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Plasma Osteopontin Velocity Differentiates Lung Cancers from Controls in a CT Screening Population

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Abstract

INTRODUCTION—As CT screening is integrated into non-small cell lung cancer (NSCLC) care, additional parameters are needed to help distinguish cancers from benign nodules. Osteopontin (OPN), a secreted phosphoprotein, has elevated plasma levels in NSCLC. We hypothesize that changes in plasma OPN over time (i.e., OPN velocity [OPNV]) can differentiate NSCLC patients from those without cancer in a CT screening population.

METHODS—A nested case-control study was conducted within a NSCLC CT screening trial. Incident cancers with serial plasma were matched to controls. OPN was measured by ELISA. Demographic, OPN, and OPNV were compared between cancers and controls using Wilcoxon Signed Rank test.

RESULTS—Ten incident cancers were identified. The pack years distributions were similar, but cancers were older (68.0 vs. 63.3, $p=0.002$) and their surveillance intervals were shorter (25.5 vs. 27.0 months, $p=0.03$) than matched controls. Baseline OPN was similar (median difference: -5.15 ng/ml, $p=0.50$), but OPNV in the cancers was significantly greater than that of matched controls, (median difference: 1.06 ng/ml/month, $p = 0.01$). Accuracy rate for prediction of disease status by OPNV (adjusted for age and surveillance) was 83%.

CONCLUSIONS—These are early evidence for utility of monitoring plasma OPN during CT screening to assist in identification of NSCLCs.

Keywords

non-small cell lung cancer; osteopontin; biomarker; early detection; CT screening

INTRODUCTION

Lung cancer is the world's leading cause of cancer death, with overall survival rates of <15%[1]. As with most cancers, detection of non-small cell lung cancer (NSCLC) at an early stage, when complete resection is possible, improves patient outcome compared to those found with locally advanced or metastatic disease. Unfortunately, although a strong correlation exists between tobacco use and the development of NSCLC, and high risk populations have been defined on the basis of tobacco consumption and COPD, most patients have locally advanced or metastatic disease at the time of diagnosis. Despite promising data from the National Lung Screening Trial (NSLT)[2], linking the use of low-dose computerized tomography (CT) screening with reductions in lung cancer mortality, implementation of wide scale screening for lung cancer awaits endorsement from third party payers. There is currently no widely accepted screening modality for the early detection of NSCLC. Nevertheless, CT provides the most promise as a screening tool for early detection of NSCLC [2-4]. The most influential study to date, the NSLT, reported a 20% decrease in NSCLC deaths in those patients who underwent yearly CT screening, compared to annual chest radiograph [2]. However, a common methodological criticism has been the difficulty in distinguishing between cancers and benign nodules using CT scan alone [5-7]. As CT screening initiatives are integrated into preventative care algorithms for individuals at high risk for the development of NSCLC, additional variables must be included in these algorithms that can help differentiate cancers from benign etiologies leading to more accurate clinical decisions.

Although in their infancy as an evidence-based approach, biomarkers for solid tumors are being increasingly used to aid in diagnosis, define response to therapy, and often to predict recurrence. The most clinically relevant of these include prostate specific antigen (PSA) in prostate cancer, carcinoembryonic antigen (CEA) in colon cancer and cancer antigen 125 (CA-125) in ovarian cancer. Likewise, a companion diagnostic NSCLC biomarker could ideally be used with CT scanning to increase diagnostic precision. This would translate into fewer unnecessary biopsies, reducing individual and system-wide costs associated with CT screening for lung cancer.

Osteopontin (OPN) is a ubiquitous phosphoprotein originally described as a secreted factor in malignant epithelial cells[8]. It is identified in a remarkable range of normal and pathologic contexts[9]. OPN is a member of the small integrin-binding ligand N-linked glycoprotein (SIBLING) family and engages a variety of membrane-associated glycoprotein, most notably the integrins and CD44, activating a variety of downstream processes associated with tumor progression or cellular transformation. Laboratory models have shown OPN to promote tumorigenesis and metastasis, related primarily to increased cell migration, invasion, and adhesion [10]. Along with its important role in malignancy, OPN is also an important bone matrix protein and mediator of immune cell recruitment, wound healing, and tissue remodeling [11, 12]. As a well-characterized extracellular protein and central regulator of the malignant phenotype in NSCLC, OPN is an attractive candidate biomarker for NSCLC.

Our laboratory previously demonstrated that plasma OPN levels are elevated in patients with early stage NSCLC when compared to smokers without cancer [13]. Moreover, elevated plasma OPN levels are associated with poor survival and an increased risk for recurrence following surgery in patients with stage I NSCLC [14], as well as decreased survival and response to therapy in stage IV disease [15]. Elevated OPN levels in early stage NSCLC are reduced following surgical resection [13], indicating that the biomarker is responsive to therapy and decreases in tumor burden.

Although OPN appears to play a critical role in NSCLC carcinogenesis [16], elevations in circulating OPN are not unique to NSCLC as they can be seen in relation to other pulmonary processes [9]. Therefore, while OPN may lack specificity as a stand-alone diagnostic biomarker for NSCLC, the addition of OPN surveillance to CT screening may improve the specificity and thus the clinical utility of the modality in discerning individuals with early-stage NSCLC from high risk individuals without cancer. We hypothesize that in the context of a longitudinal CT screening protocol, absolute elevations in plasma OPN and/or changes in plasma OPN concentration as a function of time (OPN velocity [OPNV]) can differentiate NSCLC patients from high-risk patients without cancer. The following pilot case-control trial demonstrates increased OPNV in NSCLC patients compared to high-risk smokers in a CT screening population, and provides evidence for the utility of monitoring serial plasma OPN levels in high-risk populations.

PATIENTS AND METHODS

Study Population

The NYU Lung Cancer Biomarker Center is a large scale screening trial funded by the Early Detection Research network (EDRN), a program of the National Cancer Institute (NCI) for the purpose of identifying and validating biomarkers for early detection of NSCLC. The center screens patients considered at high-risk for NSCLC. Current or former smokers over 50 years of age with at least a 20 pack year smoking history and without previous cancer are eligible. 1182 subjects have enrolled since the study began in 2001[17]. All screening visits included within this series occurred between April 2005 and September 2009. At each visit patients undergo a chest CT as well as serum, plasma and sputum collection, pulmonary function testing and complete a pulmonary symptom questionnaire. To date, 3771 CT scans have been performed with collection of 2174 matched plasma samples. Twenty-six NSCLC have been detected since 2001: 10 prevalence cancers and 16 incident cancers. We performed a nested case-control study to evaluate plasma OPN as a diagnostic biomarker for NSCLC within this screening cohort. Prevalence cancers were excluded due to the lack of serially banked plasma prior to the time of diagnosis. Incident cancers with serially banked plasma and 1-4 matched controls for each cancer were identified. Controls were matched for age, sex, pack years, and the duration of the surveillance interval over which plasma samples were collected. Cancers were verified by pathology following resection. Controls were considered free of cancer due to lack of clinical progression on CT screening for an interval of at least 24 months following evaluated surveillance interval.

Plasma OPN

Plasma OPN levels were measured by ELISA (Arbor Assays, Ann Arbor MI and are reported in ng/ml. All specimens were tested in a blinded fashion and in duplicate (Intra-assay %CV 3.7; Inter-assay %CV 9.2). OPN levels at the initiation and completion of the surveillance interval (pre-resection) and the OPNV (ng/ml/month) are reported. OPNV (the slope or rate of change over time) was estimated using linear regression over the entire surveillance interval, with no restrictions on the number of OPN values or time intervals between OPN measurements (Figure 1). This is the method originally described for PSA velocity (PSAV) by Carter in 1992.[18] The accuracy of linear regression calculation for PSAV has been validated in a multi-institutional analysis that compared PSAV by linear regression to two and three point simple restricted calculations.[19]

Statistical Methods

Demographic characteristics including gender, age, tobacco usage (pack years) and surveillance interval between the cancer cases and their matched controls were compared using non parametric Wilcoxon signed rank tests with 2-sided p values of 0.05 considered to

be statistically significant. The distributions of the key outcome variables, initial plasma OPN and OPNV were also compared between the matched cases and controls using non parametric Wilcoxon signed rank tests and are summarized in boxplots. To predict disease status based on OPNV levels, we used mixed effects logistic regression models that incorporated the matching of cases and controls [20]. Accuracy rates were estimated using leave-pair-out cross-validation (LPOCV) [21] which is useful to determine prediction accuracy, particularly with small numbers of cases/controls [21]. In this study “one pair” was represented by one case and its matched controls. The model used a mixed effect logistic model with OPNV as the independent variable and the cancer status as the outcome variable to train the data. The model was designed and executed by our biostatistical team. To further estimate the sensitivity and specificity of the prediction, receiver operator curves (ROC) [22] were calculated with R software (Version 2.13.1, Package “epicalc”). The OPNV is considered to have some ability to distinguish groups if the lower edge of the 95% confidence interval for the area under the curve (AUC), is above 0.5 [23].

RESULTS

All of the 16 incident cancers identified from the CT screening trial were AJCC Stage IA or IB. Six cases had incomplete plasma collection and therefore excluded from our analysis; the remaining 10 incident cancers were matched to between 1-4 controls each. Demographic characteristics are summarized in Table 1. As expected, each cancer case was matched for gender. There was a similar distribution of pack years smoked ($p=0.11$). Cancer cases were significantly older than their matched controls ($p = 0.002$). There was a small but significant difference in surveillance interval, which was shorter in the cancer cases than in the matched controls. ($p = 0.03$). Two to seven plasma samples were analyzed for each cancer case and two samples were analyzed for each control. OPN levels and OPNV for cancers and matched controls are outlined in Table 2. At the initiation of surveillance, distributions of plasma OPN levels did not differ between the cancer cases and the matched controls ($p=0.50$) as we would expect by observing the boxplots (Figure 2A). After the start of the surveillance, the rate of increase in OPN (OPNV) was significantly greater in the cancer cases than the controls (median difference of OPNV = 1.06 ng/ml/month, $p = 0.01$, see Figure 2B). We then used OPNV levels to predict disease status with mixed effects logistic regression models. In addition, since the distributions of age and surveillance interval were significantly different between the cases and their matched controls, we adjusted the mixed effect model for age and surveillance interval. LPOCV was used to estimate the accuracy of such prediction. Our results showed that, it is reliable (accuracy rate 83%) to use OPNV level to predict disease status by based on logistic regression models adjusted for age and surveillance interval.

ROC curves that ignore the matching of cases and controls and with adjustment for age and surveillance interval were also used to estimate the sensitivity and specificity of OPNV predictions. When matching is ignored, AUC for the ROC is 0.88 (95% CI: 0.73, 1) (Figure 4A, at cutoff point OPNV=1.59ng/ml, age=63 years and surveillance interval=19 months, the sensitivity is 80% and the specificity is 88%). To further explore the utility of OPNV to classify patients with cancers and those with abnormal CT scans, ROC curve analysis was limited to cancer cases and those controls with abnormal baseline CTs (with ground glass opacities [GGOs] or stable solitary nodules [SNs]). In this very limited subgroup the AUC= 0.91 (95% CI: (0.76, 1) (Figure 4B, at cut off point OPNV=0.16ng/ml, age=78 years, surveillance interval=29 months, the sensitivity is 80% and the specificity is 88%).

DISCUSSION

This nested case-control study demonstrated increased OPNV in patients with NSCLC compared to high risk smokers in a CT screening population, and provides evidence for the utility of monitoring serial plasma OPN levels in high-risk populations. Plasma OPN as a biomarker for the early detection of NSCLC is similar to PSA for prostate cancer in several ways: 1) both are extracellular glycoproteins integral to the malignant process, 2) circulating levels correspond to burden of disease, and 3) circulating levels decrease following successful treatment [13-15, 18, 24]. However, elevations in either biomarker are not unique to a cancer diagnosis. PSA levels are elevated with advanced age, increasing gland size, inflammation and numerous other processes. Therefore, PSAV positively correlates with a greater risk for cancer diagnosis than the absolute PSA level [18, 19]. Current National Comprehensive Cancer Network (NCCN) guidelines recommend that males with PSAV >35ng/ml/year should consider biopsy even if PSA levels are below the normal threshold for biopsy [24]. Plasma OPN for NSCLC diagnosis parallels this phenomenon. Along with being elevated in NSCLC, OPN levels are elevated to a lesser extent in smokers compared to non-smokers, and in the setting of granulomatous disease and pulmonary fibrosis [9]. The results from this series falls in line with this concept; OPN levels in the high risk control group, with a large smoking history, were 45-75 ng/ml, This is higher than reported for healthy volunteers without lung disease using a similar assay system [25, 26], and not significantly different than very early stage cancers detected in this series, but lower than that reported for advanced NSCLC[15, 26]. We found that neither the plasma OPN at initiation nor that at the completion of surveillance was different between cancers and controls (direct statistical comparison of OPN at completion was not performed to lack of control in the surveillance intervals). In this population with low volume disease, there does not appear to be any one value for OPN which is critical but rather the trend which carries significance. As with PSA, the velocity of rise appears to be more predictive of the presence of an early malignancy than any single OPN value.

The clinical value of OPNV may be best illustrated by the differences that were seen when screened cancers were compared to those controls with GGOs and SNs. The AUC for OPNV was highest in this subset which clearly represents the greatest diagnostic dilemma in CT screening, when either SNs or GGOs need to be differentiated from early cancer. This diagnostic dilemma often requires numerous CT scans over a significant time interval to differentiate cancer from benign etiologies. Algorithms for serial scanning are based primarily on nodule size, but radiographic borders, calcifications, and density also play a role [27]. Determination of OPNV in conjunction with CT scan findings could result in more expedient diagnosis of malignancy, fewer CT scans, shorter follow-up, and more benign nodules left untouched, thus saving unnecessary, expensive, and risky procedures. OPNV in the setting of an abnormal CT scan finding has tremendous potential to help guide clinical decision making.

With only 10 incident cancers, the major limitation to this study is its sample size. In this evaluation we were not able to evaluate the stability of our results over multiple surveillance intervals as apposed to only a period of two years or greater. We were also unable to estimate acceleration in OPNV. Similar to PSA, evidence suggests that plasma OPN increases with increasing NSCLC tumor burden. Studies of plasma OPN from early stage resectable NSCLC patients indicate plasma levels of 250-350ng/ml [13, 14], while work in advanced disease report higher mean OPN levels [15]. This series was limited to very small and very early stage tumors. Interestingly, the cancer cases in this series fell into two distinct subgroups, with three cases (numbers 2, 5, and 6) having surveillance intervals less than one year while the remaining seven cases have surveillance greater than two years. The cancer cases with short follow up (one GGO and two SN) demonstrated the most significant growth

of CT abnormality during their short surveillance interval, and also had the greatest OPNV. One could speculate from this very small subset that OPNV may accelerate with tumor growth, and this may be better appreciated with significant increases in tumor volume. Further evaluation of this biomarker in larger CT screening populations could address such variables. The mean OPNV in the remaining more indolent cancer cases was 1.25ng/ml/month, still greater than seen in their matched controls. Notably, we also observed a modest rise (>1ng/ml/month) in OPNV in some of the controls, but this rise was less than seen in the cancers and below the ROC cut point. Similar to nodules size on CT, it may be possible in a larger series to stratify OPNV to low, moderate and high risk ranges, to help guide clinical decisions.

This study reflects initial investigations of OPNV as a screening biomarker in NSCLC. It is essential to further explore the utility of OPNV in larger prospective screening cohorts, such as the NLST. There is a scarcity of CT screening trials, aside from the NLST, which have included biomarker collection in their protocol. The relatively small size of our screening study also resulted in imperfect matching between cancer cases and their controls with respect to important variables such as age and duration of surveillance interval. These factors would likely also be mitigated in a larger CT screening cohort.

In conclusion, this data provides early evidence that OPNV has the potential to differentiate early stage NSCLCs from non-cancers in a CT screening cohort. This would carry tremendous clinical value in the evaluation of indeterminate pulmonary nodules and to the integration of CT screening initiatives for NSCLC care.

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Figure 1.

Example of OPNV calculation for a single cancer case (case #7) demonstrating the calculation that was used for each individual cancer case. The OPNV represents the slope of the line of the linear regression of plasma OPN levels over the entire surveillance interval, with no restrictions on the number of OPN values or time intervals between OPN measurements. This is the method as it was originally described for PSAV by Carter in 1992.[18]

Figure 2: (A).

Distributions of initial OPN levels for cancer cases, means of matched controls, and differences between cases and their matched control means. Initial OPN for the cancer cases (Median: 21.8; Range:12-164.7); Initial OPN for matched controls (Median: 54.8; Range: 17.8-80.2); Difference in OPN between cancer cases and mean of matched controls (Median: -5.15; Range: -64.5-95.35). **(B)** Distributions of OPNV levels for cases, means of matched controls, and differences between cases and their matched control means. OPNV for cancer cases (Median: 1.67; Range:-0.21-7.39); OPNV for matched controls (Median: 0.33; Range:-2.69-0.89); difference in OPNV between cancer cases and mean of matched controls (Median: 1.06; Range: -0.67-8.27).

Figure 3.

OPN levels over time for 10 case-control groups. Black lines represent OPNV over the surveillance interval for each cancer case and grey lines represent OPNV for each matched control. For each cancer case, OPNV is obtained by linear regression of OPN levels vs. the surveillance time. For each of the matched controls, the line is obtained by connecting the baseline OPN and that at completion of surveillance.

Figure 4: (A).

ROC Curve for OPNV levels with the Logistic Equation (Cases vs Controls): $\text{logit (Probability of Case)} = -12.31 + 1.51 \cdot \text{OPNV} + 0.137 \cdot \text{Age} + 0.013 \cdot \text{Surveillance Interval}$. At cutoff point $\text{OPNV} = 1.59 \text{ ng/ml}$, $\text{Age} = 63 \text{ years}$ and $\text{Surveillance Interval} = 19 \text{ months}$, the sensitivity is 80% and the specificity is 88%. **(B)** ROC Curve for OPNV levels with the Logistic Equation (Cases vs /Controls with Abnormal CT Scans): $\text{logit (Probability of Case)} = -2.50 + 1.77 \cdot \text{OPNV} + 0.131 \cdot \text{Age} + 0.062 \cdot \text{Surveillance Interval}$. At cut off point $\text{OPNV} = 0.16 \text{ ng/ml}$, $\text{Age} = 78 \text{ years}$, $\text{Surveillance Interval} = 29 \text{ months}$, the sensitivity is 80% and the specificity is 88%.

Table 1

Demographic characteristics of cancer cases and matched controls

Matched Group (number of controls)	Age (years)			Tobacco (pack years)			Surveillance Interval (months)		
	Cancer	Controls Mean (SD)	Difference	Cancer	Controls Mean (SD)	Difference	Cancer	Controls Mean (SD)	Difference
Group 1 (control = 1)	77	71.0	6	50	62.0	-12	52	54.0	-2.0
Group 2 (control = 4)	71	67.5 (1.7)	3.5	60	74.0 (29.7)	-14	2	7.3 (1.2)	-5.3
Group 3 (control=4)	65	60.3 (2.7)	4.7	47	39.0 (14.5)	8	25	25.3 (4.6)	-0.3
Group 4 (control=3)	86	78.0 (1.1)	8	66	39.0(6)	27	28	27.7 (1.5)	0.3
Group 5 (control=2)	70	62.5 (3.5)	7.5	66	36.5 (0.7)	29.5	3	3.5 (0.7)	-0.5
Group 6 (control=4)	64	63.5 (4.9)	0.5	95	45.8 (13.0)	49.25	7	15.8 (4.9)	-8.7
Group 7 (control=4)	68	63.0 (4.9)	5	35	43.0 (11.5)	-8	28	26.3 (2.1)	1.7
Group 8 (control=4)	65	58.8 (3.8)	6.2	89	33.5 (6.2)	55.5	24	39.0 (2.2)	-15.0
Group 9 (controls=3)	68	66.0 (2.6)	2	45	44.0 (5.3)	1	26	28.0 (3.6)	-2.0
Group 10 (control =4)	66	60.3 (2.4)	5.7	74	50.3 (16.9)	23.7	34	39.5 (1.7)	-5.5
Median(Range) (significance Wilcoxon signed rank test)	68.0 (64-86)	63.3 (58.8-78.0)	5.4 (0.5-8.0) p=0.002	63.0 (35-95)	43.5 (33.5-74.0)	15.9 (-14.0-55.5) p=0.11	25.5 (2.0-52.0)	27.0 (3.5-54.0)	-2.0 (-15-1.7) p=0.03

Table 2

OPN and OPNV levels of cancer cases and matched controls

Matched Group (number of controls)	Initial OPN (ng/ml)			Completion OPN (ng/ml)		OPNV (ng/ml/month)		
	Cancer	Controls Mean (SD)	Difference	Cancer	Controls Mean (SD)	Cancer	Controls Mean (SD)	Difference
Group 1 (control =1)	12.0	17.8	-5.8	99.2	66.0	1.4	0.9	0.5
Group 2 (control = 4)	17.0	60.2 (21.8)	-43.2	26.9	58.8 (23.1)	4.8	-0.1(0.8)	4.9
Group 3 (control = 4)	48.5	49.4 (29.3)	-0.9	58.0	53.4 (10.5)	1.4	0.3(1.7)	1.1
Group 4 (control=3)	24.0	49 (22.5)	-25.0	77.0	62.8 (10.3)	1.4	0.5 (0.8)	0.9
Group 5 (control=2)	164.7	69.3 (12.8)	95.4	159.6	62.5 (10.5)	3.0	-2.7 (7.2)	5.7
Group 6 (control=4)	15.7	80.2 (26.7)	-64.5	67.4	73.4 (32.8)	7.4	-0.9 (2.92)	8.3
Group 7 (control=4)	19.6	75.8 (14.2)	-56.2	87.4	85.4 (18.2)	2.3	0.3 (0.56)	2.0
Group 8 (control=4)	49.1	30.5 (10.4)	18.7	42.2	48.1 (12.0)	-0.2	0.5 (0.46)	-0.7
Group 9 (control=3)	100.6	67.2 (5.0)	33.4	86.6	68.6 (8.1)	0.5	0.1 (0.36)	0.4
Group 10 (controls=4)	15.2	19.7 (17.4)	-4.5	68.7	53.9 (7.6)	1.9	0.9 (0.64)	1.0
Median (Range) (significance Wilcoxon signed rank test)	21.8 (12.0-164.7)	54.8 (17.8-80.2)	-5.1 (-64.5-95.4) p=0.50	72.9 (26.9-159.6)	62.6 (48.1-85.4)	1.6 (-0.2-7.4)	0.3 (-2.7-0.9)	1.0 (-0.7-8.2) p=0.01