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Original Articles

Vascular endothelial growth factor (VEGF) and cyclooxygenase 2 (COX 2) immunostaining in ovarian cancer

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Summary

Purpose of investigation: Vascular endothelial growth factor (VEGF) and cyclooxygenase-2 (COX 2) are markers of angiogenesis and potential therapeutic targets. Previous studies demonstrate that VEGF is upregulated in some ovarian cancers. The purpose of this study was to determine the correlation of VEGF and COX 2 staining with survival in ovarian cancer patients. *Materials and Methods:* One hundred forty-three consecutive patients with ovarian carcinoma underwent primary staging or cytoreduction prior to platinum-based chemotherapy. Their tumors were immunohistochemically stained for expression of VEGF and COX 2. FIGO stage, grade, cytoreduction status, and histology were also analyzed as prognostic factors. *Results:* Twenty-seven patients had Stage I tumors, three Stage II, 87 Stage III, and 26 Stage IV. Median follow-up was 74 months (mean 79 months). One hundred nineteen patients (83.2%) had tumors that were positive for VEGF and 110 patients (76.9%) had tumors that were positive for COX 2. Patients with tumors staining positive for both VEGF and COX 2 (68.5%) had a significantly increased risk of dying from their ovarian cancer (Chi-square $p = 0.011$, Log rank $p = 0.037$). Multivariate logistic regression analysis revealed FIGO stage, grade, cytoreduction status, and VEGF/COX 2 expression to be independent prognostic indicators of survival. *Conclusion:* VEGF and COX 2 staining are frequently positive in ovarian cancer. Patients whose tumors are positive for both VEGF and COX 2 have a decreased survival. These patients may benefit from anti-angiogenesis targeted therapy.

Key words: VEGF; COX 2; Ovarian cancer.

Introduction

Ovarian cancer is a devastating disease affecting approximately 20,000 and killing approximately 14,000 women in the United States each year [1]. Studies have indicated that the gynecologic oncology training of the surgeon, rather than biologic aggressiveness, defines whether a patient's tumor can be optimally debulked [2]. Biologic aggressiveness, however, is important in a patient's prognosis and can be studied indirectly by the immunohistochemical expression of specific proteins [3]. Vascular endothelial growth factor (VEGF) is one such protein [4]. It is a marker of biologic aggressiveness and is closely related to tumor angiogenesis [5]. VEGF participates in ovarian cancer growth, metastasis, and associated complications [6]. It is an important part of the development of pleural effusions in both the benign and malignant setting, as well as ascites [6, 7]. Elevations have been documented in the setting of acute inflammation [8,9].

Cyclooxygenase-2 (COX-2) is another surrogate marker of tumor aggressiveness, as it is involved in regulating angiogenesis as well as tumor cell proliferation [10]. It is also implicated in inflammation. In ovarian cancer, higher expression appears to decrease patients' overall survival [11]. As results have varied from study to study as to whether certain angiogenic markers alone are accurate prognostic indicators, the authors wanted to determine if combining VEGF and COX-2 would be useful in predicting survival.

Materials and Methods

Patients' records were examined to determine all patients undergoing primary cytoreductive surgery for ovarian cancer. Institutional Review Board approval was obtained. One hundred and forty-three consecutive patients with epithelial ovarian carcinoma had their tumor's VEGF and COX 2 staining studied by image analysis. The hospital, office, and tumor registry records were examined for FIGO stage, grade, histology, level of cytoreduction, and survival. No tumors of low malignant potential were included. A further criterion was that the initial surgical procedure, prior to chemotherapy, had to be performed by one gynecologic oncologist. Since only one surgeon's patients were studied, the level of optimal cytoreduction achieved during surgery remained the same throughout the years of the study and was determined by the amount of unresectable tumor left after primary surgery. Optimal cytoreduction was achieved when no gross residual tumor mass greater than one cm in diameter remained following surgery. All patients, other than those with FIGO Stage IA or IB grade 1 tumors, were treated with either six courses of platinum based chemotherapy.

VEGF and COX 2 immunohistochemical staining was performed on the primary cancer for all patients. Immunohisto-

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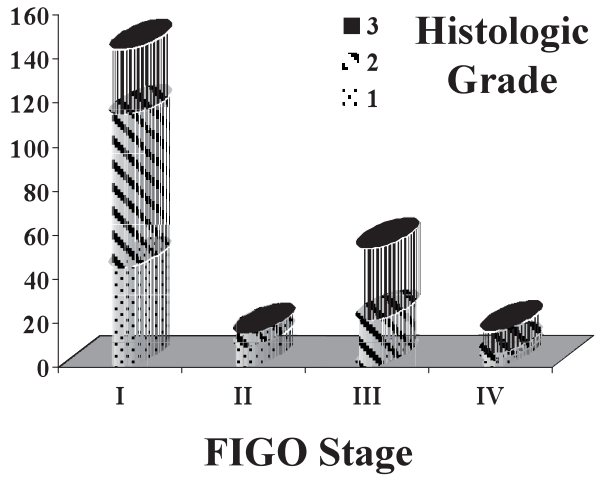

Figure 1. — FIGO stage in relation to grade.

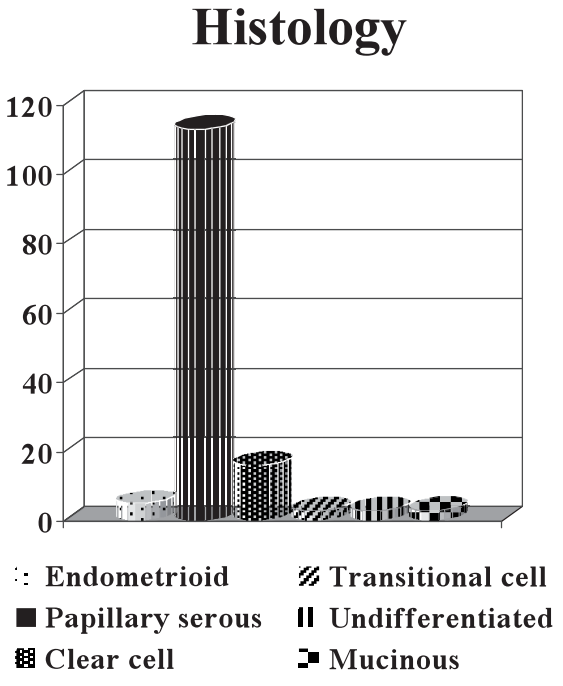

Figure 2. — Histological types.

chemical staining for the VEGF polyclonal antibody was performed according to published protocols [12,13]. Immunohistochemical staining for COX2 was also performed according to previously published protocols [14].

Statistics were performed utilizing SPSS, version 7.5, namely, Student's *t*-test, one-way analysis of variance (one-way ANOVA), or cox-regression analysis.

Results

One hundred forty-three women had epithelial ovarian cancer. Twenty-seven patients had Stage I tumors, three

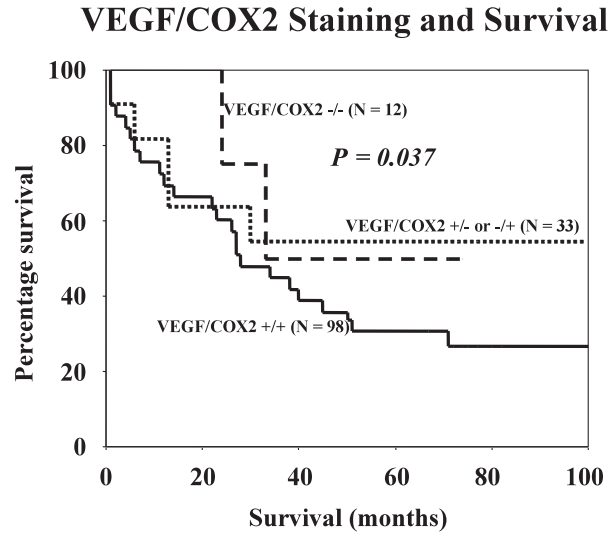

Figure 3. — Survival in relation to immunohistochemical staining.

Table 1. — *Ovarian cancer immunohistochemical staining.*

Staining	Positive (%)	Negative (%)
VEGF	114 (83.2%)	29 (16.8%)
COX2	110 (76.9%)	33 (24.1%)

Table 2. — *Multivariate analysis.*

Factor	*p* value
Grade	0.041
Stage	0.0047
Optimal cytoreduction	0.038
VEGF staining	0.048
COX 2 staining	0.021
VEGF/COX 2 staining	0.018

Stage II, 87 Stage III, and 26 Stage IV (Figure 1). Most of the patients had grade 2 or 3 disease (Figure 1). The most common histologic type was serous with the second most common being clear cell (Figure 2). Median follow-up was 74 months (mean 79 months).

One hundred nineteen patients (83.2%) had tumors that were positive for VEGF and 110 patients (76.9%) had tumors that were positive for COX 2 (Table 1). There was no difference in staining of either protein in the 114 patients that underwent optimal cytoreduction compared to the 29 that had suboptimal cytoreduction.

Patients with tumors staining positive for both VEGF and COX 2 (68.5%) had a significantly increased risk of dying from their ovarian cancer (Chi-square $p = 0.011$, Log rank $p = 0.037$). Multivariate analysis revealed FIGO stage, grade, cytoreduction status, and VEGF/COX 2 expression to be independent prognostic indicators of survival (Figure 3).

Discussion

In this study, the authors evaluated the relationship of the combination of VEGF and COX-2 positive epithelial ovarian tumors on patient survival. Their results indicate that positive VEGF and positive COX-2 staining in combination are significantly more effective in predicting patient mortality than either alone, although both are independent prognostic indicators of survival. The present results also show that FIGO stage, grade, and cytoreduction status are independent prognostic indicators as well, which supports the current literature [15, 16]. These findings are important not only for prognostic reasons, but possibly for targeted treatment as well. Research on anti-angiogenic treatments has become increasingly more common and results hold promise. For recurrent epithelial ovarian cancer, bevacizumab, a VEGF-directed antibody, improves progression-free survival significantly in women treated with it in combination with paclitaxel than paclitaxel alone [17]. Bevacizumab has also been tested in trials as an added agent with carboplatin and paclitaxel, and this combination proved to be more effective in increasing progression-free survival than chemotherapy alone [18]. Used by itself, bevacizumab had a response rate of 16-21% in phase II trials in women with epithelial ovarian cancer, and several other trials have also shown the benefit of bevacizumab as maintenance therapy [19, 20].

Studies evaluating the efficacy of VEGF inhibitors have been ongoing for some time, while those assessing the usefulness of COX-2 inhibitors are more recent in the literature. However, research in the past few years suggests that COX-2 inhibitors also have promise in the fight against ovarian cancer. In a study of mice with a human ovarian carcinoma cell line, Li *et al.* found that a combined therapy of taxol with COX inhibitors was more effective than taxol monotherapy. The authors suggested that this was due to decreased angiogenesis, decreased cell proliferation, and increased apoptosis [21]. In humans, epidemiologic studies have suggested risk reduction for ovarian cancer in women who used selective COX-2 inhibitors [22].

Conclusion

Additional research investigating the efficacy of COX inhibitors is greatly needed, as well as the possibility of combining them with a drug, such as bevacizumab and current chemotherapies. The present authors hope that the results of this study will aid in choosing patients appropriate for these targeted therapeutic options.

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