The Clinical Significance of Serum Markers of Bone Turnover in NSCLC Patients: Surveillance, Management and Prognostic Implications

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Abstract. *The purpose of this study was to investigate various serum markers of bone turnover in non-small cell lung cancer patients (NSCLC) in the presence or absence of bone metastasis. Our retrospective study included 79 newly diagnosed NSCLC patients. Group A included 51 patients with bone metastasis and group B included 28 patients that never developed bone metastasis. The measurement of bone formation markers, bone resorptive markers and osteoclastogenesis markers as well as routine biochemical analysis was determined. Patients with bone metastasis had an increase in receptor activator of nuclear factor κB ligand, osteopontin and osteoprotegerin. Patients who later developed bone metastasis had decreased osteocalcin and tartrate-resistant acid phosphatase isoform 5b levels (TRACP-5b). We also found an unusually low TRACP-5b/RANKL ratio for patients who have or later developed metastasis. In patients that never developed bone metastases, cross-linked carboxy-terminal telopeptide of type I collagen was increased. Positive correlations were found between osteopontin and TRACP-5b, and also between bone alkaline phosphatase with osteocalcin and TRACP-5b. In conclusion, serum markers of bone turnover may be able to determine the time-to-tumor progression, metastatic potential and overall survival of the NSCLC patient. In addition, they may contribute to a more accurate follow-up and tailored treatment options.*

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Patients with lung cancer often experience bone metastasis leading to skeletal complications that present a major challenge in disease management (1). It is estimated that more than 35% of patients with advanced lung cancer manifest bone metastases, while, at autopsies, up to 50% of patients with lung cancer have evidence of skeletal metastasis (2). The nature of these bone lesions is mainly osteolytic, although their pathogenesis has not been fully clarified. Recent preclinical studies indicate merely the direct effects of cancer cells on bones do not cause this osteolysis. It is more likely that interactions between lung cancer cells and bone microenvironment play an important role in the development of lytic disease, disrupting the balance between osteoclasts and osteoblasts (2).

Osteoclasts are activated through these interactions, resulting in an increased resorptive activity, without a comparable increase in bone formation; thus leading to the development of osteolytic lesions. The increased bone resorption in lung cancer patients with bone metastases is illustrated by the elevated values of the bone collagen degradation products, including the urinary *N*-terminal cross-linking telopeptide of type-I collagen (NTX), and the urinary deoxypyridinoline (D-PYD) (3, 4). Moreover, serum level of tartrate-resistant acid phosphatase isoform 5b (TRACP-5b), which is an enzyme produced only by activated osteoclasts, has been reported to be raised in lung cancer patients with skeletal metastasis (5). Bone alkaline phosphatase (bALP) and osteocalcin (OC), markers of bone formation, have also been implicated in the pathogenesis of neoplastic lesions to the bones, suggesting that bone formation is also increased in this cohort of patients but without resolving the increased bone resorption (6).

Detection and monitoring of metastatic bone disease is mainly performed with bone scintigraphy, a method that cannot accurately determine the neoplastic nature of the bone lesions found. Neither can it precisely track the clinical course of the patients following administration of bisphosphonates or during treatment of a primary tumor. On the other hand, bone remodeling activity could provide valuable information on the existence of secondary bone lesions. It can be assessed either by direct measurement of the affected bone cells (osteoblasts and osteoclasts) or by the evaluation of the metabolic products released from the bone formation and resorption.

Osteoclastic activation is mainly regulated by the receptor activator of nuclear factor κB (RANK), its ligand (RANKL) and its decoy receptor osteoprotegerin (OPG) (7) as well as by osteopontin (OPN), a glycosylated phosphoprotein implicated in tumor metastasis (8). Serum markers that reflect bone remodeling include among others *C*-terminal cross-linking telopeptide of type I collagen (CTX) and TRACP-5b (bone resorption markers), and bALP, OC, and *C*-terminal propeptide of type I collagen (CICP; bone formation markers). Serum levels of some of these markers have been investigated in patients with neoplastic bone lesions, mainly with multiple myeloma and prostate cancer, although existing studies included few bone markers and only a limited number of patients. Presently, there are no similar studies in non-small cell lung cancer patients (NSCLC).

We evaluated the serum levels of osteoclast regulators soluble RANKL (sRANKL), OPG, OPN and markers of bone turnover TRACP-5b, CTX, bALP, OC and CICP in NSCLC patients and examined their potential correlation with the existence of secondary bone lesions at diagnosis or later in the course of their disease. We also investigated whether any of the parameters studied could be used as prognostic markers in this group of patients.

Patients and Methods

Patients. Our retrospective study included 79 newly diagnosed NSCLC patients (69 males and 10 females) that were investigated and treated in the Oncology Unit of the Third Department of Medicine, Sotiria General Hospital, Athens Medical School. These consisted of two groups: group A included 51 NSCLC patients with bone metastasis either at diagnosis (subgroup A1 n=34), or later in the course of their disease (subgroup A2 n=17), and group B included 28 NSCLC patients that never developed bone metastasis. The diagnosis of the 79 NSCLC patients (median age 69, range 42-88 years) was confirmed by histological or cytological examination of specimens taken from bronchoscopy or by computerized tomography-guided fine needle biopsy. Cancer stage was assigned according to the TNM system (all patients were stage M1) and histological grade was classified as either well, moderate, or poorly differentiated. Between September 2000 and September 2002, archived (at –80˚C) serum samples that were collected before the administration of any anticancer treatment after initial diagnosis were used. The control group consisted of 29 healthy volunteers (25 males and 4 females) of comparable age (median age 66, range 40-75 years). The study was performed in accordance with the ethical standards of the Helsinki Declaration and was approved by the local ethical committees.

The medical records of all participants were reviewed to ensure that they were not taking any medication known to interfere with bone turnover, such as calcium supplementation or bisphosphonate. With regard to the NSCLC patients, evidence of bone involvement and any suspicious areas at the time of diagnosis were documented using Tc-99m bone scan and confirmed with plain radiography (baseline date was within one month before treatment). In special cases, computerized tomography or magnetic resonance imaging were used to diagnose bone metastases. Patients were considered to have bone involvement if radiographic abnormalities were consistent with bone disease, which included osteolytic lesions and fractures. Although bone pain was documented, the presence of bone pain alone was not considered to be indicative of bone disease in the absence of radiographic abnormalities. Follow-up bone scans were performed every four months, or sooner if there were clinical signs or symptoms suggestive of bone metastasis, until severe deterioration of the patient's performance status (ECOG scale PS>2) was observed. Plain radiological investigation was performed to confirm the positive findings of the bone scans, or whenever there were clinical signs or symptoms suggestive of bone metastasis, irrespective of the ECOG performance status of the patient.

Samples. Blood samples were collected in plastic tubes (Nouva Aptaca, Italy; Cat No 5995/E) between 07:30 and 09:00 a.m., stored in ice and centrifuged at 2,000 × g for 15 minutes, at 4˚C, within 2 hours from venipuncture.

Bone formation markers. Markers were all measured with the application of standard immunoenzyme assays on an ELX 800 analyzer (Bio-Tek Instruments, Winooski, Vermont, USA). Bone ALP was measured with the application of an enzyme-linked immunosorbent assay (ELISA) method (Metra® BAP; Quidel Corporation, San Diego, CA, USA), as well as Osteocalcin (OC) (N/MID® Osteocalcin ELISA; Nordic Bioscience Diagnostics A/S, Herlev, Denmark) and CICP (Metra® CICP; Quidel Corporation). The inter- and intra- assay variation in our lab was less than 7% for all bone formation markers.

Bone resorption markers. CTX was measured by a one-step ELISA (Serum CrossLaps® ELISA; Nordic Bioscience Diagnostics A/S) using two monoclonal antibodies specific for a β-aspartate form of the epitope EKAHDGGR derived from the carboxy-terminal telopeptide region of type I collagen α1 chain. The detection limit was 0.01 ng/ml. Intra- and interassay coefficients of variation (CVs) of the assay were 5.4% and 6.2%, respectively. TRACP-5b serum levels were measured using a solid phase immunofixed-enzyme activity assay (BoneTRAP® Assay, SBA; Immunodiagnostic Systems Ltd, Boldon, UK). The sensitivity of this assay was 0.06 U/l; intra- and interassay CVs were less than 6% and 8%, respectively. Normal values ranged from 0.5 to 3.8 U/l for men and premenopausal women and up to 4.8 U/l for postmenopausal women. In our laboratory the inter- and intraassay variation was less than 8.7% for both parameters.

Osteoclast regulators. Total OPG was measured by an ELISA method (Biomedica Medizinprodukte, Gesellschaft GmbH & Co KG, Vienna, Austria). Soluble RANKL was also measured with an

ELISA method (No. BI-20422H; Biomedica Medizinprodukte,). The intra- and interassay coefficients of variation (CV) were less than 10% for both OPG and RANKL according to the manufacturer, as well as in our laboratory. The detection limit for OPG was 0.14 pmol/l and for sRANKL was 0.08 pmol/l. Osteopontin (OPN) serum levels were also measured using an ELISA method (Assay Designs Inc., Ann Arbor, MI, USA). The sensitivity of the method was 3.6 ng/ml, while the intra- and interassay CVs were less than 10.3% according to the manufacturer. In our laboratory, intra- and interassay CVs were 9.8% and 11.7%, respectively.

Routine biochemistry determinations. Total alkaline phosphatase (tALP) (upper reference limit 70 U/l), alanine aminotransferase (SGPT) (upper reference limit 40 U/l) and creatinine (105 μmol/l) were measured by standard assays on an Olympus AU640 analyzer.

Statistical analysis. Statistical calculations were performed with the SPSS 11.5 for Windows (SPSS, Munich, Germany) program. Differences between patients and controls were evaluated using analysis of variance (ANOVA) or its non-parametric equivalent (Kruskal-Wallis test). When a significant association was found, post hoc bonferroni comparisons were used and mean differences along with the corresponding 95% confidence intervals (CI) were reported. The correlation between different laboratory and clinical parameters, Pearson's correlation coefficients, were computed. Furthermore, chi-squared tests were carried out to assess associations between patient groups and clinical characteristics, while Kaplan-Maier estimates for overall survival and time to progress were used. Finally, a Cox proportional model was used to assess univariate and multivariate associations between hazard ratios for death and time to disease progression and potential risk factors. All *p*-values are two-sided and confidence intervals refer to 95% boundaries.

Results

Patients. Table I summarizes the demographic and main clinical parameters of the groups studied. There were no significant differences in age and gender between the group of patients and that of the controls, nor amongst the subgroups of NSCLC patients.

Biochemical markers of bone remodeling in patients and controls. Table II shows the mean values with standard deviations (SD) of all markers studied in the control and the NSCLC patient groups. Statistical evaluation of our findings can be summarized as follows.

No significant difference was documented for bALP and CTX between any group of the NSCLC patients or the control group. OPG, when compared to controls, was found significantly increased in all subgroups of patients ($p<0.001$), including those that never developed bone metastases ($p=0.01$). Patients with bone metastasis at diagnosis had significantly increased sRANK, compared to the control group ($p=0.04$), as well as with the patients who never developed bone metastases ($p=0.05$). Subsequently, the difference between all patients and controls was also significant ($p=0.05$). No significant difference was observed for the RANKL/OPG ratio in any of the groups studied. OPN was significantly increased only in the group of patients diagnosed with bone metastases ($p<0.001$) compared to the rest of groups. OC was significantly decreased in the group of patients that developed bone metastases at some point of their disease (upon onset, or later), but not in the group of patients that never developed bone metastases ($p<0.001$). As expected, the statistical difference was more profound in the subgroup of patients that had already developed bone metastases at the time of blood sampling ($p=0.001$), than in the subgroup that developed them later ($p=0.03$). With regard to TRACP-5b, significantly lower levels were found only in the group of patients that had delayed bone metastases, when compared with the controls ($p=0.012$) and with the group of patients that never developed bone metastases ($p=0.001$). Finally, CICP was found significantly increased only in the group of patients that never developed bone metastases, both when compared with the controls ($p=0.005$) and the rest of the patients ($p=0.03$).

With regard to the interactions amongst the markers studied, OPN levels correlated positively with the bone resorption markers TRACP-5b (r=0.23, $p<0.05$) and CTX (r=0.26, $p<0.05$). Positive correlation was also found between bALP and two markers: OC (r=0.38, $p<0.001$) and TRACP-5b (r=0.32, $p<0.001$).

Correlation between markers of bone remodeling and clinical data Multivariate analysis of the serum levels of the

Table I. *Demographics of the control and the NSCLC groups studied: Group A: patients with bone metastases at diagnosis (subgroup A1), or later in the course of their disease (subgroup A2). Group B: patients that did not develop bone metastasis during the course of their disease.*

	Total (n)	Males	Females	Age (years) mean (SD)
NSCLC patients	79	66 (83%)	13 (17%)	67.7 (9.1)
Groups of Patients				
Group A	51	44 (86%)	7 (14%)	66.6 (8.8)
Subgroup A1	34	29 (85%)	5 (15%)	
Subgroup A2	17	15 (88%)	2 (12%)	
Group B	28	22 (79%)	6 (21%)	69.6 (8.7)
Degree of Differentiation				
Well	28			
Moderate	8			
Poor	43			
Performance Status (ECOG scale)				
0	46			
1	21			
2	12			
Controls	29	25 (86%)	4 (14%)	65. 3 (9.9)

Table II. *Serum values [mean (SD)] of the markers measured in control and patient groups.*

	Group A			Group B	Total NSCLC Patients Groups A + B	Controls
	Subgroup A1	Subgroup A2	Subgroups A1+ A2			
OPG (pmol/l)	8.69(5.7)[a]	7.64(3.5)[b]	8.34 (5.1)[c]	7.84 (3.7)[d]	8.17 (4.6)[e]	4.69(1.9)[a,b,c,d,e]
Srankl (pmol/l)	0.88 (0.7)[a]	0.64 (0.4)	0.8 (0.6)[b]	0.75 (0.8)	0.78 (0.7)[c]	0.38 (0.7)[a,b,c]
RANKL/OPG	14.5 (13.7)	9.5 (7.2)	12.8 (12.1)	11.6 (13.0)	12.4 (12.3)	5.94 (12.1)
OPN (ng/ml)	92.7(111.1)[a,d,e]	30.4 (16.8)[d]	69.9 (93.2)[b]	40.5(18.4)[e,f]	59.3(76.2)[c]	22.7(7.2)[a,b,c,f]
bALP (U/l)	30.3(26.7)	21.6 (7.1)	27.4 (22.4)	25.6(9.4)	26.8(18.7)	22.4(12.3)
OC (ng/ml)	7.05(7.8)[a]	9.52 (2.1)[b]	7.87 (6.5)[c]	10.5(6.7)	8.81(6.7)[d]	16.7(8.5)[a,b,c,d]
CICP (ng/ml)	56.7(79.5)	65.3 (49.1)	59.6 (70.1)[b]	93.3(93.8)[a,b]	71.5(80.1)	38.8(31.8)[a]
CTX (ng/ml)	0.96(0.6)	0.90 (0.28)	0.94(0.56)	1.09(0.46)	0.99(0.53)	0.81(0.62)
TRACP-5b (U/l)	1.83(1.0)[b]	1.2 (0.5)[a,b,c]	1.62(0.9)[d]	2.26(0.8)[c,d]	1.85(0.9)	1.95(0.82)[a]

Subgroups that share the same superscript letter are significantly different from each other ($p<0.05$). Group A: patients with bone metastases at diagnosis (subgroup A1), or later in the course of their disease (subgroup A2). Group B: patients that did not developed bone metastasis during the course of their disease. OPG: osteoprotegerin, RANKL: receptor activator of nuclear κB ligand, OPN: Oteopontin, bALP: bone alkaline phosphatase, OC: osteoclacin, CIPC: C-terminal propeptide of type I collagen, CTX: C-terminal cross-linking telopeptide of type-I collagen, TRACP-5b: tartrate-resistant acid phosphatase isoform 5b.

markers studied demonstrated the following correlations with clinical and tumor parameters at the time of diagnosis. Age was significantly associated with OPG (r=0.22, p=0.01). Weight loss was independently associated with increased OPN levels (p=0.04) and low TRACP-5b levels (p=0.02). Presence of adrenal secondary lesions at diagnosis correlated independently with increased values of the RANKL/OPG ratio (p=0.006) and increased serum levels of the bone markers bALP (p=0.001), OPN (p=0.02), CTX (p=0.02) and OC (p=0.04). Presence of secondary lesions in the liver at diagnosis correlated independently with bALP (p=0.02) and OC (p=0.04). Finally increased OPG levels correlated positively with the presence of pericardial infiltration (p=0.04).

Correlation between markers of bone remodeling and outcome. The median time to disease progression of the 79 patients included in the study was 3 months (range 0-12 months), with a mean survival of 12 months (range 3-41 months). The 51 patients of group A, in addition to chemotherapy, received intravenous zoledronic acid at some point during the course of their disease, initiating when the bone metastases were documented. None of the factors studied (histological subtype, degree of differentiation, EORTC PS, smoking exposure (number of packs per year), kind of regimen administered, or number of chemotherapy cycles administered) correlated to the response rate or to overall survival.

With regard to the bone markers investigated, the univariate analysis suggested that increased TRACP-5b and OPN serum levels at diagnosis were associated with an increased hazard of disease progression. However, in the multivariate analysis only TRACP-5b [HR 1.88 (95% CI: 1.07-3.34), p=0.03] independently predicted the time to progression (TTP). Finally, the multivariate analysis demonstrated that increased OPG levels at diagnosis adversely predict overall survival [HR: 0.89 (95% CI: 0.81-0.99), p=0.03].

Discussion

Bone scintigraphy is considered the gold standard for diagnosis of bone involvement in patients with solid tumors, including NSCLC. However, it is an expensive method with low specificity. In addition, it is not suitable for the follow-up of any existing neoplastic lesions. Although several researchers have suggested the application of bone markers for the more accurate follow-up of patients with secondary neoplastic lesions in the bones, there is a discrepancy as to which markers should be used. This is mainly due to the small number of patients studied, the types of tumor markers reported as well as the inconsistency of the results described due to methodological problems. The markers that have been studied so far have a poor stability *in vitro* and the existing assays unfortunately recognize different epitopes.

Our present study, to the best of our knowledge, includes the largest reported number of NSCLC patients and bone turnover markers reported in the literature. Of the markers studied, all osteoclastogenesis markers (sRANK, OPG, and OPN) were found to be significantly increased and the bone formation marker OC was found significantly decreased, in the group of patients with bone metastases, and the bone resorption marker TRACP-5b was reduced in the group of patients that had delayed to develop bone metastases. Discrepancies amongst the various markers and amongst the subgroups of patients could be attributed to the different time of expression of these substances in the course of the disease.

Metastatic bone disease in lung cancer patients is the result of osteoclastic activation, following the interaction of malignant cells with the bone marrow microenvironment. The attachment of lung cancer cells to stromal cells triggers a paracrine loop of cytokine pathway that leads to osteoclast activation and lytic bone disease (1, 2). The RANK/RANKL/OPG system has recently been recognized as the final, dominant mediator for osteoclastogenesis (7, 9). The ratio of RANKL/OPG wasincreased in patients with neoplastic disease indicating severe osteolysis, which is also seen in multiple myeloma patients (10, 11). As osteoclastogenesis is the connecting link between bone formation and bone resorption, so too are our results which correspond with the histopathological evidence that resorptive activity coexists with osteoblastic activity.

We demonstrated that OPG levels at diagnosis are significantly increased in all groups of NSCLC patients, including the subgroup of patients that did not develop neoplastic skeletal lesions during the course of their disease. Our finding is in accordance with similar observations in prostate cancer patients and implicates a broader biological role of OPG in NSCLC cancer patients, which suggests that OPG is not associated at all with the skeletal metastasis, rather with the tumor load per se. This suggestion is further supported by the fact that patients with bone metastases from breast, renal, pancreatic and colorectal carcinoma do not demonstrate increased levels of OPG. We were able to demonstrate that increased levels of OPG provide an adverse, independent prognostic factor in patients with NSCLC. Our finding correlates with similar observations in prostate and bladder cancer patients (12, 13).

Soluble RANKL was also increased, but only in the subgroup of NSCLC patients already experiencing bone metastasis. Even patients without a bone metastasis had an elevation of sRANKL, which did not, however, reach the level of statistical significance. This result suggests that the sRANKL elevation in NSCLC patients may not be related to bone metastasis alone but may also be associated with tumor burden. We failed to confirm an increased value of the sRANKL/OPG ratio in any subgroup of NSCLC, including those with bone metastasis, contrary to what has been described in most cases of metastatic bone disease from solid tumors. In addition, this ratio did not correlate with the serum values of any bone marker investigated, while its increased values did correlat with pericardial invasion. The above findings suggest that the sRANKL/OPG ratio in the serum may not accurately reflect the imbalance of these cytokines in the microenvironment of bone metastasis, but advocate a broader role of these cytokines in tumor spread, reflecting the tumor burden of lung cancer patients.

According to our findings, increased serum OPN levels correlated with the presence of bone metastasis at the time of diagnosis. OPN is a non-collagenous matrix protein produced by various cell types including osteoblasts, osteoclasts and tumor cells. Binding of osteoclasts to OPN deposited in the bone matrix is essential for the migration, attachment and resorptive activity of osteoclasts (8). The demonstrated correlation between increased OPN serum levels and the presence of bone metastasis suggests that OPN is an important molecule for the development of secondary lesions in the bones. Moreover, increased OPN levels were correlated with increased levels of the two markers of bone resorption (TRACP-5b and CTX) studied. This further confirms that OPN enhances bone resorption in lung cancer patients leading to osteoclast activation and lytic bone metastasis. Previous studies demonstrated that OPN was increased in the serum of patients with lung cancer and correlated with survival in curatively resected NSCLC patients (14, 15). It was also demonstrated in preclinical studies that increased OPN levels correlated with augmented metastatic potential in an isogenic model of spontaneous human breast cancer (16). According to our findings, increased levels of OPN at diagnosis predicted a shorter TTP, although not independently, but also correlated with significant weight loss and increased potential of metastasis to the bones and adrenals, suggesting that OPN is also an important marker in the pathogenesis of lung cancer.

In our study, we investigated two bone resorption markers: TRACP-5b, which is an enzyme produced only by activated osteoclasts, and CTX, a very accurate marker of bone resorption (17, 18). With regard to CTX, no difference was documented in any of the groups studied, irrespective of the presence or absence of bone metastasis. Furthermore, TRACP-5b values were not different between patients who had bone metastasis at diagnosis and those who never developed bone metastasis. Thus these two markers seem not to correlate with resorptive metastatic events in our cohort of patients. Our data is in accordance with Ebert *et al*. (4) that also failed to establish increased levels of resorption markers in NSCLC patients with or without bone metastases. In addition, our data further contribute to clarifying the existing confusion in the literature as Izumi *et al*. (3) showed an increase in collagen type-I degradation products in patients with lung cancer and bone metastases, while Brown *et al*. (19) reported increased levels of NTX and bALP. However, in our cohort of patients, increased TRACP-5b levels correlated independently with weight loss, shorter time to progression and development of distant metastases after stabilization of the disease. This suggests that this molecule may be valuable in reflecting the tumor burden as well as evaluating the metastatic potential of NSCLC patients.

Particularly noteworthy is our finding that NSCLC patients who did not have evidence of bone metastases at the onset of the disease but developed it later demonstrated significantly lower serum levels of TRACP-5b at diagnosis. The difference was statistically significant both in comparison with the controls and with the other subgroups of patients (those with bone

metastases at the start and those who never developed bone metastases, p=0.001). By monitoring the levels of TRACP-5b, we may be forewarned as to when the bone's last attempt occurs towards salvaging itself by decreasing osteoclastic activity before uncontrollable bone resorption takes place causing severe bone damage. This indeed indicates a potentially useful tool to examine and isolate the group of NSCLC patients who may develop of skeletal lesions and therefore will require a more aggressive treatment and a closer surveillance.

In addition to this, in investigating our own data, we found an unusual relationship between TRACP-5b and sRANKL. TRACP-5b represents the activity of the osteoclast and is located in the ruffled border of the cell. RANKL, on the other hand, works off of the osteoblast but encourages osteoclast formation. We noticed that even though the significantly low levels of TRACP-5b occurred in patients who later developed metastasis, the ratio of TRACP-5b/sRANKL was strikingly similar to all patients who developed bone metastasis (2.08 and 1.88). Those who never developed bone metastasis; the ratio remained at 3.01. Controls were at the highest, which was at 5.13. The absence of both of these factors causes osteopetrosis. However, the balance between both of these factors may be important in maintaining homeostasis of the bone and preventing the disruption between activity and formation to occur. When this occurs as in metastases, the bone microenvironment may be inclined to an osteolytic fate. Further studies are warranted to confirm this incidental relationship.

Finally, we investigated three bone formation markers, of which bALP was found to have no difference between cancer patients and controls, while OC levels were found to be significantly lower in NSCLC patients with bone metastasis at diagnosis, or later in their disease. Again, patients that never developed bone metastasis had no difference in the OC levels at diagnosis, providing the means to recognize those patients that are not likely to develop skeletal lesions from their disease. This particular group of NSCLC was the only group that also had a significantly increased level of the third studied bone formation marker, CICP, when compared with controls or with patients who developed bone metastases, the difference being particularly significant (p=0.005). These differences among bone formation markers reflect the complex interactions between malignant cells, bone microenvironment and osteoblast function in NSCLC, where other molecules are overproduced (CICP) by osteoblasts and others seem to be down regulated (OC) or unaffected (bALP).

Our above mentioned observations, if confirmed by further investigations, have several important clinical implications. We suggest that OPG and OPN may have a broader role in the pathogenesis and spread of NSCLC since they provide useful information regarding the metastatic potential and the overall survival of these patients. With the possibility of identifying a particular group of patients, treatments could be more tailored to prevent overtreatment or exposure to potential toxicity from drugs such as bisphosphonates, which may then be accepted and prescribed as prophylactic for patients with solid tumors and without evidence of bone metastases. In fact, our study provides the option of identifying that particular group of patients who are less likely to profit by the prophylactic administration of bisphosphonates. These particular groups of NSCLC patients are those who do not have any imaging evidence of bone metastasis at diagnosis, whose CICP serum levels are increased, while their TRACP-5b and OC levels are within the normal range. On the other hand, NSCLC patients without imaging evidence of bone metastasis, with low TRACP-5b and OC and normal CICP levels at diagnosis are more likely to develop secondary bone lesions and therefore deserve additional treatment and surveillance. Either way, the ratio of TRACP-5b/sRANKL may also be informative.

In addition to more tailored therapies, detection of serum bone markers enables the surveillance of bone resorption in the face of bone metastasis. By targeting these markers of bone metabolism, we may be able to further manage patients' disabling outcomes. As seen, for example, with the RANKL/OPG interaction, preliminary studies, notably with mice models, have focused on reducing RANKL or increasing OPG and have showed an inhibition of bone resorption (20 21). In a phase 1 study in humans, the administration of recombinant OPG (AMGN-0007, Amgen; Bonn, Germany) in patients with multiple myeloma or breast cancer led to a dramatic and sustained decrease of NTX (indicating bone resorption) without severe side-effects (22).

In conclusion, in the era of individualized approach and tailored therapies for cancer patients, our study sets the background for further studies that would exploit serum markers of bone turnover for prognostic and therapeutic purposes. In essence, it may allow us to closely survey the progress of these patients, permitting us to intervene when necessary.

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