**Vascular Endothelial Growth Factor and Cluster of Differentiation 34 for Assessment of Perioperative Bleeding Risk in Gastric Cancer Patients**

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**Abstract**

**Background:** Angiogenesis is the formation of new blood vessels to supply nutrients to tumors. Vascular endothelial growth factor (VEGF) and cluster of differentiation 34 (CD34) are important signaling proteins involved in angiogenesis. Many studies have demonstrated that VEGF and CD34 are related to tumor progression. This study focused on the relationship between VEGF, CD34, and perioperative hemorrhage in patients with gastric cancer.

**Methods:** To observe the relationship between VEGF and CD34, we tracked 112 patients with advanced gastric cancer for 5 years to assess factors related to hemorrhage, using immunohistochemistry. The results were subjected to statistical analysis using a 2 × 2 contingency table, logistic regression, and receiver operating characteristic (ROC) test.

**Results:** The concentrations of VEGF and CD34 were critically correlated with perioperative hemorrhage and neural invasion in patients with gastric cancer (P < 0.05). Expression of VEGF and CD34 was related (P = 0.05, \( \chi^2 = 6.834 \)). VEGF and CD34 co-expression strongly increased the risk of preoperative bleeding (area under the ROC curve >0.7, P < 0.05).

**Conclusions:** Expression of VEGF and CD34 was critically correlated with perioperative hemorrhage in gastric cancer patients. Co-expression of VEGF and CD34 could be an effective indicator for evaluating the risk of perioperative bleeding in gastric cancer patients.

**Key words:** Cluster of Differentiation 34; Gastric Cancer; Neural Invasion; Perioperative Hemorrhage; Vascular Endothelial Growth Factor

**Introduction**

Gastric cancer is the most common malignant tumor of the digestive tract, with a high mortality rate worldwide, especially in China. There is evidence that the morbidity rate of gastric cancer is growing worldwide. Many researchers have found that angiogenesis is essential in many physiological processes and is correlated with tumor progression, with the formation of new blood vessels for the supply of nutrients to the tumor. To promote proliferation of malignant tumors, factors related to angiogenesis such as vascular endothelial growth factor (VEGF) and cluster of differentiation 34 (CD34) are always overexpressed. Thus, these two factors could be effective prognostic indicators in many cancers.[1-7]

VEGF is an important signaling protein involved in both vasculogenesis and angiogenesis.[9] VEGF can enhance microvascular permeability of the endothelium as well as new vessel development, as involved in tumor growth, local extension, and metastasis. CD34 is a cell surface glycoprotein and functions as a cell adhesion factor, which also mediates the attachment of stem cells to bone marrow extracellular matrix and directly to stromal cells.[9] Moreover, CD34 is expressed on early hematopoietic and vascular-associated...
tissue. As an indirect marker of neoangiogenesis,\(^\text{7,10,11}\) CD34 could be an indicator of microvascular density (MVD). Both VEGF and CD34 are closely correlated with vasculogenesis and angiogenesis in tumor development.\(^\text{12}\)

However, there is a lack of data regarding the risk of perioperative bleeding and neural invasion in gastric cancer patients with expression of VEGF and CD34. In our study, we focused on the relationship between VEGF and CD34 and angiogenesis and perioperative hemorrhage. We aimed to select an effective criterion to evaluate the risk of hemorrhage in the treatment of gastric cancer.

**Methods**

**Patients and specimens**

The study was approved by the Ethics Committee of the Second Affiliated Hospital and Yuying Children’s Hospital of Wenzhou Medical University. All patients provided written informed consent. Samples from 112 consecutive patients (81 males and 31 females, median age: 57.1 years, age range 20–83 years) with pathologically confirmed gastric cancer after radical gastrectomy without distant metastasis were collected from 2008 to 2011 at the Department of General Surgery of the Second Affiliated Hospital of Wenzhou Medical University, China. The study design was retrospective, and the patients underwent six to eight cycles of standard FOLFOX6 (fluorouracil + oxaliplatin + leucovorin) chemotherapy after surgery. All specimens and fresh tissue samples were confirmed by pathological diagnosis and were staged according to the 7th American Joint Committee on Cancer TNM Classification of Malignant Tumors. The relative clinical features were collected from self-report, medical history, operative records, pathological biopsy, and biochemical tests. The definition of perioperative hemorrhage included preoperative bleeding (self-report of upper digestive tract hemorrhage, hemoglobin [Hb] <120 g/L), intraoperative bleeding (operative records of bleeding >500 ml), and postoperative bleeding (Hb <120 g/L). All the tissue samples were fixed in 4% formalin immediately after removal and embedded in paraffin for immunohistochemical staining.

**Immunohistochemistry**

Immunohistochemical staining was performed using standard procedures, and expression of VEGF and CD34 in the specimens was evaluated according to the methods described by Frietsch et al.\(^\text{13}\) Expression of VEGF and CD34 was considered as follows: 0, 0% immunoreactive cells; 1, <5% immunoreactive cells; 2, 5–50% immunoreactive cells; and 3, >50% immunoreactive cells. In addition, the intensity of VEGF and CD34 expression by the tumor cells was also determined (score 0, none; 1, low; 2, moderate; and 3, strong). The final score was calculated by adding the staining intensity and extension. The samples were classified as negative (0), weakly stained (1 or 2), moderately stained (3), and strongly stained (4–6). With the moderate and strong immunoreaction scores considered positive and the other scores negative, we identified each section using low magnification (original magnification, ×50) and high magnification (original magnification, ×200), respectively. VEGF and CD34 antibodies were purchased from Abcam (Cambridge, MA, USA).

**Statistical analysis**

The data were analyzed by SPSS version 19.0 (IBM company, USA). The correlation between expression of VEGF and CD34 and histology or clinical features was analyzed using a 2 × 2 contingency table and Pearson’s Chi-square test, with the combing indicator of perioperative hemorrhage risk analyzed by logistic regression and receiver operating characteristic (ROC) curve. Finally, all the statistical hypotheses were tested to the level of significance (\(P<0.05\)).

**Results**

Thirty-one patients (27.7%) had hematemesis during the diagnostic period, 31 (27.7%) had intraoperative hemorrhage (>500 ml), and 74 (66.1%) had anemia (Hb <120 g/L) within 1 month after surgery. Twenty-two (19.6%) patients had neural invasion. Expression of VEGF was significantly correlated with preoperative, intraoperative, and postoperative bleeding and neural invasion (\(P<0.05\)). Expression of CD34 was closely related to preoperative and intraoperative hemorrhage (\(P<0.05\)). However, expression of VEGF and CD34 was not significantly related to sex, age, TNM stage, and 5-year survival.

Thirty-five and 25 patients with gastric cancer had overexpression of VEGF and CD34, respectively, and 13 (11.6%) had VEGF and CD34 co-expression as is shown in our clinical data [Figure 1]. Co-expression of VEGF and CD34 was more critically related with perioperative bleeding than that of either single factor. The relationship between the main clinicopathological characteristics and VEGF or CD34 is shown in Table 1. The significance of VEGF and CD34 was demonstrated using the 2 × 2 contingency table and Pearson’s Chi-square test [Table 1, \(P<0.05\)].

![Image](https://via.placeholder.com/150)

**Figure 1:** Expression of CD34 and VEGF in perioperative hemorrhage in gastric cancer patients and normal individuals via immunohistochemical staining. Expression of CD34 (a and c) in normal tissue and gastric cancer tissue, respectively; and expression of VEGF (b and d) in normal tissue and gastric cancer tissue, respectively (original magnification, ×200). VEGF: Vascular endothelial growth factor; CD34: Cluster of differentiation 34.
The concentrations of VEGF and CD34 were closely related, according to the 2 × 2 contingency table and Pearson’s Chi-square test [Table 2, P < 0.05]. Expression of VEGF and CD34 was calculated to obtain an equation for combining predictors (Y) via logistic regression [Table 3].

To compare the significance of single and combined factors, we conducted statistical analysis via ROC curve [Table 4]. Co-expression of CD34 and VEGF strongly increased the risk of perioperative bleeding [Table 4]; thus, it could be a better indicator for evaluation of the risk of hemorrhage than either factor alone.

**Discussion**

Hemorrhage is the most common complication in gastric cancer, the risk of which is increased during surgery and postoperative recovery. Meanwhile, hemorrhage is always correlated with the degree of malignancy. With more attention being given worldwide to antiangiogenesis as a novel therapy, appropriate biomarkers should be found to evaluate the risk of perioperative bleeding, so as to provide more effective treatment for gastric cancer. The expression of biomarkers might be important for identifying disease severity and treatment decision-making.
In many studies, VEGF and CD34 were closely related to progression of many cancers. For instance, Kolev et al. found that VEGF was responsible for the hematogenous recurrence of early-stage gastric carcinoma. Some researchers have noted an increase in the levels of serum VEGF, and that MVD could be used to determine the morphological grade of breast carcinoma. Similarly, a correlation between overexpression of VEGF and the increasing degree of esophageal cancer has been reported. A relationship between serum level of VEGF and MVD in the grade of tumors, which showed necrosis and hemorrhage, has been reported. CD34 participates in cell recognition, signal transduction, cell proliferation, inflammation, blood coagulation, tumor metastasis, and a series of important physiological processes. Some studies have indicated that CD34 is expressed on some types of leukemia cells, solid tumor cells, endothelial cells, and hematopoietic stem/progenitor cells. Therefore, we conclude that the perioperative hemorrhage should be attributed to the functions of VEGF and CD34 promoting angiogenesis, with new vessels increasing the risk of hemorrhage during surgery and postoperative recovery.

As mentioned above, combined factors can be helpful indicators for evaluating the risk of hemorrhage when we perform regular gastroscopic biopsy before surgery. Surgeons should use elaborate ligation, blunt dissection, and more effective methods of hemostasis in patients with overexpression of bifactors. Furthermore, antiangiogenic therapy against VEGF and CD34 might be promising for patients with advanced gastric cancer in the future. It might help to interfere with initial tumor development and reduce surgical risks by blocking the angiogenic switch (VEGF and CD34) from progression to invasive cancer.

In conclusion, we showed that CD34 and VEGF are correlated with preoperative, intraoperative, and postoperative hemorrhage as well as neural invasion. There is a clear correlation between VEGF and CD34 and the risk of bleeding during treatment of gastric cancer. Co-expression of CD34 and VEGF is strongly correlated with the risk of hemorrhage during treatment, which could be a promising indicator in evaluating the perioperative hemorrhage risk, so as to provide effective and customized treatment. However, there is a lack of cases and evidence-based medicine for

### Table 3: Combining predictors of VEGF and CD34 via logistic regression

| Clinical features       | Biomarker | B     | OR   | 95% CI          | Formula             | P      |
|-------------------------|-----------|-------|------|-----------------|---------------------|--------|
| Preoperative bleeding   | CD34      | 1.436 | 4.205| 1.556–11.367    | Y = VEGF + 1.167 CD34 | 0.005  |
|                         | VEGF      | 1.230 | 3.423| 1.355–8.642     |                      | 0.009  |
| Operative bleeding      | CD34      | 0.754 | 2.126| 0.803–5.628     | Y = VEGF + 0.836 CD34 | 0.129  |
|                         | VEGF      | 0.902 | 3.902| 1.007–6.031     |                      | 0.048  |
| Postoperative hemoglobin| CD34      | 0.799 | 2.224| 0.672–7.359     | Y = VEGF + 0.832 CD34 | 0.190  |
|                         | VEGF      | 0.960 | 2.612| 0.943–7.240     |                      | 0.065  |
| Nerve invasion          | CD34      | 0.750 | 0.472| 0.132–1.688     | Y = VEGF − 0.530 CD34 | 0.248  |
|                         | VEGF      | 1.416 | 4.122| 1.500–11.325    |                      | 0.006  |

VEGF: Vascular endothelial growth factor; B: Intercept; OR: Odd ratio; CI: Confidence interval; CD34: Cluster of differentiation 34.

### Table 4: Value of VEGF and CD34 as diagnostic criteria in perioperative bleeding

| Clinical features       | Biomarker | AUC     | 95% CI  | P     |
|-------------------------|-----------|---------|---------|-------|
| Preoperative bleeding   | CD34      | 0.658   | 0.537–0.779 | 0.008 |
|                         | VEGF      | 0.663   | 0.545–0.781 | 0.010 |
|                         | VEGF + CD34| 0.706   | 0.588–0.824 | <0.001|
| Operative bleeding      | CD34      | 0.591   | 0.468–0.714 | 0.137 |
|                         | VEGF      | 0.618   | 0.498–0.739 | 0.053 |
|                         | VEGF + CD34| 0.643   | 0.522–0.764 | 0.019 |
| Postoperative hemoglobin| CD34      | 0.576   | 0.464–0.689 | 0.204 |
|                         | VEGF      | 0.608   | 0.497–0.719 | 0.073 |
|                         | VEGF + CD34| 0.626   | 0.520–0.733 | 0.035 |
| Nerve invasion          | CD34      | 0.474   | 0.342–0.607 | 0.709 |
|                         | VEGF      | 0.645   | 0.511–0.779 | 0.036 |
|                         | VEGF + CD34| 0.669   | 0.573–0.800 | 0.014 |

VEGF: Vascular endothelial growth factor; CD34: Cluster of differentiation 34; AUC: Area under receiver operating characteristic curve; CI: Confidence interval.

At present, the most important factors in the physiological and pathological regulation of angiogenesis are VEGF and CD34. Expression of VEGF and CD34 by tumors recruits new blood vessels from the existing circulation (angiogenesis) and participates in tumor invasion and metastasis. Here, we performed a retrospective study of 112 consecutive patients with pathologically confirmed gastric cancer undergoing radical surgery from 2008 to 2011. We explored the association between biomarkers and perioperative hemorrhage as well as neural invasion. We demonstrated that the roles of VEGF and CD34 extended to the assessment of risk of preoperative, intraoperative, and postoperative hemorrhage in gastric cancer patients. Although CD34 was not correlated with postoperative bleeding ($P = 0.076$) in our experiment, it might tend toward significance if the number of cases was to be increased. We found a significant correlation between VEGF and CD34 in progression of gastric cancer ($P < 0.05$, $\chi^2 = 6.834$). The correlation between co-expression of these parameters and perioperative hemorrhage in advanced gastric cancer patients seemed to be more reliable. It is suggested that both markers play significant roles in the promotion of angiogenesis within malignant tumors, and concentrations of VEGF and CD34 might also be good indicators of perioperative hemorrhage in gastric malignancy (area under the ROC curve >0.7, $P < 0.001$).
demonstrating its significance in clinical treatment. It would be better if the gastritis patients are chosen to be a control group. More randomized clinical trials should be performed to confirm our results.

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Conflicts of interest

There are no conflicts of interest.

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