RESEARCH ARTICLE

Circulating levels of TNF-α and its soluble receptors in the plasma of patients with epithelial ovarian cancer

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ABSTRACT. The significance of circulating levels of TNF-α and its soluble receptors (sTNF-Rs) in the plasma of patients with epithelial ovarian cancer (EOC) has not been fully elucidated. The present study was to investigate the relationship of pretreatment plasma levels of TNF-α, sTNFR-1 and sTNFR-2 with outcome in 126 patients with EOC. Concentrations of TNF-α and sTNF-Rs were determined by enzyme-linked immunosorbent assay (ELISA). Median TNF-α and sTNF-Rs levels were significantly higher in EOC patients than in healthy controls. High plasma levels of TNF-α and sTNF-Rs were correlated with tumor stage and with reduced mean survival time (MST). The results of the present study suggested that preoperative plasma TNF-α and sTNF-Rs levels in EOC patients correlated with the highest risk of cancer progression. Thus, the clinical value of an activated TNF system in EOC needs to be further investigated.

Keywords: tumor necrosis factor-α, soluble tumor necrosis factor receptor 1, soluble tumor necrosis factor receptor 2, plasma, epithelial ovarian cancer

Epithelial ovarian cancer (EOC) comprises the majority of malignant ovarian tumors in adult women. About 190 000 new cases and 114 000 deaths from ovarian cancer are estimated to occur annually [1].

Surgical determination of tumor stage is the most important prognostic factor. Early stage disease has a very good prognosis. Overall, five-year survival rates for all stages were in the 30-50% range. Most women however, present with late stage disease, which is associated with a five-year survival rate of about 20% [2].

The pro-inflammatory cytokine, tumor necrosis factor-α (TNF-α), is disturbed in malignant tumors compared with normal ovarian surface epithelium. Several studies have associated inflammation with ovarian tumorigenesis, with TNF-α playing a key role in modulating invasion, angiogenesis and metastasis [3, 4].

The biological activity of TNF-α can be modulated by the soluble forms of its membrane receptors (sTNF-Rs). These proteins, corresponding to the extracellular domain of p55TNF receptor (TNF-R1) and p75TNF receptor (TNF-R2), are shed from the membrane surface retaining their ability to bind TNF [5, 6].

Several reports have associated detection of abnormally high levels of TNF-α in the blood of ovarian cancer patients with a wide range of tumor types [7-9]. However, circulating TNF-α is not always detectable in cancer patients, and can vary within individual patients over time and the course of disease [10, 11].

Regulation of sTNF-Rs is critical to tumor cell responsiveness to TNF-α, and tumor tissue levels of TNF-α might be more relevant than blood levels in explaining pro-tumorigenic associations. Soluble forms of cell surface TNF-α receptors are the specific cytokine antagonists. They bind circulating TNF-α and inhibit the biological activity of TNF-α by preventing its binding to cellular receptors. High concentrations of sTNF-Rs can inhibit TNF activity and may thus represent a tumor escape mechanism from the destructive effects of TNF-α. Although the actual pathogenic role of sTNF-Rs remains controversial, they have been proposed as reliable markers of local TNF-α production [12-14].

The aim of this study was to determine whether a preoperative plasma TNF-α system in EOC is activated; the relationships between their elevations and both clinicopathological factors of patients were also investigated.

METHODS

Patients and clinical samples

A total of 126 patients with EOC (ages 18-79 years; mean age - 58.3 years), all treated at the Department of

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Gynecology District Hospital in Bialystok (Poland) between 2004 and 2008, were included in this study. The plasma from 30 healthy volunteers (ages 19-71 years; mean age - 52.8 years) served as controls. Patients were informed and gave their consent to the study. The protocol had been previously approved by the Bioethical Committee of the Medical University of Bialystok (R-I-003/229/2003).

Tumor stage and histological diagnosis of each case were established 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. Tumors were graded as: well (G_1), moderately (G_2), or poorly (G_3) differentiated. Histologically, 64 (50.8%) of the patients had a serous adenocarcinoma, 18 (14.3%) — a mucinous adenocarcinoma, 26 (20.6%) — an endometrioid adenocarcinoma, there were 18 (14.3%) other types (therein 10 cases of clear cell carcinomas and eight of different histological types). The protocol required that the pretreatment plasma specimen be collected before initial exploratory laparotomy and the initiation of frontline chemotherapy.

Blood samples were collected preoperatively into heparinized tubes as a part of other, routine blood sampling. Samples were centrifuged for 15 minutes at 1 000 x g within 30 minutes of collection; and after separation, plasma was kept frozen at - 70°C in aliquots of 2-3 mL and thawed just before use.

TNF-α, sTNF-R1 and sTNF-R2 levels were measured using; a Quantikine human TNF-α Immunoassay PDTA00C (the minimum detectable dose (MDD) of TNF-α ranged from 0.5-5.5 pg/mL. The mean MDD was 1.6 pg/mL), a Quantikine human sTNF-R1 Immunoassay PDRT100 (MDD of sTNF-R1 ranged from 0.43-1.20 pg/mL. The mean MDD was 0.77 pg/mL), and a Quantikine human sTNF-R2 Immunoassay PDRT200 (MDD of sTNF-R2 ranged from 0.2-2.3 pg/mL. The mean MDD was 0.6 pg/ml), following the manufacturer's instructions (ELISA-kit of R&D Systems, Minneapolis, USA) and with an ETI Sorin Biomedica ELISA reader (Sorin Biomedica, Bio-Tek, USA). Samples were assayed in duplicate.

Using these kits, the upper normal limit of total TNF-α in healthy subjects is 1.8 pg/mL. The upper limits of sTNF-R1 and sTNF-R2 plasma levels in healthy donors never exceed 0.7 ng/mL and 1.8 ng/mL, respectively.

Statistical analysis

Statistical analysis was performed using Statistica software version 8.0 (StatSoft, Inc., StatSoft Polska Sp. z o.o., Poland). The Mann and Whitney *U* test was used for comparison of average plasma concentrations of cytokine and cytokine receptors, correlations between TNF-α and sTNF-R1 and sTNF-R2 levels and disease severity (stage and grade), and the Fisher's exact test for comparison of prevalence of elevated values. A p-value, lower than 0.05, was considered significant.

RESULTS

Plasma TNF-α levels

The data for plasma TNF-α concentrations in EOC and healthy controls are presented in *table 1* and *figure 1A*. In the 30 volunteer blood donors, the plasma TNF-α concentration ranged between 0.6-4.5 pg/mL (mean - 1.8). The plasma TNF-α concentrations in EOC patients ranged between 0.6-9.8 pg/mL (mean - 4.1). The mean pretreatment plasma level of TNF-α was significantly higher than the mean level in the control group (*table 1*, $p < 0.001$). In general, the increase in the plasma TNF-α correlated with disease stage and grade *(tables 2, 3)*. The plasma TNF-α levels in EOC patients in clinical stages III-IV were statistically more significant than the plasma TNF-α levels in patients in early stage I-II (*table 3*, $p < 0.001$).

Plasma sTNF-Rs levels

Pretreatment plasma sTNF-R1 (mean - 1.1, range: 0.4-2.8) and sTNF-R2 (mean - 3.7; range: 2.3-9.6) levels in patients with EOC were significantly higher in comparison with the healthy controls - R1 (mean - 0.7, range: 0.0-1.3) and - R2 (mean - 1.8, range: 0.0-4.2), respectively (*figure 1B*, *C* and *table 1*, $p < 0.001$). The increase in the plasma sTNF-Rs correlated with disease stage. The plasma sTNF-R1 and sTNF-R2 levels in EOC patients in advanced stages (III-IV) were statistically more significant than the plasma sTNF-Rs levels in patients in early clinical stage (I-II) (*table 3*, $p < 0.001$).

The relationship between plasma concentrations of TNF-α and sTNF-Rs and treatment outcome was investigated. Patients with high levels of TNF-α and sTNF-Rs had a significantly shorter mean survival time (MST) than those with low levels (*table 4*, $p < 0.001$).

DISCUSSION

In the last decade, several studies have assessed the clinical relevance of different biological variables evaluated in serum/plasma samples from patients with EOC in order to detect biomarkers capable of predicting either

Table 1
Plasma concentrations of tumor necrosis factor-α, soluble tumor necrosis factor receptors 1 and 2 in patients with epithelial ovarian cancer and healthy controls

	No. of cases	TNF-α (pg/mL) median (range)	sTNF-R1 (ng/mL) median (range)	sTNF-R2 (ng/mL) median (range)
EOC	126	4.1 (0.6-9.8)	1.1 (0.4-2.8)	3.7 (2.3-9.6)
Controls	30	1.8 (0.0-4.5)	0.7 (0.0-1.3)	1.8 (0.0-4.2)
p-value		$p < 0.001$	$p < 0.001$	$p < 0.001$

EOC: epithelial ovarian cancer; TNF-α: tumor necrosis factor-α; sTNF-R1: soluble tumor necrosis factor receptor 1; sTNF-R2: soluble tumor necrosis factor receptor 2.

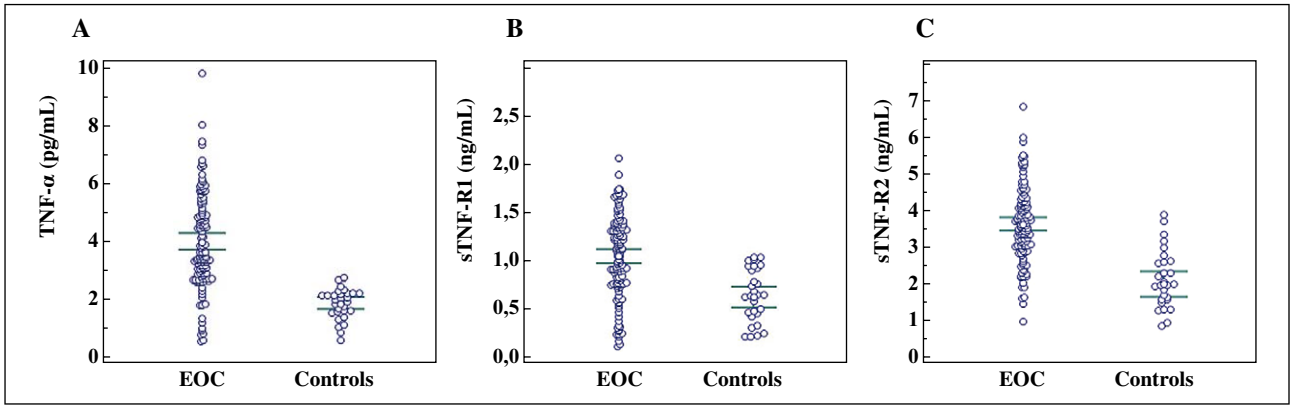

Figure 1
Scatter plots of plasma concentrations of (**A**) TNF-α, (**B**) sTNF-R1 and (**C**) sTNF-R2 in patients with epithelial ovarian cancer (EOC) and healthy controls.

Table 2
Plasma concentrations of tumor necrosis factor-α, soluble tumor necrosis factor receptors 1 and 2 in patients with epithelial ovarian cancer in different WHO grade

	Grade	No. of cases	TNF-α (pg/mL) median (range)	sTNFR-1 (ng/mL) median (range)	sTNFR-2 (ng/mL) median (range)
EOC	G_1	23	3.2 (0.6-5.6)	0.8 (0.4-1.2)	2.9 (2.3-5.1)
	G_2	67	3.9 (0.8-7.9)	1.3 (0.6-2.2)	4.1 (3.1-6.4)
	G_3	36	4.2 (1.2-9.8)	1.1 (0.9-1.3)	3.5 (2.3-9.6)
p-value			NS	NS	NS

EOC: epithelial ovarian cancer; TNF-α: tumor necrosis factor-α; sTNF-R1: soluble tumor necrosis factor receptor 1; sTNF-R2: soluble tumor necrosis factor receptor 2; NS: not significant; G_1, G_2 and G_3: grade of epithelial ovarian cancer according to World Health Organization (WHO) criteria.

Table 3
Plasma concentrations of tumor necrosis factor-α, soluble tumor necrosis factor receptors 1 and 2 in patients with epithelial ovarian cancer in a different FIGO stage

	Stage	No. of cases	TNF-α (pg/mL) median (range)	sTNFR-1 (ng/mL) median (range)	sTNFR-2 (ng/mL) median (range)
EOC	I-II	53	3.1 (0.6-6.8)	0.7 (0.4-1.6)	3.2 (2.3-6.2)
	III-IV	73	5.2 (1.3-9.8)	1.4 (0.5-2.2)	4.4 (3.1-9.6)
p-value			$p < 0.001$	$p < 0.001$	$p < 0.001$

EOC: epithelial ovarian cancer; TNF-α: tumor necrosis factor-α; sTNF-R1: soluble tumor necrosis factor receptor 1; sTNF-R2: soluble tumor necrosis factor receptor 2; I-IV: stage of epithelial ovarian cancer according to International Federation of Obstetrics and Gynecology (FIGO) criteria.

Table 4
Plasma concentrations of tumor necrosis factor-α, soluble tumor necrosis factor receptors 1 and 2 in patients with epithelial ovarian cancer in correlations with mean survival time.

MST (years)	No. of cases	TNF-α (pg/mL) median (range)	sTNF-R1 (ng/mL) median (range)	sTNF-R2 (ng/mL) median (range)
< 5	69	5.5 (1.1-9.8)	1.6 (0.7-2.2)	4.6 (3.9-9.6)
> 5	57	2.8 (0.6-6.9)	0.5 (0.4-1.2)	3.0 (2.3-5.1)
p-value		< 0.001	< 0.001	< 0.001

TNF-α: tumor necrosis factor-α; sTNF-R1: soluble tumor necrosis factor receptor 1; sTNF-R2: soluble tumor necrosis factor receptor 2; MST: mean survival time.

the response to chemotherapy or survival [15]. Numerous reports have associated detection of abnormally high levels of TNF-α in the blood of EOC patients [7-9]. Within groups of patients with the same tumor type, higher levels of TNF-α correlated with more advanced tumor stage and shorter survival time. However, circulating TNF-α is not always detectable in cancer patients and can vary among individual patients over time and the course of disease [10, 11]. The present results are in accordance with those of others, who reported higher TNF-α serum levels in women with ovarian carcinoma than in healthy subjects and women with benign ovarian cysts.

Ovarian cancer develops along a continuum of malignant transformation and promotion. Tissue expression and fluid levels of TNF-α have been associated significantly

with ovarian cancers, but not with benign tumors [10, 11]. Inflammatory conditions related to ovarian cancer include exposure to exogenous irritants and ovulation, accompanied by cell proliferation, oxidative stress, vascular permeability and overproduction of biological factors, such as prostaglandins, leukotrienes, TNF-α and other cytokines [5, 10, 16, 17].

TNF-α mediates tumor regression, and recombinant TNF-α is approved for administration locoregionally at supraphysiological levels as a treatment for experimental tumors [18-20]. On the other hand, the notion that the major effects of endogenous TNF-α in cancer are opposite to the effects observed with high dose TNF-α therapy, has gained importance recently. Instead of causing tumor regression, cancer-derived TNF-α can mediate tumor progression by causing the proliferation, invasion and metastasis of tumor cells [12, 14]. The discordance that this cytokine is both a "necrosis factor" and a "promoting factor" can be explained by the differences in levels of TNF-α in distinct settings. When TNF-α is administered therapeutically in extremely high doses, it acts as an anti-angiogenic and necrotic factor. However, when TNF-α is produced by tumors and tumor-associated macrophages or stromal cells at physiological levels, it promotes tumor growth and additional macrophage recruitment, stimulating the elaboration of angiogenic and growth factors from infiltrating cells [5, 6].

Most of the effects of TNF-α are as a result of two receptors, termed TNF-R1 and TNF-R2, identified on the surface of many cells. The extracellular domain fragments of both receptors, shed from the cell surface, can be detected as soluble forms sTNF-R1 and sTNF-R2 [21]. The role of the sTNF-Rs in EOC progression is not clear. High concentrations of sTNF-Rs can inhibit TNF-α activities and thus may represent a tumor escape mechanism, protecting the tumor from the destructive effects of TNF-α.

CONCLUSION

The observations made in the current study show higher levels of several components of the TNF system in the plasma of EOC patients. High TNF-α and sTNF-Rs levels indicated the highest risk of ovarian cancer progression, and were independently related to poor survival. However, the role of such activation in determining the prognosis of EOC remains to be elucidated.

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