High Preoperative Serum sVCAM-1 Concentration as a Predictor of Early Ovarian Cancer Recurrence

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Research

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Abstract

Background: Role of soluble vascular cell adhesion molecule-1 (sVCAM-1) in ovarian cancer is largely unclear. It was shown that mesothelial expression mediates tumour cell invasion and is associated with metastases in advanced ovarian cancer.

Results: Mean sVCAM-1 serum concentration in all patients before operation was 1564.68 ± 435.65 ng/ml while mean ascites level was 801.84 ±244.35 ng/ml. Follow up period was minimum 27 and the maximum 58 months. Patients were divided in two groups according to time to recurrence. Group A: 20 patients with disease progress or relapse within 12 months (mean serum level 1660.54±417.93 ng/ml; mean ascites level 827.92±290.36) and group B: 17 patients with tumour relapse after more than 12 months (mean serum level 451.91±441.15ng/ml; mean ascites level 771.16±179.93). There was statistically significant difference in serum concentration and not in ascites concentrations of sVCAM-1, grade, histology and stage and tumour between the groups. There was a correlation between serum and ascites concentrations in group A and not in the patients from group B. Increased sVCAM-1 concentration in serum and ascites relates to advance ovarian cancer.

Conclusions: This is the first study demonstrating that higher serum sVCAM-1 concentrations at the time of diagnosis might be predictive for early relapse. Serum sVCAM-1 can be potential marker for ovarian cancer follow-up.

Introduction

Ovarian cancer (OC) is a chronic disease, which is the most lethal gynecological cancer and in more than 70% of all cases diagnosed at an advanced stage. It has been shown that not only complete cytoreductive surgery, but also tumour chemotherapy sensitivity are crucial in terms of relapse time. Approximately 80% of the women respond to treatment. [1] However, the relapse is common even though complete clinical remission is obtained. Most of the patient will relapse within 24 months of the treatment, which has unfortunately been unchanged in recent decades despite developments in chemo pharmacy.[2] Predicting risk of recurrence will allow patients to have a better quality of life and clinicians to better approach in ovarian cancer treatment.

Several tumour markers have been studied in the serum of ovarian cancer patients to provide better disease monitoring. One such protein has been Soluble Vascular Cell Adhesion Molecule-1 (sVCAM-1).[3] Its elevated concentration is shown to be associated with tumor presence.[4] Expressed on activated endothelial and mesothelial cells VCAM-1 has been identified as an important mediator for adhesion of ovarian cancer cells to and invasion through the mesothelium.[5]

In our previous study we evidenced the positive correlation of the sVCAM-1 concentration in ovarian cancer patient's serum and ascites, which represents tumor microenvironment. The aim of the current

study was to analyze sVCAM-1 preoperative serum and ascites level the accordance with ovarian cancer recurrence.

Materials And Methods

We performed a prospective study made up of 37 patients with stage III and IV primary ovarian cancer operated on between 2011 to 2013 at the Department of Gynecology, University Medical Centre Ljubljana. The exclusion criteria were the presence of any disease demonstrated to influence sVCAM-1 concentration (active inflammation, complicated diabetic disease). Patients clinical characteristics are presented in Table 1. Disease staging was in accordance with the International Federation of Gynecology and Obstetrics (FIGO) classification for ovarian cancer staging. All patients received and signed consent documentation about the research and analysis of their blood and ascites for the purposes of the research. Clinical data was collected during planned visits. Statistical analysis was performed using statistical package SPSS version 21.

Before operation four ml of peripheral blood were collected in a vacutianer, without anticoagulant or other additives and used for sVCAM-1 analysis. Twenty ml of ascites were aspirated into a sterile syringe at the beginning of each operation and immediately transferred into a conical tube and kept on ice until centrifugation at 1000 x *g* for 10 min at 4^{O}C. Serum was separated by centrifugation at 2000 x *g* for 15 minutes at 4°C. Ascites supernatants and serum were stored in aliquots at −80°C. sVCAM-1 sample concentration was analyzed by means of flow cytometric bead-based assay and measured using a FlowCytomix Simplex Kit (eBio-science, Vienna). The kit consists of fluorescent microspheres (diameter: 4 μm, emission wavelength at 700 nm) coated with specific sVCAM-1antibodies raised.

Data are presented as mean ± SD. The normality of distribution was tested with the Kolmogorov-Smirnov test. The Mann-Whitney test was used for non-normally distributed variables. We used a chi squared test for nominal variables and ANOVA for continuous variable such as age, which are normally distributed. A *p* of <0.05 was considered significant. Statistical analysis was performed using software statistical package SPSS, version 21 (IBM Corp, Armonk, NY).

Results

Mean serum sVCAM-1 concentration in all patients before operation was 1564.68 ± 435.65 ng/ml and mean ascites sVCAM-1 concentration was 801.84±244.35. We devided all patients in two groups. Group A being patients with disease progress or tumor relapse within 12 months of treatment completion (n=20) and group B being patients with tumor relapse occuring more than 12 months after treatment completion (n=17). Mean sVCAM-1 concentration in the patient in group A was 1660.54±417.93ng/ml in serum and 827.92± 290.36 ng/ml in ascites. Mean sVCAM-1 concentration in group B was 1451.91±441.15 ng/ml in serum and 771.16±179.93 ng/ml in ascites. There was a significant (p=0.04) higher serum sVCAM-1 concentration in group A compared with group B (Graph 1). There was no significant difference in ascites sVCAM-1 between the groups (p=0.38). There was correlation between

serum and ascites sVCAM-1 concentration in group A (p=0.006) and not in group B (p=0.22). There was no statistically significant difference between the groups in disease histology, grade and stage. Group B were statistically significantly younger (p=0.01), with a higher percentage of them treated with primary cytoreductive surgery (p=0.01) compared with group A.

Follow up took place until 24 March 2016 or until patient death. The final patient in the study had surgery 19 December 2013. The minimum follow up period was 27 months, the maximum 58 months.

Discussion

In our previous study we identified the presence of sVCAM-1 in ovarian cancer patient serum and its correlation with ascites sVCAM-1 concentration. These findings further supported the idea that sVCAM-1 is measurable in serum of advanced ovarian cancer serum and reflects the ongoing ovarian cancer. In the current study, we find that there was higher pretreatment serum sVCAM-1 in patients with early tumour progress or relapse when compared with patients relapsing 12 months after the treatment.

VCAM-1 is a member of Ig superfamily expressed on activated endothelial and mesothelial cells. VCAM-1 expression and tumour presence association has been evidenced. [6,7] It has been proposed as a prognostic tumour factor in different cancers, its value being its expression of different outcome. Increased serum sVCAM-1 levels have been linked to poor survival in relation to melanoma, Hodgkin and non- Hodgkin lymphoma and breast and gastric cancer. Similar results were seen in terms of colorectal cancer.[8] In terms of renal cell carcinoma, VCAM-1 tumour cell expression is associated with better survival rate. [9]

In the first study measuring sVCAM-1 serum concentrations in 15 patients with ovarian cancer it has been found that the concentration is significantly higher if patients have ovarian cancer. [14] The opposite is presented in another study, which reported lower sVCAM-1 concentration in patients with ovarian cancer compared to those with benign ovarian conditions. The likely cause of the difference may lie in patient selection, that is the one of the studies only patients with early ovarian cancer were included.[16] Slack Davis and al. measured serum sVCAM-1 levels in ovarian cancer patient serum and state that this protein is linked to worse outcomes, whilst another recent study didn't report any correlation between sVCAM-1 and ovarian cancer outcome, but only with metastasis presence. [5,10]

In 2013 Scalici and al. suggested VCAM-1 as indicator for ovarian cancer response to platinum based chemotherapy, [11] and in a recent study, correlated serum sVCAM- 1 with mesothelial VCAM-1 expression in 18 patients with ovarian cancer and were unable to identify serum sVCAM-1 as a surrogate for mesothelium expression. [12] To the best of our knowledge we didn't find any studies concerning sVCAM-1 and ovarian cancer recurrence. The role VCAM-1 in ovarian cancer is largely unclear, but it has been shown that mesothelial expression mediates tumor cell invasion. [13] In this study we measured increased levels of sVCAM-1 before operation in patients with ovarian cancer whose disease had progressed or tumor had relapsed at a very early stage, 12 months, after treatment completion. The observed increase

in serum sVCAM-1 concentration could be attributed to disease biology and behaviour, as we were not able to find any correlation with tumour stage, disease grade or histological type between groups; we therefore measured sVCAM-1 from ascites as a fluid representing the local tumour microenvironment. It is an ideal media for evaluation of collected tumor cells and soluble proteins. The results from our measurements of patient's sVCAM-1 concentrations in ascites only evidences a correlation with serum sVCAM-1 levels in the group of patients with early relapse or disease progress, but not in the group with later disease relapse. Research carried out by Slack Davis et al. attests the importance of VCAM-1in the regulation of ovarian cancer cell mesothelial invasion and metastatic progression. A possible explanation for this might be that if the tumours are more aggressive, mesothelial invasion is stronger and peritoneum mesothelial cells around the attached tumour cells are more severly affected. Peritoneum breakdown leads to easier ascites outflow with sVCAM-1 into the blood, so correlation is visible; whilst in the other group, mesothelial cell affection is slowed down and ascites transfer into the blood is limited, so sVCAM-1 correlation is not visible. Patients from this group needs longer periods of time to relapse post treatment. We also found that most patients with early relapse and tumour progress were treated with neoadjuvant chemotherapy, while most patients from the group that relapsed more than 12 months posttreatment were treated with primary cytoreductive surgery. The decision for treatment was based on radiological and laparoscopic evaluation of resectability, which is the standard pretreatment procedure at our institution. From our findings, we can assume a preoperative concentration of serum sVCAM-1, which can be another indicator for tumor aggressiveness for predicting primary optimal citoreduction and cancer treatment response. Ovarian cancer and cancer mediator biology are not very clear. Kong in a recent review covers the role and relevance of sVCAM-1 in inflammation and cancer, and highlights the emerging potential of sVCAM-1 as a new therapeutic target in terms of immunological disorder in cancer. [14] It has been shown that sVCAM-1 is closely associated with the progression of various immunological disorders, inflammation-associated vascular adhesion and the transendothelial migration. It seems possible that inflammation around tumours can be a crucial process for the different tumour and host behavior, tumor cell shedding and consequently different sVCAM-1 concentrations.

The very small sample size is a limitation of this study that needs to be acknowledged. We are also well aware that other factors can influence sVCAM-1 concentration, but in our case, the exclusion criteria was the presence of a systemic disease, known to affect sVCAM-1expression, both groups possibly experiencing typical inflammatory responses characteristic of cancer. The small sample size restricted our ability to perform multivariate analysis and nullify other possible influence.

In correlating and evaluating serum and ascites sVCAM-1 concentration and correlation, we showed that cancer aggressiveness is reflected in the serum in terms of cancer cells secretom and probably determinate before the treatment is started. However further work needs to be done on bigger sample size to establish whether sVCAM-1 in serum can be used in clinical practice as an early indicator for treatment outcome.

Serum sVCAM-1 concentration at the time of diagnosis might be predictive for different biologic behavior and treatment resistance and associated with cancer progression or recurrence. This is the first study

demonstrating that higher serum sVCAM-1 concentrations in ovarian cancer patients is connected to early tumour recurrence or disease progression. Serum sVCAM-1 can be a potential tumor marker for ovarian cancer follow-up.

Abbreviations

sVCAM-1 – Soluble Vascular Cell Adhesion Molecule-1

FIGO – International Fderation of Gynecology and Obstetrics

Declarations

Ethics approval

The trial was approved by the National Medical Ethics Committee of Republic of Slovenia, Approval number 82/01/11 and in agreement with the Helsinki Declaration.

Consent

A written informed consent for publication was obtained from patients or patient's relatives. All patient data used in the manuscript were anonymized.

Availability of data and materials

All data used in analysis can be found at first author Marina Jakimovska MD, PhD, on request.

Competing interests

The authors declare that they have no competing interests.

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Author's contributions

MJ collected clinical and pathological data and drafted the manuscript. KČ performed analyses, using flow cytometry drafted the manuscript and revised the final version of the manuscript. IV made the statistical analysis. BK supervised the study, provided clinical and surgical information, revised the final of the manuscript

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Table

Table 1. Patient demographic and treatment characteristics

	Group A n	Group B n	All patients n
Number of patients	20	17	37
Age	63.3±10.8	53.9±10.6	59 ± 11,6
FIGO stage III	13	15	28
FIGO stage IV	7	2	9
Serouse tip	19	13	32
Endometrioid type	1	4	5
G1	2	3	5
G2	6	5	11
G3	12	9	21
Primary citoreductive operation	6	12	18
Neoadjuvant chemotherapy	14	5	19

Figures

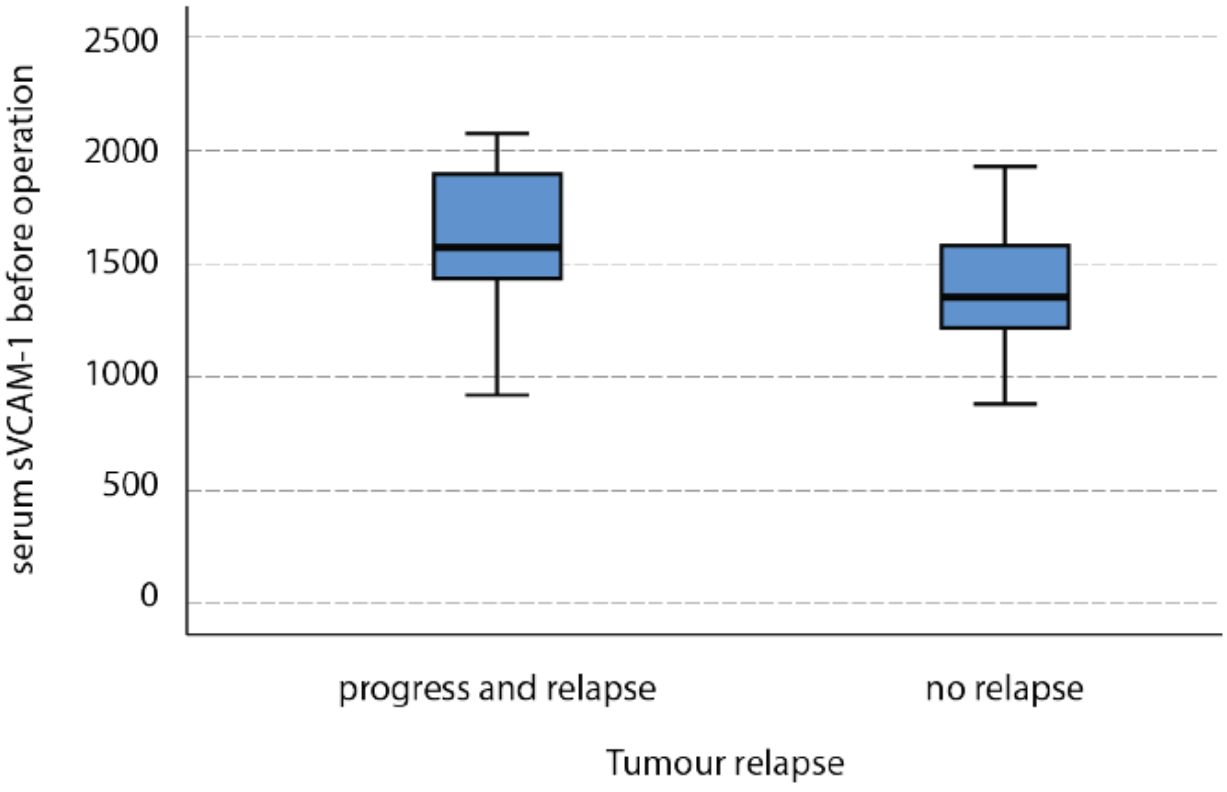

Figure 1

Graph 1. Comparison sVCAM-1 concentration in serum before operation in patients with relapse after 12 months or without relapse in the follow up period and in patients with disease progress or relapse in 12 months after the treatment