research article

Potential of osteopontin in the management of epithelial ovarian cancer

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Background. Osteopontin (sOPN) is a promising blood tumour marker for detecting epithelial ovarian cancer (EOC). However, other clinical uses of sOPN as a tumour marker in EOC are still lacking. Since sOPN concentrations in serum are not associated with those in ascites, we compared clinical value of sOPN concentrations in the two body fluids.

Patients and methods. The study included 31 women with advanced EOC and 34 women with benign gynaecological pathology. In the EOC group, serum for sOPN analysis was obtained preoperatively, after primary debulking surgery and after chemotherapy. In the control group, serum was obtained before and after surgery. Ascites and peritoneal fluid were obtained during surgery. sOPN concentrations were determined by flow cytometry bead-based assay.

Results. The sensitivity and specificity of sOPN in detecting EOC was 91.2% and 90.3% (cut-off = 47.4 ng/ml) in serum, and 96.8% and 100% (cut-off = 529.5 ng/ml) in ascites. Kaplan-Meier analysis showed a significant association between higher serum sOPN concentration and overall survival ($p = 0.018$) or progression free survival ($p = 0.008$). Higher ascites sOPN concentrations were associated with suboptimally debulked tumour and unresectable disease. Higher serum sOPN concentrations were associated with refractory disease or incomplete response to platinum-based chemotherapy.

Conclusions. The study showed that ascites sOPN level mirrors present disease and is superior to serum level for diagnostic purposes and surgical planning, although the end result of treatment is the response of the whole body in fighting the disease. The preoperative sOPN concentration in serum thus better reflects disease outcome.

Key words: advanced ovarian cancer; osteopontin; serum; ascites

Introduction

Osteopontin (OPN) is an important signalling agent in the development and progression of cancers.[1-4] As a soluble protein, sOPN is one of the promising blood tumour markers for detecting epithelial ovarian cancer (EOC).[5-8] However, practical clinical use of sOPN as a tumour marker in EOC awaits further evaluation.

Although there are encouraging reports on sOPN as a serum tumour marker for detecting primary and recurrent EOC [5,9-12], we have not found any published report on the prognostic value of preoperative serum sOPN level in EOC. It has been reported that increased expression of OPN in peritoneal metastatic lesion of EOC is associated with poor survival.[13] Additionally, we found that detecting the preoperative serum sOPN level is helpful

in assessing the prognosis of other cancer patients (*e.g.*, breast, neck, gastric).[1,14] It would therefore be worth also exploring the prognostic value of preoperative serum sOPN level in EOC patients.

Another attribute of a tumour marker is to help in selecting the best treatment for cancer patients. Treatment for advanced EOC patients consists of cytoreductive surgery and platinum-based chemotherapy.[15-17] Because of the significant survival benefit associated with successful cytoreductive surgery for advanced-stage EOC and the lack of benefit associated with an incomplete resection, attention has been directed toward developing preoperative models to predict surgical outcome. In addition to radiographic images, extent of ascites and gene expression, some of these models incorporate serum tumour marker levels, primarily cancer antigen 125 (CA125) as approved standard tumour marker in EOC.[18,19] Since OPN expression in metastasis is significantly increased compared to the primary tumour[13], including OPN in such a model may improve the accuracy of determining the extent of intra-abdominal disease for surgical planning. The clinical usefulness of pre-treatment sOPN levels for predicting the response to chemotherapy is another field for further evaluation. A faster response to chemotherapy is an independent predictor of survival for patients with advanced EOC, regardless of debulking status.[20]

The results of our previous published study showed that the increase of sOPN baseline concentration in EOC patients in comparison to patients with non-malignant gynaecological pathology was much higher for local fluid (27-fold) ascites *vs* peritoneal fluid taken from the cavum Douglasi than for serum (3-fold).[21] It is therefore worth discovering whether determination of sOPN concentrations in ascites gives additional or more accurate information about the disease than the determination of serum sOPN concentration alone. In addition to the sOPN retention tendency in local fluid, which is potentiated in malignant compared to benign conditions, we found that sOPN concentrations in serum were not associated with concentrations in ascites or peritoneal fluid.[21] It would thus be reasonable to set separate control values of this marker in the blood and in the local fluid. Determination of sOPN concentrations in local fluid may be useful in combination with cytology in order to obtain more accurate results, especially in the classification of early stage disease.

Since OPN plays a significant role in carcinogenesis, the objective of our study was further to evaluate the clinical usefulness of sOPN as a tumour marker in advanced EOC patients. The main aim of this study was to elucidate the prognostic value of preoperative serum and ascites sOPN levels. Furthermore, the usefulness of determining preoperative sOPN levels for surgical planning purposes and response to standard chemotherapy were investigated. To this end, we determined the kinetic pattern of sOPN serum concentrations after primary surgery and chemotherapy. Additionally, we examined the relationship of sOPN concentrations with various clinicopathological variables. Since sOPN concentrations in serum are not associated with concentrations in ascites, we compared the clinical usefulness of sOPN as a tumour marker in both body fluids of EOC patients.

Patients and methods

Patients

The study included 31 patients with advanced EOC [FIGO III-IV] and 34 patients with benign gynaecological pathology as a control group, who were operated between December 2011 and December 2013 at the Department of Gynaecology, University Medical Centre Ljubljana. Family, general, gynaecological and obstetric history, indication for surgery, other relevant diseases and current therapy were collected from medical records. Early-stage (FIGO I, II) and absence of ascites were exclusion criteria for enrolling patients with ovarian malignancy. The control group enrolled patients with common non-malignant gynaecological indications for surgery (*e.g.*, benign ovarian cyst, uterine myoma). Patients with malignancies and elevated standard tumour marker CA125 were excluded from the control group. The purpose of the study was explained to all patients and written informed consent was obtained prior to enrolment. The study was approved by the Commission of the Republic of Slovenia for Medical Ethics (No. 82/01/11) and in accordance with the Helsinki Declaration.

All EOC patients were intended for primary debulking surgery (PDS), but resectability was evaluated through imaging methods and diagnostic laparoscopy performed by an experienced oncologic surgeon. A patient was considered a candidate for neoadjuvant chemotherapy (NACT) in the case of wide spread of the disease in the abdominal and pelvic cavity (unresectable massive peritoneal involvement, widespread infiltrating carcinomatosis of diaphragm, mesenteral retraction, miliary car-

cinomatosis of the bowel, liver and stomach metastases). On the basis of these criteria, 13 patients underwent PDS and 18 patients underwent NACT, and 10 of the patients in the latter group also underwent interval debulking surgery (IDS). The extent of residual disease after debulking surgery was based on the diameter of the single largest lesion. Complete response to chemotherapy was defined by a normal serum CA125 level. The applied cut-off value for CA125 was 35 U/ml. A partial response was defined by a decrease of at least 50% in CA125 level. Patients with a smaller decrease or any increase in CA125 during chemotherapy were defined as non-responders. All available dimensions of the ovarian tumour were measured using imaging methods and, in the case of PDS, from the pathology report. All patients were followed to disease recurrence or death. Overall survival (OS) was measured from diagnosis until death from any cause. Progression-free survival (PFS) was defined as the time from diagnosis to first tumour recurrence.

Collection and storage of samples

In the OC group, venous blood samples for determination of sOPN concentrations were obtained preoperatively, one week after PDS, and 3-6 months after the last cycle of chemotherapy. In the control group, venous blood samples were obtained prior to surgery, and 3-6 months after surgery, when the patients were healthy and non-pregnant. Four ml of peripheral blood was collected into a vacutainer, without anticoagulant or other additives. Serum was separated by centrifugation at 2000 x *g* for 15 minutes at 4 °C. In both groups of patients, blood for analysis of standard tumour marker CA125 was collected at the same time as for sOPN analysis. Additionally, in the group of EOC patients, blood for CA125 measurement was obtained after each cycle of chemotherapy. Samples of ascites from patients with EOC were aspirated immediately after entry to the abdominal cavity, using a 50 ml syringe. In controls, samples of peritoneal fluid were collected during laparoscopy using a standard sampling protocol as previously described.[22] Samples of local fluids were transferred into a tube, which was kept on ice until centrifugation at 1000 x *g* for 10 min at 4 °C within 30 minutes. Sera and supernatants of ascites and peritoneal fluid were stored in aliquots at -80 °C. Samples of serum, ascites and peritoneal fluid for total protein measurement were obtained at the same time, and prepared and stored in the same manner as for sOPN analysis.

Analysis of sOPN and total proteins

Concentrations of sOPN were measured using a FlowCytomix Simplex Kit (eBioscience, Vienna). The kit consisted of fluorescent microspheres with an emission wavelength of 700 nm. Microspheres were coated with specific antibodies raised against each of the analytes. They also contained a biotin-conjugated second antibody and streptavidin-phycoerythrin emitting at 575 nm. Samples were run on a Cell Lab Quanta™ SC-MPL (Beckman Coulter). Samples were acquired by Cell Lab Quanta™ SC-MPL software (Beckman Coulter) and analysed using FlowCytomix™ Pro 3.0 software (eBioscience). Total protein concentration was determined using the Bradford method.

Statistical analysis

The prognostic value of sOPN concentration was examined in terms of OS and PFS, using the 50th percentile (median value) as the optimal cut-off. Surviving patients were censored at the date of last contact. Survival curves were generated using Kaplan-Meier, and the difference between the curves was analyzed by the Breslow test. Receiver operating characteristic (ROC) curve analysis was used to find the cut-off level of sOPN with optimal sensitivity and specificity. Cut-off values were calculated by Youden's index (as a criterion for selecting the optimum cut-off point). The areas under the ROC curve (AUCs) were calculated to evaluate diagnostic accuracy and to compare AUCs between sOPN in serum and in ascites. Pearson's and Spearman's correlation coefficients were used to calculate the direction and strength of the relationship between variables, as required in terms of the normality of variables. Data were compared by independent samples t-test. A p-value of < 0.05 was considered significant. All data are presented as mean ± standard error of mean (SEM). Statistical analysis was performed using software statistical package SPSS, version 19 (IBM Statistics, USA).

Results

The clinical characteristics of the investigated EOC and control patients are summarized in Table 1 and 2, respectively.

TABLE 1. Comparison between ovarian cancer patients' characteristics who underwent primary debulking surgery and those considered candidates for neoadjuvant chemotherapy (= diagnostic laparoscopy as primary event)

Parameters	Data	
	Primary event: Debulking surgery	Primary event: Diagnostic laparoscopy
Number of patients	13	18
Age (years, value ± SEM)	57.61 ± 3.27	62 ± 2.45
Age range (years)	41-76	45-85
Elevated CA125 (U/mL)		
n (%)	13 (100 %)	18 (100 %)
Value (mean ± SEM)	3936 ± 1568	3904 ± 1972
sOPN (ng/mL)		
Serum (mean ± SEM)	70.48 ± 9.95	102 ± 11.53
Ascites (mean ± SEM)	2154 ± 479.7	4515 ± 657.3
Histological type, n (%)		
Serous	10 (77 %)	17 (94 %)
Endometrioid	2 (15 %)	1 (6 %)
Serous + clear cell	1 (8 %)	0 (0 %)
FIGO stage, n (%)		
IIIB	1 (8 %)	0 (0 %)
IIIC	11 (84 %)	11 (61 %)
IV	1 (8 %)	7 (39 %)
Histological grade, n (%)		
G1	0 (0 %)	2 (11 %)
G2	5 (38 %)	7 (39 %)
G3	8 (62 %)	9 (50 %)
Ascites (mL)		
Volume (mean ± SEM)	1779 ± 728.4	3916 ± 614.7
Resection, n (%)		*
R0	5 (38 %)	9 (50 %)
R1	5 (38 %)	1 (6 %)
R2	3 (24 %)	0 (0 %)
Unresectable	0 (0 %)	8 (44 %)

* Results of interval debulking surgery.

CA125 = cancer antigen 125; FIGO = International Federation of Gynecology and Obstetrics;.G = gradus; R0 = no macroscopic residual disease; R1 = < 1 cm residual disease; R2 = > 1 cm residual disease; SEM = standard error of the mean; sOPN = soluble osteopontin

TABLE 2. Characteristics of control patients

Parameters	Data
	Control group
Number of patients	34
Age (years, value ± SEM)	41.97 ± 1.68
Age range (years)	21-69
Elevated CA125 (U/mL)	
n (%)	0
Value (mean ± SEM)	NA
sOPN (ng/mL)	
Serum (mean ± SEM)	28.12 ± 2.10
Peritoneal fluid (mean ± SEM)	132.02 ± 7.85
Benign diagnosis, n (%)	
Benign ovarian cyst	6 (17 %)
Myoma of uterus	21 (62 %)
Pelvic pain, sterilisation	5 (15 %)
Preventive adnexectomy	2 (6 %)
Peritoneal fluid (mL)	
Volume (mean ± SEM)	8.04 ± 1.22

CA125 = cancer antigen 125; SEM = standard error of the mean; sOPN - = soluble osteopontin

Diagnostic value of sOPN

The mean concentrations of sOPN in serum (88.92 ± 8.28 ng/ml) and ascites (3525 ± 475.1 ng/ml) were both significantly higher in EOC patients than in serum of patients in the control group (28.12 ± 2.15 ng/ml) and the peritoneal fluid (132.0 ± 7.85 ng/ml) of patients in the control group (p < 0.001). To identify the diagnostic power of sOPN in serum and ascites, sensitivity and specificity were calculated at various cut-off points of sOPN level. The optimum cut-off value in the diagnosis of EOC was found to be 47.4 ng/ml for serum sOPN and 529.5 ng/ml for ascites sOPN. The sensitivity and specificity of these cut-off levels were lower for serum sOPN (91.2% and 90.3%; Figure 1A) than for ascites sOPN (96.8% and 100%; Figure 1B). The AUC for sOPN in serum (Figure 1A) and in ascites (Figure 1B) were 0.964 (95% CI: 0.926 – 1.00) and 0.998 (95% CI: 0.993 - 1.00), respectively. Preoperative concentrations of sOPN in serum and ascites were not correlated with concentrations of standard tumour marker CA125 in serum (serum: r = -0.117, p = 0.530; ascites: r = 0.083, p = 0.658).

Prognostic value of sOPN

Patients were followed to disease recurrence and death. Survival status was updated in June 2016. The median follow-up was 34 months (range 0.7 - 59.2 months). During this time, 26 patients (83.8%) had developed documented disease progression and 20 (64.5%) had died.

Based on sOPN median serum concentration, patients were divided into 2 groups: group 1 = sOPN ≤ 75.39 ng/ml (n = 16) and group 2 = sOPN

> 75.39 ng/ml (n = 15). The OS curves of sOPN serum groups differed significantly (p = 0.018). The estimate median OS was 40.2 months for patients in group 1 and 14.3 months for patients in group 2 (Figure 2A). In addition, PFS curves of sOPN serum groups differed significantly (p = 0.008). The estimate median PFS was 17.7 months for patients in group 1 and 12.1 months for patients in group 2 (Figure 3A).

Based on sOPN median ascites concentration, patients were divided into 2 groups: group 1 = sOPN ≤ 2729 ng/ml (n = 16) and group 2 = sOPN > 2729 ng/ml (n = 15). The estimated median OS was longer (40.2 months) for patients in group 1 than for patients in group 2 (11.5 months). However, the OS curves of sOPN ascites groups did not differ significantly (p = 0.051) (Figure 2B). PFS curves of sOPN ascites groups were also not significantly different (p = 0.109). The estimated median PFS was 16 months for patients in group 1 and 6.5 months for patients in group 2 (Figure 3B).

Furthermore, we evaluated the prognosis of patients with sOPN levels in ascites and/or serum below the diagnostic cut-off value. Patients with an sOPN concentration in ascites below the cut-off value had no relapse during the follow-up period of 43.3 months. Two out of three patients with an sOPN concentration in serum below the cut-off value also had no relapse during follow-up periods of 43.3 and 56.1 months. The third patient with serum below the cut-off value had progression while receiving the last line of platinum dose. However, this patient had the highest ascites to serum ratio in the study group (205-fold). The mean ascites to serum ratio in EOC patients was 46-fold (range: 3 - 205).

Usefulness of preoperative sOPN level for surgical planning

After evaluation of resectability through imaging methods and diagnostic laparoscopy performed by an experienced oncologic surgeon, 13 patients underwent PDS and 18 patients underwent NACT; 10 of the patients in the latter group also underwent IDS (Table 1).

The mean preoperative serum sOPN level for patients who underwent PDS (70.45 ± 9.95 ng/ml) was significantly lower (p = 0.031) than that of patients who underwent NACT, with or without IDS (102 ± 11.53 ng/ml). The mean preoperative ascites sOPN level for patients who underwent PDS (2154 ± 479.7 ng/ml) was also significantly lower (p = 0.018) than that of patients who underwent NACT, with or without IDS (4515 ± 657.3 ng/ml) (Table 1). However, there was no significant difference (p > 0.05) in mean serum CA125 concentrations between the groups (3936 ± U/ml *vs* 3904 ± U/ml) (Table 1).

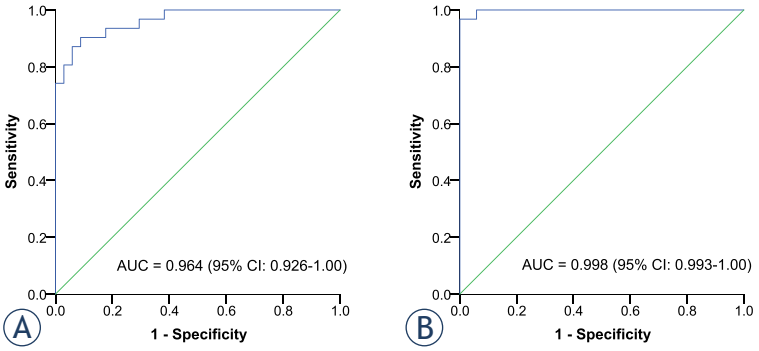

FIGURE 1. Receiver operating characteristic (ROC) curve for the diagnosis of ovarian cancer. The predictive performance of preoperative serum soluble osteopontin (sOPN) concentration **(A)** and ascites sOPN concentration **(B)**.

AUC = area under the curve

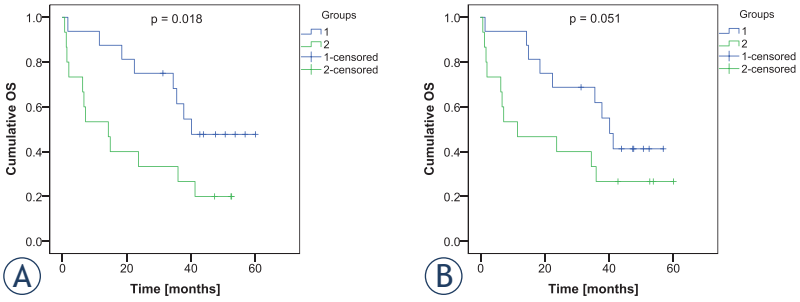

FIGURE 2. Kaplan-Meier survival curves. Overall survival (OS) according to preoperative soluble osteopontin (sOPN) concentrations in serum **(A)** and in ascites **(B)**. Serum sOPN concentrations: group 1 ≤ 75.39 ng/ml (blue line) and group 2 > 75.39 ng/ml (green line). Ascites sOPN concentrations: group 1 ≤ 2729 ng/ml (blue line) and group 2 > 2729 ng/ml (green line).

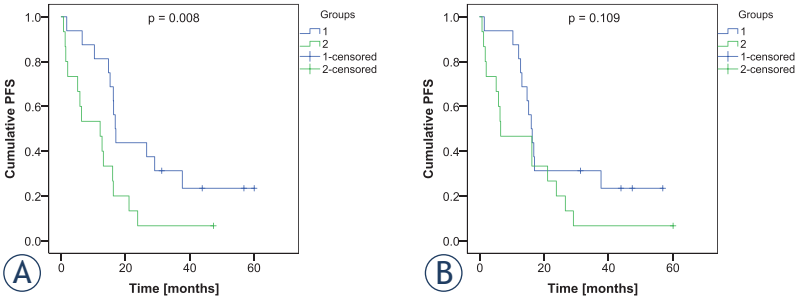

FIGURE 3. Progression-free survival (PFS) according to preoperative soluble osteopontin (sOPN) concentrations in serum **(A)** and in ascites **(B)**. Serum sOPN concentrations: group 1 ≤ 75.39 ng/ml (blue line) and group 2 > 75.39 ng/ml (green line). Ascites sOPN concentrations: group 1 ≤ 2729 ng/ml (blue line) and group 2 > 2729 ng/ml (green line).

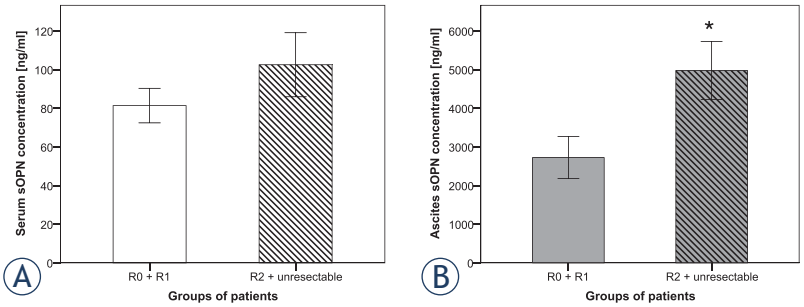

FIGURE 4. Association of surgical outcome and soluble osteopontin (sOPN) concentrations in serum **(A)** and ascites **(B)** at primary operation. Group 1: patients with complete (R0) and optimal (R1) cytoreduction. Group 2: patients with suboptimal (R2) cytoreduction and unresectable disease.

*$p < 0.05$

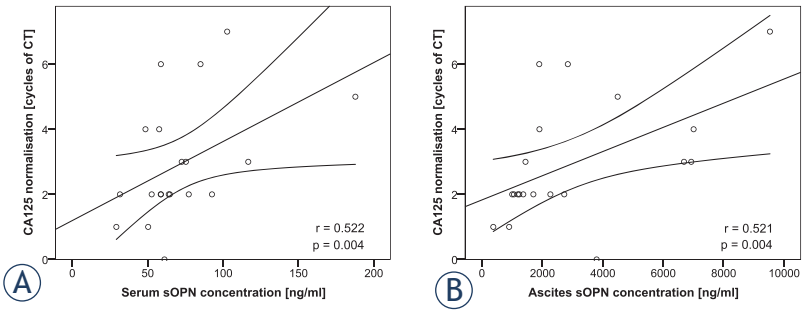

FIGURE 5. Correlation between serum cancer antigen 125 (CA125) normalisation after platinum-based chemotherapy and soluble osteopontin (sOPN) concentrations in preoperative serum **(A)** and ascites **(B)**.

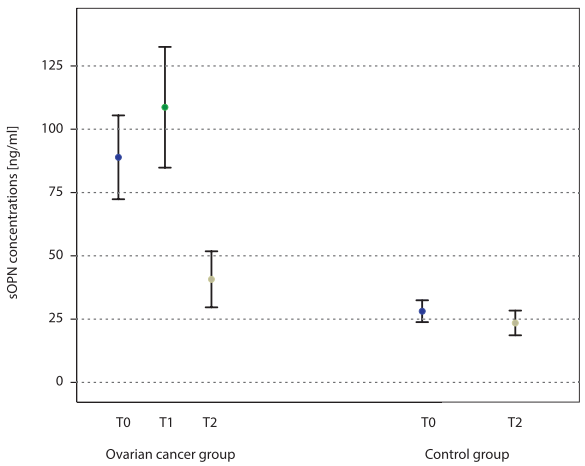

FIGURE 6. Comparison of soluble osteopontin (sOPN) concentrations in serum during treatment. Epithelial ovarian cancer (EOC) group - sOPN concentration: T0-preoperative, T1-after primary (debulking) surgery and T2–3 to 6 months after systemic chemotherapy. Control group (patients with benign gynaecological pathology) – sOPN concentrations: T0-preoperative and T2–3 to 6 months after surgery.

We also examined whether the preoperative sOPN level in serum and ascites can predict cytoreductive surgical outcome. Residual disease was assessed after PDS and after IDS. Complete cytoreduction of all macroscopic disease was achieved in 14 patients, optimal cytoreduction (< 1 cm) in 6 patients and suboptimal (> 1 cm) in 3 patients, while 8 patients had bulky unresectable disease. Due to the small numbers, patients were divided into 2 groups: group 1 = complete cytoreduction and optimal cytoreduction (n = 20) and group 2 = suboptimal cytoreduction or unresectable disease (n = 11). There was no significant difference (p = 0.086) in mean serum sOPN concentrations between the groups (73.91 ± 7.82 ng/ml vs 102.6 ± 16.5 ng/ml) (Figure 4A). In contrast to serum, the ascites sOPN concentration in group 1 (2783 ± 542.3 ng/ml) was significantly lower (p = 0.023) than in group 2 (4980 ± 748.2 ng/ml) (Figure 4B).

Due to the retention tendency of sOPN in ascites, attention should be paid to a high ascites to serum ratio, as already previously mentioned. A high ratio may indicate unresectable disease in spite of a low detected serum sOPN level in such patients. Of two patients with an extremely high ascites ratio (144 and 205), one had unresectable disease and the second had suboptimal cytoreduction after PDS.

Usefulness of preoperative sOPN level for prediction of response to chemotherapy

Twenty-nine (93%) patients received platinum-based chemotherapy and 2 died from EOC before chemotherapy was started. Thirteen patients who had PDS received a median number of 6 cycles (range 5-7) of chemotherapy with carboplatin and paclitaxel. The median number of cycles of chemotherapy in the NACT group was also 6. However, ten patients in this group who had IDS received a median number of 8 cycles (6-10) of chemotherapy with carboplatin and paclitaxel and eight patients who remained inoperable after NACT were treated with a median number of 3 cycles (range 1-6) of therapy with carboplatin/paclitaxel (4 patients), carboplatin monotherapy (1 patient), and carboplatin/pegylated liposomal doxorubicin (1 patient).

Faster CA125 normalisation was significantly associated with lower preoperative sOPN concentrations in serum (r = 0.522, p = 0.004) (Figure 5A) and ascites (r = 0.521, p = 0.004) (Figure 5B). Nine (31%) patients were partial/non-responders. The serum sOPN mean concentration in patients with an inadequate response (119.1 ± 15.24 ng/ml) was

significantly higher (p = 0.005) than in responders (72.35 ± 7.72 ng/ml). In contrast to serum, mean ascites sOPN concentrations in patients with an inadequate response (4440 ± 798.3 ng/ml) did not differ significantly (p = 0.123) when compared to responders (3022 ± 575.8 ng/ml).

On the basis of platinum-free interval, we divided our patients into two groups; group 1 = patients relapsing in less than 6 months (platinum-resistant disease), and group 2 = patients relapsing after more than 6 months or with no relapse during the follow-up period (platinum-sensitive disease). The mean concentration of sOPN in the preoperative serum of 8 patients in group 1 (104.5 ± 8.32 ng/ml) was not significantly different (p = 0.39) from the serum sOPN in group 2 (79.49 ± 8.34 ng/ml). In contrast, patients in group 1 had significantly higher (p = 0.014) ascites sOPN concentrations (54011 ± 836.1 ng/ml) than patients in group 2 (2881 ± 562.9 ng/ml).

Kinetic pattern of sOPN concentrations in serum

In the group of EOC patients, the kinetic patterns of sOPN serum levels were determined one week after PDS (n = 13) and 3-6 month after chemotherapy (n = 22) to ensure an adequate recovery time of patients from the adverse effects of cytotoxic drugs (Figure 6). We found no significant difference (p = 0.786) between mean serum sOPN concentrations in preoperative (88.56 ± 9.03 ng/ml) and postoperative (84.25 ± 12.85 ng/ml) samples. The mean serum sOPN concentration after chemotherapy (40.73 ± 5.52 ng/ml) was significantly lower (p < 0.001) than the mean sOPN concentrations in preoperative and postoperative samples. Concentrations of sOPN in postoperative serum were positively correlated with concentrations in preoperative serum (r = 0.489, p = 0.008) and with ascites concentrations (r = 0.418, p = 0.027). Concentrations of sOPN in serum after chemotherapy were not correlated with any other samples.

At the time of collecting serum after chemotherapy, nine patients had died. In the remaining 22 patients, the sOPN serum concentration decreased in 21 patients (95%) and decreased below the diagnostic cut-off level in 17 patients (77%). In terms of the total number of patients in the study group, this result corresponded to 68% and 55% patients, respectively. The patient who had an increase in sOPN serum concentration after chemotherapy had the worst prognosis in terms of OS among patients who were alive at the time of collecting serum after chemotherapy.

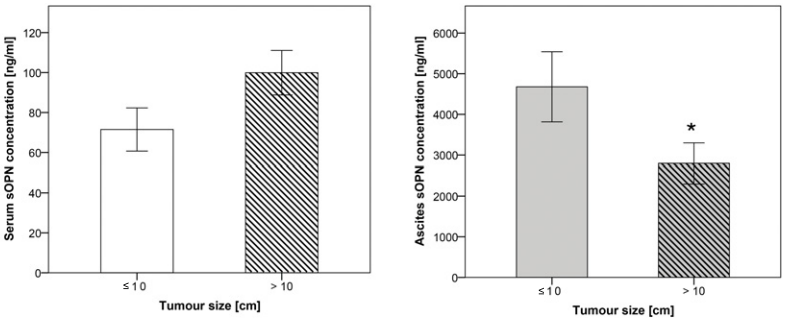

FIGURE 7. Association of tumour size and soluble osteopontin (sOPN) concentrations in preoperative serum **(A)** and ascites **(B)**. Group 1: patients with tumour size ≤ 10 cm. Group 2: patients with tumour size > 10 cm.

*p < 0.05

In the control group, kinetic patterns of sOPN serum levels were determined 6 months after surgery, when the patients were healthy (Figure 6). The mean serum sOPN concentration after treatment was 23.49 ± 2.46 ng/ml.

Biological characteristics of sOPN in different body fluids

We found that concentrations of sOPN in ascites were positively correlated with the volume of ascites (r = 0.431, p = 0.013) and with total proteins in the fluid (r = 0.985, p < 0.001). In contrast to ascites, sOPN concentrations in the peritoneal fluid of the control group were not correlated with the volume of the peritoneal fluid (r = -0.122, p = 0.552) and were negatively correlated with total proteins in the fluid (r = -0.518, p = 0.008). In serum, concentrations of sOPN were negatively correlated with total proteins (r = -0.372, p = 0.033) in EOC patients, whereas no correlation was found in patients of the control group (r = 0.227, p = 0.537).

In ascites, concentrations of sOPN were negatively correlated with tumour size (r = -0.371, p = 0.044), whereas serum sOPN concentrations were not correlated with tumour size (Pearson: r = 0.279, p = 0.135). Based on tumour size, patients were divided into two groups: group 1 = tumour size ≤ 10 cm (n = 12) and group 2 = tumour size > 10 cm (n = 19). There was no significant difference (p > 0.05) in mean serum sOPN concentrations between the groups (71 ± 10 ng/ml vs 97 ± 11 ng/ml) (Figure 7A). In contrast to serum, the ascites sOPN mean concentration in group 1 (4677 ± 862.3 ng/ml)

was significantly higher ($p = 0.029$) than in group 2 (2958 ± 530.1 ng/ml) (Figure 7B).

Discussion

sOPN has been intensively studied as a serum tumour marker in the diagnosis of EOC.[5–8,11] However, information on potential other applications of this promising tumour marker in serum and ascites in women with EOC are still lacking. One important problem with blood tumour markers is that it is questionable whether a sufficient quantity of molecules can reach the peripheral blood (a range of 0.1 to 20% of secreted protein is assumed) to detect change in the local environment of the ovaries.[23] The results of our previous study showed that the retention tendency of sOPN in local fluid represented by ascites is potentiated in malignant conditions and that serum sOPN concentrations were not associated with sOPN concentrations in ascites.[21] We thus systematically compared the clinical usefulness of serum sOPN with ascites sOPN. In addition, we evaluated whether sOPN in ascites can improve the diagnostic accuracy of serum sOPN. To the best of our knowledge, the present study is the first to indicate the usefulness of serum sOPN as a prognostic tumour marker of EOC and is also the first to demonstrate ascites sOPN usefulness in selecting the best treatment for advanced EOC patients (surgical planning and response to platinum-based chemotherapy).

The major new findings in our study were as follows: (1) The diagnostic accuracy of sOPN for detecting advanced-stage EOC was higher for sOPN in ascites than in serum. Ascites sOPN exhibited a lower false negative rate when compared to serum and no false positive rate. (2) Higher preoperative sOPN concentrations in serum were associated with significantly shorter median OS and PFS. A poor prognosis of EOC patients can thus be predicted by a high serum sOPN preoperative level. (3) Higher sOPN concentrations in ascites were associated with a worse surgical outcome and with smaller tumour size. Since high abdominal dissemination with a small primary tumour indicates biological aggressiveness, usually high-grade serous carcinoma, our findings suggest that a higher sOPN level in ascites can predict incomplete resection. (4) A very high ascites to serum sOPN ratio may identify patients who warrant further evaluation for the presence of malignant disease and also identify patients with unresectable disease and the worst prognosis, and this despite a low sOPN concentration in serum of the same patient. (5) Faster CA125 normalisation was positively correlated with lower preoperative sOPN levels in serum and ascites. Relapse in less than 6 months from the last date of platinum dose was associated with higher preoperative levels of sOPN in ascites. sOPN might therefore have predictive value for response to platinum-based chemotherapy in primary and recurrent EOC. (6) A significant positive association between concentrations of sOPN in ascites and ascites volume and total proteins, and no association of sOPN concentrations in peritoneal fluid of control group with peritoneal fluid volume and a negative correlation with total proteins in peritoneal fluid, indicated that an elevated sOPN concentration in ascites was related to the malignant process, especially to the production of ascites.

Our previous report showed that serum sOPN concentrations are not associated with sOPN concentrations in ascites, so we determined separate cut-off values for sOPN in serum and ascites.[21] The cut-off level for serum sOPN of 47.4 ng/ml was in the range of published serum cut-off values (28-60 ng/ml).[11] We found that the sensitivity and specificity of serum sOPN for detecting EOC were 91.2% and 90.3%, respectively. We then tried to elucidate whether the performance of sOPN as a diagnostic marker in ascites is better than in serum. We found that at a cut-off level 529.5 ng/ml, the diagnostic sensitivity and specificity were higher for ascites (96.8% and 100%) than for serum. The AUC under the sROC curve of sOPN was also higher for ascites than for serum. These data indicate that the diagnostic accuracy of sOPN for detection of advanced-stage EOC is higher for sOPN in ascites than in serum. Ascites sOPN exhibited a lower false negative rate and no false positive rate. Furthermore, one patient with serum below the cut-off value, who was diagnosed with FIGO stage IV disease, had the highest ascites to serum ratio (205-fold) in the study group (mean ratio was 46-fold; range: 3 - 205). So a very high ascites to serum sOPN ratio may identify patients who warrant further evaluation for the presence of malignant disease, in spite of a low sOPN concentration in the patient's serum. Higher false negative rates in serum are probably the consequence of the sOPN retention tendency in ascites potentiated in malignant conditions.[21] Moreover, sOPN levels in systemic circulation, may be influenced by noncancerous causes, which must be considered in evaluating the results.[2,3] A higher specificity of sOPN in ascites was expected, since tumour markers closer to the origin of disease are more specific. In

addition, our results on a significant association of sOPN concentration with ascites volume and with ascites total proteins indicated that elevated sOPN levels were disease specific. A greater volume of ascites and higher content of proteins in ascites are general signs of disease progression and/or poor prognosis.[24,25]

OPN mediates critical processes in cancer progression, such as cell adhesion, migration, immune response and apoptosis prevention.[4] It has also been demonstrated that an elevated serum sOPN concentration is associated with advanced FIGO stage, high grade, and the presence of ascites, thus suggesting a prognostic value of this marker.[26,27] During the median follow up of 34 months, 83% developed documented disease progression and 64.5% had died. We found that increased serum sOPN concentration was associated with significantly shorter OS and PFS when patients were grouped using an sOPN median concentration of 75.39 ng/ml. The median OS was 40.2 months for patients with sOPN of 75.39 ng/ml or less and 11.5 months for sOPN greater than 75.39 ng/ml. In addition, the median PFS was 17.7 months for patients with sOPN of 75.39 ng/ml or less and 14.3 months for sOPN greater than 75.39 ng/ml. When patients were grouped using an sOPN median concentration of 2729 ng/ml in ascites as a cut-off, the median OS was 40.2 months for patients with sOPN of 2729 ng/ml or less and 11.5 months for sOPN greater than 2729 ng/ml. In addition, the median PFS was 16 months for patients with an sOPN of 2729 ng/ml or less and 6.5 months for sOPN greater than 2729 ng/ml. A higher ascites sOPN concentration demonstrated border line statistical significance ($p = 0.51$) for shorter median OS, and no association with median PFS. However, if the prognosis of patients was evaluated in relation to sOPN levels in ascites and serum below our diagnostic cut-off value, a different insight into the prognostic usefulness of the two body fluids was obtained. Patients with an sOPN concentration in ascites below the cut-off value had no relapse during a follow-up period of 43.3 months. Two out of three patients with an sOPN concentration in serum below the cut-off value also had no relapse during follow-up periods of 56.1 and 43.3 months. However, a third patient with serum sOPN below the cut-off value had progression while receiving the last line of the platinum dose. This patient had the highest ascites to serum ratio in the study group. So a very high ascites to serum sOPN ratio may identify patients with the worst prognosis, in spite of a low sOPN concentration in the patient's serum.

In the management of advanced-stage EOC, it is essential to identify patients who are more eligible for NACT and IDS, since primary complete resection cannot be achieved.[18,19,28,29] Laparoscopy can be used as an adjuvant procedure to assess tumour spread and resectability. Laparoscopic evaluation, however, has limitations and may, in some cases, underestimate the extent of disease.[30] We investigated the usefulness of sOPN for predicting surgical outcome in order to improve the preoperative treatment strategy. Significantly higher sOPN concentrations in ascites were associated with suboptimal cytoreduction or unresectable disease. Moreover, high ascites sOPN concentrations were associated with smaller tumour size. We can therefore presume that higher sOPN concentrations in ascites demonstrate a greater extent of metastatic disease and knowing this would be useful in preoperative assessment of residual disease and, potentially, in the evaluation of neoadjuvant treatment. It has already been demonstrated that OPN expression in metastasis is significantly increased compared to the primary tumour.[13] The different influences that determine the steady-state levels of sOPN in serum and retention of sOPN in ascites might explain why no association was found between serum sOPN concentrations and surgical outcome. Bandiera *et al.* also investigated the correlation between serum sOPN and surgical outcome, and found that elevated serum sOPN levels were associated with macroscopic residual disease. However, 28% of included patients had early stage disease, when retention of sOPN in ascites is probably less pronounced. Moreover, ascites was not present in 50% of patients.[27]

In EOC patients, carboplatin/paclitaxel remains the preferred combination, with docetaxel substituted for paclitaxel in patients with preexisting neuropathy. Since EOC is a chemosensitive disease, response to therapy is an important prognostic determinant. The results of our study showed that 28% of patients with an inadequate response to primary chemotherapy had a significantly higher concentration of sOPN in serum but not in ascites, when compared to responders. However, in responders, faster CA125 normalisation was associated with lower preoperative sOPN in serum and ascites. Although EOC patients often respond (~80%) to primary therapy[31], the majority of women with advanced EOC will ultimately relapse and develop drug-resistant disease.[32] All patients received platinum-based chemotherapy, so we used platinum-free interval to assess the usefulness of sOPN for predicting response to chemo-

therapy at relapse. The traditional definition of platinum resistance as disease relapsing within 6 months and sensitive disease as recurring beyond 6 months after chemotherapy was adopted by the Gynaecologic Oncology Group (GOG).[33] We found that patients relapsing in less than 6 months had a higher ascites sOPN concentration than patients relapsing after more than 6 months or who had no relapse during the follow-up period. sOPN may contribute to chemoresistance via the antiapoptotic signal, upregulation of P-gp expression, and induction of stem-like properties and thus induces chemoresistance.[34-36] In published studies, higher expression of OPN in lung, colorectal and oral cancer was associated with resistance to platinum-based primary chemotherapy.[36–38] Tumour markers for predicting response is an attractive concept, since it permits individualized treatment, so sOPN is worth further research.

The kinetic pattern of mean serum sOPN concentrations after PDS and chemotherapy showed that sOPN did not change ~ one week after PDS, although the mean sOPN concentration 3 to 6 months after completion of chemotherapy was significantly decreased. The period ~ one week after surgery was probably too short to see the effect of cytoreduction on the sOPN level. In addition, the surgical induced stress response in a patient may also influence protein distribution.[39] This might be why our result was not in agreement with a previously published study.[10] Schorge *et al.* reported significantly decreased sOPN after PDS. However sOPN was measured before the first cycle of chemotherapy.[10] We waited 3 to 6 months after chemotherapy before we measured sOPN, in order to ensure an adequate recovery time of patients from the adverse effects of cytotoxic drugs. At the time of collecting serum after chemotherapy, nine patients had died. In the remaining patients, the sOPN serum concentration decreased in 95% of patients and below the diagnostic cut-off level in 77% of patients. The observed decreased serum sOPN concentration after treatment supports the suggestion that sOPN in serum is correlated with tumour bulk. A similar result was observed in the Schorge *et al.* study, in which an earlier increase of sOPN compared to CA125 in patients developing recurrent disease was also shown.[10]

Conclusions

Our study showed that the local fluid sOPN level, as represented by ascites, mirrors the present disease and is superior to serum sOPN level for diagnostic purposes and surgical planning, although the end result of treatment is a response of the whole body in fighting against disease and, in this respect, the preoperative sOPN concentration in systemic circulation better reflects the outcome of disease than sOPN in ascites. Nevertheless, a very high ascites to serum sOPN ratio may identify patients who warrant further evaluation for the presence of malignant, unresectable disease, and identify patients with the worst prognosis, in spite of a low sOPN concentration in the serum of the same patient.

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