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Plasma and Immunohistochemical Biomarkers of Epithelial Ovarian Cancer

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Abstract

Introduction: Epithelial Ovarian Cancer (EOC) is one of the most common malignancies. Growth factors such as Macrophage Colony-Stimulating Factor (M-CSF) and Vascular Endothelial Growth factor (VEGF) are involved in the pathogenesis and spread of cancer. Matrix Metalloproteinases (MMPs) participate in tissue re-modelling, stimulate neo-vascularisation and inflammatory response. TIMPs are protease inhibitors of MMPs.

Methods: We compared the concentration of plasma levels and tissue expression of M-CSF, VEGF, MMP-2, MMP-9, TIMP-1 and TIMP-2 in patients with Epithelial Ovarian Cancer (EOC) and in patients with ovarian cysts (50 people each). Plasma levels of tested proteins were determined by ELISA, tissue expression was evaluated by immunohistochemical technique.

Discussion: Plasma levels of M-CSF, VEGF, MMP-9 and TIMP-1 in EOC group were statistically significantly higher (in all cases $p<0.05$) when compared to the ovarian cysts group. The expression of all proteins was observed predominantly in epithelial cells. We noted significant differences when the tissue expression of MMP-2, MMP-9 and TIMP-2 in EOC patients was compared with that of ovarian cysts patients. Interestingly, that data revealed correlation of cytokine M-CSF between plasma levels and epithelial ovarian cancer cells expression.

Conclusion: Our findings suggest that M-CSF, VEGF, MMP-9 and TIMP-1 might play a role in the development of ovarian cancer. Moreover, only M-CSF showed the positive correlations between the plasma concentrations and tissue expression which can indicate a potential role of this cytokine in laboratory diagnostics of EOC.

Keywords: Growth factor; Tissue expression; MMPs; TIMPs; Ovarian cancer

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Abbreviations: ELISA: Enzyme-Linked Immunosorbent Assay; ECM: Extracellular Matrix; EOC: Epithelial Ovarian Cancer; FIGO: International Federation of Gynecology and Obstetrics; LMP: Low Malignant Potential; M-CSF: Macrophage-Colony Stimulating Factor; MMP-2: Metalloproteinase 2; MMP-9: Metalloproteinase 9; OC: Ovarian Cancer; TIMP-1: Tissue Inhibitor of Metalloproteinase 1; TIMP-2: Tissue Inhibitor of Metalloproteinase 2; VEGF: Vascular Endothelial Growth Factor; VEGFR: Vascular Endothelial Growth Factor receptor.

Introduction

Ovarian cancer remains the fifth leading cause of death in gynecological malignancies [1]. A lack of sensitive and specific biomarkers or specific symptoms in the early stages of this type of cancer considerably delays diagnosis. Unfortunately, the majority of patients with ovarian cancer are asymptomatic at the early stages of the disease and screening methods in such patients have been ineffective [2]. Early metastasis of ovarian cancer cells suggests that the formation of new blood vessels is an important step in disease progression [3]. Several studies have examined the role of growth factors, matrix metalloproteinases and tissue inhibitors of metalloproteinases in this process.

Vascular endothelial growth factor (VEGF) is a key pro-angiogenic factor which is produced and released by tumor cells in response to hypoxia. It plays an important role in tumor growth, invasion, and in the development of metastases [4]. It is normally produced by endothelial cells, myocytes, macrophages, lymphocytes (CD4), plasma cells and megakaryocytes [5]. VEGF binds to and activates VEGFR-1 and VEGFR-2, located on the endothelial cells of preexisting blood vessels. When this growth factor binds to its corresponding specific receptor, various signal pathways are activated to promote the activation of endothelial cells [6]. VEGF is the main factor that stimulates angiogenesis and its release is mediated by MMPs. In particular, binding of VEGF to extracellular matrix components appears to be the key aspect of the factor's action [7,8]. Another hematopoietic growth factor, macrophage colony-stimulating factor (M-CSF), may be a high specificity marker for ovarian cancer. It is produced in vitro by fibroblasts, endothelial cells, thymic epithelial cells, Natural Killer cells, monocyte-macrophages, marrow stromal cells, B and T-cells, osteoblasts, astrocytes, microglia, neurons, polymorph nuclear cells and keratinocytes [9]. One study has revealed that M-CSF is localized in glandular epithelial cells, stromal macrophages and the endothelial cells of ovarian cancer [10].

Numerous studies have demonstrated that the invasion and metastatic capacity of ovarian cancer cells is closely associated with the degradation of extracellular matrix and components of the basement membrane by matrix metalloproteinases (MMPs) [11]. To date, more than 20 members of the MMP family have been identified and the majority of them have been studied in various human cancers [12]. They are produced by fibroblasts, mast cells, osteoblasts, odontoblasts, dendritic cells, microglia cells, smooth muscle myocytes, keratinocytes and endothelial cells [13-15]. MMP-2 and MMP-9 are the main two enzymes responsible for the degradation of collagen and other proteins in the extracellular matrix [16]. The expression of MMP-2 and MMP-9 has been shown to be related to tumor aggressiveness and metastasis of different types of tumor cells, including ovarian cancer [17-19]. Additionally, MMP-2 expression has been demonstrated to be localized in stromal areas with maximal expression in the areas adjacent to the ovarian neoplasm. However, MMP-9 expression has been found to be associated with cells in epithelial and stromal areas, consistent with the distribution of macrophages [20].

MMPs activity is regulated at different levels including transcriptional expression, proteolytic activation and, predominantly, by tissue inhibitors of MMPs (TIMPs) [7]. There are four members of the TIMP family, TIMP-1, -2, -3 and -4, each participating in tissue re-modelling of the extracellular matrix (ECM) by inhibiting the activity of various MMPs and produced mainly by the same cells as MMPs [21-23]. TIMPs have been recognized as multifunctional enzymes in cancer and their role is controversial since they may also promote the growth of malignant cells [24,25].

Our study was designed to assess VEGF, M-CSF, MMP-2, MMP-9, TIMP-1 and TIMP-2 levels in the plasma of patients with ovarian cancer and ovarian cysts. The study also evaluated immunohistochemical staining in the stroma and epithelium of growth factors, matrix metalloproteinases and their tissue inhibitors in ovarian cancer and ovarian cysts. We also wanted to evaluate whether plasma levels of these proteins reflect their expression in tissues and whether this expression is associated with protein production by cancer cells, stromal cells or whether it is associated with an entirely different process, unrelated to pathological changes (e.g. disintegration of normal cells).

Materials and methods

Human Subjects: The study included 50 patients with ovarian cancer and 50 patients with ovarian cysts who had been referred to the Department of Gynecology. The clinical stage and histological classification are determined in accordance with the International Federation of Gynecology and Obstetrics (FIGO) criteria in all cases (Table 1). The study was approved by the local Ethics Committee (R-I-002/239/2014) and all the patients gave their informed consent for study participation.

ELISA method: The tested parameters (M-CSF, VEGF, MMP-2, MMP-9, TIMP-1, TIMP-2) were measured in plasma with enzyme-linked immunosorbent assay (ELISA) (Quantikine Human M-CSF Immunoassay; R&D systems, Abingdon, United Kingdom) according to the manufacturer's protocols and duplicate samples were assessed for standard and samples. This assay employs the quantitative sandwich enzyme immunoassay technique. The intra-assay coefficient of variation (CV%) of M-CSF is reported to be 3.4% at a mean concentration of 227 pg/mL, SD=7.7. VEGF is reported to be 4.5% at a mean concentration of 235 pg/mL (SD = 10.6). MMP-2 is reported to be 3.8% at a mean concentration of 11.20 ng/mL, SD=0.42, MMP-9 is reported to be 1.9% at a mean concentration of 2.04 ng/mL, SD=0.039. TIMP-1 is reported to be 3.9% at a mean concentration of 1.27 ng/mL, SD=0.05; TIMP-2 is reported to be 6.0% at a mean concentration of 2.90 ng/mL, SD=0.173.

The inter-assay CV% of M-CSF is reported to be 3.1% at a mean concentration of 232 pg/mL (SD = 7.3); VEGF is reported to be 7.0% at a mean concentration of 250 pg/mL (SD = 17.4). MMP-2 is reported to be 6.6% at a mean concentration of 11.10 ng/mL, SD=0.738, MMP-9 is reported to be 7.8% at a mean concentration of 2.35 ng/mL, SD=0.184. TIMP-1 is reported to be 3.9% at a mean concentration of 1.28 ng/mL, SD=0.05; TIMP-2 to be 6.7% at a mean concentration of 2.79 ng/mL, SD=0.188.

The value of intra- and inter- assay CVs were calculated by the manufacturers and enclosed in the reagent kits. The assay does not exhibit cross-reactivity or interference with numerous human cytokines and other growth factors.

Immunohistochemistry: Formalin-fixed and paraffin-embedded tissue specimens cut on a microtome into 4 µm sections were deparaffinized in xylenes and hydrated in alcohols. In order to retrieve antigens, sections were heated in a water bath in EDTA buffer pH = 9 (M-CSF, VEGF and TIMP-1 antigens) and citrate buffer pH = 8 (MMP-2 and TIMP-2 antigens) at 99.5°C for 20 minutes. For the MMP-9 antibody, the step of antigen retrieval was not required. Endogenous peroxidase was blocked in 3% H_2O_2 for 5 minutes. Next, they were incubated with anti-human antibodies: rabbit polyclonal antibody of M-CSF (clone ab9693, Abcam, dilution 1:100) for 60 minutes; mouse monoclonal antibody of VEGF (clone #26503, R&D Systems, UK; dilution 1:100) for 60 minutes; mouse monoclonal antibody of MMP-2 (clone 17B11, Leica, UK; dilution 1:60) overnight; mouse monoclonal antibody of MMP-9 (clone 15W2, Leica, UK; dilution 1:80) for 60 minutes; mouse monoclonal antibody of TIMP-1 (clone 6F6a, Leica, UK; dilution 1:750) for 15 minutes; mouse monoclonal antibody of TIMP-2 (clone 46E5, Leica, UK; dilution 1:20) for 60 minutes.

The reaction was visualised with a detection kit NovoLink Polymer (Novocastra, Poland) and DAB chromogen (Novocastra, Poland). Cellular nuclei were stained with hematoxylin. Following that, the slides were dehydrated in alcohols, rinsed in xylenes and closed in a DPX medium. Positive and negative controls were performed according to the manufacturer's recommendations. Stained preparations were observed under a light microscope. The positive reaction of the investigated proteins was observed in the cells'cytoplasm (magnification x200).

Expression was evaluated using an immunoreactive score that represented the percentage of positive cells (0, none; 1, <25%; 2, 25–50%; and 3, 50–100%) and a staining intensity (0, no staining; 1, light, yellow staining; 2, moderate yellow staining; and 3, strong, brown staining). The reported score for each patient was the average of the scores in five areas of the slide. The total score ranged from 1 to 12 and protein expression was considered as none (-), 0; weak (+), score1-4; moderate (++), score 5-6; and strong (+++), 9-12 score.

Statistical analysis: Statistical analysis was performed by using STATISTICA 13.0 (StatSoft, Tulsa, OK, USA). A preliminary statistical analysis revealed that the levels of the growth factors, metalloproteineses and their inhibitors did not follow a normal distribution. Consequently, statistical analysis between the examined groups of patients was performed using the U Mann-Whitney test and Kruskal-Wallis test, and a multivariate analysis of various data using the post-hoc Dwass-Steele-Crichlow-Flinger test. The data were presented as median and range.

Comparative analyses of tissue expression in epithelial cells and stromal cells was performed using Wilcoxon matched-pairs test. Pearson's chi-squared test was used to perform analyses between tissue expression of all the tested parameters in endothelial cells in the tested groups. Statistically significant differences were defined as comparisons resulting in $p<0.05$.

Results

Plasma levels of M-CSF, VEGF, MMP-2, MMP-9, TIMP-1 and TIMP-2 in ovarian cysts group vs. ovarian cancer group: The median and range of the investigated parameters' plasma levels (M-CSF, VEGF, MMP-9 and TIMP-1) showed statistically significantly lower concentrations ($p<0.05$ in all cases) in ovarian cysts patients when compared to ovarian cancer patients. In the case of MMP-2 and TIMP-2, we did not observe a statistical dependence (Table 2).

Immuno-histochemical expression of tested parameters in epithelial ovarian cancer cells and in epithelial cysts cells: We observed statistically significantly higher expression of all the tested parameters in epithelial cells in comparison to stromal cells in ovarian cysts cases. Moreover, we observed statistically significantly higher expression of M-CSF, MMP-2, MMP-9, TIMP-1 and TIMP-2 in epithelial cells in comparison to stromal cells in ovarian cancer cases (Table 3; Figures 1-2). Additionally, we noticed statistically significantly lower expression of MMP-2 and TIMP-2 and statistically significantly higher expression of MMP-9 in epithelial cells of ovarian cysts patients in comparison to ovarian cancer patients ($p=0.001$; <0.001; 0.001, respectively). In the case of stromal cells, we observed statistically significantly higher expression of MMP-9 in cysts when compared to ovarian cancer ($p=0.002$) (Table 3; Figure 2).

The Spearman's rank correlation: The correlations between plasma concentrations of the tested proteins are presented in Table 4. We found a positive correlation between VEGF and MMP-9, VEGF and TIMP-1, MMP-9 and TIMP-1, MMP-2 and TIMP-2 in the plasma of both ovarian cysts and ovarian cancer patients (cysts: $R=0.41$, $p=0.003$; $R=0.57$, $p<0.001$; $R=0.39$, $p=0.006$; $R=0.47$, $p=0.001$, ovarian cancer: $R=0.40$, $p=0.005$; $R=0.49$, $p=0.001$; $R=0.38$, $p=0.009$; $R=0.40$, $p=0.006$, respectively). Additionally, we found a positive correlation between M-CSF and MMP-9, M-CSF and TIMP-1 in ovarian cysts patients ($R=0.46$, $p=0.001$; $R=0.42$, $p=0.002$, respectively) and between M-CSF and VEGF ($R=0.43$, $p=0.003$) in the plasma of ovarian cancer patients.

Correlations between the expression of the tested parameters in tissues are presented in Table 4. We observed a positive correlation between MMP-2 and TIMP-2, M-CSF and TIMP-2, MMP-9 and TIMP-2 ($R=0.60$, $p<0.001$; $R=0.39$, $p=0.006$; $R=0.32$, $p=0.026$, respectively) and a negative correlation between MMP-9 and TIMP-1 ($R=-0.33$, $p=0.024$) in epithelial cells of ovarian cyst tissues. Moreover, we found a positive correlation between M-CSF and MMP-2, VEGF and MMP-2 ($R=0.52$, $p=0.019$; $R=0.51$, $p=0.022$, respectively) in epithelial ovarian cancer tissues. In the case of stromal cells, we found a positive correlation between M-

CSF and MMP-2 (R=0.35, p=0.016) in cysts and a negative correlation between both TIMPs (R=-0.58, p=0.008) in ovarian cancer tissues.

Our analysis revealed a statistically positive correlations of M-CSF between plasma levels and epithelial ovarian cancer cells expression of (R=0.27; p=0.044) (Table 5).

Discussion

Angiogenesis occurs due to a dynamic series of events which commences with the disruption of the endothelial cell basement membrane by proteolytic enzymes including MMPs. Proteolysis becomes pathological when the normal balance between proteases and their tissue inhibitors (TIMPs) is disrupted. MMPs contribute to angiogenesis by degrading ECM components, allowing endothelial cells to migrate out of existing blood vessels and by releasing proangiogenic factors (VEGF) [26]. It has been proven that angiogenesis is essential for the growth and metastatic spread of solid tumors [27]. Proangiogenic factors,particularly VEGF, whose high serum levels are a well-established indicator of poor prognosis in carcinoma patients,appear to be the most promising markers [28]. Several other candidate markers have also been indicated, particularly those involved in tumor invasion. M-CSF is a hematopoietic growth factor that stimulates the proliferation and differentiation of monocytes to macrophages and its increased expression is correlated with poor prognosis in ovarian cancer [29].

In the present study we measured the levels of VEGF, M-CSF, MMP-2 and MMP-9, TIMP-1 and TIMP-2 in the plasma of ovarian cysts and ovarian tumor patients and reported correlations between the concentrations of these parameters.

Serum VEGF levels are elevated in a number of tumors, including ovarian tumor [30]. A series of studies have attempted to determine the role of VEGF in the angiogenesis, metastasis, and proliferation of ovarian cancer. We showed that plasma levels of VEGF and M-CSF were statistically significantly lower in ovarian cysts patients when compared to ovarian cancer patients. The results reported in the available literature regarding VEGF and M-CSF [30,31] correspond to the results of the current study. Similar relationships have also been observed in other cancers, with breast cancer being one of them [32,33].

In our study, which was based on the ELISA method, statistically lower plasma levels of MMP-9 and TIMP-1 were also observed in ovarian cysts patients compared to ovarian cancer patients. We were able to establish these parameters as differentiation markers between malignant and non-malignant disease. Unfortunately, we did not find any papers comparing the concentrations of the studied parameters in ovarian cysts patients. Similar findings were noted by Määttä et al. [34] who observed higher serum TIMP-1 levels in patients with malignant ovarian tumors and lower in the control group (patients with benign ovarian tumors). However, the authors failed to show any usefulness of serum MMP-2 and MMP-9 in differentiating between benign, LMP (low malignant potential) and malignant ovarian tumors. In opposition to these findings, Zhang et al. [35] observed higher serum MMP-9 levels in patients with malignant ovarian tumors in comparison to the benign tumor group.

In our study the Spearman's rank correlation was used in the dependence analysis between the investigated parameters. The study proved dependence between the tested proteins. The obtained data showed a significant correlation between VEGF concentration and M-CSF, MMP-9 and TIMP-1 concentrations in the ovarian cancer group. The relationship between MMPs and TIMPs has previously been described in numerous publications. According to the current knowledge, TIMP-1 inhibits the proteolytic effect of most MMPs, including pro-MMP-9 and MMP-9 [16,36]. Additionally, pro-MMP-2 is activated by the membrane-tethered type 1 metalloproteinase (MT1)-MMP in a process regulated by TIMP-2 [37]. In our study, we observed that MMP-9 concentration correlated with TIMP-1, while MMP-2 concentration correlated with TIMP-2 in both tested groups. Several studies have indicated correlations between serum levels of growth factors, MMPs, TIMPs and patients' clinicpathological parameters. Smerdel et al. [38] demonstrated a significant relationship between VEGF expression and the stage of ovarian cancer. Results of a study by Wu et al. [39] revealed that high levels of MMP-9 and TIMP-1 correlated significantly with lymph node metastasis and advanced stage of breast cancer.

The immunohistochemical tests performed during the present study revealed a positive reaction of M-CSF, VEGF and TIMP-1 in epithelial cyst cells, and VEGF and TIMP-1 in stromal cyst cells. In the case of ovarian cancer cells, we observed a positive reaction of all the tested parameters in epithelial cells and a positive reaction of VEGF and TIMP-1 in stromal cells. We also noted significantly enhanced expression of all the tested parameters in epithelial cells in comparison to stromal cells, both in the ovarian cysts and ovarian cancer cases (with the exception of VEGF in ovarian cancer). Our findings are in line with those of Wang et al. [40]. The authors demonstrated increased MMP-2 expression in ovarian cancer cells and suggested that it may be related to the invasion and metastasis of ovarian cancer. The work of Brun et al. [41] also indicates that MMP-2, MMP-9, TIMP-1 and TIMP-2 are significant factors in tumor proliferation and invasiveness as their epithelial expression was found to be relatively high. Kinose et al. [42] also showed high VEGF expression in ovarian cancer. In the case of M-CSF, Baiocchi et al. [43] demonstrated the expression of this growth factor in 78% of all ovarian cancer cases. However, Vos et al. [44] and Brun et al. [41] found that despite their tissue expression, MMP-2, MMP-9, TIMP-1 and TIMP-2 have no significant prognostic value in patients with ovarian cancer.

It is well known that both aforementioned TIMPs are responsible for controlling the activity of MMPs, thus maintaining the correct balance in the degradation and re-modelling of extracellular matrix [45]. In the present study we observed correlations between MMP-2 and TIMP-2, and MMP-9 and TIMP-1 only in epithelial cyst tissues. We failed to observe similar correlations in ovarian cancer, which may indicate that in the course of cancer development there is an imbalance between MMPs and TIMPs. This is in accordance with a paper of Swellam et al. [46] on breast cancer.

We also found correlations between M-CSF and TIMP-2 in epithelial cyst tissues, M-CSF and MMP-2, VEGF and MMP-2

in epithelial ovarian cancer tissues, M-CSF and MMP-2 in stromal cyst tissues, and between both TIMPs in stromal ovarian cancer tissues. We have not found any papers demonstrating the same correlations between the tested parameters in ovarian cancer patients. Wefound only one paper [47] which describes correlations between these parameters in blood plasma.

To our knowledge, we are also the first researchers to have evaluated correlations between plasma concentrations and tissue expression of M-CSF, VEGF, MMP-2, MMP-9, TIMP-1 and TIMP-2. This is a crucially important part of this paper since it demonstrates that serum levels of the tested proteins do not reflect their expression in tissue. This may be due to their different origin, e.g. from disintegrating cells outside the lesion or peritumoral infiltration.

As is well known, MMPs and TIMPs play an important role in tissue re-modelling and the development of the inflammatory process. However, their additional functions also include cell repair, wound healing and an immunological response. It is probable that their participation in these processes, which also accompany cancerous processes, may, to a certain degree, explain their enhanced serum concentrations in contrast to the absence of such a significant difference in cancerous tissue expression. Since M-CSF and VEGF are produced by similar cells, including cells associated with the immune response, it can be assumed that in this case they are produced outside the tumor. The concentration of VEGF, the main proangiogenic factor, may increase beyond the boundaries of the tumor due to its production by platelets in blood vessels. Its production is caused primarily by the stimulation of cells by previously secreted HIF-1, presumably produced by cancer cells [48]. In the case of M-CSF, its production can also occur in vessels located near the tumor, where it affects the differentiation of hematopoietic stem cells into macrophages or other related cell types [49].

Conclusion

Our findings confirm that plasma levels of M-CSF, VEGF, MMP-9 and TIMP-1, and tissue expression of all the tested parameters may be useful in the diagnosis of epithelial ovarian cancer. Moreover, only M-CSF showed the positive correlations between the plasma concentrations and tissue expression which can indicate a potential role of this cytokine in laboratory diagnostics of EOC.

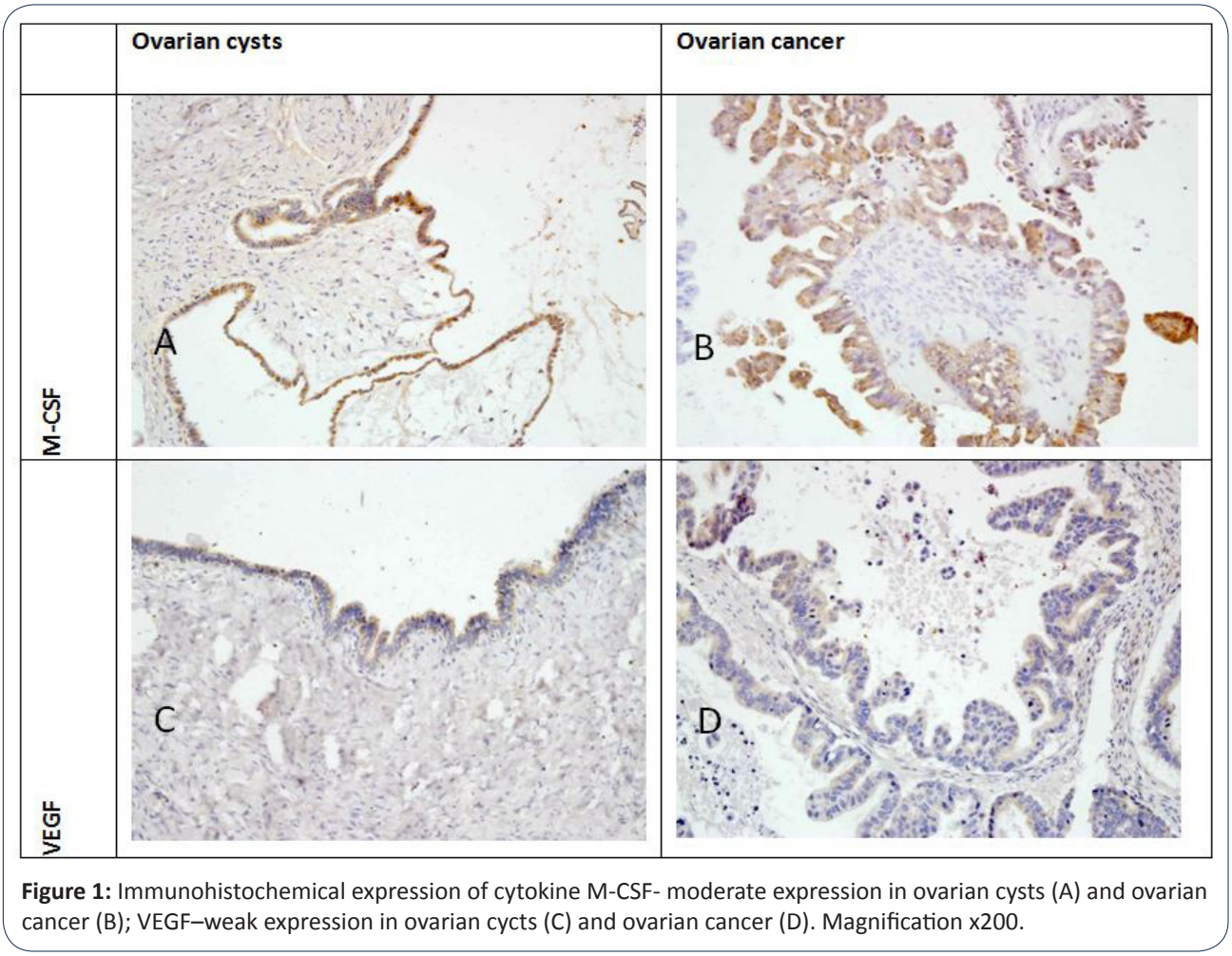

Figure 1: Immunohistochemical expression of cytokine M-CSF- moderate expression in ovarian cysts (A) and ovarian cancer (B); VEGF–weak expression in ovarian cycts (C) and ovarian cancer (D). Magnification x200.

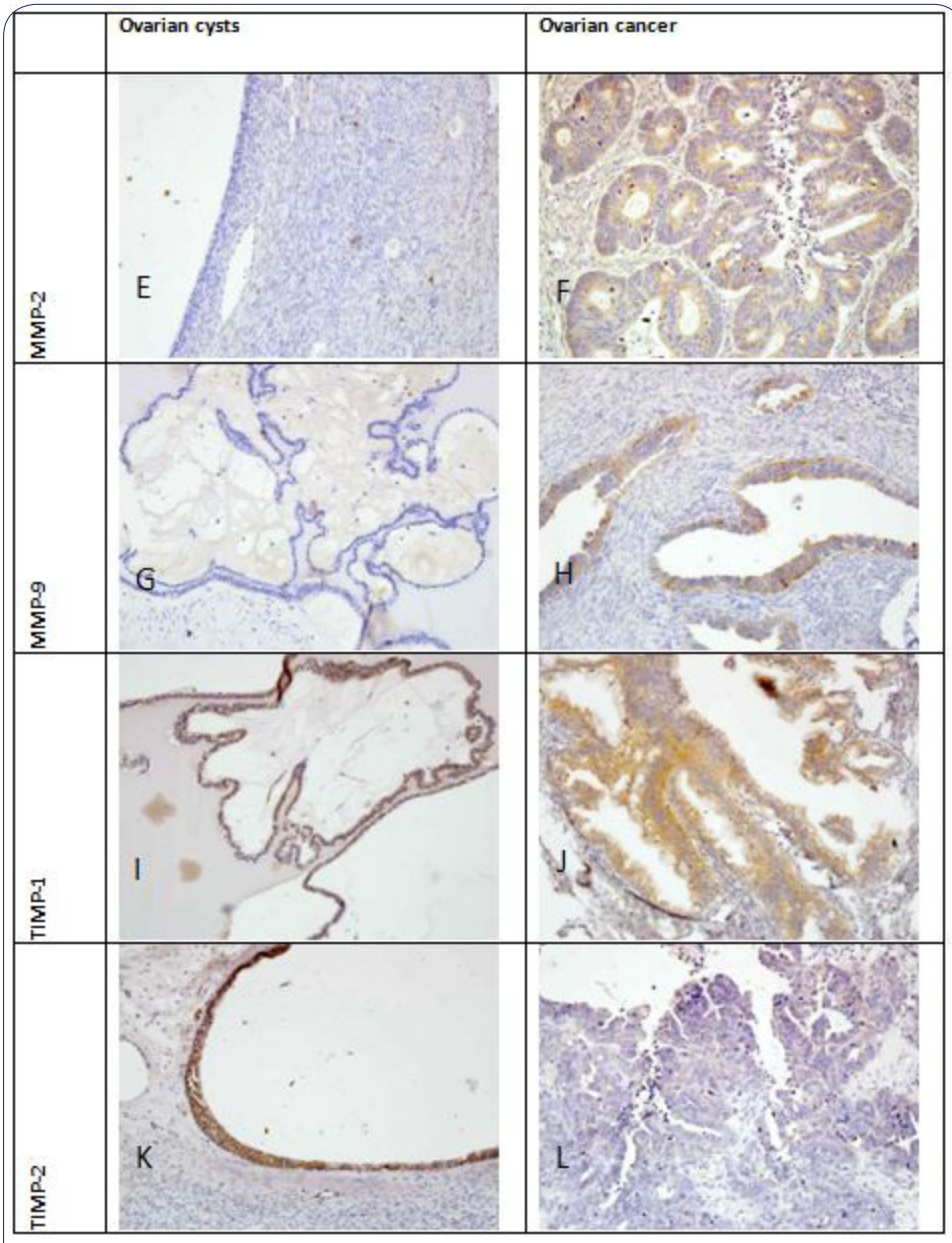

Figure 2: Immunohistochemical expression of MMP-2 - lack of expression in ovarian cysts (E) and weak/moderate expression in ovarian cancer (F); MMP-9 -non-homogenous expression in ovarian cysts (G) and weak/moderate expression in ovarian cancer (H); TIMP-1 - moderate/strong expression in ovarian cysts (I) and ovarian cancer (J); TIMP-2 - moderate/strong expression in ovarian cysts (K) and ovarian cancer (L). Magnification x200.

Table 1: Characteristics of ovarian cysts and ovarian cancer patients.

Study group			Number of patients
Tested group	Epithelial ovarian cancer patients		**50 (100%)**
	- sub-type *serous epithelial*		29 (58.0%)
	- sub-type *endometrioid epithelial*		21 (42.0%)
	Median age (range)		55 (46-81)
	Tumor stage	IA-IIC	28 (56.0%)
		IIIA-IV	22 (44.0%)
	Menopausal status:		
	- postmenopausal		50 (100%)
Control group	Ovarian cysts patients		**50 (100%)**
	- type *serous*		22 (44%)
	- type *endometriosis*		28 (56%)
	Median age (range)		53 (50-74)
	Menopausal status:		
	- postmenopausal		50 (100%)

Table 2: Plasma levels of tested proteins in ovarian cysts and ovarian cancer patients.

Groups tested	**M-CSF** (pg/mL)	**VEGF** (pg/mL)	**MMP-2** (ng/mL)	**MMP-9** (ng/mL)	**TIMP-1** (ng/mL)	**TIMP-2** (ng/mL)
Ovarian cancer Median (Range)	638.6 (210.7-3791.1)	227.7 (8.9-2701.1)	195.8 (108.4-299.4)	292 (7.1-880)	169.4 (4.6-839)	69.8 (27.5-350)
Ovarian cysts patients Median (Range)	380.5 (125.3-2009.3)	70 (9-403.5)	208.5 (122.5-288.3)	194 (9.2-512)	87 (6.7-304.8)	73.6 (37.5-115.8)
p	**<0.001**	**<0.001**	0.367	**0.003**	**<0.001**	0.491

Table 3: Immunohistochemical expression of tested proteins in epithelial and stromal cells in tissues of cysts and ovarian cancer.

OVARIAN CANCER CELLS									p
	Epithelial cells (% of cases)				Stromal cells (% of cases)				
	-	+	++	+++	-	+	++	+++	Ephitelial vs. Stromalcells
M-CSF	5	45	30	20	85	10	5	0	**<0.001**
MMP-2*	30	50	20	0	70	30	0	0	**0.003**
MMP-9*	25	50	25	0	75	15	10	0	**0.012**
TIMP-1	0	20	45	35	20	80	0	0	**<0.001**
TIMP-2*	15	65	20	0	75	25	0	0	**<0.001**
OVARIAN CYSTS CELLS									
M-CSF	25.5	40.5	8.5	25.5	66	19.1	2.1	12.8	**0.001**
VEGF	12.8	61.7	14.9	10.6	42.6	42.6	8.4	6.4	**0.013**
MMP-2*	48.9	29.8	0	21.3	83	17	0	0	**<0.001**
MMP-9*	51.1	48.9	0	0	100	0	0	0	**<0.001**
TIMP-1	2.1	12.8	44.7	40.4	21.2	68.1	6.4	4.3	**<0.001**
TIMP-2*	44.7	19.1	2.1	34.1	63.8	36.2	0	0	**<0.001**

Proteinsexpression:(-): absent, (+): weak, (++): moderate, and (+++): strong
* Epithelial MMP-2 (cysts) vs epithelial MMP-2 in ovarian cancer, p=0.001
* Epithelial MMP-9 (cysts) vs epithelial MMP-9 in ovarian cancer, p=0.001
* Epithelial TIMP-2 (cysts) vs epithelial TIMP-2 in ovarian cancer, p<0.001
* Stromal MMP-9 (cysts) vs stromal MMP-9 in ovarian cancer, p=0.002

Table 4: Correlations between tested proteins in plasma levels or tissue expression.

Groups/parameters		VEGF		MMP-2		MMP-9		TIMP-1		TIMP-2	
		R	p	R	p	R	p	R	p	R	p
PLASMA LEVELS											
Ovarian Cysts Patients	**M-CSF**	0.24	0.098	0.13	0.386	0.46	**0.001**	0.42	**0.002**	-0.21	0.151
	VEGF			0.15	0.307	0.41	**0.003**	0.57	**<0.001**	0.03	0.829
	MMP-2					0.22	0.124	0.18	0.210	0.47	**0.001**
	MMP-9							0.39	**0.006**	-0.05	0.745
	TIMP-1									0.04	0.775
Ovarian cancer patients	**M-CSF**	0.43	**0.003**	0.00	0.999	0.18	0.227	0.18	0.230	0.09	0.561
	VEGF			-0.02	0.897	0.40	**0.005**	0.49	**0.001**	0.19	0.217
	MMP-2					-0.10	0.507	0.01	0.953	0.40	**0.006**
	MMP-9							0.38	**0.009**	0.00	0.984
	TIMP-1									-0.07	0.653
EPITHELIAL CELLS											
Ovarian Cysts	**M-CSF**	0.22	0.141	0.25	0.088	-0.01	0.947	-0.01	0.955	0.39	**0.006**
	VEGF			0.09	0.551	-0.20	0.182	0.26	0.077	-0.12	0.429
	MMP-2					0.23	0.123	0.10	0.518	0.60	**<0.001**
	MMP-9							-0.33	**0.024**	0.32	**0.026**
	TIMP-1									0.04	0.797
Ovarian Cancer	**M-CSF**	0.21	0.379	0.52	**0.019**	0.13	0.582	0.15	0.523	0.13	0.583
	VEGF			0.51	**0.022**	0.05	0.849	-0.03	0.910	-0.04	0.856
	MMP-2					0.41	0.069	0.22	0.361	-0.10	0.685
	MMP-9							-0.19	0.418	-0.12	0.605
	TIMP-1									-0.26	0.273
STROMAL CELLS											
Ovarian Cysts	**M-CSF**	0.22	0.134	0.35	**0.016**	0.22 0.867 -0.27	0.32 0.419 0.454	0.00	0.997	0.07	0.640
	VEGF			0.12	0.403			-0.17	0.254	-0.05	0.740
	MMP-2							-0.02	0.919	0.25	0.093
	TIMP-1									0.07	0.652
Ovarian Cancer	**M-CSF**	0.12	0.604	0.06	0.799	0.03	0.888	-0.12	0.608	0.42	0.066
	VEGF			0.09	0.709	0.32	0.175	0.16	0.504	0.24	0.307
	MMP-2					0.20	0.399	-0.22	0.355	0.38	0.100
	MMP-9							0.29	0.222	-0.03	0.912
	TIMP-1									-0.58	**0.008**

Table 5: Correlations between tissue expression and plasma levels of tested proteins.

Expression of tested proteins			Plasma level vs. tissue expression	
			R	P
Ovarian cysts	Epithelial cells	VEGF	0.04	0.775
	Stromal cells		-0.27	0.071
Ovarian cancer	Epithelial cells		-0.12	0.617
	Stromal cells		-0.03	0.885
Ovarian cysts	Epithelial cells	M-CSF	-0.09	0.544
	Stromal cells		-0.21	0.150
Ovarian cancer	Epithelial cells		**0.27**	**0.044**
	Stromal cells		-0.11	0.644
Ovarian cysts	Epithelial cells	MMP-2	0.05	0.741
	Stromal cells		0.06	0.696
Ovarian cancer	Epithelial cells		-0.31	0.189
	Stromal cells		-0.21	0.379
Ovarian cysts	Epithelial cells	TIMP-1	0.15	0.307
	Stromal cells		0.19	0.205
Ovarian cancer	Epithelial cells		-0.22	0.356
	Stromal cells		0.21	0.383
Ovarian cysts	Epithelial cells	MMP-9	-0.10	0.509
	Stromal cells		0,14	0.440
Ovarian cancer	Epithelial cells		-0.20	0.407
	Stromal cells		-0.02	0.924
Ovarian cysts	Epithelial cells	TIMP-2	0.11	0.465
	Stromal cells		0.11	0.444
Ovarian cancer	Epithelial cells		-0.05	0.826
	Stromal cells		0.11	0.644
	Stromal cells		-0.10	0.509

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