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# Immunohistochemical expression of vascular endothelial growth factor (VEGF) in intestinal type gastric carcinoma

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**Abstract**

*Background.* Gastric cancer still represents a difficult problem in the field of oncology, in terms of morbidity and mortality. The local progression and systemic spread is significantly influenced by tumor angiogenesis and lymphangiogenesis. In spite of many studies on the topic, data about the significance of growth factors in gastric cancer is controversial. *Aim*: to investigate the immunohistochemical expression of VEGF and to evaluate the relationships with the tumors stage and grade. *Material and methods.* The immunohistochemical expression of VEGF was investigated on 80 patients with intestinal type gastric carcinoma. Specimens were fixed in buffer formalin, embedded in paraffin, and sections were stained with Hematoxylin–Eosin and immunohistochemistry was performed for VEGF (clone VG-1). Evaluation was performed using the VEGF score, based on the intensity of reaction and percent of positive cells. *Results.* The reaction for VEGF was positive in 52 from 80 cases (70%). The final product of reaction was found in the cytoplasm of tumor cells, with granular pattern. Positive reaction was also found in eight from 28 cases with associated intestinal metaplasia, and in six from nine cases with gastric dysplasia. In the adjacent apparently normal mucosa, the reaction was positive in hyperplastic gastric pits and parietal cells. A strong correlation was found between VEGF expression and lymph node status and grade of the primary, but not with the stage of the tumor. *Conclusions.* The investigation of the immunohistochemical expression of VEGF in the intestinal type of gastric carcinoma showed positive reaction in 70% of the cases. It was demonstrated the expression of VEGF in intestinal metaplasia and gastric dysplasia, which could signify an early angiogenic switch during tumorigenesis.

***Keywords*:** gastric cancer, angiogenesis, vascular endothelial growth factor (VEGF), immunohistochemistry, prognosis.

## Introduction

Recent acquisitions in the field of diagnosis and treatment induced the improvement of prognosis in patients with superficial gastric cancer, but in advanced stages of the disease, the rate of mortality was not significantly changed. The rate of mortality is mainly due to the tumor spread by both lymphatic and blood vessel route, and finally leads to systemic metastases. Investigations performed on gastric carcinoma in last 15 years brought new insights in some steps of the local invasion and metastasis. To produce metastasis, tumor cells pass through a sequence of stages: detachment, local invasion, acquires motility, followed by the angiogenic switch, vascular invasion, survival in the circulation, and finally, growth in distant organs [1, 2].

There were identified adhesion molecules, growth factors and motility factors that were correlated with these sequences, and can be regarded as individual prognostic factors [3, 4]. Even with these new data, gastric cancer remains a major problem in oncology, mainly because these tumors are usually insensitive to radio-/chemotherapy. This is why many authors focused on early stages of invasion, characterized by the formation of new blood and lymphatic vessels in the tumor stroma.

Angiogenesis is the process of new blood vessel formation from preexisting vessels. This process was demonstrated to be extremely active in malignant tumor, which cannot growth more than 3 mm in the absence of blood vessels, as Folkman demonstrated many years ago [5]. This is why many malignant tumors have angiogenic phenotype, and this means that tumor cells are able to secrete angiogenic factors. Proangiogenic factors stimulate proliferation of endothelial cells of the host [6] and can recruit circulating endothelial cells that are integrated in the wall of newborn vessels [7].

Data about lymphangiogenesis are significantly reduced as compared with angiogenesis, but in gastric cancer it was already demonstrated the presence of proliferating lymphatic capillaries [8].

The demonstration of the angiogenic phenotype could be difficult, and for many years, the only method was represented by the counting of blood vessels in the tumor area, well known as microvessel density, performed on specimens stained for an endothelial marker [9]. Many authors in almost all known human tumors calculated microvessel density, but its prognostic value remains controversial. Divergent results were mainly due to the working system, reagents, and finally yet importantly, this method does not discriminate between blood and lymphatic vessels. In these conditions, microvascular density does not reflect the angiogenic phenotype of the tumor, but the intercapillary distance. For this reason, microvessel density is no longer accepted as an angiogenic indicator and does not reflect the ability of the tumor to respond to anti-angiogenic therapy. For this purpose, is essential to identify angiogenic factors secreted by tumor cells and/or cells of the host. The best known and the most efficient growth factor involved in tumor angiogenesis is vascular endothelial growth factor (VEGF), which was investigated in this paper.

VEGF has a mitogenic effect on endothelial cells and is the major angiogenic factor *in vivo*. It was identified in tumor cells of the gastric cancer more than 10 years ago [10, 11]. VEGF represents a family of growth factors with some isoforms, and from them, VEGF-A was shown to be pro-angiogenic, and VEGF-C seems to be the most potent lymphangiogenic agent [12]. Data about the expression of VEGF in gastric carcinoma are restricted to few publications, and therefore, its prognostic value, the expression in precancerous lesions and the normal stomach adjacent to the tumor as well are controversial. Our purpose was to investigate the immunohistochemical expression of VEGF in the intestinal and diffuse gastric carcinoma, the most frequently found pathological forms.

## Material and methods

There were investigated 80 patients admitted with gastric carcinoma (Table 1), without any prior chemotherapy.

**Table 1 –** ***Case distribution based on the stage of the tumor (T) and grade (G)***

| T/G* | T2 | T3 | T4 |
|---|---|---|---|
| *G1* | 8 | 16 | – |
| *G2* | 4 | 16 | 12 |
| *G3* | – | 20 | 4 |

*Note: G4 was not included in this study; only cases with typical characters of intestinal carcinoma were selected.

Forty-four from 80 cases showed enlarged regional lymph nodes, with metastases detected during microscopic examination. In eight cases were found liver metastases, detected by ultrasound. Specimens removed by open surgery were fixed in buffer formalin and embedded in paraffin, using the routine histological technique. From each case there were performed step sections stained with standard Hematoxylin–Eosin for the pathologic diagnosis and grade. Diagnostic criteria and grade were established according Rosai J [13] and Fletcher CDM [14]. All cases included in the study were intestinal type gastric carcinomas, selected from a consecutive series of 136 consecutive patients. Additional sections were stained for VEGF (clone VG-1), using the following protocol: antigen retrieval with microwave in EDTA pH 9 buffer, 25 minutes, inhibition of peroxidase with hydrogen peroxide 3%, incubation with the primary antibody VG-1, dilution 1:30, for 40 minutes; catalyzed signal amplification was the working system and diaminobenzidine was used as chromogen. Nuclei were stained with Lillie's modified Hematoxylin. All reagents were from Dako (Denmark). Examination was performed with Eclipse i80 Nikon, and the presence and distribution of the final product of reaction was correlated with the stage, grade, and presence of precancerous lesions.

VEGF scoring was based on the presence, intensity and percent of positive cells and it is shown in Table 2.

**Table 2 –** ***VEGF scoring***

| Score | Staining | Positive tumor cells [%] | Intensity |
|---|---|---|---|
| *0* | No | <1 | – |
| *1* | + | 1–25% | Weak |
| *2* | ++ | 26–50% | Moderate |
| *3* | +++ | >50% | Strong |

Adapted after Terris B *et al.*, 1998 [15].

## Results

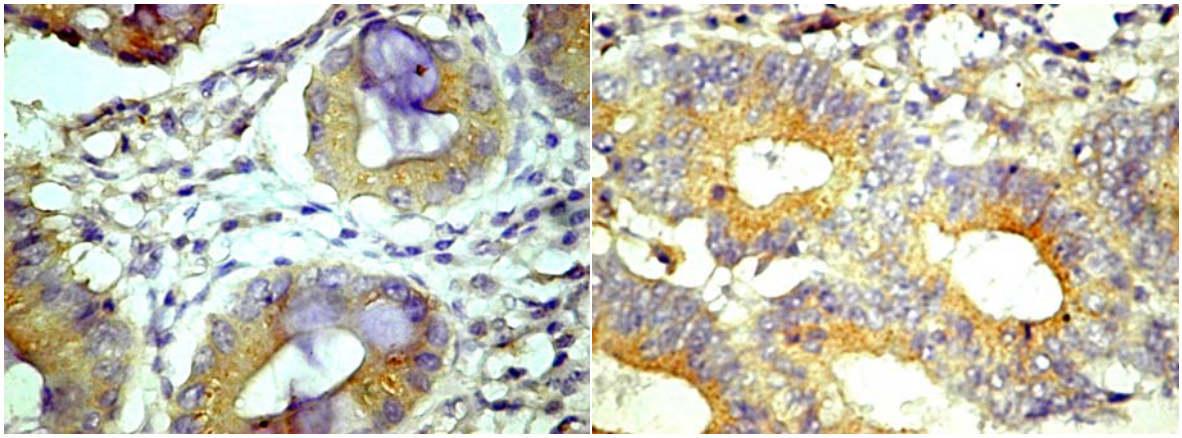
The final product of the immunohistochemical reaction for VEGF was cytoplasmic with granular patter and homogeneous distribution. The intensity of reaction was variable from a case to another, and even in different areas of the same lesion. The immunoreaction was found positive in 52 (70%) from 80 cases included in the study. The covering epithelium and epithelium of the gastric pits located at distance from the malignant proliferation were constantly negative. There were also negative all cells of the tumor stroma. Intestinal metaplasia was identified in 28 from 80 cases, and from these, eight were positive for VEGF in the cytoplasm of absorptive cells and negative in goblet cells (Figure 1a). Gastric dysplasia was found in the periphery of carcinoma in nine cases, and six from them were positive for VEGF (Figure 1b). In three cases, mucus-secreting glands of the gastric antrum with normal histological aspect but located close to the tumor, were positive for VEGF (Figure 2). The final reaction product was preferentially distributed in the basal cytoplasm.

In 52 cases in which the VEGF immunoreaction was positive, the final product of reaction was found in the cytoplasm of tumor cells, with diffuse distribution and granular pattern (Figure 3a). The intensity of reaction was significantly higher in the deepest part of the tumor than in the superficial area. In cases with weak or moderate positive reaction there were found small cell groups intensely stained (Figure 3b).

The cut-off point between positive and negative cases was a minimum of 1% positive tumor cells, according to VEGF scoring, showed in Table 2.

All 44 cases with lymph node metastasis showed a strong positive reaction for VEGF, and the correlation was significant for $p<0.001$. The strongest intensity was noticed in cases less differentiated (Figure 4). No correlation was found between the expression of VEGF and the stage of the tumor ($p<0.23$).

*Positive reaction was noticed in the normal mucosa adjacent to the tumor. On one hand, there were positive columnar cells, stained in the basal part of the cytoplasm, with moderate intensity* (Figure 5a). *To the best of our knowledge, this aspect was not reported in the literature. On the other hand, parietal cells from the same area showed strong positivity* (Figure 5b). *These aspects were noticed only in tumors extended to the gastric body, and somehow surprising, mucus-secreting cell of the gastric antrum did not express VEGF when located close to positive-tumor cells* (Figure 6a). *The intensity of reaction in apparently normal mucosa was not significantly different from that found in tumor cells* (Figure 6b).



**(a)** **(b)**

**Figure 1 –** ***Intestinal metaplasia, with VEGF-positive absorptive cells and negative goblet cells (a). Severe dysplasia. All epithelial cells show positive reaction (b). Anti-VEGF, ×400***

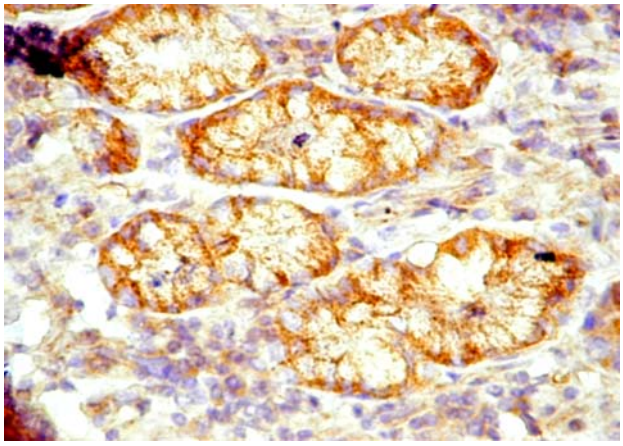
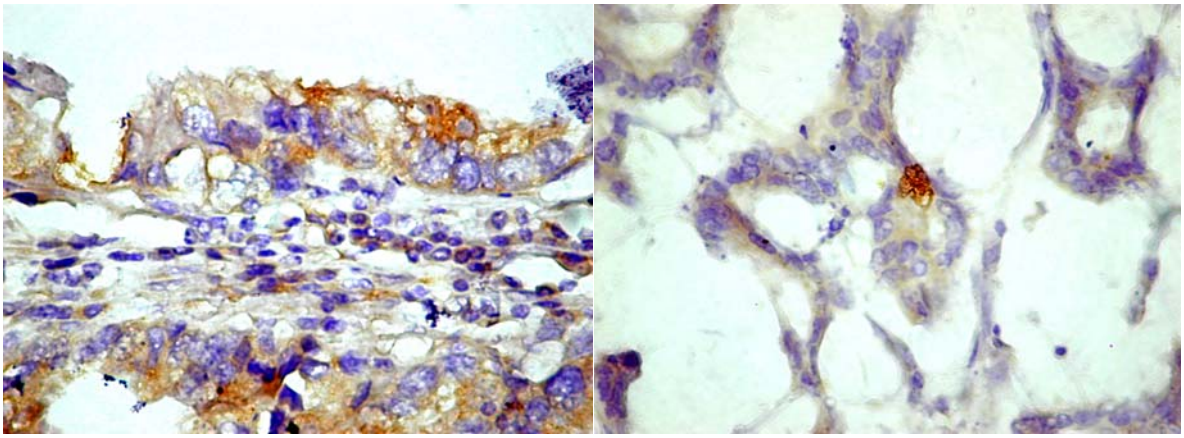


**Figure 2 –** ***Expression of VEGF in mucus-secreting glands of the gastric antrum (×400)***



**(a)** **(b)**

**Figure 3 –** ***Gastric carcinoma with tumor cells intensely stained, but heterogeneous (a). Mucus-secreting gastric carcinoma with focal intense positive reaction (b). VEGF, ×400***

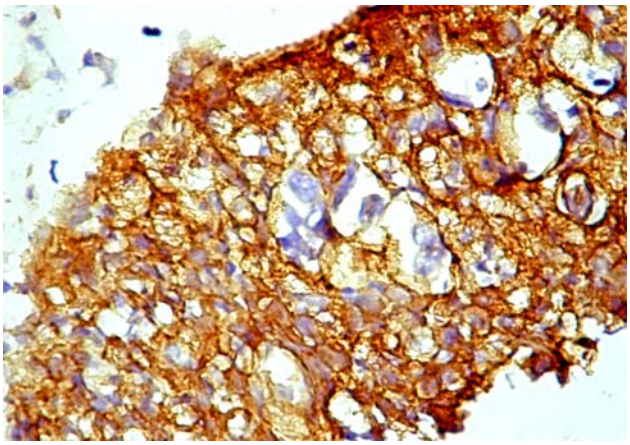
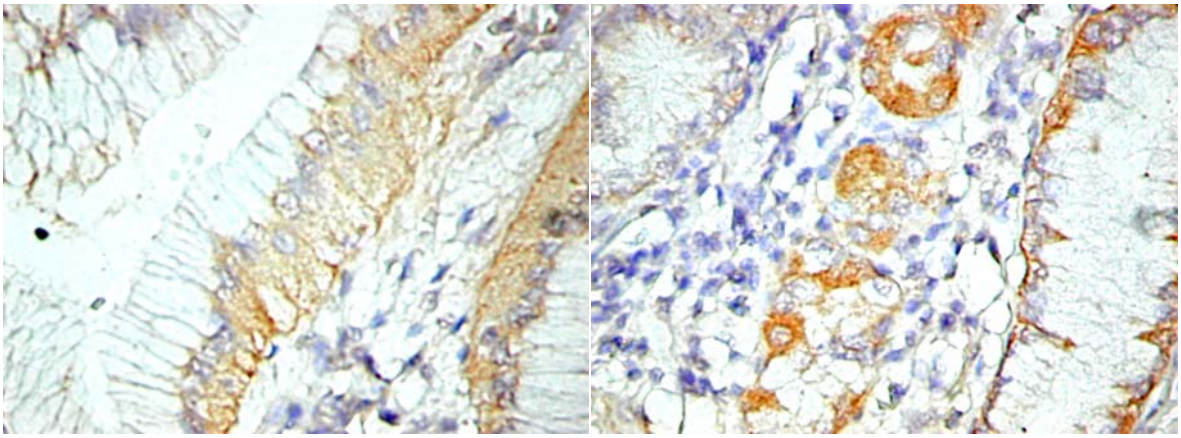
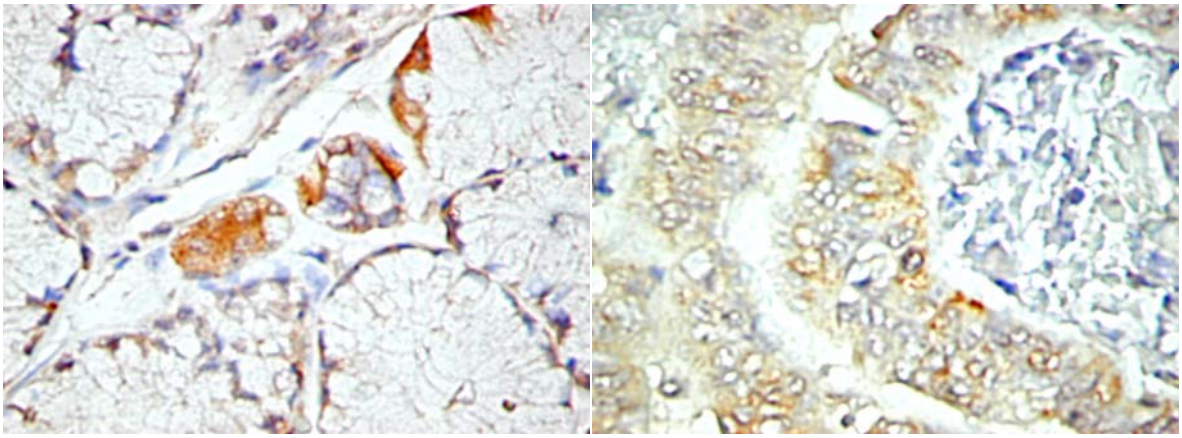


**Figure 4 –** ***Undifferentiated gastric carcinoma, with strong positive reaction. VEGF, ×400***



**(a)** **(b)**

**Figure 5 –** ***Apparently normal mucosa, close to the tumor. VEGF expression in hyperplastic pits (a), and parietal cells (b)***



**(a)** **(b)**

**Figure 6 –** ***Gastric carcinoma, antrum. Positive tumor cells and negative mucus secreting cells (a). Intestinal type gastric carcinoma with all tumor cells positive for VEGF, and moderate intensity (b). VEGF, ×400***

## Discussions

The immunohistochemical expression of VEGF was investigated in a large spectrum of malignant tumors. Some of them are included in Table 3, and significant differences can be noticed considering the incidence of positive reaction for VEGF. In the study published by Choi JH *et al*. [16], there were found 68.5% positive cases, and confirmed by results of the present finding (70%).

Studies of molecular biology have a crucial importance in the knowledge of the natural evolution of the gastric carcinoma. Although the most important nowadays, surgical therapy is far to be the ideal method for the management of gastric cancer, excepting for very rare cases with early carcinoma. Moreover, studies on the plasma levels of growth factors after surgery noticed a significant increase of VEGF after surgery [24].

The angiogenic phenotype of malignant tumors is given by the ability of tumor cells to secrete growth and mitogenic factor that target the endothelial cell. The immunohistochemical expression of VEGF in gastric carcinoma was demonstrated bay many other authors [10, 11, 25–27].

**Table 3 – *Immunohistochemical expression of VEGF in carcinoma***

| Authors | Year | Carcinoma | Patients | %VEGF+ |
|---|---|---|---|---|
| Terris B *et al*. [15] | 1998 | Digestive, neuroendocrine | 28 | 75 |
| Paradis V *et al.* [17] | 2000 | Renal | 74 | 35 |
| Hemmerlein B *et al.* [18] | 2001 | Renal | 58 | 70 |
| Liu D *et al*. [19] | 2001 | Gastric | 60 | 73.3 |
| Rosa AR *et al*. [20] | 2003 | Esophagus | 86 | 40.4 |
| Lee JS *et al*. [21] | 2006 | Ovary – border<br>– invasive | 23<br>54 | 47.8<br>74.1 |
| Choi JH *et al*. [16] | 2006 | Gastric | 137 | 68.6 |
| Raica M *et al*. [22] | 2007 | Renal cell | 54 | 75.5 |
| Lieto E *et al* [23] | 2007 | Gastric | 69 | 48 |

Our observations confirm data published until now, which show that the main source of VEGF is represented by tumor cells. Opposite to carcinoma from other sites, in which it is thought that approximately 10% of tumor cells produce VEGF, we found positive reaction in their large majority in gastric cancer. We tried to make evident the global expression of this growth factor, using the polyclonal anti-VEGF antibody. Previous studies on gastric carcinoma revealed the expression of both VEGF-A and VEGF-C [28, 29]. Isoforms of VEGF correlate with the expression of connective tissue growth factor, which indicates the risk for lymph node metastasis and poor survival [30]. The expression of VEGF is supported by the high density of small blood vessels, found in all invasive gastric carcinomas [3, 31, 32]. Identification of VEGF seems to be of major importance in early gastric carcinoma, and its expression correlates with regional lymph node metastasis [33].

Our results confirm the relationship between secretion of VEGF by tumor cells and lymph node metastasis, because all cases with this condition were positive. This finding supports the individual prognostic value of VEGF in gastric carcinoma. Some authors found a correlation between the expression of VEGF and incidence of micrometastasis in the bone marrow [34], but the significance of these aspects needs further investigations concerning survival.

In our study, the immunohistochemical expression of VEGF was restricted to epithelial cells. Opposite to other authors [35], we did not found positive reaction in cells of the stroma, previously reported as potential sources of VEGF, like mast cells or macrophages. We found positive reaction in epithelial cells of the intestinal metaplasia and in glands of the antrum. It could be hypothesized that this aspect is the expression of an early event of tumor angiogenesis during the natural evolution from the normal mucosa to carcinoma. Overexpression of VEGF enhances the sensitivity of the tumor to chemotherapy based on cisplatin [36].

Active tumor angiogenesis and VEGF secretion by tumor cells make the gastric cancer an almost "ideal" target for antiangiogenic therapy. Therapy in this instance refers to both angiogenesis and lymphangiogenesis, as postulated by others [12]. However, first experimental data demonstrated a significant decrease of the primary and of the incidence of metastasis, using an anti-VEGF antibody [37, 38].

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## Conclusions

The investigation of the immunohistochemical expression of VEGF in 80 cases of intestinal type gastric carcinoma revealed the following aspects: the reaction was positive in 52 cases (70%); positive reaction was demonstrated in intestinal metaplasia, gastric dysplasia, and apparently normal mucosa adjacent to the tumor; VEGF expression correlates with the status of regional lymph nodes, and does not correlate with tumor stage and grade.

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