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ORIGINAL RESEARCH

Effect of zoledronic acid on tartrate-resistant acid phosphatase isoform type 5b and other bone markers in lung cancer patients with bone metastases

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Background: Up to 44% of lung cancer patients eventually develop bone metastases. Zoledronic acid has been shown to prevent skeletal-related complications in these patients. Bone metabolic markers play an important role in the prediction and diagnosis of bone metastasis. Measurement of serum tartrate-resistant acid phosphatase (TRAP) type 5b and other markers of bone formation and resorption might determine the response of bone metastasis to zoledronic acid therapy. Here we report the effect of zoledronic acid on bone metabolic markers in lung cancer patients.

Methods: Patients with lung cancer metastatic to bone undergoing monthly treatment with zoledronic acid were enrolled in this study. Serum markers of activity of bone resorption and formation were collected at baseline, during chemotherapy, before and after zoledronic acid treatment, and every 2 weeks for the first 6 weeks, and then monthly for a total of 6 months or earlier, if bone metastases progressed radiologically. Resorption markers included N-terminal telopeptide of type I collagen, TRAP type 5b, C-terminal telopeptide of type I collagen, and deoxypyridinoline. Bone-specific alkaline phosphatase was the only bone formation marker included. The data were analyzed using the linear mixed-effects model and paired *t*-test.

Results: Twenty-eight participants were enrolled. Thirteen patients could not be evaluated because of low-quality samples (n = 7), noncompliance (n = 5), or withdrawal of consent (n = 1). During chemotherapy without zoledronic acid, levels of TRAP type 5b remained stable. Fourteen days after zoledronic acid treatment, mean N-terminal telopeptide of type I collagen, TRAP type 5b, and bone-specific alkaline phosphatase levels decreased by 41.4% ($P = 0.003$), 44.9% ($P = 0.014$), and 12.9% ($P = 0.031$), respectively. These markers remained significantly decreased during monthly zoledronic acid. C-terminal telopeptide of type I collagen and deoxypyridinoline levels did not change with zoledronic acid treatment.

Conclusion: TRAP type 5b, N-terminal telopeptide of type I collagen, and bone-specific alkaline phosphatase levels decreased significantly with zoledronic acid treatment in lung cancer patients with bone metastasis. The rapid response of TRAP type 5b levels to bisphosphonate therapy makes it an attractive tool for monitoring the efficacy of bone-targeted therapy in lung cancer patients.

Keywords: lung cancer, bone metastasis, biphosphonates, bone markers

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Introduction

Lung cancer is the most common cause of bone metastasis after breast and prostate cancers.[1] Between 19% and 44% of lung cancer patients are affected, with the most common sites being the vertebral bodies. The malignant activation of osteoclasts

results in disruption of normal bone remodeling, and the relative increase in osteoclast activity results in a net loss of bone, causing severe bone pain, spinal cord compression, pathological fractures, and hypercalcemia.[2,3]

Bisphosphonates are effective inhibitors of osteoclastic bone resorption, and have demonstrated therapeutic efficacy in the treatment of hypercalcemia of malignancy, and lytic and blastic bone metastases.[4,5] Zoledronic acid, a third-generation bisphosphonate, is a potent inhibitor of osteoclast activity. Several large, randomized, Phase III trials have indicated the safety and efficacy of zoledronic acid in preventing or delaying skeletal-related events in multiple myeloma, and breast, prostate and lung cancers with bone metastases.[6–8] In addition, when zoledronic acid was compared with another bisphosphonate (pamidronate), the incidence of skeletal-related events, such as pathological bone fracture and spinal cord compression, was lower in those who received zoledronic acid.[7] A significant delay in median time to first skeletal-related event and reduction of the annual incidence of skeletal-related events have also been reported in nonsmall cell lung cancer patients with bone metastasis.[9]

Early detection of bone metastases in cancer patients may provide a rationale for early intervention to prevent or delay skeletal-related complications. The standard approach to diagnosis of metastatic bone disease is imaging studies. In addition to the significant costs involved, these radiological tests often fail to detect early metastatic bone disease, and may be ineffective when trying to differentiate healing from progressive lesions.[10,11]

Biochemical markers of bone turnover are technically easy to evaluate, relatively inexpensive, and can assess changes in the bone remodeling process associated with osteoporosis and metastatic bone disease.[12,13] Four markers of bone formation and about 13 markers of bone resorption have been studied for diagnosis, prognosis, and the monitoring of response to antitumor therapy.[14] Two bone formation markers, ie, bone-specific alkaline phosphatase and osteopontin, have shown diagnostic value in the detection of bone metastases as well as prognostic value in prediction of survival in cancer patients.[15,16] As biomarkers of bone resorption, urinary or serum N-telopeptide of type I collagen (NTx), deoxypyridinoline, and serum carboxyterminal cross-linked telopeptide of type I collagen (ICTP) have been widely investigated in a variety of cancers. Patients with a biochemical response to bisphosphonate treatment have fewer skeletal-related events, whereas increases in marker levels predict disease progression.[17–19]

Tartrate-resistant acid phosphatase (TRAP) type 5b is a specific serum marker of osteoclast activity and bone resorption.[20] Studies in breast cancer have shown the clinical value of TRAP type 5b for diagnosis, prognosis, and monitoring of response to bone-targeted treatment.[21,22] Although high TRAP type 5b activity has been correlated with bone metastasis and worsening pain levels in advanced-stage lung cancer, other studies have not confirmed this association.[23–27]

We hypothesized that TRAP type 5b and other markers of bone formation and resorption could determine the response to zoledronic acid and chemotherapy in lung cancer patients with metastases to bone. In this study, we measured the bone formation marker, bone-specific alkaline phosphatase, and the bone resorption markers, NTx, ICTP, deoxypyridinoline, and serum TRAP type 5b, in lung cancer patients with bone metastasis, and evaluated the usefulness of such biochemical bone markers as a disease response monitoring tool in lung cancer patients treated with zoledronic acid.

Patients and methods

Patient population and study design

This was a prospective, open-label, single-arm clinical study designed to evaluate the value of TRAP type 5b and other bone turnover markers as early indicators of biochemical response to zoledronic acid in lung cancer patients.

After obtaining informed consent, 28 patients with histologically proven small/nonsmall cell lung cancer and lytic bone metastases were enrolled into this study at the James Graham Brown Cancer Center, University of Louisville, between July 2005 and June 2009.

All patients received monthly intravenous injections of zoledronic acid 4 mg. The zoledronic acid dose was adjusted according to calculated creatinine clearance. All patients also received supplements containing 400–500 IU of vitamin D and 500 mg of calcium once a day. Chemotherapy, radiation therapy, targeted therapy, and best supportive care were given as standard of care.

A serum sample was collected prior to zoledronic acid therapy for baseline determination of TRAP type 5b activity and other bone turnover markers. Bone marker levels were tested every 2 weeks after the first zoledronic acid infusion for the first 6 weeks, and then monthly for 6 months or earlier in the event of radiological progression of bone metastases, whichever came first. In patients receiving chemotherapy, TRAP type 5b was tested with every cycle of chemotherapy.

Bone marker tests and assessment

All blood samples were collected into 6 mL EDTA-containing tubes, except for samples required for bone-specific alkaline phosphatase testing which were collected into heparinized tubes as per protocol. All bone turnover marker assessments were performed at one central laboratory (Veterans Affairs Central Laboratory, Louisville, KY). Bone-specific alkaline phosphatase and TRAP type 5b were measured in serum using commercial assay kits. Deoxypyridinoline levels were measured using immunoassay kit procedures (Metra Biosystems Inc, Mountain View, CA), as were serum NTx levels (Ostex International, Seattle, WA). Baseline creatinine clearance was calculated using the Cockroft-Gault formula.

Statistical analysis

All results are expressed as the mean ± standard deviation. The R 2.10.1 statistical software program was used to analyze these longitudinal data. This program can be downloaded from http://www.r-project.org. Mixed-effects regression, specifically, the linear mixed-effects model, was used for data analysis. A paired *t*-test was used to compare treatment effects on TRAP type 5b levels as well as other bone turnover markers between consecutive times and baseline. A *P* value of <0.05 was considered statistically significant.

Results

Twenty-eight patients were enrolled from July 5, 2009 to June 9, 2009, and 140 blood samples were tested. Seven samples were excluded from the final analysis because of low quality. One patient withdrew their consent to be included in the study. Samples from a further five patients were missing due to their noncompliance with follow-up. Data from 15 eligible patients were analyzed.

Statistical analysis using the mixed-effects regression model and paired *t*-testing showed that the mean serum TRAP type 5b level decreased by 44.9% from baseline (4.86 U/L to 2.66 U/L, $P = 0.014$) by week 2, and remained in the lower range if the patient continued to receive monthly zoledronic acid (Figure 1).

Average serum NTx decreased significantly by 41.4% from a baseline level of 34.50 nM to 20.21 nM ($P = 0.003$) by week 2 after initiating zoledronic acid. Levels decreased in all but one patient, possibly representing disease progression (Figure 2). NTx levels continued to be suppressed to lower levels by zoledronic acid treatment until month 5. Two of the 15 patients showed a decrease in their TRAP type 5b and NTx levels of more than 80%. Levels of the other two serum bone resorption markers, ICTP and deoxypyridinoline, did not change significantly with zoledronic acid treatment ($P = 0.890$ and $P = 0.999$, Figures 3 and 4). After treatment with zoledronic acid at week 2, the mean bone-specific alkaline phosphatase level decreased by 12.9% from a baseline level of 36.47 µg/L to 31.76 µg/L ($P = 0.031$) and continued to decrease significantly to 17.80 µg/L by month 5 ($P < 0.0001$, Figure 5).

Discussion

After years of research on markers of bone resorption and formation, good predictors of response to bone-targeting treatments are still lacking in practice. In several studies, patients with a biochemical response to bisphosphonate treatment had fewer skeletal-related events, whereas increases in marker levels predict disease progression.[15–17,28]

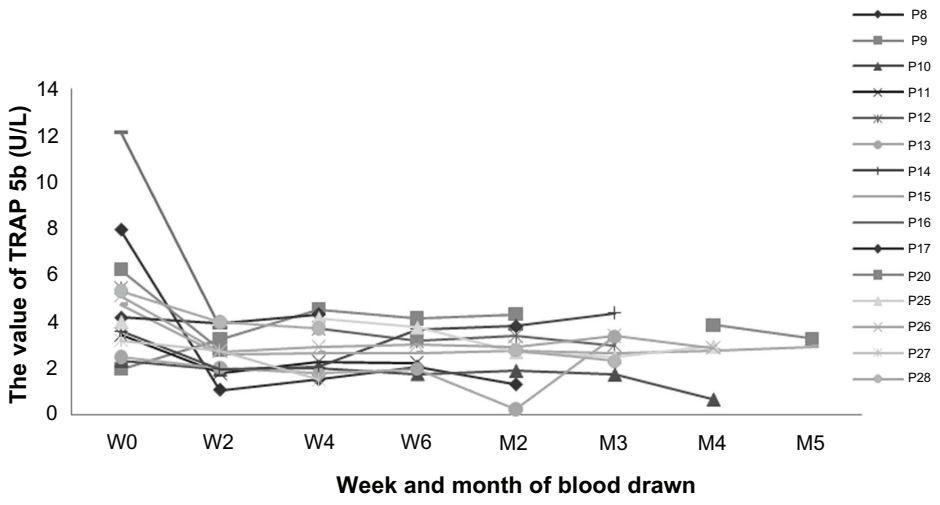

Figure 1 Effect of zoledronic acid on TRAP 5b levels in lung cancer patients with bone metastases.
Abbreviation: TRAP, tartrate-resistant acid phospatase.

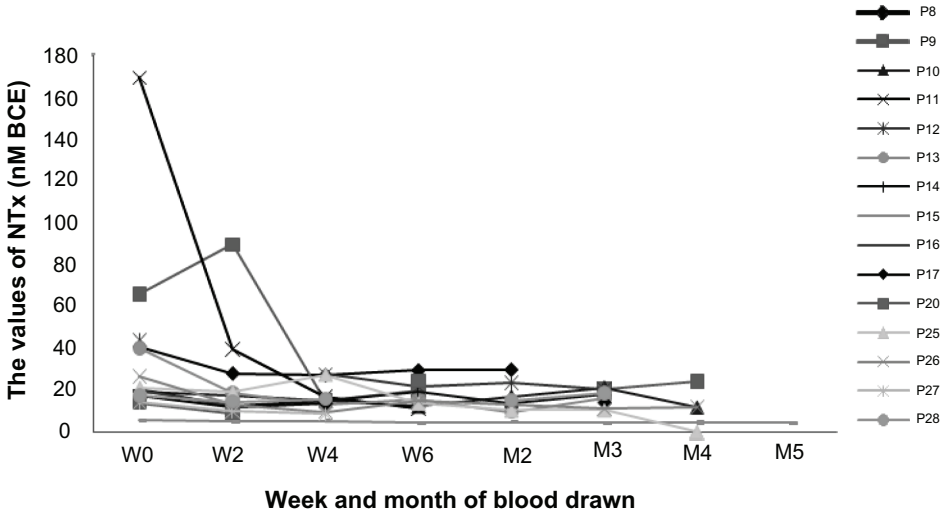

Figure 2 Effect of zoledronic acid on NTx levels in lung cancer patients with bone metastases.
Abbreviations: NTx, N-terminal telopeptide of type I collagen; nM BCE, nano molar bone collagen equivalents.

A commonly used marker of bone formation is bone-specific alkaline phosphatase. High bone-specific alkaline phosphatase levels in metastatic malignancy correlate with increased osteoblastic activity and increased risk of skeletal-related events.[13,14,29] Despite elevated bone-specific alkaline phosphatase levels in lung cancer patients with bone metastases, there is conflicting evidence regarding the association between bone-specific alkaline phosphatase and skeletal-related events.[27,30,31] The discrepancy between studies may be due to differences in patient population, sample size, time of specimen collection, and the method used to analyze the samples. In our study, lung cancer patients with bone metastasis had elevated serum bone-specific alkaline phosphatase levels, and zoledronic acid significantly decreased these levels, which continued to drop steadily with continuation of zoledronic acid use (Figure 5).

Elevated NTx, a marker of bone resorption, correlates with an increased risk of skeletal-related events, disease progression, and death in prostate and lung cancer patients compared with patients with low NTx levels.[28] Treatment with zoledronic acid in patients with nonsmall cell lung cancer and bone metastasis tends to reduce NTx levels within a couple of months, and has been associated with inhibition of osseous metastases and decreased mortality.[32,33] Patients with nonsmall cell lung cancer and elevated NTx levels treated with zoledronic acid have a reduced risk of death and improved palliation compared with placebo.[34] NTx levels in our lung cancer patients with bone metastases also decreased sig-

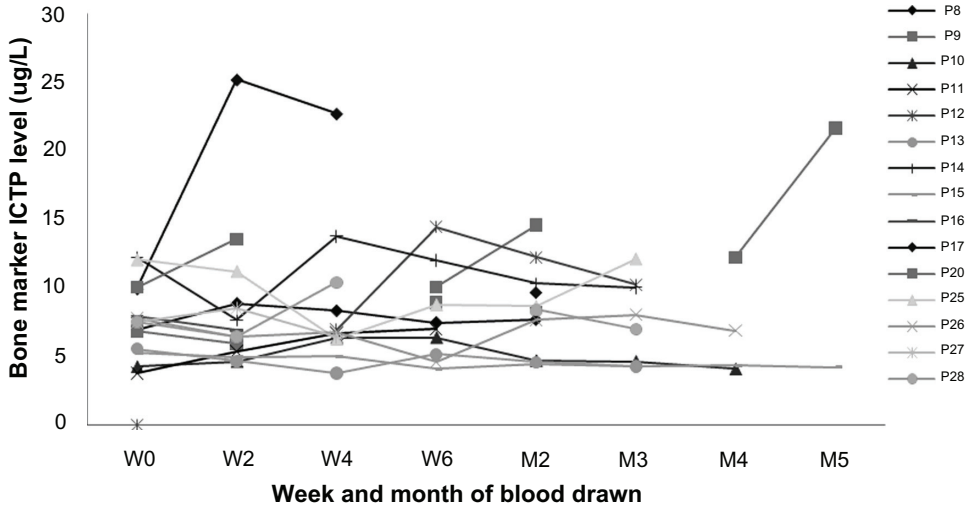

Figure 3 Effect of zoledronic acid on serum carboxyterminal cross-linked telopeptide of type I collagen (ICTP) level in lung cancer patients with bone metastases.

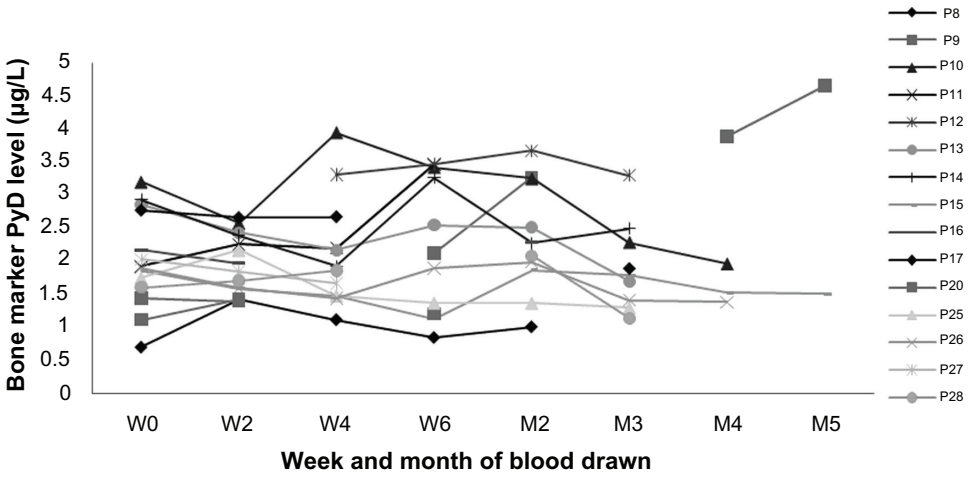

Figure 4 Effect of zoledronic acid on deoxypyridinoline levels in lung cancer patients with bone metastases.
Abbreviation: PyD, deoxypyridinoline.

nificantly during zoledronic acid treatment. Zoledronic acid continued to suppress this bone marker during continuation of treatment (Figure 1). The response of NTx to zoledronic acid treatment showed a faster decrease than bone-specific alkaline phosphatase in this study. These results are consistent with previous studies suggesting that both NTx and bone-specific alkaline phosphatase are useful markers for monitoring the response to zoledronic acid. However, the response of NTx to zoledronic acid is more rapid than that of bone-specific alkaline phosphatase, which may make NTx a more clinically attractive marker.[27,30,34]

ICTP and deoxypyridinoline are sensitive bone turnover markers in patients with multiple myeloma.[35] ICTP and deoxypyridinoline levels have been found to be significantly increased in lung cancer patients with bone metastases compared with those without metastases.[24,36,37] However, ICTP has not been as good as NTx as a predictive marker in lung cancer patients with bone metastases.[38] Even though ICTP and deoxypyridinoline levels were significantly higher in lung cancer patients with bone metastases compared with those without metastases, the data on response of ICTP and deoxypyridinoline to zoledronic acid in lung cancer patients are limited.[21,37] In our study, neither ICTP nor deoxypyridinoline predicted response to bisphosphonates in lung cancer patients (Figures 3 and 4). Karapanagiotou et al also reported that ICTP was not elevated in lung cancer patients.[27] The reason for this is not clear.

Serum TRAP type 5b is a specific marker of bone resorption and represents increased osteoclast activity.[39] As a new

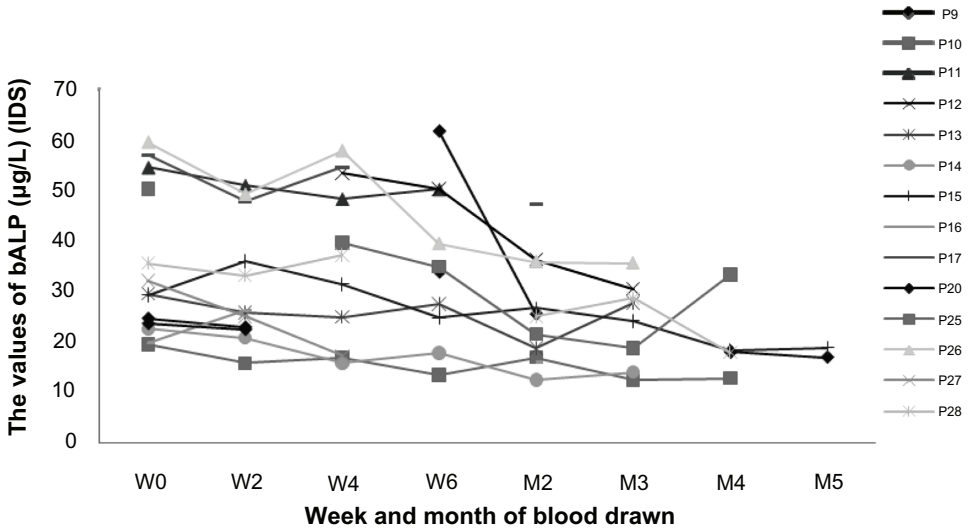

Figure 5 Effect of zoledronic acid on bone-specific alkaline phosphatase levels in lung cancer patients with bone metastases.
Abbreviations: bALP, bone-specific alkaline phosphatase levels; IDS, measured by IDS-iSYS Ostase® bone alkaline phosphatase assay (Immunodiagnostics Systems Limited, Tyne and Wear, UK).

specific marker of bone metastases, it may play an important role in diagnosis, evaluating treatment response, and predicting recurrence or worsening bone metastases in cancer patients.[40] Although some studies in lung cancer patients have shown that TRAP type 5b may be a useful diagnostic and prognostic marker for bone metastasis, others have not confirmed these findings.

Ebert et al reported that TRAP type 5b activity was elevated in lung cancer patients in comparison with healthy controls, but the mean serum TRAP type 5b level did not differentiate bone metastases from nonbone metastases in lung cancer patients.[24] Yao et al investigated the association of TRAP type 5b and lung cancer with bone metastases in 35 newly diagnosed patients and found that high TRAP type 5b activity was correlated significantly with bone metastatic status and worsening pain level.[23]

Studies of the diagnostic value of TRAP type 5b levels in lung cancer with bone metastases have failed to show its bone specificity.[25,41] Most recently, a retrospective study investigated a panel of bone markers, including two bone formation markers, ie, bone-specific alkaline phosphatase and osteocalcin, two bone resorption markers, ie, collagen type 1 cross-linked C-telopeptide and carboxyterminal propeptide of type I collagen, and four osteoclast regulatory proteins, ie, osteoprotegerin, receptor activator of nuclear factor kappa-B ligand, osteopontin, and TRAP type 5b. The results showed no increase in serum levels of bone-specific alkaline phosphatase, carboxyterminal propeptide of type I collagen, or TRAP type 5b in any of the lung cancer groups. However, the results did show an independent association between TRAP type 5b levels and increased risk of disease progression.[27]

Although TRAP type 5b may not be a good diagnostic marker, the present study found a decrease in mean levels by 44.9% within 2 weeks and persistent suppression in patients who continued to receive monthly zoledronic acid (Figure 1). However, the effect of zoledronic acid on metabolic markers was very variable between patients and often inconsistent between markers, possibly reflecting independent effects on bone resorption and formation (eg, chemotherapy, radiation, renal clearance). This study adds to the evidence that TRAP type 5b levels help to monitor bisphosphonate treatment in lung cancer patients. The short response time of TRAP type 5b levels would make them attractive for clinical decision-making. Considering the variability in serological response, larger studies are needed to correlate clinical outcomes with changes in TRAP type 5b levels.

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