RESEARCH ARTICLE

Serum levels of IL-6, IL-8 and CRP as prognostic factors in epithelial ovarian cancer

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ABSTRACT. In the present study, associations between pretreatment interleukin 6 (IL-6), interleukin 8 (IL-8) and C-reactive protein (CRP) serum levels and epithelial ovarian cancer (EOC) were analyzed using commercially available, enzyme-linked immunosorbent assay (ELISA) in 118 patients and 64 control subjects. Values were correlated with clinicopathological characteristics and outcomes. Control variables included age, stage, grade, histological type and residual tumor size. Kaplan-Meier plots and univariate and multivariate Cox proportional hazards models were used to study the associations between IL-6, IL-8 and CRP levels, control variables, overall survival and disease-free survival.The median IL-6, IL-8 and CRP serum levels in EOC were significantly higher than in the normal control group; 11.5 pg/mL (range, 3.4-62.6) *versus* 2.9 (1.1-12.3) pg/mL ($p<0.001$) and 21.8 pg/mL (range, 16.4-105.3) *versus* 9.3 (4.3-32.4) pg/mL ($p<0.001$) and 9.51 mg/L (range, 0.3-129.2) *versus* 1.2 (0.1-11.5) mg/L ($p = 0.001$), respectively. High levels of IL-6, IL-8 and CRP were associated with reduced overall survival ($P = 0.003$, $P = 0.035$, $P = 0.046$) and disease-free survival ($P<0.001$, $P = 0.026$, $P = 0.043$), respectively. Multivariate analyses showed that IL-6, IL-8 and CRP serum levels independently predicted disease-free survival ($P = 0.011$, $P = 0.001$ and $P = 0.021$), and overall survival ($P = 0.004$, $P = 0.014$ and $P = 0.016$), respectively.EOC is associated with extensive changes in the serum cytokine environment, highlighting the importance of further investigations of relative cytokine level changes. Preoperative serum IL-6, IL-8, and CRP levels seem promising for distinguishing EOC patients from healthy controls; however, their clinical value is still to be confirmed. High levels of IL-6, IL-8, and CRP in EOC patients have been suggested to be a poor prognostic factor for OS and DFS.

Key words: interleukin-6 (IL-6), interleukin-8 (IL-8), C-reactive protein (CRP), prognostic factors, epithelial ovarian cancer (EOC)

Epithelial ovarian cancer (EOC) is the prevailing malignant ovarian tumor in adult women. About 190,000 new cases and 114,000 deaths from ovarian cancer are estimated to occur annually. The highest rates are reported in Scandinavia and Eastern Europe, the USA, and Canada. The age-adjusted incidence rate in the USA is 12.48 per 100,000 women per year [1]. Low rates are found in Africa and Asia [2]. In Poland, the age-adjusted incidence is 10.8 per 100,000 women per year [3].

The etiology of EOC is poorly understood. Over the years, a variety of agents including hereditary, reproductive, hormonal, inflammatory, dietary, surgical, and geographic factors have been implicated, but not yet proven to play a role in influencing ovarian cancer risk [4-6].

A number of theories have been postulated in order to explain the etiology of EOC. These theories however, are not mutually exclusive, as they all in their way tend to conclude that inflammation promotes ovarian tumorigenesis and cancer progression. Several cytokines, such as IL-6 and IL-8, produced by the tumor itself and/or activated by immune cells, in addition to cancer cell growth stimulation, are known to influence clinical disease status and prognosis [5, 7].

EOC arises in ovarian surface epithelium cells. These are flatto-cuboidal epithelial cells, which constitute a single layer, with some distinguishing features, that cover the ovary. This modified mesothelium may also have the ability to secrete bioactive inflammatory cytokines such as IL-1, IL-6 and 8, which play an important role in lysis and repair of the ovarian surface during ovulation. IL-6 and IL-8 have been demonstrated to be involved in autocrine growth of ovarian cancer cells and in the regulation of tumor cell proliferation, invasion and angiogenesis [8, 9].

The physiological activity of IL-6 is complex, and comprises proinflammatory and anti-inflammatory effects. Depending on the duration of its activity, IL-6 can

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either act as an activator or an inhibitor of T-cell responses. This combination of pro-inflammatory and anti-inflammatory effects suggests that IL-6 might have a direct influence on immune system activation during the various phases of EOC evolution, and might modulate the transcription of several, liver-specific genes during acute inflammatory states, especially C-reactive protein (CRP) [10].

It has been reported in a number of studies that there is an association between serum levels of IL-6 and prognosis, furthermore elevated serum levels correlated with a poor, relapse-free, overall survival [11, 12]. However, some reports do not prove such a correlation between elevated serum levels of IL-6 and survival time [13].

IL-8, a pro-inflammatory factor of the CXC chemokine family that was originally classified as neutrophil chemoattractant, has recently been reported to play an important role in cancer invasion, angiogenesis and metastasis. Moreover, the cancer cells themselves can secret IL-8 in an autocrine or paracrine manner, as is seen in ovarian cancer [9].

The progress of EOC is also thought to be related to a local immune deficiency because of its easy dissemination into the peritoneal cavity and its tendency to remain confined to the cavity even in advanced stages of the disease. For example, IL-6 and IL-8 were expressed by over 90% of samples of EOC [14]. Increased IL-6 expression and reduced Th1 cytokines were found to coincide with a reduced survival rate of EOC patients in advanced stages of the disease [15]. Constitutive expression of IL-6, IL-8 by EOC cells may be a major component of immune escape [16].

During inflammatory processes, IL-6 and IL-8 participate in tumorigenesis by acting directly on epithelial cells via signaling through the nuclear factor of kappa light polypeptide gene enhancer in B-cells 1 (NFKB1 pathway) [17]. Moreover, expression of IL-6 and IL-8 by cancer cells stimulates cancer cell proliferation, migration, and invasion. With dual paracrine or autocrine mechanisms, serum IL-6 and IL-8 contribute to or reflect cancer progression and biology, and are also likely to have a dual effect on tumor initiation and additional inhibition of the immune response directed against the tumor [18].

Circulating serum CRP, which is an acute phase protein involved in the response to an inflammatory stimulus, has been used for decades in clinical laboratories as a marker of various acute and chronic inflammatory diseases. As a result, it is nowadays widely available and cheap [19]. Serum CRP is also produced during carcinogenesis, suggesting production by tumor cells, a result of a host-defense reaction by the immune system against malignant cells, or both [20].

High circulating levels of proinflammatory cytokines such as IL-6, IL-8, and CRP have been found in EOC patients with advanced stages of disease and an unfavorable prognosis. The prognostic role of various cytokines has been studied; however, firm conclusions cannot be drawn [11-14, 19, 21-23].

The purpose of the present study was to investigate the prognostic significance of serum IL-6, IL-8, and CRP levels for disease-free and overall survival in a cohort of 118 EOC patients.

DONORS AND METHODS

Patients and clinical samples

A total of 118 patients with EOC (aged 19–78 years; mean age 57.6 years), all treated in the Department of Gynecology at District General Hospital in Bialystok (Poland) between 2003 and 2007, were included in this study. The serum from 64 healthy volunteers (aged 19-69 years; mean age 55.8 years) served as controls. The protocol had been previously approved by the Local Bioethics Committee of the Medical University of Bialystok. All patients gave their informed consent to participate in the study. Follow-up data were collected until December 2012. At a median follow-up of 24.63 months (range 0.84-58.16), 62 patients had died as a consequence of cancer progression.

Primary treatment generally consisted of surgery, which entails total abdominal hysterectomy, omentectomy, multiple peritoneal and lymph node samplings as well as peritoneal washings for cytology. Adjuvant chemotherapy consisted of different platinum-based treatment regimens. Bilateral tumors were resected from 46 patients, and remaining tumors were found unilaterally. Tumor stage and histological diagnosis of each case were determined according to the criteria of the International Federation of Gynecology and Obstetrics (FIGO) and the histological typing system of the World Health Organization (WHO), respectively. Patients were categorized as having limited disease (stage I or II) or advanced disease (stage III or IV). Tumors were graded as: well (G1), moderately (G2), or poorly (G3) differentiated. Histologically, 57 patients (48.3%) had serous cystadenocarcinoma, 18 (15.3%) mucinous cystadenocarcinoma, 12 (10.2%) endometrioid carcinoma and 31 (26.3%) other carcinomas (8 cases of clear-cell carcinomas and 23 of different histological types). Evaluation of the histological appearance of all tissue samples was performed by two experienced pathologists in a blinded fashion.

According to the protocol, the pretreatment serum specimens must have been collected before initial exploratory laparotomy and initiation of frontline chemotherapy. Blood samples were immediately stored on ice until centrifuged at 2.500 g for 15 min at 4°C. Serum samples were kept frozen at -80°C and then thawed shortly before determination of interleukinn (IL-6 and IL-8) and CRP levels. Quantification of serum protein levels was performed using an enzyme-linked immunosorbent assay (ELISA) with a commercially available ELISA kit (Quantikine Human Immunoassay, R&D Systems, Minneapolis, MN, USA; D6050 and Q8000B, respectively). Serum samples from all patients were incubated for 2 hours at room temperature, in duplicate (100 μL), on microtiter plates coated with a monoclonal antibody specific for interleukins. Any unbound substances were washed away, and an enzyme-linked polyclonal antibody, specific for IL-6 or IL-8, was introduced. After incubation for 2 hours at room temperature, the plates were washed, a substrate solution was added, and color development was stopped after 25 minutes at room temperature. A microplate LabSystems Multiskan Spektrum Microplate Reader (Thermo Scientific, USA) was then used to determine colorimetric densities at 570 nm and 450 nm for each sample. Results were calculated from a standard curve generated by a parametric logistic

curve fit and expressed in pg/mL of serum. The minimum detectable dose (MDD) of IL-6 is typically less than 0.70 pg/mL as determined by the manufacturer (assay range 3.12-300 pg/mL). The MDD of IL-8 ranged from 1.6-5.000 pg/mL. The mean MDD was 0.97 pg/mL.

Serum CRP concentration was determined using an immunoturbidimetric Protiline CRP assay kit (bio-Merieux, Lyon, France), in accordance with the manufacturer's instructions. The intra-assay CV% reported by the manufacturer was 2.49% at a mean concentration of 10 mg/L.

All test runs were duplicated. The patients' clinical status was not known by the persons running the assays, and the results of these assays were disclosed to the surgeons only after the patients' disease status was recorded.

Statistical analysis

Results are presented as the median and range. The cohorts were defined by age (<50 or ≥50 years), clinical FIGO stage, tumor grade and residual tumor size. The associations between clinical status and the clinicopathological characteristics were evaluated using the Mann-Whitney U-test and the Kruskal Wallis test. For the univariate analysis, survival rates were compared according to the clinicopathological parameters.

Receiver-operating characteristic (ROC) curves were constructed to determine the optimal sensitivity and specificity of IL-6, IL-8 and CRP levels for the purpose of outcome prediction. The ROC curve analysis was subjected to the selection of optimal cut-off values for IL-6, IL-8 and CRP in all patients. The specificity and the sensitivity for the outcome (overall survival and disease-free survival at each level were plotted to generate the ROC curve. The concentration that was the closest to the point at both maximum sensitivity and specificity, the point (0.0, 1.0) on the curve, was selected as the optimal cut-off value leading to the highest serum levels of IL-6, IL-8 or CRP, which were correctly classified as having or not having the outcome. Statistical differences in overall survival and disease-free survival for prognostic markers were determined by the log-rank test, and Kaplan-Meier survival curves were performed. Overall survival was defined as the period from primary surgery to death due to EOC or the date of the last follow-up. disease-free survival was defined as the period from primary surgery until the date of progression or relapse of the disease. Multivariate analysis was done using the Cox's proportional hazard model.

All statistical analyses were performed using Statistica software version 9.0PL (StatSoft, Inc., StatSoft Polska Sp. z o.o., Poland). $P < 0.05$ was considered as statistically significant.

RESULTS

Patient characteristics are presented in *table 1*. The EOC cohort comprised 57 (48.3%) serous, 18 (15.3%) mucinous, 12 (10.2%) endometrioid, 8 (6.8%) clear cell, and 23 (19.4%) tumors of other types, including predominantly mixed forms and adenocarcinomas with no specific histotype. A total of 18 (15.2%) were diagnosed with stage I disease, 25 (21.2%) with stage II, 61 (51.6%) with stage III and 14 (12%) with stage IV disease. The samples were grouped by histological grade: 23 (19.5%) EOC were classified as grade 1, 46 (40%) were grade 2, and 49 (41.5%) grade 3 (*table 1*).

Table 1
Patient characteristics.

	n (%)
Number of patients	118
Age at diagnosis, median (yrs)	57.6
Range (yrs)	19–78
Histological type	
Serous	57 (48.3)
Mucinous	18 (15.3)
Endometrioid	12 (10.2)
Clear cell	8 (6.8)
Others	23 (19.4)
Grade	
1	23 (19.5)
2	46 (40.0)
3	49 (41.5)
Stage	
I	18 (15.2)
II	25 (21.2)
III	61 (51.6)
IV	14 (12.0)
Residual tumor	
≤2 cm	51 (43.2)
>2 cm	67 (56.8)

Table 2
Median serum concentrations of IL-6, IL-8 and CRP in EOC patients and the control group.

	Patients (n = 118)	**Control (n = 64)**	P-value
	Median (range)	**Median (range)**	
IL-6	11.5 (3.4-62.6) pg/mL	2.9 (1.1-12.3) pg/mL	<0.001
IL-8	29.8 (16.4-105.3) pg/mL	9.3 (4.3-32.4) pg/mL	<0.001
CRP	9.51 (0.3-129.2) mg/L	1.2 (0.1-11.5) mg/L	0.001

The distribution of serum concentrations of CRP, IL-6 and IL-8 in individual patient samples is shown in *figure 1*. Circulating concentrations of all biomarkers measured showed a significant difference ($p<0.001$) between normal control samples and patients with EOC (*table 2*).

Pretreatment levels of CRP in the EOC group were significantly higher than in the normal control group (median – 9.51, range: 0.3-129.2 mg/L *versus* median – 1.2, range: 0.1-11.5 mg/L) ($p = 0.001$) (*figure 1A*, *table 2*). The increase in the serum CRP levels correlated with age ($p = 0.002$), disease stage ($p<0.001$) and residual tumor size ($p<0.001$) (*table 3*).

The data for serum IL-6 concentrations in EOC patients and healthy controls are presented in *table 2* and *figure 1B*. In the 64 volunteer blood donors, the serum IL-6 concentration ranged between 1.1-12.3 pg/mL (median – 2.9). The serum IL-6 concentrations in EOC patients ranged

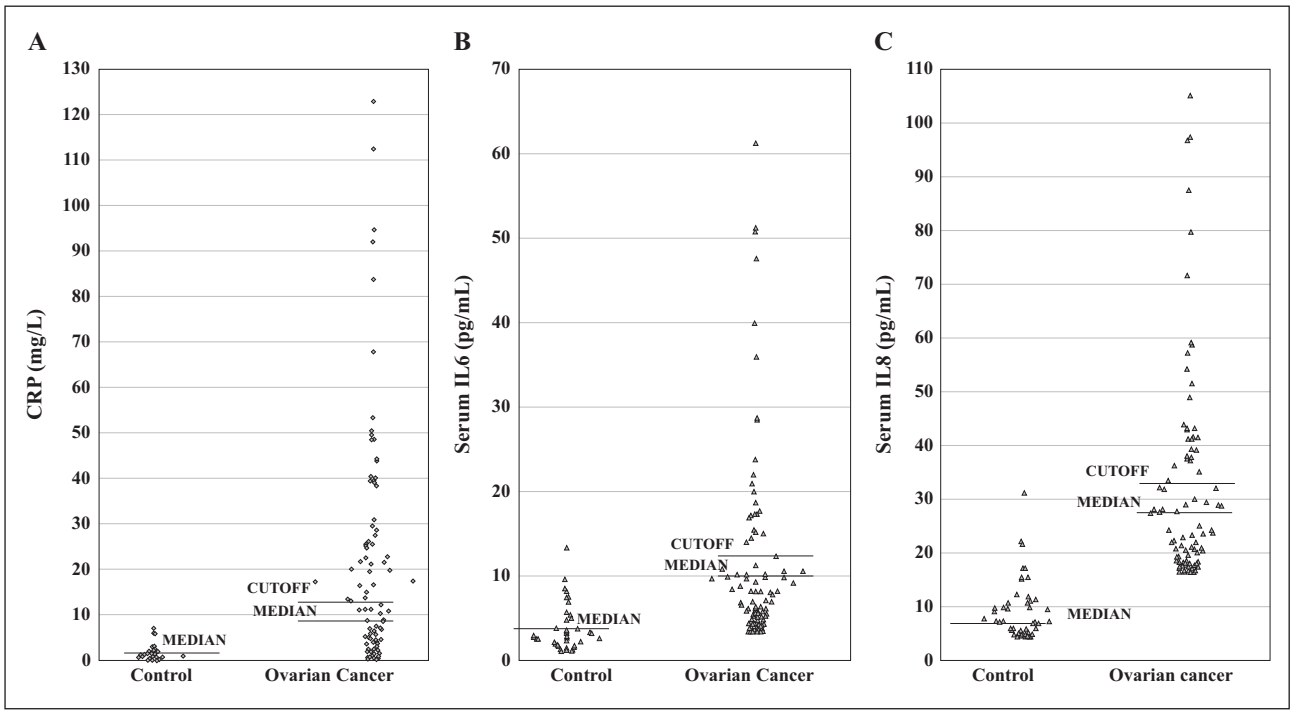

Figure 1

A) Comparison of serum CRP concentrations across all patient groups. The overall shape indicates the individual values for each patient measurement shown on a log scale, and horizontal lines show the median concentration for each group and cut-off value. **B)** Comparison of serum IL-6 concentrations across all patient groups. The overall shape indicates the individual values for each patient measurement shown on a log scale, and horizontal lines show the median concentration for each group and cut-off value. **C)** Comparison of serum IL-8 concentrations across all patient groups. The overall shape indicates the individual values for each patient measurement shown on a log scale, and horizontal lines show the median concentration of each group and cut-off value.

between 3.4-62.6 pg/mL (median – 11.5). In general, the increase in the serum IL-6 correlated with age ($p = 0.011$), disease stage ($p<0.001$) and residual tumor size ($p<0.001$) (*table 3*).

Pretreatment serum IL-8 levels in patients with EOC (median – 29.8, range: 16.4-105.3 pg/mL) were significantly higher in comparison with the healthy controls (median – 9.3, range: 4.3-32.4 pg/mL) (*figure 1C* and *table 2*; $p<0.001$). The increase in the serum IL-8 levels correlated with age ($p = 0.021$), histological type ($p = 0.011$), grade ($p = 0.023$), disease stage ($p<0.001$) and residual tumor size ($p<0.001$) (*table 3*).

The relationship between serum IL-6, IL-8 and CRP concentrations and treatment outcome was investigated. In univariate analysis of all patients, age, high grade, high stage, residual tumor size >2 cm, high preoperative serum concentrations of IL-6, IL-8 and CRP were associated with short overall survival and disease-free survival.

For respective outcomes of progression to disease and death, separate receiver-operating characteristic analyses were used in order to estimate sensitivity and specificity for IL-6, IL-8 and CRP cut-offs for each EOC patient. The optimal cut-off value of IL-6 for predicting disease-free survival in our patients with EOC was 12.38 pg/mL (sensitivity, 87.3%; specificity, 56.4%). For predicting overall survival, the optimal cut-off was the same as the value for predicting disease-free survival, with a sensitivity of 82.4% and specificity of 51.2%. The optimal cut-off value of IL-8 for disease-free survival in patients was 32.19 pg/mL (sensitivity, 88.6%; specificity, 69.2%). For predicting overall survival, the optimal cut-off value of IL-8 was the same as a value for predicting disease-free survival, with a sensitivity of 71.8% and specificity of 76.8%. The optimal cut-off value of CRP for disease-free survival was 11.19 pg/mL (sensitivity, 78.9%; specificity, 72.4%). For predicting overall survival, the optimal cut-off value of CRP was the same as a value for predicting disease-free survival with sensitivity of 70.9% and specificity of 73.8%. Patients above the cut-off levels were classified as having high concentrations; patients below or at the cut-off levels were classified as having low concentrations. Using the cut-off value, high IL-6 and IL-8 levels were found in 25 (21.2%) and 31 (26.3%) patients with EOC, respectively. High levels of CRP were found in 32 (27.1%) patients.

Seven prognostic factors (pretreatment serum levels of IL-6, IL-8, CRP, age, histological type, grade, and FIGO stage) were analyzed using Cox proportional multivariate regression analyses and with the overall survival and disease-free survival rate as the dependent variable. Residual tumor size was excluded from multivariate analysis due to high multicollinearity.

In the multivariate analysis, six of these factors were independent and statistically highly significant. Age, stage and grade were independent prognostic factors for overall survival ($p<0.001$, $p = 0.023$ and $p<0.001$, respectively), but only age and grade was significant for disease-free survival ($p<0.001$ and $p<0.001$, respectively).

The elevated pretreatment serum IL-6 and IL-8 concentrations also predicted adverse overall survival (HR = 2.63; 95%CI 1.94-4.26; $p = 0.004$, HR = 2.32; 95%CI 1.53-3.64; $p = 0.014$, respectively) and disease-free survival (HR = 1.64; 95%CI 1.11-2.32; $p = 0.011$, HR = 0.94;

Table 3
Correlation between IL-6, IL-8, CRP levels, and clinicopathological parameters.

Parameter	IL-6 pg/ml		IL-8 pg/ml		CRP mg/L	
	Median (range)	p	Median (range)	p	Median (range)	p
Age (yrs)						
≤50	9.3 (3.4-43.8)	0.011	17.9 (16.4-69.7)	0.021	8.4 (0.3-64.6)	0.002
>50	20.2 (12.4-62.6)		36.5 (21.2-105.3)		18.2 (1.2-129.2)	
Histological type						
Serous	13.8 (4.6-62.6)	0.524	45.8 (36.2-105.3)	0.011	19.6 (6.8-129.2)	0.429
Mucinous	7.8 (5.2-48.9)		18.6 (16.4-41.2)		21.3 (0.3-38.6)	
Endometrioid	9.4 (8.2-56.8)		26.2 (21.4-56.1)		14.8 (3.8-111.2)	
Clear cell	8.6 (6.4-62.2)		30.6 (28.2-62.4)		8.5 (5.2-98.4)	
Others	7.2 (3.4-38.4)		24.8 (15.8-49.2)		7.8 (6.5-98.2)	
Grade						
1	9.2 (3.4-52.1)	0.436	20.4 (16.4-56.2)	0.023	9.4 (3.5-129.2)	0.213
2	11.4 (6.2-62.6)		31.9 (22.4-68.1)		13.2 (0.3-98.6)	
3	9.8 (5.1-58.2)		36.4 (26.1-105.3)		15.8 (6.5-113.4)	
Stage						
I	6.7 (3.4-18.9)	<0.001	18.6 (16.4-72.4)	<0.001	7.8 (0.3-15.8)	<0.001
II	13.2 (8.4-36.8)		32.8 (28.2-69.1)		14.5 (1.2-39.4)	
III	28.9 (21.2-62.6)		49.2 (36.8-105.3)		22.4 (9.8-129.2)	
IV	19.4 (5.8-46.2)		39.6 (32.2-96.4)		30.8 (11.2-110.2)	
Residual tumor						
≤2 cm	8.4 (3.4-38.2)	<0.001	14.8 (16.4-76.2)	<0.001	8.3 (0.3-96.3)	<0.001
>2 cm	18.6 (11.2-62.6)		34.4 (25.6-105.3)		15.9 (16.8-129.2)	

95%CI 0.86-3.58; p = 0.001, respectively). High levels of CRP were also considered to be an independent prognostic factor for overall survival (HR = 1.87; 95%CI 0.58-2.87; p = 0.016) and disease-free survival (HR = 0.84; 95%CI 0.72-2.84; p = 0.021).

To determine, whether pretreatment serum IL-6, IL-8 and CRP levels could serve as prognostic factors for EOC, Kaplan-Meier survival curves and the Cox proportional hazard model were used to evaluate the association between high and low serum concentrations and survival outcome in EOC patients. Kaplan-Meier survival curves showed that patients with EOC, and with high IL-6, IL-8 and CRP levels had substantially shorter overall survival (*figure 2A*; p = 0.003, *figure 2C*; p = 0.035 and *figure 2E*; p = 0.046, respectively) and disease-free survival (*figure 2B*; p<0.001, *figure 2D*; p = 0.026 and *figure 2F*; p = 0.043, respectively), than patients with low levels of IL-6, IL-8 and CRP.

DISCUSSION

Ovarian cancer is ranked as the fifth leading cause of cancer deaths in women, and is the most lethal gynecological malignancy [24]. The poor prognosis for ovarian cancer is mainly due to the high percentage of cases diagnosed at an advanced stage. The prognosis for EOC is determined by several factors (of the type of cancer, the patient and the treatment) [4]. The most popular prognostic factor is the clinical stage. However, clinical progress and prognosis for the same clinical stage could be different, as they are determined by the origin of the tumor, the biological characteristics of the cancer cells, and the differences in the cellular/microscopic environment. Several molecular markers used for EOC classification have been investigated; however, there are only a few that have a significant importance in predicting the prognosis. It is therefore crucial to find some new molecular factors that might serve as prognostic markers for EOC. One approach for finding a more profound insight into the biology of EOC is the evaluation of circulating cytokines, which have already been investigated as potential diagnostic and prognostic markers in EOC patients [25, 26].

A number of theories have been postulated in an effort to explain the etiology of EOC, but these theories are not mutually exclusive, as they all in their way tend to conclude that inflammation promotes ovarian tumorigenesis and cancer progression. The tumor milieu in which ovarian carcinoma develops has been described as one enriched with a broad spectrum of pro-inflammatory cytokines and chemokines. In particular, several of these cytokines, such as tumor necrosis factor (TNF)-α, interleukin (IL)-1β and

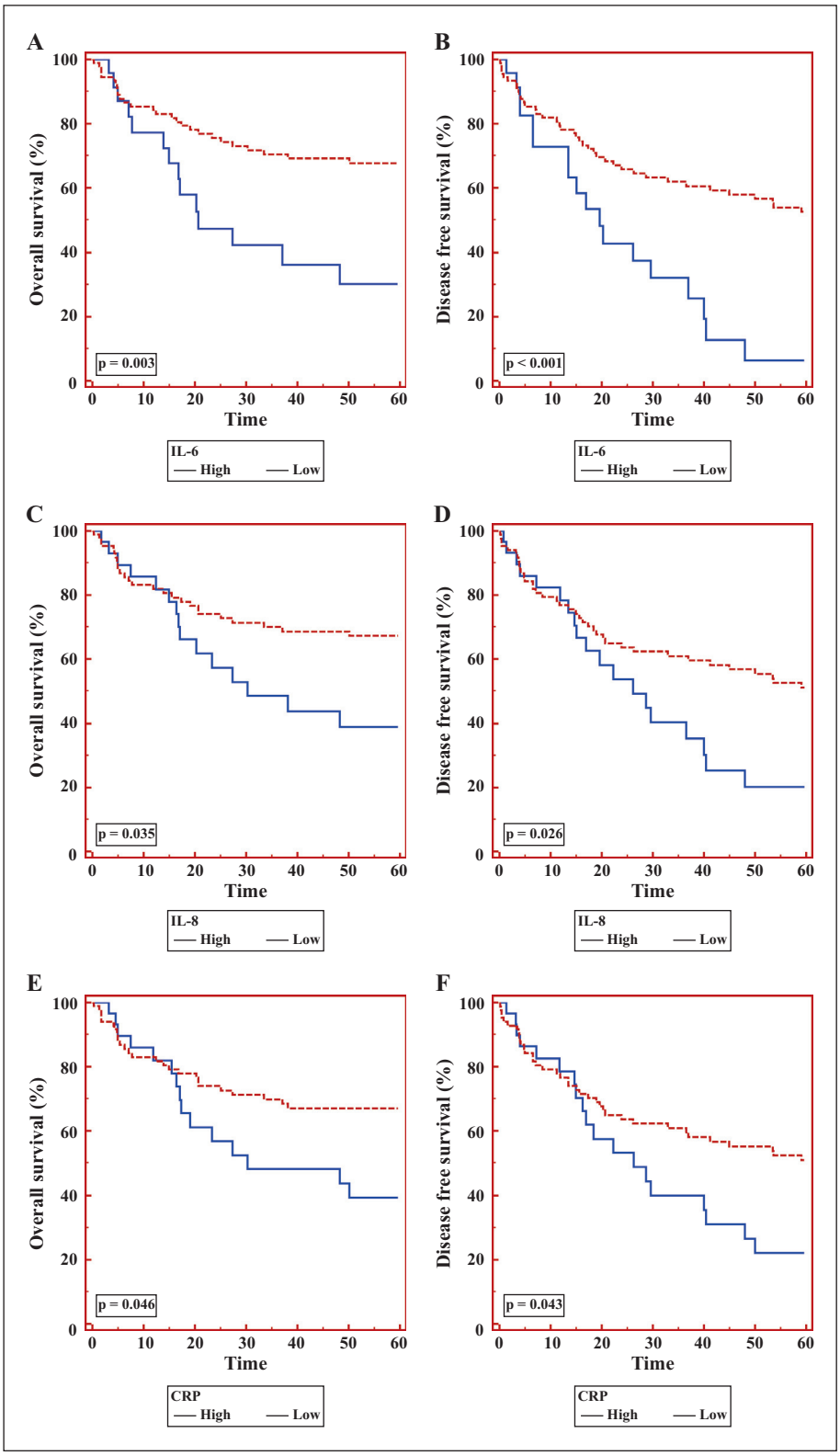

Figure 2

A) Kaplan-Meyer survival estimates of overall survival in EOC patients with low (dotted line) and high (continuous line) preoperative serum levels of IL-6 (p=0.003). **B)** Kaplan-Meyer survival estimates of disease-free survival in EOC patients with low (dotted line) and high (continuous line) preoperative serum levels of IL-6 (p<0.001). **C)** Kaplan-Meyer survival estimates of overall survival in EOC patients with low (dotted line) and high (continuous line) preoperative serum levels of IL-8 (p=0.035). **D)** Kaplan-Meyer survival estimates of disease-free survival in EOC patients with low (dotted line) and high (continuous line) preoperative serum levels of IL-8 (p=0.026). **E)** Kaplan-Meyer survival estimates of overall survival in EOC patients with low (dotted line) and high (continuous line) preoperative serum levels of CRP (p=0.046). F. Kaplan-Meyer survival estimates of disease-free survival in EOC patients with low (dotted line) and high (continuous line) preoperative serum levels of CRP (p=0.043).

IL-6, produced by tumor itself and/or activated by immune cells, in addition to cancer cell growth stimulation, are known to influence clinical disease status and prognosis [7, 22, 27].

In our study, serum levels of IL-6, IL-8, and CRP were found to be elevated in EOC patients when compared to healthy controls. The temporal association between pretreatment serum IL-6, IL-8 and CRP levels and both recurrence and survival confirm that serum IL-6, IL-8 and CRP concentrations might be used as valuable prognostic biomarkers in such a population. Patients with high levels of pretreatment serum IL-6, IL-8 and CRP levels may benefit from closer follow-up and therefore have a better chance of being identified as having recurrent disease, at an early stage. Those with higher pretreatment serum IL-6, IL-8 and CRP levels could be candidates for more aggressive intervention in order to prolong survival.

Our results are in accordance with previous reports on ovarian cancer. In addition, they confirm, in an independent sample set, that a blood-based, multianalyte assay has significant advantages over CRP measurements for distinguishing symptomatic women with EOC from controls or women with benign disease [22, 25, 28, 29].

A later cancer stage may be associated with greater inflammation and a greater inflammatory response, thus later cancer stages were more likely to have higher serum levels of IL-6, IL-8 and CRP than earlier stages, which has also been demonstrated in other studies [29-33].

Tumor angiogenesis plays an important role in progression and metastasis in EOC. The angiogenesis and disruption of the vascular barrier contribute to EOC progression. Jee *et al*. [34] reported that IL-6 played a role in mediating angiogenesis. IL-6 enhances tumor cell proliferation, motility and invasion, upregulates anti-apoptotic genes, stimulates angiogenesis by VEGF induction and promotes chemoresistance by MDR1 upregulation. The association between IL-6 and EOC tumorigenesis principally involves the activation of the JAK/STAT signaling pathways that leads to evasion of apoptosis. Thus, IL-6 is a growth-promoting and anti-apoptotic factor, and high serum levels of IL-6 in advanced stage EOCs correlate with poor prognosis.

Many studies have focused on the role of IL-8 in promoting cell proliferation, migration, and cancer cell invasion, and more recently, IL-8 has been identified as assisting cancer cells to evade stress-induced apoptosis. Consistent with the preponderance of effects seen in *in vitro* studies, IL-8 expression has been shown to correlate with the angiogenesis, tumorigenicity, and metastatic potential of EOC [9, 35-38].

Both multivariate analysis and univariate analysis showed that pretreatment serum levels of IL-6, IL-8, CRP, and age, histological grade and FIGO stage were important prognostic factors in our study . Although the levels of IL-6, IL-8, and CRP were increased in clinically detected EOC, the levels of these biomarkers were also increased prior to the cancer diagnosis, suggesting that they might be useful biomarkers for cancer screening [20, 39-42].

Bertenshaw *et al*. [43] reported that CRP and IL-8 concentrations are two of the most informative serum biomarkers for ovarian cancer in a multianalyte profiling study. Moreover, increased levels of IL-6 and IL-8 reported in the serum of patients with ovarian cancer are used in multimarker panels for the detection of ovarian cancer [29, 44-46].

Several other studies have reported increased serum concentrations of CRP associated with ovarian cancer [42, 47, 48]. High serum concentrations of CRP and IL-6 are reported as being used as significant factors in the prognosis of ovarian cancer [21, 28]. Indeed, high CRP levels seem to be a risk factor for developing ovarian cancer [42].

EOC is associated with extensive alterations to the serum cytokine environment, highlighting the importance of further investigations of relative cytokine level changes. Preoperative serum IL-6, IL-8, and CRP levels seem promising in distinguishing EOC patients from healthy controls; however, their clinical value is still to be confirmed. High levels of IL-6, IL-8, and CRP in EOC patients have been suggested to be a poor prognostic factor for overall survival and disease-free survival.

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