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Research paper

Sub-optimal primary surgery leads to unfavorable immunological changes in ovarian cancer patients

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ABSTRACT

Primary cytoreduction, followed by chemotherapy, is a standard treatment of patients with epithelial ovarian cancer (EOC). However, the effectiveness of this treatment depend on various elements e.g. type of operation. It is accepted that optimal surgery correlates with longer survival of patients. The other element, an efficiency of immune system after surgical intervention although important is less elucidated. The aim of this study was to establish the impact of optimal and sub-optimal operation on immunological status of EOC patients regarding also their overall survival (OS). On the day of primary cytoreduction and 7 days after, the selected serum immunological parameters were determined in 49 patients with confirmed EOC. We found that, the level of immunosuppressive (interleukin 10; transforming growth factor-β – TGF-β1) and pro-inflammatory (interleukin-6 and 8) cytokines was significantly higher in the group of patients with advanced stage of disease, compared to early stage. However, the number of circulating $CD3^+$, $CD4^+$ or $CD8^+$ cells, $CD19^+$ and NK cells was similar in both group of EOC patients. The overall survival of patients who underwent optimal cytoreduction was significantly higher than that in whom only sub-optimal surgery was performed. Sub-optimal cytoreduction only partially weakened the serum level of TGF-β1 and IL-8 and what is more enhanced the number of circulating $CD4^+CD25^{+high}$ cells in patients with advanced stage of disease. Sub-optimal surgery and high post-operative level of TGF-β1 increased the hazard ratio for patients. Besides, we noticed that the high pre-operative concentration of TGF-β1 could distinguish all EOC patients (independently of FIGO classification) for whom optimal or sub-optimal surgery would be applied. Sub-optimal debulking resulted in higher immunosuppression and lower OS of EOC patients.

1. Introduction

Cytokines play a predominant role in facilitating the tumor growth and have a modulatory effect on the immune and tumor cells activity, both in the tumor microenvironment and peripheral blood. Among many cytokines that are involved in cancer progression, those mentioned below are worth being distinguished (Nash et al., 1999; Dunlop and Campbell, 2000). Interleukin 10 (IL-10) is a suppressor cytokine that is produced and released by monocytes, macrophages and different T-cell subsets, as well as by tumor cells. It down-regulates the monocyte-macrophages function (e.g. antigen presentation), inhibits the production of IL-2 and interferon γ (IFN-γ) by Th1-cells, as well as decreases the expression of MHC class I molecule on tumor cells, resulting in the development and promotion of cancer (Sabat, 2010; Zhou et al., 2007; Taylor et al., 2006; Mocellin et al., 2005). Transforming growth factor β is a family of factors that were originally reported as a potent inhibitor of epithelial cell growth and tumor suppressor. However, along with cancer development, TGF-β1 loses their ability to inhibit tumor cells proliferation and favors tumor growth and metastasis. This growth factor have also strong immunosuppressive activity through blocking of antigen presenting cells maturation, as well as inhibition of natural killer cells cytotoxicity and T-cell proliferation. It also induces maturation of naive T-cells into $CD4^+$ $CD25^+$ suppressor/regulatory T-cells subset (Taylor et al., 2006; Muraoka-Cook et al.,

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2005; Jakowlew, 2006). IL-6 is a multifunctional cytokine produced mainly by monocytes and macrophages. It stimulates B-cell differentiation into plasma cells and activates T-cells to the production of IL-2 as well as inhibits a pro-inflammatory response of macrophages. In solid tumors IL-6 can act both as a tumor cells growth factor and as a immunosuppressive cytokine that inhibits maturation of dendritic cells (Łukaszewicz et al., 2007; Fisher et al., 2014). Lastly, IL-8 (also known as CXCL8) is a pro-inflammatory cytokine produced by various normal and tumorigenic cells. Its constitutive expression has been observed in many human cancers. It mainly acts as a chemotactic factor that induces recruitment of neutrophils to the site of inflammation e.g. tumor microenvironment and their activation. What is more, IL-8 enhances adhesion of cancer cells to endothelium and promotes their migration and invasion (Xie, 2001; Waugh and Wilson, 2008).

In addition to immunosuppressive soluble factors, abnormal behavior of T-cells plays a key role in the inhibition of anti-tumor immunity. It was found in patients with malignant diseases that circulating T-cells and NK cells have a functional dysfunction that is connected with down-regulation of CD3-zeta protein expression. Additionally, the proportion of $CD4^+$ T-cells and $CD8^+$ T-cells is altered in cancer patients. Furthermore, tumor microenvironment is characterized by a high level of population of $CD4^+/CD25^+$ suppressor/regulatory T-cells (Treg-cell). However, they are also detectable in peripheral circulation. Treg-cells are known to contribute to tumor escape from the host immune system presumably *via* secretion of IL-10 and TGF-β1 (Whiteside, 2006; Whiteside, 2014; Cheriyan et al., 2009).

Ovarian cancer has the highest mortality rate of all gynecological malignancies among women. Current treatment of epithelial ovarian cancer (EOC) consists of debulking surgery, followed by a chemotherapy with carboplatin/paclitaxel. Cytoreduction diminishes the number of cancer cells and decreases the likelihood of the cancer developing a resistance to chemotherapy. Clinical studies have shown that patients in whom optimal surgery was possible to apply live longer than those with sub-optimal surgical procedure. However, the available information regarding the impact of debulking surgery on the immunological status of EOC patients is still insufficient (Burges and Schmalfeldt, 2011; Lavoué et al., 2013). Therefore, the goal of our studies was to examine the immunological status of EOC patients before and after optimal or sub-optimal primary cytoreductive surgery. The serum cytokines levels as well as the subpopulation of circulating lymphocytes were determined. The second aim was to find correlation with type of surgery, immunological parameters and overall survival (OS).

2. Patients and methods

2.1. Patients and clinical procedures

Our study group consisted of 49 patients with confirmed epithelial ovarian cancer that underwent operative treatment and histopathological examination in the Polish Mother's Memorial Hospital − Research Institute. All patients were operated before 2011 year and in all cases 5-year overall survivals (OS) were calculated. The average age of patients was 58.4. The clinical stage of ovarian cancer was established after laparotomy and pathologic examination, according to the International Federation of Gynecology and Obstetrics (FIGO) protocol and classification (Benedet et al., 2000). During every laparotomy in ovarian cancer the effort was made to perform total abdominal hysterectomy with bilateral salpingo-oophorectomy (TAH + BSO), omentectomy, appendectomy and remove all possible metastases. Optimal cytoreduction was defined as leaving no residual disease greater than 1 cm in diameter after primary surgery. The characteristics of cancer patients is listed in Table 1.

Control group consisted of 17 age-matched generally healthy women treated with total abdominal hysterectomy with bilateral salpingo-oophorectomy (TAH + BSO) according to benign ovarian tumors of epithelial origin (12 patients) or uterine myomas (5 patients).

Table 1
Ovarian cancer patients characteristics.

	Number of patients	%
Total	49	100
FIGO stage		
I/II	13	26.5
III/IV	36	73.5
Ovarian cancer type		
Type 1	23	46.9
Type 2	26	53.1
Histology		
serous	21	42.9
mucinous	6	12.2
endometrioid	11	22.4
clear cell	2	4.1
undifferentiated	9	18.4
Grade		
1	8	16.3
2	13	26.5
3	28	57.2
Cytoreduction		
FIGO I/II opt	12	92.3
FIGO III/IV opt	10	27.8
FIGO III/IV sub-opt	26	72.2

Patients with any previous malignant neoplasia or gynecologic operation, transplantation, autoimmunological disease, thyroid problems or any signs of infection were excluded from the study. Inclusion criterion for both groups was also noncomplicated postoperative period (no relaparotomies, postoperational bleeding, infection, ileus, etc.).

2.2. Serum cytokine level

Peripheral venous blood was obtained in the morning at the day of surgical operation and at 7th day after operation. All blood samples were centrifuged (1500*g*, 10 min) and stored at −80 °C until use in tests. The amounts of serum IL-10, IL-6, and IL-8 were determined using BD OptEIA Human Elisa (Becton Dickinson, USA). The sensitivity of kits was on the level of 1 pg/ml. The level of TGF-β1 in sera was measured with Human TGF-β1 Elisa Kit (Bender, Austria) with the sensitivity of 23.76 pg/ml.

2.3. Determination of white blood cell subsets

Heparinized peripheral venous blood samples were obtained from control group and from EOC patients in the morning at the day of surgical operation and at 7th day after operation. The subpopulation of cells were determined using Simultest IMK Plus (Beckton-Diskinson, USA) according to the manufacturer procedure. Briefly, 100 µl of blood was incubated with fluorochrome-labeled antibodies that bind specifically to antigens on the surface of leucocytes, for 30 min at room temperature. The stained samples were then treated with Lysing Solution to remove erythrocytes and next samples were washed with Cell Wash Buffer and then fixed with 1% paraformaldehyde. The cells were analyzed using FACS Calibur flow cytometer (Beckton Dickinson, USA) equipped with software SimulSet v 3.1 (Beckton Dickinson, USA). Following cell populations were determined: T ($CD3^+$) and activated T ($CD3^+$ HLA-DR) lymphocytes, B ($CD19^+$) lymphocytes, helper/inducer ($CD4^+$) and suppressor/cytotoxic ($CD8^+$) lymphocytes, and natural killer (NK) ($CD16^+$ or $CD56^+$ or both) lymphocytes, as well as the helper/suppressor ratio ($CD4^+/CD8^+$). The data were presented as a percentage of appropriate cell population.

In some experiments, we also determined the percentage of $CD4^+/CD25^{+\,high}$ T-cells in peripheral blood of woman with control group and EOC patients at the day of surgical operation and at 7th day after