Serum TRAIL levels in patients with epithelial ovarian cancer or primary peritoneal cancer treated with neoadjuvant chemotherapy. A pilot study

A. Gąsowska-Bodnar[1], L. Bodnar[2], A. Synowiec[2], M. Jerzak[1], G. Wcislo[2], M. Smoter[2], C. Szczylik[2], W. Baranowski[1]

[1]Department of Gynecology and Gynecologic Oncology, Military Institute of Medicine;
[2]Department of Oncology, Military Institute of Medicine, Warsaw (Poland)

Summary

Aims: The study attempted to evaluate the kinetics of changes in serum TRAIL levels as a potential predictive and prognostic factor in patients with epithelial ovarian cancer (EOC) or primary peritoneal carcinoma (PPC), eligible for an interval debulking surgery (IDS). *Material and Methods:* 17 patients with primary inoperable EOC or PPC in FIGO Stage IIIC or IV who underwent an exploratory operation were enrolled to the study. Serum TRAIL levels were determined by ELISA method (DIACLONE, Besancon Cedex, France) before and after two courses of neoadjuvant chemotherapy (NAC) based on paclitaxel and platinum analogue (cisplatin or carboplatin). The control group consisted of six healthy volunteers. The median difference in concentration of TRAIL (dTRAIL) between the initial marking and after two courses of NAC in each patient was 192 pg/ml and it was used for dichotomization of the test group. *Results:* Suboptimal interval debulking surgery (IDS) was performed in 23.5% (4/17) and optimal IDS in 76.5% (13/17) patients. TRAIL concentration before chemotherapy did not differ significantly between patients with EOC or PPC [1426.96 ± 321.06 pg/ml (mean ± SD) (U = 26, p = 0.08)] and the control group [1160.40 ± 256.39 pg/ml (mean ± SD. After two courses of NAC serum TRAIL concentration level was 1247.49 ± 378.46 pg/ml (mean ± SD). The difference was significant (Z = 2.44, p = 0.0147). Statistical analysis showed that dTRAIL did not significantly influence either extent of IDS (U = 35, p = 0.0962) or time to progression (log-rank test, p = 0.1185), overall survival (log-rank test, p = 0.1973) and response to treatment according to RECIST criteria (U = 35.5, p = 0.9616). *Conclusions:* Serum TRAIL concentration levels changed significantly during NAC. However, it seems that the concentration of this protein has no critical value as a predictive or prognostic factor in patients with EOC or PPC.

Key words: Ovarian cancer; TRAIL; Neoadjuvant chemotherapy; Interval debulking surgery.

Introduction

Ovarian cancer remains a major diagnostic and therapeutic problem despite significant progress in oncologic gynecology. It is the sixth most common malignancy as well as the seventh leading cause of cancer-related death in women worldwide [1-3]. One of the major reasons for the dismal prognosis is the fact that nearly 70% of cases are diagnosed at an advanced stage (i.e., tumor already spread beyond the ovary, in spite of great efforts to develop reliable screening and prevention strategies. The main reason for this phenomenon is the lack of both symptoms and specific testing [4].

The current standard treatment for advanced ovarian cancer is primary debulking surgery followed by postsurgical chemotherapy. Better prognosis can be expected in cases in which optimal debulking can be achieved. Unfortunately, optimal debulking in the primary surgery can be achieved in only 30-60% of FIGO Stage III/IV ovarian cancers in average institutions [5, 6], and the prognosis for patients with advanced ovarian cancer is poor. Neoadjuvant chemotherapy has been recognized as a possible approach to improve the prognosis of these patients. In initial studies, neoadjuvant chemotherapy (NAC) was chosen for patients with apparently unresectable bulky tumors or poor performance status as an alternative treatment to primary debulking surgery (PDS). The main problem arising in selecting patients with advanced ovarian cancer for neoadjuvant chemotherapy is defining the patient selection criteria to determine those who could potentially benefit from interval debulking surgery (IDS).

Tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) has the ability to induce apoptosis in cancerous cells without causing toxicity in healthy cells. This points to the role of TRAIL as a member of the tumor necrosis factor (TNF) cytokine family which can induce apoptosis upon binding to its death domain containing two receptors: TRAIL receptor 1 (death receptor 4, DR4) and TRAIL receptor 2 (death receptor 5, DR5) [7]. Both TRAIL receptors, TRAIL-R1 and TRAIL-R2, are prevalent in many cancer cells including ovarian cancera [8, 9]. Lancaster *et al*. [10] reported that relative TRAIL expression was 2.3-fold higher (1.52 vs 0.66) in ovarian cancers from women who lived five years than in ovarian cancers from those who died in within one year (p = 0.03). The high TRAIL expression in ovarian tumor specimens was associated with better 5-year survival perhaps resulting from increased sensitivity to platinum-based chemotherapy among these tumors.

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The aim of our study was to evaluate the kinetics of changes in serum TRAIL levels as a potential predictive and prognostic factor in patients with epithelial ovarian cancer (EOC) or primary peritoneal carcinoma (PPC), eligible for IDS.

Materials and Methods

We analyzed 17 patients treated at the Department of Gynecology and Gynecologic Oncology and Department of Oncology at the Military Institute of Medicine in Warsaw, Poland between January 2003 and August 2007 meeting the following criteria: (1) histologically confirmed ovarian carcinoma or primary peritoneal serous carcinoma (2) advanced ovarian cancer or primary peritoneal serous carcinoma in Stage IIIC or IV (presence of malignant cells in pleural fluid but no metastases in parenchymal organs), (3) the patients were qualified for neoadjuvant chemotherapy containing paclitaxel and platinum analogues. The control group consisted of six healthy women. The study was approved by the Local Bioethics Committee, and each patient signed a written consent to participate in the study.

In the study, laparotomy or laparoscopy was defined as an initial surgery with the collection of specimens for histopathological examination (diagnostic excision of both ovaries was allowed). IDS was allowed, as an option, when residual tumor larger than 1 cm had been left at PDS. In such cases, IDS was performed three to six weeks after administering the third (the last cycle) of NAC unless there was disease progression occurring during the chemotherapy. The disease was defined unresectable when our team of surgeons, comprising at least two experienced gynecologists, stated that optimal debulking (tumor less than 1 cm) was impossible to obtain in a standard procedure.

NAC was defined as the initial treatment prior to the deferred cytoreductive surgery. It consisted of paclitaxel and platinum analogue and in ten cases also liposomal doxorubicin was added. The deferred debulking surgery was performed three to six weeks after administering of the last cycle of NAC unless disease progression occurred during NAC. Standard procedures of IDS consisted of a total abdominal hysterectomy, bilateral salpingo-oophorectomy, omentectomy, systematic pelvic and/or aortic lymphadenectomies and the maximal debulking of metastatic tumors.

The surgery was defined as optimal when the tumor remaining in the abdominal cavity did not exceed 1 cm in diameter; the outcome of surgery was defined as suboptimal if the dimension of residual changes exceeded 1 cm.

Blood draws from patients, taken from the basilic vein, were left at room temperature for 30-60 min in order to form a clot. Serum was centrifuged at 4°C at the speed of 1000 x g for 15 min, then frozen in Eppendorf tubes at the temperature of –80°C, where they were stored until the time of marking. TRAIL concentrations in serum were determined by ELISA (DIACLONE, Besancon Cedex, France) before and after two courses of NAC based on paclitaxel and platinum analogue (cisplatin or carboplatin). The procedure was performed according to the manufacturer's instructions. The control group consisted of six healthy women.

The median difference in concentration of TRAIL (dTRAIL) between the initial marking and after two courses of NAC in each patient was 192 pg/ml and was used for dichotomization of the test group.

Table 1. — *Characteristics of patients (n = 17).*

Age, years, median (range)	56 (39-69)
ECOG performance status:	
0	11.8% (2/17)
1	76.4% (13/17)
2	11.8% (2/17)
BSA (m^2)	1.72 (95% CI; 1.63-1.8)
Stage at diagnosis (FIGO)	
IIIC	88.2% (15/17)
IV	11.8% (2/17)
Neoadjuvant chemotherapy:	
Paclitaxel/Cisplatin	29.4% (5/17)
Paclitaxel/Carboplatin/PLD	58.8% (10/17)
Paclitaxel/Carboplatin	11.8% (2/17)
Histopathological type	
Serous EOC	72.6% (12/17)
Endometrial EOC	17.6% (3/17)
PPC	11.8% (2/17)
CA 125, average level, 95% CI (U/ml)	
Before NAC	1433.26 (626.31-2240.21)
After second cycle of NAC	181.94 (51.57-312.32)
TRAIL, average level, 95% CI (pg/ml)	
Before NAC	1426.96 (1261.89-1592.94
After second cycle of NAC	1247.49 (1052.90-1442.08)
Optimal IDS	76.4% (13/17)
Suboptimal IDS	23.6% (4/17)
RECIST response:	
CR	41.2% (7/17)
PR	41.2% (7/17)
SD	11.8% (2/17)
PD	5.9% (1/17)

FIGO: International Federation of Gynecology and Obstetrics; PS: performance status; ECOG: Eastern Cooperative Oncology Group; CI: confidential interval; RECIST: response evaluate criteria in solid tumors; CR: complete response; PR: partial response; SD: stable disease; PD: progression of disease; NAC: neoadjuwant chemotherapy; IDS: interval debulking surgery; PLD: Pegylated liposomal doxorubicin; BSA: body surface area.

Statistical analysis

Progression-free survival (PFS) was defined as time elapsed between date of start of NAC and date of disease progression or date of last follow-up. Overall survival (OS) was defined as time elapsed between date of start of NAC and date of death or date of last follow-up. The cut-off date for our analysis was established on April 2010. Median and life tables were computed using the product-limit estimate by the Kaplan and Meier method and the log-rank test was employed to assess statistical significance; p values less than 0,05 were considered to be statistically significant. To compare the results which did not meet the criteria of a normal distribution, a nonparametric Mann-Whitney test (U test) was used. We performed the analysis using the statistical package STATISTICA PL (7.00 version).

Results

The characteristics of the 17 patients included in the study are presented in Table 1. The median age of patients was 56 (range 39-69). Suboptimal cytoreductive operation was performed in 23.5% (4/17) of patients and optimal in 76.5% (13/17) of patients.

TRAIL concentration did not differ significantly between the control group [1160.40 ± 256.39 pg/ml (mean ± SD)] and patients with the EOC or PPC, in which the value of the concentration before chemotherapy was 1426.96 ± 321.06 pg/ml (mean ± SD) (U = 26, $p = 0.08$).

Fig. 1

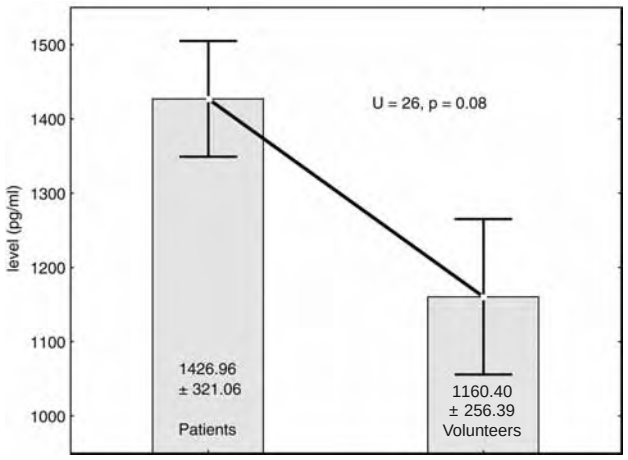

Figure 1. — Serum TRAIL levels before chemotherapy and the control group.

Fig. 2

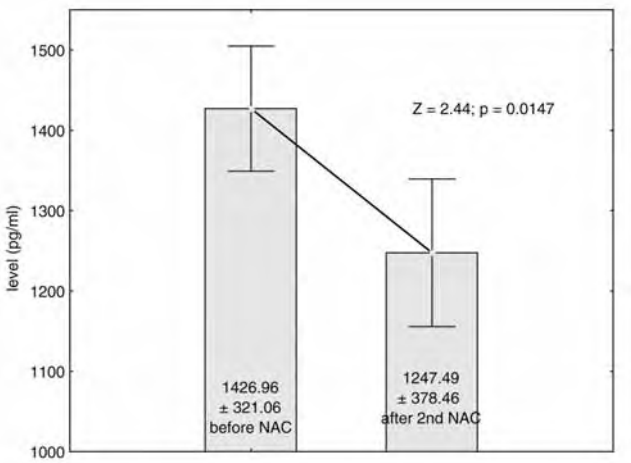

Figure 2. — Serum TRAIL levels before and after 2nd course of neoadjuvant chemotherapy (NAC).

Fig. 3

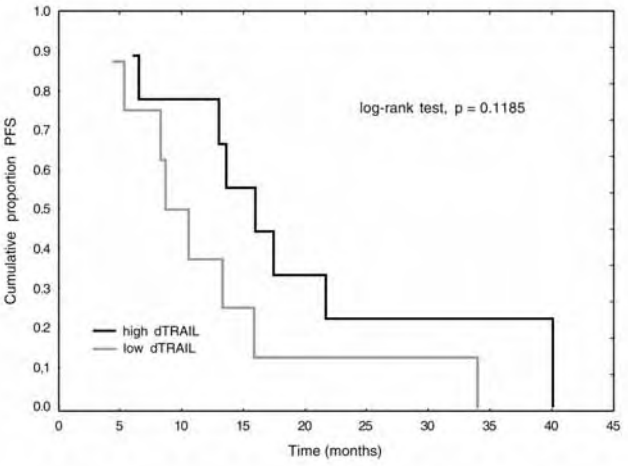

Figure 3. — Progression-free survival (PFS) in months for all patients.

Fig. 4

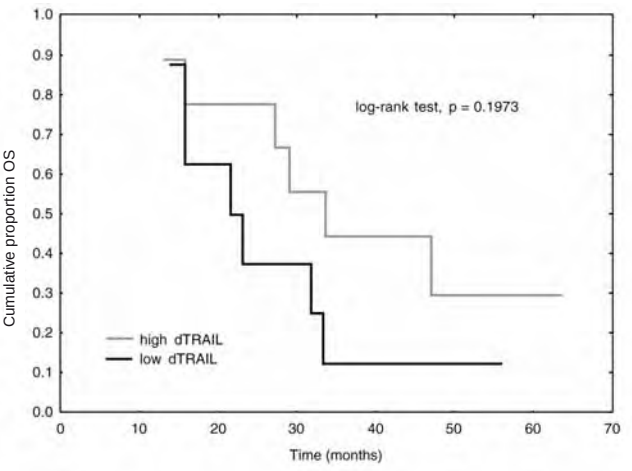

Figure 4. — Overall survival (OS) in months for patients.

After two courses of NAC the concentration of TRAIL was 1247.49 ± 378.46 pg/ml (mean ± SD) and the difference was significant (Z = 2.44, p = 0.0147).

There was no impact of dTRAIL on: the extent of the IDS (U = 35, p = 0.0962) and PFS (log-rank test, p = 0.1185). Median PFS in the high dTRAIL group was 14.7 months, while in the low dTRAIL group 8.8 months.

dTRAIL had no effect on overall survival (log-rank test, p = 0.1973) and obtained response to treatment according to RECIST criteria (U = 35.5, p = 0.9616). Median OS in the high dTRAIL group was 31.2 months, while in the low dTRAIL group it was 21.4 months.

Discussion

The size of residual disease after debulking surgery is the most important prognostic factor. Optimal tumor debulking is achieved in only 40-50% of advanced ovarian cancer cases. Additionally, the POS rate differs from 40% up to 90% among the sites and even individual physicians within the same institution [11]. In our study, the optimal debulking surgery in initially unresectable patients was 76.4%, and in the remaining 23.6% of women the surgery was suboptimal.

With regard to the prospective phase II study conducted in 63 patients with advanced ovarian cancer, Kuhn *et al*. [12] obtained a higher (84%; 26/31) optimal debulking, but 2 cm of residual tumor was taken as the parameter of optimal treatment. These researchers showed statistically significant improvement in the percentage of optimal surgery obtained in the group receiving NAC before the appropriate surgical treatment compared to the conventionally treated group and a significant improvement in median survival 42 months vs 23 months in the group treated conventionally.

Similar results were obtained in a prospective phase III trial by Vergote *et al*. [13], who compared the effects of treatment according to two protocols in patients with ovarian cancer, peritoneal and fallopian tube in Stage IIIC/IV. The first group of patients had undergone primary cytoreductive surgery followed by adjuvant chemotherapy (6 cycles paclitaxel/carboplatin). Patients

eligible for the second arm of the study were treated with three courses of NAC (paclitaxel/carboplatin) followed by cytoreductive surgery and than adjuvant chemotherapy (three courses of the same regimen.) Results in both arms were comparable in terms of OS time and PFS. Percentage of optimal surgeries was significantly different in the arms and amounted to 48% in the first group and 84% in the second. Fewer complications were observed in the arm treated with NAC compared to the standard treatment group, i.e. fewer deaths in the perioperative period (2.7% vs 6%), fevers, (2% to 8%), hemorrhage (1%-7%) and thrombotic-embolic complications (0.3% vs 2.4%).

The results of dTRAIL had no statistically significant impact on the range of the debulking surgeries, response to treatment by RECIST criteria and PFS. Despite the differences found between serum concentrations of TRAIL baseline and after two courses of NAC (p = 0.0147), this parameter has no predictive significance for the result of IDS.

Searching for new predictors of the outcome of the operations Levine *et al*. [14], who defined the role of the gene expression microarray test, assessed the ability of the RNA oligonucleotide effect on maximum debulking during the original surgery in 70 patients with advanced serous ovarian cancer (FIGO IIIB-IV). Optimal cytoreduction was obtained in 45 patients, and suboptimal treatment was performed in 25 patients. Analysis of gene expression in both operated groups showed no statistically significant differences and did not affect the possibility of obtaining optimal cytoreducton.

There was no noticeable impact of dTRAIL on OS. Median OS in patients with high dTRAIL was 31.2 months, while in the group with low dTRAIL it was 21.4 months. The resultant difference did not reach statistical significance. This parameter has no prognostic significance in patients undergoing NAC or the deferred cytoreductive surgery.

Duiker *et al*. [15] found an immunohistochemical assessment of TRAIL expression in 17.3% (59/340) of patients with ovarian cancer. TRAIL expression was associated with a lower degree of histological malignancy, at an earlier stage of cancer, and longer duration of PFS. In previous studies on the effects of TRAIL on the course of ovarian cancer, the relationship between expression of this protein and a low degree of histological malignancy [16] and an early stage of disease was observed, but no prognostic significance of this protein was proven [17, 18].

In the study by Lane *et al*. [19], a cell viability test was employed to evaluate the effect of peritoneal fluid collected from 54 patients with ovarian cancer during primary debulking surgery. The authors demonstrated that peritoneal fluid has an inhibitory effect on cell apoptosis induced by TRAIL. These results suggest that the presence or concentration of prosurvival factors differ in different ovarian cancer ascites. Ascites that have a protective effect on TRAIL cytotoxicity are often protective against cisplatin. This data show that the prosurvival activity of ascites against TRAIL is associated with a shorter disease-free interval. This may partly explain the phenomenon of resistance to chemotherapy based on paclitaxel/cisplatin in patients with concomitant ascites. The results of this study suggest that ascites may contain factors affecting the increase of cell survival that protect against TRAIL and chemotherapy. The parameter of concomitant ascites was not assessed in our study.

In conclusion the concentration of TRAIL during neoadjuvant chemotherapy varies significantly. Our preliminary results indicate that the concentration of this protein has no predictive and prognostic significance in patients with ovarian cancer or primary peritoneal carcinoma.

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Address reprint requests to:
L. BODNAR, M.D.
Department of Oncology,
Military Institute of Medicine
4-141 Warsaw
128 Szaserow Str. (Poland)
e-mail: lubo@esculap.pl