EJGO European Journal of Gynaecological Oncology

Serum MCP-1 (CCL2), MCP-2 (CCL8), RANTES (CCL5), KI67, TNF-β levels in patients with benign and borderline ovarian tumours

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Summary

Objective: The purpose of this study was to assess, among others, monocyte chemoattractant protein type-1 MCP-1 [also called chemokine ligand 2 (CCL2)], MCP-2 (CCL8), regulated on activation, normal T cell expressed and secreted (RANTES) (CCL5), Ki-67, and transforming growth factor beta (TNF-β) levels in the serum of patients with benign and borderline ovarian lesions. *Study Design:* Patients who underwent laparoscopy and or laparotomy in the Gynecology and Obstetrics Clinical Hospital, Poznan Medical University, Poland, for adnexal changes in the form of endometrial cysts (n=11) and mature teratomas (n=11), mucinous cysts (n=16), borderline tumors of the ovary (n=17), simplex (serosa) cysts (n=19), or hemorrhagic cysts (n=5) were included in this study. *Results:* The differences in the medians of MCP-1, MCP-2, RANTES, Ki-67, and TNF-β were non-statistically significant. Spearman's correlation analysis performed on the group of women with endometriomas showed a statistically significant and positive dependence on the level of RANTES with Ki-67 ($r = 0.6$; $p = 0.0467$). Moreover, Spearman's correlation analysis performed on the group of women treated surgically for mature teratomas was statistically significant for the level of MCP-1 with TNF-β ($r = 0.7$; $p = 0.0301$). *Conclusion:* The study of serum levels of MCP-1 (CCL2), MCP-2, RANTES (CCL5), Ki-67, and TNF-β in patients with benign and borderline ovarian tumors revealed correlations between the level of RANTES and Ki-67 and of MCP-1 and TNF-β, which suggests that chemokines as the markers of benign and borderline ovarian tumors are questionable.

Key words: MCP-1 (CCL2); MCP-2; RANTES (CCL5); KI67; TNF-β; Benign ovarian tumor; Borderline ovarian tumor.

Introduction

The classification of ovarian tumors includes three main groups: benign, borderline, and malignant tumours. In women of reproductive age, benign tumors or cysts constitute the majority of ovarian lesions. Endometriosis most frequently takes the form of endometrial cysts (chocolate cysts) [1, 2]. Mature teratomas (*teratomata adultum*) being one of the most frequent types of ovarian tumour, and for over 58% of benign ovarian lesions [3]. Simplex (serosa) cysts are very common single-celled unilateral lesions, which are filled with a transparent and usually light-colored fluid; however, when a blood vessel ruptures within the cyst wall, they may transform into hemorrhagic cysts, similar to other tumor-like lesions [4]. Malignant ovarian tumors of epithelial or stromal origin account for around 80-90% of all malignant ovarian tumors and rarely occur in patients below 21 years of age [5]. Borderline ovarian tumors have an epithelial origin with a low rate of growth; they have a low potential to transform into a malignant state and to invade or metastasize. Borderline ovarian tumors are often associated with a significantly better prognosis than that of epithelial ovarian cancer [6].

Diagnostic tests of ovarian lesions include physical examination and ultrasonography, along with the determination of biochemical markers, which primarily include cancer antigen 125 (CA125). In recent years, literature data has indicated the significance of monocyte chemoattractant protein type-1 [MCP-1, also called chemokine ligand 2 (CCL2)], MCP-2 (CCL8), regulated on activation, normal T cell expressed and secreted - RANTES (CCL5), and tumor necrosis factor beta (TNF-β) in diagnosing malignant ovarian cancer. Cytokines are a large group of soluble proteins that play a significant role in the induction of immune response against foreign invaders. These proteins are involved, among others, in inflammatory responses and immune defences and also in the growth and differentiation of cells [7]. However, they are usually limited to a reaction site. They are transiently produced (after activation stimulus) by locally activated cells [7]. However, the role of chemokines in ovarian cysts remains unclear; however, chemokines may be responsible for the development of endometrial changes in postmenopausal women. Chemokines are alkaline proteins of low molecular weight, high biological activity, and have specific function as chemoattrac-

Revised manuscript accepted for publication October 26, 2017

tants. They also participate in the processes of angio- genesis, hemo- and lymphopoiesis, as well as in carcinogenesis and wound healing [7]. Antigen Ki-67 is a nuclear protein that is associated with and may be necessary for cellular proliferation. Therefore, the purpose of this study was to assess the concentrations of, *inter alia*, MCP-1 (CCL2), MCP-2 (CCL8), RANTES (CCL5), and also the levels of Ki67 and TNF-β in the serum of patients with benign and borderline ovarian tumors.

Materials and Methods

In this study, patients treated by laparoscopy and or laparotomy in the Gynecology and Obstetrics Clinical Hospital, Poznan Medical University, Poland from September 2012 to December 2014 due to the following reasons were recruited: adnexal changes in the form of endometrial cysts and mature teratomas, mucinous cysts, borderline ovarian tumors, simplex (serosa) cysts, or hemorrhagic cysts [7].

The following were the inclusion criteria: intraoperative diagnosis of an endometrial cyst without macroscopic peritoneal endometriosis and/or ovarian tumors in the form of mature teratoma, mucinous cysts, borderline ovarian tumors, simplex (serosa) cysts or hemorrhagic cysts, unremarkable obstetric history, and good health without comorbidities. None of the patients must have been previously treated for infertility. Preoperative laboratory tests did not demonstrate any coagulation abnormalities. In all the patients with endometrial cysts, mature teratomas, mucinous cysts, simplex (serosa) cysts, or hemorrhagic cysts, laparoscopy must have been performed in the first phase of the cycle. In all the patients with borderline ovarian tumors, laparotomy with hysterectomy, and adnexectomy was performed. The following were the exclusion criteria: coexistence of various ovarian lesions in one patient (e.g., an endometrial cyst and mature teratoma), history of obstetric complications, or coagulation disorders.

After intraoperative histopathological verification of the obtained tissue fragments, the patients were divided into six study groups: group E (endometrial cysts) included women with histologically confirmed endometrial cysts (n = 11), without macroscopic foci of peritoneal endometriosis after laparoscopic and or laparotomy, group T (mature teratoma) comprised patients after laparoscopic and or laparotomy treatment of ovarian mature teratomas (n = 11), group M (mucinous cysts) comprised patients after laparoscopic and or laparotomy treatment of a mucinous cyst (n = 6), group BOT (borderline ovarian tumors) comprised patients after laparoscopic and or laparotomy treatment for borderline ovarian tumors (n = 17), group S (simplex (serosa) cysts) comprised patients after laparoscopic and or laparotomy treatment of ovarian simplex (serosa) cysts (n = 19), and group H (hemorrhagic cyst) comprised patients after laparoscopic and or laparotomy treatment of a hemorrhagic cyst (n = 5).

After the patients were admitted to the hospital, blood samples were collected in the morning on an empty stomach on the day before the surgery. Blood sample for MCP-1, MCP-2, RANTES, Ki67, and TNF-β analysis was centrifuged and frozen at −20°C. The levels of MCP-1 (CCL2), MCP-2 (CCL8), RANTES (CCL5), Ki67, and TNF-β in serum were determined by enzyme-linked immunosorbent assay (ELISA) (measurements were performed twice to verify the errors). Concentrations were determined in ng/mL for Ki67 and pg/mL for other biomarkers.

The statistical analysis for differences of distribution of MCP-1, MCP-2, RANTES, Ki67, and TNF-β between the study groups was performed using the Kruskal–Wallis One-way analysis of variance on ranks tests. The Spearman rank correlation coefficients were calculated to assess the relationships between individual variables and the power of these relationships. The significance level assumed for all of the tests was $p \leq 0.05$. Statistical calculations were made using the STATISTICA software.

Table 1. — *Levels of MCP-1 (CCL2), MCP-2, RANTES (CCL5), KI67, and TNF-β levels in patients with benign and borderline ovarian tumours.*

Group	Median (min-max)	*p*-value
CCL-2 [pg/mL]		
Ovarian mature teratomas	45.1(31-156.5)	NS
Endometrial cysts	71.2 (31-137.6)	
Simplex (serosa) cysts	54.6 (34,1 -108.7)	
Borderline ovarian tumours	96 (36.9 -361.4)	
Mucinous cysts	58.2 (41.1-77.7)	
Haemorrhagic cysts	59.2 (37.4-137.6)	
KI67 [ng/mL]		
Ovarian mature teratomas	8.1 (0.3-12.1)	NS
Endometrial cysts	4.1 (0 – 10.3)	
Simplex (serosa) cysts	6.5 (0 – 11.9)	
Borderline ovarian tumours	5.6 (0 – 10.2)	
Mucinosa cysts	4.4 (0.2- 10.1)	
Haemorrhagic cysts	7.41 (0-10.4)	
CCL-5 [pg/mL]		
Ovarian mature teratomas	357.4 (279.6 – 421.2)	NS
Endometrial cysts	395.2 (163.3 – 408.6)	
Simplex (serosa) cysts	309.5 (180.4 – 449.9)	
Borderline ovarian tumours	389.5 (204.3-444.4)	
Mucinosa cysts	302.7 (121.3-396.7)	
Haemorrhagic cysts	333.6 (278.3-403.7)	
MCP-2 [pg/mL]		
Ovarian mature teratomas	709.8 (272.2 – 1587.2)	NS
Endometrial cysts	614.9 (401.5-1314)	
Simplex (serosa) cysts	839 (26.7-1906.9)	
Borderline ovarian tumours	816.7 (312.5 – 1777)	
Mucinous cysts	504,4 (174,3 – 644,2)	
Haemorrhagic cysts	501.4 (317.6 – 833.4)	
TNF – beta [pg/mL]		
Ovarian mature teratomas	69.3 (22.8 – 337.7)	NS
Endometrial cysts	66 (11.2 – 277.4)	
Simplex (serosa) cysts	72 (8.1 – 1702.2)	
Borderline ovarian tumours	69.3 (12.3 – 414.4)	
Mucinous cysts	103.69 (2.95 - 309.2)	
Haemorrhagic cysts	363.99 (76.96 – 3445.2)	

Patient enrollment methods, ways of obtaining the research material, and its storage were previously approved by the Bioethics Committee at the Poznan University of Medical Sciences (specifically approved only for this study on 8 January 2009; Resolution No. 10/2009). The patients provided written informed consent for this study. The Ethical committee approved this consent procedure.

Results

Group E (n = 11) comprised patients (aged 34.27 ± 13.54 years, Median = 31 years) and with a 5.32 ± 1.96 cm lesion in the right ovary or with a 5.26 ± 3.18 cm lesion in the left ovary. Group T (n = 11) comprised patients (aged 32 ± 6.74

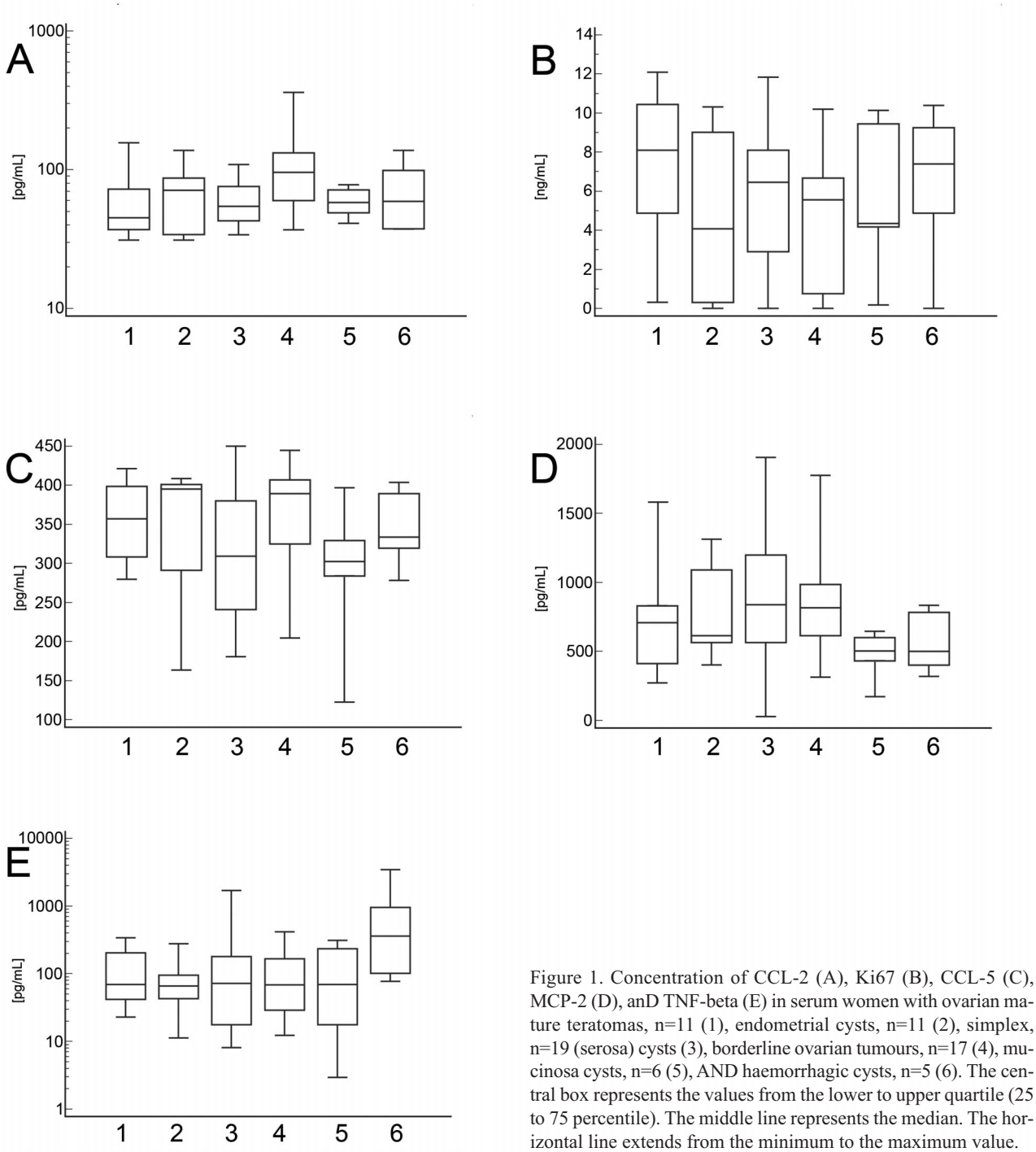

Figure 1. Concentration of CCL-2 (A), Ki67 (B), CCL-5 (C), MCP-2 (D), anD TNF-beta (E) in serum women with ovarian mature teratomas, n=11 (1), endometrial cysts, n=11 (2), simplex, n=19 (serosa) cysts (3), borderline ovarian tumours, n=17 (4), mucinosa cysts, n=6 (5), AND haemorrhagic cysts, n=5 (6). The central box represents the values from the lower to upper quartile (25 to 75 percentile). The middle line represents the median. The horizontal line extends from the minimum to the maximum value.

years, median = 30 years) and with a 4.96 ± 3.12 cm lesion in the right ovary or with a 5.57 ± 3.47 cm lesion in the left ovary. Group M (n = 6) comprised patients (aged 46.74 ± 13.71 years, median = 44 years) and with a 6.32 ± 3.57 cm lesion in the right ovary or with a 6.89 ± 2.52 cm lesion in the left ovary. Group BOT (n = 17) comprised patients (aged 61.12 ± 12.24 years, median = 59 years) and with a 7.91 ± 3.64 cm lesion in the right ovary or with an 11.11 ± 1.41 cm lesion in the left ovary. Group S (n = 19) comprised patients (aged 43.00 ± 14.48 years, median = 46 years) and with a 5.63 ± 2.21 cm lesion in the right ovary or with a 6.12 ± 4.34 cm lesion in the left ovary. Group H (n = 5) comprised patients (aged 31.8 ± 7.53 years, median = 33 years) and with a 5.85 ± 0.64 cm lesion in the right ovary or with a 3.70 ± 0.56 cm lesion in the left ovary.

Table 2. — *Correlation MCP-1 (CCL2), MCP-2, RANTES (CCL5), KI67, and TNF-β levels in patients with benign and borderline ovarian tumours.*

Group			KI67	MCP2	CCL5	TNFbeta
E	**CCL2**	Correlation coefficient	0.345	-0.0273	-0.0364	0.123
		p value	0.283	0.924	0.903	0.693
	KI67	Correlation coefficient		0	0.6	0.137
		p value		0.989	0.0467*	0.673
	MCP2	Correlation coefficient			-0.127	-0.542
		p value			0.693	0.0762
	CCL5	Correlation coefficient				-0.0456
		p value				0.881
T	**CCL2**	Correlation coefficient	0.0753	0.417	-0.3	0.7
		p value	0.809	0.243	0.407	0.0301*
	KI67	Correlation coefficient		-0.343	-0.234	0,251
		p value		0.331	0.52	0.491
	CCL5	Correlation coefficient			0.4	0.0833
		p value			0.264	0.809
	MCP2	Correlation coefficient				-0.517
		p value				0.138
M	**CCL2**	Correlation coefficient	0.427	-0.0961	-0.227	0.264
		p value	0.0759	0.699	0.356	0.281
	KI67	Correlation coefficient		0.147	-0.193	0.268
		p value		0.552	0.436	0.278
	CCL5	Correlation coefficient			-0.166	-0.2
		p value			0.503	0.416
	MCP2	Correlation coefficient				-0.142
		p value				0.569
BOT	**4_CCL2**	Correlation coefficient	0.273	0.274	0.471	-0.135
		p value	0.298	0.298	0.0637	0.609
	4_KI67	Correlation coefficient		0.12	0.0578	-0.0719
		p value		0.648	0.822	0.788
	4_CCL5	Correlation coefficient			-0.0324	0.0957
		p value			0.9	0.713
	4_MCP2	Correlation coefficient				-0.422
		p value				0.0988
S	**5_CCL2**	Correlation coefficient	0.371	-0.429	-0.143	-0.2
		p value	0.497	0.419	0.803	0.714
	5_KI67	Correlation coefficient		0.6	0.143	-0.0857
		p value		0.242	0.803	0.919
	5_CCL5	Correlation coefficient			0.429	0.429
		p value			0.419	0.419
	5_MCP2	Correlation coefficient				0.771
		p value				0.103
H	6_CCL2	Correlation coefficient	-0.1	0.9	0.6	-0.6
		p value	0.95	0.0833	0.35	0.35
	6_KI67	Correlation coefficient		-0.2	0.3	0.7
		p value		0.783	0.683	0.233
	6_CCL5	Correlation coefficient			0.3	-0.7
		p value			0.683	0.233
	6_MCP2	Correlation coefficient				0.2
		p value				0.783

* *p<0.05 Spearman's correlation analysis*

The differences in the medians of MCP-1, MCP-2, RANTES, KI67, and TNF-β were non-statistically significant. Table 1 and Figure 1 present the blood levels of MCP-1, MCP-2, RANTES, Ki-67, and TNF-β in patients with benign and borderline ovarian tumors. Spearman's correlation analysis performed on a group of women with endometriomas showed a statistically significant, positive dependence on the level of RANTES with Ki-67 ($r = 0.6$; $p = 0.047$). In the group of women treated surgically for mature teratomas, Spearman's correlation analysis showed statistically significant results for the level of MCP-1 with TNF-β ($r = 0.7$; $p = 0.03$) (Table 2).

Discussion

In recent years, literature on ovarian tumors has indicated the significance of MCP-1 (CCL2), MCP-2 (CCL8), RANTES (CCL5), and TNF-β, KI67 in diagnosing benign and borderline ovarian tumors.

MCP-1 (CCL2) and MCP-2 (CCL8) are small cytokines from the family of CC cytokines [8, 9]. It has been suggested that MCP-1 has a direct angiogenic effect on vascular endothelial cells and an influence on the migration of epithelial cells. It has also been suggested that it shows increased activation of endometrial cancer cells [10, 11]. MCP-2 has chemotactic effects, taking part in the immune response to an inflammatory factor [12, 13]. It was observed that the concentration of the MCP-1 chemokine is high in the peritoneal fluid of women with endometriosis, and the values correlate with the stage of the disease [14]. It may be that estradiol (E_2) indirectly increases the expression of MCP-1 and RANTES in ectopic endometrium. Tao *et al.* [15] observed significantly increased levels of MCP-1 and aleptin in the peritoneal fluid of patients treated for infertility coexisting with endometriosis [15]. Gmyrek *et al.* [16] studied whether the serum MCP-1 level correlated with endometriosis in infertile women [16]. In the study group of women treated surgically for mature teratomas, Spearman's correlation analysis showed statistically significant results for the level of MCP-1 with TNF-β ($r = 0.7$; $p = 0.03$). MCP-1 is also an important mediator of monocyte infiltration in various solid tumors of epithelial origin and might play a functional role in the natural history of ovarian cancer [17]. The study of serum levels of chemokines in patients with epithelial ovarian cancer identified a down-regulation in CCL2/MCP-1 and CCL4/MIP-1β. This suggests that the two chemokines play an important role in the pathophysiology of ovarian cancer [18]. Functional polymorphisms in MCP-1 are associated with increased susceptibility to ovarian cancer, in which rs1024611A/G may increase the serum MCP-1 level in the Chinese population [19]. Mortality rates for epithelial ovarian cancer are high. The identification of biomarkers for this cancer could contribute to earlier diagnosis and increased survival rates. Kristjánsdóttir *et al.* [20] showed that MCP-1/CCL2 and interleukin (IL)-8/CXCL8 were significantly higher in the malignant versus benign tumor cysts. MCP-1, IL-8, and GROα were detected at 5- to 100-fold higher concentrations in case of cystic fluids than in case of serum. The aforementioned biomarkers are proinflammatory cytokines and promoters of tumor growth [20].

RANTES is a chemokine that stimulates chemotaxis and adhesion of lymphocytes T CD4+ [7]. RANTES is actively synthesized by cells of eutopic endometrium and its ectopic foci. Thus, RANTES levels in the peritoneal cavity of women with endometriosis have been found to be increased, which show a correlation with the stage of the disease [14, 21, 22]. Margari *et al.* [23] studied the level of chemokines in the peritoneal fluid of patients with endometriosis versus a control group with an idiopathic cause of infertility. No statistically significant differences were observed in the concentrations of MCP-3 and RANTES, but the level of MCP-1 in patients with Stage II endometriosis was significantly lower than in patients with Stages III and IV of the disease [23]. Tsukishiro *et al.* [24] suggested that preoperative serum RANTES levels may be useful in differentiating benign ovarian tumors from malignancy correlating with the extent of the disorder [24]. In the study group of women treated surgically for endometrial cysts, Spearman's correlation analysis showed statistically significant results for the level of RANTES with Ki-67 ($r = 0.6$; $p = 0.047$).

Ki-67 is a marker of cellular proliferation. It is an excellent marker to determine the growth fraction of a given cell population. The fraction of Ki-67-positive tumor cells is often correlated with the clinical course of cancer. Henzen-Logmans *et al.* [25] studied the immunoreactivity of Ki-67 in relation to immunohistochemically assessed expression of epidermal-growth-factor receptor (EGFR), estrogen receptor (ER), and progesterone receptor (PgR) in advanced human ovarian adenocarcinomas, borderline, and benign cystadenomas and normal ovaries. A significantly higher number of Ki-67-positive cells were found in metastatic tumors that of primary adenocarcinomas and in the total group of adenocarcinomas versus benign/borderline cystadenomas [25]. Giurgea *et al.* [5] found that Ki-67 was positive in 61.53% of malignant cases, with higher percentage observed in advanced clinical stages. Ki-67 immunoreactions were also positive in borderline and benign ovarian tumors, with a lower percentage, 13.3% and 9.09%, respectively, but proliferative activity, as assessed by Ki-67 staining, does not explain any possible relationship of serous borderline tumors to epithelial ovarian cancer [5]. Ki-67 as a marker of proliferation has been used to detect proliferating cells in tissue. Epidemiological studies show that anti-inflammatory drugs reduce the incidence and mortality of several types of cancer, indicating the potential role of pro-inflammatory factors in carcinogenesis [26]. The expression of most of the studied factors depended on the histological tumor subtype and the degree of malignancy. Expression of IL-1 and TNF-α is increased with the stage of the disease in both serous and mucinous tumors. The highest level of TGF-β expression is observed in serous borderline tumors.

In the present study, the differences in the medians of MCP-1, MCP-2, RANTES, Ki-67, and TNF-β were non-statistically significant. Spearman's correlation analysis performed on the group of women with endometriomas showed a statistically significant and positive dependence on the level of RANTES with Ki-67 ($r = 0.6$; $p = 0.0467$). Moreover, Spearman's correlation analysis performed on the group of women treated surgically for mature teratomas was statistically significant for the level of MCP-1 with

TNF-β (r = 0.7; p = 0.0301). Because these biomarkers were increase in benign and borderline as well as in malignancy, the benefit to use these markers to distinguish these tumors might be not useful. The role of investigated serum biomarkers, such as MCP-1 (CCL2), MCP-2 (CCL8), RANTES (CCL5), KI67, and TNF-β, as the markers of benign and borderline ovarian tumors is questionable.

Conclusion

The study of serum levels of MCP-1 (CCL2), MCP-2, RANTES (CCL5), Ki-67, and TNF-β in patients with benign and borderline ovarian tumors revealed correlations between the level of RANTES and Ki-67 and of MCP-1 and TNF-β, which suggests that chemokines as markers of benign and borderline ovarian tumors are questionable.

Acknowledgements

The study was financed with the funds for education as a research project 502-01-0111-01-38-038554 entitled: "Study of specific chemokines and non-chemokine factors and apoptotic proteins affecting the occurrence of benign neoplasms of female reproductive tract: endometrial cysts, mature teratomas, uterine sarcoma, and minor pelvic pains".

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