ORIGINAL

High serum ALP level is associated with increased risk of denosumab-related hypocalcemia in patients with bone metastases from solid tumors

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Abstract. Metastatic bone disease is one of the most common complications of advanced cancers. Pathological fractures, spinal cord compression, and radiotherapy or surgery to the bone are collectively called skeletal-related events (SREs), which cause severe pain, increase hospitalization rates, and impair the quality of life (QOL) of patients with bone metastases. The receptor activator of nuclear factor-kB ligand (RANKL)/RANK pathway is critical in the progression of bone metastases. Previous studies have demonstrated that an anti-RANKL antibody (denosumab) was superior to zoledronic acid in prolonging time to first SRE in patients with bone metastases from prostate and breast cancers. However, severe hypocalcemic events occur more frequently after treatment with denosumab compared with zoledronic acid. In this study, 368 administrations of denosumab in 219 patients with metastatic bone disease from solid tumors were analyzed to clarify the risk factors for developing hypocalcemia. The results showed that grade 2/3 hypocalcemia was observed in 10.4% of the total number of denosumab administrations. Patients with higher baseline serum ALP, higher performance status (PS), or gastric cancer were at higher risk for developing hypocalcemia. The cut-off value for ALP to predict denosumab-related hypocalcemia was 587 U/L with a sensitivity of 0.77 and a specificity of 0.81. Close monitoring of serum calcium, especially after the first treatment with denosumab, is strongly recommended in these patients.

Key words: Hypocalcemia, Denosumab, Alkaline phosphatase, Performance status

METASTATIC bone disease is one of the most common complications of advanced cancers. The incidence of bone metastases varies among cancer types, but is estimated to be 70-80% in patients with advanced breast and prostate cancers, and 15-30% in patients with lung cancer or other solid tumors [1]. Pathological fractures, spinal cord compression, and radiotherapy or surgery to the bone are collectively called skeletal-related events (SREs), which cause severe pain, increase hospitalization rates, and impair the quality of life (QOL) of patients with bone metastases [2].

There are two classical phenotypes of bone metastases, osteoblastic and osteolytic bone metastases, according to its pathology and radiographic findings. Additionally, there is another form of bone metastases which is undetected by radiograph. It is called intertrabecular metastases, in which tumor cells infiltrate bone marrow space without trabecular bone alteration [3]. Although it is widely believed that prostate and breast cancers produce the prototypic osteoblastic and osteolytic metastases, respectively, bone metastases generally show more heterogeneous nature [4, 5]. Patients can simultaneously have osteosclerotic and osteolytic lesions, or even mixed lesions containing both elements.

Bone remodeling process is dysregulated in each phenotype and an activation of osteoclasts through the receptor activator of nuclear factor-kB ligand (RANKL)/RANK pathway is critical in the progression of bone metastases [6]. Therefore, bisphosphonates and an anti-RANKL antibody (denosumab) are the mainstream pharmacological treatments for bone

metastases. According to the previous clinical studies, denosumab was superior to zoledronic acid in prolonging time to first SRE in patients with bone metastases from prostate and breast cancers [7, 8]. Moreover, a combined analysis of three identically designed phase 3 trials including 5,723 patients with breast cancer, prostate cancer, other solid tumors, or multiple myeloma has demonstrated that denosumab was superior to zoledronic acid in delaying time to first SRE by a median of 8.21 months, reducing the risk of first SRE by 17% [9]. There are other advantages of denosumab over bisphosphophates, such as the subcutaneous rout of administration, no need for dose adjustment according to renal function, and less acute-phase reactions. However, serious hypocalcemia following denosumab treatment has been reported [10-12]. Although oral and intravenous bisphosphonates can induce hypocalcemia, the combined analysis of three phase 3 trials demonstrated that the incidence of grade 2-4 hypocalcemia was higher with denosumab (12.4%) than with zoledronic acid (5.3%) [13]. Hypocalcemia leads to paresthesia, tetany, and sometimes life-threatening complications such as laryngospasm and cardiac arrhythmia. Therefore, patients with severe hypocalcemia need to be hospitalized for close monitoring and intravenous calcium replacement, which adversely affects their QOL.

In September 2012, the risk of severe hypocalcemia related to denosumab for treatment of bone metastases was announced in Japan. Since then, supplementation of calcium (at least 500 mg/day) and vitamin D_3 (at least 400 IU/day) is required in all patients unless corrected serum calcium levels are high. For patients with renal impairment, activated vitamin D_3 and calcium are alternatively prescribed. In spite of these prophylaxes, some patients still develop severe hypocalcemia which requires extended hospitalization. Therefore, we retrospectively analyzed patients with metastatic bone disease who were treated with denosumab at our hospital to clarify the risk factors for developing hypocalcemia.

Patients and Methods

Patients

We retrospectively analyzed 219 patients who received subcutaneous injections of 120 mg denosumab for bone metastases from solid tumors at the University of Tokyo Hospital between May 2012 to May 2015. The use of denosumab was determined by either doc-

tors in charge or members of bone metastasis board of the University of Tokyo Hospital. Patients were eligible for the study if they had been checked for serum calcium concentrations before and after the administrations of denosumab. Patients who received denosumab to treat diseases other than bone metastases from solid tumors, such as multiple myeloma and giant cell tumor of the bone, were excluded. Since it has been reported that the first episode of grade 2 or higher hypocalcemia occurred mostly within 6 months after initiating denosumab [14], data from the initial to the third course were collected in patients who received multiple injections of denosumab. This study was approved by the internal review board of the University of Tokyo.

Data collection

All data including patients' sex, age, type of cancer, Eastern Cooperative Oncology Group performance status (ECOG PS), serum albumin, serum calcium, serum phosphate, serum alkaline phosphatase (ALP), and estimated glomerular filtration rate (eGFR) were collected from the medical record. Serum calcium concentration was corrected by albumin. The reference ranges for adults are 8.4-9.7 mg/dL for corrected calcium, 2.7-4.6 mg/dL for phosphate, 106-332 U/L for ALP. Hypocalcemia was defined as serum corrected calcium less than 8.0 mg/dL in this study, which is the grade 2 hypocalcemia according to the Common Terminology Criteria for Adverse Events. Daily supplementation of calcium carbonate, vitamin D_3, and magnesium carbonate was recommended for patients unless their corrected calcium concentrations are high. For patients with chronic kidney disease with eGFR below 60 mL/min/1.73m^2, calcium lactate and activated vitamin D_3 were used as an alternative.

Statistical analyses

Student's *t* test was used to compare values between the groups. Univariate and multivariate logistic regression analyses were performed to identify the factors associated with hypocalcemia following denosumab administration. In the multivariate logistic regression analysis, the forced entry method was employed using the factors that were $P < 0.05$ in the univariate analyses. Receiver-operator characteristics (ROC) curve was used for ALP to estimate sensitivity, specificity, and cut-off value to predict hypocalcemia. Data were analyzed using JMP11.0 (SAS Institute Inc.). *P* values < 0.05 were considered statistically significant.

Results

Patient characteristics

The cumulative number of denosumab administrations analyzed in this study was 368 (Table 1). The number of patients who received the first, the second, and the third course of denosumab treatment was 180, 103, and 85, respectively. Grade 2/3 hypocalcemia, which is serum corrected calcium level less than 8.0 mg/dL and more than 6.0 mg/dL, was observed 37 times in 368 administrations (10.4%), most of which occurred during the initial course of denosumab treatment (Table 1). The number of patients who developed hypocalcemia for the first time was 5 out of 6 and 1 out of 2 during the second and the third course

of treatment, respectively. Serum ALP and ECOG PS were significantly higher in patients with grade 2/3 hypocalcemia compared to those without hypocalcemia (ALP, 1,362.9±1,252.0 *vs* 469.5±485.2, $P <$ 0.01; ECOG PS, 1.7±1.2 *vs* 1.0±1.1, $P < 0.01$) (Table 1). Baseline serum albumin, serum corrected calcium, serum phosphate, and eGFR were not significantly different between groups. Among different types of primary cancer, lung and prostate cancers had a large number of patients, 66 and 62, respectively (Table 2). Univariate logistic regression analyses showed that the odds ratio to develop hypocalcemia was highest in gastric cancer [odds ratio (OR), 12.768; confidence interval (CI), 4.298-38.901; $P < 0.0001$] (Table 2).

Table 1 Demographics and baseline characteristics of patients

Characteristics	Without hypocalcemia[a] (n=331[b])	With hypocalcemia[a] (n=37[b])	*P* value
Sex, n (male/female)	209/122	27/10	N.S.
Age, years	64.7 ± 14.1 (21-89)	65.6 ± 12.6 (29-90)	N.S.
Serum albumin, g/dL	3.7 ± 0.6 (1.9-4.9)	3.5 ± 0.6 (2.2-4.5)	N.S.
Serum corrected calcium, mg/dL	9.2 ± 0.7 (7.7-16.2)	9.2 ± 1.1 (8-13.4)	N.S.
Serum phosphate, mg/dL	3.2 ± 0.7 (1.4-5.0)	3.4 ± 0.7 (1.9-4.8)	N.S.
eGFR, mL/min/1.73mm^2	76.6 ± 28.2 (7.9-235)	78.3 ± 43.0 (14.3-257.2)	N.S.
Serum ALP, U/L	469.5 ± 485.2 (92-3,872)	1,362.9 ± 1,252.0 (190-5,125)	<0.01
ECOG PS	1.0 ± 1.1 (0-4)	1.7 ± 1.2 (0-4)	<0.01
0	123	7	
1	138	10	
2	27	9	
3	34	10	
4	9	1	
Timing of occurrence of hypocalcemia			
1st course (n=180)	151 (84% [c])	29 (16% [c])	
2nd course (n=103)	97 (94% [c])	6 (6% [c])	
3rd course (n=85)	83 (98% [c])	2 (2% [c])	

[a] Defined as a laboratory grade 2/3 hypocalcemia. [b] The cumulative number of denosumab administrations. [c] % indicates the incidence in each course. Data are shown as means±SD (range).

Table 2 Types of primary cancer

Types of cancer	Without hypocalcemia (n=331) n (%[b])	With hypocalcemia (n=37) n (%[b])	Odds ratio (95% CI)	*P* value
Lung cancer	60 (90.9)	6 (9.1)	0.874 (0.318-2.054)	0.7713
Prostate cancer	54 (87.1)	8 (12.9)	1.415 (0.577-3.133)	0.4273
Colorectal cancer	23 (88.5)	3 (11.5)	1.182 (0.271-3.627)	0.7979
Gastric cancer	7 (46.7)	8 (53.3)	12.768 (4.298-38.901)	< 0.0001
(with gastrectomy)	5	4		
(without gastrectomy)	2	4		
Breast cancer	47 (100)	0 (0)		
Hepatocellular cancer	32 (100)	0 (0)		
Renal cell cancer	28 (100)	0 (0)		
Others[a]	80 (87.0)	12 (13.0)		

[a] Including cancers of bile duct, bladder, ureter, pancreas, uterine, thyroid, colorectal, gastroesophageal junction, duodenum papilla, and cancers of unknown primary origin. [b] % indicates the incidence in each type of cancer.

Factors associated with hypocalcemia

Univariate logistic regression analyses showed that higher serum ALP (OR, 1.134; CI, 1.084-1.195; *P* < 0.0001) and higher ECOG PS (OR, 1.651; CI, 1.244-2.193, *P* = 0.0006) were associated with grade 2/3 hypocalcemia after denosumab administration (Table 3). In multivariate analysis, increased risk of developing hypocalcemia was observed in patients with higher serum ALP (OR, 1.102; CI, 1.048-1.163; *P* < 0.0001), higher ECOG PS (OR, 1.763; CI, 1.237-2.532, *P* = 0.0018), and gastric cancer (OR, 9.110; CI, 2.257-35.951, *P* = 0.0024) (Table 3).

The area under the ROC curve (AUC) obtained by univariate logistic regression analysis of ALP was 0.813 (Fig. 1). The cut-off value for ALP to predict denosumab-related hypocalcemia was 587 U/L with a sensitivity of 0.77 and a specificity of 0.81.

Discussion

In this study, we analyzed baseline characteristics of patients with bone metastases from solid tumors to clarify the risk factors for denosumab-related hypocalcemia. Grade 2/3 hypocalcemia was observed in approximately 10% of the total number of denosumab administrations. As expected, hypocalcemia occurred mostly during the first course of denosumab treatment. In general, serum calcium concentrations were measured 7, 14, and 28 days after denosumab administration in inpatients, and 28 days after administration in outpatients. Therefore, we might have overlooked asymptomatic hypocalcemia in this retrospective analysis, and the incidence of hypocalcemia might be higher. Multivariate logistic regression analysis showed that higher baseline serum ALP levels,

higher ECOG PS, and gastric cancer were risk factors for denosumab-related hypocalcemia.

Bone turnover markers are useful to diagnose bone metastases and to estimate the prognosis of patients with bone metastases [14]. A previous study of patients with prostate cancer with bone metastases has reported that the basal level of bone-specific alkaline phosphatase (BSAP) before the first administration of zoledronic acid was a predictor of mortality [15]. Moreover, a study of ovariectomized monkeys has shown that both higher osteoclast activity and greater
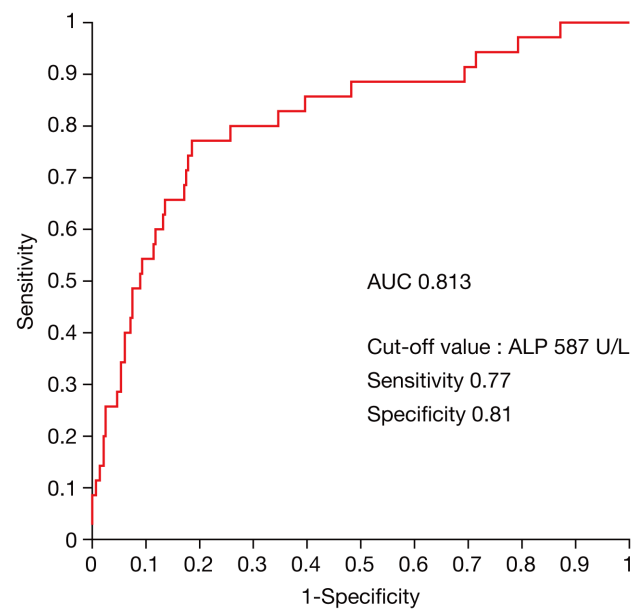

Fig. 1 The area under the ROC curve (AUC) obtained by univariate logistic regression analysis of baseline serum ALP was 0.813. The cut-off value for baseline serum ALP to predict denosumab-related hypocalcemia was 587 U/L with a sensitivity of 0.77 and a specificity of 0.81.

Table 3 Risk factors for denosumab-related hypocalcemia

Baseline characteristics	Univariate analysis		Multivariate analysis	
	Odds ratio (95% CI)	*P* value	Odds ratio (95% CI)	*P* value
Male	1.576 (0.759-3.522)	0.2279		
Age, years	1.005 (0.981-1.032)	0.6760		
Serum albumin, g/dL	0.617 (0.349-1.108)	0.1044		
Baseline serum corrected calcium, mg/dL	1.124 (0.711-1.601)	0.5740		
Baseline serum phosphate, mg/dL	1.412 (0.723-2.805)	0.3135		
eGFR, mL/min/1.73mm^2	1.002 (0.990-1.012)	0.7381		
eGFR (< 60 *vs* ≥60 mL/min/1.73mm^2)	1.377 (0.643-2.811)	0.3987		
Serum ALP (per 100 U/L increase)	1.134 (1.084-1.195)	<0.0001	1.102 (1.048-1.163)	<0.0001
ECOG PS	1.651 (1.244-2.193)	0.0006	1.763 (1.237-2.532)	0.0018
Gastric cancer	12.768 (4.298-38.901)	< 0.0001	9.110 (2.257-35.951)	0.0024

osteoid volume indicated by increased serum BSAP related to greater reduction of calcium after denosumab administration [16]. In human, higher baseline bone turnover markers such as urinary N-telopeptide of type I collagen (uNTx) and serum BSAP have been shown to be risk factors for developing denosumab-related hypocalcemia [13]. We used ALP as an alternative to BSAP, because we could not afford to collect bone turnover markers in this retrospective analysis, and ALP is more accessible than BSAP in daily practice. At the same time, bone turnover markers should be examined in patients with metastases in the liver or hepatobiliary diseases, since increased ALP may not reflect bone metastases in these patients.

The ECOG PS is used to quantify the functional status of cancer patients [17]. PS is an important prognostic factor for survival which is frequently assessed in clinical trials of cancer treatment. In our study, we showed that higher PS with more restricted ordinary life is a risk factor for developing denosumab-related hypocalcemia.

We also found that patients with gastric cancer had the highest odds ratio to develop hypocalcemia among all types of cancer, which is in contrast to the previous studies showing higher risk for patients with prostate or lung cancer [13]. Although patients with gastric cancer showed higher serum ALP levels compared to those with other types of cancer (mean±SD, 1,600.4±1,422.8 *vs.* 517.2±574.6), multivariate analysis demonstrated that gastric cancer is an independent risk factor for developing hypocalcemia after adjusting for ALP levels. While gastrectomy is a well-known risk factor for osteopenia and osteomalacia [18], gastrectomy did not increase risk for developing hypocalcemia in patients with gastric cancer in our study. Therefore, malnutrition from gastrointestinal malfunction in the setting of subclinical vitamin D deficiency may cause hypocalcemia in patients with gastric cancer, irrespective of a history of gastrectomy. It has been reported that hypocalcemia following antiresorptive agents is associated with vitamin D deficiency and insufficient calcium intake [19, 20]. Vitamin D

deficiency is defined as serum 25-hydroxy vitamin D [25(OH)D] values below 20 ng/mL by the Endocrine Society [21], which is widespread in Japan where oral vitamin D supplementation and food fortification are not common. Although we could not evaluate 25(OH)D levels in our subjects, we believe that patients with advanced cancers are especially at high risk for vitamin D deficiency because of inadequate sun exposure and malnutrition. Our results suggest that patients with advanced disease, high tumor burden, and comorbid conditions such as gastrointestinal malfunction are highly likely to develop hypocalcemia, and correction of vitamin D deficiency before starting denosumab treatment might be effective to prevent hypocalcemia in these patients.

Baseline eGFR values in our study were not significantly different between patients with or without hypocalcemia, in contrast to previous studies which have indicated that renal impairment is one of the risk factors to develop hypocalcemia [10, 11, 13]. The reason is probably because prophylactic administrations of calcium lactate and activated vitamin D_3 in patients with impaired kidney function at our hospital was effective to prevent hypocalcemia. At the same time, there is a possibility that eGFR calculated from serum creatinine is overestimated in patients with malnutrition and low muscle mass.

In summary, hypocalcemia was observed in approximately 10% of the total number denosumab administrations in our study. Patients with higher baseline serum ALP, higher ECOG PS, or gastric cancer were at higher risk for developing hypocalcemia. In these patients, conventional prophylactic administration of vitamin D_3 and calcium could not always prevent hypocalcemia, and close monitoring of serum calcium, especially after the first treatment with denosumab, is strongly recommended.

Disclosure

None of the authors have any potential conflicts of interest associated with this research.

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