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Journal of Cancer Science and Therapeutics

Assessment of circulating VEGF as a predictive biomarker of peritoneal carcinomatosis in gastric cancer

Pedro Villarejo-Campos[1*], David Padilla[2], Natalia Bejarano[3], Victor Baladrón[4], Patricia Faba[4], Mercedes Molina-Roblese[5], Pablo Menéndez[6] and Francisco-Javier Redondo[4]

[1]Department of Peritoneal Cancer Surgery, Fundación Jiménez Díaz University Hospital, Spain
[2]Department of Surgery, Ciudad Real University Hospital, Spain
[3]Department of Critical Care Medicine, Ciudad Real University Hospital, Spain
[4]Department of Anesthesiology and Critical Care Medicine, Ciudad Real University Hospital, Spain
[5]Department of Internal Medicine, Ciudad Real University Hospital, Spain
[6]Department of Surgery, Hospital Gutiérrez Ortega, Spain

Abstract

Introduction: *Early diagnosis of peritoneal carcinomatosis is difficult in patients with gastric cancer who are at high risk of developing peritoneal metastases. The measurement of serum Vascular Endothelial Growth Factor (VEGF) has proven to be a useful prognostic factor in gastric cancer, but it also could be a predictive factor for peritoneal metastases since the VEGF signalling pathway is directly involved in the development of peritoneal metastases.*

Methods: *This is a retrospective study from 2005 to 2017. We reviewed the peritoneal recurrence pattern of a cohort of 59 gastric cancer patients in whom serum VEGF was measured before surgery and after completion of adjuvant treatment*

Results: *Preoperative serum VEGF (pre-VEGF) level was identified as an independent prognostic factor for developing peritoneal metastases. The optimal cut-off value of pre-VEGF levels was 507 pg/mL, which presented a sensitivity of 66 % and a specificity of 78% to predict the development of peritoneal metastases. Patients with high pre-VEGF levels (>507 pg/mL) were at greater risk of developing peritoneal metastases than patients with low pre-VEGF levels (<507 pg/mL) (p=0.023).*

Conclusions: *VEGF plays a crucial role in the development of peritoneal metastases, and serum VEGF meets the requirements of a potential predictive marker for peritoneal carcinomatosis. Therefore, the measurement of serum VEGF levels could be useful during the follow-up of patients with advanced gastric cancer.*

Keywords: *vascular endothelial growth factor (VEGF), serum, overexpression, gastric cancer, peritoneal metastases, peritoneal carcinomatosis.*

Abbreviations: *AUC: Area Under The Curve; CI: Confidence Interval; ELISA: Enzyme-Linked Immunosorbent Assay; HIPEC: Hyperthermic Intraperitoneal Chemotherapy; HR: Hazard Ratio; p: p-value; PCI: Peritoneal Carcinomatosis Index; pg/mL: picogram/millilitre; Post-VEGF: Post-Treatment Vascular Endothelial Growth Factor; Pre-VEGF: Preoperative Vascular Endothelial Growth Factor; ROC: Receiver Operating Characteristic Curve; VEGF: Vascular Endothelial Growth Factor; VEGFR-2: Vascular Endothelial Growth Factor Receptor 2*

Introduction

In Western countries, advanced gastric cancer has a poor prognosis without new therapies to improve survival outcomes. Only 30% of patients with a regional stage at first diagnosis survive at 5 years [1].

Moreover, the 5-year survival rates for patients with curative resection range from 15% to 35% [2].

The development of peritoneal metastases is a frequent occurrence in gastric cancer and is associated with a dreadful prognosis, as the following data show:

- -There are 14% of patients with peritoneal metastases at the time of initial diagnosis (synchronous metastases) [3].
- -After surgical resection with curative intent (R0), 15-29% of patients may develop peritoneal metastases (metachronous metastases) in a median time of 17-18 months [4,5].
- -Regardless of the timing (metachronous or synchronous), peritoneal metastases in gastric cancer are associated with a median survival of 2-4 months [3,4].

The main factors associated with a high risk of peritoneal metastases in gastric cancer are depth of tumor invasion (T4), signet ring histology, undifferentiated grading (G3/G4) and young age [4,6].

Identifying gastric cancer patients at high risk of peritoneal metastases is important to develop close follow-up programs and to provide comprehensive treatments, especially in young patients [7].

Numerous signaling pathways have described in the carcinogenesis process over recent years, but among these,

vascular endothelial growth factor (VEGF) has been an important source of research on prognostic factors in gastric cancer [8].

VEGF was originally described as a vascular permeability protein secreted by tumor cells [9]. VEGF (also known as VEGF A to differentiate it from the rest of the family members of vascular growth factors) plays a critical role in angiogenesis through activating the vascular endothelial growth factor receptor 2 (VEGFR-2) on vascular endothelial cells [8]. The process of angiogenesis is crucial for both primary tumor growth and peritoneal spread [10].

VEGF is produced and secreted mainly by cancer cells, but also by endothelial cells and immune cells, such as macrophages and fibroblasts, from the tumor microenvironment [11]. In addition, VEGF could be synthesized by non-tumor peritoneal mesothelial cells, outside of this microenvironment [10].

The role of VEGF signaling is not limited to vascular permeability and activation of angiogenesis; it is also related to critical aspects of carcinogenesis. VEGF regulates immune cells (such as dendritic cells, T cells, regulatory T cells, and myeloid-derived suppressor cells) in the tumor microenvironment to suppress the antitumor response [8].

Even more recently, it has been reported that VEGF increases the invasiveness and motility of tumor cells in serosa-infiltrated gastric cancer, which may facilitate the migration and exfoliation of cancer cells into the peritoneal cavity [12].

This comprehensive knowledge of the role of VEGF in gastric cancer, leads us to consider the following question: Could VEGF levels be useful as a marker to detect gastric cancer patients at high risk of developing peritoneal metastases?

For this purpose, we have reviewed our database of 59 gastric cancer patients in whom we performed pre-treatment and post-treatment determination of serum VEGF. We have analyzed associations between serum VEGF levels and the occurrence of peritoneal metastases in gastric cancer patients.

Materials and methods

Patients

We have reviewed the recurrence pattern of a prospective cohort of 59 gastric cancer patients with a regional stage at diagnosis who underwent surgery from 2005 to 2007 at the General Hospital of the Ciudad Real. Adjuvant chemo-radiotherapy was administered 4 weeks after surgery. None of the patients received neoadjuvant treatment.

This research was conducted in accordance with the Declaration of Helsinki. The study protocol was approved by the Ethics Committee of Ciudad Real General Hospital (Acta 03/2004.Fecha 13-Abril-2004). All enrolled patients signed the informed consent form.

Patients were followed-up every four months in the first two years and every six months afterwards. If recurrence was suspected, histological confirmation was sought in all instances. After ten years, surveillance was conducted annually in the surviving patients.

Blood samples and assays

Peripheral venous samples were collected before surgery (pre-VEGF) and after postoperative chemo-radiotherapy (post-VEGF). These serum levels of VEGF were measured by sandwich enzyme-linked immunosorbent assay (ELISA), using a commercial kit, Quantikine, from R&D System (Minneapolis, USA) according to the manufacturer's instructions. Results were expressed in picograms per millilitre (pg/mL).

Statistical analysis

The association of preoperative serum VEGF levels (pre-VEGF) and post-treatment serum VEGF levels (post-VEGF) with the development of peritoneal metastases were evaluated using the Mann-Whitney U test. Univariate and multivariate Cox analyses were performed to identify independent prognostic factors.

Receiver operating characteristic curve (ROC) and the area under the ROC curve (AUC) were calculated to test the diagnostic performance of the VEGF measures. The best cut-off value was established with the use of Youden's index. Time to peritoneal carcinomatosis, defined as the time from the date of the surgery to the date of the development of peritoneal metastases (histologically confirmed), was considered in the survival analysis. To perform survival analyses, we used the Kaplan-Meier estimator with the log-rank test and Cox proportional hazards regression models to estimate Hazard Ratios (HRs).

All statistical tests were performed with a significance of 0.05 and estimated with a 95% confidence interval (CI). Continuous data were expressed as mean and the standard deviation. Analyses were carried out using the SPSS statistical package version 23.0 software for Windows (SPSS, Chicago, Illinois, USA). The data that support the findings of this study are available on request from the corresponding author.

Results

We completed 11 years of follow-up in our study cohort, and the main characteristics and outcomes are shown in Table 1.

Up to 85% of gastric cancer patients were at an advanced stage at initial diagnosis, and the overall survival at 5 and 10 years was 19% and 12%, respectively.

A total of 9 patients (15.2%) during this long time presented a peritoneal relapse as the first recurrence.

Patients who developed peritoneal metastases showed higher pre-VEGF than patients who did not develop peritoneal metastases (mean 643.55±296.34 versus 368.14±298.78 respectively, p=0.026).

Univariate Cox analysis revealed that pre-VEGF (p=0.024) and deep tumour infiltration (T4) (p=0.034) were significant prognostic factors affecting the occurrence of peritoneal metastases.

Subsequently, in multivariate analysis, only pre-VEGF remained an independent prognostic factor for developing peritoneal metastases (p=0.042).

To determine the diagnostic performance of pre-VEGF and post-VEGF in peritoneal metastases, we performed a ROC analysis. The area under the curve of pre-VEGF demonstrated a statistically significant value of 0.804 (CI 95%=0.630-0.977; p=0.021). These results allowed us to consider that pre-VEGF is a useful marker for predicting the occurrence of peritoneal metastases in gastric cancer [13].

According to Youden's Index, the optimal cut-off value for pre-VEGF was 507 pg/mL, which presented a sensitivity of 66% and a specificity of 78% to predict the development of peritoneal metastases. So, in accordance with this cut-off value, we have defined high (>507) and low (<507) pre-VEGF levels.

The probability of developing peritoneal metastases in patients grouped according to pre-VEGF was calculated by a Kaplan-Meier analysis. Patients with high pre-VEGF levels were at greater risk for developing peritoneal metastases than patients with low levels (p value for the log rank test=0.023), shown in Figure 1.

Estimation of Hazard Ratios (HRs) with a Cox proportional hazards regression model showed that low pre-VEGF was a protective prognostic factor, with a relative risk of 0.229 (p=0.039), which represents a risk reduction of 73% for the occurrence of peritoneal metastases, shown in Table 2.

Table 1. Demographics and mean serum VEGF levels of the cohort.

Age at surgery			
< 71	31	404.52 ± 57.48	329.17 ± 51.24
> 71	28	416.39 ± 58.42	298.29 ± 51.81
P-value		0.885	0.674
Sex			
Male	42	442.52 ± 52.70	294.04 ± 46.37
Female	17	330.17 ± 51.43	361.00 ± 50.99
P-value		0.214	0.405
Localización			
Cardia	9	346.33 ± 130.09	286.60 ± 124.97
Fundus	5	373.00 ± 126.51	229.50 ± 31.83
Body	11	464.09 ± 86.06	350.62 ± 68.73
Antrum	30	424.43 ± 58.46	324.17 ± 54.86
Undefined	4	130	--
P-value		0.605	0.755
Histologic Grade			
G1	26	438 ± 65.37	322.47 ± 51.10
G2	17	470.44 ± 82.56	320.22 ± 81.49
G3	10	351.10 ± 83.01	343.83 ± 76.88
P-value		0.578	0.82
Primary Tumor			
Tis	1	271	212
T1	4	203.75 ± 81.23	186.50 ± 39.21
T2	3	491.67 ± 151.20	350.50 ± 183.50
T3	41	428.68 ± 50.50	337.48 ± 44.83
T4	10	406.20 ± 107.08	285.50 ± 183.5
P-value		0.489	0.745
Regional lymph nodes			
N0	8	493.00 ± 178.39	292.00 ± 114.17
N1	20	327.05 ± 44.03	296.83 ± 48.41
N2	14	465.43 ± 85.08	302.22 ± 69.41
N3	17	423.41 ± 76.23	390.17 ± 82.83
P-value		0.664	0.571
Recurrence (locoregional or metastatic disease)			
No	37	411.89 ± 353.43	332.37 ± 220.90
Yes	22	407.22 ± 234.83	282.07 ± 56.83 (14)
P-value		0.351	0.47
Developing of peritoneal carcinomatosis			
No	50	368.14 ± 298.78	309.53 ± 214.08
Yes	9	643.55 ± 296.34	333.33 ± 206.04
P-value		0.026	0.806

Discussion and conclusion

VAlthough, in recent decades, there has been a trend towards improved global survival and a decreased mortality rate of gastric cancer [14], the percentage of patients who develop peritoneal metastases, remains high and unchanged [4].

On the other hand, the trend in gastric cancer incidence in young adults (less than 40 years old) is slightly increasing in western countries, such as the United States [7]. Furthermore, there is a direct relationship between the risk of developing metachronous peritoneal metastases and young age in gastric cancer [6,15]. Based on these data, an increase in the incidence of peritoneal carcinomatosis in young patients with gastric cancer is expected.

Currently, the treatment of peritoneal metastases of gastric origin has poor outcomes. Only patients with limited peritoneal carcinomatosis (PCI below 7) have improved outcomes by cytoreductive and HIPEC approaches [16]. So, early detection of peritoneal metastases is critical. However, imaging tests have limited efficacy in the early diagnosis of peritoneal carcinomatosis [17].

The search for a measurable risk marker to support the early diagnosis of peritoneal metastases in gastric cancer patients led us to consider the usefulness of circulating VEGF. Over-expression of VEGF in gastric cancer cells and circulating levels of VEGF have been shown to be useful prognostic factors in gastric cancer [18-20].

The relationship between peritoneal metastases and overexpression of VEGF in surgical samples of gastric cancer has also described [21,22], but the main problem of the immunohistochemical studies is their lack of usefulness as risk markers in the follow-up of patients. However, serum VEGF meets the suitability criteria of biomarkers, since it comes from a reliable source, is readily available by non-invasive methods, is easily detectable by simple laboratory methods and can be quantified during patient follow-up.

Our results show that high levels of preoperative serum VEGF is a prognostic factor for the development of peritoneal carcinomatosis. Moreover, these results have a clear physio-pathological support described in previous studies.

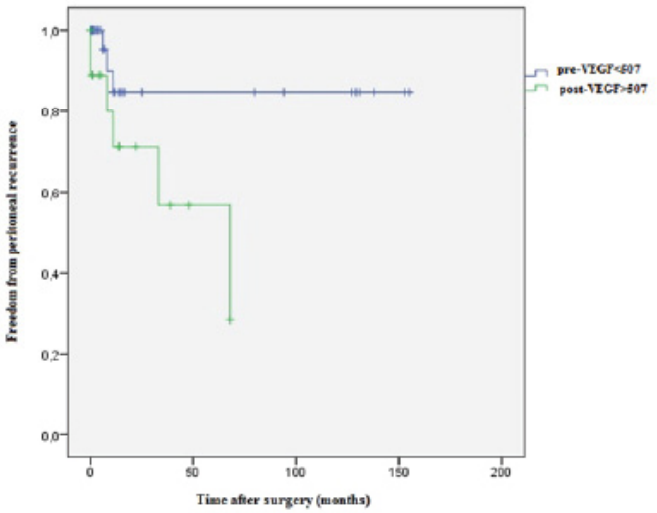

Figure 1. Kaplan–Meier estimates of the probability of peritoneal metastases according to preoperative serum vascular endothelial growth factor (pre-VEGF) levels (low, 507 pg/mL or less; high, over 507 pg/mL) P = 0.023.

Table 2. Estimation of relative risk by univariate Cox proportional hazards regression model. Pre-VEGF: Preoperative Serum VEGF, SE: Standard Error, df: degree freedom, P: P-value, CI: Confidence Interval, HR: Hazard Ratio.

Pre-VEGF<507	β	SE	Wald	df	P	HR	95% C.I. for HR Lower	Upper
	-1.473	0.714	4.256	1	0.039	0.229	0.057	0.929

The spread of peritoneal metastases is determined by the reciprocal interaction between the tumour microenvironment (which includes tumour-cells and tumour-stroma) and peritoneal mesothelial cells existing in healthy peritoneal tissues. This interaction is regulated by growth factors, such as VEGF, which pre-condition the peritoneal surfaces for the settlement of tumour cells, according to the current interpretation of Paget's "seed and soil" theory [10,23].

In this way, VEGF acts by triggering angiogenesis and promoting tumour cell migration in the tumour microenvironment, but also by conditioning the pre-metastatic niche in distant peritoneal tissues. The VEGF signalling pathway also regulates the immune response of the host tissue and promotes the survival of the stem cells. [24,25]. Ultimately, the VEGF pathway modulates the relationships between tumour cells and peritoneal tissue and plays an important role in the development of peritoneal metastases.

Study Strengths and Limitations: The major limitations of this study are its retrospective and single-centre nature, as well as the limited number of patients.

In conclusion, preoperative serum VEGF levels meet the requirements of a predictive prognostic factor for the development of peritoneal metastases in gastric cancer and may be useful for close follow-up of patients with gastric cancer at high risk of peritoneal carcinomatosis.

Acknowledgements

The authors would like to thank Dr. Teodoro Palomino-Muñoz (Clinical Analysis Service, Ciudad Real University Hospital) for his scientific and technical support.

Statement of Ethics

All patients have given their written informed consent. This study was approved by the Clinical Research Ethics Committee of the Ciudad Real General Hospital. The research was conducted ethically in accordance with the World Medical Association Declaration of Helsinki.

Funding Sources

PThis study was funded by the Castilla La Mancha Health Service (SESCAM)..

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*Correspondence: Pedro Villarejo-Campos, Department of Peritoneal Cancer Surgery, Fundación Jiménez Díaz University Hospital, Spain, Tel: +34 665664830; E-mail: villarejocampos@yahoo.es

Rec: 05 Jun 2020; **Acc**: 26 Jun 2020; **Pub**: 29 Jun 2020

J Cancer Sci Therap. 2020;3(2):21

DOI: 10.36879/JCST.20.000121