range < 12.5 - > 4445 pg/ml). They tend to be higher in estrogen receptor-positive disease, age > 50 years, bone metastases, or more than one disease site while they were lower in patients with liver metastases or with prior chemotherapy. Levels > 32.6 pg/ml were significantly associated with a shorter time to progression and no instance of extended tumor control compared to levels ≤ 32.6 pg/ml ($p = 0.003$) [23]. During the E4599 phase II/III study, 878 NSCLC patients were randomized to receive carboplatin and paclitaxel with or without bevacizumab. Based on the fact that VEGF, basic fibroblast growth factor (bFGF), soluble intracellular adhesion molecule (ICAM) and E-selectin were increased in several tumors, researchers performed a prospective biomarker assessment and their correlation to treatment outcomes. Plasma levels were measured before cycle 1 and after cycle 2. Patients with high baseline VEGF levels (> 35.7 pg/ml) had increased probability of a response if bevacizumab was added to their treatment regimen (33% vs. 7.7%, $p = 0.01$). In patients with baseline VEGF ≤ 35.7 pg/ml, the response was similar, 28.6% and 29% for bevacizumab/chemotherapy and chemotherapy only arm respectively. Low VEGF levels were also significantly associated with progression-free survival (PFS) (6 vs. 4.5 months, $p = 0.04$). While VEGF was predictive of response, low ICAM levels (≤ 260.5 ng/ml) were prognostic for survival ($p = 0.00005$) and predictive of response to treatment (32% vs. 14%, $p = 0.02$) and 1-year survival (65% vs. 25%) [24]. Sathornsumetee S., *et al.* used tumor specimens collected at diagnosis from 45 patients (27 glioblastoma multiforme and 18 anaplastic astrocytoma), who were treated with bevacizumab and irinotecan. They retrospectively evaluated tumor vascularity and expression of components of VEGF pathway and hypoxic response as predictive markers for radiographic response and survival in Tumor expression of VEGF, VEGFR-2, CD31 hypoxia-inducible cardonic anhydrase 9 (CA9) and hypoxia-inducible factor-2a (HIF-2a) were semi quantitatively assessed by immunohistochemistry (IHC). High VEGF expression (mean positive area > 5000 pixels/x400 field) was associated with increased likelihood of radiographic response ($p = 0.024$). Moreover, high CA9 expression (mean positive area>10000 pixels/high-powered field) was associated with poor 1-year survival after initiation of bevacizumab treatment (37 vs. 74 weeks, $p = 0.02$). Similarly, a trend was observed between HIF-2a expression and poor 1-year survival ($p = 0.07$). No significant differences in radiographic response or survival were reported for VEGFR-2 and CD31 ($p > 0.1$) [25]. During another phase II trial assessing efficacy and safety of bevacizumab and cisplatin, etoposide combination in 63 patients with extensive stage small-cell lung cancer, correlative studies were performed in order to explore any potential relationship between treatment outcome and plasma levels of VEGF, soluble vascular cell adhesion molecules (VCAM), ICAM, E-selectin and bFGF. Blood samples collected before cycle 1 and after completion of cycle 2. High baseline VCAM levels predicted survival as they were associated with higher risk of progression ($p = 0.05$) and death ($p = 0.01$) compared to low levels. High bFGF and ICAM levels also showed a trend toward higher risk of death ($p = 0.06$ both). The response was not associated significantly with baseline VEGF ($p = 0.43$) or any other biomarker [26]. Another group assessed several biomarkers at baseline, 3 and 12 days after a dose of bevacizumab monotherapy, 32 days after initiation of neoadjuvant bevacizumab, fluorouracil and radiotherapy and 1 week before surgery (8 to 9 weeks after completion of preoperative treatment). Notably, patients who experienced greater than 2-fold increases in plasma PIGF after bevacizumab monotherapy had a minimal disease at surgery ($p < 0.05$). Furthermore, bevacizumab alone or in combination with chemoradiotherapy increased plasma PIGF, VEGF and soluble VEGFR ($p < 0.0001$). Thus, the researchers concluded that mainly PIGF and VEGF might serve as generic pharmacodynamics biomarkers for anti-VEGF therapy as the chemoradiotherapy alone did not seem to change VEGF or PlGF [27]. In the AVF2119g trial were enrolled 462 patients with mBC treated with capecitabine or capecitabine plus bevacizumab, 223 of them received bevacizumab and their primary tissue samples were available for analysis. The panel of the examined biomarkers included VEGF-A, VEGF-B, THBS-2, Flt4, VEGF-C, PDGF-C, neuropilin-1, delta-like ligand D114, Bv8, p53, and thymidine phosphorylase. Of them, only VEGF-A expression showed a prognostic significance ($p = 0.01$) for improved PFS when bevacizumab was added [28]. In a large retrospective study 1254 (713 received bevacizumab) plasma samples from mCRC patients who were enrolled in phase III HORIZON II (FOLFIRINOX plus cediranib or placebo) and HORIZON III

(mFOLFOX6 plus cediranib or bevacizumab) were evaluated for baseline levels of VEGF, soluble VEGFR-2 and carcinoembryonic antigen (CEA). In both studies, high baseline VEGF (> 98 pg/ml) and CEA levels were associated with worse outcomes in terms of both PFS and overall survival (OS) independent of treatment. In addition, sVEGFR-2 was not prognostic for PFS or OS, but in HORIZON III that included patients with bevacizumab, the subgroup of high sVEGFR-2 had slightly increased median PFS and OS compared with the low (10.4 vs. 9.6 months PFS, 22.6 vs. 21.4 months OS) [29]. ABIGAIL was a phase II, open-label, randomized, international and multicenter study, which investigated the correlation between biomarkers (VEGF-A, VEGFR-1, VEGFR-2, bFGF, E-selectin, ICAM-1, PIGF) in plasma samples at baseline and through treatment with response to bevacizumab. 303 chemo naïve NSCLC patients were randomized to receive bevacizumab 7.5 mg/kg (154 patients) or 15 mg/kg (149) in combination with chemotherapy (carboplatin and gemcitabine or carboplatin and paclitaxel). Primary specimen tumor samples were also analyzed for VEGF-A, VEGFR-1, VEGFR-2, bFGF, E-selectin, PIGF, neuropilin (NRP), ICAM-1 and CD31. Baseline and dynamic changes in plasma levels of VEGFR-1, VEGFR-2, bFGF, E-selectin, ICAM-1 and PIGF did not correlate with response to bevacizumab. However, low baseline plasma VEGF-A levels were correlated with longer PFS (7.4 vs. 6.1 months, p = 0.002) and longer median OS (19.8 vs. 11.1 months, p = 0.004). As a result, authors reported that VEGF-A might be promising biomarker [30].

Conclusion

To sum up, there are enough data from various types of tumors to support the rationale for use of VEGF levels as prognostic or predictive factors for the clinical outcomes of anti-angiogenic treatment. Our results support the correlation of VEGF-A only with treatment-or disease-related factors and further research in the field is warranted.

Conflict of Interest

There are no competing interests to declare.

Bibliography

1. Folkman J. "Tumor angiogenesis: therapeutic implications". *New England Journal of Medicine* 285.21 (1971): 1182-1186.
2. Ferrara N., *et al*. "The biology of VEGF and its receptors". *Nature Medicine* 9.6 (2003): 669-676.
3. Hicklin DJ and Ellis LM. "Role of the vascular endothelial growth factor pathway in tumor growth and angiogenesis". *Journal of Clinical Oncology* 23.5 (2005): 1011-1027.
4. Ellis LM and Hicklin DJ. "VEGF-targeted therapy: mechanisms of anti-tumor activity". *Nature Reviews Cancer* 8.8 (2008): 579-591.
5. Veikkola T and Alitalo K. "VEGFs, receptors and angiogenesis". *Seminars in Cancer Biology* 9.3 (1999): 211-220.
6. Tolnay E., *et al*. "Hepatocyte growth factor/scatter factor and its receptor c-Met are overexpressed and associated with an increased microvessel density in malignant pleural mesothelioma". *Journal of Cancer Research and Clinical Oncology* 124.6 (1998): 291-296.
7. Zucker S., *et al*. "Vascular endothelial growth factor induces tissue factor and matrix metalloproteinase production in endothelial cells: conversion of prothrombin to thrombin results in progelatinase A activation and cell proliferation". *International Journal of Cancer* 75.5 (1998): 780-786.
8. Prager GW., *et al*. "Vascular endothelial growth factor (VEGF) induces rapid prourokinase (pro-uPA) activation on the surface of endothelial cells". *Blood* 103.3 (2004): 955-962.
9. Nagy JA., *et al*. "Vascular permeability factor/vascular endothelial growth factor induces lymphangiogenesis as well as angiogenesis". *Journal of Experimental Medicine* 196.11 (2002): 1497-1506.
10. Kerbel RS. "Tumor angiogenesis". *New England Journal of Medicine* 358.19 (2008): 2039-2049.
11. Fischer C., *et al*. "FLT1 and its ligands VEGFB and PIGF: drug targets for anti-angiogenic therapy?" *Nature Reviews Cancer* 8.12 (2008): 942-956.
12. Carmeliet P., *et al*. "Synergism between vascular endothelial growth factor and placental growth factor contributes to angiogenesis and plasma extravasation in pathological conditions". *Nature Medicine* 7.5 (2001): 575-583.

13. Werther K., *et al*. "Soluble vascular endothelial growth factor levels in patients with primary colorectal carcinoma. The Danish RANX05 Colorectal Cancer Study Group". *European Journal of Surgical Oncology* 26.7 (2000): 657-662.

14. Broll R., *et al*. "Vascular endothelial growth factor. (VEGF)--a valuable serum tumour marker in patients with colorectal cancer?" *European Journal of Surgical Oncology* 27 (2001): 37-42.

15. Bremnes RM., *et al*. "Angiogenesis in non-small cell lung cancer: the prognostic impact of neoangiogenesis and the cytokines VEGF and bFGF in tumours and blood". *Lung Cancer* 51.2 (2006): 143-158.

16. Salgia R. "Prognostic significance of angiogenesis and angiogenic growth factors in nonsmall cell lung cancer". *Cancer* 117.17 (2011): 3889-3899.

17. Karayiannakis AJ., *et al*. "Circulating VEGF levels in the serum of gastric cancer patients: correlation with pathological variables, patient survival, and tumor surgery". *Annals of Surgery* 236.1 (2002): 37-42.

18. Karayiannakis AJ., *et al*. "Serum vascular endothelial growth factor levels in pancreatic cancer patients correlate with advanced and metastatic disease and poor prognosis". *Cancer Letters* 194.1 (2003): 119-124.

19. Avastin® Summary of Product Characteristics, EMA (2015).

20. Gerber HP and Ferrara N. "Pharmacology and pharmacodynamics of bevacizumab as monotherapy or in combination with cytotoxic therapy in preclinical studies". *Cancer Research* 65.3 (2005): 671-680.

21. Larsson A., *et al*. "Serum and plasma levels of FGF-2 and VEGF in healthy blood donors". *Angiogenesis* 5.1-2 (2002): 107-110.

22. Quantikine® human VEGF immunoassay. "R&D Systems Inc., Minneapolis, USA (2014).

23. Burstein HJ., *et al*. "VEGF as a Marker for Outcome Among Advanced Breast Cancer Patients Receiving anti-VEGF Therapy with Bevacizumab and Vinorelbine Chemotherapy". *Clinical Cancer Research* 14.23 (2008): 7871-7877.

24. Dowlati A., *et al*. "Cell Adhesion Molecules, Vascular Endothelial Growth Factor, and Basic Fibroblast Growth Factor in Patients with Non Small Cell Lung Cancer Treated with Chemotherapy with or without Bevacizumab an Eastern Cooperative Oncology Group Study". *Clinical Cancer Research* 14.5 (2008): 1407-1412.

25. Sathornsumetee S., *et al*. "Tumor Angiogenic and Hypoxic Profiles Predict Radiographic Response and Survival in Malignant Astrocytoma Patients Treated With Bevacizumab and Irinotecan". *Journal of Clinical Oncology* 26.2 (2008): 271-278.

26. Horn L., *et al*. "Phase II Study of Cisplatin Plus Etoposide and Bevacizumab for Previously Untreated, Extensive-Stage Small-Cell Lung Cancer: Eastern Cooperative Oncology Group Study E3501". *Journal of Clinical Oncology* 27.35 (2009): 6006-6011.

27. Willett CG., *et al*. "Efficacy, safety, and biomarkers of neoadjuvant bevacizumab, radiation therapy, and fluorouracil in rectal cancer: a multidisciplinary phase II study". *Journal of Clinical Oncology* 27.18 (2009): 3020-3026.

28. Jubb AM., *et al*. "Impact of exploratory biomarkers on the treatment effect of bevacizumab in metastatic breast cancer". *Clinical Cancer Research* 17.2 (2011): 372-381.

29. Jürgensmeier JM., *et al*. "Prognostic and predictive value of VEGF, sVEGFR-2 and CEA in mCRC studies comparing cediranib, bevacizumab and chemotherapy". *British Journal of Cancer* 108.6 (2013): 1316-1323.

30. Mok T., *et al*. "A correlative biomarker analysis of the combination of bevacizumab and carboplatin-based chemotherapy for advanced nonsquamous non-small-cell lung cancer: results of the phase II randomized ABIGAIL study (BO21015)". *Journal of Thoracic Oncology* 9.6 (2014): 848-855.

Volume 7 Issue 6 June 2019