Expression of IL-10 in Patients with Ovarian Carcinoma

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Abstract. *Background: Cytokines are involved in the pathogenesis of different gynecological malignancies. Additionally, they stimulate the spread of cancer cells. Interleukin 10 (IL-10) was described as a pro-inflammatory factor and seems to be implicated in the immune deficiency of patients with cancer. The aim of this study was to determine whether the level of IL-10 in the serum and ascites was associated with the prognosis of advanced ovarian cancer (OC). Materials and Methods: In a prospective study from 2001 to 2003, the concentration of IL-10 in the serum and ascites of 117 consecutive patients with advanced OC and 30 women with benign disease who underwent surgery as a control group (CG), was analyzed by the enzyme-linked immunosorbent assay. For statistical analyses, the Chi-square test by Pearson, Fisher's exact test and the Mann-Whitney test were employed. Results: The concentrations of IL-10 were a median of 9.87 pg/ml (range 7.8 to 500 pg/ml) in the serum and a median of 43.70 pg/ml (range 7.8 to 389.4 pg/ml) in the ascites of the OC patients. The IL-10 level in the sera of the CG was a median of 7.80 pg/ml (range 7.8 to 62.8 pg/ml) and 18.34 pg/ml (range 7.8 to 88.72 pg/ml) in the peritoneal fluid. A significant association was observed between the IL-10 serum levels (p=0.003) and levels in the peritoneal fluid (p=0.03) in both OC and the CG. IL-10 was significantly more expressed in the ascites of patients with OC than in their sera (p=0.003). The concentration of IL-10 correlated significantly with proven conventional prognostic factors such as recurrence status (p=0.005), volume of (ascites, p<0.001, serum, p=0.03), histological grading (p=0.053) and histological type (ascites p=0.005/ serum p=0.09). There was no significant correlation between the levels of IL-10 in the ascites and/or serum and FIGO stage, residual tumor mass or age. The cut-off value of 8.0 pg/ml for IL-10 serum levels had a positive predictive value of 84% (95% CI: 76-91) and a negative predictive value of 29% (95% CI: 16-41), with a specificity and sensibility of 47% (95% CI: 29-65) and 70% (95% CI: 62-78), respectively. Conclusion: Due to the fact that the levels of IL-10 were significantly higher in the ascites and serum of OC patients than in those of the CG, IL-10 may play an important immunosuppressive role in the pathogenesis of OC. The association between high IL-10 levels in ascites and serum and the histological type of the tumor, as well as between the levels in the peritoneal cavity and grading, suggest that IL-10 could be a prognostic factor in OC.*

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Ovarian cancer (OC) is the leading cause of death in all gynecological malignancies (1). The 5-year survival rate remains poor, despite significant improvements in surgical treatment and chemotherapy (2). Different studies suggest that immunological components play a key role in the development of cancer (3, 4). Cytokine levels, for example, were described to be increased in different solid tumors (4-6). Cytokines are involved in the regulation of the immune system and have been implicated in the pathogenesis of different gynecological malignancies, including OC (5-10).

A typical feature of cytokines is their pleiotropy and redundancy. Every cytokine has numerous functions, and one function is often mediated by several different cytokines to create a cytokine network. (11) Cytokines may contribute to metastases by promoting angiogenesis, enhancing tumor cell adhesion and inducing proteolytic enzymes (12-14, 16). They activate natural killer cells and tumor-specific cytotoxic T-lymphocytes, enhancing the host immune defense against malignant cells (14-16).

Interleukin 10 (IL-10) is an immunosuppressive cytokine produced by a variety of cells including T- and B- lymphocytes, monocytes and macrophages. It was demonstrated that IL-10 is secreted by different cancer types, *e.g.*, ovarian carcinoma, melanoma, neuroblastoma, non-small cell lung cancer, lymphoma and gastrointestinal cancer (4-6, 17). IL-10 is also considered to be a potent negative regulator of immunoproliferative and inflammatory responses.

Table I. *Patients' characteristics.*

Parameters	Value
Age at treatment in years, median (range)	57 (19-88)
Tumor status, no. (%)	
primary	63 (55.3)
reccurence	51 (44.7)
Histology, no. (%)	
serous-papillary	92 (80.7)
endometrioid	10 (8.8)
mucinous	8 (7.2)
mixed/others	4 (3.6)
Grading, no. (%)	
I-II	54 (47.6)
III-IV	60 (52.6)
FIGO stage, no. (%)	
I	10 (8.8)
II	8 (7)
III	63 (55.3)
IV	33 (28.9)
Ascites volume, no. (%)	
none	3 (2.6)
≤500 ml	54 (47.4)
>500 ml	57 (50)
Postoperative residual tumor mass, no. (%)	
macroscopically tumor-free	48 (42.1)
≤2 cm	45 (39.4)
>2 cm	21 (18.4)
Lymph node status, no. (%)	
positive (N1)	28 (24.6)
negative (N0)	28 (24.6)
not known	58 (50.8)

Gianoti *et al.* showed that high levels of IL-10 are correlated with aggressive tumor growth (17). Furthermore, IL-10 is overexpressed in ascites and sera of patients with advanced OC (9, 10, 17). In most advanced cases of OC (FIGO III), the tumor remains in the peritoneal cavity. It has been suggested that this typical feature of OC could be related to a local phenomenon of immunosuppression (17-19). An explanation of this observation could be that tumor-associated lymphocytes and tumor-infiltrating lymphocytes from OC patients have been shown to be defective in several immunological functions: the ability to proliferate in the presence of mitogens, cytotoxic activity against autologous or allogeneic tumor cells, natural killer function and expression of the cytoplasmic CD3- z chain, which is essential for T cell receptor signaling (18, 19).

The responsible mechanisms for the impaired immune response remain unclear, but some possible mechanisms have been proposed: the production of tumor-derived inhibitory factors (20, 21), tolerogenic or deleting presentation of antigens by ovarian tumor cells or activation of inhibitory regulatory elements of the immune system (22).

In the present study, the production of IL-10 in the ascites and sera of patients with advanced OC was examined and quantified. Its expression was correlated with relevant clinical data.

Table II. *Control group characteristics.*

Parameters	Value
Age at treatment, median (range)	50.46 (24-76)
Diseases, no. (%)	30 (100)
Benign ovarian tumor	17 (56.7)
Endometriosis	3 (10)
Myoma	2 (6.7)
Benign cyst	8 (26.6)
Healthy women	4 (13.3)

Materials and Methods

This prospective, mono-institutional study was reviewed and approved by the Clinical Review Board and Ethics Committee of the Charité Medical University, Berlin, Germany. All samples were obtained from Tumor Bank Ovarian Cancer (TOC) (23). Patients with histologically confirmed OC were allocated to this trial. Written informed consent was provided by each patient. Borderline ovarian tumors are different tumor entities from epithelial OC and, therefore, were excluded from this study (24)

The control group (CG) consisted of women with no history of cancer. Blood drawing and ascites collection were performed at the time of the surgical procedure. Ten ml of blood were drawn from the antecubital vein from each patient. Serum and ascites were centrifuged at 3,000 rpm for 10 min and were then stored at –80˚C until analysis.

IL-10 serum and ascites concentrations were measured with an enzyme-linked immunosorbent assay kit (ELISA) according to the manufacturer's protocol (Quantikine, R&D Systems Inc., Minneapolis, MN, USA). All samples were measured in duplicate.

The clinical data were collected and entered in an SPSS database. The statistical analyses were performed using the SPSS statistical software package (11-0/2000), and significance was analyzed by the Chi-square test by Pearson and Fisher's exact tests. Statistical significance was determined by the Mann-Whitney test (two-tailed). All two-tailed p values were calculated, as well as all 95% confidence intervals (CI). A two-tailed p value <0.05 was considered statistically significant. The overall survival probability was estimated using the Kaplan-Meier product limited method. Log-rank test statistics for analysis of the equality of survival distribution were performed.

Results

From August 2002 to November 2003, 114 women with OC (Table I) and 30 women without any malignancy (Table II) were enrolled in this study.

The median age of the patients with OC was 57 years (range 19-88). Of the 114 patients, only 8.8% were diagnosed in FIGO stage I, and 84.2% in FIGO stages III-IV. The most common histological type was serous-papillary (80.7%). To enhance the relevance of the statistical analysis, the following subgroups were created: stage I+II and III+IV; histological grading, I+II and III+IV.

IL-10 levels were detected in the serum (median= 10.5 pg/ml; range 7.8 to 500 pg/ml) and in the ascites

Table III. *IL-10 serum level cut-off of 8.0 pg/ml.*

Parameters	Value (%)	95% Confidence interval
Sensitivity	70	62-78
Specificity	47	29-65
Positive predictive value	84	76-91
Negative predictive value	29	16-41

(median=40.5 pg/ml; range 7.8 to 389.4 pg/ml) of the OC patients, and in the serum samples (median=7.80 pg/ml; range 7.8 to 62.8 pg/ml) and peritoneal fluid (median=18.34 pg/ml; range 7.8 to 88.72 pg/ml) of the CG.

A significant correlation between the IL-10 concentration in ascites ($p=0.029$) and the serum level of IL-10 ($p=0.002$) in the OC and the CG was observed. The correlation between the IL-10 levels in the ascites and sera of the OC group ($p=0.002$) was highly significant.

The cut-off level of 8.0 pg/ml for IL-10 concentrations in serum was reported to be useful for the diagnosis of cancer (8), and was thus adopted in this study. In our analyses, the IL-10 serum level of 8.0 pg/ml reached a positive predictive value of 84% (95% CI: 76-91) and a negative predictive value of 29% (95% CI: 16-41), with a specificity and sensitivity of 47% (95% CI: 29-65) and 70% (95% CI: 62-78), respectively (Table III).

All the clinical data relevant to the IL-10 levels in serum and ascites were also compared (Table IV). Significant correlations between the serum and ascites IL-10 concentrations and the histological type ($p=0.005$), as well as between recurrent status ($p<0.005$) and ascites volume ($p<0.001$), were observed. No statistically significant differences were found in the correlation between IL-10 levels in ascites and serum and the FIGO stage (ascites $p=0.76$, serum $p=0.84$), recurrence status and serum ($p=0.36$), post-operative residual tumor mass (ascites $p=0.403$, serum $p=0.54$), histological grading and ascites ($p=0.09$) or age (ascites $p=0.703$, serum $p=0.528$).

Table IV. *Correlation between "conventional" prognostic factors and IL-10 ascites and serum levels.*

Parameters (no. of patients)	Serum, *p*-value	Ascites, *p*-value
Age (114)	0.528	0.703
FIGO stage (114) I and II *vs.* III and IV	0.84	0.76
Histological type (114) Serous-papillary *vs.* others	<0.005	0.09
Grading (114) I and II *vs.* III and IV	0.053	0.364
Recurrence status (92)	0.36	0.005
Ascites volume (114) ≤ *vs.* ≥ 500 ml	0.03	<0.001
Residual tumor mass (111) macroscopically tumor-free	0.54	0.043
Lymph node status (56) N0 *vs.* N1	0.23	0.46

Discussion

In this study, the presence of IL-10 in the serum and ascites of OC patients was analyzed. It was observed that IL-10 was present in all serum and ascites samples at high levels. In contrast, the IL-10 levels in the serum and peritoneal fluid derived from the CG were lower.

The histological type of tumor may play a specific role in IL-10 production, *e.g.*, our study patients with serous-papillary tumors had significantly elevated IL-10 serum levels. It is known that different histological types of OC display different biological behaviors and prognoses, *e.g.*, clear cell carcinoma (24) We can also suggest that higher concentrations of IL-10 in the peritoneal cavity led to an enhanced production of ascites ($p<0.001$). This result can be compared to that of Santini *et al.*, who analyzed the IL-10 plasma and ascites concentrations in 28 patients with OC and in 10 patients without any malignancy (control group) (10). Elevated IL-10 levels were detected in the plasma (mean 12 pg/mL; range 8 to 23 pg/mL) and in the ascites (mean 165 pg/mL; range 50 to 556 pg/mL) of the OC patients, while the IL-10 levels were not elevated in the control plasma samples. In all patients, the IL-10 levels were signifcantly higher in ascites than in the plasma ($p<0.001$).

Gotlieb *et al.* reported that ascites from patients with ovarian or other intra-abdominal cancers contained significantly elevated levels of IL-10 (542±77 pg/ml, n=35), compared with the peritoneal fluid from patients with benign gynecological conditions (34.2±7.5 pg/ml, n=63) ($p<0.001$) (7). Peritoneal fluid IL-10 levels did not correlate with histology, tumor stage, grade or prognosis.

Local and systemic secretion of immunosuppressive cytokines may play an important role in the impaired antitumor immune function commonly observed in advanced OC. The secretion of different cytokines, *e.g.*, IL-10, directly by tumor cells or by tumor-associated macrophages or lymphocytes, was suggested as a possible mechanism for modulating the antitumor immune response of the host (11-15). IL-10 has been demonstrated to have powerful immunosuppressive activities affecting the building components of immune-response pathways (13-16, 19-22). IL-10 also decreases the maturation of dendritic cells (the most

potent antigen-presenting cells known in humans for triggering the induction of an antigen-specifc immune response). In studies with other tumor models (20-22), the main source of IL-10 detected in OC patients may be derived from lymphoid or myeloid cells infiltrating or associated with ovarian tumors.

To date, only limited data are available on the circulating levels of IL-10 in patients with advanced OC (7, 9, 10) indicating that a large percentage of such patients have abnormal serum and ascites IL-10 levels. The levels in ascites were significantly higher than in serum samples, suggesting that cells in the abdominal cavity are the major source of IL-10 in OC patients.

The presence of cytokines, such as IL-10, in the peritoneal cavity of OC patients could be important for the growth and development of cancer, more specifically in relation to host immune responsiveness. In conclusion, the results of this study confirmed the presence of IL-10 in serum and ascites and suggest an important causal role for IL-10 in the progressive decrease of immune function.

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