

## Evaluation of Bone Turnover / Quality Markers and Bone Mineral Density in Prostate Cancer Patients Receiving Androgen Deprivation Therapy with or without Denosumab

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**Abstract.** *Background/Aim:* Androgen deprivation therapy (ADT) is a mainstay therapy for prostate cancer (PCa). ADT induces bone loss and increases the risk of osteoporosis and fractures. Recently, loss of bone quality has received attention as a factor that causes loss of bone strength independent of bone mineral density (BMD). Pentosidine has been identified as a surrogate marker of bone quality. Therefore, bone quality markers were evaluated retrospectively in PCa patients receiving ADT with or without denosumab. *Patients and Methods:* This study included 46 PCa patients. Twenty patients received denosumab. We measured pentosidine as bone quality marker and TRACP-5b as bone turnover marker. Pre- and 12-month BMD was measured in the lumbar spine and femoral neck. *Results:* In the denosumab group (D+), BMD at the lumbar spine was increased by 6.7% compared with the group that did not receive denosumab (D-) at 12 months ( $p=0.0015$ ). BMD at the femoral neck was increased by 3.1% at 12 months ( $p=0.0076$ ). The mean value of TRAP-5b was lower in the D+ group than the D- group at 12 months ( $p<0.001$ ). The mean serum levels of pentosidine in the D+ group were decreased by -39.6% compared with the D- group at 12 months ( $p=0.0036$ ). *Conclusion:* Denosumab increased BMD during ADT for PCa and inhibited the increasing levels of serum pentosidine in PCa patients undergoing ADT.

Prostate cancer (PCa) is the most common cancer in males in Western countries and its incidence has increased in Japan (1). Androgen deprivation therapy (ADT) with gonadotropin-

releasing hormone (GnRH) agonists, GnRH antagonists or bilateral orchiectomy is a well-established treatment for advanced PCa (2). ADT is also used to manage cases of localized PCa when radical treatment cannot be administered (3). The administration of ADT improves disease-free survival and overall survival in men with locally advanced PCa treated with radiation therapy (4). ADT functions by decreasing testosterone to castrate levels. ADT also markedly decreases serum estradiol levels because estradiol is derived from the peripheral conversion of testosterone by aromatase, also called estrogen synthase. Therefore, side-effects associated with estrogen deficiency are also observed. Estradiol plays a role in bone formation and bone resorption in men (5). Therefore, ADT decreases bone mineral density (BMD) and increases the incidence of clinical fractures (6, 7). The risk of fracture increases with an increasing duration of ADT (6, 7). Patients with prostate carcinoma bone metastases are faced with the risk of skeletal complications, including pathologic fractures, which are collectively termed skeletal-related events (SREs) (8). SREs are associated with mortality, increased pain, decreased quality of life and increased treatment costs (9).

Although several drugs, including bisphosphonates and selective estrogen-receptor modulators, can prevent a decreased BMD, clinical trials of those drugs have revealed that the effects on fracture prevention are insufficient (10, 11). In contrast, denosumab was found to increase BMD at all sites and decrease the incidence of new vertebral fractures among men receiving ADT for non-metastatic PCa (12). Furthermore, denosumab decreased the risk of skeletal complications compared to zoledronic acid in castration-resistant prostate cancer (CRPC) patients with bone metastases (13). The administration of denosumab decreased the serum levels of bone turnover markers (BTMs), including type-1 C-teropeptide (sCTX) and tartrate-resistant alkaline phosphatase 5b (TRAP-5b) compared to placebo (14). Therefore, BTMs are often used to predict the effects of denosumab during CRPC patient care.

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**Key Words:** Prostate cancer, denosumab, bone quality, pentosidine.

Table I. Clinical characteristics of all patients.

Characteristics	All	Denosumab (–)	Denosumab (+)	
No. of patients	46	26	20	
Age (mean±S.D.)	73.8±7.0	74.6±5.9	72.1±8.0	<i>p</i> =0.239
Initial PSA (median±S.D.)	11.0±494.1	9.59±55.0	12.7±735.4	<i>p</i> =0.129
Stage				
T1cN0M0	9	4	5	
T2N0M0	14	11	3	
T3N0M0	8	6	2	
T4N0M0	2	1	1	
TanyN1M0	3	2	1	
TanyN0M1	5	1	4	
TanyN1M1	5	1	4	
Metastasis				
All distant metastasis	10	2	8	
Bone metastasis	7	1	6	
Visceral metastasis	3	1	2	
Gleason score				
GS 6	6	6	0	
GS 7	12	5	7	
GS ≥8	28	15	13	
BMD of lumbar spine (g/cm <sup>3</sup> )	1.026±0.249	1.064±0.181	0.982±0.321	<i>p</i> =0.198
BMD of femoral neck (g/cm <sup>3</sup> )	0.699±0.098	0.731±0.106	0.671±0.094	<i>p</i> =0.044

S.D., Standard deviation; PSA, prostate-specific antigen; BMD, bone mineral density.

Bone strength is determined by BMD and bone quality (15). The bone matrix consists of a two-phase composite material in which the mineral phase provides stiffness and collagen fibers provide tensile strength. Collagen cross-linking plays important roles in the biological and biomechanical features of bone. Serum or urine pentosidine, a non-enzymatic collagen cross-linking element, and homocysteine levels are used to estimate the future fracture risk in osteoporosis (16, 17).

In the current study, bone quality markers, BTMs and BMD were evaluated retrospectively in PCa patients receiving ADT with or without denosumab.

## Patients and Methods

**Patients.** This study included 46 patients with histologically confirmed PCa who were diagnosed at Gunma University Hospital (Maebashi, Japan) and evaluated retrospectively. Table I lists the clinical characteristics of the enrolled patients. Patients' ages ranged from 60-87 years with a mean of 73.8 years. The clinical stage was T1cN0M0 in nine, T2N0M0 in 14, T3N0M0 in eight, T4N0M0 in two, TanyN1M0 in three, TanyN0M1 in five and TanyN1M1 in five patients. Ten patients had metastasis of which seven had bone and three visceral metastases. All patients received a monthly subcutaneous injection of degarelix (240 mg for the first month followed by maintenance doses of 80 mg). Four patients were treated with bicalutamide as part of a combined anti-androgen blockade. We

administered denosumab for patients who had bone metastasis or were diagnosed with osteoporosis at baseline. Twenty patients received denosumab (the D+ group): 16 of them received 60 mg every 6 months (Pralia®; Daiichi-Sankyo, Tokyo, Japan) to treat osteoporosis and prevent loss of BMD, while four received 120 mg/month (Ranmark®; Daiichi-Sankyo) to treat bone metastasis. The Institutional Review Board of the Gunma University Hospital approved this study and written consent was obtained from all enrolled patients.

**Measurement of BMD.** BMD was measured in the lumbar spine and femoral neck at baseline and after 12 months using dual X-ray absorptiometry with the Horizon DXA system (Hologic, Marlborough, MA USA). Bone metastasis was not observed at the measured site in all patients.

**Blood samples and measurement of bone turnover and quality markers.** Baseline blood samples were collected from all patients, whereas post-treatment samples were taken after 6 and 12 months of treatment. Serum was stored at –80°C until measurements. BTM and bone quality markers were assessed by SRL Inc. (Tokyo, Japan). TRACP-5b was measured using enzyme immunoassays (EIAs) and pentosidine was measured using enzyme-linked immunosorbent assays (ELISAs).

**Statistical analysis.** Values are expressed as means±standard deviations and were compared using Student's *t*-tests. A *p*-value <0.05 was considered significant. Analysis of variance (ANOVA) and the Tukey-Kramer method were used to analyze changes in bone markers between pretreatment and the other time points.

Table II. Changes in BMD and bone turnover and quality markers.

	Denosumab (–) (n=26)		Denosumab (+) (n=20)	
	BMD (g/cm <sup>3</sup> , mean±S.D.) (mean percent changes from baseline, %)			
	Lumbar	Femoral neck	Lumbar	Femoral neck
Baseline	1.064±0.181	0.731±0.106	0.982±0.321	0.671±0.094
12 months	1.024±0.174* (–3.3%)	0.713±0.114* (–3.3%)	1.011±0.322* (+3.4% <sup>†</sup> )	0.673±0.095 (+0.43% <sup>†</sup> )
	TRACP-5b (mU/dl, mean±S.D.)			
Baseline	219.1±166.2 mU/dl		328.8±462.3 mU/dl	
6 months	368.4±175.1 mU/dl*		399.2±318.8 mU/dl	
12 months	426.1±167.1 mU/dl*		198.9±190.4 mU/dl*	
	Pentosidine (µg/ml, mean±S.D.) (mean percent change from baseline, %)			
Baseline	0.0798±0.024		0.1059±0.053	
6 months	0.0950±0.027 (+25.4%)		0.0882±0.023 (–4.35% <sup>†</sup> )	
12 months	0.0986±0.027* (+34.7%)		0.0859±0.019 (–4.94% <sup>†</sup> )	

BMD, Bone mineral density; TRAP-5b, tartrate-resistant alkaline phosphatase 5b; S.D., standard deviation. \* $p < 0.05$  vs. baseline, <sup>†</sup> $p < 0.05$  vs. denosumab (–) group.

## Results

All 46 patients received the luteinizing hormone-releasing hormone (LHRH)-antagonist degarelix. The median prostate-specific antigen (PSA) level at baseline was 11.0 ng/ml, which declined to 0.44 ng/ml after 12 months of ADT treatment. There was no prostate carcinoma progression in the 46 cases at 12 months. There was a significant change in the BMD of the femoral neck between the group that did not receive denosumab (D–) and the one that received denosumab (D+) group at baseline (0.731 g/cm<sup>3</sup> vs. 0.671 g/cm<sup>3</sup>, respectively;  $p = 0.044$ ).

**BMD.** Denosumab was associated with an increased BMD at the lumbar spine and femoral neck (Table II). In the D+ group, BMD at the lumbar spine was increased by 6.7% compared with the D– group at 12 months (+3.4% vs. –3.3%, respectively;  $p < 0.001$ ). The BMD at the femoral neck was increased by 3.7% in the D+ group compared with the D– group at 12 months (+0.43% vs. –3.3%, respectively;  $p = 0.007$ ). In the D+ group, the BMD of the lumbar spine was increased significantly at 12 months compared with baseline (baseline, 0.981 g/cm<sup>3</sup>; 12 months, 1.011 g/cm<sup>3</sup>,  $p = 0.013$ ). However, there was no significant increase at the femoral neck (baseline, 0.671 g/cm<sup>3</sup>; 12 months, 0.673 g/cm<sup>3</sup>;  $p = 0.755$ ). In the D– group, the BMD of the lumbar spine and femoral neck was decreased significantly at 12 months

compared to baseline (lumbar: baseline, 1.064 g/cm<sup>3</sup> and 12 months, 1.024 g/cm<sup>3</sup>,  $p < 0.001$ ; femoral neck: baseline, 0.731 g/cm<sup>3</sup> and 12 months, 0.713 g/cm<sup>3</sup>;  $p < 0.001$ ).

**Changes in bone turnover and quality markers.** Table II lists the changes in bone turnover and quality markers. After 12 months of treatment with denosumab, the mean TRACP-5b levels decreased significantly in the D+ group from baseline (328.8 mU/dl vs. 198.9 mU/dl,  $p < 0.05$ ). In contrast, the mean TRACP-5b levels in the D– group increased significantly between baseline and 6 months (219.1 mU/dl to 368.4 mU/dl  $p < 0.05$ ) and between baseline and 12 months (219.1 mU/dl to 426.1 mU/dl  $p < 0.05$ ). The mean serum levels of pentosidine in the D+ group decreased between baseline and 12 months, but not significantly (0.1059 µg/ml to 0.0859 µg/ml,  $p = 0.168$ ). In contrast, the serum pentosidine levels increased significantly in the D– group (from 0.0798 µg/ml at baseline to 0.0986 µg/ml at 12 months,  $p = 0.023$ ). When the percent change was examined, serum pentosidine levels were decreased by 4.9% in the D+ group compared with the D– group at 12 months (–4.94% vs. +34.7%,  $p < 0.001$ ).

## Discussion

In this study of men receiving ADT for PCa, patients who also received denosumab for 12 months experienced a significant increase in BMD at the lumbar spine and femoral

neck compared to patients who did not receive denosumab. A significant decline in the levels of the bone turnover marker TRACP-5b and the bone quality marker pentosidine was observed in patients receiving denosumab compared to patients who did not receive denosumab.

Smith *et al.* have reported many effects of denosumab administration in PCa patients receiving ADT. For example, denosumab significantly increased BMD and decreased the incidence of new vertebral fractures among men receiving ADT for non-metastatic PCa (12). The current study found a similar percent change in BMD at 12 months. Smith *et al.* also reported that the levels of bone turnover markers declined (12). The current study confirmed these results by measuring the change in TRACP-5b levels. Inhibiting osteoclast activity with denosumab prevented a decrease in bone density and reduced levels of bone turnover markers, such as TRACP-5b, thus reflecting a decrease in osteoclast activity. Therefore, denosumab plays a major role in the bone management of PCa patients treated with ADT and functions by preventing a reduction in BMD (14).

Recent research has reported that bone quality is an important factor determining bone strength, independent of BMD (15). The proposed determinants of bone quality are the accumulation of micro-damage and the formation of collagen cross-links (18). Both of these processes are regulated by cellular activities and tissue turnover rate (15). Impaired enzymatic cross-linking and/or increased non-enzymatic cross-links in bone collagen have been proposed as determinants of impaired bone mechanical properties during aging, osteoporosis and diabetes mellitus (19). Reduced bone turnover allows the formation of additional collagen cross-links *via* non-enzymatic means, resulting in the accumulation of non-enzymatic advanced glycation end-products (AGEs) in bone tissue (20). Bone collagen glycation allows micro-damage in bones to spread more easily, thereby increasing the total crack of surface density and making bone tissue more brittle and more likely to fracture (21). Pentosidine, one of several AGEs' cross-links, has been quantified in bone and serum (22). Its accumulation is associated with age-related degradation in the mechanical properties of bone (17, 23). Pentosidine levels are higher in the bones of patients with femoral neck fractures compared with age-matched controls (24, 25). Serum pentosidine concentrations were positively correlated with the radiographic severity of lumbar spondylosis in a cross-sectional study involving Japanese subjects (26). Higher urine pentosidine levels represent a risk factor for fracture in older adults with diabetes and may also account, in part, for the reduced bone strength observed in type 2 diabetes (27). Yamamoto *et al.* suggested that serum pentosidine levels may be associated with prevalent vertebral fractures in post-menopausal women with type 2 diabetes and serum pentosidine might reflect bone quality (28). Vaculik *et al.*

reported that patients with hip fractures had higher serum and bone pentosidine concentrations than those with advanced-stage hip osteoarthritis. Therefore, they suggested that serum pentosidine is a potential biomarker for identifying subjects with impaired bone quality and strength (29). Together, these findings suggest a correlation between the concentration of serum, bone or urine pentosidine and an increased risk of fracture.

This study had several limitations. First, the investigated cohort of patients was small, meaning that this research has to be expanded using a larger study population. Second, the D+ group contained patients who received different doses of denosumab. However, when we analyzed the percent change in BMD, we observed similar trends between TRACP-5b and pentosidine, in patients treated by denosumab without metastases (n=12) and patients without denosumab treatment (data not shown). Third, since this is a retrospective study, there is a possibility that treatment intervention by denosumab might have affected the patients' lifestyle, behavior and metabolism. It may be that the changes of patients' lifestyle, behavior and metabolism indirectly influenced the dynamics of pentosidine.

In summary, the current study demonstrated that serum pentosidine increased significantly during 12 months in PCa patients undergoing ADT. Additionally, denosumab increased BMD and inhibited bone quality markers, such as serum pentosidine as compared to patients who never took denosumab.

## Conflicts of Interest

Yoshiyuki Miyazawa, Takahiro Syuto, Yoshitaka Sekine, Masashi Nomura, Hidekazu Koike, Hiroshi Matsui, Yasuhiro Shibata and Kazuto Ito declare that there are no conflicts of interest that could be perceived as prejudicing the impartiality of the research reported. Kazuhiro Suzuki is a recipient of research grants and honoraria from Daiichi-Sankyo Co. Ltd.

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