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Case Report

Multicentric carpotarsal osteolysis syndrome (MCTO) with generalized high bone turnover and high serum RANKL: Response to denosumab

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ABSTRACT

MCTO is a rare disorder, caused by mutations in the *MafB* gene, a negative regulator of receptor activator of nuclear factor-κB ligand (RANKL). Manifestations include carpal and tarsal osteolysis and renal failure. Pathophysiology is poorly understood, and no effective treatment is available.

In this case report we describe a patient with MCTO (*MafB*, mutation c.206C>T, p.Ser69Leu), diagnosed at the age of 5 years. At 7 years, skeletal survey showed diffuse osteopenia. BMD was mildly reduced, and bone turnover markers increased. He was treated with denosumab, a human monoclonal RANKL inhibitor for two years. Each injection was followed by a marked reduction in C-telopeptide (CTX). Following denosumab his BMD and bone symptoms improved and the osteolysis stabilized. At the age of 13 years, osteoporosis was diagnosed using high resolution peripheral quantitative computed tomography (HRpQCT) and serum RANKL was found to be markedly increased.

This initial experience suggests that the associated osteoporosis may be ameliorated by denosumab, although further study will be needed to understand the appropriate dose, frequency, and the extent of efficacy. Monitoring of CTX and bone specific alkaline phosphatase will be especially useful in this regard. Further study in other MCTO patients is also needed to determine whether high bone turnover is specific to this mutation or more common than previously appreciated. We propose a model in which osteolysis in this condition is strongly associated with the systemic osteoporosis.

1. Introduction

Multicentric Carpotarsal Osteolysis Syndrome (MCTO) (OMIM Entry #166300) is a rare skeletal disorder characterized by progressive osteolysis of the carpal and tarsal bones, and nephropathy (Park et al., 2018). Clinical manifestations include progressive osteolysis of the carpal and tarsal bones producing pain, deformity and loss of joint function. Although the carpal and tarsal bones are predominantly affected, other skeletal sites including the elbows, knees, hips and thoracic vertebrae may also be affected (Mehawej et al., 2013). Kidney

Abbreviations: MCTO, Multicentric Carpotarsal Osteolysis Syndrome; MafB, gene v-maf musculoaponeurotic fibrosarcoma oncogene ortholog B; RANKL, receptor activator of nuclear factor-κB ligand; OPG, osteoprotegerin; HRpQCT, high resolution peripheral quantitative computed tomography; CTX, C-telopeptide; ACR, albumin to creatinine ratio; ESKD, end stage kidney disease.

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involvement ranges from proteinuria to end stage renal failure. Craniofacial anomalies and mental impairment have also been observed including triangular shaped face, protruding eyes, and micrognathia (Carnevale et al., 1987). There are suggestions in the literature that this disorder may be related to Gorham Stout Disease (Park et al., 2018). MCTO patients are most often followed by rheumatologists since the presentation mimics juvenile idiopathic arthritis and is often treated with glucocorticoids and anti-inflammatory agents. To date no treatment has proven effective in mitigating the progression of osteolysis or of the renal disease.

MCTO is caused by an autosomal dominant mutation in the *MafB* gene encoding the *MafB* protein. It is an autosomal dominant condition with high frequency of sporadic cases (Upadia et al., 2018). The *MafB* protein negatively regulates the receptor activator of nuclear factor-κB ligand (RANKL). Reduced *MafB* expression consequently results in excessive RANKL stimulation of osteoclasts and increased bone resorption (Upadia et al., 2018). It is unclear why mutations in *MafB* predominantly impact carpal and tarsal bones. Furthermore, there have been no reports suggesting that MCTO may be associated with osteoporosis.

In this case report, we present a patient with genetically confirmed MCTO who early in the course of the disease presented with generalized high bone turnover. He was treated with denosumab, a human monoclonal RANKL inhibitor, which stabilized the osteoporosis and may have arrested further osteolysis. More recently, the high RANKL in the serum was found to be the likely mechanism for the high bone turnover and osteoporosis.

2. Case report

A 20-month-old boy presented to Rheumatology at The Hospital for Sick Children (SickKids) with symptoms typical of arthritis. He was born to a 32-year-old mother G1P1 at 38 weeks and weighed 7 lbs. Pregnancy was uncomplicated, and no abnormalities were noted on ultrasound. Delivery was by C-section secondary to decreased amniotic fluid. His parents are non-consanguineous. His father has a history of galactosemia and psoriasis. His mother was diagnosed at 1-year of age with arthritis of unknown etiology involving her knees, feet, hands, and lower back.

At 20 months of age, his mother was concerned about his gait. He had started to walk at 16-months of age, but was limping and unstable, and tended to fall more than other infants. Apart from reactive airway disease, he did not experience any other medical or surgical disorders. His mother was particularly concerned with the appearance of his tapering fingers since they appeared similar to hers as an infant. Physical examination at 20 months was normal apart for significant shortening and distal tapering of all his digits. There was appropriate range of motion of all small and large joints with no evidence of effusion or active joints. Hand and feet x-ray were normal and a serological panel for rheumatological disorders showed no abnormalities.

At 5-years of age he developed pain in his right (R) wrist with limitation in wrist movement. Physical examination revealed a well appearing boy, with diffuse swelling in the region of his R wrist joint, most prominently on the dorsal aspect of the radial side. He had reduced range of motion, particularly with extension. The wrist was warm to touch. All other joints were normal. Ultrasound showed normal wrist joints with no signs of fluid or tenosynovitis. With a provisional diagnosis of juvenile idiopathic arthritis he was started on naproxen. MRI showed evidence of pan-carpal synovitis with significant joint effusion as well as extensor tenosynovitis and lateral flexor tenosynovitis, all involving the R wrist. There was no improvement with naproxen and he was subsequently treated with several other medications including intraarticular corticosteroid injections, intravenous pulse methylprednisolone and subcutaneous methotrexate, with limited improvement. Plain films showed central loss of his entire proximal row of carpal bones, suggesting MCTO. Genetic testing revealed an *MafB* gene mutation in both the patient and his mother (c.206C>T, p.Ser69Leu). At the time of the MCTO diagnosis, his mother had experienced progressive osteolysis in both wrists and ankles. She had been noted to have proteinuria during childhood and as an adult developed end stage kidney disease that required transplantation at the age of 40.

At 7-years of age, assessment by rheumatology revealed R wrist movement limitation with significantly reduced active and passive range of motion. There was some muscle wasting and marked reduction in grip strength. There was also mild stress related pain of the R elbow, and mild range of motion measuring approximately 10 degrees less than the contralateral side. He also had inconsistent and mild stress tenderness of the R ankle. The other joints showed no abnormalities.

He was referred for nephrology assessment at 7 years of age given the MCTO and his mother's history of ESKD. Other than the naproxen taken intermittently since the age of 5 years and a 3-month trial of methotrexate, he had no history of nephrotoxic medications. Urinalysis was negative and renal function tests and kidney ultrasound were normal. First morning urine tested positive for protein on dipstick but was normal for albumin to creatinine ratio (ACR).

He was further assessed at this age in our Bone Health Clinic for consideration of anti-resorptive therapy. Skeletal survey showed diffuse osteopenia and spine x-ray mild height loss at T1. DXA adjusted for height and age (L1-L4) *Z*-score was −0.7 (Lunar Prodigy, General Electric Healthcare Company, USA). Indices of calcium phosphate metabolism, including 25 OHD and PTH, were within reference ranges as was bone specific alkaline phosphatase 237 (reference range for age (U/L; <335 U/L for males aged 1–10 yrs, Sebia HYDRAGEL 15 ISO-PAL agarose gel electrophoresis) and CTX markedly increased (1440 ng/L; 90–750 ng/L)). Growth was consistent along the 90th percentile for height and 50–75th percentile for weight.

Given the evidence for high bone turnover in addition to the osteolysis, he was offered a trial of denosumab (0.5–0.75 mg/kg) subcutaneously every 4-months. He was treated for 2 years. Denosumab was the sole therapeutic agent used during this period. Response of the bone turnover markers to these injections is detailed in Table 1. Each injection was followed by a marked reduction in CTX and a mild decrease in bone specific alkaline phosphatase. He had a consistent decrease in serum calcium and increase in his PTH above the reference range. This was managed with calcium supplementation and calcitriol. No rebound hypercalcemia was noted during these two years.

Clinically, he experienced less pain with increased participation in activities of daily living with improved function of his R wrist. On completion of the treatment course, the osteolysis in the R wrist showed no further progression and no osteolysis was noted in the left (L) wrist or tarsal bones of either ankle. DXA (L1-L4) *Z*-score was −0.2. A year following this treatment he received two additional injections of denosumab 4-months apart for pain and movement restriction in the R elbow, R knee and L ankle (no osteolysis found). He declined further injections for reasons unrelated to medication side effects.

At 13-years of age he fell and was assessed for possible fracture of his R knee. X ray showed osteopenia without fracture. Physical exam

Table 1
Bone turnover markers pre- and post- treatment with denosumab.

Denosumab® treatment	C-Telopeptide (ng/L)		Bone specific alkaline phosphatase, BSAP (U/L)	
	Pre-	Post-	Pre-	Post-
1	1130	529	266	269
2	1390	–	266	–
3	1300	742	279	264
4	1610	461	256	249
5	1690	393	214	187
6	>2000	338	282	269
7	>2000	539	–	262

Denosumab® Tx was given to patient every 3 to 4 months over the course of an approximately 2 year period, 2014–2015. Bone turnover markers were measured 2 to 4 weeks post Denosumab® Tx.

revealed R wrist deformity. His R upper limb musculature was wasted compared with the left, his shoulder and elbow strength were preserved. No other joints were involved. His BMD Z-score was −1.2. X ray of the wrists showed osteopenia in the L carpal bones and an almost complete destruction of the R carpal bones. Both ankles showed osteopenia without evidence of osteolysis. Serum calcium, 25OH vitamin D and PTH were normal. CTX (1470 ng/L; 90–750 ng/L) was elevated.

To further investigate the high bone turnover state, he underwent HRpQCT using Xtreme CT II (Scanco Medical AG, Brüttisellen, Switzerland) to assess the volumetric density (total, trabecular and cortical density) and microarchitecture. Both the L and R distal radii and corresponding distal tibiae were assessed, given the more severe involvement on the right side. Semi-automated software was used to separate cortical and trabecular regions based on a threshold-based algorithm. Total, trabecular, and cortical bone density (Tot.vBMD, Tb.vBMD, Ct.vBMD) as well as microarchitecture data including cortical thickness (Ct.Th, mm), cortical porosity, trabecular thickness (Tb.Th, mm), trabecular number (Tb.N,mm1), and trabecular separation (Tb.Sp, mm) were evaluated (Lenherr-Taube et al., 2020; Nishiyama and Shane, 2013).

Results for the L distal radius and tibia are presented in Table 2. Results were compared with 7 healthy boys, aged 13.5–15.5y. Findings for the right distal radius and tibia were similar.

Tot.vBMD, Tb.vBMD, and Ct.vBMD in the distal radius and Tot.vBMD, and Ct.vBMD in the distal tibia were below the 3rd percentile of the aged-matched healthy volunteers. The Tb.N in distal radius and Ct.Th, and cortical porosity in the distal tibia were also reduced, and radial trabecular separation was increased compared with the healthy controls comparing with the healthy controls. Although the BMD was only mildly reduced on DXA scan, the reduced Tot.vBMD and Tb.vBMD on HR pQCT indicates osteoporosis and bone loss. The reduced Tb.N and increased Tb.sp. on HR pQCT indicates that the trabecular microstructure is also affected.

RANKL was measured using Luminex xMAP technology for multiplexed quantification of Human Receptor Activator for Nuclear Factor κB Ligand (RANKL) protein The analysis was performed using the Luminex™ 200 system (Luminex, Austin, TX, USA) by Eve Technologies Corp. (Calgary, Alberta). A single biomarker was measured in the samples using a MILLIPLEX Human RANKL Single Plex Kit (Millipore, St. Charles, MO, USA) according to the manufacturer's protocol. The 1-plex consisted of RANKL. The assay sensitivity of this biomarker is 5.0 pg/mL. OPG was measured using a MILLIPLEX Human Bone 13-Plex Kit (Cat. # HBNMAG-51K, Millipore, St. Charles, MO, USA) according to the manufacturer's protocol. The essay sensitivity of this biomarker is 1.9 pg/mL.

Serum RANKL and OPG were measured at 7 and 10 weeks after treatment with Denosumab. RANKL was markedly increased at 7 weeks after treatment in undiluted (35.4 pmol/L) and averaged diluted (83.73 pmol/L) samples when compared with age matched healthy control children (0.3 pmol/L (0.21–0.41)) (Akhtar Ali et al., 2019). At 10 weeks similar results were obtained (26.63, and 58.9 pmol/L, respectively). OPG was moderately increased at 7 weeks (8.65 pmol/L) and at 10 weeks (7.56 pmol/L) when compared with healthy controls (3.39 pmol/L (2.88–4.04)) (Akhtar Ali et al., 2019). RANKL/OPG ratio was markedly increased at 7 and 10 weeks (4.09 and 3.51, respectively) when compared with age matched controls 0.09 (0.06–0.14) (Akhtar Ali et al., 2019).

A more comprehensive renal assessment was completed, which was overall normal with no evidence of renal impairment, proteinuria or tubular dysfunction. He was normotensive. Kidney function showed normal serum electrolytes, urea, creatinine, and estimated GFR by Schwartz equation of 150 mL/min/1.73 m^2. Urinalysis showed pH of 7 was significant only for trace haemoglobin, was negative for glucose, ketones, leukocytes, nitrites and protein, and a specific gravity of 1.020. There was a mildly elevated albumin/creatinine ratio in urine 6.6 mg/mmol (<3.5 mg/mmol) and urinary protein to creatinine ratio of 24 mg/mmol (normal <20 mg/mmol). Urinary beta 2 microglobulin was below the detection limit at <100 μg/L (normal range 0–300 μg/L). There was no hypercalciuria, urinary citrate to creatinine ratio was normal. Urine osmolality was measured at 590 mmol/kg H_2O. On ultrasonography both kidneys were of a normal size for the patient's age and had normal corticomedullary differentiation with no evidence of nephrocalcinosis or nephrolithiasis.

On completion of these investigation, treatment with denosumab was resumed on a 2 monthly basis using a dose 0.5 mg/kg.

3. Discussion

In this case report, we present a 13-year old male with MCTO and osteoporosis. He was diagnosed with MCTO at age 5 years. At age 7 he had evidence of generalized high bone turnover. Osteoporosis at this age was suggested by generalized osteopenia, a mildly reduced BMD and high bone turnover markers, particularly, the resorption marker CTX. Given these findings, he was treated with denosumab every 4 months for two years. A year later he received two additional 4 monthly injections. Following these two courses of denosumab, bone related symptoms and

Table 2
HRpQCT characteristics of the L Radius and L Tibia compared with seven aged-matched healthy controls.

HRpQCT parameters	Radius				Tibia			
	Patient	Healthy controls (median)	3rd percentile	97th percentile	Patient	Healthy controls (median)	3rd percentile	97th percentile
Density parameters								
Tot.vBMD (mg HA/cm^3)	193.4[a]	277.2	202.6	336.5	191.7[a]	262.8	198.2	306.4
Tb.vBMD (mg HA/cm^3)	115.7[a]	201.4	129.7	262.8	174	215.5	154.2	271.1
Ct.vBMD (mg HA/cm^3)	372.3[a]	698.2	664.3	807.4	528.8[a]	709.3	636.5	792.5
Microarchitecture parameters								
Ct.Th (mm)	1.071	0.927	0.698	1.217	0.474[a]	1.026	0.757	1.207
Ct.Po (%)	0.006	0.015	0.006	0.023	0.011[a]	0.026	0.014	0.043
Tb.N (mm^{-1})	0.896[a]	1.626	1.281	1.874	1.557	1.687	1.372	2.081
Tb.Sp (mm)	1.161[b]	0.578	0.458	0.761	0.617	0.558	0.430	0.705
Tb.Th (mm)	0.241	0.237	0.216	0.274	0.234	0.250	0.232	0.270

Tot.vBMD = total volumetric bone mineral density; Tb.vBMD = trabecular vBMD; Ct.vBMD = cortical vBMD; Ct.Th = cortical thickness; Ct.Po = cortical porosity; Tb.N = trabecular number; Tb.Sp = trabecular separation; Tb.Th = trabecular thickness.

[a] Reduction.

[b] Increase.

BMD improved modestly. There was, however, progressive destruction of the R carpal bones. Over the following 2–3 years in the absence of any specific treatments, he continued to show evidence of a high bone turnover state. At age 13, osteoporosis was diagnosed using HRpQCT. Serum RANKL was found to be high.

MCTO is a rare genetic disease in which the carpal and tarsal bones undergo progressive osteolysis. While involvement of the carpal and tarsal bones is the most common feature of this condition, there are several reports which describe involvement of other skeletal sites, such as the elbow, shoulder, knees, hips, thoracic vertebrae and cervical spine. The radiological findings at these skeletal sites include changes such as resorption of the capitellum, enlargement in the semilunar notch, dislocation of the elbow, destruction of the trochlea and dysplasia of the olecranon and coracoid process, destruction of the humeral epiphysis and the subchondral zone and swollen soft tissue (Klein et al., 2018).

Long-term mild and non-progressive proteinuria is commonly described as a feature of this disease. Nephropathy progressing to end stage kidney disease over a variable time period occurs in up to 50% of cases. Proteinuria and hypertension are the most typical renal manifestations documented (Zagury and Neto, 2001). Focal segmental glomerulosclerosis (FSGS) was reported in some children when biopsy was done early in the disease course (Connor et al., 2007; Zhuang et al., 2017). A case report described non-specific changes in the glomerular basement membrane without changes on light microscopy raising the possibility of the nephropathy being a primary issue with the glomerular basement membrane, similar to Alport syndrome (Bakker et al., 1996; Bakker et al., 1996). The possibility of this being a vasculopathy has also been raised in the past with the consistent finding of intimal media proliferation and hypertrophy of renal arteries on biopsy, however these biopsies were done in patients with advanced kidney disease and are more likely explained by hypertension (Bennett et al., 1980). Many of these patients have been successfully transplanted without recurrence of nephropathy after transplantation (Bennett et al., 1980).

MCTO is caused by an autosomal dominant mutation in the *MafB* gene encoding the *MafB protein*. *MafB* is a member of the MaF family of transcription factors, expressed in the kidney (Moriguchi et al., 2006) and in monocyte/macrophage lineage, but not in other hematopoietic cells (Kim et al., 2007; Mumm et al., 2014). During macrophage differentiation, *MafB* expression is undetectable in pluripotential progenitors, but is expressed at moderate levels in myeloblasts, and strongly up-regulated in monocytes and macrophages. *MafB* contributes to the establishment and maintenance of the myelomonocytic phenotype by preventing erythroid-specific gene expression. Overexpression of *MafB* in transformed myeloblasts stimulates the rapid formation of macrophages, suggesting that *MafB* is important for macrophage differentiation (Kim et al., 2007). *MafB* interferes with the DNA-binding domains of transcription factors including, c-Fos, Mitf and NFATc1; thereby inhibiting the transactivation of NFATc1 and OSCAR. A positive feedback circuit involving RANKL, NFATc1, and OSCAR appears to be important for efficient differentiation of osteoclasts. Therefore, *MafB* acts as a key modulator of RANKL-induced osteoclastogenesis by means of its ability to attenuate the expression of NFATc1 and OSCAR (Kim et al., 2007). In turn, reduced *MafB* expression results in increased RANKL levels, excessive activation of osteoclasts and bone resorption (Upadia et al., 2018).

MafB is important for renal development, podocyte differentiation, podocyte foot process formation and tubular genesis. *MafB* knockout mice had fewer mature glomeruli and had renal tubular dysgenesis on histopathological assessment. Mice had significantly reduced *nephrin*, *podocin* and *CD2AP* mRNA most likely due to their failed activation by the transcription factor *MafB*. Nephrin and podocin are two of the most important proteins that make up the slit diaphragm between interdigitating podocyte foot processes and associated themselves with severe forms of congenital nephrotic syndrome and FSGS (Moriguchi et al., 2006; Sadl et al., 2002).

The mutations in *MafB* that cause MCTO are concentrated in a small region of the Pro-Ser-Thr rich acidic transcriptional activation domain in the amino terminal transactivation domain of the transcription factor (Wang et al., 1999). Zankl et al. (2012) noted that these mutations lie within a region of *MafB* that is phosphorylated. Therefore, MCTO mutations may affect *MafB* phosphorylation and alter the protein's action (Mumm et al., 2014). Mumm et al. (2014) reported in 9 patients, including our patient and his mother that all mutations were located within a 36 bp (13 amino acid) segment of the single exon *MafB*, suggesting that only a few domain specific defects within *MafB* cause MCTO. In 2013, Dworshak et al. (2013) had reported a case of a novel missense *MafB* variant mutation present in a patient and other unaffected relatives demonstrating incomplete penetrance for some *MafB* mutations, suggesting other modifier genes, epigenetic mechanisms or environmental factors that may modulate the MCTO phenotype (Dworschak et al., 2013).

RANKL is a type II transmembrane protein belonging to the tumor necrosis factor (TNF) superfamily that activates its receptor RANK. RANKL is broadly expressed in different tissues, including bone (osteoblasts and osteocytes), spleen, heart, lung, brain and the immune system (thymus, T and B lymphocytes). It is also found as a soluble form. Following binding of RANKL to its receptor RANK, a signal cascade is activated that is essential for the differentiation, activity and survival of osteoclasts.

The initial rationale for using denosumab in this case was based on the evidence for a systemic high bone turnover state and a potential role for RANKL in this process. Other interventions for MCTO reported in the literature to date have proved to be disappointing. The goals of these therapies have been to arrest progression of the carpal and tarsal osteolysis. In particular, the efficacy of bisphosphonates has been questioned (Park et al., 2018; Carmichael et al., 2007). Furthermore, bisphosphonates are potentially nephrotoxic (Domschke and Schuetz, 2014), which raises an additional concern in the context of a condition in which renal failure is an outcome. Some cases of MCTO have been treated with a TNF-α monoclonal antibody, methotrexate, salicylates, and non-steroidal anti-inflammatory agents. None of these agents have proven to be effective (Upadia et al., 2018; Klein et al., 2018).

Denosumab is a fully human monoclonal antibody to RANKL that blocks RANKL binding to RANK. This in turn inhibits both the development and activity of osteoclasts, decreases bone resorption, and increases bone density (Boyce, 2017; Cummings et al., 2009). It is a useful medication in the treatment of osteoporosis especially when bone turnover is high, with adjunct uses in malignancies and other skeletal disorders. Denosumab has a short half-life and positive benefits may be reversed after treatment is discontinued. The pharmacokinetics of denosumab have been studied extensively in adults, and similar to that seen for other fully human monoclonal antibodies, exhibits dose-dependent, non-linear elimination. The pharmacokinetics and pharmacodynamics of denosumab in children are largely unknown (Boyce, 2017).

The safety and efficacy of Denosumab have not been established in the pediatric patients. However, denosumab has been used in children in several clinical research settings. There is an ongoing international therapeutic trial in Osteogenesis Imperfecta (AMGEN, 2020)(AMGEN, 2020). There are also reports of its use in juvenile idiopathic osteoporosis with high bone turnover, hematopoietic stem cell transplantation with osteopetrosis and refractory hypercalcemia, Giant cell tumor of bone, fibrous dysplasia, central giant cell granuloma and spinal aneurysmal bone cysts (Boyce, 2017). We were able to find only one other report of the use of denosumab in a patient with MCTO (Zhuang et al., 2017). This patient had progressive bone destruction and low grade focal segmental glomerulosclerosis on renal biopsy and was treated with a single dose of denosumab (60 mg). Reduction of inflammation in the affected joint was demonstrated with magnetic resonance imaging 9 months later.

Our patient was started on a relatively low dose of denosumab. This

dose was repeated every 4 months as it was unclear whether he would experience hypocalcemia and or hypercalcemia as has been reported in patients treated for other conditions after stopping denosumab (Grasemann et al., 2013; Hoyer-Kuhn et al., 2016). Monitoring of his serum calcium levels showed a consistent early decline in serum calcium levels with an increase in serum PTH. We attributed this response to a significant bone mineral deficit as suggested by generalized osteopenia and the hypocalcemia was managed with calcitriol and calcium supplementation. Our patient did not experience urinary tract infections, which has been documented with denosumab in vulnerable/immunosuppressed individuals (Bonani et al., 2017; Ferrari-Lacraz and Ferrari, 2011).

The evidence for a generalized high bone turnover state/osteoporosis in association with very high serum RANKL levels in our patient is strong (Akhtar Ali et al., 2019). Additional support for this conclusion was found in the literature in the case reported by Zhuang et al. (2017); a tarsal bone biopsy at age 18 showed enhanced bone resorption and fibrosis. Further, in the study by Rinotas et al. (2014), transgeneic mice were generated carrying the human RANKL genomic region. Mice developed early onset osteoporosis and the levels of RANKL expression correlated with bone resorption and disease severity (Rinotas et al., 2014).

In our patient, the use of denosumab diminished bone related symptoms, mildly improved BMD and possibly arrested progression of the osteolysis. Therefore, it appears from this initial experience that osteoporosis may be ameliorated by denosumab. Further study will be needed to understand the appropriate dose, frequency and the extent of efficacy. We anticipate that monitoring of CTX and bone specific alkaline phosphatase will be especially useful in this regard.

Given our patient has both osteoporosis and osteolysis (Fig. 1), an important future step will be to explore the relationship between these two findings. Several observations allow for the construct of a model of how these two processes might be linked. Short bones such as the central vertebra, carpal and tarsal bones, are known to have very thin cortices and trabecular bone that is mainly responsible for diffusing biomechanical forces along the length of the bone (Currey, 1984). In our patient, radiology showed very thin cortices and with HRpQCT cortical volumetric density was significantly reduced. Osteolysis in this condition may therefore represent the end point of a compressive process in which bone resorption far exceeds bone formation. Given that vertebral compression fractures but not osteolysis in the carpal/tarsal bones are common in other cases of severe osteoporosis, specific biomechanical forces or impact of the *MafB* mutation in bone development in the wrist, ankles and other affected sites may explain the severity of the process in these sites. In support of an underlying developmental impact, a number of the radiological findings suggest dysplasia in certain sites (Klein et al., 2018).

Long-term use of denosumab is a clinical approach to exploring the link between the osteoporosis and osteolysis. Ideally denosumab should be used in those patients with mild to moderate osteolysis, and as a sole medication to avoid the potential for drug interactions with other agents. In the event these studies show arrest of the osteolysis with improvement in the osteoporosis, this would provide strong support for the dominance of the RANKL driven osteoporosis in the genesis of the osteolysis.

4. Conclusions/clinical lessons

MCTO is a rare genetic condition, which in our patient it is associated with a high turnover skeletal phenotype (osteoporosis) driven by very high levels of serum RANKL. Osteoporosis may be ameliorated by denosumab, although further study will be needed to understand the dose, frequency and the extent of efficacy. Further study in other MCTO patients is also needed to determine whether high bone turnover is specific to this mutation or more common than previously appreciated. We propose a model in which osteolysis in this condition is strongly

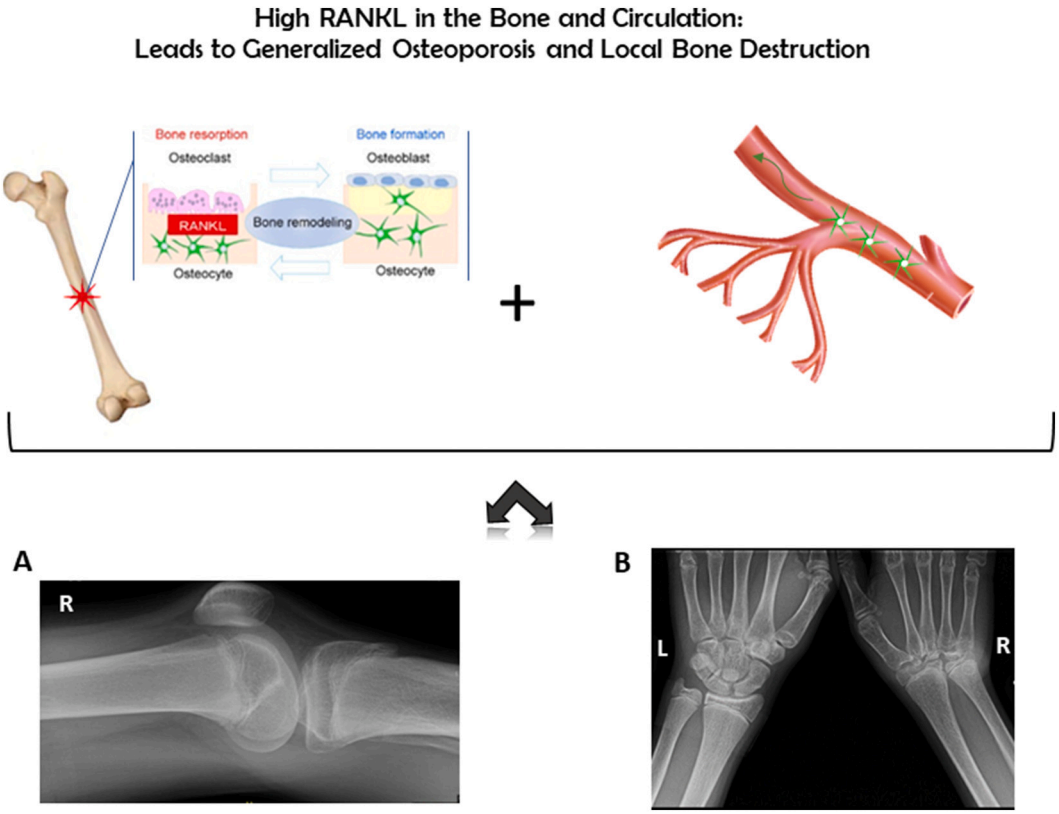

Fig. 1. High RANKL in serum and bone leads to: A. Rt. knee showing generalized osteoporosis. B. Rt. And Lt. hand showing generalized osteoporosis and local destruction of the Rt. Carpal bones (biomechanical forces and/or dysplasia)

associated with the systemic osteoporosis. Specific biomechanical forces in the wrist and ankles and or developmental problems related to the *MafB* mutation are proposed to explain the severity of the process in the carpal and tarsal bones. Should further work confirm this model, MCTO may represent a form of idiopathic juvenile osteoporosis in which short bones are more severely affected than long bones.

Author contributions

All authors have contributed to the manuscript in accordance with the guidelines that have been provided.

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Declaration of competing interest

None.

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

We declare that the manuscript is being submitted only to Bone, that it will not be submitted elsewhere, while under consideration, that it has not been published elsewhere, and, should it be published in Bone, that it will not be published elsewhere—either in similar form or verbatim—without permission of the editors. These restrictions do not apply to abstracts or to press reports of presentations at scientific meetings.

References

Akhtar Ali, S., et al., 2019. Quantifying RANKL and OPG levels in healthy children: a large cross-sectional analysis. Bone 127, 215–219. https://doi.org/10.1016/j.bone.2019.06.012.

AMGEN, 2020. Multicenter, single-arm study to evaluate efficacy, safety, & pharmacokinetics of denosumab in children w/ OI (OI). Protocol Number: 20130173. ClinicalTrials.gov Identifier: NCT02352753 May. Available from: https://clinicaltrials.gov/ct2/show/NCT02352753.

Bakker, S.J., et al., 1996. Abnormal glomerular basement membrane in idiopathic multicentric osteolysis. Pediatr. Nephrol. 10 (2), 200–202. https://doi.org/10.1007/BF00862077.

Bennett, W.M., Houghton, D.C., Beals, R.C., 1980. Nephropathy of idiopathic multicentric osteolysis. Nephron 25 (3), 134–138. https://doi.org/10.1159/000181769.

Bonani, M., et al., 2017. Infections in De novo kidney transplant recipients treated with the RANKL inhibitor denosumab. Transplantation 101 (9), 2139–2145. https://doi.org/10.1097/TP.0000000000001547.

Boyce, A.M., 2017. Denosumab: an emerging therapy in pediatric bone disorders. Curr. Osteoporos. Rep. 15 (4), 283–292. https://doi.org/10.1007/s11914-017-0380-1.

Carmichael, K.D., Launikitis, R.A., Kalia, A., 2007. The orthopedic and renal manifestations of idiopathic carpal tarsal osteolysis. J. Pediatr. Orthop. B 16 (6), 451–454. https://doi.org/10.1097/BPB.0b013e3282e1c667.

Carnevale, A., et al., 1987. Idiopathic multicentric osteolysis with facial anomalies and nephropathy. Am. J. Med. Genet. 26 (4), 877–886. https://doi.org/10.1002/ajmg.1320260415.

Connor, A., et al., 2007. Multicentric carpal-tarsal osteolysis with nephropathy treated successfully with cyclosporine A: a case report and literature review. Am. J. Kidney Dis. 50 (4), 649–654. https://doi.org/10.1053/j.ajkd.2007.06.014.

Cummings, S.R., et al., 2009. Denosumab for prevention of fractures in postmenopausal women with osteoporosis. N. Engl. J. Med. 361 (8), 756–765. https://doi.org/10.1056/NEJMoa0809493.

Currey, J., 1984. Comparative mechanical properties and histology of bone. Am. Zool. 24 (1), 5–12. https://doi.org/10.1093/icb/24.1.5.

Domschke, C., Schuetz, F., 2014. Side effects of bone-targeted therapies in advanced breast cancer. Breast Care (Basel) 9 (5), 332–336. https://doi.org/10.1159/000368844.

Dworschak, G.C., et al., 2013. An incompletely penetrant novel MAFB (p.Ser56Phe) variant in autosomal dominant multicentric carpotarsal osteolysis syndrome. Int. J. Mol. Med. 32 (1), 174–178. https://doi.org/10.3892/ijmm.2013.1373.

Ferrari-Lacraz, S., Ferrari, S., 2011. Do RANKL inhibitors (denosumab) affect inflammation and immunity? Osteoporos. Int. 22 (2), 435–446. https://doi.org/10.1007/s00198-010-1326-y.

Grasemann, C., et al., 2013. Effects of RANK-ligand antibody (denosumab) treatment on bone turnover markers in a girl with juvenile Paget's disease. J. Clin. Endocrinol. Metab. 98 (8), 3121–3126. https://doi.org/10.1210/jc.2013-1143.

Hoyer-Kuhn, H., et al., 2016. Safety and efficacy of denosumab in children with osteogenesis imperfect—a first prospective trial. J. Musculoskelet. Neuronal Interact. 16 (1), 24–32.

Kim, K., et al., 2007. MafB negatively regulates RANKL-mediated osteoclast differentiation. Blood 109 (8), 3253–3259. https://doi.org/10.1182/blood-2006-09-048249.

Klein, C., et al., 2018. Multicentric carpotarsal osteolysis syndrome: long-term follow-up of three patients. Skelet. Radiol. 47 (7), 1015–1019. https://doi.org/10.1007/s00256-018-2874-z.

Lenherr-Taube, N., et al., 2020. Severe primary hyperparathyroidism caused by parathyroid carcinoma in a 13-year-old child; novel findings from HRpQCT. JBMR Plus 4 (3), e10324. https://doi.org/10.1002/jbm4.10324.

Mehawej, C., et al., 2013. The identification of MAFB mutations in eight patients with multicentric carpo-tarsal osteolysis supports genetic homogeneity but clinical variability. Am. J. Med. Genet. A 161A (12), 3023–3029. https://doi.org/10.1002/ajmg.a.36151.

Moriguchi, T., et al., 2006. MafB is essential for renal development and F4/80 expression in macrophages. Mol. Cell. Biol. 26 (15), 5715–5727. https://doi.org/10.1128/mcb.00001-06.

Mumm, S., et al., 2014. Multicentric carpotarsal osteolysis syndrome is caused by only a few domain-specific mutations in MAFB, a negative regulator of RANKL-induced osteoclastogenesis. Am. J. Med. Genet. A 164A (9), 2287–2293. https://doi.org/10.1002/ajmg.a.36641.

Nishiyama, K.K., Shane, E., 2013. Clinical imaging of bone microarchitecture with HR-pQCT. Curr. Osteoporos. Rep. 11 (2), 147–155. https://doi.org/10.1007/s11914-013-0142-7.

Park, P.G., et al., 2018. Three cases of multicentric carpotarsal osteolysis syndrome: a case series. BMC Med. Genet. 19 (1), 164. https://doi.org/10.1186/s12881-018-0682-x.

Rinotas, V., et al., 2014. Novel genetic models of osteoporosis by overexpression of human RANKL in transgenic mice. J. Bone Miner. Res. 29 (5), 1158–1169. https://doi.org/10.1002/jbmr.2112.

Sadl, V., et al., 2002. The mouse Kreisler (Krml1/MafB) segmentation gene is required for differentiation of glomerular visceral epithelial cells. Dev. Biol. 249 (1), 16–29. https://doi.org/10.1006/dbio.2002.0751.

Upadia, J., et al., 2018. A familial case of multicentric carpotarsal osteolysis syndrome and treatment outcome. J. Pediatr. Genet. 7 (4), 174–179. https://doi.org/10.1055/s-0038-1657760.

Wang, P.W., et al., 1999. Human KRML (MAFB): cDNA cloning, genomic structure, and evaluation as a candidate tumor suppressor gene in myeloid leukemias. Genomics 59 (3), 275–281. https://doi.org/10.1006/geno.1999.5884.

Zagury, A., Neto, J.G., 2001. Idiopathic carpotarsal osteolysis with nephropathy. Pediatr. Nephrol. 16 (2), 121–126. https://doi.org/10.1007/s004670000515.

Zankl, A., et al., 2012. Multicentric carpotarsal osteolysis is caused by mutations clustering in the amino-terminal transcriptional activation domain of MAFB. Am. J. Hum. Genet. 90 (3), 494–501. https://doi.org/10.1016/j.ajhg.2012.01.003.

Zhuang, L., et al., 2017. Identification of a MAFB mutation in a patient with multicentric carpotarsal osteolysis. Swiss Med. Wkly. 147, w14529. https://doi.org/10.4414/smw.2017.14529.