

UPDATE

# The Importance of Vascular Endothelial Growth Factor as a Marker of Angiogenesis and Lymphangiogenesis in Non-small Cell Lung Cancer

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Lung cancer is the main cause of cancer death both in men and women. In spite of progress seen in the early diagnosis of lung cancer, and implementation of new treatment principles for these patients, 5 year survival of non-small cell lung cancer patients undergoing surgery is low. Introduction of anti-angiogenic therapy administered concomitantly with conventional chemotherapy agents represented practically the first success seen in the treatment of lung cancer in the last 20 years. The aim of this paper is to review the literature informations about the importance of VEGF (vascular endothelial growth factor) as a marker of angiogenesis in patients with non-small cell lung cancer. Therefore, we practiced a literature review about these topics : the importance of VEGF in tumor angiogenesis and lymphangiogenesis in patients with non-small cell lung cancer and his importance as a prognostic factor at these patients, the prognostic impact of serum levels of VEGF and of the cellular expression of VEGF at these patients and also we reviewed the value of the antiangiogenic therapy.

**Keywords:** angiogenesis, lymphangiogenesis, non-small cell lung cancer

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## Introduction

Lung cancer is the main cause of cancer death both in men and women [1]. According to 2006 European statistical data, 1.7 million people died of cancer in Europe, and approximately 20% of these deaths were caused by lung cancer [2]. With the increase of the incidence of smoking in developing countries, the incidence of lung cancer increased exponentially [3]. In spite of progress seen in oncologic therapies during the last decades, average survival of patients undergoing surgery for non-small cell lung cancer showed an average increase of 1-2 years, but the 5 year survival did not change. One of the most important achievements implemented during the last 20 years in the treatment of non-small cell lung cancer was the introduction of anti-angiogenic therapy and tyrosine-kinase inhibitors.

In spite of progress seen in the early diagnosis of lung cancer, and implementation of new treatment principles for these patients, 5 year survival of non-small cell lung cancer patients undergoing surgery is low (7% to 14%) [4]. Introduction of anti-angiogenic therapy administered concomitantly with conventional chemotherapy agents represented practically the first success seen in the treatment of lung cancer in the last 20 years [5].

## Material and methods

In order to perform this study we search for articles in sciencedirect.com. We used articles published in English. Searches was conducted on the following terms : non-

small cell lung cancer, angiogenesis, vascular endothelial growth factor. We used 68 studies published between 2000 and 2015.

## The importance of VEGF in tumor angiogenesis and lymphangiogenesis

Tumor angiogenesis is the development of new vessels from pre-existing vessels in the tumor [6]. The importance of angiogenesis in cancer pathogenesis has been demonstrated by several authors, who have shown that development of tumors over the size of 2 mm depends on the presence of tumor neovascularization [7]. At the same time, the presence of multiple cytokines has been demonstrated that are produced by tumor cells and stimulate tumor angiogenesis. Besides stimulating development of new vessels in the tumor, these cytokines increase capillary permeability, enhance tumor invasion and development of tumor metastases [8]. Recent studies demonstrate that the mechanisms that trigger tumor angiogenesis are similar to those that trigger tumor lymphangiogenesis [9,10].

The major cytokine that stimulates tumor angiogenesis is VEGF (vascular endothelial growth factor). Currently 5 different types of VEGF (A,B,C,D and E) are known. Of these 5 different types of VEGF, the most important role in angiogenesis is played by VEGF-A [8]. Certain authors demonstrated that VEGF is also involved in modulation of lymphangiogenesis (through VEGF-C and VEGF-D) [11]. In approximately 30-40% of the non-small cell lung cancer patients enhanced VEGF-A expression is seen in tumor cells, accompanied by increased angiogenic activity [8,12,13]. Regarding the role of VEGF-C in modulat-

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ing lymphangiogenesis, there is no unanimously accepted agreement, even if most of the authors admit its role in tumor lymphangiogenesis modulation [14].

Structurally VEGF is a glycoprotein. VEGF is secreted by endothelial cell, macrophages, stromal cells and tumor cells as well. It stimulates endothelial cell proliferation and migration, increases vascular permeability, and stimulates stromal degradation through activation of certain proteolytic enzymes [15,16]. The role of VEGF in stimulation of the development of new vessels is important both during physiological, and pathological processes [17].

VEGF binds to three types of receptors: VEGFR-1, -2 and -3 (vascular endothelial growth factor receptor) [18,19]. While VEGF-A and VEGF-B bind VEGFR-1 and VEGFR-2, VEGF-C and VEGF-D bind to VEGFR-2 and VEGFR-3 [20]. This group of receptors belongs to the tyrosine-kinase receptor group [21]. VEGFR-2 is the main pro-angiogenic receptor, and plays an important role in triggering tumor neo-angiogenesis [22]. Regarding non-small cell lung cancer, the existence of VEGFR-2 has been demonstrated in the membranes of tumor cell, in their cytoplasm and in newly developed vessels inside the tumor; these facts demonstrate the role of VEGF, and tumor angiogenesis in lung cancer pathogenesis [23]. Nevertheless, even if an intense VEGFR-2 expression has been demonstrated in lung cancer patients, most of the studies have shown that this marker does not correlate with the prognosis of these patients [24]. On the contrary, other studies have demonstrated that VEGFR-2 and VEGFR-3 blockade may have beneficial effects on the invasive and metastatic properties of the tumors [25,26]. In vivo and in vitro studies demonstrate that VEGF-C binding to the VEGFR-3 stimulates motility of tumor cells, which enhances metastatic properties [27].

The first study to demonstrate the importance of VEGF as marker of tumor angiogenesis in non-small cell lung cancer patients was published by Mattern in 1996 [28]. Since then, several reports have been published demonstrating the relationship between cellular expression and blood levels of VEGF, as prognosis factors in non-small cell lung cancer patients. The results were contradictory as in certain studies cellular expression of VEGF correlated with a poor prognosis for these patients [29,30], while other authors concluded that there is no correlation with the long term prognosis of these patients [29,31]. Additionally, there are significant differences regarding the importance of VEGF as prognosis factor, and the histological type of lung cancer. Some authors suggested that VEGF is a negative prognosis factor only for patients with lung adenocarcinomas, and not those with squamous cell carcinomas [32]. After reviewing the literature we found that there are more studies that show the relationship between cellular expression or blood levels of VEGF that correlate with a poor prognosis for non-small cell lung cancer patients, compared to the number of studies demonstrating that VEGF expression does not correlate with the prognosis of these patients [29-31].

### **The importance of VEGF as prognosis factor in non-small cell lung cancer patients**

Currently there are two published meta-analysis studies that deal with the importance of VEGF as prognosis marker in non-small cell lung cancer patients; the first was authored by Delmotte (2002, 1549 patients) and the other by Zhan (2009, 4499 patients). Both meta-analysis studies conclude that cellular expression of VEGF, and its blood levels are poor prognosis factors for patients diagnosed with lung cancer [33,34].

There are differences in the importance as prognosis factors of different VEGF subtypes, as well as in the importance of disease stage in non-small cell lung cancer patients. Therefore, certain authors suggest that VEGF-A is a negative prognosis factor for lung adenocarcinoma patients, while VEGF-C is a negative prognosis factor for squamous cell carcinoma patients [35]. Currently there is no consensus regarding the correlation between the histological type of lung cancer and cellular expression of a certain VEGF subtype [36].

Unlike VEGF-A that only stimulates tumor angiogenesis, VEGF-C stimulates lymphangiogenesis as well, and thus the lymphatic metastatic properties of the tumor [37]. Certain authors suggest that VEGF-B and D are factors of poor prognosis for patients with stage I and II of the disease, but for stage III and IV patients, cellular expression of VEGF-D represents a factor of good prognosis [38].

Currently it is unanimously accepted that invasive or metastatic properties of a tumor are associated with a poor prognosis. Numerous authors observed the fact that binding of VEGF to its receptor is a crucial moment of the tumor metastasis process [39,40]. Other authors suggest that the importance of VEGF-A as a negative prognosis factor for these patients is also due to the fact that cellular expression of VEGF-A correlates with lymph node metastases [41].

### **Measurement of serum levels and identification of cellular expression of VEGF in non-small cell lung cancer patients**

Numerous debates surround the methods of approach to the impact of VEGF and angiogenesis in non-small cell lung cancer patients. These controversies are especially about the best method of EGFR expression detection [46]. Certain authors recommend immunohistochemical methods for detection of EGFR expression in tumor cells. The critics of these methods argue with the fact that immunohistochemical methods are semi-quantitative and most often, exact evaluation of angiogenesis is difficult in these cases [46,47]. This is one of the reasons that were incriminated by some studies for not being able to obtain direct correlations between cellular expression of VEGF and prognosis of patients included in the study [47]. On the other hand, other authors think that immunohistochemical methods are very useful for the study of tumor microvascular density, and of tumor neo-angiogenesis [48].

Other authors recommend measurement of serum VEGF levels, which allows a more exact quantification of VEGF [8]. This method is also not accepted unanimously, because coagulation occurring after blood sampling may alter serum VEGF levels. During coagulation, large quantities of VEGF are released from the leucocytes and platelets. Thus, certain authors suggest that serum VEGF levels returned by the assays are made up rather by VEGF contents of blood cell components, and in a less proportion by cellular expression [49,50]. Nevertheless, certain studies observed that serum VEGF levels represent a negative prognosis factor for non-small cell lung cancer patients, even if these observations are currently not accepted unanimously [51]. Likewise, certain authors suggest that preoperatively decreased serum VEGF levels correlate with a favorable response to chemotherapy with platinum derivatives, and it also represents a good prognosis for these patients [51,52]. There are studies that look into a possible relationship between serum VEGF levels and tumor stage in non-small cell lung cancer patients. There controversies in the literature about the existence of such relationship; some studies suggest that serum VEGF levels correlate with tumor stage, but others negate that [53,54]. It is unanimously accepted that serum VEGF levels correlate with tumor size; this claim is supported by the fact that practically VEGF is secreted by the tumor cells [55].

Certain authors observed that in lung cancer patients with paraneoplastic pleural effusion (i.e. advanced disease stage), VEGF levels of the pleural fluid were increased, and correlated with the disease stage and long term survival of these patients [56]. Increased VEGF titers of the pleural fluid are caused by increased cellular expression of VEGF in mesothelial cells of the pleural membranes [57]. Consequently, certain authors recommend measurement of pleural fluid VEGF titers in case of such patients.

Currently, the molecular mechanisms of metastatic spreading of tumor cells in non-small cell lung cancer patients are not fully understood [58]. One of the possible mechanisms contributing to metastasis formation is the existence of intensive angiogenic and lymphangiogenic processes in the tumor [59,60]. Tumor cell migration into lymph nodes is stimulated by the activation of VEGF-C and its receptor, VEGFR-3 [61,62]. A crucial role in lymphangiogenesis stimulation is played by VEGF-D; together with VEGF-C, this molecule causes dilatation of peritumoral lymphatics, which may lead to development of tumor metastases [63,64].

### Antiangiogenic therapy

The first studies about the significance of angiogenesis in patients with neoplastic disease were published by Folkman in 1971. He suggested that tumor growth can be stopped by inhibition of the development of new vessels [7]. The interest for angiogenesis studies in patients with neoplastic disease increased dramatically with the introduction of anti-angiogenic therapies. Thus, certain authors

demonstrated that medications capable of blocking VEGF binding to its receptor (VEGFR), inhibit tumor growth and have beneficial effects in cancer patients [65]. The first angiogenesis inhibitor introduced into medical practice was bevacizumab (a monoclonal antibody that binds to VEGF) [66]. Concomitant administration of bevacizumab with conventional chemotherapy has lead to a global increase of approximately 1 year in survival of non-small cell lung cancer patients [67]. These results lead to the introduction of bevacizumab administered concomitantly with platinum derivatives as first line medications in non-small cell lung cancer patients both in the European Union and the United States [68].

### Conclusions

The majority of published studies consider that serum VEGF levels, and VEGF expression in tumor cells is a negative prognosis factor for non-small cell lung cancer patients. Beneficial effects of anti-angiogenic therapies seen in some non-small cell lung cancer patients calls for new randomized studies aimed at identification of patients as candidates of routine anti-angiogenic therapy.

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