Short Communication

Comparison of Plasma Osteopontin Levels between Patients with Borderline Ovarian Tumours and Serous Ovarian Carcinoma

(osteopontin / OPN / CA125 / epithelial ovarian cancer / borderline ovarian tumour)

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Abstract. Osteopontin (OPN) is a novel biomarker of various cancers including ovarian carcinoma. OPN is a promising adjunct to a major biomarker of ovarian cancer, CA125, in diagnosis, differential diagnosis and prognosis. The aim of our study was to measure the plasma level of OPN and CA125 in patients with borderline ovarian tumours (BOTs), serous ovarian carcinoma, and controls to determine its potential role in the differential diagnosis between serous ovarian carcinoma and BOT. The plasma samples of 66 women were analysed using Luminex technology, designed to simultaneously measure multiple specific protein targets. The mean OPN plasma level for the control group was 23.3 ng/ml; for BOT 26.3 ng/ml; and for patients with serous ovarian carcinoma 59.5 ng/ml. Specifically, there was a significant difference between the OPN levels in patients with ovarian carcinoma and BOT (P < 0.001) as well as controls (P < 0.001). There was no difference between the mean levels of OPN in patients with BOT and the control group (P = 0.286). Using the receiver operating characteristic (ROC), we determined the utility of OPN and CA125 to differentiate between BOT and serous ovarian carcinoma. The area under the ROC curve (AUC) for OPN was 0.793 (95% confidence interval (CI) 0.669–0.917, P < 0.001) and for CA125 0.766 (95% CI 0.626–0.907, P = 0.002). Based on our data, we suggest that OPN can be used as a possible differential diagnostic biomarker to distinguish between malignant serous ovarian carcinoma and BOT.

Introduction

Epithelial ovarian cancers (EOCs) are leading causes of death among gynaecological malignancies in the Western world. EOCs represent a heterogeneous group of clonal proliferative diseases with common origins in ovarian or tubal surface epithelium or epithelial inclusion cysts. Borderline ovarian tumours (BOTs), also known as tumours of low malignant potential or atypical proliferative tumours, represent an independent disease entity among EOCs (Bagade et al., 2012; Fischerova et al., 2012).

Diagnosis of epithelial ovarian cancers is based on the combination of imaging methods, namely transvaginal sonography, and measurement of the ovarian cancer biomarker CA125 (MUC16) in the plasma/serum. These methods have sufficient sensitivity and specificity to identify pelvic pathologic processes, but in most cases they are not sufficient to distinguish among individual types of pelvic tumours (Rosenthal et al., 2006). For definitive diagnosis the histopathologic examination of tumour tissue following the surgical procedure is essential (Jelovac and Armstrong, 2011; Díaz-Padilla et al., 2012). Approximately 90 % of women with advanced ovarian cancer have an elevated serum CA125 level. Its overall diagnostic sensitivity and specificity for EOCs are approximately 0.80 and 0.75, respectively (Medeiros et al., 2009).

BOTs are typically diagnosed in a younger age group than their invasive counterparts and often at an earlier

Abbreviations: AUC – area under the curve, BOT – borderline ovarian tumour, CI – confidence interval, EOC – epithelial ovarian cancer, FIGO – Federation of Gynaecologists and Obstetricians, OPN – osteopontin, ROC – receiver operating characteristic, SEM – standard error of mean.

stage, resulting in an excellent prognosis. However, it is agreed that complete surgical resection of the tumour is the best curative method for BOTs. Nevertheless, especially in younger women with BOT, compared to other types of epithelial ovarian cancers, there might be a possibility to reduce the extent of surgery and the postoperative chemotherapy in order to preserve fertility (Zanetta et al., 2001; Trillsch et al., 2010; Uzan et al., 2010). Differential diagnosis between BOTs, which represent 15–20 % of all ovarian malignancies, and other EOCs before surgery is difficult.

Osteopontin (OPN), also known as secreted phosphoprotein 1 (SPP1), is a candidate diagnostic and prognostic biomarker for ovarian cancer and many other cancers (Rittling and Chambers, 2004). OPN levels have been reported to be elevated in cancers of the breast, prostate, lung, colon, pancreas, multiple myeloma, as well as ovarian cancer (Fedarko et al., 2001; Kim et al., 2002; Brakora et al., 2004; Standal et al., 2004; Mor et al., 2005; Nakae et al., 2006). The value of OPN in the diagnosis and prognosis of epithelial ovarian cancer has been intensively studied. Serum OPN levels are generally elevated in ovarian neoplasm patients, indicating that OPN is a potential diagnostic marker for ovarian cancer (Kim et al., 2002; Schorge et al., 2004; Milivojevic et al., 2013). Several studies have suggested that preoperative analysis of the serum/plasma OPN level positively correlate with ovarian neoplasm progression and might be a clinically useful biomarker for ovarian cancer prognosis (Kim et al., 2002; Brakora et al., 2004; Bao et al., 2007; Hu et al., 2015). However, there are only a few reports that focus on the plasma/serum level of OPN in individual histopathologic types of ovarian cancer and benign ovarian tumours (Moszynski et al., 2013). OPN is a secreted extracellular matrix glycoprotein that is involved in various cellular processes, including cell migration and adhesion, wound healing, tumorigenesis, metastasis, angiogenesis, inflammation, immune response, and apoptosis (Liaw et al., 1995; Coppola et al., 2004; Chakraborty et al., 2006). OPN is expressed in a variety of normal and tumour tissues, including bone, breast, prostate, lung, kidney, stomach, ovary, and uterine endometrium (Sodek et al., 2000). It has been suggested that OPN may be involved in tumour invasion and metastasis through integrin-mediated signalling (Song et al., 2008; Kothari et al., 2016).

We analysed the level of OPN in patients with specific types of EOCs: serous epithelial ovarian carcinoma and BOT, to evaluate the utility of the OPN plasma levels for the diagnosis of BOT and/or for differential diagnosis between BOT and serous ovarian carcinoma. Although several studies have focused on the role of OPN in ovarian cancer screening, the utility of OPN for differentiating between malignant ovarian carcinoma (e.g., serous epithelial ovarian cancer) and BOT, a tumour with low malignant potential, has not been evaluated.

Material and Methods

Patient selection

The study included 66 women whose age ranged from 19 to 72 with epithelial serous ovarian carcinoma (N = 30), borderline ovarian tumour (N = 20) and controls (N = 16). Plasma samples were collected at the Department of Obstetrics and Gynaecology of the First Faculty of Medicine and General Teaching Hospital after informed consent from both patients and healthy age-matched and weight-matched women. Patient samples were collected at the time of preliminary diagnosis before surgery and chemotherapy. The diagnosis was confirmed histologically after the surgery and only the samples from patients with confirmed EOC subtype were included in the study. Tumour typing and staging were performed by the Institute of Pathology, First Faculty of Medicine, Charles University and General University Hospital in Prague according to the criteria of the International Federation of Gynecologists and Obstetricians (FIGO) and the International Union against Cancer (IUCC) (Prat, 2014).

Plasma collection

Blood was collected into BD Vacutainer tubes (Becton, Dickinson and Co., Franklin Lakes, NJ) with acid citrate dextrose. The tubes were kept at room temperature and gently agitated. Platelet-free plasma was collected within 2 h by centrifugation of blood at $2700 \times g$ for 15 min at 10 °C followed by the second spin of collected plasma at $2700 \times g$ for 10 min at 10 °C. Collected plasma was then aliquoted into 2 ml screw-cap tubes (Axygen, Thermo Fisher Scientific, Waltham, MA) and stored at –80 °C.

Biomarker assay

OPN and CA125 levels were measured using multiplex Luminex® xMAP® based magnetic bead assay, HCCBP1MAG-58K MILLIPLEX MAP Human Circulating Cancer Biomarker Magnetic Bead Panelx Assay and analysed in the MAGPIX® System (Merck Millipore, Darmstadt Germany) at the Institute of Medical Biochemistry and Laboratory Diagnostics, First Faculty of Medicine, Charles University in Prague and General University Hospital in Prague, Czech Republic.

Statistical evaluation

The Mann-Whitney U-test was performed to determine the statistical significance between the groups tested. Spearman rank correlation was used to determine the relationship between the biomarkers. Receiver operator characteristic (ROC) curves were constructed and the areas under the curve (AUC) with binomial exact 95% confidence intervals (95% CI) were calculated. Statistical significance was set at value of $P < 0.05$.

Table 1. Characteristics of serous carcinoma patients, BOT patients and controls

Patients		Serous ovarian carcinoma (N = 30) mean ± SEM	BOT (N = 20) mean ± SEM	Controls (N = 16) mean ± SEM
Age (years)		56.3 ± 1.6	49.6 ± 3.7	50.6 ± 0.9
BMI (kg/m^2)		27.0 ± 1.0	29.1 ± 1.1	28.0 ± 1.3
Stage (N)	I	2	16	-
	II	0	2	-
	III	25	2	-
	IV	3	0	-

Results and Discussion

Table 1 shows characteristics of serous carcinoma patients, BOT patients and controls included in the study. The median age of the serous carcinoma group was 58 years (range: 40–72 years), of the BOT group 51 years (range: 19–86), and the control group median age was 50 (range: 40–69). The BOT tumours are in general diagnosed in younger women compared to serous ovarian carcinoma. To match patients for age we selected younger patients with serous ovarian cancer. Most of the serous ovarian carcinomas were histopathologically graded as high-grade tumours (N = 28) and staged according to FIGO classification as stage III malignant tumours (N = 25). Most of the BOT tumours were stage I tumours (N = 16). Histopatologically, all tested BOT tumours except for two were described as serous (N = 18). Two of the BOT tumours were classified as mucinous tumours. No significant differences in age and BMI were detected among the tested groups.

The blood levels of biomarkers OPN and CA125 were determined in the platelet-free plasma of patients with serous ovarian carcinoma, BOT, and controls (Fig. 1). As expected, we found that both markers, OPN and CA125, were significantly higher in serous ovarian carcinoma patients compared to controls (P < 0.001 for both biomarkers). In patients with serous ovarian carcinoma the mean ± SEM CA125 plasma level was 481.7 ± 160.7 U/ml and the mean ± SEM OPN plasma level was 59.5 ± 8.9 ng/ml. We detected significant correlation between the plasma levels of CA125 and OPN in patients with serous ovarian carcinoma and OPN (Spearman correlation coefficient ρ = 0.628, P = 0.002). In patients with BOT, however, the mean level of OPN was not significantly different from that of controls, 26.3 ± 2.7 ng/ml and 23.3 ± 1.9 ng/ml (P = 0.286; mean ± SEM), respectively.

The plasma levels of OPN in serous and mucinous BOT were similar. Previous studies have shown high levels of OPN in malignant ovarian cancers, irrespective of histological type of the cancer (Kim et al., 2002). Compared to malignant ovarian tumours, in benign ovarian tumours, such as serous and mucinous cystadenoma, endometrionic cysts, adult teratoma, and functional cysts, the OPN level in the plasma is low (Moszynski et al., 2013). The finding of nearly physiological levels of plasma OPN in patients with BOT and in benign ovarian

tumours is in agreement with experimental studies showing that OPN is rather a marker of tumour invasiveness and metastatic potential than a general tumour marker (El-Tanani et al., 2006; Wai and Kuo, 2008). The plasma levels of CA125 were significantly higher in patients with BOT compared to controls, 110.7 ± 50.6 U/ml and 6.5 ± 2.3 U/ml, respectively (P = 0.008). Using ROC we determined the utility of OPN and CA125 to differentiate between BOT and serous ovarian carcinoma (Fig. 2). The AUC for OPN was 0.793 (95% CI 0.669–0.917, P < 0.001) and for CA-125, AUC = 0.766
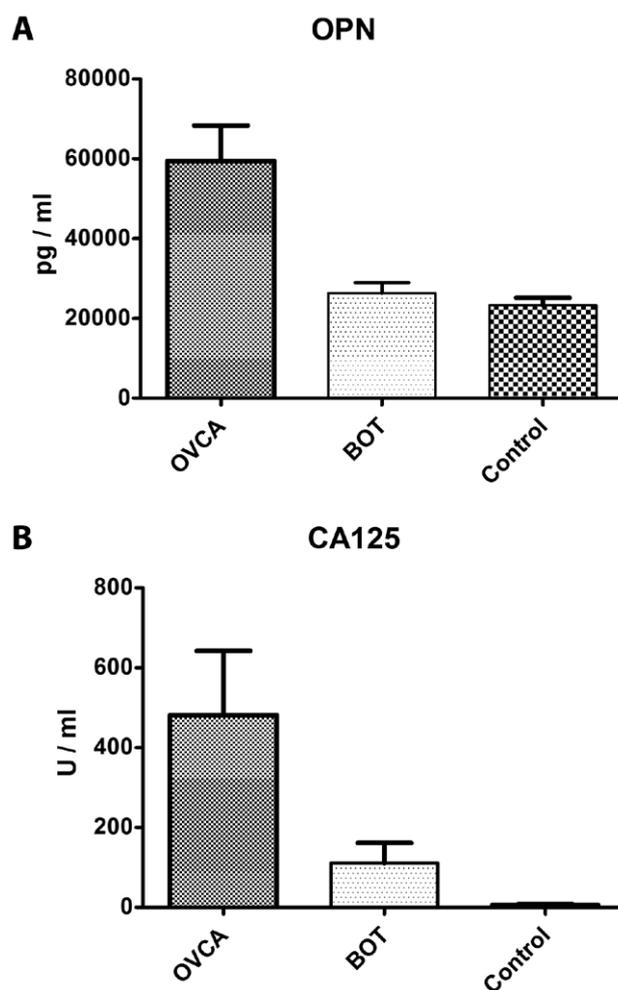

Fig. 1. Mean concentration with SEM of plasma OPN (**A**) and CA125 (**B**) in patients with serous ovarian carcinoma (OVCA), BOT, and controls
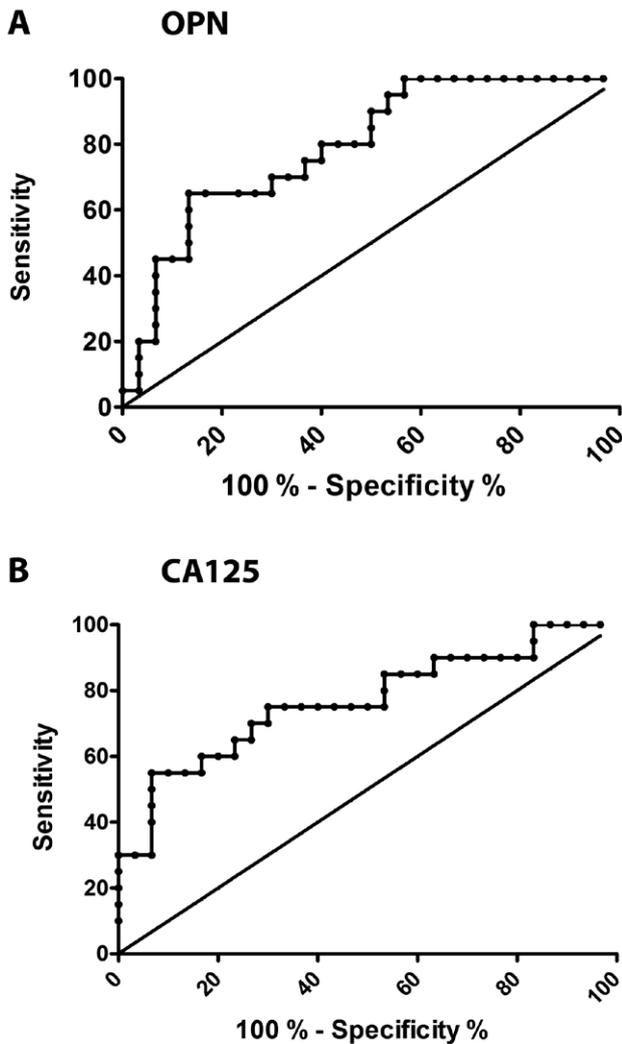
Fig. 2. ROC to determine the utility of OPN and CA125 to differentiate between BOT and serous ovarian carcinoma. AUC for OPN (**A**) and CA125 (**B**) plasma concentrations discriminate patients with BOT and serous ovarian carcinoma.

Fig. 3. ROC to determine the utility of OPN and CA125 to differentiate between BOT and controls. AUC for OPN (**A**) cannot discriminate patients with BOT and healthy controls. AUC for CA125 (**B**) can discriminate patients with BOT from healthy controls.

(95% CI 0.626–0.907, P = 0.002), indicating that both markers are able to discriminate between serous ovarian carcinoma and BOT. Further, ROC analysis revealed the ability of CA125 to discriminate between controls and BOT (Fig. 3); the AUC for CA125 was 0.761 (95% CI 0.606–0.915, P = 0.008). Contrary to CA125, OPN did not display sufficient ability to discriminate between controls and BOT; the AUC for OPN was 0.606 (95% CI 0.416–0.796, P = 0.279). At present, there is no reliable preoperative diagnostic method (imaging or laboratory), except for the presence of ascites, to differentiate between BOT and primary invasive tumours or benign lesions. Due to the limitations of diagnostic imaging methods and the lack of useful biomarkers, BOTs are often preoperatively misdiagnosed; correct preoperative diagnosis of BOT is reported in 29–69 % of cases (Sokalska et al., 2009). Our data suggest that OPN, when used as a biomarker of ovarian cancer in a multi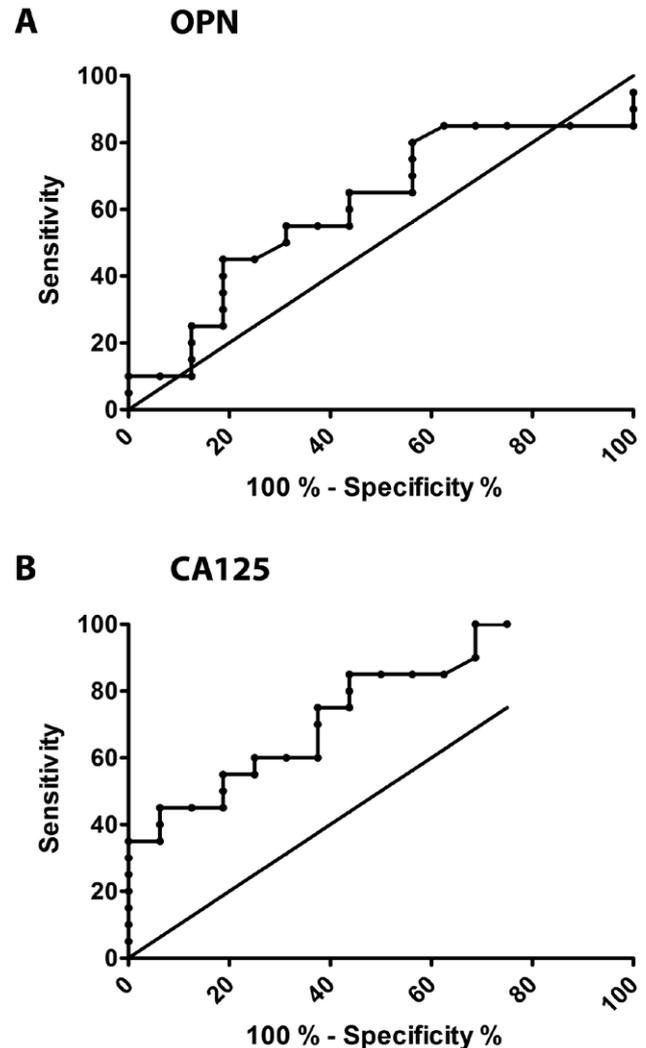plex marker panel, would be useful in differential diagnosis between tumours with high malignant potential (e.g., serous ovarian carcinoma) and borderline ovarian tumour.

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