Vascular Endothelial Growth Factor Expression and Microvessel Density – Two Useful Tools for the Assessment of Prognosis and Survival in Gastric Cancer Patients

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Abstract

Background and aims. The role of angiogenesis in progression and metastasis of gastric cancer has been studied over the last years. The aim of our study was to assess the microvessel density and vascular endothelial growth factor (VEGF) expression in correlation with prognosis, survival and the risk for upper gastrointestinal (GI) bleeding as well. Method. We prospectively assessed angiogenesis in 40 patients with gastric carcinoma. Microvessel density was calculated using CD31 and CD34 markers, and VEGF expression was assessed in biopsy samples. The tumor stage was established using imaging methods: CT scan for M and N stage and endoscopic ultrasound for T and N stages. The correlation between pathological markers and tumor stage, survival rate and risk of upper gastrointestinal bleeding was assessed. Results. The study included 40 patients with gastric cancer; among them 8 patients presented with upper GI bleeding. The average microvessel density was 10.21 for CD31 and 11.85 for CD34 in all patients VEGF was positive only in 45% of patients. The microvessel density was higher in patients with advanced TNM stage, and a correlation with the risk of UGIB and survival rate was also found. VEGF expression correlated with TNM stage and with the risk of upper GI bleeding. Conclusions. Microvessel density (estimated by CD34) was involved in locally advanced disease, while VEGF was correlated with loco-regional extension and distant metastasis in gastric cancer patients. There was a clear correlation between angiogenic parameters, survival rate and the risk of upper GI bleeding.

Key words

Gastric cancer – microvessel density – prognosis – survival – upper GI bleeding – VEGF.

Introduction

Gastric cancer is one of the most frequent malignancies, with a poor prognosis and dismal survival in advanced cases. Many studies were designed to uncover the mechanisms of carcinogenesis, as well as the role of various markers in the development and progression. The researchers focused on the study of the prognostic markers for gastric cancer, with emphasis on angiogenesis. Angiogenesis represents the process of new vessel development, being involved in the tumor growth, local extension and metastasis [1, 2], as well as prognosis [3, 4]. Angiogenic growth factors involved in the formation of new vessels are vascular endothelial growth factor (VEGF), platelet-derived growth factor (PDGF), and transforming growth factor β1 (TGF-β1). The expression of these factors is well correlated with a poor prognosis [5-8]. The role of microvessel density (MVD) in tumor extension and metastasis has also been studied. The immunohistochemical markers used for MVD quantification are anti-factor VIII related antigen polyclonal antibody, CD31, CD34, and CD105 [3, 9-11]. However, there is a lack of data regarding the risk of upper gastrointestinal bleeding (UGIB) in patients with gastric cancer and the role of angiogenesis in these patients.

The aim of our study was to assess the expression of intra-tumoral MVD, as well as VEGF, in patients with gastric cancer, in correlation with the risk for UGIB.

Material and methods

The study included 40 consecutive patients with gastric cancer (32 males and 8 females) examined by upper GI endoscopy, with 8 patients presenting for UGIB. Angiogenesis was prospectively assessed in all 40 patients with gastric carcinoma admitted consecutively in the Gastroenterology Department and the demographical, clinical and pathological parameters were included in a database. The cancer diagnosis
was established by upper GI endoscopy completed with conventional pathologic examination of biopsy samples. At least 4 samples from the tumor were taken in all patients. Cancer stage was established using imaging methods: abdominal ultrasound as an initial examination, CT scan for M and N stage and endoscopic ultrasound (EUS) for T stage and N stage (completed with fine needle aspiration for a better characterization of lymph nodes). In operable patients, the final stage was established by surgery and complete pathological examination of the resection piece. MVD was assessed by immunohistochemistry using CD 31 and CD 34 markers. By using low-power magnification hot spots were found. After that, a high-power magnification (400X) was used for counting the vessels in three different fields and an average was calculated.

CD 31 clone JC 70A (DakoCytomation, Denmark) and CD 34 clone QBEnd 10 (DakoCytomation, Denmark), diluted 1:50 in PBS, were used after 20 minutes pre-treatment of tissues with heat induced epitope retrieval (MW) in DakoCytomation target retrieval solution High pH and 30 minutes incubation at room temperature with primary antibody, with visualization by Dako EnVision+/HRP. Negative control was DakoCytomation Mouse IgG, diluted in the same concentration as primary antibody.

The expression of VEGF was considered positive in the samples where the marker was evident in at least 5% of tumoral cells. VEGF clone VG1 (DakoCytomation, Denmark), diluted 1:50 in PBS, was used after 20 minutes pre-treatment of tissues with heat induced epitope retrieval (MW) in DakoCytomation target retrieval solution pH 9 and 30 minutes incubation at room temperature with primary antibody, with visualization by Dako LSAB+/HRP. Negative control was DakoCytomation Mouse IgG, diluted in the same concentration as primary antibody. External control was the human colon, which was positive in all run IHC.

Finally, a correlation between pathological parameters, tumor stage, survival rate for a period of two years of follow up, and risk of UGIB was established. Univariate analysis and calculations of a 2x2 contingency table was used for statistical analysis, p value being obtained using Fischer’s test. A p value below 0.05 was considered significantly statistical.

Results

The median age of the patients was 65.93 with a standard deviation (SD) of 10.84 years (range 33 to 82 years). The cut-off point used in subsequent statistical analysis was 66. The TNM score [12] was used for staging: only 9 patients were in the first two stages, while 32 patients were classified as stage 3 or 4.

The intestinal type of carcinoma was found in 32 patients: only 8 patients had diffuse types. Histological grading of carcinoma revealed that 3 patients had G1 grade (well differentiated), 9 patients had G2 grade (medium differentiated), and 28 patients had G3 grade (low differentiated). The average microvessel density was 10.21 for CD31 and 11.85 for CD34 in all patients, while VEGF was positive only in 18 patients (45%) (Figs. 1, 2). These means were chosen to be the cut-off values for discrimination of prognosis and for the univariate analysis of the study group.

The majority of patients were in advanced stage of diseases (77.5% in 3rd and 4th TNM stages), only 3 patients were in the first TNM stage and 6 patients in second TNM stage. Distant metastases were found in 17 patients: liver metastasis (in 17), peritoneal spreading (in 6), bone metastasis (in 2) and distant lymph nodes (mediastinal mass and/or supraclavicular malignant lymph nodes) in 4 patients.

The MVD was higher in patients with advanced TNM stage, as assessed by both methods (CD31, CD34). Moreover, there was a correlation between MVD and the risk of UGIB (p=0.0436 for CD31 and p=0.0138 for CD34, respectively).

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VEGF expression was correlated with TNM stage (stronger with the M stage, \( p<0.0001 \)) and also with the risk of upper GI bleeding (\( p=0.0138 \)). Survival rate after first year of follow-up was 37.5% and 30% respectively after two years of follow-up. Low survival rate was positively correlated with MVD (reaching statistical significance). VEGF expression was not correlated with survival (Table I).

**Discussion**

Microvessel density is a useful tool for the assessment of tumor-associated blood vessels, as well as for stratification of the prognosis in patients with gastric cancer. In various studies, the MVD was proven to be involved in tumor metastasis and progression [2, 3, 13], although in other studies MVD did not seem to have influenced the survival rate [14]. We found a clear correlation between the MVD and the TNM stage, the major role being in local advanced disease, when MVD was estimated by CD34. In our patients, the advanced T stage was dominant. In other studies, patients in stage I and II were highly represented. The MVD is involved in lymph node extension [3] but the role of MVD is more evident in distant metastasis [2, 13]. Some studies compared the different ways of assessment of angiogenesis. It was found that MVD measured by CD34 expression might not be very useful, the authors recommending the use of CD31 [14].

VEGF expression in tumor samples was proven to be another useful marker for the assessment of angiogenesis [5, 6, 15] and of prognosis [4, 13, 16]. Kolev et al found that VEGF was expressed in 50.3% of the patients, in concordance with our data (45%). Moreover, VEGF is responsible for the haematogenous recurrence of early-stage gastric carcinoma [17]. The existing differences between various studies could be due to VEGF polymorphisms, which may contribute to gastric tumor characteristics [18]. In our study, the role of VEGF and MVD was extended for the assessment of UGIB risk in gastric cancer patients. A clear correlation between these parameters and UGIB was found. Future studies should be performed, including an increased number of patients, in order to confirm our results.

Beyond the role in tumor progression, MVD and VEGF

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could represent two important markers for assessing the efficacy of anti-tumoral treatment. Anti-angiogenic therapy has been introduced in various digestive cancers, being now the new standard of care in advanced colorectal cancer, while it is still being tested in gastric cancer due to its convincing clinical benefit and its tolerability and combinability with multiple chemotherapeutic agents [19]. Recent publications on targeted therapies in gastric cancer are limited to non-randomized 1st or 2nd phase trials. Adequately powered, randomized phase III trials are necessary to define the clinical role of targeted therapies in advanced gastric cancer. Biomarker studies correlated with treatment outcomes will be critical in order to identify patients who mostly benefit from chemotherapy and targeted therapy [20]. In a study on mice, the intraperitoneal administration of an anti-angiogenic agent, bevacizumab, was extremely useful in peritoneal metastases of gastric cancer [21]. Immunohistochemical analysis of disseminated peritoneal nodules stained with CD31 showed that the vessel area in the bevacizumab group was significantly lower as compared with that in the control group (P < 0.001) [21]. Thus, CD31, CD 34 and other markers used for the assessment of MVD could become routine markers for the assessment of efficacy of anti-angiogenic treatment.

There are only scarce data regarding the risk of UGIB in gastric cancer patients. A recent case report revealed that thalidomide, an anti-angiogenic agent, was used to stop the bleeding in gastric cancer patients, thus angiogenesis could be an important factor in bleeding gastric cancer [22]. However, there are reasons for a moderate enthusiasm, because the anti-angiogenic therapy itself could represent a pro-bleeding factor [23, 24]. Anyway, most of the adverse events of VEGF inhibitors are modest and manageable [25].

The correlation between VEGF expression and MVD, in gastric cancer patients with UGIB seems to be feasible, allowing the assessment of angiogenesis before and after specific anti-angiogenic treatment. In the future, new markers for angiogenesis could be found and the studies must be extended in this direction, for a better characterization which allows development of new therapeutic agents.

Conclusions

According to the results of our study, MVD estimated by CD34 was involved in local advanced disease, while VEGF was correlated to distant metastasis as well as to loco-regional extension in gastric cancer patients. There was a clear correlation between VEGF, MVD and the risk of upper GI bleeding. The results of our study should be confirmed in future studies with a larger number of patients included, preferably with a multicentric design.

Acknowledgement

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Conflicts of interest

None to declare.

References


