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Case Report

Acute hypercalcemia and excessive bone resorption following anti-RANKL withdrawal: Case report and brief literature review

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ABSTRACT

Denosumab is an anti-RANKL antibody that is commonly used for the treatment of osteoporosis; in oncology, bisphosphonates and denosumab have become the standard therapies for the treatment and prevention of skeletal complications in patients with myeloma and solid tumors. In recent years, excessive bone remodeling following the discontinuation of denosumab has raised concerns. Several cases of hypercalcemia have been reported after the discontinuation of high-dose denosumab (120 mg every 4 weeks), mainly in children. In this study, we report a new case of severe refractory hypercalcemia in a 54-year-old woman who received high-dose denosumab for 5 years as an adjuvant therapy for breast cancer. She is currently in remission and undergoing treatment with anastrazole, an aromatase inhibitor. The peculiarities of this case are the presence of associated bone pain with subperiosteal bone resorption on hand X-rays, and diffuse, long bone diaphyseal uptake on a bone scan. Hyperparathyroidism has been ruled out, and existing evidence suggests that this high-level of bone remodeling could be due to a rebound hyperactivation of the RANKL pathway. In addition to rehydration, repeated use of i.v. bisphosphonates was required to control recurrent hypercalcemia. As hypercalcemia is a serious metabolic complication, a gradual dose reduction should be considered when interruption of high dose denosumab therapy is planned.

1. Introduction

Major advances in the understanding of the molecular regulation of bone physiology have benefited from the identification of the receptor activator of NF-κB ligand (RANKL) pathway as a central modulator of bone resorption. RANKL is a membrane-bound or soluble glycoprotein that belongs to the TNFα family, and is expressed in bone tissue by osteocytes and osteoblasts. By cell contact, or by binding of the soluble ligand, the trimeric RANKL interacts with the RANK receptor expressed by osteoclasts to play an essential role in the differentiation, survival, and bone-resorbing activity of these cells. The fine-tuning of bone resorption also involves osteoprotegerin (OPG), a secreted decoy RANKL receptor that competes with RANK. Owing to its regulatory role in bone resorption, RANKL has rapidly garnered attention as a therapeutic target in osteoporosis and malignant osteolysis [1].

Denosumab, a human monoclonal antibody against RANKL, is being used clinically to reduce the risk of osteoporotic fractures at low doses (60 mg s.c. every 6 months). However, occurrences of rapid bone loss and multiple vertebral fractures have recently been noted after the discontinuation of denosumab in postmenopausal women treated for osteoporosis [2,3]. Denosumab is also currently used at high doses (120 mg every 4 to 12 weeks) for the prevention of skeletal-related events in patients with myeloma or metastatic bone disease [4,5]. A few cases of asymptomatic hypercalcemia have been reported following the discontinuation of denosumab at high doses in children, but only one case has been reported in a postmenopausal woman with osteoporosis [6].

We report a case of severe hypercalcemia following the discontinuation of denosumab at high doses in an adult who presented with bone pain associated with subperiosteal bone resorption. We believe that the bone involvement described here illustrates the excessive bone remodeling observed following the sudden discontinuation of

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high-dose denosumab. This case also raises the question about how this drug should be discontinued to maintain its benefits and avoid this unique side effect.

2. Case report

A 54-year-old woman was evaluated at the metabolic bone disease clinic in 2017 for refractory hypercalcemia. Her main personal medical history was breast cancer (T1cN1M0) diagnosed in November 2011 (at age 48). Her cancer was an estrogen receptor-positive tumor, which was treated by partial mastectomy and axillary dissection, followed by chemotherapy [5-fluorouracil, epirubicin, and cyclophosphamide, followed by docetaxel (in 2012)] and adjuvant radiotherapy. Tamoxifen was prescribed from 2011 to 2013, then anastrazole (Arimidex®) has been prescribed until 2018. As part of the management of her cancer, she also participated in a phase III clinical trial evaluating denosumab as an adjuvant therapy to prevent bone metastasis in patients with early breast cancer, and she received denosumab (XGeva®) at a dose of 120 mg delivered subcutaneously every month for 6 months, then every 3 months for a total of 5 years (last dose in February 2017). Follow-up reports have included bone scans (January 2013 and December 2016), which were normal and did not show evidence of bone metastases. The last breast MRI (February 2016) and mammogram (December 2016) did not show any evidence of recurrence. Chest and abdominal CT scans did not indicate any significant abnormalities (December 2016).

The patient was also known to suffer from migraines and cervical dystonia, fibrocystic breast disease, and had one episode of acute pyelonephritis. She stopped smoking in 2012 (10 pack-years), and her regular medication list included 500 mg calcium with 400 IU vitamin D3 per day (after chemotherapy-induced menopause), and citalopram.

Starting June 2017, the patient reported diffuse, mixed pattern pain, which occurred predominantly in the thighs and also in the arms, forearms, and hands, and required regular analgesia. Concomitantly, she experienced polyuro-polydipsic syndrome, as well as severe constipation and asthenia. On physical examination (June and August 2017), no synovitis was noted, and her weight was stable (55 kg). A biological assessment (June 2017) showed a total calcium level, corrected for albumin, of 3.1 mmol/L (normal range (N) 2.07–2.55), an ionized calcium level of 1.55 mmol/L (N 1.02–1.31), a serum phosphorus level of 1.3 mmol/L (N 0.87–1.45), and a phosphate reabsorption rate of 91%. The parathormone (PTH) level was low (1.5 pmol/L, N 1.6–6.9), PTH-related protein (PTHrP) level was normal, as were the levels of thyroid-stimulating hormone, alkaline phosphatase, and lactate dehydrogenase. The serum 25-OH vitamin D level was 115 nmol/L (N 75–117), the level of 1,25(OH)2D was low, 22 pmol/L (N 63–228), and an analysis of serum and urinary proteins by electrophoresis was normal, with no monoclonal components or light chains. A slight change in renal function was observed (clearance 70 cc/min, 100 cc/min after hydration).

A FDG PET-CT (June 2017) did not show any evidence of neoplasia or bone lesions. The ^{99m}Tc-MDP bone scan performed in August 2017 showed diffuse cortical uptake of the long bones, mostly of the lower limbs, with no bone metastases or identifiable lesions seen on the low-dose CT (Fig. 1).

Hand X-rays revealed subperiosteal bone resorption of the phalanges (radial side) and a discreet resorption of the phalangeal tuft of the fourth finger of each hand (Fig. 2). Slight periosteal thickening was found on the right proximal femur diaphysis, but the tibias appeared normal.

In June 2017, hypercalcemia was treated with rehydration and bisphosphonates (90 mg pamidronate i.v.). Bisphosphonates were administered in August, September, and October at a dose of 90 mg i.v. because of recurrent increases in serum calcium levels (Fig. 3). Biochemical markers of bone resorption and bone formation were evaluated in November 2017, and both were elevated (C-telopeptide of type I collagen: 0.669 µg/L, and osteocalcin: 64 µg/L). Calcium levels normalized in October 2017 (Fig. 3) and remained stable since.

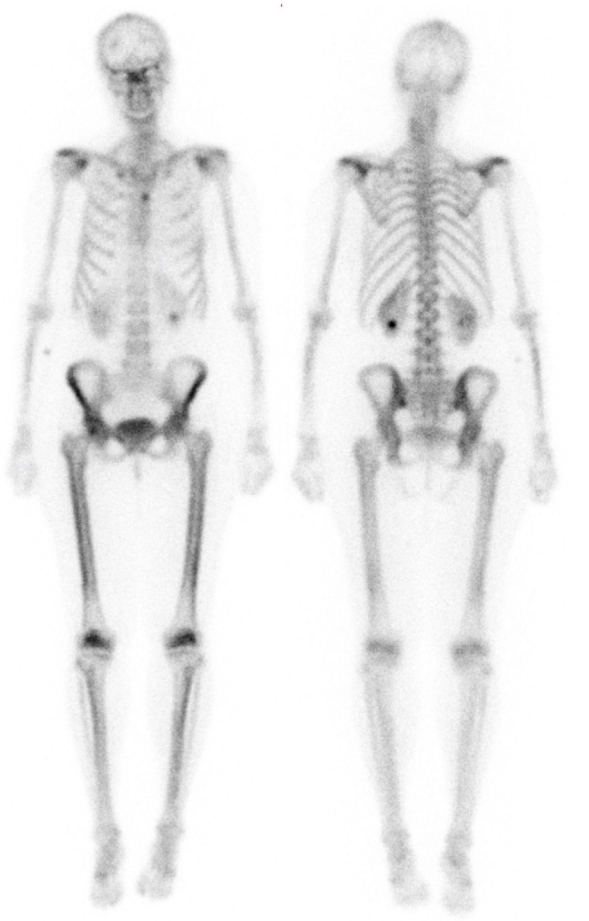

Fig. 1. Bone scintigraphy (^{99m}Tc-MDP): Diffuse cortical uptake of the femoral and tibial diaphyses, the distal ends and heads of the femurs, the pelvic bones, both radii, both humeri and the glenoids.

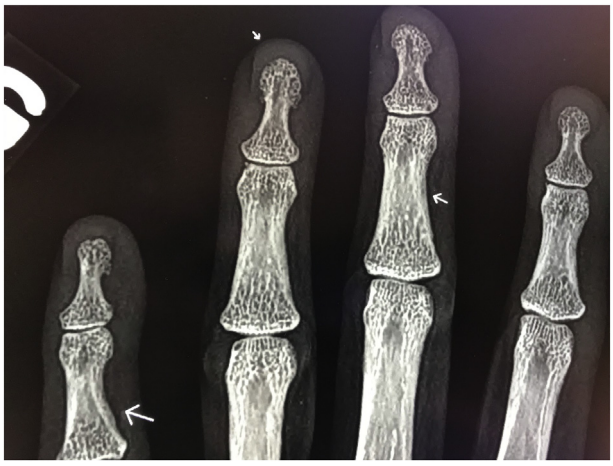

Fig. 2. X-Rays: Discreet resorption of the phalangeal tuft of the fourth finger (small arrow), and subperiosteal bone resorption of the radial side of the phalanges (large arrows).

The patient clinically improved after 3 months of bisphosphonate treatment and good hydration. Control scintigraphy (December 2017) did not reveal bone metastases but showed a decrease in cortical bone remodeling. In July 2018, bone mineral density (BMD) was found significantly decreased compared to values obtained in 2016: decreasing by 16% at the lumbar spine (T score: −2.9; 0.849 g/cm^2) and 10.7% at

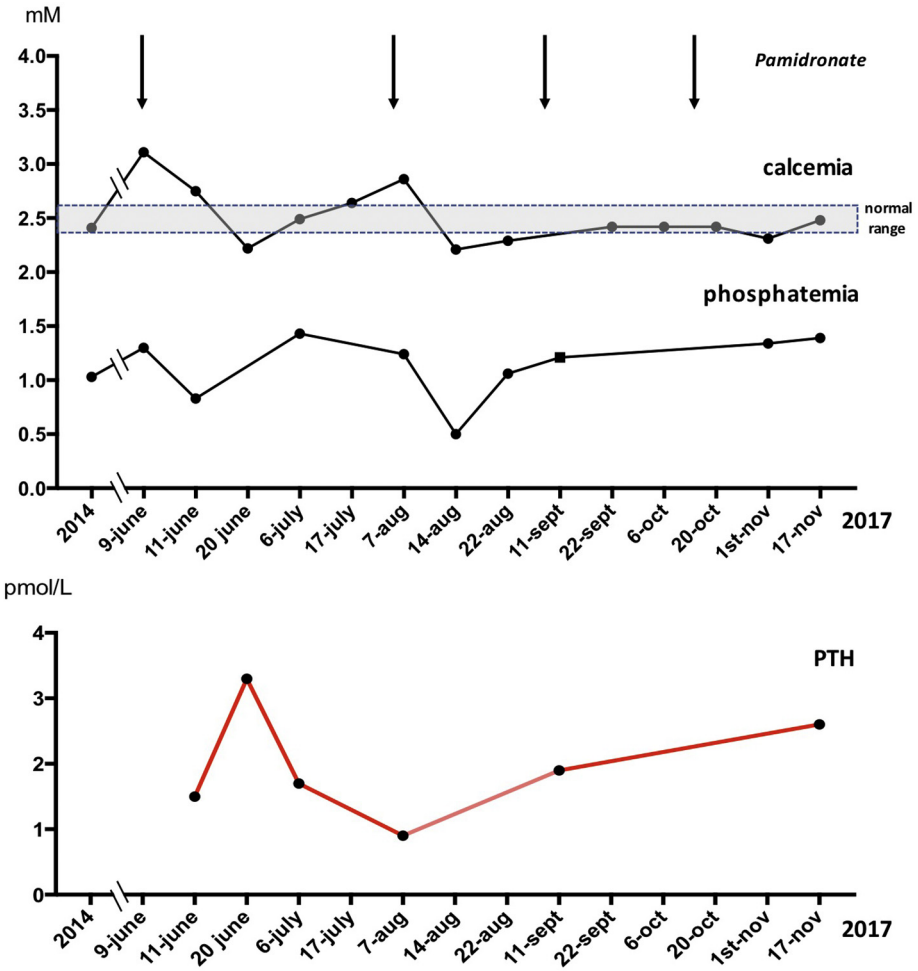

Fig. 3. Treatment of hypercalcemia with pamidronate: A rebound hypercalcemia was observed following denosumab discontinuation. Graphs with serum calcium levels (corrected for albumin), phosphorus levels and PTH levels are presented, infusions of pamidronate 90 mg i.v. are indicated by vertical arrows.

the femoral neck (T score: −0.9; 0.870 g/cm^2). No vertebral fractures were observed.

The diagnosis was RANKL-related hypercalcemia with high bone resorption and a major increase in bone turnover likely due to a rebound effect following the discontinuation of long-term treatment with high-dose denosumab. No other cause of hypercalcemia was found (no hyperparathyroidism, bone metastases, or paraneoplastic hypercalcemia).

3. Discussion

We report a case of a 54-year-old female with breast cancer in remission (without bone metastasis) whose treatment included long-term, high-dose denosumab (120 mg s.c. every 3 months for 5 years after an initial loading with 120 mg every month for 6 months) and who developed severe refractory hypercalcemia 4 months after the last dose, and a drastic bone loss in the ensuing year, for which the intake of aromatase inhibitor could have contributed.

On the basis of the clinical and paraclinical observations associated with this case of hypercalcemia, primary, secondary, and paraneoplastic hyperparathyroidism were all excluded from the differential diagnosis, as were the presence of bone metastases or progressive neoplasia. The observed increase in bone remodeling was at the expense of both the cortical and trabecular bone, as illustrated by the huge decrease in BMD at both sites, and was associated with subperiosteal bone resorption on hand X-rays, along with diffuse, increased uptake on the bone scan, suggestive of high-level bone remodeling. Subperiosteal bone resorption is typical, and sometimes thought to be diagnostic, of hyperparathyroidism and has also been described in adult T-cell leukemia/lymphoma with an overproduction of PTHrP [7,8]. In our case, no hyperparathyroidism was observed, but the RANKL pathway appeared to be involved, directly or indirectly, as the rebound of bone remodeling and hypercalcemia followed the stopping of its prolonged inhibition by denosumab treatment. Interestingly, PTH is a potent inducer of RANKL expression; the signals induced by PTH receptor activation have been shown to induce bone resorption in the adult skeleton by directly upregulating RANKL gene expression in osteocytes [9]. Thus, the overproduction of RANKL caused by an excessive production of PTH is responsible for PTH-induced bone lesions [10]. These data help to understand that hyperactivation of the RANKL pathway may induce bone lesions that mimic hyperparathyroidism. The administration of exogenous RANKL to mice was shown to cause severe hypercalcemia and drastic increases in bone resorption [11]. Transgenic mice expressing human RANKL have severe osteoporosis-associated trabecular bone loss and severe intracortical porosity, particularly in the mid-diaphyseal cortex [12]. Therefore, an excessive activation of the RANKL–RANK pathway may be involved in the observed rebound effect on bone turnover following the discontinuation of denosumab, but the precise molecular mechanism responsible remains unknown.

The deleterious impact of the discontinuation of denosumab on bone was initially reported in cases of postmenopausal osteoporosis, with increases in bone remodeling and bone loss observed during the year following denosumab withdrawal after long-term, low-dose

therapy (60 mg every 6 months), and an increase in the risk of multiple vertebral fractures in a small number of patients [2,3]. High doses of denosumab are typically used in oncology protocols (120 mg monthly to every 3 months). Asymptomatic hypercalcemia has previously been reported in children after the discontinuation of high-dose denosumab when prescribed as an adjuvant therapy in giant cell tumors of bone (GCTB) [13–15]. Other pediatric cases of hypercalcemia have been reported after the discontinuation of denosumab prescribed for juvenile Paget's disease [16], fibrous dysplasia [17], and osteogenesis imperfecta type VI [18]. A young adult treated for GCTB also experienced hypercalcemia after the discontinuation of denosumab [15]. Interestingly, hypercalcemia as a rebound effect has been reported in diseases involving the aberrant expression of RANKL or OPG: overexpression of RANKL by the mesenchymal tumor fraction of GCTBs, which leads to the formation of numerous osteoclasts [19], or an *opg* gene mutation in juvenile Paget's disease [20]. Therefore, some conditions may cause a predisposition to the excessive bone remodeling and hypercalcemia associated with the cessation of RANKL inhibition, especially in children with an actively growing skeleton. Only one case of hypercalcemia 6 months after the discontinuation of denosumab has been reported in a woman treated with low-dose denosumab for 10 years for postmenopausal osteoporosis [6]. A bone scan was only reported in this case, without any evidence of diffuse bone hypermetabolism [6].

Denosumab is a human anti-RANKL IgG2 antibody that does not exert cytotoxicity after binding to its membrane-associated ligand [21]. Pharmacological studies of denosumab in patients with multiple myeloma or breast cancer metastases have shown that denosumab has a rapid onset of action (24 h), which can last up to 84 days at high doses following injection, and a half-life that increases five-fold with increasing dosage (0.1 vs. 3 mg/kg), reaching 33 and 46 days at a dose of 3 mg/kg in patients with myeloma and breast cancer, respectively [21]. In postmenopausal women, the half-life of denosumab is approximately 30 days after a 60-mg dose, with a significant decrease in serum NTX levels (N-telopeptide of type I collagen) up to 6 months compared to placebo [22]. Elimination of the drug includes receptor-mediated endocytosis and linear pharmacokinetics involving protein degradation by reticuloendothelial cells, antibody opsonization, and binding to the Fcγ receptor [22]. Many interactions exist during the synthesis [23], trafficking [24] and degradation [25,26] of the components of the RANKL pathway, with an inverse regulation of the expression of RANKL and OPG in osteoblasts [27]. Denosumab binding to membrane-bound or soluble RANKL competes with soluble OPG and membrane-bound RANK, and a new equilibrium is thus established between these different components [28]; the impact of stopping denosumab on both the RANKL pathway and bone remodeling after long-term treatment may depend on both the treatment protocol and the type of underlying bone pathology, but also the preexisting steady state between RANKL, RANK, and OPG. Outcomes may therefore differ between individuals, and may occur at variable times after the discontinuation of anti-RANKL therapy. After high-dose denosumab, hypercalcemia has been observed from 7 weeks to 7 months after the final treatment [13–18]. In the present case report, symptoms appeared 4 months after the last injection. In nearly all reported cases, hypercalcemia was often associated with acute renal failure and typically responded to hydration and repeated administration of bisphosphonates. In one case, calcitonin-refractory hypercalcemia recurrent despite i.v. bisphosphonates was controlled by low-dose injections of denosumab [13].

Bone lesions and hypercalcemia were associated with high levels of bone markers (resorption and formation) even several months after the acute episode, and even after bisphosphonate therapy (pamidronate). This suggests that the bone imbalance caused by stopping denosumab persisted for several months. In addition, the biological activity of bisphosphonates is related to their relative potency, but also to their uptake and retention in the skeleton, and may be influenced by the rate of bone turnover [29,30]. A limitation to the interpretation of bone markers however remains that we have no initial values (baseline prior to denosumab and at time of the hypercalcemia).

Our case report and results from previous studies suggest that precautions must be taken when discontinuing denosumab, especially when used at high doses, and during the first year after the treatment is terminated. As reported by Gossai et al., a gradual decrease in the dose of denosumab and/or the timing of the injections may alleviate the rebound effect. This approach must be validated for the different indications of denosumab. However, the recommendations of the American Society of Clinical Oncology on bone-modifying agents used to treat myeloma suggest not abruptly discontinuing denosumab therapy [4]. For patients with osteoporosis, the discontinuation of denosumab is also a concern; experts have suggested that, when the discontinuation of denosumab is considered, an alternative, short-term antiresorptive treatment should be considered as well [3,31].

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