Osteopontin as an Adjunct to CA125 in Detecting Recurrent Ovarian Cancer

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ABSTRACT

Purpose: **CA125 is currently the only tumor marker to have a validated role in the postoperative monitoring of ovarian cancer. Osteopontin (OPN) is a putative plasma biomarker that was recently identified using high-throughput cDNA microarray technology. The purpose of this study was to test the hypothesis that OPN is a clinically useful adjunct to CA125 in detecting recurrent ovarian cancer.**

Experimental Design: **Thirty-eight ovarian cancer patients had a single pretreatment blood sample and 200 postoperative specimens were prospectively collected during chemotherapy and follow-up. OPN measurements were performed using an enzyme-linked immunoassay, and CA125 levels were concurrently obtained. Wilcoxon's signed rank-sum test was used to perform paired comparisons between pretreatment and postoperative OPN and CA125 measurements. Longitudinal mixed effects polynomial models were used to determine whether OPN and CA125 levels correlated with the development of recurrent ovarian cancer.**

Results: **The median pretreatment OPN level was 178 ng/ml (range, 12–3468) and the median CA125 measurement was 812 units/ml (range, 12–81,500). There was a trend for OPN levels to decline after treatment was initiated ($P = 0.07$), but decreasing CA125 measurements were more consistently observed ($P = 0.0009$). The quadratic functional trends of OPN and CA125 were each highly significant ($P < 0.0001$). Although inferior to CA125 in predicting clinical response to therapy, OPN rose earlier in 90% (95% confidence interval, 56–100%) of the patients developing recurrent disease (median lead time, 3 months).**

Conclusions: **OPN may be a clinically useful adjunct to CA125 in detecting recurrent ovarian cancer.**

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INTRODUCTION

Ovarian cancer accounts for 14,300 deaths in the United States each year, more than all other gynecological malignancies combined (1). Three quarters of patients present with International Federation of Gynecology and Obstetrics stage III-IV disease, chiefly because there is no reliable screening technique for early detection (2, 3). CA125 is currently the only tumor marker to have a well-defined and validated role in the monitoring of ovarian cancer (4). Decreasing levels are associated with response to therapy and increasing levels with tumor progression (5, 6). Despite this correlation, most patients with a normal CA125 (<35 units/ml) and no clinical evidence of disease at the completion of primary chemotherapy still have microscopic residual cancer (7). Another clinically useful biomarker would greatly enhance the ability to make treatment decisions during treatment and surveillance.

High-throughput cDNA microarray techniques offer great potential for identifying specific biomarkers of several types of cancer (8, 9). Numerous candidate tumor markers of ovarian cancer have been reported (10, 11). Recently, osteopontin (OPN) was shown to exhibit 184-fold overexpression by comparing pooled ovarian cancer cell lines to healthy human ovarian surface epithelium (12). OPN is a secreted glycoprotein that is detectable in human body fluids. Plasma levels of this protein have been shown to correlate with disease status in several types of carcinoma (13, 14).

Mean OPN levels are higher ($P < 0.001$) in epithelial ovarian cancer patients compared with healthy controls (15). However, this putative biomarker has not been directly compared with CA125 in monitoring patients postoperatively during treatment and follow-up. The purpose of this study was to test the hypothesis that OPN is a clinically useful adjunct to CA125 in detecting recurrent ovarian cancer.

PATIENTS AND METHODS

Biosamples. Institutional review board approval was obtained to collect plasma samples from women undergoing primary treatment for ovarian cancer at the University of Texas

Southwestern Medical Center. Thirty-eight epithelial ovarian cancer patients diagnosed between December 2000 and December 2002 had pretreatment specimens obtained and 200 additional samples prospectively collected before each course of chemotherapy and subsequent follow-up visits. All plasma samples were centrifuged at 2000 × *g* at 4°C for 15 min. The separated plasma was removed, aliquoted, and frozen at −80°C for future analysis.

Enzyme-Linked Immunosorbent Assay. Levels of OPN were measured in plasma samples with a solid-phase sandwich ELISA using a commercially available kit (Code 17158, Immuno-Biological Laboratories) as described previously (15). Briefly, microplates were first precoated with antihuman OPN rabbit IgG [100 μl of 20 μg/ml in 0.1 M carbonate buffer (pH, 9.5)] and blocked with 1% BSA and 0.05% Tween 20. Plasma and standard OPN samples were diluted with 1% BSA and 0.05% Tween 20 in PBS and incubated for 1 h at 37°C. After seven washes with 0.05% Tween 20 in phosphate buffer, horseradish peroxidase-labeled conjugated antihuman OPN (10A16) mouse monoclonal antibody (100 μl of 2 ng/ml) was added and incubated for 30 min at 4°C. After nine washes, 100 μl of tetramethyl benzidine buffer was added, and the signal was allowed to develop for 30 min at room temperature. The reaction was stopped with 100 μl of 1 N sulfuric acid. The absorbance at 450 nm was measured by an automatic ELISA reader (Bio-Rad, Hercules, CA).

Results were converted from the mean absorbance of duplicate wells after subtraction of background values. Recombinant human OPN protein (IBL) was used as a standard. The standard curve was prepared simultaneously with the measurement of test samples. Reagent blank, test-sample blank, and internal controls of plasma samples were used to normalize OPN values obtained from each experiment.

Clinical Data Collection. Clinical data were prospectively recorded, including patient age, race, Federation of Gynecology and Obstetrics stage, type of adenocarcinoma, histological grade, primary therapy, residual tumor after surgical cytoreduction, type of chemotherapy, CA125 level at each visit, and response to therapy and clinical outcome. All patients were followed to disease recurrence, death, or April 2003. Surviving patients had a median follow-up of 11.5 months (range, 2–27).

Complete response was defined by a normal physical examination, a normal computed tomographic scan of the abdomen and pelvis, and a normal serum CA125 level. The applied cutoff value for CA125 was 35 units/ml as recommended by Bast *et al.* (5). A partial response was defined by a decrease of at least 50% in the sum of the largest dimensions of tumors as measured by computed tomographic scanning. A smaller decrease or any increase in tumor size during primary chemotherapy was defined as a nonresponder. The duration of overall survival was the interval between initiation of treatment (surgery or chemotherapy) and death.

Statistical Analysis. Wilcoxon's signed rank-sum test was used to perform paired comparisons between pretreatment and postoperative OPN and CA125 measurements. Longitudinal mixed effects polynomial models (quadratic functions of months after initial surgery) were used to describe the longitudinal behavior of OPN and CA125 over time. Each model allowed random intercept and random coefficients of all polynomial terms and was fit to the following two subgroups: complete clinical responders and partial/nonresponders. The Federation of Gynecology and Obstetrics stage of disease was included as the adjusting covariate. χ^2 or the Fisher's exact test was used for dichotomous variable analysis. The level of critical significance was assigned at $P < 0.05$. All analyses were performed using SAS version 8.2 (SAS Institute, Cary, NC), SPSS version 9.0 (SPSS Inc., Chicago, IL), and S-PLUS (Insightful Inc., Seattle, WA).

Table 1 Clinical characteristics of 38 ovarian cancer patients monitored during treatment and follow-up

Clinical characteristic	No. of patients
Race	
White	21
Black	8
Hispanic	6
Asian	3
Stage	
I	2
II	1
III	25
IV	10
Type of adenocarcinoma	
Papillary serous	22
Endometrioid	4
Clear cell	4
Transitional cell	1
Mucinous	1
Mixed	6
Histologic grade	
1	0
2	6
3	32
Postsurgical residual disease	
<0.5 cm	17
0.5–1.0 cm	7
1.0–2.0 cm	8
>2.0 cm	3
No primary surgery	3
Primary chemotherapy	
Platinum-based	34
None	4
Clinical response	
Complete	26
Partial	11
None	1
Disease status	
Alive/no evidence of cancer	19
Alive with disease	11
Dead of disease	8

RESULTS

The mean patient age was 55 years. Other clinical characteristics are shown in Table 1. The median pretreatment OPN level was 178 ng/ml (range, 12–3468) and the median CA125 measurement was 812 units/ml (range, 12–81,500).

There was a trend for OPN levels to decline after treatment was initiated ($P = 0.07$), but decreasing CA125 measurements were more consistently observed ($P = 0.0009$). Among the 35 patients who underwent primary cytoreductive surgery, OPN levels decreased postoperatively in 27 (77%), compared with 31 (89%) CA125 measurements ($P = 0.20$). Two of the four

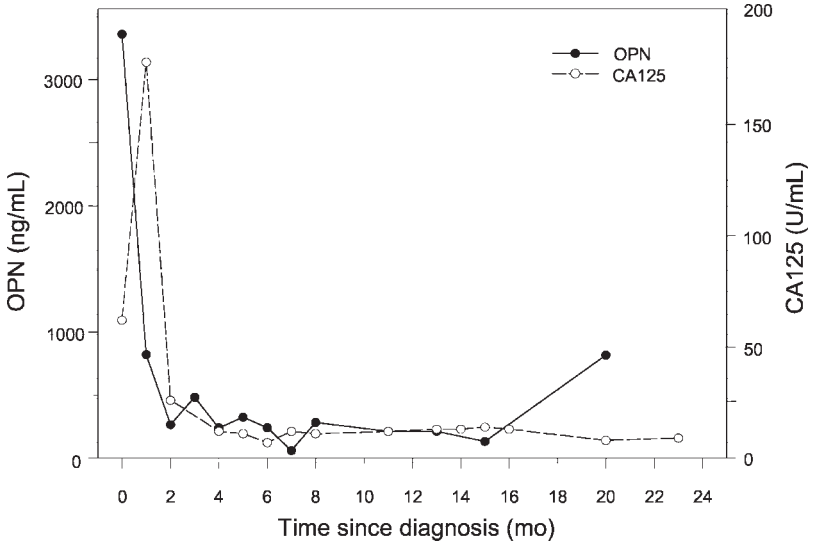

Fig. 1 Postoperative decrease in osteopontin (OPN) but a transient postoperative rise in CA125. Clinically, there was a complete response to surgical cytoreduction (1–2 cm residual disease) and primary chemotherapy. OPN level was elevated at 20 months, but there was still no evidence of disease 23 months after surgery.

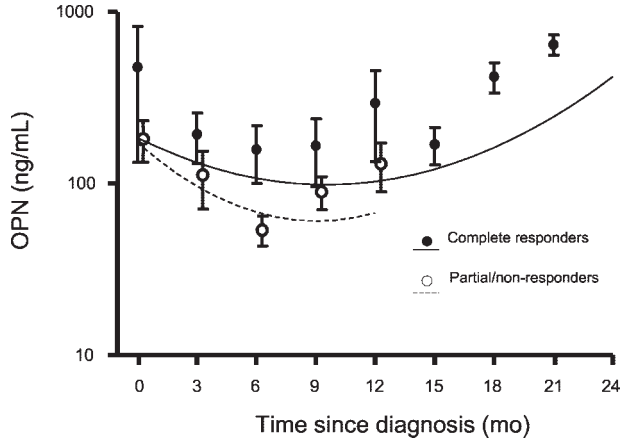

Fig. 2 Osteopontin (OPN) levels (median and interquartile range) of complete clinical responders and partial/nonresponders at every 3 months of follow-up since diagnosis as predicted by the longitudinal mixed effects models. Partial/nonresponders had shorter survival and follow-up.

women exhibiting a paradoxical postoperative increase in CA125 had declining OPN levels (Fig. 1). Overall, 33 (94%) patients had a postoperative decrease in either OPN or CA125 after tumor debulking. The combination was more accurate than using OPN alone ($P = 0.04$) but not statistically improved over CA125 ($P = 0.39$).

Median levels of OPN fell during the first 6–9 months after surgery, and both complete clinical responders and partial/nonresponders rose thereafter (Fig. 2). The overall quadratic functional trend of OPN was significant ($P < 0.0001$), but the difference between the two subgroups was of borderline significance ($P = 0.03$ for the coefficient of the linear term and $P = 0.06$ for the quadratic term). The longitudinal behavior of OPN was inferior to CA125 in predicting complete clinical responders *versus* partial/nonresponders.

Median levels of CA125 also fell postoperatively for 6–9 months in both groups (Fig. 3). The CA125 in those patients with a complete clinical response stayed low until 24 months whereas those of the partial/nonresponders rose back again by 12 months. The overall fit of the quadratic model was significant ($P < 0.0001$), and the trend of the patients with complete clinical response differed significantly from that of the partial/nonresponders ($P < 0.0001$ for both the differences in coefficients of the linear and quadratic terms).

Overall, rising or falling OPN levels correlated with progression or regression of disease in 27 (71%) of 38 patients *versus* 34 (90%) CA125 measurements simultaneously performed ($P = 0.04$; Fig. 4). Either OPN or CA125 correlated with disease status in 36 (95%) women. The combination was more accurate than OPN alone ($P = 0.006$), but not superior to CA125 ($P = 0.39$). OPN levels rose earlier than CA125 in 90%

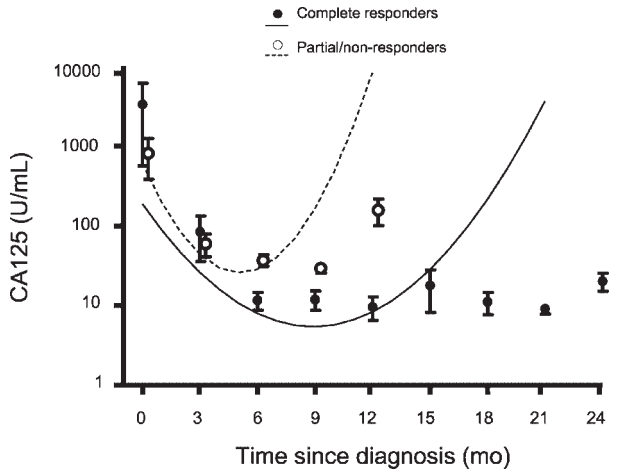

Fig. 3 CA125 levels (median and interquartile range) of complete clinical responders and partial/nonresponders at every 3 months of follow-up since diagnosis as predicted by the longitudinal mixed effects models. Partial/nonresponders had shorter survival and follow-up.

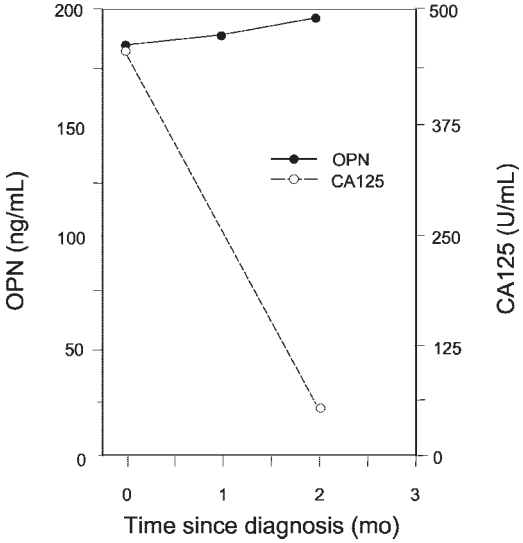

Fig. 4 Postoperative OPN level increase and CA125 decrease in patient with gross residual disease >2 cm at the completion of primary surgery. One course of platinum-based chemotherapy was administered, but progressive disease resulted in patient death within 2 months.

(95% confidence interval, 56–100%) of the patients developing progressive or recurrent epithelial ovarian cancer during the study period (median lead time, 3 months; range, 1–7; Fig. 5).

DISCUSSION

OPN may be a clinically useful adjunct to CA125 in detecting recurrent ovarian cancer. After primary surgery and platinum-based chemotherapy, most patients with advanced disease achieve a complete clinical remission (no evidence of disease on physical examination or radiographic studies and normalization of CA125). However, 75–80% of these women will eventually relapse and neither consolidation therapy nor maintenance therapy has been shown to extend survival. CA125 is currently used exclusively to monitor patients during therapy and surveillance. Another clinically useful biomarker might improve postoperative decision-making in several ways. First, the potential for protracted paclitaxel therapy to have a considerable impact on progression of the disease process has recently been demonstrated (16). CA125 alone is not reliable for identifying patients at risk for early relapse who would be most likely to benefit. Second, earlier detection of platinum-resistant patients (recurring <6 months after the completion of primary therapy) might hasten a therapeutic change to a research protocol or a less toxic palliative agent. Finally, earlier detection of platinum-sensitive disease (recurring >6 months after primary chemotherapy) should increase the number of patients benefiting from secondary cytoreductive surgery (17, 18). The efficacy of measuring OPN during relapse therapy as an aid to decision-making about continuation of treatment was not evaluated in this study. Additional investigation is warranted to determine the potential clinical utility of adding OPN to routine CA125 measurement during treatment and surveillance of ovarian cancer.

Postoperative OPN levels decreased in 77% of patients undergoing primary cytoreductive surgery. Significantly reduced circulating plasma levels would be expected after tumor debulking if ovarian cancer cells actively secrete OPN. Microdissected ovarian tumors and cell lines have been shown to exhibit OPN mRNA overexpression. In addition, OPN protein expression is increased in ovarian carcinoma (15). Correlation of OPN levels and surgical outcome in the majority of patients suggests the potential clinical utility of this marker during primary treatment. Serum human kallikrein 6 (68%) and prostasin (88%) concentrations have also been reported to drop after ovarian cancer debulking, but not β1,4-galactosyltransferase (10, 19, 20). Interestingly, one of the patients in this study with a postoperative OPN increase (Fig. 4) had significant postsurgical residual disease and expired within 8 weeks despite a declining CA125. Although no putative marker has yet been shown to be clinically superior to CA125, OPN may be a biologically plausible adjunct to evaluate surgical response.

OPN and CA125 each declined for 6–9 months after ovarian cancer diagnosis, but OPN measurements were inferior to CA125 in predicting complete clinical responders. Although the combination of markers was similar to CA125 alone, our sample size may not have been sufficient to identify a clinically meaningful advantage by adding OPN. Serum levels of tissue polypeptide-specific antigen and β1,4-galactosyltransferase have also been combined with CA125 to determine whether recurrent ovarian cancer can be detected earlier (20, 21). The utility of adding OPN should be prospectively evaluated in a larger prospective study (22).

OPN measurements began rising earlier than CA125 with a median lead time of 3 months in this study. Elevations of CA125 alone have been shown to predate clinical relapse by a median of 4 months in approximately 70% of patients (23, 24). There is no evidence that reinstituting treatment for a rising CA125 without other evidence of recurrence will improve survival, but

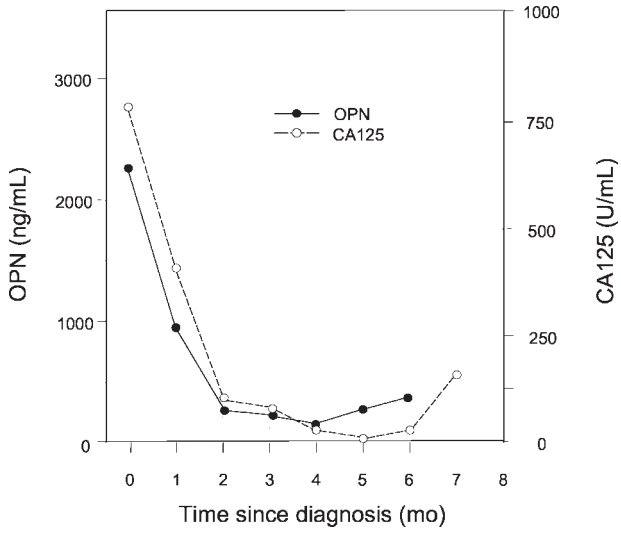

Fig. 5 Postoperative marker decrease of both osteopontin (OPN) and CA125. The OPN level began to rise 5 months after surgical cytoreduction (1–2 cm residual disease). CA125 did not increase until 6 months, when recurrent disease was confirmed.

surgical exploration may identify a group of patients with subclinical macroscopic disease that benefit from cytoreduction at the time of lowest tumor burden (17). A prospective randomized trial is currently underway to determine whether early institution of second-line treatment for a rising CA125 produces a survival benefit as well as an enhancement in quality of life, compared with treatment at the time of clinical or symptomatic recurrence.

OPN in blood may derive from several tissue sources and many different tumor cell types (25). On the basis of transcriptional profiling, OPN has been proposed as the leading candidate clinical marker of colon cancer (26, 27). Elevated OPN concentrations are also associated with increased tumor burden and decreased survival in breast and prostate cancer (13, 28). Different histological subtypes of ovarian carcinoma exhibit different patterns, as follows: serous tumors appear to actively secrete OPN to psammoma bodies whereas mucinous variants exhibit high levels of OPN in the cytoplasm (15, 29). The apparent clinical utility of OPN in several human cancers supports our observation that this marker has potential applications during the treatment and follow-up of patients with ovarian cancer. OPN undergoes extensive posttranslational modification, and at least three transcriptional variants of OPN have been described (30). Additional studies may define isoforms of OPN that are more unique in ovarian cancer.

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