ORIGINAL ARTICLE

Proinflammatory and immunosuppressive serum, ascites and cyst fluid cytokines in patients with early and advanced ovarian cancer and benign ovarian tumors

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Abstract

OBJECTIVE: To analyze the profiles of interleukin-2 (IL-2), IL-6, IL-8, IL-10, tumor necrosis factor-α (TNF-α), transforming growth factor-β1 (TGF-β1) and interferon-γ (IFN-γ) in serum and the tumor microenvironment (cyst fluid, ascites) in women with ovarian cancer or benign ovarian tumors to find the differences in their immunological status. We also estimated serum cytokines as biomarkers to distinguish preoperatively between malignant or benign character of tumors.

DESIGN: Prospective study. **SETTING:** Tertiary referral hospital. **POPULATION:** 51 women with epithelial ovarian cancer, 26 with benign ovarian tumors of epithelial origin and 21 healthy controls. **METHODS:** The levels of cytokines were measured using ELISA sets.

RESULTS: We did not found differences in the levels of IFN-γ, TNF-α and IL-2 in all fluids isolated from patients with malignant or benign tumors. Women with advanced cancer had significantly higher serum IL-6, IL-10 and TGF-β1 levels than women with early stages or benign tumors. Moreover, women with very advanced cancer in whom the optimal cytoreduction was disabled had the highest serum levels of IL-10, TGF-β1 and IL-8. The concentrations of IL-6 and IL-8

were higher in ascites of cancer patients than in ascites of women with benign tumors. The areas under curves constructed for the selected cutoff serum cytokines levels (AUC-ROC) showed good predictive values for IL-6 (0.87), IL-10 (0.836) and IL-8 (0.797).

CONCLUSIONS: Our results indicate on intensified inflammatory process in women with ovarian cancer (accompanied by their immunosuppression). Preoperative analysis of serum IL-6, IL-10 and IL-8 may improve the differential diagnosis of ovarian tumors.

INTRODUCTION

In the last few decades, our knowledge of tumor-host interactions and cancer development has improved. The fundamental processes of cancer progression, tissue invasion, angiogenesis and metastasis are inherently proinflammatory and thus should activate innate and adaptive antitumor immunity. To elude immune surveillance, tumors must develop mechanisms that block the elaboration and sensing of proinflammatory danger signals, thereby shifting the balance from activation to tolerance induction (generally, shifting from Th1 type response mediated by interleukin-2 (IL-2), IL-12, IL-18, interferon-γ (IFN-γ) to Th2 type response mediated by IL-4, IL-5, IL-6, IL-10, IL-13). In fact, many cancer cells (include ovarian cancer) produce immunosuppressive (IL-10, tumor growth factor-β1 (TGF-β1)) and proinflammatory (IL-6, IL-8, tumor necrosis factor-α (TNF-α)) cytokines, which can weaken host antitumor immunity (Berger *et al.* 2001; Carr *et al.* 2008; Lin & Karin 2007; Toutirais *et al.* 2003). Tumor cells undergo immune escape and grow rapidly when their immunosuppressive activity is stronger than the host- mediated antitumor response. In the opposite situation, when the host- mediated antitumor immunity is stronger, tumor cells are eliminated (Lin & Karin 2007).

Ovarian cancer is still the leading problem in gynecological oncology. It accounts for about 25% of gynecological malignant neoplasms, but is responsible for almost 50% of deaths caused by these malignancies (Benedet *et al.* 2000; Piver & Hempling 1997). The diagnosis of ovarian cancer is always a very serious but not homogenic situation. The combined 5-year survival rate for all patients with ovarian cancer is approximately 50%, but is much higher (90%) when the disease is diagnosed and properly treated early (stage I by FIGO) and radically decreases (18%) in the advanced stage (stage IV by FIGO) (Heintz *et al.* 2006). The prognosis for women with ovarian cancer is also dependent on the histopathological type and grade of the tumor and the residual disease left after the primary surgery (DiSaia & Creasman 1997; Heintz *et al.* 2006).

Ovarian cancer is often called a "silent killer" because almost 75% of patients are diagnosed in the advanced stage of the disease (stage III and IV) due to the absence of or nonspecific clinical symptoms at the beginning and the lack of screening methods for diagnosis (Benedet *et al.* 2000; Piver & Hempling 1997). Up till now, the gold standard in the diagnosis of pelvic masses is still a bimanual gynecological examination supplemented by transvaginal sonography (with color Doppler) and eventually, serum markers. The most common serum marker used is CA 125. However, it is not specific for this disease. It is elevated in about 80% of patients with ovarian cancer, but also in about 60–80% of cases of liver cirrhosis with ascites or endometriosis, in 15–20% of benign and borderline ovarian tumors and in 1–5% of healthy women (DiSaia & Creasman 1997; Piver & Hempling 1997; Rustin *et al.* 1993). In the last few decades, many serologic biomarkers have been evaluated during the diagnosing of ovarian cancer, including those based on tumor-host immunologic interactions (various cytokines and antibodies) (Bertenshaw *et al.* 2008; Gorelik *et al.* 2005; Kavsak *et al.* 2008; Lambeck *et al.* 2007; Lokshin *et al.* 2006; Visintin *et al.* 2008a,b). Up till now none of them has been applied to the general practice.

Preoperative assessment of the ovarian tumor is very important especially, when we consider laparoscopic procedures, because they are connected with a higher risk than laparotomy of damaging the cyst capsule and the dissemination of cancer cells. On the other hand, laparoscopy is less traumatic, guarantees faster patient recovery and is widely used in gynecologic surgery. Many authors proposed the use of cytokines determinations in the preoperative differential diagnosis of the ovarian cancer and benign ovarian cysts or wider – benign pelvic masses (and in comparison to healthy controls), but they usually did not report the clinical and ultrasound signs of those tumors (Bertenshaw *et al.* 2008; Gorelik *et al.* 2005; Kavsak *et al.* 2008; Lambeck *et al.* 2007; Lokshin *et al.* 2006; Visintin *et al.* 2008a,b).

Thus, the first aim of our study was to analyze the cytokine profiles (proinflammatory: IL-6, IL-8, tumor necrosis factor-α (TNF-α), immunosuppressive: IL-10, TGF-β1 and Th1/Th2 balance: IFN-γ, IL-2/IL-6, IL-10) in serum and the tumor microenvironment (cyst fluid, ascites) in women with ovarian cancer or benign ovarian tumors to find the differences in their immunological status. According to main prognostic factors for women with ovarian cancer listed above, we also analyzed the differences in the cytokines profiles in relation to the clinical stage of the disease, histological grading of the tumor and the result of the primary surgery. Secondarily, we estimated the use of the selected (in the first part of this study) serum cytokines determinations to discriminate between malignant and benign character of ovarian tumors clinically and sonographically assessed as "suspected" in order to improve the preoperative differential diagnosis in these patients.

MATERIALS AND METHODS

Patients

Our study, approved by local Ethical Committee, was carried out in the Polish Mother's Memorial Hospital - Research Institute, Lodz, Poland. We checked the patients with a pelvic mass suspected to be an ovarian tumor admitted to the gynecologic departments. Patients with a history of any previous malignant neoplasia or gynecologic operation, transplantation, autoimmunologic disease, diabetes, thyroid problems or any signs of infection were excluded from the study. After examining the patients we qualified for further prospective evaluation women with ovarian tumors suspected of malignancy (solid or mixed, cystic multiocular with thick septa, low mobility, pathologic vascularisation, presence of ascites etc). After operative treatment and histopathologic examination, we finally collected 77 patients for further analysis and divided them into two main groups: 51 women with epithelial ovarian cancer (mean age: 58.4 years; range: 26–79 yr) and 26 with benign ovarian tumors of epithelial origin (16 serous and 10 mucinous) (mean age: 55.8 years; range: 39–71). Ascites was present in 37 cases of ovarian cancer (73%) and in 5 patients with benign ovarian tumors (19%). The control group consisted of 21 healthy age-matched (mean: 52.1 yr; range: 19–76) women from the outpatient clinic. The stage of ovarian cancer was established after laparotomy and pathologic examination, according to the International Federation of Gynecology and Obstetrics (FIGO) classification and protocol (Benedet *et al.* 2000). All tissues removed during surgery were examined by a pathologist: tumor grade, histological type and the presence of metastases was established, according to FIGO and WHO classifications (Benedet *et al.* 2000). Optimal cytoreduction was defined as leaving no residual disease greater than 1 cm in diameter after primary surgery.

The clinical data of ovarian cancer patients is listed in Table 1. In our study group, 15 women (29%) were diagnosed and operated on in the early stage of the disease (stage I and II) and 36 patients (71%) were suffering from advanced ovarian cancer (stage III and IV). The age of patients with early ovarian cancer (mean: 58.1 yr; range: 26–79) was similar when compared to advanced stage of the disease (mean: 59.0 yr; range: 37–78). There where no significant differences in tumor grade and histology according to the clinical stage of the disease (Table 1). It is obvious that in the early stage of ovarian cancer, optimal cytoreduction was possible in almost every patient (93%), while in the advanced stage it was possible in 28% of women.

Cytokines determinations

Peripheral venous blood from patients with epithelial ovarian tumors was obtained in the morning of the day of the surgery or during routine laboratory tests in the control group. Ascites and cyst fluids were collected during surgery. All body fluids were transported to the laboratories within one hour of collection. Body fluid samples were centrifugated at 1 500 × g for 10 minutes and then stored at –80 °C until analyzed. We determined the levels of interferon-γ (IFN-γ), tumor necrosis factor-α (TNF-α), intereukin-2 (IL-2), IL-10, IL-6, IL-8 using BD OptEIA Human ELISA sets (Becton-Dickinson) for every listed cytokine. The sensitivity of these tests was 1 pg/ml. The data are presented in pg/ml. The concentration of tumor growth factor-β1 (TGF-β1) was measured by means of Human TGF-β1 ELISA sets (Bender) with the sensitivity 23.8 pg/ml. The results are presented in ng/ml.

Statistics

For the continuous data we calculated mean, median, standard deviation (SD) and standard error of the mean (SEM). Verification of the distributions was made with the Shapiro-Wilk test. To compare the differences among groups we used the Students' t test or Mann-Whitney U test where appropriate. For dependent values (various body fluids from the same patient) we applied the Wilcoxon signed-rank test. For the categorical variables the χ^2 test was used. In the ovarian cancer group a multivariate analysis of variances (ANOVA)

Tab. 1. Ovarian cancer patients characteristics.

	Ovarian cancer	FIGO I/II	FIGO III/IV	*p*-value
	n (%)	n (%)	n (%)	
	51 (100)	15 (29.4)	36 (70.6)	
Age (mean ± SEM)	58.4±1.7	58.1±4.2	59.0±1.8	0.85
Stage				
I	13 (25.0)	13 (86.6)		
II	2 (4.0)	2 (13.4)		
III	33 (65.0)		33 (91.7)	
IV	3 (6.0)		3 (8.3)	
Grade				
1	9 (18.0)	5 (33.3)	4 (11.1)	
2	14 (27.0)	4 (26.7)	10 (27.8)	0.30
3	28 (55.0)	6 (40.0)	22 (61.1)	
Histology				
Serous	22 (43.0)	5 (33.3)	17 (47.2)	
Mucinous	7 (13.5)	2 (13.3)	5 (13.9)	
Endometrioid	11 (21.5)	6 (40.0)	5 (13.9)	0.51
Clear cell	2 (4.0)	1 (6.7)	1 (2.8)	
Undifferentiated	9 (18.0)	1 (6.7)	8 (22.2)	
Cytoreduction				
Optimal (≤ 1cm)	24 (47.0)	14 (93.3)	10 (27.8)	<0.001
Suboptimal (> 1cm)	27 (53.0)	1 (6.7)	26 (72.2)	<0.001

was also performed. We checked the influence of FIGO stage (2 levels: stage I/II and III/IV) and tumor grade (3 levels: G1, G2, G3) or FIGO stage (2 levels) and the result of cytoreduction during primary surgery (2 levels: optimal and suboptimal) on the results of the investigated cytokines. After this, ANOVA post hoc tests were used (NIR test).

We also estimated the use of serum cytokines determinations in preoperative distinguishing between benign and malignant ovarian tumors. For this purpose we calculated sensitivity, specificity, positive and negative predictive values and accuracy for multiple cutoff values using the results of all ovarian cancer and benign tumor patients. Then, we constructed receiver operator characteristic (ROC) curves for the selected cutoff levels and calculated the areas under the curves (AUC).

The differences were considered as statistically significant for p-value ≤ 0.05.

RESULTS

Thirty-three (33.7%) of 98 women investigated had detectable serum IFN-γ levels. TNF-α was almost not detectable in serum (only 9.2 % positive results (9/98)). IL-2 was detectable in 25.5% of cases (25/98). In analyzing the levels of these cytokines in ascites and cyst fluids no significant differences in the investigated groups of patients were observed (data not shown). Thus, IFN-γ, TNF-α and IL-2 were excluded from the further analysis.

Almost all the women (>95%) had detectable serum levels of IL-6, IL-8, IL-10 and TGF-β1. The results are presented on the Figure 1. In patients with ovarian cancer serum levels of IL-6, IL-8 and IL-10 were the highest of all investigated groups (p<0.05), whereas the levels of these cytokines didn't differ in patients with benign ovarian cysts and healthy controls. Serum TGF-β1 levels were similar in patients with all investigated kinds of ovarian tumors and all significantly higher than in healthy controls (Figure 1). By analyzing of serum cytokines levels we found significant differences between early and advanced ovarian cancer. Serum IL-10 concentrations in patients with III/IV FIGO stage of ovarian cancer were significantly higher than in all other investigated groups (p<0.05), when in I/II FIGO stage IL-10 levels did not differ from the results noted in women with benign ovarian tumors and healthy controls (Figure 1). The same differences were observed in serum TGF-β1 levels according to FIGO stage of ovarian cancer with one exception: patients in the early stages of the disease had the results similar to healthy controls and lower than all other groups of ovarian tumors. Serum IL-6 concentrations in women with advanced cancer were higher than in all other groups (Figure 1).

The levels of IL-6, IL-8, and IL-10 in ascites were significantly higher in patients with advanced ovarian cancer than in women with the early stage of this disease (except IL-8) and benign ovarian cysts (except IL-10) (Table 2). Patients with I/II FIGO stage of ovarian cancer had elevated levels of IL-8 in the ascites when compared to the benign group. Cyst fluid obtained from advanced ovarian cancer had significantly higher TGF-β1 concentrations than in the early stage of the disease, when IL-6, IL-8 and IL-10 were similar in all investigated stages of ovarian cancer and benign tumors (Table 2).

Figure 2 presents the comparison of mean levels of the cytokines in serum, ascites and cyst fluid of patients from whom all three fluids were present and collected.

Tab. 2. Ascites and cyst fluid proinflammatory and immunosuppressive cytokines concentrations in investigated groups of patients (results are presented as mean ± SEM in pg/ml).

	Benign	Ovarian Cancer	Stage I/II	Stage III/IV
Ascites	n = 5	n = 37	n = 9	n = 28
IL-6	2 415.8 ± 1 580.6	9 337.6 ± 1 619.7[&]	4 145.7 ± 1 734.2	11 006.4 ± 1 977.9[&]
IL-8	502.9 ± 118.2	5 546.1 ± 1 827.7[#]	6 962.8 ± 3 612.0[#]	5 090.7 ± 2 147.4[#]
IL-10	200.1 ± 92.3	258.1 ± 38.9	102.5 ± 21.8	302.5 ± 46.4*
TGF-β1	–	14 435.3 ± 2 269.7	12 560.1 ± 4 301.1	14 837.1 ± 2 649.3
Cyst Fluid	n = 7	n = 20	n = 5	n = 15
IL-6	12 301.1 ± 7 572.8	25 403.6 ± 6 766.7	13 504.7 ± 6 350.3	28 519.8 ± 8 286.7
IL-8	12 020.5 ± 7 571.1	12 816.4 ± 3 115.3	9 681.3 ± 3 021.4	13 705.0 ± 3 871.8
IL-10	114.2 ± 40.6	195.4 ± 29.6	119.1 ± 60.5	221.5 ± 32.1
TGF-β1	–	13 056.6 ± 3 160.9	8 200.4 ± 2 092.2	13 549.8 ± 3 451.4*

[&] higher than stage I/II of ovarian cancer and benign, p<0.05
[#] higher than benign, p<0.05
* higher than stage I/II of ovarian cancer, p<0.001

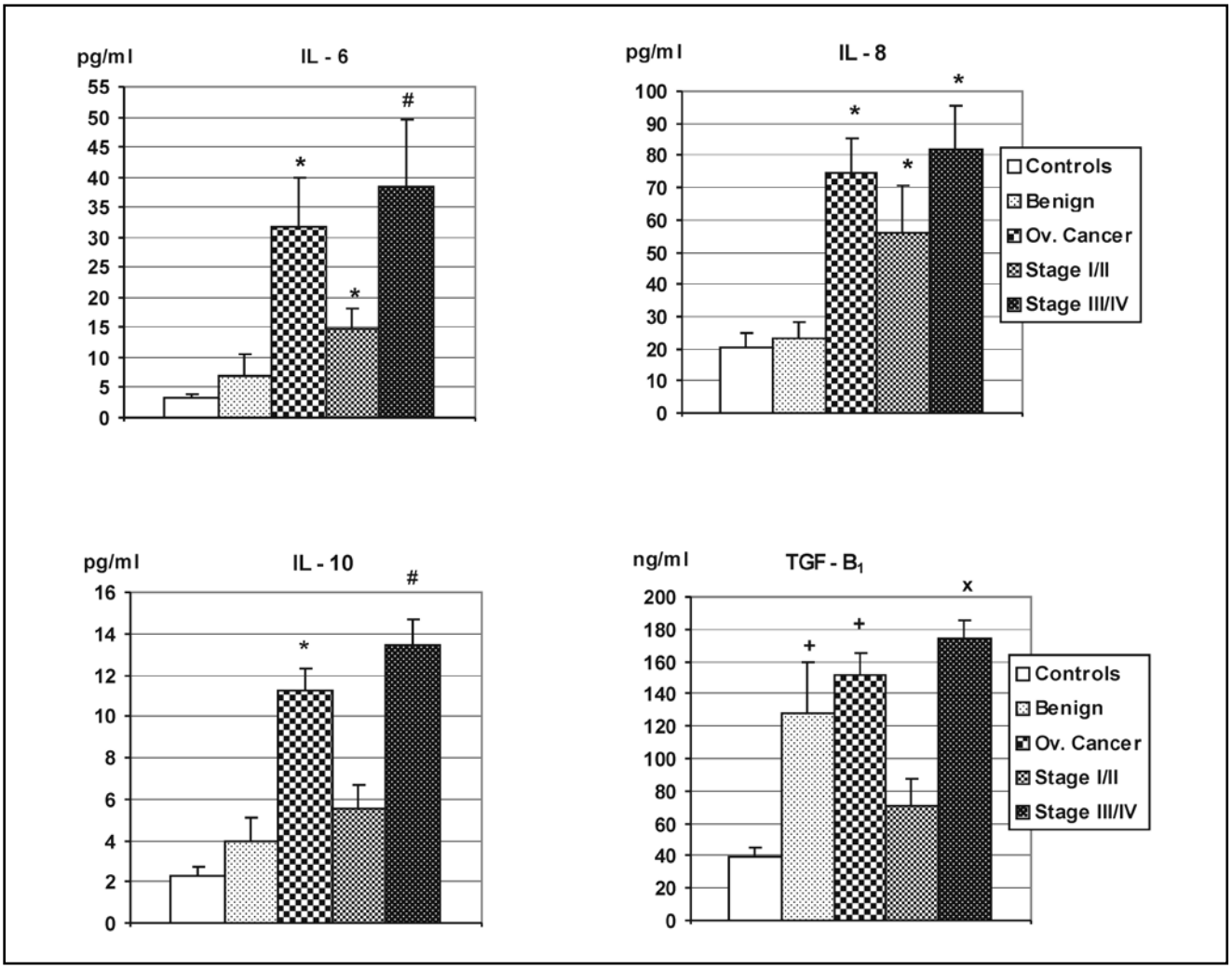

Fig. 1. Serum inflammatory, immunosuppressive and Th2 type cytokines concentrations in groups of patients investigated (data presented as mean ± SEM).
* higher than controls and benign, $p<0.001$ for IL-6, IL-10 and IL-8 (in ovarian cancer and stage III/IV); $p<0.05$ for IL-8 in stage I/II
# higher than controls, benign and stage I/II of ovarian cancer ($p<0.001$)
+ higher than controls, $p<0.05$ for IL-6, IL-8, IL-10 and TGF-β1 (in benign); $p<0.005$ for TGF-β1 in ovarian cancer
x higher than controls and stage I/II of ovarian cancer, $p<0.05$

It was possible in 17 women with ovarian cancer (5 with early and 12 with advanced stage of the disease) and five with benign ovarian tumors. IL-10 reached significantly higher levels in ascites than in serum of ovarian cancer patients ($p<0.001$) and slightly lower in cyst fluid, but still higher than in serum ($p<0.01$). The same tendencies were observed in other groups of patients (Figure 2). Another pattern of the cytokines concentrations was found in the results of IL-6 and IL-8 measurements. In patients with ovarian cancer (and advanced subgroup), we noted a significant higher IL-6 and IL-8 levels in ascites when compared to serum ($p<0.001$) and then higher in cyst fluid in comparison with ascites ($p<0.01$). Similar tendencies were observed in cases of early ovarian cancer and benign ovarian cysts (Figure 2).

We have also performed an analysis of serum, ascites and cyst fluid cytokines levels in ovarian cancer patients according to the result of the primary surgery and the tumor grade. In the group of patients where advanced cancer process resulted in suboptimal cytoreduction, we found higher serum TGF-β1 ($p<0.005$), IL-10 ($p<0.01$) and IL-8 ($p<0.05$) concentrations than in patients with optimal surgery performed (Figure 3). Ascites and cyst fluid cytokines levels did not differ significantly according to the type of cytoreduction, except IL-6. It achieved higher levels in ascites (mean ± SEM: 12 296±9 972 pg/ml vs. 8 492±3 707 pg/ml, $p<0.05$) and cyst fluid (37 703±9 972 pg/ml vs. 8 282±3 687 pg/ml, $p<0.05$) of patients with suboptimal surgery vs. patients with optimal cytoreduction. No significant differences in the cytokines levels in all three investigated fluids according to the tumor grading were found.

In the ovarian cancer patients we also analyzed the interrelations between FIGO stage and tumor grade or FIGO stage and the type of cytoreduction on the levels

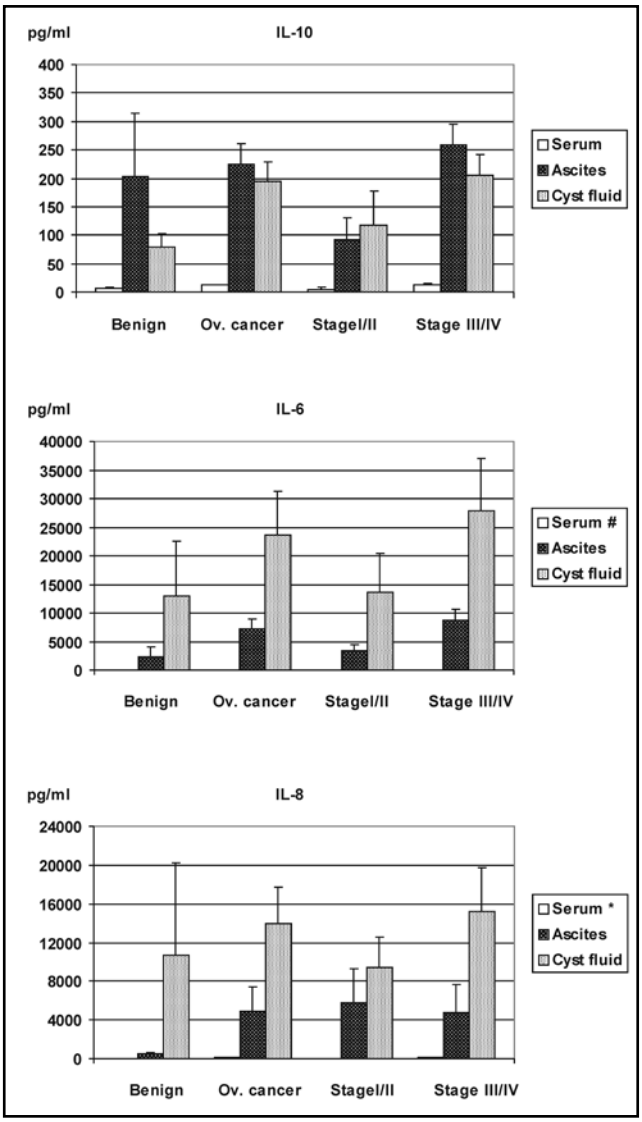

Fig. 2. Comparison of serum, ascites and cyst fluid cytokines levels. Presented data are means (± SEM) of the results taken only from patients from whom all three fluid samples were collected (results commented in text).
serum levels of IL-6 in benign was 9.25±7.6 pg/ml, in ovarian cancer 23.4±6.6, in stage I/II 12.3±6.5 and in stage III/IV was 26.5±7.9 pg/ml
* serum levels of IL-8 in benign was 37.4±8.6 pg/ml, in ovarian cancer 74.9±15.7, in stage I/II 47.4±16.6 and in stage III/IV was 81.0±19.0 pg/ml

of the investigated serum cytokines by means of multivariate analysis of variances (ANOVA). We found only one interrelation: FIGO stage and the type of cytoreduction on the serum TGF-β1 levels (Figure 4).

According to our results showing the significant differences in serum IL-6, IL-8 and IL-10 levels in patients with ovarian cancer when compared to women with benign ovarian tumors, we estimated the use of serum determinations of these cytokines in preoperative differentiation between benign and malignant ovarian lesions. For IL-10 > 5.3 pg/ml the sensitivity was 86%, the specificity was 82%, the positive and negative predictive values were 96% and 56% and the accuracy was 85% in predicting of malignancy. At a cut-off IL-6 level at 5.7 pg/ml, the sensitivity was 86%, the specificity was 77%, the positive and negative predictive values were 90% and 71% and the accuracy was 83%. For IL-8 > 20 pg/ml, the calculated sensitivity was 84%, the specificity was 68%, the positive and negative predictive values were 86% and 65% and the accuracy was 79%. The receiver operator characteristic (ROC) curves for the cutoff values listed above are shown on the Figure 5. The areas under curves showed good predictive values of IL-6 (AUC = 0.866), IL-10 (0.836) and IL-8 (0.797) in the preoperative distinguishing between malignant and benign ovarian tumors.

DISCUSSION

The Th1 type cytokines were detected in sera of only a few patients in all groups investigated. The low concentration of these cytokines (below the limits of detection) in serum was also noted by the others investigators (Gorelik *et al.* 2005; Kavsak *et al.* 2008). Serum proinflammatory (IL-6, IL-8), immunosuppressive (IL-10, TGF-β1) and Th2 type cytokines (IL-6, IL-10) were detectable in almost all patients. IL-6, IL-8, IL-10 had the highest serum concentrations in ovarian cancer patients. The Th1 type cytokines concentrations in ascites were similar in all groups of women investigated. Ascites IL-6, IL-8, IL-10 reached the highest levels in ovarian cancer patients (in cyst fluid no differences were noted). We also found significant differences in the immunological status of patients with early and advanced ovarian cancer. Women suffering from the advanced cancer process had higher levels of Th2 and immunosuppressive cytokines (in serum and ascites) then those in the early stage of the disease or with benign ovarian tumors. Patients in the early stages of ovarian cancer showed a cytokine pattern (except IL-8) in ascites similar to women with benign tumors. Moreover, we noted the worse status of women with the advanced disease leading to inefficient cytoreduction during primary surgery – they had the highest levels of serum IL-10, TGF-β1 and IL-8, what may be one of the factors of their poor prognosis. These results may indicate the inflammatory character of ovarian cancer process and immunosuppression of these women. Serum TGF-β1 results also showed that patients with benign ovarian tumors could be in a kind of immunosuppression when compared to healthy controls.

The comparison of the serum, ascites and cyst fluid cytokines levels from the same patients showed a proinflammatory situation in the cancer microenvironment (the highest levels of IL-6 and IL-8 in the cyst fluid) accompanied by a high concentration of immunosuppressive IL-10 (ascites and cyst fluid).

Thus, the results of our study support the thesis about the crucial role of inflammation in cancer development and progression. A tumor inflammatory microenviron-

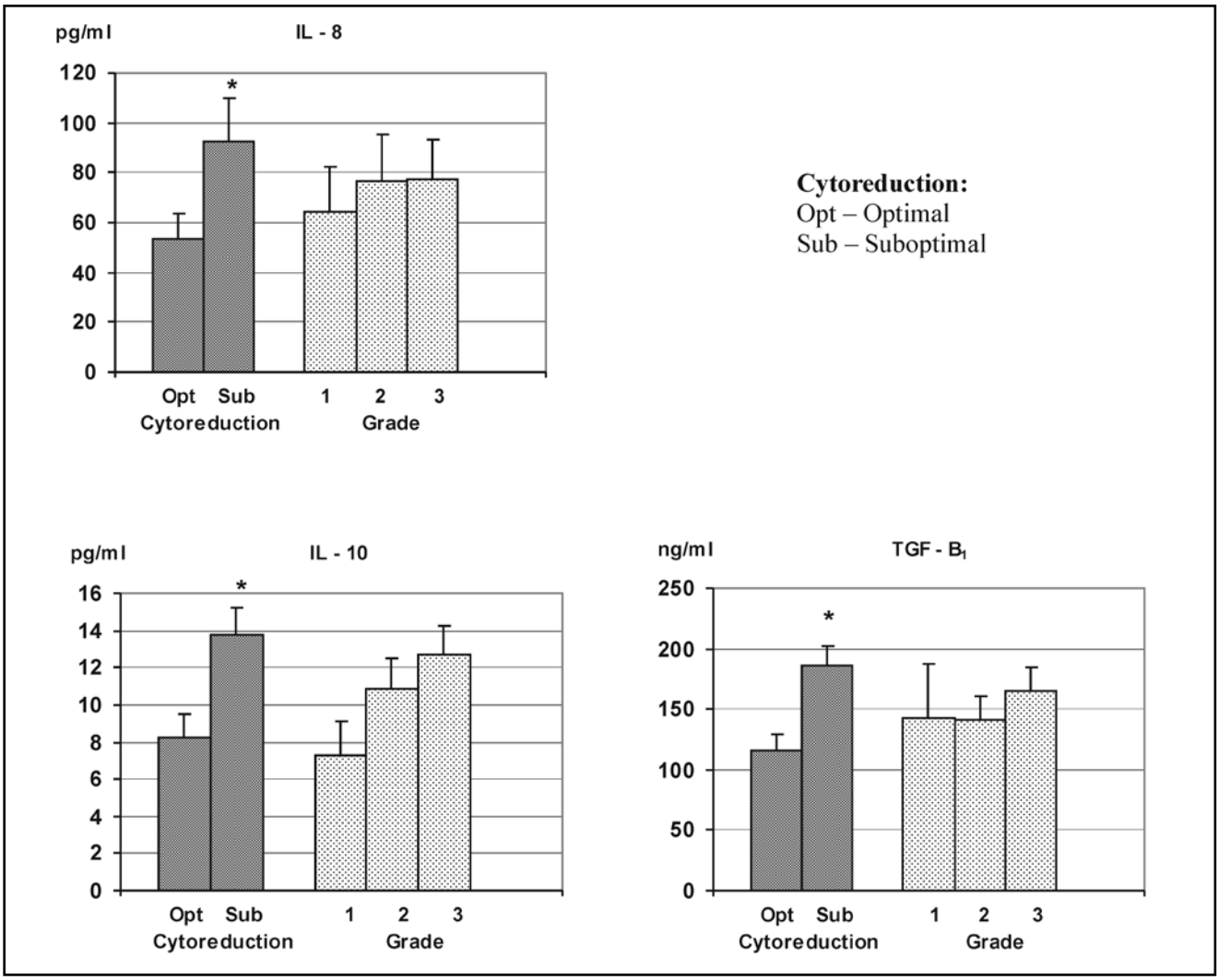

Fig 3. Serum cytokines levels in ovarian cancer patients according to the result of the primary surgery and the tumor grade (data presented as mean ± SEM).
* higher than optimal cytoreduction, $p<0.05$ for IL-8, $p<0.01$ for IL-10 and $p<0.005$ for TGF-β1

ment facilitates the breakage of the basal membrane in the process of cancer invasion of the surrounding tissues and metastases (Coussens & Werb 2002; Freedman *et al.* 2004). *In vitro* studies revealed that ovarian cancer cells produce an array of cytokines and chemokines (e.g. IL-6, IL-8) attracting inflammatory cells: neutrophils, macrophages, dendritic cells, eosinophils, mast cells and lymphocytes (Berger *et al.* 2001; Carr *et al.* 2008; Toutirais *et al.* 2003; Lu *et al.* 2006). The immunocytes infiltrating the tumor produce cytokines, proteases, matrix metalloproteinases which help tumor cells to proliferate and shift the host response to immunosuppression (Lin & Karin 2007; Lu *et al.* 2006). Moreover, ovarian cancer cells also produce the cytokines that exert immunosuppressive effects (e.g. IL-10, TGF- β1) (Berger *et al.* 2001; Carr *et al.* 2008; Toutirais *et al.* 2003; Poggi & Zocchi 2006).

The second goal of our study was to estimate the use of serum cytokines as biomarkers for the preoperative differential diagnosis of malignant and benign character of an ovarian tumor which had been clinically and ultrasonographically assessed as 'suspected'. Our results revealed that the highest accuracy was for IL-10, IL-6 and IL-8. The use of serum determinations of these cytokines may improve preoperative differentiation of the suspected ovarian tumors (AUC for IL-6 was 0.87, 0.84 for IL-10 and 0.8 for IL-8) but is insufficient for ovarian cancer screening. Even more complex immunologic determinations are still inadequate for this purpose. The use of a novel multianalyte LabMAP profiling technology for early detection of ovarian cancer was reported by Gorelik *et al.* (2005). A panel of 24 serologic markers (CA 125, cytokines, chemokines, and growth and angiogenic factors) was analyzed in blood of women with early ovarian cancer, benign pelvic masses and healthy individuals. Serum concentrations of IL-6, IL-8 and CA 125 were the highest in ovarian cancer patients. They have also constructed a classifica-

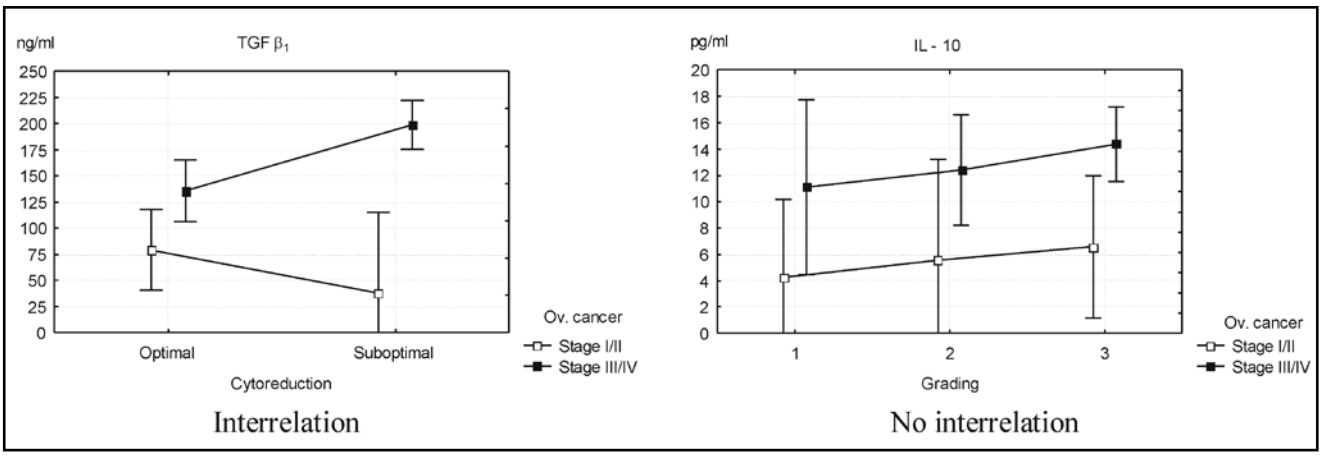

Fig. 4. Serum cytokines levels in ovarian cancer patients – multivariate analysis of variances (ANOVA) – the influence of FIGO stage and cytoreduction or FIGO stage and tumor grading. We found one interrelation: serum TGF-β1/FIGO Stage/Cytoreduction (p=0.031); for others there were no interrelations – for example: serum IL-10/FIGO Stage/Grading. The data are presented as means ± 95% confidence intervals.

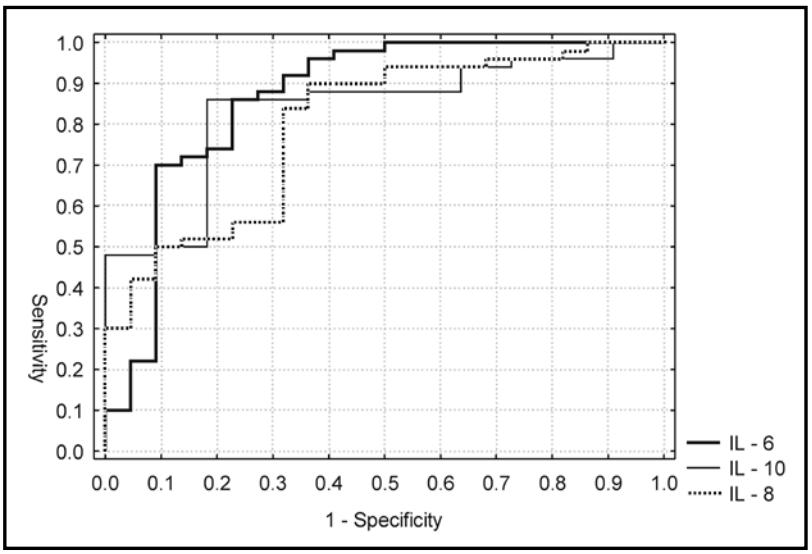

Fig. 5. Receiver operator characteristic (ROC) curves for the selected cytokines in predicting of the malignancy of ovarian tumors. The curves are constructed for the cutoff value of 5.7 pg/ml for IL-6; 5.3 pg/ml for IL-10 and 20 pg/ml for IL-8.

tion tree for comparison of benign tumors and cancer (using CA 125, granulocyte colony-stimulating factor (G-CSF), IL-6, epidermal growth factor (EGF) and vascular endothelial growth factor (VEGF)) which resulted in a sensitivity of 84.1% and specificity of 75.7% (80.2% of patients were correctly classified). For comparisons of early stages of ovarian cancer versus healthy controls, the classification tree was composed of CA 125, EGF, VEGF, IL-6 and IL-8. It resulted in specificity at 91%, sensitivity at 96% (93% of subjects correctly classified) and area under the ROC curve was 0.966 (Gorelik *et al.* 2005). Lokshin *et al.* (2006) reported that the combination of serum IL-8, anti-IL-8 antibodies and CA 125 had a sensitivity of 87.5% and specificity 98% of ovarian cancer detection (compared to healthy controls). This three marker composite panel resulted in 98% specificity but only 42% sensitivity in distinguishing between malignant and benign ovarian tumors (Lokshin *et al.* 2006). Lambeck *et al.* (2007) reported the use of cytokine bead array for the simultaneous analysis of 14 serum cytokines in sera of women with ovarian cancer, benign ovarian tumors and healthy controls. They found that serum CA 125, IL-6, IL-7, IL-10 was elevated in ovarian cancer patients and had the highest diagnostic value in discriminating between malignant and benign lesions or healthy controls (AUC were: 0.7 to 0.88). The highest predictive value was achieved by combining IL-7 and CA 125: 69% of the patients with ovarian cancer was accurately classified without falsely classifying women with a benign ovarian tumor (Lambeck *et al.* 2007). Bertenshaw *et al.* (2008) analyzed serum concentrations of 204 analytes representing 104 antigens, 44 autoimmune and 56 infectious disease molecules measured using a set of proprietary multiplexed immunoassays in patients with ovarian cancer. The control group was not homogenic and consisted of women with benign ovar-

ian conditions (71%), other gynecologic cancers (10%) and healthy individuals (19%). The highest discriminatory power for ovarian cancer was noted for CA 125 (AUC = 0.966), C-reactive protein (0.756), epidermal growth factor receptor (0.733), IL-10 (0.725) and IL-8 (AUC = 0.715) (Bertenshaw *et al.* 2008). The recent data about the new panel of biomarkers published by Visintin *et al.* (2008a) seemed to be very useful in the ovarian cancer screening. This novel multiplex blood biomarker test (leptin, prolactin, osteoponin, insulin-like growth factor II, macrophage inhibitory factor and CA 125) had a sensitivity of 95.3% and specificity of 99.4% for the detection of ovarian cancer in the investigated group of women. The chosen model correctly classified 221 out of 224 specimens in the test set, with a classification accuracy of 98.7%, but after recalculating the obtained results with the real incidence of ovarian cancer in the population the predictive value of this test decreased to 6.5% (Visintin *et al.* 2008a,b).

In conclusion, our results suggest that the inflammatory character of the ovarian cancer process and immunosuppression are parallel in women suffering from this disease. Women suffering from an advanced ovarian cancer had higher levels of inflammatory and immunosuppressive cytokines (in serum and ascites) then those in the early stage of the disease or with benign ovarian tumors. Moreover, women with advanced ovarian cancer resulting in inefficient cytoreduction had the highest levels of IL-10, TGF-β1 and IL-8 what may be the additional factor of their poor prognosis. The analysis of serum IL-6, IL-10 and IL-8 may improve the preoperative differential diagnosis in patients with suspected ovarian tumors.

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REFERENCES

1 Benedet JL, Bender H, Jones III H, Ngan HYS, Pecorelli S (2000). FIGO staging classifications and clinical practice guidelines in the management of gynecologic cancers. Int J Gynecol Obstet. **70:** 209–62.
2 Berger S, Siegert A, Denkert C, Kobler M, Hauptmann S (2001). Interleukin-10 in serous ovarian carcinoma cell lines. Cancer Immunol Immunother. **50:** 328–33.
3 Bertenshaw GP, Yip P, Seshaiah P, Zhao J, Chen TH, Wiggins WS et al (2008). Multianalyte profiling of serum antigens and autoimmune and infectious disease molecules to identify biomarkers dysregulated in epithelial ovarian cancer. Cancer Epidemiol Biomarkers Prev. **17:** 2872–81.
4 Carr TM, Adair SJ, Fink MJ, Hogan KT (2008). Immunological profiling of a panel of human ovarian cancer cell lines. Cancer Immunol Immunother. **57:** 31–42.
5 Coussens LM, Werb Z (2002). Inflammation and cancer. Nature. **420:** 860–7.
6 DiSaia PJ, Creasman WT (1997). Epithelial ovarian cancer. In: DiSaia PJ, Creasman WT, editors. Clinical gynecologic oncology. 5th ed. St Louis, Mosby-Year Book. p. 282–350.
7 Freedman RS, Deavers M, Liu J, Wang E (2004). Peritoneal inflammation – A microenvironment for epithelial ovarian cancer (EOC). J Transl Med. **2:** 23–33.
8 Gorelik E, Landsittel DP, Marrangoni AM, Modugno F, Velikokhatnaya L, Winans MT et al (2005). Mulitplexed immunobead-based cytokine profiling for early detection of ovarian cancer. Cancer Epidemiol Biomarkers Prev. **14:** 981–7.
9 Heintz AP, Odicino F, Maisonneuve P, Quinn MA, Benedet JL, Creasman WT et al (2006). Carcinoma of the ovary. FIGO 6th Annual Report on the Results of Treatment in Gynecological Cancer. Int J Gynaecol Obstet. **95:** Suppl1:161–92.
10 Kavsak PA, Lee A, Hirte H, Young E, Gauldie J (2008). Cytokine elevations in acute coronary syndrome and ovarian cancer: A mechanism for the up-regulation of the acute phase proteins in these different disease etiologies. Clin Biochem. **41:** 607–10.
11 Lambeck AJA, Crijns APG, Leffers N, Sluiter WJ, ten Hoor KA, Braid M et al (2007). Serum cytokine profiling as a diagnostic and prognostic tool in ovarian cancer: a potential role for interleukin 7. Clin Cancer Res. **13:** 2385–91.
12 Lin WW, Karin M (2007). A cytokine-mediated link between innate immunity, inflammation, and cancer. J Clin Invest. **117:** 1175–83.
13 Lokshin AE, Winans M, Landsittel D, Marrangoni AM, Velikokhatnaya L, Modugno F et al (2006). Circulating IL-8 and anti-IL-8 autoantibody in patients with ovarian cancer. Gynecol Oncol. **102:** 244–51.
14 Lu Haitan, Ouyang W, Huang C (2006). Inflammation, a key event in cancer development. Mol Cancer Res. **4:** 1–13.
15 Piver MS, Hempling RE (1997). Ovarian cancer: etiology, screening, prophylactic oophorectomy, and surgery. In: Rock JA, Thompson JD, editors. Te Linde's operative gynecology. 8th ed. Philadelphia, New York: Lippincott-Raven Publishers. p. 1557–68.
16 Poggi A, Zocchi MR (2006). Mechanisms of tumor escape: role of tumor microenvironment in inducing apoptosis of cytolitic effector cells. Arch Immunol Ther Exp. **54:** 323–33.
17 Rustin GJS, van der Burg MEL, Berek JS (1993). Tumour markers. Ann Oncol. **4:** 71–7.
18 Toutirais O, Chartier P, Dubois D, Bouet F, Levegue J, Catros-Quemener V et al (2003). Constitutive expression of TGF-beta1, interleukin-6 and interleukin-8 by tumor cells as a major component of immune escape in human ovarian carcinoma. Eur Cytokine Netw. **14:** 246–55.
19 Visintin I, Feng Z, Longton G, Ward DC, Alvero AB, Lai Y et al (2008a). Diagnostic markers for early detection of ovarian cancer. Clin Cancer Res. **14:** 1065–72.
20 Visintin I, Feng Z, Longton G, Ward DC, Alvero AB, Lai Y et al (2008b). Correction: Diagnostic markers for early detection of ovarian cancer. Clin Cancer Res. **14:** 5308.