

**VASCULAR ENDOTHELIAL
GROWTH FACTOR AND ITS
RECEPTORS IN TUMOR
TISSUE AND BLOOD SERUM
OF PATIENTS WITH
OVARIAN NEOPLASMS:
CLINICO-PATHOLOGIC
ANALYSIS**



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Background and introduction

- ❖ Angiogenesis has a vital role in tumour growth and metastasis. *Vascular endothelial growth factor (VEGF) acting through its specific tyrosine kinase receptors (VEGFR1 and VEGFR2) is a potent endothelial cell mitogen* and is an important component of the angiogenic stimulus in a range of human neoplasms.
- ❖ The influence of VEGF in ovarian cancer remains controversial. But in experimental studies it was demonstrated that VEGF-A and its main signaling receptor VEGFR2 are coexpressed in primary ovarian tumors, ascitic cells and metastases, suggesting the existence of an autocrine VEGF-A/VEGFR2 loop in ovarian cancer cells and its role in protecting them from anoikis and apoptosis.
- ❖ Hence, it was proposed that treating ovarian cancer patients with VEGF blocking agents may potentially reduce peritoneal dissemination by decreasing vascular permeability as well as inducing apoptosis of shed ovarian cancer cells in ascites.
- ***The aim of this study*** was to compare tumor and serum levels of VEGF and its type 1 (VEGFR1) and type 2 (VEGFR2) receptors in ovarian cancer, borderline and benign tumors, and to analyze associations of these markers with ovarian cancer clinico-pathologic features.

Patients and methods

- ❖ The study enclosed 92 patients with ovarian tumors (60 malignant, 11 borderline and 21 benign), and 32 control female patients. Blood serum was obtained by standard procedure before the onset of treatment.
- ❖ Cancer tissues obtained at surgery were lysed in 1:3 ratio in *20 mM Tris-HCl pH 7,5 buffer containing 150 mM NaCl, 1 mM EDTA, 1 mM EGTA, 1% Triton X-100, 2,5 mM sodium pyrophosphate, 1 mM β -glycerophosphate, 1 mM sodium orthovanadate, and 1 μ g/ml leupeptin*, and centrifuged for 30 min at 20000 rpm, 4°C. The supernatant was used for ELISA tests, and the results were normalized using protein concentration in the tissue extracts.
- ❖ **Marker levels in tissue extracts and sera were determined with standard ELISA kits (*Quantikine®*, R&D Systems) listed below:**
 - **«Human VEGF Immunoassay»**
 - **«Human VEGF R1 Immunoassay»**
 - **«Human VEGF R2 Immunoassay»**
 - **«Human MMP-2 total»**

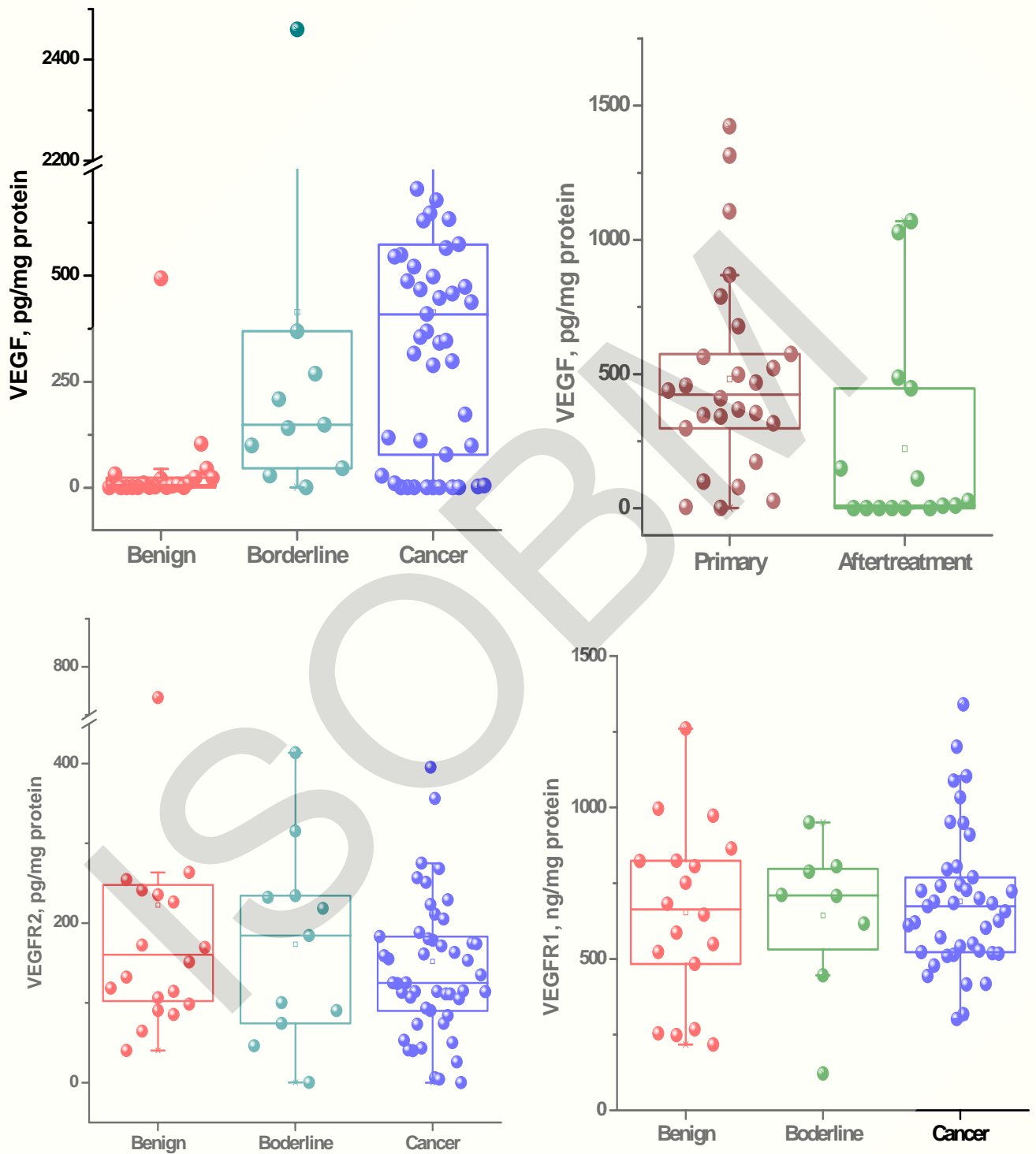


Figure 1. VEGF, VEGFR1 and VEGFR2 levels in ovarian cancer, borderline and benign tumor lysates.

Results (1): general observations

- ❖ **VEGF** content was significantly increased in malignant and borderline as compared to benign ovarian tumors ($p < 0.001$), while no differences were observed for **VEGFR1** and **VEGFR2** (**Fig. 1**).
- ❖ Serum **VEGF** concentration in ovarian cancer patients was significantly higher than in control ($p < 0.001$) and in borderline ($p < 0.01$) groups, but the difference with benign group was not statistically significant. (**Fig. 2**).
- ❖ On the contrary, serum **VEGFR2** level in ovarian cancer and benign tumor groups was decreased as compared to control ($p = 0.05$) and **VEGFR1** levels in all the groups were similar (**Fig. 2**).
- ❖ Weak **positive association between tumor and circulating marker levels** was demonstrated only for **VEGF** ($R = 0.22$) in the general group, but it was mostly pronounced in patients with **benign tumors** ($R = 0.49$; $p < 0.05$), and negligible in ovarian cancer patients.

Results (2): clinico-pathologic data

- ❖ 67% of ovarian cancer patients were newly diagnosed and their markers were studied before the onset of any treatment. **VEGF content in the tumors** (but not in blood serum) of these patients was significantly higher than in those investigated after treatment (**Fig. 1**). No other differences between these groups were revealed.
- ❖ The markers' levels in the tumors and serum were analyzed in relation to the main clinico-pathologic features of ovarian cancer: **FIGO stage, tumor size, histologic type and differentiation, lymph node involvement, presence of distant metastases and/or ascites**, but **no significant associations were revealed**.
- ❖ 79% of primary ovarian cancer patients had **CA125** serum levels >100 U/ml, and other 12% had CA125 between 36 and 100 U/ml. **No correlations** were found between serum CA125 levels and any of the markers of VEGF signaling cascade studied.

Conclusions

- ❖ A prominent increase of **VEGF concentration** is observed both in the tumors and blood sera of ovarian cancer patients not depending on clinical stage, tumor grade or histology and other disease manifestations including the level of classical ovarian cancer marker CA125.
- ❖ Soluble **VEGFR2** level in peripheral blood is decreased in patients with both malignant and benign ovarian tumors.
- ❖ Thus, none of the parameters studied can be regarded as a diagnostic marker for ovarian cancer.
- ❖ Though we could not find any associations between **VEGF/VEGFRs** levels in tumor tissue confirming the existence of autocrine loop demonstrated in experimental studies, the increase of VEGF may indicate to its possible role as a **target for specific therapy** of ovarian cancer.

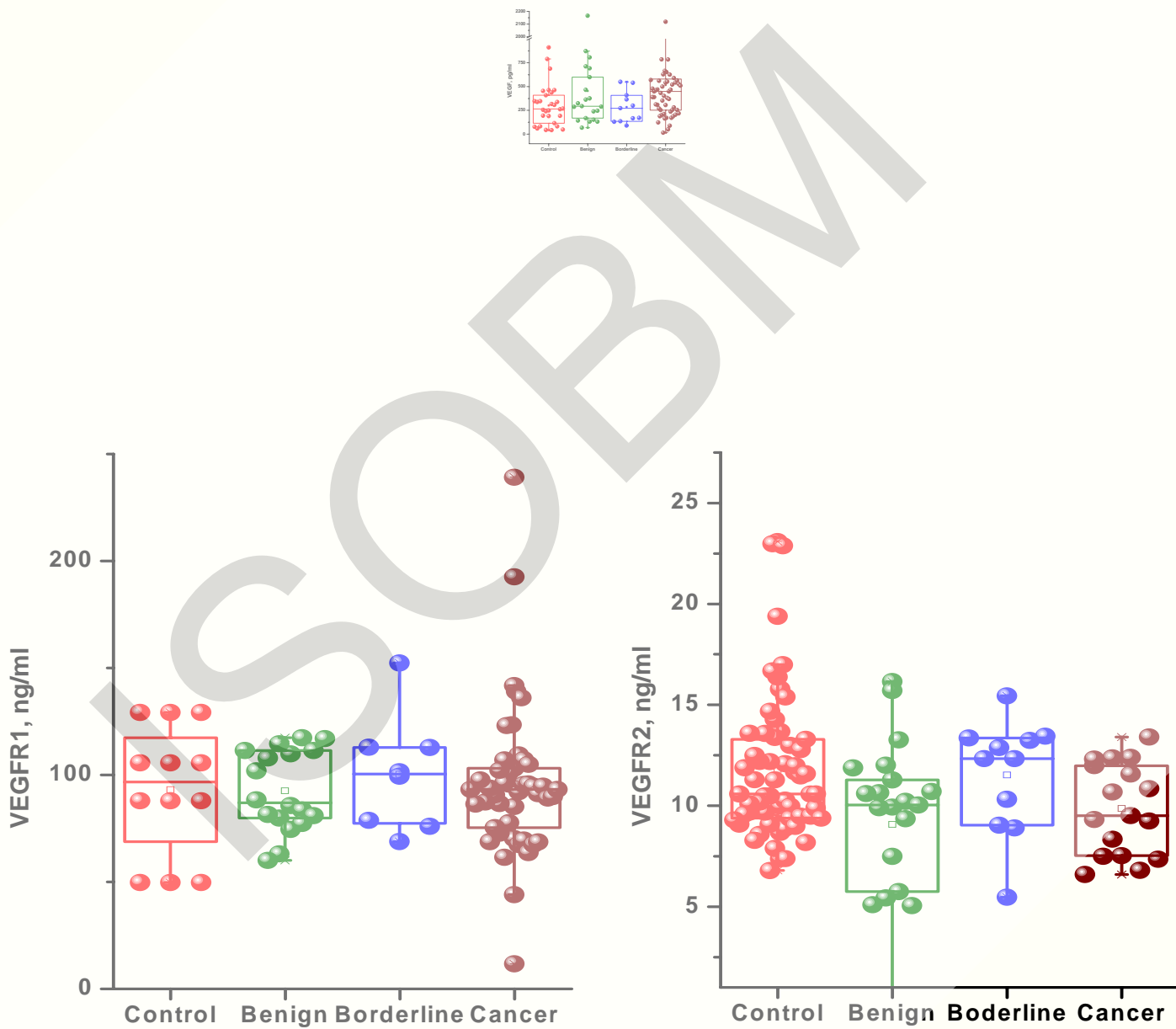


Figure 2. VEGF, VEGFR1 and VEGFR2 concentrations in blood serum of ovarian cancer, borderline and benign tumor patients, and in control group.

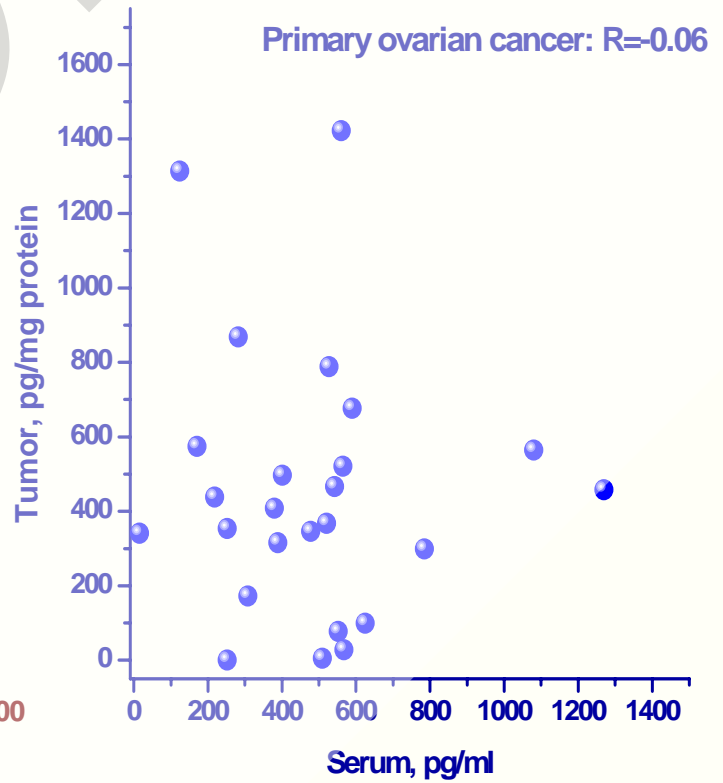
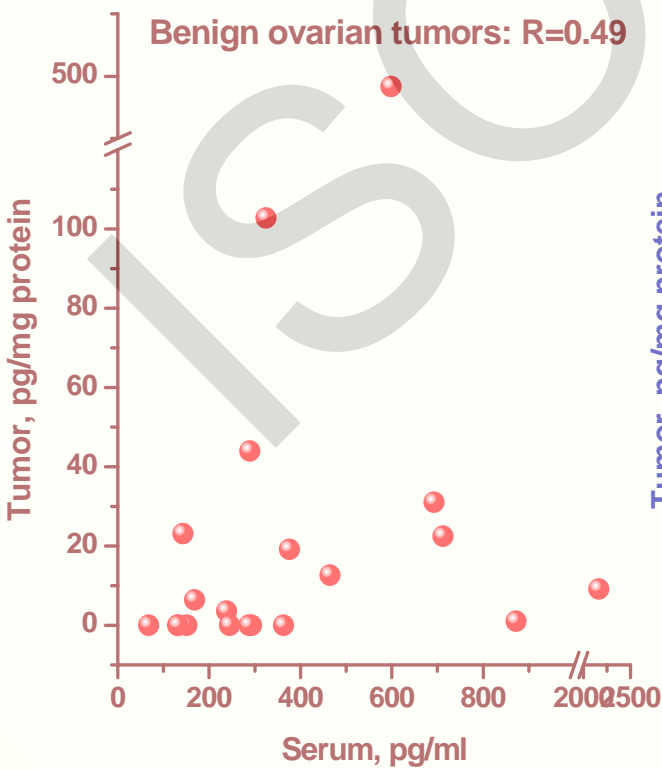
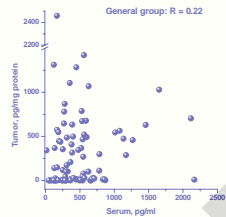
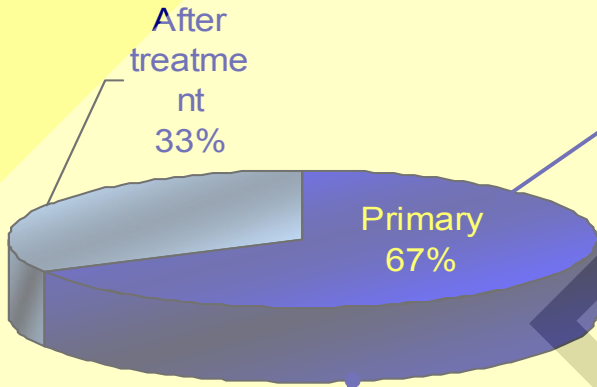


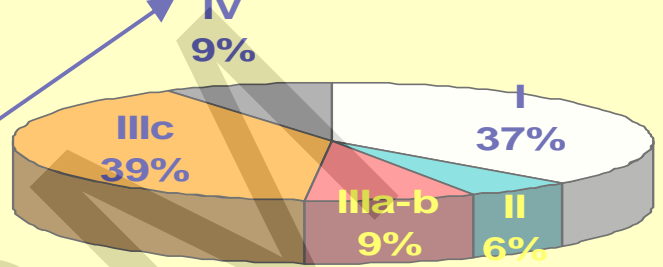
Figure 3. Relationship between tumor and serum VEGF concentrations in various patients' groups.

Main clinico-pathologic characteristics of ovarian cancer group

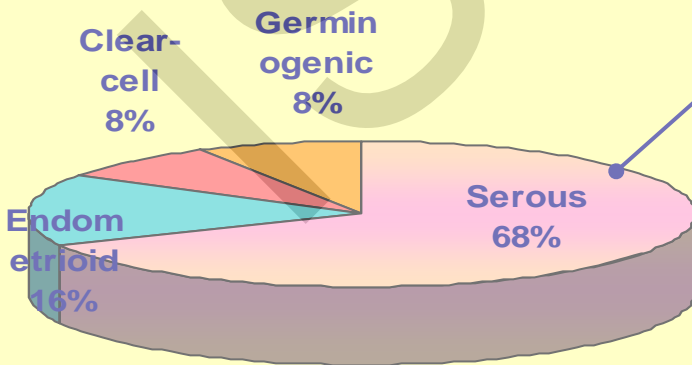
Treatment before examination



FIGO stage



Histologic type



Differentiation

