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Successful Ageing Symposium

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Ageing successfully without high levels of disability is becoming more important as the life expectancy of Australians continues to increase slowly, and more older people are surviving into late old age. There are many disease conditions that are more prevalent in older age including dementia, osteoporosis, and frailty. These result in high levels of disability, and preventing or mitigating the effects of these conditions can markedly improve independence and quality of life in older age. The presence of frailty has a greater effect on outcomes for older people after surgery, trauma, stroke or infection than age alone, yet its presence is often unrecognised. Dementia is also often a hidden problem despite the fact that at 80 years of age, one in four people will have it. This presentation reviews evidence for what can be done in terms of prevention, recognition, and management of frailty, and discusses how to address the modifiable risk factors that account for 40% of all cases of dementia. It will summarise actions that can be taken to improve the ability of older Australians to age successfully.

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Identifying the proteins secreted by bone marrow mesenchymal precursor cells responsible for negatively regulating fat mass in male mice.

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Publish consent withheld

1. Russell, P. K., Mangiafico, S., Fam, B. C., Clarke, M. V., Marin, E. S., Andrikopoulos, S., Wiren, K. M., Zajac, J. D., & Davey, R. A. (2018). The androgen receptor in bone marrow progenitor cells negatively regulates fat mass. Journal of Endocrinology, 237(1), 15-27. Retrieved Jul 20, 2023, from https://doi.org/10.1530/JOE-17-0656

3

Co-localisation between genome-wide association and gene expression data identifies *ATP6V1A* as a potential regulator of bone mineral density that acts through osteoclasts

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Background: Whole-body bone mineral density (WB-BMD) is used to assess peak bone mass acquisition and bone health. It is highly heritable, with >50% of BMD variance accounted for by genetic factors. More than 80 genomic regions (loci) in the human genome have been associated with WB-BMD variation, and >500 with ultrasound-derived heel eBMD. However, underlying cell types and effector genes that regulate these loci remain largely unknown.

Aims: To conduct the largest genome-wide association study (GWAS) meta-analysis for WB-BMD and integrate the results with gene expression data from bone cells to identify genes and cellular mechanisms that regulate BMD.

Methods: GWAS meta-analysis included 11 cohorts and 107,000 individuals. WB-BMD associated loci were identified using GCTA-Cojo and deemed novel if located >1Mb from any known BMD-associated locus. Co-localisation analyses incorporating eQTL data from human osteoclast-like cells were used to screen WB-BMD loci and identify candidate effector genes. Effector genes were followed up in a single-cell RNA expression dataset encompassing 34 bone/marrow cell-types isolated from mice, as well the MGI mouse mutant database.

Results: 196 association signals for WB-BMD ($P<5\times10^{-8}$) were detected and 9 were novel. Co-localisation highlighted several potential effector genes acting through osteoclasts, including the V-ATPase proton pump subunit gene *ATP6V1A* (Fig.1A). The *G*-allele at the lead GWAS variant (rs2305545, MAF=0.35), was associated with increased WB-BMD and reduced *ATP6V1A* expression (Fig.1B). Differential gene expression analysis demonstrated up-regulation of *ATP6V1A* during differentiation of human osteoclast-like cells ($P<0.0001$, Fig.1C). Single-cell RNA-seq revealed that *Atp6v1a* is highly expressed in mouse osteoclasts relative to other cells (Fig.1D). Mice lacking *Atp6v1a* present with increased bone mineral content.

Conclusion: GWAS of WB-BMD identified 9 loci that have not been identified by much larger GWAS of ultrasound-derived heel eBMD. Co-localisation analysis implicated *ATP6V1A* as a potential effector gene that may act through osteoclasts to regulate BMD.

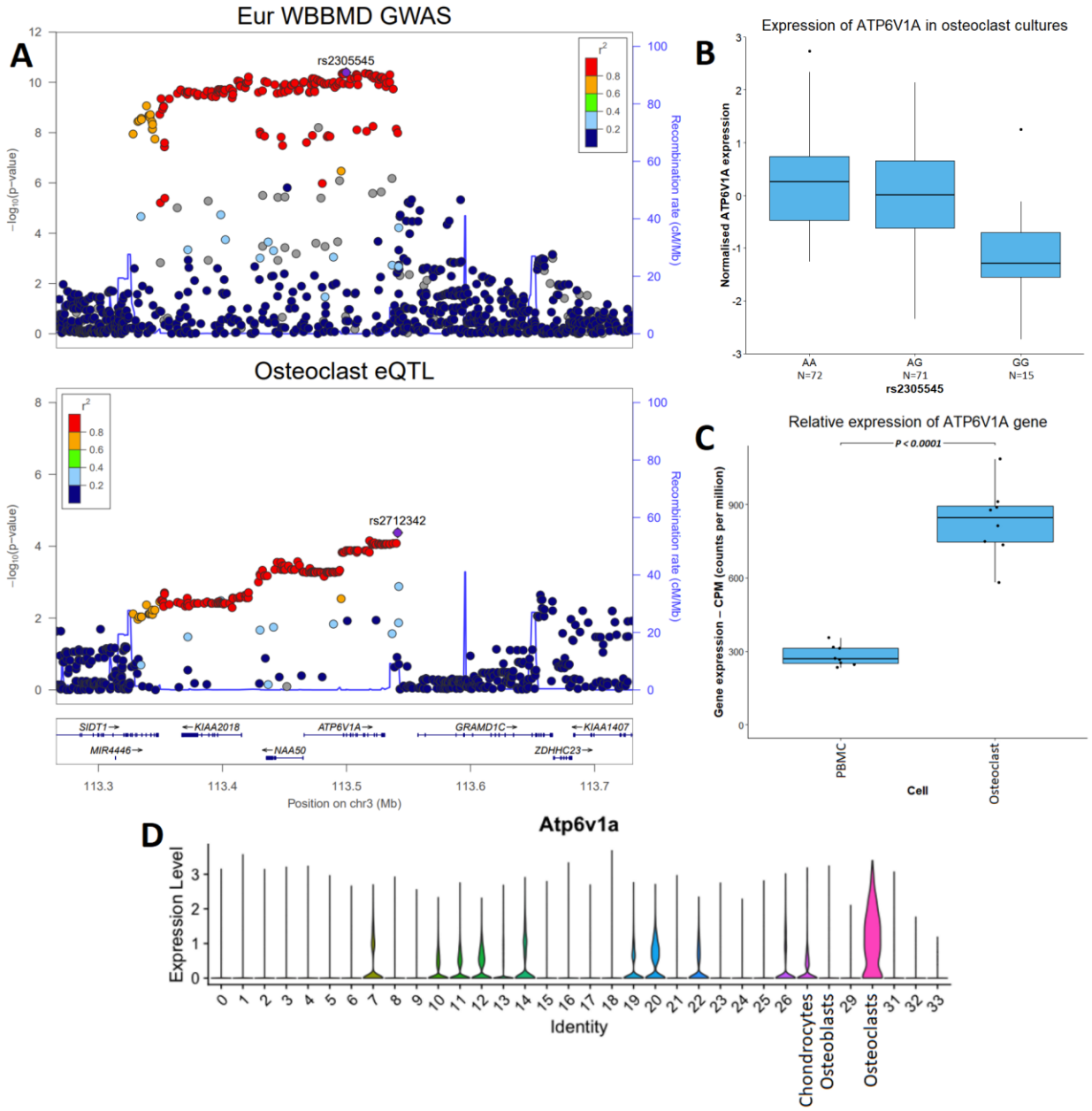

Figure 1. A) Co-localised association signals for WB-BMD and ATP6V1A expression; B) ATP6V1A expression for genotype groups of rs2305545; C) relative expression of ATP6V1A in osteoclast-like cells and their peripheral blood mononuclear cell (PBMC) precursors; D) single-cell RNA-seq data showing high expression of Atp6v1a in mouse osteoclasts relative to 34 other bone/marrow cell types.

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Reducing hip and non-vertebral fractures in institutionalised older adults by restoring inadequate intakes of protein and calcium is cost-saving

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Background: Older adults in aged care homes account for 30% of the population burden of hip fractures. Nutritional interventions to correct protein and calcium inadequacies reduce these and other debilitating fractures, perhaps partly by reducing falls and slowing deterioration in bone morphology. We aimed to determine whether a nutritional approach to fracture risk reduction in aged care homes is cost-effective.

Methods: Costing was estimated based on results of a prospective two-year cluster-randomised controlled trial involving 3313 residents in 27 aged care homes (intervention using high dairy menus), 3911 residents in 29 aged care homes (controls consuming from normal menus) and cost of ambulance, hospital, rehabilitation, and residential care incurred after fracture. The incremental cost-effectiveness ratios per fracture averted within a 2-year time horizon were estimated from the Australian healthcare perspective applying a 5% discount rate on costs after the first year.

Results: Intervention resulted in a total of 3.5 servings of milk, yoghurt and/or cheese daily, achieving 1,142mg calcium and 69g protein versus usual daily intakes of 700mg calcium and 58g protein consumed by controls. This intervention reduced all fractures by 33% at a daily cost of AU$0.66 per resident. The base-case results showed that intervention was cost-saving per fracture averted, with robust results in a variety of sensitivity and scenario analyses. Scaling the benefits of intervention to the Australian community equated to a saving of AU$66,780,000 annually in Australia and remained cost saving up to a daily food expenditure of AU$1.07 per aged care resident.

Conclusions: Averting hip and other non-vertebral fractures in older adults in aged care homes by restoring nutritional inadequacies of protein and calcium is cost saving and supports the wide-spread implementation of this type of nutritional intervention in similar settings.

Acetylcholinesterase inhibitors reduce fracture and mortality risk in older patients with dementia syndromes

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Background: Older people are at high risk of osteoporosis and dementia. We reported that in vitro and in vivo mouse models suggest that antidementia medications acetylcholinesterase inhibitors improve bone health through its impact on inhibiting osteoclasts and a potential anabolic effect on osteoblasts (Ref1). In this study we a looked at the impact of acetylcholinesterase in fracture risk and mortality in patients with dementia syndromes.

Methods: In this aged care, dementia clinic, cohort study we follow-up 744 patients annually for 4 years. The main outcomes assessed were, incidence of new fracture and death. Data was entered into SPSS with analysis of frequency and comparison of groups using Chi-Square Test. Main analysis was demographics and risk factors at baseline, diagnosis of dementia, prevalence of baseline osteoporosis and fracture, falls risk, dementia medications, osteoporosis medications and follow up incidence of fracture and mortality.

Results: There were 744 patients followed up. At baseline fifty-nine percent were female and mean age was 81 years (SD6.8). Twenty one percent had prior hip fracture Fifty six percent had dementia and 113 (15.2%) were on dementia medications at baseline. During 4 years of annual follow up 137(18%) reported new fractures (some multiple and hip). Overall mortality rate was 267/744 (36%). Patients with dementia were more likely to die (180/415 vs 87/329; p <0.001) with a trend to more fractures (73/415 vs 64/329; p = 0.078). Patients on dementia medications were less likely to fracture 15/113 vs 122/631 (p = 0.029).

Risk factor	Fracture (1 year)	Fracture (4 years)	Deceased(1 year)	Deceased(4 year)
Dementia (yes)	26/299	73/415	27/415	180/415
Dementia (no)	17/241	64/329	14/329	87/329
Pearson Chi-Square	0.183	**0.078**	0.181	**<0.001**
Dementia Treatment (yes)	2/78	15/113	3/113	43/113
Dementia Treatment (no)	41/462	122/631	38/631	224/631
Pearson Chi-Square	0.150	**0.029**	0.149	0.602

Conclusions: Patients with dementia have a higher risk of recurrent fractures and mortality. Treating dementia with acetylcholinesterase inhibitors appear to reduce the risk of all fractures. This clinical finding is consistent with our recently published in vitro and in vivo mouse model confirming the benefit of cholinesterase inhibitors on bone biology, histology and biomarkers.

1. Shangfu Li, Dian Teguh, Depeng Wu, Lesong Liu, Chaofeng Hu, Jinbo Yuan, Inderjeeth Charles and Jiake Xu. Anti-dementia medication acetylcholinesterase inhibitors may have therapeutic benefits on osteoporotic bone by attenuating osteoclastogenesis and bone resorption. Journal of Cellular Physiology. 19 May 2023 http://doi.org/10.1002/jcp.31057

6

Effects of antipsychotics on embryonic bone development

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Introduction: Antipsychotics are a class of psychotropic medication used in the treatment of psychosis. Given the potential harm of not treating psychosis, administration is recommended during pregnancy; however, resultant congenital abnormalities and preterm birth have been described. To date there is no clear evidence on the effects of antipsychotics on fetal bone development. Therefore, we aimed to investigate the effects of antipsychotics on embryonic bone formation *in-vivo* using zebrafish as a model.

Methods: The effect of first- (haloperidol; FGA), second- (olanzapine; SGA) and third- (aripiprazole; TGA) generation antipsychotics on early zebrafish bone development was measured using alizarin red staining. Osteoblast development marker expression (*runx2b*, *col10a1*, *spp1*) was measured using whole mount in-situ hybridization, with the total area of staining measured in pixel/µM. Embryos were treated from 36-50 hpf for *runx2b* and 36-72 hpf for *col10a1* and *spp1*. Dopamine (*drd1b* and *drd2a*), serotonin (*htr2b*) and adrenergic receptor (*adrb2b*) expression profiles were measured along with a marker of apoptosis (*casp3a*).

Results: Each antipsychotic inhibited zebrafish bone formation in a dose-dependent manner, where haloperidol was the most potent inhibitor, followed by aripiprazole and then olanzapine. Expression of osteoblast genes were decreased with treatment of 10µM for haloperidol and aripiprazole, whereas olanzapine reduced bone development at 30µM. There was no effect on *casp3a* expression upon antipsychotic exposure, or on dopamine or serotonin receptor expression. However, higher concentration of olanzapine increased *adrb2b* expression, while lower concentrations had no effect.

Conclusion: Each antipsychotic dose dependently inhibited bone development, with haloperidol and aripiprazole being the most potent inhibitors compared to olanzapine. Haloperidol- and aripiprazole-induced bone loss was not due to apoptosis nor did antipsychotic exposure effect dopamine, serotonin or adrenergic receptor expression, while olanzapine-induced bone loss could be due to increased *adrb2b* expression. Further work into the potential signalling pathways is needed to understand the mechanisms involved in antipsychotic induced bone loss.

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Lysosomal proteins Legumain and Cathepsin B expressed within osteocytes cleave Collagen Type I

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During lactation (breast-feeding), to meet the neonate's nutritional demands the maternal calcium is provided from the skeleton through bone resorption by osteoclasts on the bone surface. It has been suggested that osteocytes are also capable of removing their surrounding bone. This osteocytic osteolysis is activated by parathyroid hormone (PTH) and/or parathyroid hormone related-protein (PTHrP) signalling through PTH receptor 1 (PTHR1) on osteocytes. We sought to determine whether osteocytes, like osteoclasts, contribute to lactation-induced bone loss via lysosomal enzymes.

To identify osteocytic lysosomal enzymes, microarray datasets obtained from two previously published studies ((1) Kusa4b10s (osteoblasts) treated with PTH and PTHrP and (2) bones from age-matched lactating and unmated mice), were checked against 151 known lysosomal genes. Two were strongly upregulated by PTH and PTHrP: legumain and cathepsin B, the latter of which was also upregulated in lactating samples.

Legumain and cathepsin B were detected by immunohistochemistry in tibial samples from (1) 6-week-old male C57BL/6 mice injected with 30 µg/kg hPTH(1-34) or vehicle and (2) lactating 10-week-old Black Swiss mice and age-matched controls. Both enzymes were detected in newly-embedded osteocytes in both sets of samples. Cathepsin B staining showed greater intensity in lactating bones compared to non-mated mice, and there was a 3-fold increase in the number of positively-stained osteocytes with PTH treatment compared to vehicle.

To assess collagenolytic activity, recombinant legumain and cathepsin B were added to pure rat-tail collagen I and incubated at 37°C. Cleaved products were separated by gel electrophoresis and visualised by Coomassie stain. Both enzymes dose-dependently cleaved collagen I within 15 minutes.

These data identify legumain and cathepsin B as collagen cleaving lysosomal enzymes that are expressed in osteocytes under normal physiological conditions and upregulated by PTH/PTHrP treatment and lactation. We conclude that osteocytes may use these lysosomal enzymes to conduct osteocytic osteolysis.

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Elevated High-sensitivity Cardiac Troponin I is Associated with Fall and Fracture-Related Hospitalizations in Older Women

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Publish consent withheld

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Engineering cell and animal models of dominant-negative osteogenesis imperfecta using CRISPR

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Osteogenesis imperfecta (also known as brittle bone disease or OI) is a debilitating bone fragility disease often leading to frequent fractures. Although numerous genes are reported to be involved in the pathogenesis of OI, 85-90% of OI cases are attributed to mutations in the genes *COL1A1* and *COL1A2,* encoding for the α1 and α2 chains of type 1 collagen, respectively. We report a patient mutation featuring a 20bp deletion (Δ20) in *COL1A1,* producing a readthrough in the C-terminal pro-peptide of the α1 chain of type 1 collagen. While this mutation is a candidate for gene therapy, these therapeutic strategies require distinct cell and animal models for pre-clinical testing.

Using CRISPR gene editing, we have developed a HEK293T cell line harbouring the Δ20 mutation. An SpCas9 nickase approach with homology directed repair (HDR) yielded a 73.6% editing efficiency, with 26.4% cells incorporating other DNA edits. In parallel, we used CRISPR to generate a genetically modified mouse line harbouring an analogous Δ20 mutation. With evidence for Δ20 homozygosity being lethal, the bone phenotype of Δ20 heterogeneous mice (Δ20/+) was characterized by MicroCT. Both trabecular and cortical bone parameters were reduced in the Δ20/+ male and female mice including trabecular BV/TV (-24.6% males, -26.9% females $p < 0.05$) and cortical thickness (-19.7% males, -12.4% females $p < 0.05$). Predicted bone strength (polar moment of inertia) was less, which will be confirmed by mechanical testing.

These models will enable further understanding of the OI disease pathobiology in C-terminal pro-peptide mutations, whilst also serving as *in vitro* and *in vivo* testing platforms for CRISPR-based gene therapies for disease rescue.

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Bone mineral density and trabecular bone score values in novel subgroups of adult-onset diabetes

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Background:

Individuals with diabetes are heterogenous and recently new, more subgroupings have been proposed (1). These are: mild age-related diabetes (MARD), mild obesity-related diabetes (MOD), severe insulin-resistant diabetes (SIRD), severe insulin-deficient diabetes (SIDD), and severe autoimmune diabetes (SAID). Little is known about how bone health varies between these groups. This study investigated differences in bone health between these subgroups and normoglycaemia.

Methods:

Male participants (n=895, 20-97yr) were drawn from the Geelong Osteoporosis Study. Diabetes (n=105) was defined as fasting plasma glucose≥7.0mmol/L, self-report and/or use of antihyperglycaemic medication. Using hierarchical clustering, men with diabetes were categorised into the SAID subgroup (positive glutamic acid decarboxylase antibodies, n=3), and using K-means clustering, those remaining were categorised into the other subgroups. The Lunar DPX-L and the GE-Prodigy were used to measure bone mineral density (BMD). Lumbar spine DXA scans were assessed for trabecular bone score (TBS) using TBS iNsight software (Version 2.2). ANOVA and linear regression were used to identify differences in BMD and TBS. The SAID group was excluded from regression analyses due to low numbers.

Results:

The subgroups in this study were defined as MARD (n=25, mean age±SD, 80.2±4.5yr), MOD (n=30, 68.4±3.8yr), SIRD (n=31, 58.2±3.1yr), SIDD (n=16, 45.8±6.0yr), and SAID (n=3, 27.0±11.5yr) (Table). Unadjusted femoral neck BMD was lower in the MARD group compared to normoglycaemia; this was not significant after adjusting for age (Table). Adjusting for weight lowered BMD values in the subgroups. No other intergroup differences were observed for BMD. Unadjusted TBS was lower in all subgroups compared to normoglycaemia. Only the SIDD group remained lower (1.557(1.440-1.675)vs1.672(1.586-1.758), p=0.003) after adjusting for age and weight.

Conclusion:

Differences were observed for unadjusted TBS, however, only the SIDD group sustained this difference after adjustment. These results may guide diabetes management strategies regarding bone health, focussing interventions on the subgroups with poorer bone health.

Table: Bone mineral density (BMD) and trabecular bone score (TBS) values for the subgroups compared to normoglycaemia.

	Normoglycaemia (n=790)	MOD (n=25)	MARD (n=30)	SIRD (n=31)	SIDD (n=16)	SAID (n=3)	p
Age (y)	57.0 ± 19.4	73.3 ± 5.6	82.6 ± 4.7	65.0 ± 7.3	58.6 ± 12.5	39.1 ± 10.6	<0.001
BMI (kg/m^2)	26.6 ± 4.0	30.2 ± 5.2	27.1 ± 3.8	29.4 ± 4.7	28.6 ± 3.7	25.9 ± 1.9	<0.001
FN BMD (g/cm^2)	0.998 ± 0.159	0.937 ± 0.145	0.903 ± 0.128	0.990 ± 0.148	0.979 ± 0.120	1.007 ± 0.123	0.048
L1-L4 BMD (g/cm^2)	1.250 ± 0.188	1.318 ± 0.221	1.275 ± 0.199	1.247 ± 0.190	1.235 ± 0.188	1.168 ± 0.058	0.607
TBS	1.286 ± 0.118	1.230 ± 0.096	1.210 ± 0.134	1.233 ± 0.125	1.233 ± 0.149	-	0.008

*MOD=Mild obesity-related diabetes, MARD=Mild age-related diabetes, SIRD=Severe insulin resistant diabetes, SIDD=Severe insulin deficient diabetes, SAID=severe autoimmune diabetes.

1. 1. Ahlqvist E, Storm P, Käräjämäki A, Martinell M, Dorkhan M, Carlsson A, Vikman P, Prasad RB, Aly DM, Almgren P, Wessman Y, Shaat N, Spégel P, Mulder H, Lindholm E, Melander O, Hansson O, Malmqvist U, Lernmark Å, Lahti K, Forsén T, Tuomi T, Rosengren AH, Groop L. Novel subgroups of adult-onset diabetes and their association with outcomes: a data-driven cluster analysis of six variables. Lancet Diabetes Endocrinol 2018;6:361-369

Development of Artificial Intelligence System for Predicting Areal Bone Mineral Density from Plain Radiographs

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Background and Aim: Dual-energy X-ray absorptiometry (DXA) is gold standard method for measuring bone mineral density (BMD) and diagnosing osteoporosis, but DXA is not widely available in low-resource settings. In this study, we developed an AI system to estimate BMD from plain radiographs for osteoporosis screening.

Methods: The development of the AI was based on 5134 plain X-rays, and testing was based on 1926 plain X-rays from the Vietnam Osteoporosis Study. Anteroposterior and oblige digital X-ray of hip and spine were taken by FCR Capsula XLII (Fujifilm Corp., Tokyo, Japan). We used seven Deep Convolution Neural Network to estimate areal BMD at the lumbar spine, total hip, and femoral neck. We termed the estimated BMD as 'xBMD'. We then compared xBMD with DXA-based areal BMD (aBMD) measured at femoral neck, total hip, and lumbar spine (Hologic Horizon, Hologic Corp., Bedford, MA, USA). The concordance between xBMD and aBMD was assessed by the coefficient of correlation and the Bland-Altman approach.

Results: The correlation between xBMD and aBMD was 0.90 (CI 95%: 0.88 – 0.91), 0.91 (CI 95%: 0.89 – 0.92), and 0.87 (CI 95%: 0.85 – 0.88) for femoral neck, total hip and lumbar spine, respectively. The correlation was greater in women than men, but the difference was not statistically significant. When aBMD was used to classify into osteoporosis vs non-osteoporosis, the discrimination of xBMD was high, with area under the ROC curve being 0.94 (CI 95%: 0.92 – 0.96) for femoral neck, 0.95 (CI 95%: 0.93 – 0.97) for total hip and 0.93 (CI 95%: 0.89 – 0.96) for lumbar spine.

Conclusion: These results suggest that it is possible to accurately predict areal BMD from plain radiographs, and that the AI system developed here can be used as an effective tool for opportunistic screening osteoporosis in low-resourced and high-volume settings.

The Tyr Phenomenon: A Hypocalcaemic Response in High-Volume Treatment Responders to ^{177}Lu-PSMA Therapy

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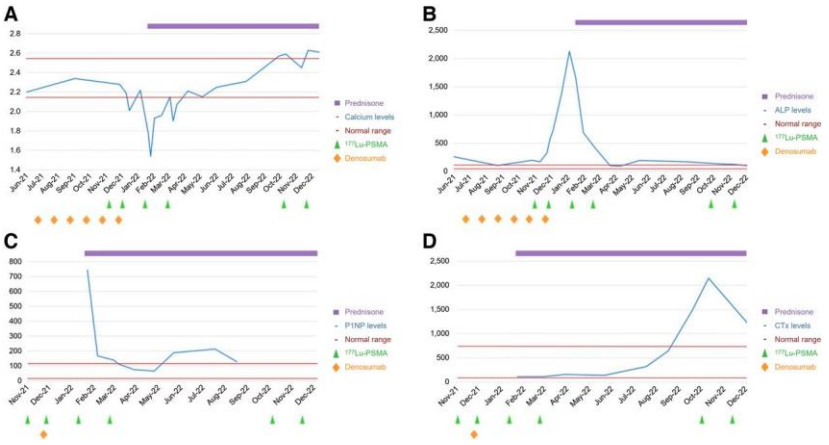
Background: ^{177}Lutetium-prostate-specific membrane antigen (^{177}Lu-PSMA) is an effective treatment for metastatic castration-resistant prostate cancer (mCRPC) which is generally well-tolerated. Clinically significant hypocalcaemia has not been reported during ^{177}Lu-PSMA treatment.

Methods: A clinical dataset of men with progressive mCRPC (n = 127) receiving a minimum of two doses of ^{177}Lu-PSMA-I&T at 6-week intervals was evaluated to estimate the incidence, severity and clinical associations with hypocalcaemia. A median of 8 GBq was administered at each dose with blood collected at baseline and 3-week intervals including corrected calcium, alkaline phosphatase (ALP) and prostate specific antigen (PSA) concentrations.

Results: Forty-one of the 127 men (32%) experienced a reduction in serum calcium and 6/127 (5%) developed laboratory-defined hypocalcaemia within 12-weeks of commencing ^{177}Lu-PSMA. Baseline SPECT total tumour volume was significantly higher in those who developed hypocalcaemia (median 3,249 cm^3 [interquartile range 1,856-3,852] vs 465 [interquartile range 135-1,172]; p = 0.002). The mean PSA response was 78% ± 24% in those who developed hypocalcaemia and some developed marked osteosclerosis. Two patients experienced severe hypocalcaemia (1.54 mmol/L, 1.68 mmol/L) with appropriately elevated parathyroid hormone (PTH) concentrations despite prior cessation of denosumab and required treatment with ≥50mg daily prednisone. Hypocalcaemia was associated with elevated ALP (2,049 U/L, normal-range 35-110) and P1NP (744 ug/L, normal-range 15-115) concentrations in one patient.

Conclusion: Clinically significant hypocalcaemia and osteosclerosis are rare but important side effects of ^{177}Lu-PSMA in men with high-volume osseous metastatic disease and significant treatment response. Given markedly elevated bone formation markers and response to high-dose glucocorticoids in the most severe case, we hypothesise Lu-PSMA triggered an exaggerated osteoblastic response resulting in depletion of circulating calcium stores and subsequent hypocalcaemia. Prospective evaluation of ^{177}Lu-PSMA-induced hypocalcaemia and histopathological examination of the tumour microenvironment is required to better understand the underlying mechanisms, optimal treatment, and consequences of any associated osteosclerotic response.



15

In elderly women, bone deposited in regions of high cortical porosity is less mineralised and has a higher carbonate content than that of younger women

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Age-related bone fragility is associated with increased cortical porosity, which varies regionally across the mid-femoral cortex. However, biomechanical studies have shown that bone fragility is not fully explained by this bone loss, and altered material composition is thought to play a role. Whether compositional defects in aged bone reflect a degradation in the quality of existing bone tissue, or an inherent difference in the bone deposited is unknown. We aimed to determine whether newly deposited osteonal bone differs in composition between older and younger women, and whether this difference is exaggerated in regions of bone loss.

Cadaveric femoral midshaft samples from 5 healthy young women (20-40 years old) and 5 healthy elderly women (77-95 years old) were obtained from the Melbourne Femur Research Collection. Cortical bone wedges from the posterior octant (which exhibits the greatest age-related increase in porosity) and lateral octant (which exhibits the least) were assessed by micro-computed tomography. Synchrotron-based Fourier-transform infrared (FTIR) microspectroscopy was then used to measure mineral:matrix and carbonate:phosphate ratios within newly deposited osteons, identified by histology and low resolution scanning.

In elderly women, mean osteonal wall thickness was 37.5% lower than young women in both regions measured, even though porosity was only significantly increased in the posterior region. Within this region, new bone deposited in older women had a significantly (4.5%) lower mineral:matrix ratio and 10% higher carbonate:phosphate ratio than bone built in the same region by young women. Contrastingly, the lateral region did not exhibit any differences in composition between the two age groups.

In summary, osteonal bone built by older women in a region of bone loss is less mineralised and has a higher carbonate content than that built by younger women. This region-specific change in bone material quality in older women may contribute to their higher susceptibility to fragility fractures.

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Reciprocal interactions of bone marrow macrophages and mesenchymal stromal cells impact skeletal homeostasis

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Pre-clinical studies show that senescent bone marrow-derived mesenchymal stromal (a.k.a. stem) cells (MSCs) and osteolineage cells contribute to age-dependent bone loss and bone marrow failure. Therefore, the identification of novel mechanisms that accelerate MSC dysfunction could enable mechanistic approaches to degenerative processes that impact the skeleton. While a handful of in vitro studies previously demonstrated MSCs' ability to phagocytose apoptotic cells (also known as efferocytosis), matrix, pathogens and metal particles, whether efferocytosis by MSCs impacts their function and bone maintenance is not known. We found that bone marrow MSCs indeed efferocytose apoptotic neutrophils in vivo. In aged mice, where bone marrow macrophages are defective, efferocytosis by MSCs is significantly increased. Transcriptional and functional data in vitro show that excessive efferocytosis by MSCs decreases osteoblastic differentiation and promotes senescence. We hypothesized that phagocytosis by MSCs, when pathologically increased in aging or in the setting of macrophage dysfunction, causes MSC oxidative stress, mitochondrial dysfunction and senescence, thus contributing to bone loss. We are using aging and genetic models to determine the mechanism of MSC efferocytosis, define the pathogenic mechanisms induced by efferocytosis in MSCs, and establish the role of efferocytosis by MSCs in normal osteoimmunology and in aged bone. Adult mice lacking the critical receptor for efferocytosis in MSCs (Axl) demonstrate increased bone. In contrast, mice with transgenic overexpression of the direct phosphatidyl serine receptor BAI1 in MSCs demonstrate bone loss. Since efferocytosis is accompanied by oxidative stress and mitochondrial changes, which we previously found to modulate osteoblastic differentiation, we tested metabolic function in efferocytic MSCs. We found that mitochondrial disruption mediates functional changes in MSCs that clear high numbers of apoptotic cells. In summary, efferocytosis induces metabolic changes and senescence in MSCs, and may therefore represent a novel, targetable mechanism of accelerated skeletal aging.

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Delayed skeletal development in CSF1R-deficient rats highlights independent contributions of osteal macrophages and osteoclasts to postnatal bone development

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The osteopetrosis associated with colony stimulating factor 1 (*Csf1*) and Csf1 receptor (*Csf1r*) mutations has been primarily attributed to the loss of osteoclasts and deficiency in bone resorption. The rodent *Csf1r* knockout (*Csf1rko)* phenotype is more severe, with postnatal lethality common, restricting opportunity to study impacts on postnatal skeletal development. We recently characterized delayed postnatal skeletal ossification in an inbred homozygous *Csf1rko* rat model that survives into adulthood and that this could be rescued by intraperitoneal cell transfer of whole bone marrow (BMT) without donor-derived haematopoietic conversion (1). Here, we have examined the cellular basis for the skeletal phenotype in the *Csf1rko* rat up to 7 weeks of age and myeloid cell dynamics associated with BMT rescue. We verified continuing osteoclasts deficiency in *Csf1rko* rat bones that was associated with persistent ineffective removal of growth plate primary spongiosa, ineffective remodelling of woven bone in spongiosa and failed excavation of the medulla. Osteogenesis at secondary ossification centres (SOC) and sites of subarticular ossification was delayed in *Csf1rko* rats but not stalled with notable progression observed between 3-7 weeks of age. Temporal micro-CT and in situ analysis indicated that at these anatomical sites in control rats, osteomac infiltration preceded osteoclasts during excavation of the cartilage anlagen. Interestingly, breakthrough re-emergence of osteomacs in *Csf1rko* corresponded with delayed ossification initiation. Accelerated phenotype reversal following BMT in *Csf1rko* rats was characterised by osteomacs acting as pioneering cells at all sites of ossification. Osteomacs persisted throughout phenotype reversal, being juxtaposition to both osteoblast and osteoclast at sites of elevated bone remodelling/modelling. Osteomac and osteoclast repopulation persisted at least 5 weeks post-BMT, suggesting prolonged engraftment of non-haematopoiesis-derived precursors within the peritoneum and/or throughout the skeleton. These observations extend evidence that osteomacs independently contribute to bone dynamic events underpinning normal postnatal bone growth and morphogenesis.

1. Keshvari S, Caruso M, Teakle N, Batoon L, Sehgal A, Patkar OL, Ferrari-Cestari M, Snell CE, Chen C, Stevenson A, Davis FM, Bush SJ, Pridans C, Summers KM, Pettit AR, Irvine KM, Hume DA. CSF1R-dependent macrophages control postnatal somatic growth and organ maturation. PLoS Genetics. 2021: 17 (6), e1009605.

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Nonmelanoma skin cancer is associated with fewer incident fractures, more vitamin D sufficiency, greater BMD and improved bone microarchitecture in older adults

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Introduction

Nonmelanoma skin cancer (NMSC), a biomarker of cumulative lifetime sun exposure, is associated with reduced fracture risk later in life, but the mechanism is unknown.

Methods

Prospective cohort analysis of 1,099 community-dwelling adults aged 50-80 years with baseline and 10 year follow up assessments. Histopathologically-confirmed NMSC diagnosis was established by linkage with the Tasmanian Cancer Registry. BMD and vertebral deformity were by quantified by DXA, 25(OH)D by radioimmunoassay, bone microarchitecture by high resolution peripheral quantitative CT, melanin density by spectrophotometry and skin photosensitivity and clinical fracture by questionnaire. 25(OH)D <50 nmol/L was considered deficient.

Results

Participants with prior NMSC at baseline were less likely to sustain an incident vertebral deformity over 10 years (RR=0.73, *p*=0.018). There were similar reductions for other fracture types but these did not reach significance. Prior NMSC was associated with baseline (RR=1.22, *p*=0.007) and 10 year longitudinal (RR=6.2, *p*=0.011) vitamin D sufficiency and greater total body BMD (β=0.20g/cm2, *p*=0.046), but not falls risk (β=-0.0, *p*=0.62). The relationship between NMSC and bone microarchitecture was age dependent ($p_{interaction}$<0.05). In the oldest age tertile, prior NMSC was associated with higher volumetric BMD (β=55.6–60.5, *p*=0.002–0.01) and less porosity (β= -4.1 – -5.1, *p*=0.002–0.009) at cortical, compact cortical and outer transitional zones. Associations were independent of sex, BMI and skin phenotype.

Conclusion

Prior NMSC was associated with fewer incident fractures in community-dwelling older adults. This protective association is most likely mediated by modifiable fracture risk factors associated with an outdoor lifestyle, including 25(OH)D, BMD and bone microarchitecture.

Bacterial heat shock protein: A new crosstalk between T lymphocyte and macrophage via JAK2/STAT1 pathway in chronic osteomyelitis

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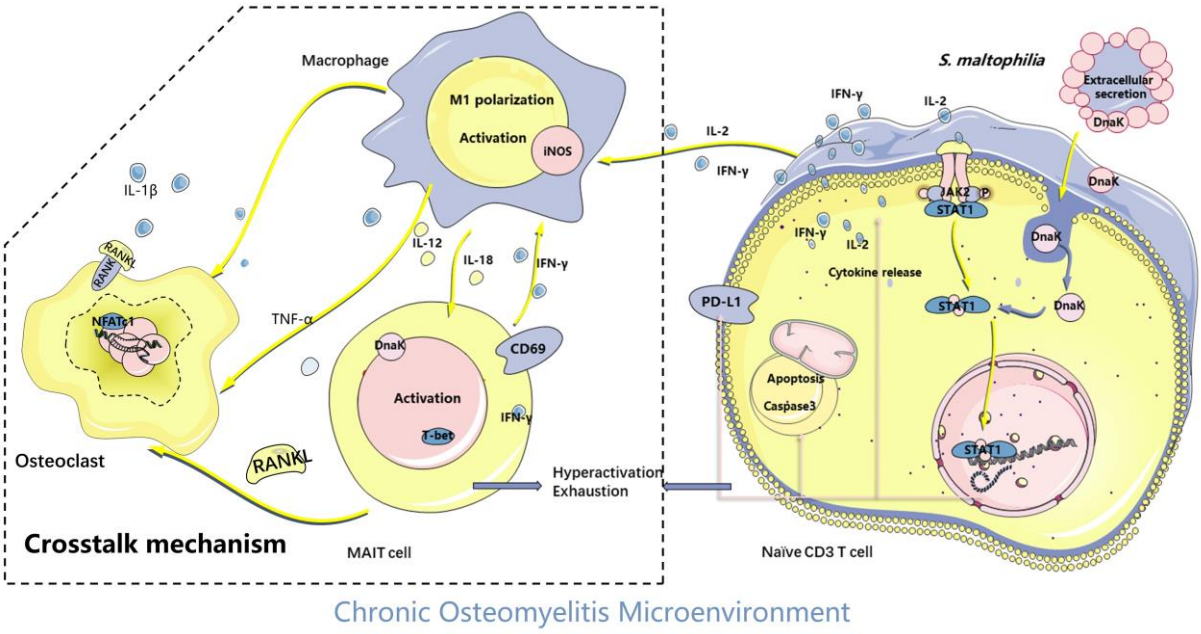
Introduction: Osteomyelitis (OM) is an infection of the bone, a systemic infection leading to multiple bone tissue dysfunction, coupled with drug resistance, bloodstream infection, and limited clinical treatment options. Symptoms may include pain in a specific bone with overlying redness, fever, and weakness. Objectives: This work aims to further investigate the new interplay between bacterial exocrine regulatory protein and host immune cells in the chronic osteomyelitis microenvironment. Whether interfering with related regulatory signaling pathways can reverse the inflammatory disorder of bone immune cells. Methods: In-depth analysis of single-cell sequencing results in patients for potential immunodeficiency factors. Analysis of key proteins enriched by host cells and key pathways using proteomics. Cell models and animal models validate the pathological effects of bacterial heat shock protein (DnaK) on T cells, MAITs, macrophages, and osteoclasts. Results: We identified that *S. maltophilia*-DnaK was enriched in immunodeficient T cells. The activation of the JAK2/STAT1 axis initiated the exhaustion of T cells. Patients with Gram-negative bacterial infections exhibited deficiencies in MAITs, which correspond to IFN-γ. Cellular and animal experiments confirmed that DnaK could facilitate MAIT depletion and M1 polarization of macrophages. Additionally, Fludarabine mitigated M1 polarization in mice. Interestingly, DnaK also repressed osteoclastogenesis of macrophages stimulated by RANKL. Conclusions: DnaK prompts the activation of the JAK2/STAT1 axis in T cells and the M1 polarization of macrophages. Targeting the DnaK's crosstalk can be a potentially effective approach for treating the inflammatory disorder in the chronic osteomyelitis microenvironment.



Chronic Osteomyelitis Microenvironment

Cardiovascular risk factors and bone mineral density: Data from the Geelong Osteoporosis Study

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Objective

Osteoporosis and cardiovascular disease (CVD) share common risk factors; both often remain undiagnosed until a major life-threatening event occurs. We investigated the associations between rate pressure product (RPP), a surrogate of cardiac workload, or pulse pressure (PP), a measure of arterial stiffness, and bone health in Australian men and women.

Method

Participants were men (n=832) and women (n=764) from recent follow-up visits of the Geelong Osteoporosis Study (men:2006-2011; women:2010-2014). DXA scans (Lunar Prodigy) were performed at the hip and spine (L2-L4). Blood pressure and heart rate were measured (seated) to calculate RPP and PP. All variables were mean-standardised by sex. Linear regression models were sex-stratified; BMD at the total hip or spine were the dependent variables and RPP or PP the independent variables. Adjustments were made for sex, age, height, weight, alcohol consumption, current smoking, physical activity and use of medications (glucocorticoids, bisphosphonates, antihypertensives, statins).

Results

Median age was 60.4yr (IQR 47.6-73.4) for men and 55.7yr (IQR 43.1-68.0) for women. Data for all models are shown in Table 1. In women, RPP was positively associated with BMD at both sites in unadjusted models, and this association persisted at the lumbar spine after adjustment for covariates. No associations were observed between RPP and BMD in men. In women, PP was inversely associated with hip BMD in unadjusted models and positively associated with BMD at both sites after adjustment. In men, PP was negatively associated with total hip BMD when unadjusted, which attenuated after adjustment. PP was positively associated with spine BMD, before and after adjustment.

Conclusion

Cardiovascular risk factors are associated with BMD in adults. Associations are seen at the spine and hip in women, and at the spine only in
men.

Table 1. Associations between Rate Pressure Product (RPP), Pulse Pressure (PP) and bone mineral density (BMD) at the total hip or lumbar spine in both men and women. Significant p values ($p<0.05$) are marked in bold.

	Women		*Men*	
	Unadjusted	*Adjusted#*	*Unadjusted*	*Adjusted#*
RPP vs Total Hip	β=0.080 **p=0.029**	β=0.041 p=0.196	β=0.041 p=0.259	β=-0.018 p=0.594
RPP vs Lumbar spine	β=0.086 **p=0.017**	β=0.083 **p=0.024**	β=0.015 p=0.657	β=-0.033 p=0.347
PP vs Total Hip	β=-0.132 **p<0.001**	β=0.101 **p=0.003**	β=-0.101 **p=0.005**	β=-0.026 p=0.468
PP vs Lumbar spine	β=-0.050 p=0.171	β=0.114 **p=0.004**	β=0.139 **p<0.001**	β=0.078 **p=0.033**

#Models adjusted for: age (cubic in women, linear in men), height, weight, alcohol consumption, mobility, smoking, glucocorticoids, bisphosphonates, antihypertensives (anti-adrenergics, diuretics, betablockers, calcium channel blockers, renin-angiotensin-aldosterone system inhibitors), and statins.

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Macrophage barnacles muddy the waters of single cell precision profiling of bone and bone marrow cell populations.

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Bone tissues and bone marrow (BM) contain multiple tissue macrophages that support distinct functional niches: erythroblastic island macrophages support red blood cell production, haematopoietic stem cell (HSC) niche macrophages preserve a life-long pool of HSC, and osteal macrophages (osteomacs) promote bone development, health and regeneration. Bone and BM macrophages represent a virtually untapped common cellular target with demonstrated potential to simultaneously promote positive outcomes for both bone and BM health. To realise this opportunity, precision molecular profiling of the signatures underpinning macrophage distinct functional subsets is needed. Characterisation of bone and BM macrophage molecular profiles has been elusive, even when using sophisticated single cell approaches[1]. We exposed that cell events rationally gated as macrophages using flow cytometry analysis of bone-BM single cell suspensions were unrelated cells with membrane-encapsulated macrophage-remnants attached to their surface. Importantly, the macrophage-remnants contain high amounts of macrophage-derived proteins, intracellular reporter molecules and RNAs. The major implication is that the state of knowledge regarding bone and BM macrophage molecular profiles needs to be re-examined and validated. We next used in situ staining or imaging flow cytometry to interrogate whether reported expression of either other cell lineage specific markers on osteomacs or expression of myeloid/macrophage markers on other cells within BM accurately reflects intrinsic expression. Immunohistochemistry showed that CD110 (thrombopoietin receptor), reported to differentiate osteomacs from other BM macrophages[2], is not expressed by osteomacs. Using *Csf1r*- or *Siglec1*-promoter driven reporter models combined with appropriate marker antibody panels, we show that reported HSC expression of the macrophage markers CD115 and VCAM1 is entirely derived from fragmented macrophage-remnants. In contrast, HSC expression of MHC class II was due to both HSC intrinsic and attached macrophage remnant expression. These data highlight that elevated scrutiny is required when applying ex vivo single cell analysis strategies to profile bone and BM cells.

1. Romano L, et al. Erythroblastic islands foster granulopoiesis in parallel to terminal erythropoiesis. Blood. 2022;140 (14):1621-34.
2. Mohamad S.F., et al. Neonatal osteomacs and bone marrow macrophages differ in phenotypic expression and function. J Bone Miner Res. 2021; 36 (8): 1580-93.

FGF23-mediated hypophosphataemic osteomalacia with low bone turnover

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Case Summary

A 29-year-old female presented with chronic bilateral proximal thigh pain on a background of fibroblast growth factor 23 (FGF23) mediated hypophosphataemic osteomalacia with atypically suppressed bone turnover markers (BTM). The patient was initially diagnosed with hypophosphataemia at the age of 19 and has experienced brittle dentition, multiple fragility and insufficiency fractures with bilateral femur Looser zones, right wrist fracture, rib fractures, bilateral cuboid stress fractures and metacarpal fractures requiring multiple operations. These fractures have been complicated by non-union/markedly delayed healing and chronic pain. The patient is a highly functioning medical practitioner, with no smoking or alcohol history and no dysmorphic features. There is no history of nephrolithiasis, hearing impairment or family history of osteoporosis. Her height is 163 cm and weight is 65 kg. Other medical history includes juvenile arthritis on leflunomide and methotrexate, and narcolepsy.

Serum phosphate level was low (0.35 mmol/L [refence range 0.75-1.50 mmol/L]) with evidence of urinary phosphate wasting (22 mmol/24h [15-60 mmol/24h]) and low renal tubular maximum reabsorption rate of phosphate relative to glomerular filtration rate (TmP/GFR) (0.53 mmol/L [0.84-1.23 mmol/L]). FGF23 was inappropriately normal (78 ng/L [23-95 ng/L]). Serum calcium levels, 25-hydroxy vitamin D, 1,25-dihydroxyvitamin D3 and estimated GFR were normal. There was no metabolic acidosis to suggest Fanconi's syndrome. Interestingly, parathyroid hormone (PTH) was normal (5.3 pmol/L, [1.7-10.0 pmol/L]) and bone turnover was atypically low with alkaline phosphatase (ALP) of 39 U/L (30-110 U/L), bone-specific ALP of 8.8 microg/L (5.5-24.6 microg/L), C-terminal telopeptide of type 1 collagen (CTX) of 172 ng/L (150-800 ng/L), and procollagen type I N-propeptide (PINP) of 21 mcg/L (15-70 mcg/L) in the setting of multiple fractures and poor healing. A bone biopsy demonstrated increased osteoid thickness consistent with osteomalacia and decreased number of double labels relative to bone surface. No pathogenic variants were found on targeted whole exome sequencing of genes related to hypophosphataemic rickets or osteogenesis imperfecta (Table 1). Bone mineral density on dual x-ray absorptiometry scan was normal. MRI brain revealed no evidence of Chiari malformation. A Ga-68 DOTATATE positron emission tomography scan demonstrated no mesenchymal tumours.

After confirming FGF23-mediated hypophosphataemic osteomalacia and satisfying Pharmaceutical Benefits Scheme (PBS) criteria, burosumab was commenced one week after cessation of calcitriol and phosphate supplementation. Recommended weight-based dosing of burosumab was complicated by hyperphosphataemia (1.91 mmol/L [0.75-1.50 mmol/L]) two weeks post-dose and after resolution a halved dose of burosumab was recommenced without further adverse events.

Within a month of recommencement of burosumab, a bone scan to investigate new proximal bilateral thigh pain demonstrated focally increased osteoblastic activity in the medial cortices of the proximal femoral shafts bilaterally, suggestive of new insufficiency fractures. Other areas osteoblastic activity were identified throughout the skeleton, corresponding with previous fracture sites and delayed healing (Figure 1). Surgical management was sought by the patient with bilateral intramedullary nails inserted after her orthopaedic surgeon determined the risk of fulminant fracture was high without intervention. After 3 doses of burosumab, TmP/GFR has now normalised, although symptomatic benefits are yet to occur. Clinical and radiological benefits with a repeat bone scan after 6 months of burosumab will be presented at the ANZBMS meeting.

Discussions

Hypophosphataemic osteomalacia has a wide range of clinical effects, including varus deformity of the lower limbs, gait disturbance, muscle weakness, enthesopathy, nephrocalcinosis and dental necrosis affecting morbidity and quality of life.[1] FGF23 is a key regulator of phosphate homeostasis and increases renal phosphate excretion and decreases the production of 1,25 dihydroxyvitamin D in renal proximal tubules.[2, 3] The ratio of maximal total reabsorption of phosphate compared to glomerular filtration rate (TmP/GFR) utilising fasting paired plasma and second-morning void urine phosphate levels,[4] is reduced in FGF23-mediated hypophosphataemic osteomalacia.[5]

The most common cause of FGF23-mediated hypophosphatemia is X-linked hypophosphatemia (XLH) secondary to a loss of function pathogenic variant in PHEX gene affecting 1/20,000.[6] Other inherited causes of FGF23-mediated hypophosphataemia include autosomal dominant and autosomal recessive hypophosphataemic rickets, alpha-klotho translocation, fibrous dysplasia-McCune-Albright syndrome, cutaneous skeletal hypophosphataemia syndrome, osteoglophonic dysplasia, and SGK3 mutations.[1] Acquired forms of FGF23-mediated hypophosphataemia include tumour-induced osteomalacia (TIO) and intravenous ferric carboxymaltose use.[1] Our patient's extensive genomic screen did not find pathogenic gene defects, and mesenchymal tumours were excluded on a PET scan, suggestive of yet-to-be-identified pathogenic genes.

Historically, phosphate and 1,25 dihydroxyvitamin D supplementation have been the cornerstone of therapy. However, burosumab, a recombinant human IgG1 monoclonal antibody against FGF23, has demonstrated superior efficacy in achieving serum phosphate control,[7, 8] improvements of physical ability, pain control and mineralisation defects compared to the conventional treatment.[1] Fasting phosphate level should be checked at two weeks post-injection to assess for hyperphosphataemia and titration of the medication.[9] Other adverse events include minor injection site reactions, pain in the extremities, fever, myalgia and rash.[7] Currently, burosumab is subsidised by the PBS for XLH and those with FGF23-mediated hypophosphataemic osteomalacia without PHEX gene mutation.

ALP and BTM are typically elevated in patients with hypophosphataemia and rickets,[10] which reflects accelerated bone remodelling.[11] Our case is an atypical case of FGF23-mediated hypophosphataemic osteomalacia with inappropriately normal BTM despite evidence of osteomalacia on bone biopsy, atraumatic poor healing fractures and chronic bony pain. ALP and BTM may be normal in TIO,[1] however, mesenchymal tumours have been excluded on a PET scan in our case. Whole genome sequencing of the patient and her parents may be warranted to determine the cause of hypophosphataemic osteomalacia with low BTMs in this case.

Take home messages:

- FGF23-mediated hypophosphataemic osteomalacia has various clinical implications, including varus deformity of the lower limbs, gait disturbance, muscle weakness, enthesopathy, nephrocalcinosis and dental necrosis, and is typically associated with elevated bone turnover markers.
- The most common FGF23-mediated hypophosphatemia is XLH secondary to a loss of function pathogenic variant in the PHEX gene. However, other genetic causes are possible, and more are yet to be identified.
- Burosumab, an FGF23 antibody, leads to significant improvement in serum phosphate and fracture healing in XLH and may be an effective treatment for other causes of FGF23-mediated hypophosphatemia
- A novel gene abnormality may explain this unusual case of hypophosphataemic osteomalacia with low bone turnover markers.

1. Trombetti A, Al-Daghri N, Brandi ML, et al. Interdisciplinary management of FGF23-related phosphate wasting syndromes: a Consensus Statement on the evaluation, diagnosis and care of patients with X-linked hypophosphataemia. Nat Rev Endocrinol. 2022;18(6):366-84.
2. Shimada T, Hasegawa H, Yamazaki Y, et al. FGF-23 is a potent regulator of vitamin D metabolism and phosphate homeostasis. J Bone Miner Res. 2004;19(3):429-35.
3. Shimada T, Yamazaki Y, Takahashi M, et al. Vitamin D receptor-independent FGF23 actions in regulating phosphate and vitamin D metabolism. Am J Physiol Renal Physiol. 2005;289(5):F1088-95.
4. Walton RJ, Bijvoet OL. Nomogram for derivation of renal threshold phosphate concentration. Lancet. 1975;2(7929):309-10.
5. Fukumoto S. FGF23-related hypophosphatemic rickets/osteomalacia: diagnosis and new treatment. J Mol Endocrinol. 2021;66(2):R57-r65.
6. Endo I, Fukumoto S, Ozono K, et al. Nationwide survey of fibroblast growth factor 23 (FGF23)-related hypophosphatemic diseases in Japan: prevalence, biochemical data and treatment. Endocr J. 2015;62(9):811-6.
7. Carpenter TO, Whyte MP, Imel EA, et al. Burosumab Therapy in Children with X-Linked Hypophosphatemia. N Engl J Med. 2018;378(21):1987-98.
8. Imel EA, Glorieux FH, Whyte MP, et al. Burosumab versus conventional therapy in children with X-linked hypophosphataemia: a randomised, active-controlled, open-label, phase 3 trial. Lancet. 2019;393(10189):2416-27.
9. Haffner D, Emma F, Eastwood DM, et al. Clinical practice recommendations for the diagnosis and management of X-linked hypophosphataemia. Nat Rev Nephrol. 2019;15(7):435-55.
10. Schini M, Vilaca T, Gossiel F, et al. Bone Turnover Markers: Basic Biology to Clinical Applications. Endocr Rev. 2023;44(3):417-73.
11. Michałus I, Łupińska A, Woch I, et al. Bone Turnover Markers and Bone Mineral Density in Children with Hypophosphatemic Rickets. J Clin Med. 2022;11(15).

Diagnostic challenges in a case of refractory severe hypercalcemia: case report

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Introduction:

Hypercalcemia is a common clinical abnormality with 90% of cases attributed to either malignancy or primary hyperparathyroidism[1]. Other causes of hypercalcaemia often require careful consideration, particularly where preliminary tests are inconclusive or calcium levels are refractory to initial therapy for the suspected underlying cause. Although hypercalcemia is a known metabolic complication of sarcoidosis, it is rarely a presenting manifestation and affects up to 10% of cases[2-5]. The primary mechanism of hypercalcaemia is attributed to increased activity of 1α-hydroxylase enzyme from activated granuloma macrophages, resulting in uncontrolled synthesis of 1,25-dihydroxyvitamin D3(1,25-OHD) from 25-hydroxyvitamin D(OHD)[6]. However, utilising vitamin D metabolites to assess disease activity remains challenging. We present a complex diagnostic and management case of sarcoidosis diagnosed on splenic biopsy, in a 60-year-old Caucasian female who presented with asymptomatic severe hypercalcemia, refractory to conventional therapy, on a background of chronic stage IV diabetic nephropathy.

Case presentation:

Our patient presented with severe asymptomatic hypercalcemia with paired CrCa 3.50mmol/L/ PTH 1.1pmol/L on routine serology. She had a normal 25-OHD 68nmol/L, and mildly elevated 1,25-OHD 206pmol/L [60-200] on a background of chronically impaired renal function (creatinine 247umol/L). Serum angiotensin-converting enzyme (ACE) level (enzymatic assay) and parathormone-related peptide (PTHrP) were undetectable. A 24-hour urinary calcium was low 2.0mmol/24h [2.5-7.5]. Whilst undergoing further investigations to ascertain the cause of her PTH-independent hypercalcemia, her corrected calcium level remained persistently elevated above 3.0mmol/L for over five weeks despite intravenous fluids and multiple doses of pamidronate, denosumab, and four aliquots of calcitonin. The patient subsequently underwent a PET scan, which revealed FDG-avid lymphadenopathy and splenic uptake suggestive of lymphoma. A splenic biopsy revealed sarcoid-like well-formed, diffuse granulomas and was subsequently diagnosed with extrapulmonary sarcoidosis.

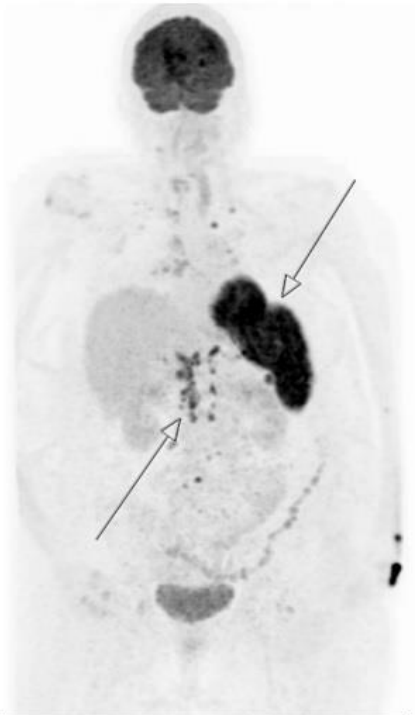

Figure 1. PET scan revealing FDG avid lymphadenopathy above and below the diaphragm coupled with avid FDG uptake in the spleen.

Treatment:

Despite high-dose prednisolone therapy for two-weeks, severe hypercalcaemia persisted and deteriorated to 3.41mmol/L. Moreover, judicious use of required intravenous fluid therapy was limited due to her chronic renal impairment. Given her hypercalcemia was not responsive to steroid therapy, coupled with a now normalised repeat 1,25-OHD 161pmol/L, the diagnosis of sarcoid-induced hypercalcemia was questioned. Conversely, while ACE activity level was undetectable using the routine enzymatic assay, ACE mass level using immunoassay showed high-normal level of 189ug/L [37-221] despite steroid therapy and on an ACE-inhibitor. After multi-disciplinary team discussion, second-line ketoconazole therapy was trialled. There was nil initial improvement in serum calcium until two weeks of ketaconazole therapy (dosed at 200mg before increasing to 600mg), which improved her corrected calcium level to 2.74mmol/L. Currently, after five weeks of ketoconazole and seven weeks of prednisone her calcium levels have now normalised (2.20mmol/L).

Discussion:

Hypercalcaemia is a common clinical entity, however sarcoidosis as an aetiology for hypercalcaemia is relatively uncommon. The incidence of hypercalcaemia as the presenting symptom in sarcoidosis is 3%[7], which makes the diagnosis challenging particularly when refractory to conventional therapy. Our case is unique, as there was no other clinical feature or radiographic evidence of systemic sarcoidosis, and diagnosis was suggested only by splenic biopsy. Our case displayed important features: (1)markedly elevated serum calcium levels 3.50mmol/L in the absence of overt systemic sarcoidosis, (2)decreased levels of urinary excretion of calcium as well as normal serum ACE and minimally elevated 1,25-OHD, and (3)hypercalcemia unresponsive to corticosteroid therapy.

Sarcoidosis is an idiopathic, multisystem, granulomatous disease, with extrathoracic splenic involvement occurring in up to 40-60% of cases[8]. Hypercalcemia in sarcoidosis is attributed to three potential mechanisms: (1)systemic extrarenal conversion of 25-OHD to 1,25-OHD by 1α-hydroxylase produced by granulomatous macrophages and the consequential increase in intestinal calcium absorption, (2)granuloma production of PTHrP causing increased renal calcium absorption and bone resorption, similar to humoral hypercalcemia of malignancy, and (3)tissue-level conversion of 25-OHD to 1,25-OHD by local granulomatous macrophages[9]. Interestingly, our case demonstrated only mildly elevated 1,25-OHD levels, in addition to undetectable PTHrP levels.

There have been similar case reports in the literature illustrating hypercalcemia with inappropriately normal 1,25-OHD levels in the setting of granuloma-forming disorders[10-12]. Proposed contributing factors for normal 1,25-OHD levels may include increased oral calcium intake, dehydration, and decreased calcium excretion, particularly in renal insufficiency[13], as seen in our patient with a baseline serum creatinine of 247umol/L and decreased calcium excretion. The clinical utility of serum ACE levels in sarcoidosis is also limited due to poor sensitivity and specificity, with high ACE levels occurring in up to 75% of cases[14].

Corticosteroids are considered first-line therapy in treating sarcoidosis-associated hypercalcemia due to their effectiveness in treating granulomatous inflammation and rapidly correcting hypercalcemia due to a relatively swift decrease in 1,25-OHD and calcium levels typically within 3-5 days[15]. As in our case of steroid-resistant sarcoidosis, ketoconazole may be effective in treating hypercalcemia as it inhibits 1α-hydroxylase required in 1,25-OHD synthesis[16]. Additional steroid-sparing agents, such as infliximab, methotrexate and azathioprine, may also help in treating refractory hypercalcemia by reducing granuloma formation[17].

Conclusion:

Our case demonstrates an unusual presentation of extrathoracic sarcoidosis, presenting as prolonged, severe hypercalcemia with inappropriately normal or minimally elevated 1,25-OHD, which was refractory to conventional therapies including fluid therapy, anti-resorptive therapies and high dose steroid therapy. This case highlights the importance of multi-displinary team input in managing uncommon causes such as sarcoidosis as a differential diagnosis for PTH-independent hypercalcaemia, as it has a multisystemic and non-specific presentation, with biopsy and radiographic findings as key to diagnosis. Timely recognition and appropriate treatment with second-line therapy may be necessary to avoid prolonged effects of first-line steroid therapy particularly in cases with prolonged refractory hypercalcemia with multiple co-morbidities such as type 2 diabetes and chronic renal failure.

Learning Points:

1. Sarcoidosis-induced hypercalcemia is uncommon and presents diagnostic challenges.
2. Persistent levels of severe hypercalcaemia can occur in sarcoidosis despite inappropriately normal or minimally elevated 1,25-OHD levels.
3. Medications such as ACE-inhibitors and prednisolone should be taken into consideration when interpreting ACE activity levels. Utilising ACE mass assay (immunoassay) rather than an enzymatic assay, may be considered more clinically reliable.
4. Biopsy and radiographic findings are key to diagnosing sarcoidosis, even without overt systemic features.
5. Refractory hypercalcemia requires timely recognition and consideration of second-line therapies, such as ketoconazole or other steroid-sparing agents. Multidisciplinary input is vital for managing complex cases effectively.

1. Walker, M.D. and E. Shane, Hypercalcemia: A Review. Jama, 2022. 328(16): p. 1624-1636.
2. Adams, J.S., Vitamin D metabolite-mediated hypercalcemia. Endocrinol Metab Clin North Am, 1989. 18(3): p. 765-78.
3. Sharma, O.P., Vitamin D, calcium, and sarcoidosis. Chest, 1996. 109(2): p. 535-9.
4. Soto-Gomez, N., J.I. Peters, and A.M. Nambiar, Diagnosis and Management of Sarcoidosis. Am Fam Physician, 2016. 93(10): p. 840-8.
5. Karnchanasorn, R., et al., Severe hypercalcemia and acute renal failure: an unusual presentation of sarcoidosis. Case Rep Med, 2010. 2010: p. 423659.
6. Zhou, Y. and E.E. Lower, Balancing Altered Calcium Metabolism with Bone Health in Sarcoidosis. Semin Respir Crit Care Med, 2020. 41(5): p. 618-625.
7. Baughman, R.P., et al., Clinical characteristics of patients in a case control study of sarcoidosis. Am J Respir Crit Care Med, 2001. 164(10 Pt 1): p. 1885-9.
8. Raber, E.L., J. Haba, and P. Beck, Splenic sarcoidosis: a case report and review of the imaging findings of multiple incidental splenic lesions as the initial presentation of sarcoidosis. Can J Gastroenterol, 2011. 25(9): p. 477-8.
9. Berlin, J.L., et al., Serum vitamin D levels may not reflect tissue-level vitamin D in sarcoidosis. BMJ Case Rep, 2014. 2014.
10. Falk, S., et al., Hypercalcemia as a result of sarcoidosis with normal serum concentrations of vitamin D. Med Sci Monit, 2007. 13(11): p. Cs133-136.
11. Gwadera, Ł., et al., Sarcoidosis and calcium homeostasis disturbances-Do we know where we stand? Chron Respir Dis, 2019. 16: p. 1479973119878713.
12. Unsal, A., et al., Renal sarcoidosis with normal serum vitamin D and refractory hypercalcemia. Int Urol Nephrol, 2013. 45(6): p. 1779-83.
13. Volpicelli, G., A. Mussa, and M. Frascisco, A case of severe hypercalcemia with acute renal failure in sarcoidosis: a diagnostic challenge for the emergency department. Eur J Emerg Med, 2005. 12(6): p. 320-1.
14. Ungprasert, P., et al., Diagnostic Utility of Angiotensin-Converting Enzyme in Sarcoidosis: A Population-Based Study. Lung, 2016. 194(1): p. 91-5.
15. Sharma, O.P., Hypercalcemia in granulomatous disorders: a clinical review. Curr Opin Pulm Med, 2000. 6(5): p. 442-7.
16. Adams, J.S., et al., Ketoconazole decreases the serum 1,25-dihydroxyvitamin D and calcium concentration in sarcoidosis-associated hypercalcemia. J Clin Endocrinol Metab, 1990. 70(4): p. 1090-5.
17. Kiltz, U. and J. Braun, Use of methotrexate in patients with sarcoidosis. Clin Exp Rheumatol, 2010. 28(5 Suppl 61): p. S183-5.

Polyostotic fibrous dysplasia: will denosumab reduce his bone pain?

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Case Summary

A 43 year-old Scottish self-employed carpenter presented to Endocrinology Clinic in 2019 for management of polyostotic fibrous dysplasia. He migrated to Australia in 2012 and had a medical history of polyostotic fibrous dysplasia since childhood.

Multiple skeletal sites including both lower limbs, left clavicle, left sided ribs, proximal humerus, scapula, and skull were involved. These resulted in complications of severe restrictive ventilatory defects from the left thoracic cage deformity, dilated cardiomyopathy with ejection fraction of 46% and compression of the left superior vena cava and internal jugular vein. His main symptoms were dyspnoea on exertion, severe pain around left clavicle especially in the context of carpentry work. Pertinent examinations findings included facial asymmetry, a large rounded bony lesion on the left clavicle and oedematous left upper limb. (Figure 1).

His fasting metabolic bone study at initial endocrinology assessment (Table 1) demonstrated ALP of 466U/L (30-110unit/L) and elevated bone turnover markers (CTX 2590ng/L [100-600ng/L]). There was no evidence of renal phosphate wasting. His FEV1 was of 49% with reduced lung volumes. The biopsy of the left clavicle showed no malignant cells on histopathology in 2014 and 2017 but did show fibrous tissue.

CT chest showed left sided reduced size of the hemithorax (Figure2) and CT venogram confirmed vascular compression of the left SVC and IJV. Bone scan showed increased osteoblastic activity throughout the entire skeleton apart from the right hemithorax, and more intense uptake in the calvarium, the left clavicle, left ribs and scapula (Figure 3).

5mg of intravenous zoledronic acid was given in July 2021. The patient experienced a severe reaction including worsening bone pains. The repeat bone scan performed 6 months post zolendronic acid did not result in significant reduction in the intensity of the bone activity (Figure 4). The patient did not receive further IV bisphosphonate given his severe reaction, minimal changes to his pain and no improvement radiologically.

His case was discussed at the orthopaedic oncology multidisciplinary meeting with cardiothoracic surgeon for consideration of total claviclectomy given the extent of his respiratory compromise. The operation was abandoned due to the high risks of surgery including major vascular damage and uncontrollable bleeding into the thorax. The consensus was that forequarter amputation would be necessary if the lesion of the clavicle continued to expand with neurovascular compromise.

The patient continues to be reviewed by endocrinology, orthopaedic oncology and respiratory. More recently, the use of RANKL inhibitor, denosumab has been discussed as case reports suggest successful response to the treatment of patients with fibrous dysplasia. He is planned to receive the first dose of denosumab in upcoming weeks.

Brief outline of the literature

Fibrous dysplasia of bone (FD) is a rare bone disease that accounts for approximately 5 to 7 percent of benign bone tumours and is caused by the postzygotic mutation in the guanine nucleotide stimulatory protein (GNAS) gene. This leads to the abnormal cellular response leading overproduction of IL-6 and receptor activator of nuclear factor-kB ligand (RANKL) resulting in high proliferative partially mineralised fibrous osseous tissue. FD may occur in single (monostotic) or multiple bones (polyostotic fibrous dysplasia) and is associated with other endocrinopathies. People with FD commonly suffer from pain from the affected skeletal sites, fractures, and disability. Management options for FD have been mainly surgical targeting stabilising impending fractures and correcting deformities. The first line pharmacological management has been supplementation of hypophosphatemia if present and vitamin D repletion. Otherwise, analgesics have been recommended (1).

Bisphosphonates are suggested with a hypothesis of normalising bone turnover and thereby reducing pain symptoms and preventing the progression of FD lesions. However, previous open-label studies have shown that those with high burden of skeletal disease, bisphosphonate treatment was not adequate to control the pain nor reducing the bone-turnover markers. Continuation of bisphosphonate is not recommended if there is no clinical improvement (2).

This case highlights the challenge in managing FD where surgery is not an option, and bisphosphate therapy being ineffective.

The use of denosumab may provide clinical benefits in patients not responding to bisphosphonate therapy. Studies have suggested the positive correlation with the upregulation of RANKL expression in skeletal lesions with skeletal burden score in patients with FD (3, 4). Positive outcomes including improvement in pain and lung function in those with thoracic involvement. A 60mg dose of denosumab resulted in sustained reduction of bone turn over markers however, 6 monthly injections may be insufficient to maintain the suppressed bone turnover markers in patients with FD. Three-monthly intervals were shown to be efficient in maintaining the decreased BTMs by more than 50% from baseline values. Case studies have shown that this was well tolerated. The optimal duration of denosumab therapy and further management once in clinical and biochemical remission remains unclear. Another study trialling bisphosphonate to aid cessation of denosumab showed rebound hypercalcaemia to be present in a few patients post cessation of denosumab particularly in those with high disease burden, requiring an additional dose of denosumab (5). Close monitoring of calcium, vitamin D and renal function is recommended to prevent severe hypo and hypercalcaemia.

Take home messages:

- Polyostotic fibrous dysplasia is rare bone disease affecting either one or multiple skeletal sites (monostotic and polyostotic fibrous dysplasia).

- There is yet no approved medical treatment to control pain and disease activity in FD.

- Bisphosphonates have been used in the management of patients with FD related bone pain however, those with high skeletal burden, may not respond to treatment with these agents.

- Denosumab been shown to be effective in those with FD refractory or intolerant to bisphosphonates therapy. Larger controlled studies are needed until then, this should be used with cautions.

Figure 1. Physical appearance of patient *published with permission from patient

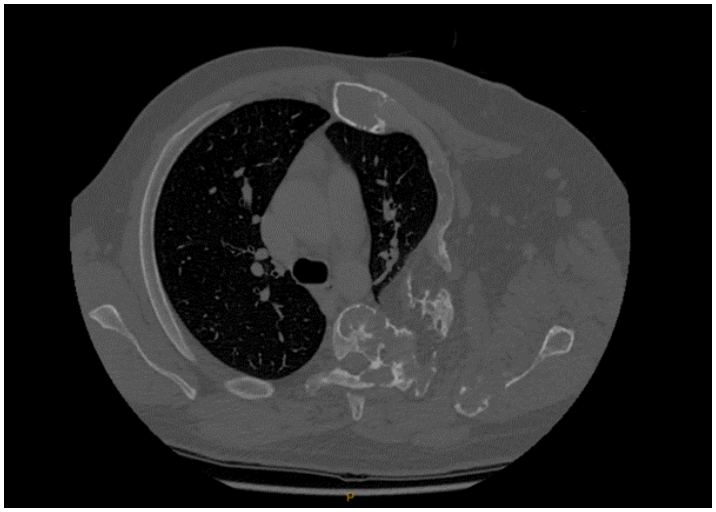

Figure 2. CT of chest showing left sided hemithorax and fibrous dysplasia involvement in vertebral bodies and sternum.

Table 1. Biochemistry including bone turnover markers

	18/06/2021	26/06/2023	Reference Range
ALP	466	285	30-110(unit/L)
P1NP		1648	15-80ug/L
CTX	2590	2070	100-600ug/L
25-OH vitamin D	59	78	>50nmol/L
eGFR	>90		>90
PTH	7.1	5.7	1.9-8.5pmol/L
Serum phosphate	0.92		0.70-1.50
Urinary phosphate	0.81		0.75-1.35nmol/L

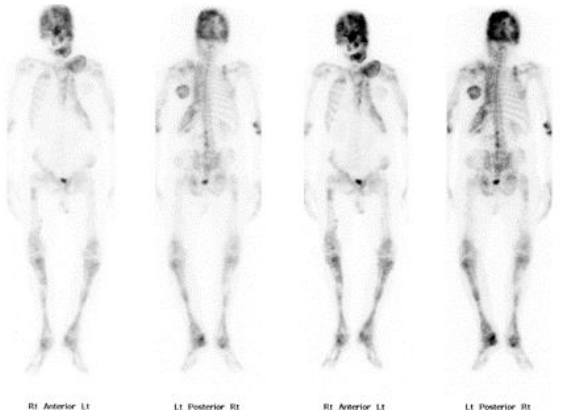

Figure 3. Nuclear medicine Bone scan (945MBq Tc-9mM HDP) done on 24/02/2021 (pre-bisphosphonate treatment) showing intense activity throughout mainly on the left side.

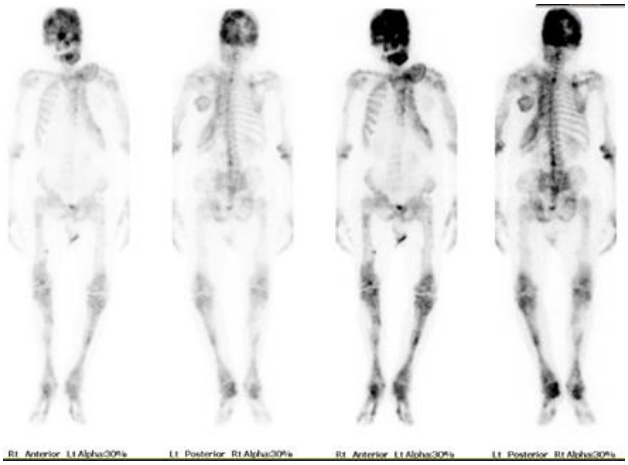

Figure 4. Repeat Nuclear bone scan (6 months post the zoledronic acid)

1. Javaid, M.K., Boyce, A., Appelman-Dijkstra, N. et al. Best practice management guidelines for fibrous dysplasia/McCune-Albright syndrome: a consensus statement from the FD/MAS international consortium. Orphanet J Rare Dis 14, 139 (2019).
2. Majoor BC, Appelman-Dijkstra NM, Fiocco M, van de Sande MA, Dijkstra PS, Hamdy NA. Outcome of Long-Term Bisphosphonate Therapy in McCune-Albright Syndrome and Polyostotic Fibrous Dysplasia. Journal of Bone and Mineral Research. 2017;32(2):264-76.
3. Majoor BCJ, Papapoulos SE, Dijkstra PDS, Fiocco M, Hamdy NAT, Appelman-Dijkstra NM. Denosumab in Patients With Fibrous Dysplasia Previously Treated With Bisphosphonates. J Clin Endocrinol Metab. 2019;104(12):6069-78.
4. Meier ME, van der Bruggen W, van de Sande MAJ, Appelman-Dijkstra NM. Regression of fibrous dysplasia in response to denosumab therapy: A report of two cases. Bone Reports. 2021;14:101058.
5. de Castro LF, Michel Z, Pan K, Taylor J, Szymczuk V, Paravastu S, et al. Safety and Efficacy of Denosumab for Fibrous Dysplasia of Bone. New England Journal of Medicine. 2023;388(8):766-8.

Persistent hyperparathyroidism in pregnant women with a prior renal transplant: challenges and treatment strategies.

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Case summary:

A 35-year-old primiparous woman, who had previously undergone a kidney transplant 4 years ago for stage 5 chronic kidney disease (CKD) caused by IgA nephropathy, was referred by her nephrologist to the endocrine clinic for evaluation of her parathyroid hormone (PTH)-dependent hypercalcaemia during her 11th week of pregnancy. Prior to her transplant, she had undergone haemodialysis for 13 months. Her regular medications include prednisone, tacrolimus, azathioprine, aspirin and nifedipine.

Upon reviewing her test results, it was found that a plasma corrected calcium level was 2.82 mmol/L (reference range 2.15-2.55), with an elevated serum PTH level of 7.2 pmol/L (reference range 1.6-6.9). A serum 25-hydroxyvitamin D level was sufficient at 60 mmol/L. Phosphate was 0.87 mmol/L (reference range 0.8-1.5), creatinine level 70 umol/L, and estimated glomerular filtration rate (eGFR) >90 ml/min/1.73m^2. 24-hour urine calcium:creatinine ratio was 0.0159 ruling out familial hypocalciuric hypercalcaemia. Pre-transplant test results showed significantly elevated serum PTH levels of 103-136.4 pmol/L and post-transplant there were intermittent episodes of mild hypercalcaemia (range 2.60-2.69 mmol/L) and PTH elevation (7.9-9.2 pmol/L).

The patient was advised to drink at least 3 litres of fluids per day. A parathyroid sestamibi scan and neck ultrasound were planned for the second trimester, but the patient expressed hesitation despite reassurance of the low radiation risk. Unfortunately, due to the development of pre-eclampsia, the patient delivered her baby prematurely at 28 weeks gestation. Postpartum plasma calcium levels remained persistently elevated at 2.81 mmol/L, with serum PTH levels also elevated at 10.6 pmol/L.

Seven months later, the patient returned to the clinic with a strong desire to undergo curative parathyroidectomy surgery in order to plan her second pregnancy and reduce the risk of pre-eclampsia. Parathyroid sestamibi scan revealed delayed uptake in both the left and right inferior poles of the thyroid, while the neck ultrasound showed a multinodular goitre with five colloid nodules and a solid nodule measuring 1.0cmx0.7cm in the left inferior aspect, suggesting the presence of a single adenoma.

The patient was referred to an experienced head and neck surgeon who performed a parathyroid neck exploration. All four parathyroid glands were visibly enlarged, and the surgeon proceeded to subtotal parathyroidectomy (removing 3.5 of the 4 hyperplastic glands). The postoperative PTH level remained mildly elevated at 8.5 pmol/L. However, considering the normalised serum calcium level and risk of postoperative hypoparathyroidism, it was decided not to remove any additional parathyroid tissue.

During a post-operative clinic visit, the patient was advised that a high-calcium diet might help to suppress the serum PTH level during a period of enhanced calcium uptake into the skeleton. Bone Mineral Density (BMD) by DXA (Lunar Prodigy) revealed osteopenia in the lumbar spine (L2-L4; BMD 1.012g/cm^2, T-score -1.6 SD) and normal bone densities in the hip and forearm. These bone densities were stable when compared to a previous DXA in 2018. Fasting bone turnover markers were not elevated. Despite the persistently elevated serum PTH level, the plasma calcium level remained within the normal range. Due to excessive weight gain, the patient was advised to switch from dairy products to calcium supplements in order to reduce caloric intake.

Literature review:

Post transplant hyperparathyroidism (PT-HPT) is a common occurrence in kidney transplant patients, and while it usually resolves within a year, recent studies have shown that it can persist in a significant percentage of recipients at the one-year mark (17-50%) [1].

Several risk factors contribute to PT-HPT including prolonged dialysis, the use of calcimimetic drugs, pre-transplant levels of PTH exceeding 31.8 pmol/L, and hypercalcaemia at the time of transplantation [2]. The clinical manifestations of PT-HPT mimic primary hyperparathyroidism and differ from the secondary hypoparathyroidism observed in non-transplant chronic kidney disease (CKD) patients. Complications of PT-HPT include increased mortality, higher risk of graft failure, decreased bone density and increased fracture rate [3].

Managing hypercalcaemia resulting from PT-HPT is challenging due to the lack of large-scale randomised controlled trials to guide treatment decisions. While imaging studies may occasionally identify a single parathyroid adenoma, the more common scenario is that of diffuse hyperplasia. Cinacalcet can be considered as a treatment option since it reduces serum PTH and calcium levels without negatively impacting graft function. In cases of moderate to severe hypercalcaemia or when patients experience symptoms, parathyroidectomy is the preferred treatment option [4].

Limited data is available on managing hypercalcaemia in pregnancy related to PT-HPT. Hypercalcaemia poses significant risks for both the mother and the foetus, including pre-eclampsia, miscarriage, nephrolithiasis, pancreatititis and neonatal hypocalcaemia [6]. The treatment approach for PT-HPT in pregnancy would be similar to that of primary hyperparathyroidism and involves maintaining an adequate fluid intake (at least 3 litres per day). Depending upon the severity of the condition, parathyroidectomy surgery may be considered during the second trimester [5].

Key messages:

- Post transplant hyperparathyroidism (PT-HPT) is a common occurrence with incidence ranging from 17-50% at the one-year mark.
- PT-HPT is associated with poor patient outcomes including increased mortality, allograft loss, and metabolic bone diseases.
- Treatment options for PT-HPT include medication with an oral calcimimetic e.g. cinacalcet or parathyroidectomy in cases where patients experience symptoms or have moderate to severe hypercalcaemia.
- Hypercalcaemia during pregnancy is linked to higher risk of pre-eclampsia, as well as miscarriage and neonatal hypocalcaemia. Initial treatment involves adequate fluid intake, but parathyroidectomy surgery may be considered during the second trimester.

References

1. Evenepoel P. Recovery versus persistence of disordered mineral metabolism in kidney transplant recipients. Seminars in Nephrology. 2013;33(2):191-203.
2. Sutton W, Chen X, Patel P, Karzai S, Prescott JD, Segev DL, McAdams-DeMarco M, Mathur A. Prevalence and risk factors for tertiary hyperparathyroidism in kidney transplant recipients. Surgery. 2022;171(1):69-76.
3. Pihlstrøm H, Dahle DO, Mjøen G, Pilz S, März W, Abedini S, Holme I, Fellström B, Jardine AG, Holdaas H. Increased risk of all-cause mortality and renal graft loss in stable renal transplant recipients with hyperparathyroidism. Transplantation. 2015;99(2):351-9.
4. Delos Santos R, Rossi A, Coyne D, Maw TT. Management of post-transplant hyperparathyroidism and bone disease. Drugs. 2019;79:501-13.
5. Dochez V, Ducarme G. Primary hyperparathyroidism during pregnancy. Archives of Gynecology and Obstetrics. 2015;291:259-63.

Severe osteoporosis secondary to systemic mastocytosis exacerbated by pregnancy and breastfeeding

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Case summary

A 38-year-old woman was referred to endocrinology with an 8 month history of atraumatic lower back pain, beginning 2 months post-partum. An earlier lumbar spine MRI had revealed mild compression fractures of all lumbar superior endplates with bony edema indicating recency. DXA scan showed severe bone loss (L1-4 Z-scores all < -4.0 SD, Left and right femoral neck, -3.0 SD and -3.4 SD respectively.) There was no evidence of gastrointestinal, renal or endocrinological disorder. Family history revealed osteoporosis in her mother, aged in her 60s. She was advised to cease breastfeeding immediately, based on a presumptive diagnosis of pregnancy and lactation induced osteoporosis (PLO). As future pregnancy was being considered, anti-resorptive agents were not immediately commenced, hoping cessation of lactation might lead to improvement.

Subsequently, a further history of recent onset stress-induced vomiting, palpitations and flushing and prior episodes of angioedema to multiple analgesic agents and shellfish-induced anaphylaxis came to light. This prompted measurement of tryptase, which was elevated at 27.4 ug/L (N<11.4). Skin biopsy of discrete maculopapular lesions (Fig 1) present since her early twenties revealed mast cells >15 per high powered field. Bone marrow biopsy and flow cytometry revealed 100% of mast cells expressing CD2 and/or CD25 consistent with an abnormal phenotype. She was diagnosed with systemic mastocytosis and is undergoing subtype differentiation. She has lost 8cm in height in a year and her back pain remains debilitating, though imaging reveals no new fracture development. She commenced IV zoledronic acid, as her priority was to prevent further debilitating fractures, and pregnancy was now not desired in the next few years.

Background

Systemic mastocytosis (SM) is the pathological accumulation of mast cells in tissues and release of vasoactive substances such as histamine and prostaglandins, leading to symptoms and signs consistent with allergy or anaphylaxis. Of the subtypes, indolent systemic mastocytosis is the most common, accounting for more than 80% of all SM[1].

There is a varying clinical spectrum of bony manifestations in SM, from bony pain, osteopenia, osteoporosis with fragility fractures, osteolytic lesions and osteosclerosis[3]. Osteoporosis is usually a feature of ISM, with increased BMD and osteosclerosis more common in the aggressive subtype[4]. The lumbar spine is most commonly involved, as mast cells tend to colonise the more metabolically active axial skeleton, rather than the fatty marrow in the appendicular skeleton[5] . In a large German retrospective cohort of over 8000 patients with osteoporosis, the prevalence of ISM was 0.5%. However, in a subgroup of young male patients, the prevalence was more than 5%[2].

The pathogenesis of osteoporosis in SM is complex but is thought to be due to the effect of inflammatory mediators that directly activate osteoclasts through the RANK-L system, leading to bone resorption[6,7]. However, not all patients with systemic mastocytosis develop osteoporosis (prevalence 18 – 31% in various studies), and increased BMD/osteosclerosis positively correlates with tryptase levels in the aggressive form. Other pathways may be involved, including the canonical Wnt-signalling pathway. Elevations in either DKK1 or sclerostin have been demonstrated in ISM versus control, though study results are somewhat conflicting[8,9].

A previous Australian case report describes two women presenting with multiple thoracolumbar vertebral fractures in the lactation period. Both had worse spinal versus femoral neck BMD and were eventually diagnosed with systemic mastocytosis[10]. We postulate that in these cases, and ours, vertebral fractures resulted from the combined effect of lactation-associated bone resorption and SM on the axial skeleton.

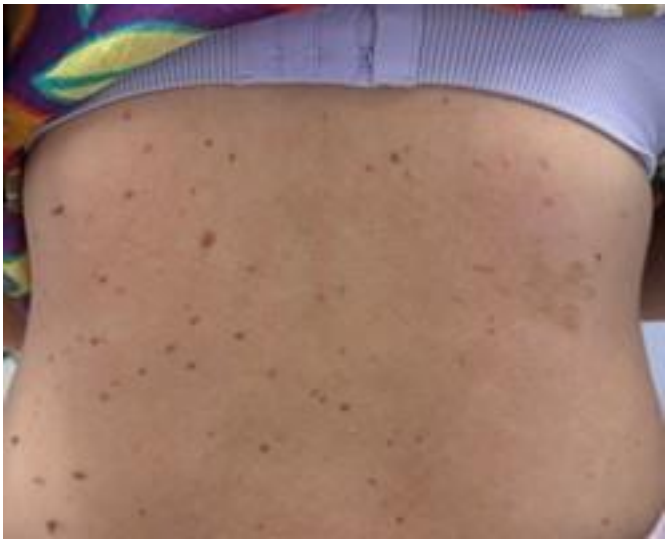
Bisphosphonates are considered first line treatment for SM-induced osteoporosis. Small studies have shown improved BMD at the lumbar spine, reduced bone turnover and improved pain with bisphosphonate use[5]. However, no randomized placebo-controlled trials exist, to conclusively demonstrate fracture risk reduction. One study of bisphosphonate use in ISM-induced osteoporosis demonstrated ongoing fracture risk, with 5 and 10-year fracture free survival of only 81.9% and 67% respectively[11]. There are limited data on alternative therapies such as denosumab[12], and no data on the use of anabolic agents. Teriparatide is said to potentially induce growth and proliferation of mast cells[4], though mouse studies show teriparatide decreased mast cells numbers[13].

Osteoporosis treatment in women of childbearing potential is a complex issue. No drug has proven safety in pregnancy. Animal studies suggest reproductive toxicity[14,15], but small case series of bisphosphonate use prior to pregnancy have described non-specific complications without consistent signal for teratogenicity[16,17]. The extended half-life of bisphosphonates in bone is problematic, no guidelines exist on appropriate washout periods for different agents prior to pregnancy.

Take home messages

- Systemic mastocytosis is a rare but important differential to consider in younger patients with osteoporosis, presenting with vertebral fractures, without the typical risk factors for osteoporosis. This diagnosis should even be considered in the absence of allergy/anaphylaxis symptoms.
- Bisphosphonates are first-line in the management of SM-induced osteoporosis however there is limited data in its prevention of further fractures. The relative role of denosumab, teriparatide or romosozumab remains undetermined.
- Treatment decisions for premenopausal women at high risk of fracture requires an individualised approach, balancing morbidity from fracture with potential reproductive harm.

Figure 1: maculopapular lesions over trunk



Baseline blood tests Aug 2022

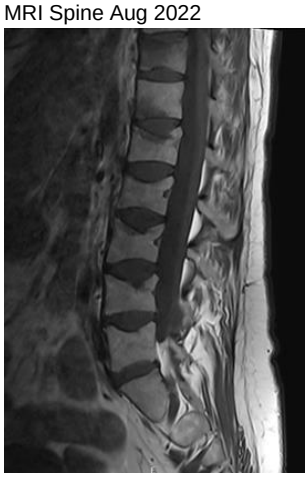
Results	Value	Units	Range
Hb	148	g/L	119 - 160
WCC	7.7	x10^9/L	4.0 - 11.0
Creatinine	63	umol/L	45 - 85
eGFR	90	ml/min/1.73m2	> 59
cCa	2.48	mmol/L	2.15 – 2.55
PO4	1.23	mmol/L	0.8 – 1.5
ALP	95	U/L	20 - 105
TSH	0.84	mIU/L	0.40 – 3.50
25 OH-Vitamin D	68	nmol/L	50 - 140
HbA1c	5.4	%	

Further tests Dec 2022

Results	Value	Units	Range
cCa	2.37	mmol/L	2.15 – 2.55
PTH	6.6	pmol/L	1.6 – 6.9
25 OH-Vitamin D	96	nmol/L	50 - 140
Urine creatinine	15.3	mmol/L	
Urine calcium	7.01	mmol/L	
Urine Ca:Cr	458	mmol/mol	0-450
Coeliac serology		Negative	
Myeloma screen		Negative	

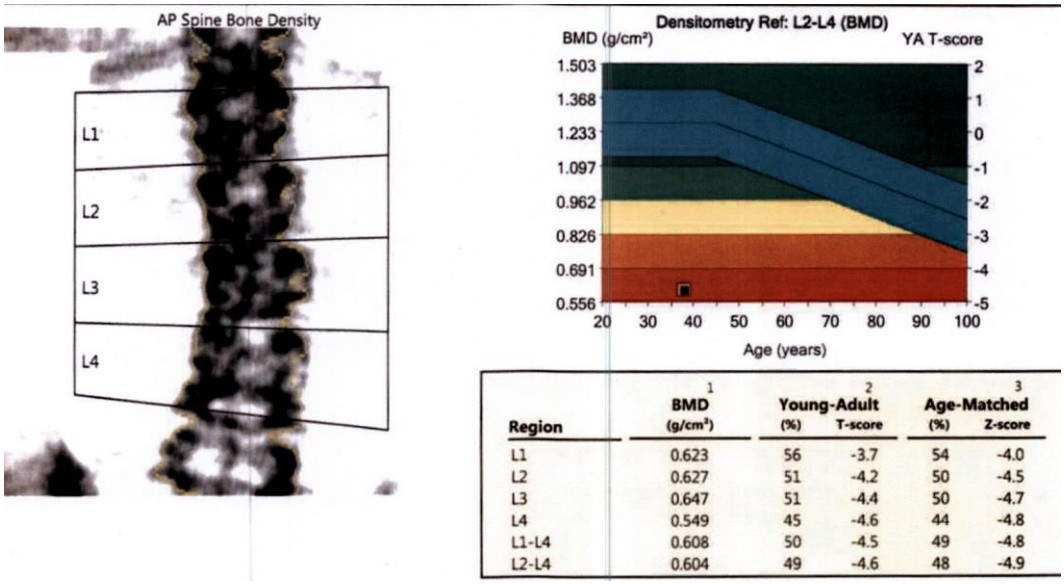
0

MRI Spine Aug 2022



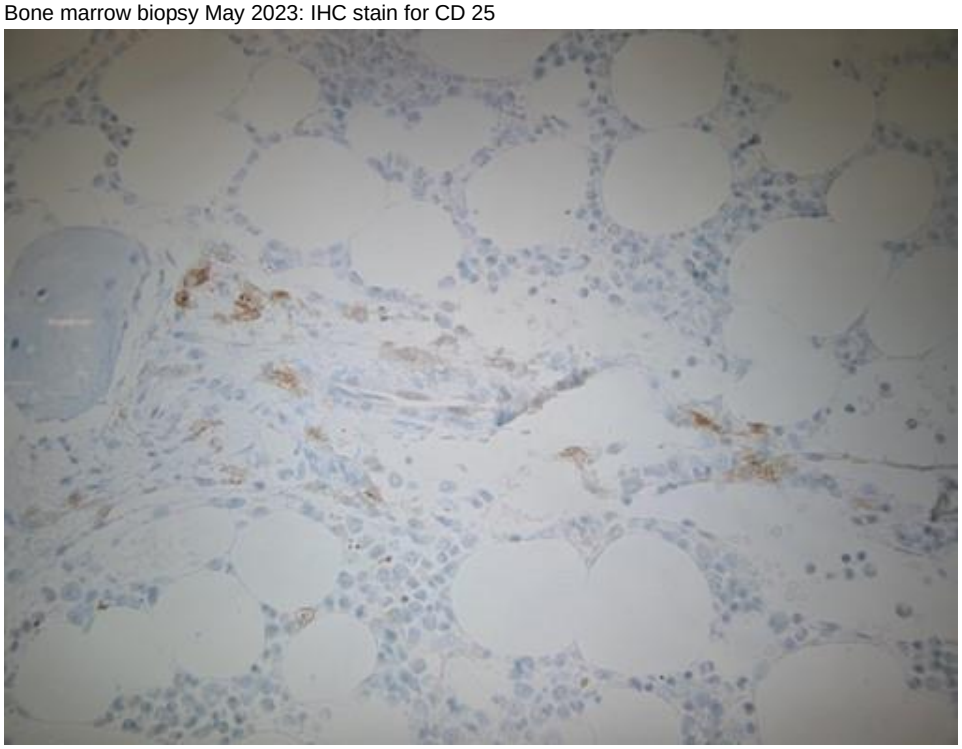
DXA Nov 2022

I-Med Radiology 11/2022			
L-spine (L2-4)	0.604	-4.6	-4.9
L FN	0.641	-3.0	-3.0
L total	0.657	-2.9	-3.2
R FN	0.593	-3.3	-3.4
R total	0.619	-3.2	-3.5



Region	1 BMD (g/cm²)	2 Young-Adult (%)	2 Young-Adult T-score	3 Age-Matched (%)	3 Age-Matched Z-score
L1	0.623	56	-3.7	54	-4.0
L2	0.627	51	-4.2	50	-4.5
L3	0.647	51	-4.4	50	-4.7
L4	0.549	45	-4.6	44	-4.8
L1-L4	0.608	50	-4.5	49	-4.8
L2-L4	0.604	49	-4.6	48	-4.9

Bone marrow biopsy May 2023: IHC stain for CD 25



1. 1. Cohen, S. S., Skovbo, S., Vestergaard, H., Kristensen, T., Møller, M., Bindslev-Jensen, C., Fryzek, J. P., & Broesby-Olsen, S. (2014). Epidemiology of systemic mastocytosis in Denmark. British journal of haematology, 166(4), 521–528.
2. 2. Gehlen, M., Schmidt, N., Pfeifer, M., Balasingam, S., Schwarz-Eywill, M., Maier, A., Werner, M., & Siggelkow, H. (2021). Osteoporosis Caused by Systemic Mastocytosis: Prevalence in a Cohort of 8392 Patients with Osteoporosis. Calcified tissue international, 109(6), 685–695.
3. 3. Greene LW, Asadipooya K, Corradi PF, Akin C. Endocrine manifestations of systemic mastocytosis in bone (2016). Rev Endocr Metab Disorder, 17(3):419–31.
4. 4. Asadipooya, Kamyar & Greene, Loren. (2020). Chapter 8: Systemic Mastocytosis and Bone-Related Events. In Akim, C (Eds) Mastocytosis: A comprehensive guide (pp. 123-140) Springer Nature Switzerland
5. 5. Rossini, M., Zanotti, R., Orsolini, G., Tripi, G., Viapiana, O., Idolazzi, L., Zamò, A., Bonadonna, P., Kunnathully, V., Adami, S., & Gatti, D. (2016). Prevalence, pathogenesis, and treatment options for mastocytosis-related osteoporosis. Osteoporosis international : a journal established as result of cooperation between the European Foundation for Osteoporosis and the National Osteoporosis Foundation of the USA, 27(8), 2411–2421.
6. 6. Theoharis C. Theoharides, William Boucher, Kathleen Spear; Serum Interleukin-6 Reflects Disease Severity and Osteoporosis in Mastocytosis Patients. Int Arch Allergy Immunol 1 August 2002; 128 (4): 344–350.
7. 7. Biosse-Duplan, M.; Baroukh, B.; Dy, M.; de Vernejoul, M.C.; Saffar, J.L. Histamine promotes osteoclastogenesis through the differential expression of histamine receptors on osteoclasts and osteoblasts. Am. J. Pathol. 2009, 174, 1426–1434.
8. 8. Rossini, M.; Viapiana, O.; Zanotti, R.; Tripi, G.; Perbellini, O.; Idolazzi, L.; Bonifacio, M.; Adami, S.; Gatti, D. Dickkopf-1 and sclerostin serum levels in patients with systemic mastocytosis. Calcif. Tissue Int. 2015, 96, 410–416.
9. 9. Rabenhorst A, Christopeit B, Leja S, Gerbaulet A, Kleiner S, Forster A, Raap U, Wickenauser C, Hartmann K (2013) Serum levels of bone cytokines are increased in indolent systemic mastocytosis associated with osteopenia or osteoporosis. J Allergy Clin Immunol 132:1234–1237
10. 10. Zhu, J. J., Mahendran, D., Lee, M. H., Seah, J., Fourlanos, S., Varadarajan, S., Ghasem-Zadeh, A., MacIsaac, R. J., & Seeman, E. (2018). Systemic mastocytosis identified in two women developing fragility fractures during lactation. Osteoporosis international : a journal established as result of cooperation between the European Foundation for Osteoporosis and the National Osteoporosis Foundation of the USA, 29(7), 1671–1674
11. 11. Onnes, M. C., van Doormaal, J. J., van der Veer, E., Versluijs, J. B., Arends, S., & Oude Elberink, H. N. G. (2020). Fracture Risk Reduction by Bisphosphonates in Mastocytosis?. The journal of allergy and clinical immunology. In practice, 8(10), 3557–3564.
12. 12. Orsolini, G., Gavioli, I., Tripi, G., Viapiana, O., Gatti, D., Idolazzi, L., Zanotti, R., & Rossini, M. (2017). Denosumab for the Treatment of Mastocytosis-Related Osteoporosis: A Case Series. Calcified tissue international, 100(6), 595–598.
13. 13. Zhang, L., Wang, T., Chang, M., Kaiser, C., Kim, J. D., Wu, T., Cao, X., Zhang, X., & Schwarz, E. M. (2017). Teriparatide Treatment Improves Bone Defect Healing Via Anabolic Effects on New Bone Formation and Non-Anabolic Effects on Inhibition of Mast Cells in a Murine Cranial Window Model. Journal of bone and mineral research : the official journal of the American Society for Bone and Mineral Research, 32(9), 1870–1883.
14. 14. Patlas, N., Golomb, G., Yaffe, P., Pinto, T., Breuer, E., & Ornoy, A. (1999). Transplacental effects of bisphosphonates on fetal skeletal ossification and mineralization in rats. Teratology, 60(2), 68–73.
15. 15. Graepel, P., Bentley, P., Fritz, H., Miyamoto, M., & Slater, S. R. (1992). Reproduction toxicity studies with pamidronate. Arzneimittel-Forschung, 42(5), 654–667.
16. 16. Sokal, A., Elefant, E., Leturcq, T., Beghin, D., Mariette, X., & Seror, R. (2019). Pregnancy and newborn outcomes after exposure to bisphosphonates: a case-control study. Osteoporosis international : a journal established as result of cooperation between the European Foundation for Osteoporosis and the National Osteoporosis Foundation of the USA, 30(1), 221–229.
17. 17. Levy, S., Fayez, I., Taguchi, N., Han, J. Y., Aiello, J., Matsui, D., Moretti, M., Koren, G., & Ito, S. (2009). Pregnancy outcome following in utero exposure to bisphosphonates. Bone, 44(3), 428–430.

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Food for thought on the therapeutic regimen after stopping denosumab in postmenopausal women

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Upon cessation of denosumab (DMAB) treatment, there is a marked acceleration in bone remodeling leading to notable bone degradation. This intensified bone remodeling has been quite often associated with a rebound increase in fracture risk. Consequently, some clinicians recommend continuous and indefinite DMAB administration. However, potential adverse effects or certain conditions might necessitate the discontinuation of DMAB. Additionally, in certain patients, bone mineral density (BMD) may have reached a threshold where the continuation of DMAB no longer offers significant reductions in fracture risk. It is imperative for patients discontinuing DMAB to transition to alternative anti-osteoporosis therapy, with meticulous monitoring, particularly within the initial year when the majority of bone loss typically occurs. Current research is investigating the efficacy of treatments such as zoledronate and oral bisphosphonates post-DMAB cessation. There is also emerging real-world evidence highlighting the impact of sequential medications, like raloxifene or romosozumab on preserving BMD in these patients. The benefits of sequential treatment can vary based on the duration of prior DMAB therapy. In all scenarios, transitioning to another anti-osteoporosis therapy is essential, although the ideal therapeutic regimen remains to be elucidated.

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An implementation science approach to community pharmacy osteoporosis screening

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Introduction. Osteoporosis and poor bone health impact a significant proportion of the Australian population. Yet over 60% of Australians have misconceptions about it and 50% don't take their osteoporosis medications as prescribed. Various interventions have been done in the past to combat this increasingly prevalent condition with various degrees of efficacy. Implementation science approaches are used to reduce the gap in time between research and practice.

Aims. To describe the development and pilot implementation of community pharmacy screening for osteoporosis and the barriers and facilitators in its implementation.

Methods. Semi-structured interviews were completed with a convenience sample of pharmacy stakeholders including patients, pharmacists, and pharmacy staff. Community pharmacies were invited to implement the screening service via social media advertising and networks and training was provided. Community pharmacy staff and consumers were interviewed after the service. The implementation process was documented using the REAIM (reach, effectiveness, adoption, implementation, maintenance) framework.

Results. An osteoporosis screening service was developed using stakeholder interviews. 25 community pharmacies were recruited and commenced a screening service. 251 pharmacy consumers (average of 10 people/pharmacy) were screened for osteoporosis during the study period (1 week in each pharmacy). Participants reported that osteoporosis was not a major disease that pharmacists often focused on, however, both patient and pharmacist participants felt that it is important and that community pharmacies are suited towards screening. Most pharmacists reported time, remuneration, and COVID were major barriers to implementation.

Discussion. Consulting stakeholders is an important part of developing new pharmacy services to ensure an intervention's success. This study gathered insights into the current state of pharmacy knowledge and practice around osteoporosis and may assist future service development.

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Rate of bone loss is associated with fracture risk: The Study of Osteoporotic Fractures

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Objective. Although the link between low bone mineral density (BMD) and fracture is well established, the association between aged-related bone loss and fracture remains controversial. In this study, we aimed at testing the hypothesis that excessive bone loss is associated with increased fracture risk in elderly women.

Methods. This study involved 5581 women who were a part of the Study of Osteoporotic Fractures and had undergone a minimum of three BMD assessments. Femoral neck BMD was measured using DXA (Hologic QDR 1000) between 1992 and 2008. The rate of BMD change was determined by a linear mixed-effects regression model for each woman. The incidence of fractures (after 3 BMD measurements) was ascertained by reviewing hospital discharge records or physician reports. The Cox's proportional hazards model was utilized to assess the association between bone loss and fracture risk, while also accounting for pre-defined covariates such as baseline BMD, age, smoking, drinking, physical activity, prior and family history of fractures.

Results. During the median follow-up of 13 years (IQR: 9-17), there were 1470 incident fractures (including 679 hip fractures), yielding fracture incidences of 25 per 1000 person-years (95% CI: 23-26). The rate of bone loss was significantly associated with an increased risk of any fracture (hazard ratio: 1.18; 95% CI: 1.07-1.30) and hip fracture (1.30; 95% CI: 1.13-1.50). Importantly, those with an excess annual bone loss (e.g., at least 2% per year) were associated with a 2-fold (95% CI: 1.01-4.0) increase in hip fracture risk.

Conclusion. These data support the hypothesis that bone loss at the femoral neck is a risk factor for fracture, independent of age and baseline BMD. This suggests that repeated measurements of BMD can be useful in identifying individuals who are at high risk of fracture.

Skeletal effects of neratinib treatment and *Blautia luti* supplementation in tibial trabecular bone of albino Wistar rats

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Background

Cancer treatment-induced bone loss (CTIBL) is a longer-term consequence of chemotherapy and endocrine therapy[1,2]. However, skeletal effects of neratinib, a tyrosine kinase inhibitor approved for breast cancer[3], remain unknown. Preclinical evidence of interactions between the gut microbiome (GM) and bone[4], and protective effects of GM modulation in bone disorders[5], suggests probiotic supplementation may mitigate CTIBL. This study aimed to determine the effect of neratinib treatment and probiotic supplementation with *Blautia luti* on tibial trabecular bone microarchitecture and histomorphometry in albino Wistar (AW) rats.

Methodology

Female AW rats (n=40) were randomly allocated to; vehicle control (VC; 0.5% hydroxypropyl methylcellulose; n=4), neratinib (N; 50mg/kg; n=4), *Blautia luti* (B; 10^7 CFU; n=8), neratinib and *Blautia luti* pre-treatment (NBp; n= 8), neratinib and *Blautia luti* pre- and post-treatment (NBppo; n=8), and neratinib and *Blautia luti* post-treatment (NBpo; n=8). Dosing schedules of neratinib and *Blautia luti* for each group are shown in figure 1. Tibia were scanned *ex-vivo* by micro-computed tomography and bone structural indices assessed; bone volume (BV), trabecular number (Tb.N), trabecular thickness (Tb.Th) and trabecular separation (Tb.Sp). Serial decalcified sections were stained with hematoxylin and eosin, and tartrate-resistant acid phosphatase for histomorphometric analysis of osteoblasts and osteoclasts respectively.

Results

BV (p=0.017), Tb.N (p=0.003) and Tb.Th (p=0.000) significantly decreased in N compared with VC; consistent with increased osteoclast (p=0.163) and significantly decreased osteoblast (p=0.014) numbers in N rats. BV increased in B compared to VC rats (p=0.605), whilst *Blautia luti* supplementation significantly increased Tb.Th in NBp (p=0.013), NBppo (p=0.011) and NBpo (p=0.001) rats, compared to N alone.

Conclusion

Loss of BV, increased osteoclast and decreased osteoblast numbers in the tibia indicates neratinib's negative effect on bone microarchitecture. Although *Blautia luti* alone caused an increase in BV, supplementation failed to provide a significant mitigative effect on neratinib-induced bone loss.

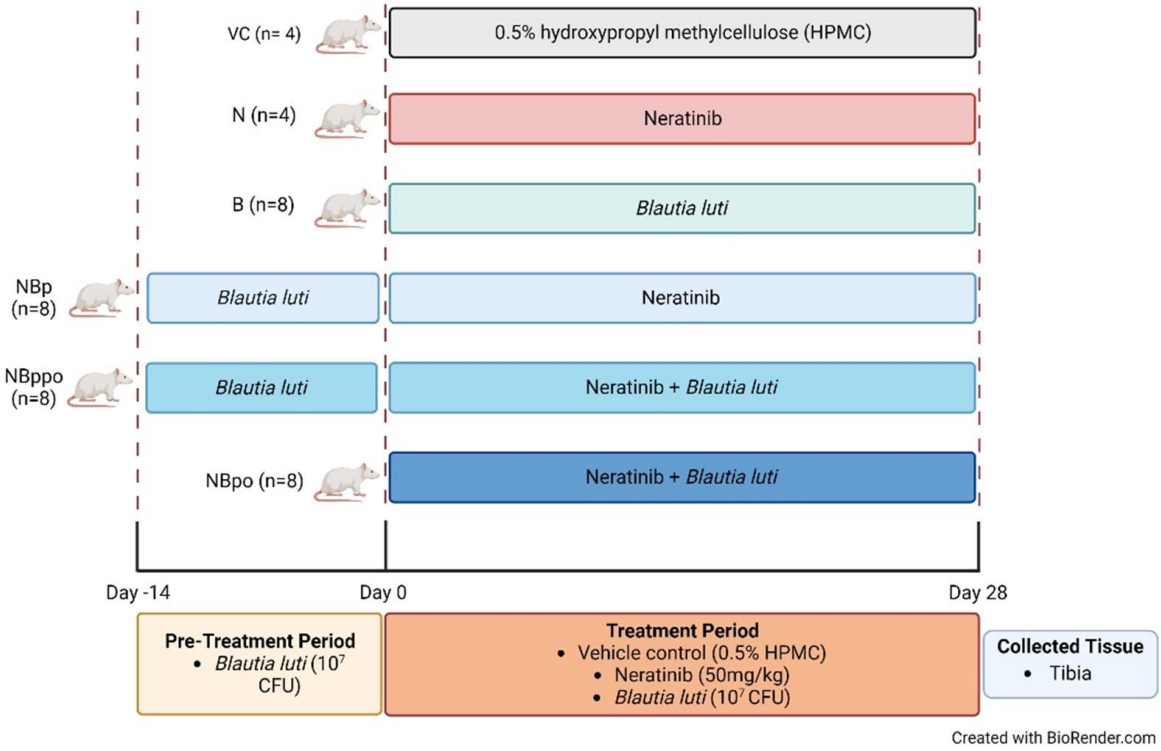

Figure 1. Experimental design of AW rat model

1. Doo L & Shapiro CL (2013). Skeletal manifestations of treatment of breast cancer on premenopausal women. Curr Osteoporos Rep 11, 311-8.
2. Axelsen CT, Jensen AB, Jakobsen EH & Bechmann T (2018). Bone loss during neoadjuvant/adjuvant chemotherapy for early stage breast cancer: A retrospective cohort study. Mol Clin Oncol 8, 767-772.
3. Chan A, Delaloge S, Holmes FA, Moy B, Iwata H, Harvey VJ, Robert NJ, Silovski T, Gokmen E, von Minckwitz G, Ejlertsen B, Chia SKL, Mansi J, Barrios CH, Gnant M, Buyse M, Gore I, Smith J, 2nd, Harker G, Masuda N, Petrakova K, Zotano AG, Iannotti N, Rodriguez G, Tassone P, Wong A, Bryce R, Ye Y, Yao B & Martin M (2016). Neratinib after trastuzumab-based adjuvant therapy in patients with HER2-positive breast cancer (ExteNET): a multicentre, randomised, double-blind, placebo-controlled, phase 3 trial. Lancet Oncol 17, 367-377.
4. Sjögren K, Engdahl C, Henning P, Lerner UH, Tremaroli V, Lagerquist MK, Bäckhed F & Ohlsson C (2012). The gut microbiota regulates bone mass in mice. J Bone Miner Res 27, 1357-67.
5. Nilsson AG, Sundh D, Bäckhed F & Lorentzon M (2018). Lactobacillus reuteri reduces bone loss in older women with low bone mineral density: a randomized, placebo-controlled, double-blind, clinical trial. J Intern Med 284, 307-317.

Severe Low Bone Mineral Density is Common in Patients with Beta Thalassaemia Major: A Single Centre Experience

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Background: Advances in Thalassaemia treatment have improved patient survival rates [1], however complications, including osteoporosis, require ongoing consideration. Data on osteoporosis prevalence and risk factors are limited, while underlying causes are complex [2,3]. We determined prevalence, risk factors and treatment of low bone mineral density (BMD) among individuals with Beta Thalassaemia major at a tertiary referral centre.

Methods: Individuals with Beta Thalassaemia major with DXA results at the same institution were included. Data collected included DXA derived BMD, endocrinopathy comorbidities, chelating therapy, vitamin D, and ferritin levels. Osteoporosis was defined as DXA T-score <-2.5 SD in individual above 25 years of age.

Results: 35 patients (14 males, 21 females) with mean age 40.1 ± 10.0 years (range 21 to 64 years) were included. Common endocrinopathies included hypogonadism (29%), hypothyroidism (20%), growth hormone/IGF-1 deficiency (20%), diabetes mellitus (9%), and hypoparathyroidism (9%). Based on DXA results 43% (15) had osteoporosis (T-scores <-2.5 SD) with an average age at diagnosis of 28.5 ± 5.4 years. Additionally, 43% (15) had osteopenia (T-scores between -2.5 and -1 SD). Three individuals reported minimal trauma fractures (MTF) of which 2 had osteopenia. Out of the 17 patients diagnosed with osteoporosis via DEXA or MTF history, only 41% (7) had received antiresorptive therapy (6 with Zoledronic acid, 1 with Denosumab followed by Risedronate) with a mean duration of 4.7 ± 3.9 years. 24% (4) were on concurrent testosterone or hormonal replacement therapy. There were no significant correlations between osteoporosis and risk factors such as age, comorbid endocrinopathies, iron chelation therapy, vitamin D, or ferritin levels (all p >0.05).

Conclusion: Osteoporosis is common and presents early in individuals with Beta Thalassaemia major, but treatment uptake is low. Systematic early screening, risk factor identification and initiation of antiresorptive and/or hormone replacement therapy are needed.

1. References: 1. Farmakis D, Porter J, Taher A, Cappellini MD, Angastiniotis M, Eleftheriou A. 2021 Thalassaemia International Federation guidelines for the management of transfusion-dependent thalassemia. Hemasphere. 2022;6(8). 2. Wong P, Fuller PJ, Gillespie MT, Kartsogiannis V, Kerr PG, Doery JC, Paul E, Bowden DK, Strauss BJ, Milat F. Thalassemia bone disease: a 19-year longitudinal analysis. Journal of Bone and Mineral Research. 2014;29(11):2468-73. 3. Pollak RD, Rachmilewitz E, Blumenfeld A, Idelson M, Goldfarb AW. Bone mineral metabolism in adults with β-thalassaemia major and intermedia. British Journal of Haematology. 2000;111(3):902-7.

Quantification of abnormal cortical bone surface remodelling in preclinical models of arthritis

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Abnormal bone remodelling at the outer cortical surface can lead to features such as bone erosion, cortical vascularisation, and osteophyte formation in arthritis. These pathological features disrupt the integrity of the cortical surface and lead to an abnormally coarse surface which can be observed using micro-computed tomography (microCT). However, quantifying these activities is challenging as commonly used measurements of the cortical bone (e.g cortical thickness) are insensitive to local remodelling activities at the surface. This study develops a novel approach, combining traditional image processing and empirical modelling, to automatically assess abnormal cortical surface remodelling through the observation of osteophytes in preclinical microCT datasets. MicroCT scans (SCANCO Medical) were obtained from previous rabbit and rat studies of osteoarthritis consisting of 8 rabbits and 11 rats [1,2]. For each animal, ACL transection was performed on the right knee and the left knee was a contralateral control. For each image of the joint, the cortical bone of the femur and tibia were segmented, and 3D thickness mapping was performed using a sphere-fitting distance transform [3]. A one-voxel-thick outer layer was segmented from the resulting thickness map, and the histogram empirically estimated by a series of statistical distributions. Parameters describing the best-fit distribution, determined with negative log-likelihood, were analysed to determine sensitivity to osteoarthritis and the presence of osteophytes using paired two-sided t-tests. Visual inspections reveal clustering of surface voxels with small thickness values around areas with osteophytes (Fig.1a), as compared to those without (Fig.1b). In both rats and rabbits, surface thickness optimally fitted to a Gamma distribution, whose shape parameter was sensitive to the presence osteoarthritis ($p < 0.01$) and osteophytes ($p < 0.05$). Future work will focus on characterising underlying biological processes of abnormal bone remodelling to gain insights into the emergence of the fitted Gamma distribution observed in this study.

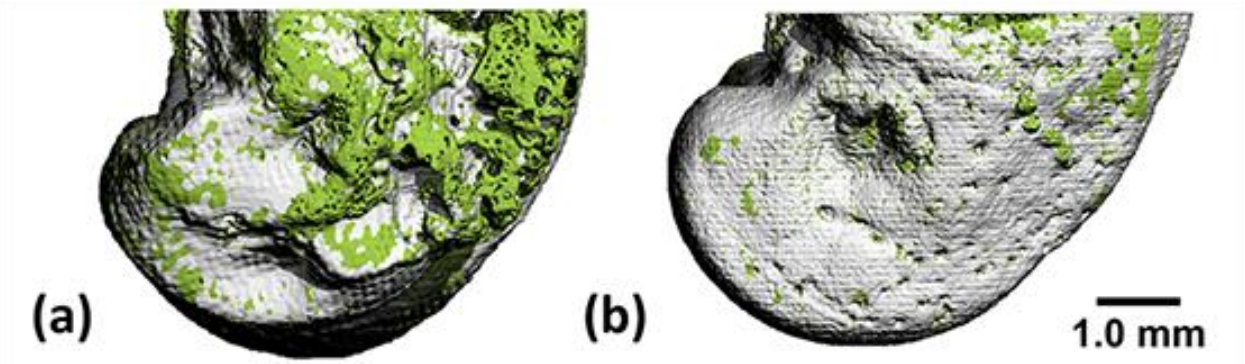

Figure 1: *Exemplar cortical surface analysis results showing medial femurs of a rat sample (a) with and (b) without osteophytes. Large thickness surface voxels are shown in white and small thickness surface voxels are shown in lime.*

1. Stok, K. S. et al., PLOS One. 11(1): e0147564, 2016.
2. Besler, B. A. et al., Bone. 146, 2021.
3. Hildebrand, T. & Rüegsegger, P., Journal of Microscopy. 185, 1997.

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Association between knee osteoarthritis and volumetric bone mineral density

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Backgrounds: Although patients with radiographic knee osteoarthritis (OA) have a higher areal bone mineral density (BMD) compared to non-OA individuals, their fracture risk was not significantly different. This study sought to define the association between radiographic knee OA and volumetric BMD.

Methods: The study was part of the Vietnam Osteoporosis Study, in which 944 men and 1506 women aged ≥ 40 years were randomly recruited from Ho Chi Minh City (Vietnam). Radiographs of the knee were evaluated using the Kellgren and Lawrence scale, with grades ranging from 0 to 4. Knee OA was defined as the presence of radiographic grades of 2 or higher in a knee joint. Trabecular and cortical volumetric bone density (vBMD) was measured in the tibia bone by a pQCT XCT2000 (Stratec, Germany). Linear regression model was used to analyze the association between pQCT parameters and knee OA.

Results: The prevalence of radiographic knee OA was approximately 31% (n = 755), and it increased with advancing age. In comparison to non-OA individuals, those with knee OA exhibited higher femoral neck aBMD (effect size [ES] = 0.05, 95% CI: 0.01 to 0.09; P = 0.02 in men vs. ES = 0.02, 95% CI: 0.0004 to 0.04; P = 0.02 in women); however, they had lower vBMD at the cortical tibia bone (ES = -16, 95% CI: -27 to -4.3; P < 0.001 in men vs. ES = -11, 95% CI: -2.2 to -2.6; P = 0.01 in women).

Conclusion: These data indicate that approximately a third of Vietnamese people have radiographic knee OA and that the cortical vBMD was lower in knee OA patients.

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Gestational vitamin D and offspring fracture risk: Do associations persist into mid adolescence?

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Introduction: Previous studies report that maternal vitamin D exposure during pregnancy is associated with offspring bone health in later life. A previous study in the Vitamin D in Pregnancy (VIP) cohort reported on maternal levels of 25-hydroxyvitamin-D (25(OH)D) and offspring fracture risk and found sexual dimorphism of fracture profiles at 10 years of age. We therefore aimed to determine associations between maternal 25(OH)D status and offspring fracture risk at 16 years of age in this cohort.Methods: In total, 475 mother-child pairs were recruited to the VIP study in southeastern Australia. Maternal serum samples were obtained at recruitment (<16 weeks gestation) and/or 28-32 weeks gestation and analysed for 25(OH)D. Radiologically-confirmed incident fractures in children were ascertained from date of birth (2002-2004) until July 16, 2019. Cox proportional hazard models were used to determine associations between maternal 25(OH)D and childhood fracture risk, and final models included maternal age at recruitment, offspring sex, birth weight, gestation length and season of 25(OH)D sample.Results: Complete follow-up data were available for 400 children (mean age 16.1 years at end of follow-up). There were 122 (30.5%) children who had sustained at least one fracture. Higher maternal 25(OH)D (per 10 nmol/L) in early gestation was associated with a decreased fracture risk in boys (HR 0.87; 95% CI 0.77, 0.99); in contrast, the pattern was reversed in girls (HR 1.10; 95% CI 1.00, 1.22). At late gestation, higher maternal 25(OH)D was associated with an increased fracture risk in girls (HR 1.14; 95% CI 1.04, 1.24) but not boys (HR 0.96; 95% CI 0.87, 1.06).Conclusion: The contradictory risk profiles observed at early childhood in this cohort remain in adolescence: while higher maternal 25(OH)D at recruitment was associated with lower fracture risk in boys, higher maternal 25(OH)D at 28-32 weeks gestation was associated with a higher fracture risk in girls.

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Estimating “Skeletal Age” by Bone Loss in Elderly Men and Women

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'Skeletal Age' is defined as the age of the skeleton resulting from fractures or exposure to risk factors that elevate the risk of fracture. Excessive bone loss is associated with an increased risk of fracture. This study sought to use the skeletal age to quantify the impact of bone loss on mortality.

We analyzed data from the Study of Osteoporosis Fractures (SOF) and Osteoporotic Fractures in Men (MrOS). Bone mineral density (BMD) at the femoral neck was measured at baseline and subsequent visits, and this analysis was limited to those with at least 3 BMD measurements (n=3848 women and n=2925 men). Mortality was ascertained from death certificates and hospital records. The association between BMD change and mortality was assessed by a multivariable Cox’s proportional hazard model, adjusting for age, body mass index, smoking status, alcohol consumption, and dietary calcium intake. The magnitude of association between bone loss and mortality was converted to years of life lost using Gompertz's law of mortality and the US life table. Skeletal age was determined as the sum of chronological age and years of life lost for each individual.

The average annual percent of change in BMD was -0.62±1.2%(mean±SD) in women and -0.16±0.9% in men. During the study period, 1942 women and 1949 men died. For the same rate of bone loss, men experienced a high risk of death than women. Each SD increase in bone loss was associated with a 1.2-fold (95%CI,1.1-1.3) in women and 1.4-fold (95%CI,1.3-1.5) in men after adjusting for age, baseline FNBMD, BMI, smoking and drinking status. Specifically, a 70-year-old individual with BMD loss exceeding 2% would have skeletal age of 74.6years in women and 75.9years in men. These data indicate that excess femoral neck bone loss is associated with an increased loss of years of life and accelerated bone fragility.

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Does long-term antiresorptive administration lead to atypical fractures at other skeletal sites excluded from the ASBMR atypical femur fracture (AFF) case definition? A systematic review

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Osteoporosis affects > 1.3 million Australians. Bisphosphonates and denosumab are approved treatments for osteoporosis with the rare complications of jaw osteonecrosis and AFFs. Cases of AFFs were first reported in 2005, highlighting a state of suppressed bone turnover on biopsy (1). In 2010 and 2013, an American Society of Bone and Mineral Research (ASBMR) Task Force proposed a case definition for these atypical fractures affecting the femoral diaphysis (2, 3). Subsequently, reports of similar atypical fractures at other skeletal sites have been published.

Aim

We aimed to systemically identify cases of atypical fractures, excluded from the ASBMR AFF case definition in patients receiving anti-resorptive medication (duration > 3 years).

Method

A structured search of electronic databases (PubMed, Medline, Embase, Cochrane, Web of Science) and hand-searching of conference abstracts/reference lists was completed. All full-text articles written in English describing atypical fractures were screened for: 1) cases of atypical fractures, excluded from the ASBMR AFF case definition in patients (aged > 18 years) receiving long-term antiresorptives, 2) cases published 2005 – 2023.

Results

7954 citations were identified. 65 articles fulfilled the inclusion criteria. Fractures were more common in females (112/120, 93%). Most frequent fracture sites included the ulna (n=32), tibia (n=12), pelvis (n=10), vertebral pedicle (n=8), sacrum (n=6) and femoral neck (n=5). One atypical fracture was reported in a monogenetic bone disorder (hypophosphatasia). Fractures were commonly atraumatic, with prodromal pain and typically transverse, non-comminuted with evidence of cortical thickening and sclerosis. Non-union was more frequent following conservative management. In most cases, anti-resorptive medication was ceased (41/47, 87%).

Conclusion

Atypical fractures at sites other than the femoral diaphysis in patients receiving long-term anti-resorptive treatment are important to recognise and may provide insights into the pathogenesis of AFF. A review of the current AFF case definition could be undertaken to include other skeletal sites.

1. Odvina CV, Zerwekh JE, Rao DS, et al. Severely suppressed bone turnover: a potential complication of alendronate therapy. J Clin Endocrinol Metab. 2005;90(3):1294-301.
2. Shane E, Burr D, Abrahamsen B, et al. Atypical subtrochanteric and diaphyseal femoral fractures: second report of a task force of the American Society for Bone and Mineral Research. J Bone Miner Res. 2014;29(1):1-23.
3. Shane E, Burr D, Ebeling PR, et al. Atypical subtrochanteric and diaphyseal femoral fractures: report of a task force of the American Society for Bone and Mineral Research. J Bone Miner Res. 2010;25(11):2267-94.

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In vivo phosphoproteomics and functional genomics of insulin signalling in bone

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Insulin signalling in bone plays a critical role in development and energy metabolism. Here we present the first mouse bone phosphoproteome study of 8- and 73-week-old mice following acute *in vivo* insulin stimulation. Hundreds of phosphorylation sites were regulated between young and old bone revealing dramatic rewiring and defects in insulin signalling. We next developed a zebrafish CRISPR/Cas9 loss-of-function screen targeting insulin-regulated phosphoproteins to assess their role in bone mineralisation and development. Several interesting candidates were identified including AFF4, a core scaffold of the Super Elongation Complex (SEC) responsible for driving transcriptional elongation. We show S831 phosphorylation of AFF4 is a novel P70S6K substrate that is dysregulated in aged and insulin-resistant bone, and correlates with reduced transcriptional elongation. Phosphorylation promotes association of ENL/AF9 to the SEC and drives transcription of insulin-dependent target genes required for bone development.

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Single-cell RNA sequencing: unravelling the bone one cell at a time

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Bone is a complex tissue populated by a highly heterogeneous mix of cell types in different compartments. The endosteal compartment is a key site for bone remodelling, a crucial process that determines and maintains bone mass. Despite the key role of the endosteal compartment in bone homeostasis and disease, the specific identity of the cells that comprise the compartment remains unclear. Our work focuses on charting the cellular and molecular landscape in bone to identify cellular mechanisms underlying the regulation of bone mineral density (BMD). Using single cell RNAseq (scRNAseq), we have identified multiple cell types in both mouse and human bones, including multiple clusters of myeloid, lymphoid, osteoblastic, chondrocytic and vascular cells, each with distinct transcriptional profiles. Integrated analysis with findings from human genetic studies of BMD identified osteoblasts, chondrocytes, and vascular cells to be enriched for BMD-associated genes. Cell type-specific BMD-associated genes were more likely to result in abnormal BMD when deleted in mice. In-depth skeletal phenotyping validated the critical functions of novel BMD-associated genes, including the endothelial cell-specific gene *SLC9A3R2*, which when deleted in mice resulted in reduced trabecular bone mass. Our integrative approach identifies cell types present in the endosteal compartment, including bone resident cells and vascular cells, in which genetic determinants of BMD may function to influence pathogenesis of monogenetic and polygenetic skeletal disorders with abnormal BMD. This provides a resource to prioritise genes to accelerate development of patient-centred therapeutics for skeletal diseases.

Frailty is associated with greater long-term risk for fall and fracture-related hospitalisations as well as mortality in community-dwelling older Australian women

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Introduction: Frailty is associated with declines in physiological capacity across sensory, neurological and musculoskeletal systems with the underlying assumption being the frailer an individual is, the more likely they are to fall and fracture. We examined whether grades of frailty can assess the long-term risk of hospitalised falls, fractures and all-cause mortality in 1261 community-dwelling older women (75.1 ± 2.7 years) over 14.5 years.

Methods: Frailty was operationalised using a frailty index (FI) of cumulative deficits from 33 variables across multiple health domains (physical, mental, comorbidities) at baseline. The total score across these variables were summed and divided by 33 to obtain the FI. Participants were graded as either fit (FI ≤0.12), mildly frail (FI >0.12-0.24), moderately frail (FI >0.24-0.36) or severely frail (FI >0.36). Fall- (n=498), any fracture- (n=347) and hip fracture-related hospitalisations (n=137) and deaths (n=482) were obtained from linked health records over 14.5 years. Associations between FI grades and each of the clinical outcomes were analysed using multivariable-adjusted Cox-proportional hazard models including age, treatment (calcium/placebo), body mass index, smoking history, socioeconomic status, plasma 25-hydroxyvitamin D status plus season obtained, physical activity, self-reported prevalent falls and fractures.

Results: At baseline, 713 (56.5%), 350 (27.8%), 163 (12.9%) and 35 (2.8%) of women were classified as fit, mildly-moderately- and severely-frail, respectively. Women with mild, moderate and severe frailty had significantly higher hazards for a fall- (46%, 104%, 168%), any fracture- (88% for moderate frailty, 193% for severe frailty), hip fracture-related hospitalisation (93%, 127%, 129%) and all-cause mortality (47%, 126%, 242%) (Figure). When hip BMD was included as an additional covariate, results remained unchanged.

Conclusion: The FI identified community-dwelling older women at risk for the most serious falls and fractures, and may be incorporated into relevant risk assessment tools to identify individuals with poorer clinical prognosis.

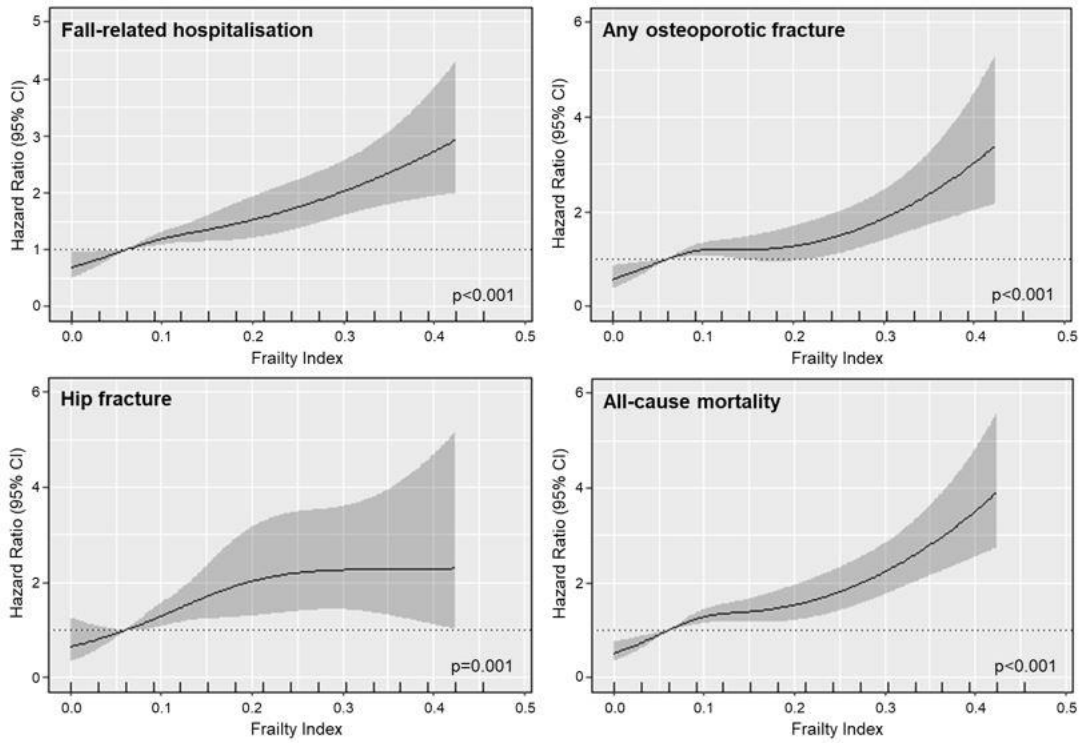

Figure. Multivariable-adjusted relationship between frailty index, fall-, any fracture- and hip fracture-related hospitalisations and all-cause mortality. Hazard ratios are obtained in comparison to the median frailty index score for mildly frail (0.16), frail (0.28) and severely frail (0.41) women, compared to fit women (0.06).

RANKL inhibition creates a pro-osteoclastic environment, leading to an overshoot in serum TRAP and accelerated bone resorption following treatment withdrawal.

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Denosumab withdrawal triggers rapid bone mineral density (BMD) loss via accelerated bone resorption. Development of optimal sequential therapy is hindered by poor understanding of the cellular mechanisms and sub-optimal serum turnover marker assessment. We compared temporal changes in RANKL, serum TRAP5b and osteoclast precursors to CTX, P1NP and BMD, to define alternative tools to guide sequential treatment.

Seven week female C57BL/6 mice received 2-weeks of saline or thrice-weekly OPG:Fc (10mg/kg) to inhibit RANKL, then withdrawn from therapy (OPG-W). Following longitudinal BMD and serum measurement, mice were harvested at weeks 2, 8, 11 and 13 for RANKL, TRAP5b, P1NP and CTX. Week 8, marrow-flushed, long-bone samples were assessed for RANKL mRNA. Week 6 bone marrow samples were analysed for osteoclast precursors ($NK1.1^-$ $Ter119^-$ $CD3^-$ $Ly6G^-$, $B220^-$, $CD11b^{lo}$, $CD117^{int}$, $CD115^+$).

Following OPG:Fc withdrawal, BMD increased 24% at week 8 in OPG-W ($p<0.01$), declining at week 10 and normalised to vehicle at week 13. At week 8, serum TRAP, CTX and P1NP were all suppressed in OPG-W ($p<0.001$). At week 11, serum TRAP was elevated in OPG-W ($p=0.01$), P1NP and CTX remained equivalent to vehicle. At week 13, serum TRAP, P1NP and CTX were all greater in OPG-W ($p<0.01$).

Serum RANKL levels at week 2 were elevated with OPG:Fc ($p<0.001$), peaking 13-fold higher at week 8 ($p<0.0001$), returning to vehicle at week 11. Prior to the overshoot in serum TRAP levels in OPG-W (at week 11), bone RANKL mRNA was elevated at week 8 ($p<0.01$) and osteoclast precursors were increased at week 6 ($p<0.05$).

Rebound BMD decline following OPG withdrawal preceded the increase in clinical turnover markers (P1NP, CTX). Overshoot in TRAP occurred earlier than CTX and P1NP, and may better guide timing of sequential therapy. Increased serum and bone RANKL levels and osteoclast precursors were detected prior to the overshoot in TRAP.

Preventing Bone and Muscle Injury and Reducing Costs in Australian Army Recruit Training with Bone-Targeted Pre-Conditioning: The PREFIT Study

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Purpose

Bone adapts to mechanical loading but overly rapid loading increments, as during Army recruit training (ART), cause bone stress injuries. We aimed to 1. determine whether bone-targeted preconditioning could reduce musculoskeletal injuries and costs during ART (Australian Army Recruit Training Centre, Kapooka, NSW), and 2. identify factors associated with musculoskeletal injury during ART.

Methods

PREFIT was a controlled proof-of-concept trial offering 45mins 5d/wk bone-targeted preconditioning to candidates to the Australian Army enlisting within 5 months. We collected biometrics, health history, medications, heel BUA (QUS, Achilles, GE); muscle strength (TTM Muscle Meter), muscle power (GymAware); functional reach; #push-ups, #sit-ups, shuttle run performance, vitamin D; prior physical activity (BPAQ); calcium (AusCal) and prior injuries. Preconditioning adherence and injuries during 12-week ART were monitored. Rates and costs of injury in recruits who did and did not undertake pre-conditioning and predictors of injury were examined.

Results

We enrolled 91 candidates into PREFIT training of whom 37 entered ART (54 decided not to enlist). 25 completed sufficient PREFIT training to qualify as 'pre-trained'. 349 platoon-mates consented to be controls (overall mean age, 22±5.4yrs). 19% of the PREFIT-group sustained an injury versus 61.2% of non-PREFIT trained recruits. 10.7% of the PREFIT group sustained a musculoskeletal injury versus 37.7 % of the non-PREFIT group. There was insufficient power to compare bone injuries, but for every 1-unit increase in BUA or #push-ups, the odds of bone injury was 0.97 and 0.96 times lower, respectively (p=0.033;p=0.041). Lower extremity injury and bone injury were negatively associated with height, BUA, leg power, #push-ups, #sit-ups and shuttle run, and positively associated with smoking, and previous medication. Medical costs were $1,308 for non-PREFIT versus $2,241 for PREFIT recruits.

Conclusions

A bone-targeted preconditioning program reduced musculoskeletal injury and costs to the Army for recruit training. Taller, stronger, fitter recruits are less at risk.

Deletion of the chondrocyte glucocorticoid receptor attenuates cartilage degradation through suppression of early synovial activation in murine posttraumatic osteoarthritis

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Objective: We have previously shown that disruption of endogenous glucocorticoid signaling in bone cells attenuates osteoarthritis in aged mice, however, the role of endogenous glucocorticoids in *chondrocytes* is unknown. Here, we investigated whether deletion of the glucocorticoid receptor, specifically in chondrocytes, also alters osteoarthritis progression.

Design: Knee osteoarthritis was induced by surgical destabilization of the medial meniscus (DMM) in male 22-week-old chondrocyte (Col2a1-Cre^{ERT2}) glucocorticoid receptor knockout (chGRKO) mice and their wild-type (WT) littermates (n=7-9/group). Mice were harvested at 2, 4, 8 and 16 weeks after DMM-surgery to examine spatiotemporal histological and molecular joint changes.

Results: Cartilage damage was attenuated in chGRKO compared to WT mice at all timepoints following DMM (Fig.1A-B). At 2 weeks post-DMM, WT mice exhibited extensive synovial activation characterized by synovial thickening and increased IL-1β expression that subsided over time. Intriguingly in chGRKO mice, synovial thickening and IL-1β expression were significantly less pronounced at 2 weeks post-DMM compared to WT mice (Fig.1A,C-D). To determine how GRKO in chondrocytes reduced synoviocyte activation, we analysed expression of Hypoxia Inducible Factor (HIF)-2α, a protein known to regulate chondrocyte crosstalk with synoviocytes during osteoarthritis. Immunohistochemistry revealed that chondrocyte HIF-2α expression was significantly reduced in chGRKO compared to WT mice at 2 weeks post-DMM, suggesting a mechanism by which chondrocyte glucocorticoid signaling induces synoviocyte activation through HIF-2α (Fig.1E). Consequently, downstream catabolic signaling was reduced in chGRKO compared to WT mice, characterized by high YAP1 and decreased MMP-13 expression, at 2 and 4 weeks post-DMM. Notably, chondrocyte senescence was also reduced in chGRKO compared to WT mice (Fig.1F-H).

Conclusion: Glucocorticoid signaling in chondrocytes promotes synovial activation, chondrocyte senescence and cartilage degradation by upregulation of catabolic signaling through HIF-2α in murine posttraumatic osteoarthritis. These findings indicate that inhibition of glucocorticoid signaling early after injury may present a promising way to slow osteoarthritic cartilage degeneration.

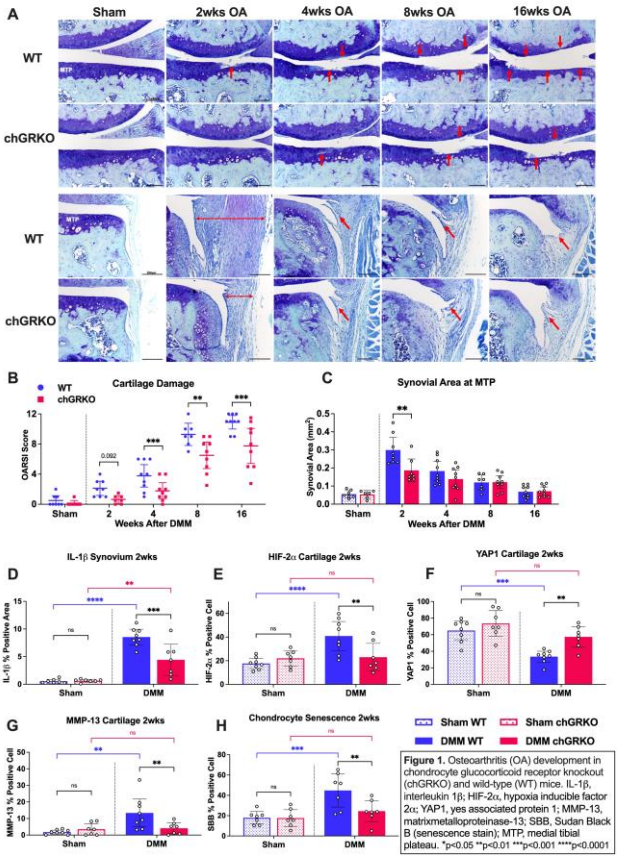

Figure 1. Osteoarthritis (OA) development in chondrocyte glucocorticoid receptor knockout (chGRKO) and wild-type (WT) mice. IL-1β, interleukin 1β; HIF-2α, hypoxia inducible factor 2α; YAP1, yes associated protein 1; MMP-13, matrixmetalloproteinase-13; SBB, Sudan Black B (senescence stain); MTP, medial tibial plateau. *p<0.05 **p<0.01 ***p<0.001 ****p<0.0001

Effects of high-intensity resistance and impact exercise on changes in body composition and metabolic and musculoskeletal health during weight loss in older adults with obesity: A pilot randomised controlled trial

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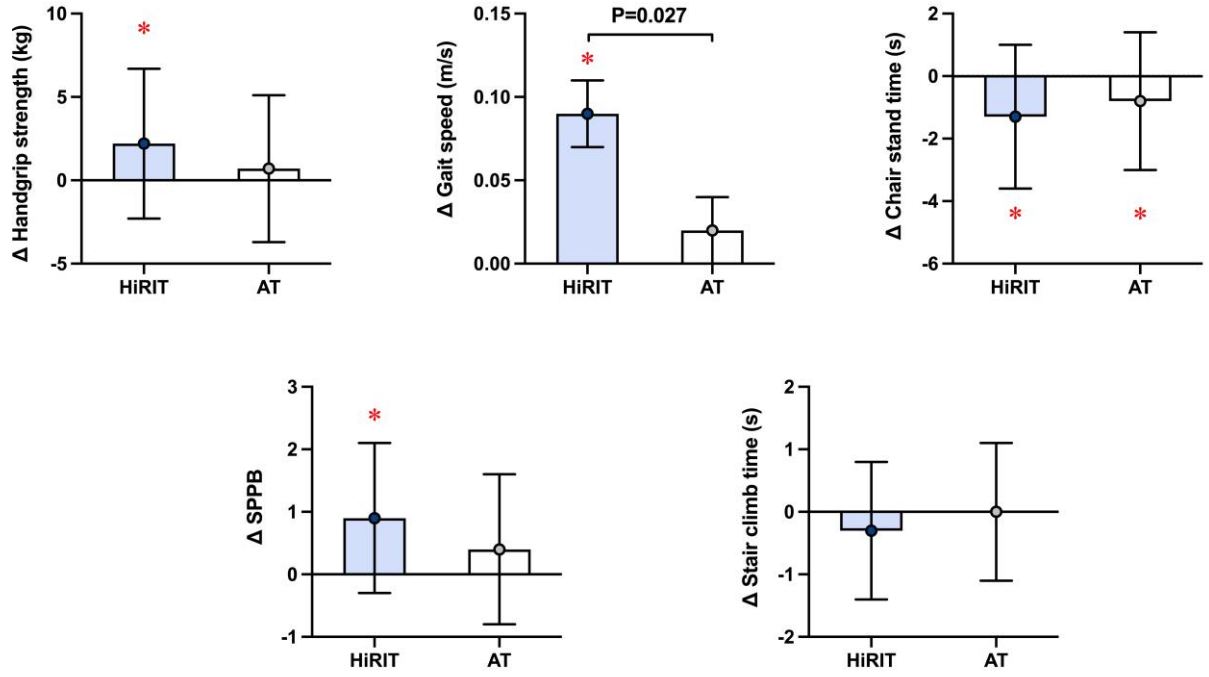
Introduction: Weight loss achieved via energy restriction leads to muscle mass and bone mineral density (BMD) loss. High-intensity resistance and impact training (HiRIT) might attenuate weight loss-induced musculoskeletal declines. Our objective was to compare changes in body composition and metabolic and musculoskeletal health in older adults with obesity undertaking weight loss combined with HiRIT or aerobic training (AT).

Methods: Sixty older adults with obesity (aged >60 years; body fat percentage ≥30% in men and ≥40% in women) and a mobility limitation (short physical performance battery score [SPPB] <11) were randomly assigned to either 12 weeks of supervised, centre-based HiRIT, or self-directed, home-based AT, with energy restriction (750-1000kcal reduction in energy intake). Changes in physical function (primary outcome: gait speed), body composition, and metabolic and bone health were compared within and between groups.

Results: Gait speed increased in HiRIT compared with AT, chair stand times decreased in both groups, and handgrip strength and SPPB scores increased in HiRIT, but not AT (Figure). Similar decreases in total body mass (HiRIT: -5.1±4.6kg versus AT: -4.9±4.5kg), fat mass (HiRIT: -3.6±3.7kg versus AT: -3.3±3.6kg), visceral fat (HiRIT: -32.1±41.6cm^2 versus AT: -31.4±39.9cm^2) and appendicular lean mass (HiRIT: -0.8±1.6kg versus AT: -1.2±1.6kg) were observed. Only HiRIT reduced fasting glucose (HiRIT: -0.4±0.8mmol/L versus AT: -0.2±0.8mmol/L), insulin (HiRIT: -3.3±4.7mU/L versus AT: -1.5±4.7mU/L) and glycated haemoglobin (HiRIT: -0.2±0.4% versus AT: -0.1±0.4%). There were no significant within- or between-group differences in BMD. HiRIT was well-tolerated and accepted with only 7 minor adverse events (AT=5) and 6 participants lost to follow-up (AT=5).

Conclusion: HiRIT appears to be safe and more effective than AT for improving physical performance in older adults with obesity during weight loss. Additional trials with larger sample sizes and longer durations are warranted to explore whether HiRIT can improve metabolic health and attenuate weight loss-induced bone loss.



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Use of denosumab in non-osteoporotic conditions including bone tumours and paediatric use

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Denosumab is a potent anti-resorptive agent that inhibits osteoclast maturation and activity by binding to receptor activator of nuclear factor kappa-B ligand (RANKL). Its use in adult osteoporosis is well established, using six-monthly dosing, which results in minimal drug accumulation with recovery of bone turnover between doses. Monthly dosing leads to continuous suppression of bone turnover and is used for treatment of giant cell-rich bone tumours, bone metastases from solid tumours and hypercalcaemia of malignancy. There is also an emerging role for treatment of symptomatic fibrous dysplasia lesions refractory to standard therapy.

The efficacy and safety of the monthly, higher-dose denosumab regimen has been shown in adults and skeletally-mature adolescents in phase 2 and 3 clinical trials, leading to its regulatory approval in these populations. Evidence for its use in children and adolescents before skeletal maturity is much scarcer. The case reports and series available describe similar efficacy when used for giant cell-rich bone tumours, but safety concerns have been far greater. The most significant of these is the hypercalcaemia observed during the rebound of bone turnover after denosumab cessation, which can be severe and invariably requires treatment with intravenous bisphosphonates or further denosumab. Nevertheless, for unresectable bone tumours, the benefits may outweigh the risks and international collaboration is underway to improve the evidence base for the paediatric use of denosumab.

This presentation will cover the use of denosumab in non-osteoporotic conditions with a focus on RANKL-mediated focal bone lesions and the management considerations for paediatric use.

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Hypophosphatasia in the age of miracles

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Hypophosphatasia (HPP) is a rare genetic condition due to *ALPL* loss-of-function mutations. The broad phenotypic spectrum ranges from absence of bone mineralisation and in-utero fetal death to mild musculoskeletal disturbances in adulthood. For those most severely affected, enzyme replacement therapy with asfotase alfa in combination with a multidisciplinary approach at any age can be life-changing.

Impact of HIV on radial bone density, geometry, and strength in midlife Zimbabwean women and association between pQCT outcomes and fracture

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Background: HIV and its treatment are associated with deficits in bone and increased fracture risk; however, limited data are available from sub-Saharan African women during midlife, and importantly during the menopause transition. We used peripheral QCT to measure radial BMD, geometry, and strength in pre-, peri-, and postmenopausal Zimbabwean women living with (WLWH) and without HIV (WLWOH) and determined whether pQCT measures were associated with self-reported fractures.

Methods: Scans were obtained from 384 women aged 40-61 years (n=191[49%] WLWH). pQCT outcomes were: 4% trabecular volumetric BMD (Trab.vBMD, mg/cm^3), total area (Tot.A4, mm^2), and compressive bone strength (BSIc, g^2/cm^4); 33% cortical vBMD (Ct.vBMD, mg/cm^3), total area (Tot.A33, mm^2), and Stress-Strain Index (SSI, mm^3). Height (m), weight (kg) and menopause status based on last menstrual period were recorded. Linear regression investigated differences adjusting for age, menopause stage, and height, and then additionally for weight. In all women, odds ratios were calculated to explore associations between pQCT measures and self-reported 1) any fracture; 2) major osteoporotic fracture.

Results: Women were of mean(SD) age 49.6(5.8) years and BMI 29.0(6.1) kg/m^2. WLWH women had lower weight and BMI (both p<0.001). All pQCT outcomes were lower in WLWH before adjustment (all p<0.05, Table 1), and robust to adjustment for age, menopause stage, and height (all p<0.05) apart from Tot.A33. Further adjustment for weight attenuated Tot.A4 and SSI between-group differences (Table 1). Tot.A33 and SSI were associated with lower odds of previous major osteoporotic fracture (OR[95%CI]: Tot.A33, 0.57[0.34;0.94]; SSI, 0.58[0.34;0.97].

Conclusion(s): In perimenopausal women HIV impacts BMD and strength at the distal radius, a common osteoporotic fracture site. These relationships were robust to initial adjustment, though the addition of weight to the models reduced the effect size. Numbers of reported fractures were low, but in the whole group analysis, radius cortical parameters were associated with fracture.

Table 1. Linear regression to determine the extent of between-group differences between perimenopausal Zimbabwean women aged 40-61 years

N=384	Unadjusted model		Adjusted for age, menopause, and height		Adjusted for age, menopause, height, and weight	
Radius 4%	MD [95% CI]	p-value	MD [95% CI]	p-value	MD [95% CI]	p-value
Trabecular vBMD (mg/cm^3)	-14.8 [-22.9; -6.7]	**<0.001**	-15.2 [-23.1; -7.2]	**<0.001**	-9.67 [-17.80; -1.57]	**0.019**
Tot.A4 (mm^2)	-13.5 [-23.4; -3.6]	**0.008**	-9.76 [-18.80; -0.73]	**0.034**	-9.36 [-18.80; 0.09]	0.052
BSIc (g^2/cm^4)	-0.0538 [-0.0743; -0.0333]	**<0.001**	-0.0491 [-0.0688; -0.0295]	**<0.001**	-0.0298 [-0.0491; -0.0104]	**0.003**
Radius 33%	MD [95% CI]	p-value	MD [95% CI]	p-value	MD [95% CI]	p-value
Cortical vBMD (mg/cm^3)	-8.91 [-15.5; -2.3]	**0.008**	-7.26 [-13.10; -1.44]	**0.015**	-8.67 [-14.70; -2.59]	**0.005**
TotA.33 (mm^2)	-3.85 [-6.74; -0.96]	**0.009**	-2.37 [-4.86; 0.12]	0.062	-1.44 [-4.02; 1.14]	0.273
SSI (mm^3)	-13.8 [-22.5; -5.0]	**0.002**	-8.40 [-15.70; -1.11]	**0.024**	-6.29 [-13.90; 1.31]	0.105

MD = mean difference; CI = confidence interval. Women without HIV as the reference group, such that negative values mean those with HIV have lower values.
vBMD = volumetric bone mineral density, TotA = total area, BSIc = bone strength index of compression, SSI = stress-strain index

Reversing high bone porosity in NF1 bone using dietary supplements

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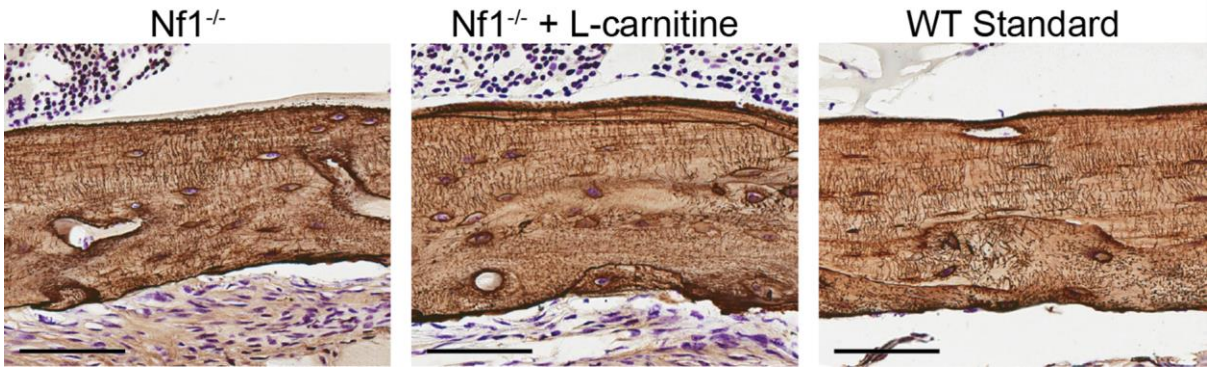
Background: Neurofibromatosis type 1 (NF1) is a genetic disorder associated with tumour susceptibility, but it can also have profound effects on bone and muscle. In children, NF1 is associated with osteopenia and focal bone dysplasias. Studies of NF1 bone microarchitecture have revealed fundamental differences in bone density and cortical porosity. Our research has found that the muscle manifestations of NF1 – low muscle tone associated with aberrant metabolism and long-chain lipid accumulation – are responsive to dietary intervention with L-carnitine + medium-chain fatty acid (MCFA) supplemented chow. This study aimed to test whether this intervention could rescue the NF1 bone phenotype in mice.

Methods: N=70 female *$Nf1^{flox/flox}$:Prx1-Cre ($Nf1_{Prx1}^{-/-}$)* NF1 limb-KO mice were given 8 weeks of standard chow or one of six modified diets altered to reduce long-chain fatty acid intake and/or improve lipid metabolism with L-carnitine supplementation. MicroCT analysis was performed on the cortical bone to look at standard parameters (bone volume, tissue mineral density, cortical thickness) and specific porosity measures (closed pores corresponding to osteocyte lacunae and larger open pores). Histological staining was used to further visualize these changes.

Results: *$Nf1_{Prx1}^{-/-}$* bones were found to have inferior properties to wild type bones, with a 4-fold increase in the porosity attributed to open pores. The high cortical bone porosity was significantly reduced via dietary interventions including L-carnitine + MCFA chow previously shown to improve muscle histology function. Dietary intervention also produced significant increases in cortical tissue mineral density and cortical thickness. A reduction in large cortical pores was further reflected by tissue histology.

Discussion: These data support the concept that lipid metabolism may have an important mechanistic effect on bone porosity and quality in NF1. Emerging interventions for NF1 muscle health may thus produce secondary benefits in bone.



Assessing the robustness of evidence for the efficacy of anti-fracture medications

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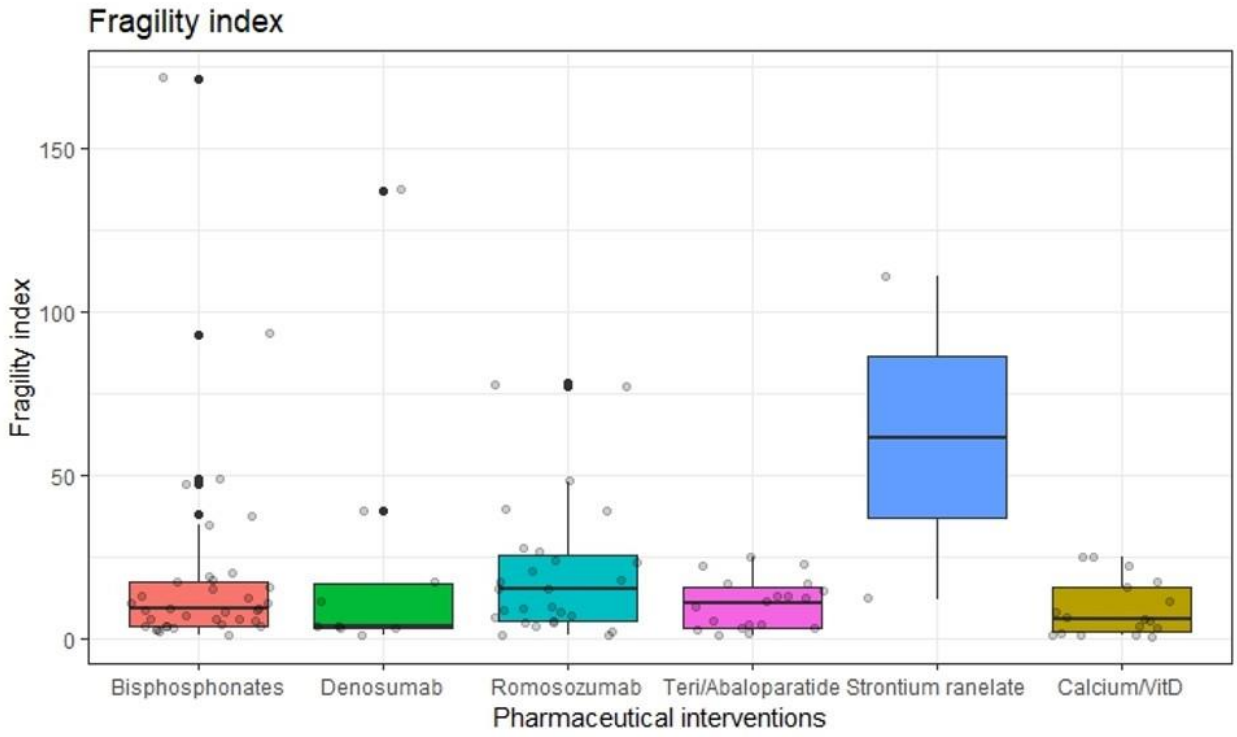
The evidence of the efficacy of anti-fracture medications is conventionally derived from randomized controlled trials (RCTs) with P-values< 0.05 being the gold standard. This study sought to quantify the fragility of the RCT evidence for anti-fracture medications.

This analysis included 22 RCTs in high-impact medical journals which generated 110 statistically significant results (P< 0.05) for at least one fracture site. The fragility of the evidence was assessed by the *'Fragility Index'* (FI), which is defined as the minimum number of patients whose condition would need to change from non-fracture to fracture to invalidate a statistically significant outcome.

The overall median FI was 9 (IQR: 3, 11), indicating that adding as few as 9 fracture patients (~0.4% of the study size) to the intervention group would eliminate the previously documented evidence of fracture prevention efficacy. Notably, in 65% of these analyses, the number of participants lost to follow-up exceeded the corresponding FI. Among the 34 results with P< 0.001, the median FI was 25 (17, 47) which was lower than the number of participants lost to follow-up in 25 results. Specifically, the evidence of anti-fracture efficacy of denosumab and calcium/vitamin D supplementation would be lost if only additional 4 (3, 17) and 6 (2, 16) patients respectively, sustained a fracture during the follow-up period (Figure). Among 37 positive results (~34%) that used fracture as the primary outcome measure, the evidence for anti-fracture efficacy remained fragile, with a median FI being only 15 (8, 25). In approximately 90% of these positive results, the number of participants lost to follow-up was higher than that required to render the results statistically non-significant.

These data indicate that the existing RCT evidence of anti-fracture efficacy is fragile. In order to increase the robustness of the anti-fracture evidence, the P-value threshold should be lower than 0.001.

Figure. Robustness of scientific evidence in fracture prevention efficacy by pharmaceutical interventions



***Notes**: dots indicate individual analyses that examine fracture events as a binary or time-to-event endpoint.*

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Sclerostin, a marker for mature fibrochondrocytes, modulates the stiffness gradient to maintain tissue integrity of the fibrocartilaginous enthesis.

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The enthesis is the attachment site of tendon and ligament to bone. Fibrocartilaginous entheses are located at the epiphysis or the apophysis of the bone. Primordial entheses arise from *Scx*$^+$/*Sox*9$^+$ progenitors during embryonic development to form as the junction between tendons/ligaments and hyaline cartilage. Fibrocartilaginous entheses consist of four graded tissue layers including tendon, unmineralized and mineralized fibrocartilage, and subchondral bone with varying degrees of stiffness. The mineral gradient of the enthesis is thought to be particularly important for limiting stress concentrations at the bone-tendon interface. However, it remains uncertain how such fine structure is constructed during postnatal growth. Taking advantage of the cryofilm method reported by Kawamoto, we performed histological and atomic force microscopy analyzes on cryosections of non-decalcified hard tissues to examine formation of the fibrocartilaginous enthesis. Development of mineralized fibrocartilage was followed by the expansion of unmineralized fibrocartilage after the decreased ALP activity in the mineralization front. Calcein labelling revealed that the mineralization front in the enthesis extends unidirectionally towards the midsubstance of the Achilles tendon. We found that sclerostin, which antagonizes canonical Wnt/b-catenin and BMP signaling, is expressed in mature mineralized fibrocartilage adjacent to subchondral bone. In *Scx* deficient mice with decreased mechanical loading due to defective tendon formation, both fibrocartilage and hyaline cartilage formation was impaired and sclerostin expression was markedly decreased. Loss of the *Sost* gene, which encodes sclerostin, resulted in increased bone mineral density and higher stiffness in the fibrocartilaginous enthesis. Thus, sclerostin marks mature fibrochondrocytes to modulate the stiffness gradient to maintain tissue integrity of the fibrocartilaginous enthesis.

“Skeletal Age” for mapping the impact of fracture on mortality using clinical data

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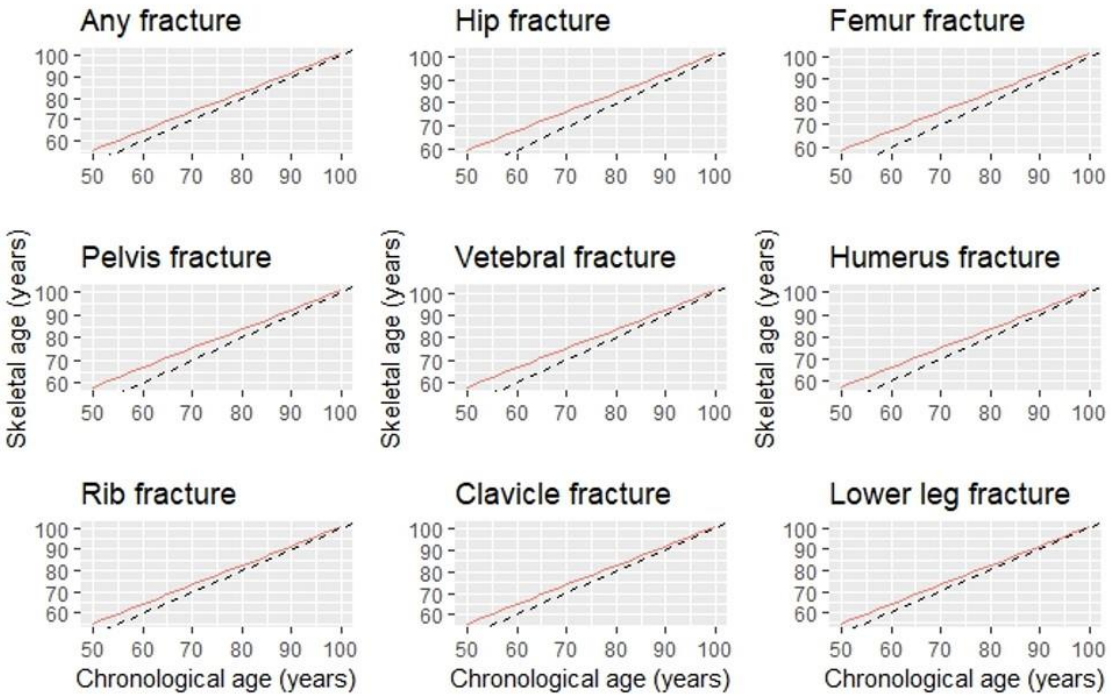
We used registry-based data to propose a new metric called “Skeletal Age” (SA) for conveying the combined risk of fracture and fracture-related mortality in patient-doctor risk communication[1]. This study sought to estimate SA for specific fracture sites using clinically measured data.

Skeletal Age is conceptually defined as the age of an individual's skeleton resulting from a fragility fracture. Thus, for an individual with a fracture associated with increased mortality risk, the SA would be expected to be higher than the individual's chronological age. SA is estimated as the sum of chronological age and the number of years of life loss associated with each fracture site for an individual with a given clinical risk profile, including age, BMD, BMI, lifestyle factors, and comorbidities.

The study involved 5994 community-dwelling elderly men in the Osteoporotic Fractures in Men Study with an average age of 73.6 (±5.9) years. During a median follow-up of 13.9 years (IQR: 8.5, 17.5), 1085 men sustained a fragility fracture followed by 694 deaths. Hip, other proximal, and lower leg fractures were associated with a significantly increased risk of death. On average, a fragility fracture was associated with 1 to 9 years of life lost, with the loss being greater in younger patients with a hip, femur, or pelvis fracture (Figure). A 60-year man with a hip fracture is estimated to have a SA of 68.1 (95% CI: 66.9, 69.2); whereas the estimated SA for a 70-year man with a hip fracture is 76.3 (75.4, 77.1).

Our results reemphasize that most fractures are associated with increased mortality risk and hence reduced life expectancy. The findings were consistent in both registry-based and clinical data. The proposed Skeletal Age supplements the traditional relative risk as a metric for conveying the mortality consequence of fracture, making the patient risk communication more intuitive.

Figure: Estimated “Skeletal Age” for each specific fracture risk using clinical data



Notes: *dashed lines indicate the perfect concordance between chronological age and skeletal age; red lines indicate the estimated skeletal age for men.*

doctor-

1. 1. Tran T, Ho-Le T, Bliuc D, et al. 'Skeletal Age' for mapping the impact of fracture on mortality. Elife 2023;12:e83888

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Bone marrow adipose tissue under control of nutrient sensors

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Obesity and type 2 diabetes accompanied by increased accumulation of bone marrow adipose tissue (BMAT) affect bone homeostasis. Using anti-diabetic drugs such as thiazolidinediones (TZDs) has a different effect on bone and fat physiology, pointing out a dual role of common factors present in periphery and bone marrow. Insulin signaling, lipid handling, oxidoreductase activity are nutrient-sensing pathways, which are affected by metabolic disturbances and play a critical role in the regulation of glucose metabolism and bone homeostasis. Here I will review our current findings on metabolic changes in BMAT phenotype and its effect on bone marrow microenvironment and stem cell properties in obesity. Also, I will present our recent data with different interventional approaches in animal models of obesity and its effect on BMAT and bone phenotype.

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Pth1r Signalling in Adipoq+ Bone Marrow Cells (MALPs) Decreases Bone Mass and Restricts the Anabolic Response to PTH

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Minimally invasive longitudinal intravital imaging of cellular dynamics in intact long bone

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Intravital two-photon microscopy enables deep tissue imaging at high temporospatial resolution in live animals. However, the endosteal bone compartment and underlying bone marrow pose unique challenges to optical imaging as light is absorbed, scattered and dispersed by thick mineralised bone matrix and the adipose-rich bone marrow. Early bone intravital imaging methods exploited gaps in the cranial sutures to bypass the need to penetrate through cortical bone. More recently, investigators have developed invasive methods to thin the cortical bone or implant imaging windows to image cellular dynamics in weight-bearing long bones. Here we provide a step-by-step protocol for the preparation of animals for minimally-invasive, non-destructive longitudinal intravital imaging of the murine tibia. This method involves the use of mixed bone marrow radiation chimeras to unambiguously double-label osteoclasts and osteomorphs. The tibia is exposed by a simple skin incision and an imaging chamber constructed using thermoconductive T-putty. Imaging sessions up to 12h long can be repeated over multiple timepoints to provide a longitudinal time window into the endosteal and marrow niches. The protocol can be used to investigate cellular dynamics in bone remodelling, cancer cell life cycle, haematopoiesis and long-lived humoral and cellular immunity.

Bone Disorganization: A Novel Biomarker Unrelated to Bone Density and Structure that May Hold the Key to The Diagnosis of Atypical, Stress Fractures, and Other Unexplained Bone Diseases.

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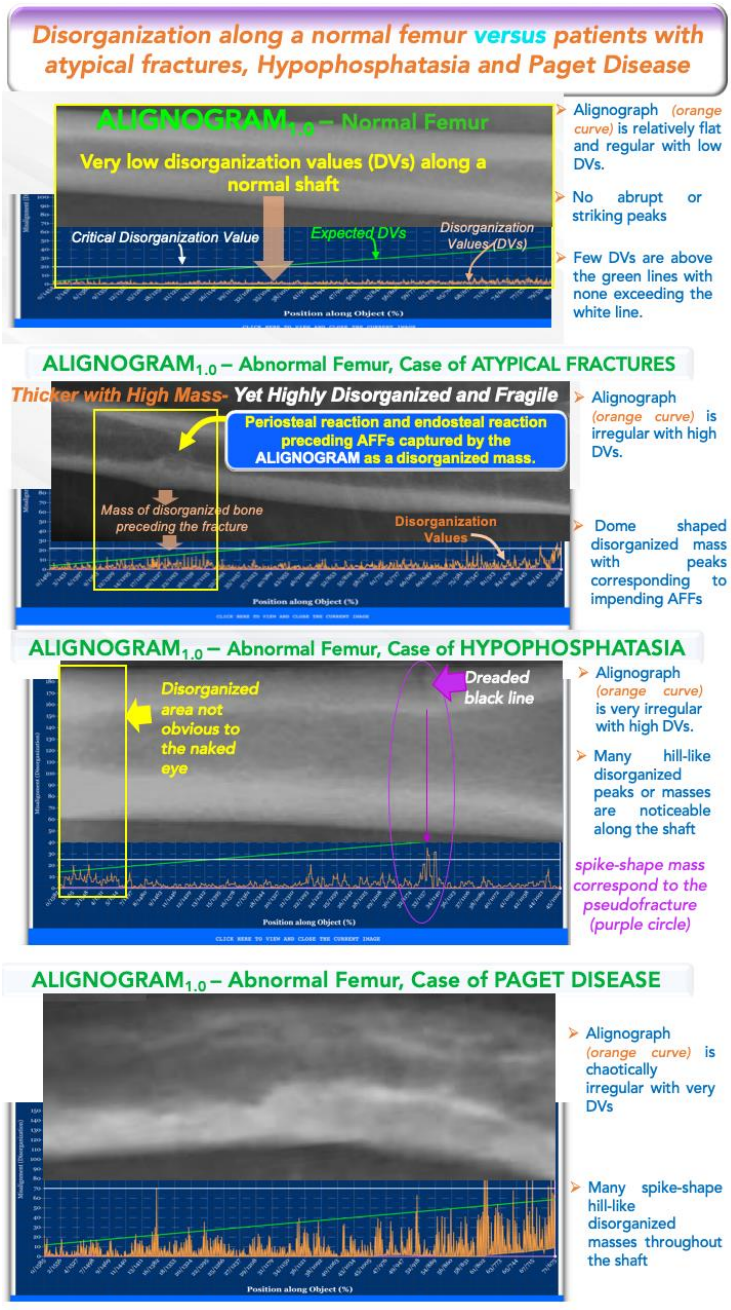

Background–The cause of many bone diseases and fractures remains a mystery. To address this enigma, we recently proposed that the abnormality in these patients is an incorrect arrangement (or disorganization) of an otherwise sufficient or even high amount of bone which by abnormally transferring loads cause damage; triggering inflammation, and a vicious cascade of events leading to diseases including fractures[1]. Hence, we developed and validated a tool to quantify this novel biomarker (disorganization)[2]. Here, we test the hypothesis that measures of disorganization are unrelated BMD or decay, *YET* identifies patients with diseases such as Atypical Femoral Fractures (AFFs), Paget Disease of Bone (PDB), and Hypophosphatasia (HPP).

Methods–We studied 45 women (10 AFFs and fracture-free peers) mean(SEM) age 68.1(1.83) years. In addition, we studied patients PDBs(02) and HPPs(02). Curves displaying the extent disorganization along the femoral shaft(**ALIGNOGRAM**) were produced. Metrics of disorganization metrics were quantified as previously reported[2]. Correlations between disorganization metrics, bone structure, and density were assessed.

Results– In normal subjects, ALIGNOGRAMs were relatively flat, regular with low Disorganization values (DVs); no abrupt or striking peaks. In all patients with AFFs, PDBs, and HPPs ALIGNOGRAMs were irregular, chaotic with higher DVs and spike-shaped peaks corresponding to the most disorganized sites within the bones (**Fig 1**). The mean DV (MDV) distinguished AFFs from controls [36.3 (IQR 23.9-60.8) *vs* 3139 (IQR 1212-14788)]; $p<0.0001$. However, the MDV was not correlated with lateral and medial cortical thicknesses, or periosteal diameter (All $R^2<0.001$; NS). The MDV was also unrelated to density ($R^2=0.0002$; $p=0.17$).

Conclusion– Disorganization is a novel mechanism and biomarker completely unrelated to bone mass and microarchitecture. This novel biomarker, readily quantifiable from standard X-rays, may hold the key to the identification of fractures that occur in individuals without reduced bone density or structural decay such as AFFs, HPP or PDB.

1. 1. Zebaze R, Ebeling PR. Disorganization and Musculoskeletal Diseases: Novel Insights into the Enigma of Unexplained Bone Abnormalities and Fragility Fractures. Curr Osteoporos Rep. 2022 Dec 10. doi: 10.1007/s11914-022-00759-2. 2. Zebaze R, Shore-Lorenti C, Nguyen HH, Chiang C, Milat F, Ebeling PR. A Quantification Method for Disorganized Bone Components: Application to the Femoral Shaft. JBMR Plus. 2023;7(2):e10713.

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Histological changes to the osteocyte peri-lacunocanalicular bone matrix that distinguish aseptic loosening from periprosthetic joint infection

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Introduction

Previous analysis of bone from patients with periprosthetic joint infection (PJI) revealed widespread lack of intact or mature collagen, commonly surrounding osteocytes [1]. This was attributed to increased expression of matrix degrading enzymes by osteocytes and was independent of infective organism. It has been proposed that aseptic loosening may represent cases of subclinical infection. We sought to examine possible means of distinguishing between these pathologies at the bone histological level.

Methods

We examined histology of bone taken from patients with aseptic loosening due to osteolysis with no clinical signs of infection (n = 15) and compared these with bone from confirmed PJI cases (n = 50) and control bone taken from patients undergoing primary hip arthroplasty (n = 10). Samples were formalin fixed, demineralised, paraffin embedded and sectioned, and then subjected to Masson's trichrome and Ploton silver stains.

Results

PJI specimens all showed characteristic type I collagen degradation. A modified trichrome stain highlighted perilacunar 'collars' of degraded collagen in PJI. Aseptic loosening cases also showed areas of degraded collagen although less severe. Aseptic loosening bone also had a distinctive 'mottled' appearance, with circular clearings in the matrix often aligned along canaliculi. Overall, aseptic loosening bone showed many more prominent canaliculi than control bone, while PJI bone had a relative deficit of canaliculi. Silver stain confirmed an increased canalicular size in aseptic loosening bone, with circular lesions corresponding to apparent nodules along these.

Conclusions

Aseptic loosening bone in all cases displayed characteristic prominent canaliculi with associated nodules due likely to increased pericanalicular osteolysis/remodelling. This was distinct from PJI bone where more widespread matrix degradation was evident with a relative lack of canalicular structures emanating from osteocyte lacunae. Histological examination of bone collagen composition and osteocyte lacunocanalicular morphology may therefore provide a useful means of distinguishing cases of aseptic loosening from PJI.

1. Ormsby RT, Zelmer AR, Yang D, Gunn NJ, Starczak Y, Kidd SP, et al. Evidence for osteocyte-mediated bone-matrix degradation associated with periprosthetic joint infection (PJI). Eur Cell Mater 2021; 42: 264-280.

Sarcopenia definitions and their association with non fracture injurious falls in older Swedish women from the SUPERB study

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8. Centre for Metabolic Bone Diseases, University of Sheffield Medical School, UK., UK

9. MRC Versus Arthritis Centre for Integrated research in Musculoskeletal Ageing, Mellanby Centre for Musculoskeletal Research, University of Sheffield, UK., UK

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Purpose: To investigate the prevalence and predictive value of three commonly used sarcopenia definitions for the risk of injurious falls in a population of older Swedish women.

Methods: 2,883 older women aged 75 to 80 years were included. Sarcopenia was defined based on the Sarcopenia Definitions and Outcomes Consortium (SDOC) (low handgrip strength and gait speed), revised European Working Group on Sarcopenia in Older People (EWGSOP2) and Asian Working Group for Sarcopenia (AWGS) (low appendicular lean mass index (ALMI; appendicular lean mass (kg)/height (m^2)) and hand grip strength (kg)) definitions. ALMI was obtained from dual-energy X-ray absorptiometry (DXA) (Hologic Discovery A). Self-reported questionnaires captured the occurrence of falls in the past 12 months and fracture risk assessment (FRAX)-based clinical risk factors. Incident injurious falls, without concurrent fracture, were identified using national registers and ICD-10 codes. Cox regression (hazard ratios (HR) and 95% confidence intervals (CI)) analyses were performed without adjustment and after adjustment for age, FRAX variables and previous falls.

Results: Sarcopenia prevalence was 4% (n=129) defined by SDOC, 12% (n=360) for EWGSOP2 and 10% (n=296) for AWGS. During a median (IQR) follow-up time of 6.6 (5.7-7.3) years there were 491 injurious falls without fracture. Sarcopenia according to EWGSOP2 and AWGS was not associated with an increased risk of injurious falls, regardless of adjustment ($p>0.05$). Individuals with sarcopenia defined by SDOC had more than 2-folds increased risk of injurious falls compared with women without sarcopenia (HR: 2.27; CI:1.64-3.15). After adjusting for confounders there was a slightly attenuated association between SDOC prevalence and injurious falls (HR:1.85; CI:1.32-2.60).

Conclusion: These findings suggest that sarcopenia definitions confined to muscle function and strength such as SDOC, rather than including DXA-based ALMI (EWGSOP2 and AWGS), lowers the prevalence but improves prediction of injurious falls in this population of Swedish older women.

Consumer Engagement for Better Outcomes

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Background/Significance:

Overcoming chronic health issues to improve outcomes in any setting is challenging. It is critical to involve patients and consumers in planning and developing health services, as a means to improving quality of services. A process through which health care professionals or relevant organisations can improve the dialogue with patients and consumers to express their needs , present their concerns , devise strategies for involvement in decision -making process should achieve better political, social and cultural outcomes to meet their needs. Hearing the Consumer voice and translating it to the appropriate action is a key priority of Healthy Bones Australia.

Setting/Methods:

Whilst Patients Advocacy can play a significant role in many areas of health care system, Healthy Bones Australia is undertaking to enhance participation of consumers and patients who are living with poor bone health or may be at risk of osteoporosis. The approach focuses on guiding principles of patients/consumer participation and taking responsibility for decision making and input into health promotion, including a significant voice in support of Healthy Bones Australia developing strategies for improving identification of patients at risk of bone fragility fracture and better management of patients with osteoporosis. Additionally, consumers and patients play a critical role in "pushing "policy makers for action to improve access to diagnosis and treatment .

Outcomes.

In 2020 Healthy Bones Australia convened the Inaugural National Consumer and Community Forum to produce Position statement with specific recommendations and actions for the broader healthcare system to improve diagnosis and management of osteoporosis, and overall bone health across community, representing a range of patients living with osteoporosis and key stakeholders.

The agreed priorities for action:

Increase Community Awareness and Education

Improve Risk Factor Identification and Diagnosis

Improve Fracture Identification and Management

Enhance Bone Health Strategic Engagement and Advocacy

Where to Next

Healthy Bones Australia incorporated the Forum's action into 21-23 Action plan and utilising Patient advocates to build effective partnership and collaborations with key stakeholder organisations and groups, as well as Federal Government.

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Fracture Care and Prevention Program - Enhancing Clinical Care

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Introduction: The Fracture Care and Prevention Program is a comprehensive healthcare initiative established in 2016 at Western Health to enhance clinical care for fragility-fractured patients. The program integrates the Fracture Liaison Service (FLS), DXA assessments, Falls and Fracture Clinic, and Gait and Balance Gym, aiming for early identification, investigation, and treatment initiation of fragility fractures to promote bone health and reduced fracture incidence.

Methods: This multidisciplinary nurse-led program employs a systematic approach to identify fragility fractured patients. Early identification is facilitated through the Fracture Liaison Service, allowing timely assessment and intervention. Comprehensive fracture risk assessments, including DXA scans and falls risk evaluations, are conducted to tailor personalised care plans. The multidisciplinary team collaborates to implement evidence-based interventions, ensuring the most effective fracture management.

Results: Over seven years, the Fracture Care and Prevention Program assessed a total of 3838 patients, revealing a distribution of fractures: 41.31% with hip fractures, 6.48% with spine fractures, and 52.21% with fractures in other sites. The program actively participated in care planning for 3108 patients and provided comprehensive assessments for 746 individuals via the Falls and Fracture Clinic. The program has received positive feedback from primary care providers, patients, researchers, and clinicians, reflecting the program's effectiveness.

Outcomes: The Program has achieved significant positive outcomes including: improved accessibility to Bone Density assessments, and strong collaborations with primary care providers. Patients empowerment through education and personalised interventions, has led to increased engagement in fracture prevention and management resulting in improved bone health and reduced future fracture incidents.

Conclusion: The Fracture Care and Prevention Program's multidisciplinary and proactive approach plays a pivotal role in enhancing clinical care for fragility-fractured patients. Early identification, comprehensive assessments, targeted interventions, and patient education collectively contribute to improved patient outcomes and reduced healthcare costs. As the positive impact of the program becomes evident, the integration of similar comprehensive care models within healthcare systems is encouraged to further prioritise evidence-based fracture prevention. The Fracture Care and Prevention Program stands as an exemplar of effective fracture management and underscores the significance of proactive and patient-centered care in reducing fracture incidence and enhancing overall clinical care.

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A Consumer Advocate Perspective

Carol David[2]

1. Consumer Advocate, Australia

Details unavailable at time of print

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A digital voice assistant-supported exercise, nutrition and medication self-management program for older women with osteoporosis

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Objective: To determine the feasibility and preliminary effectiveness of a 24-week digital voice assistant (DVA)-delivered intervention for improving osteoporosis-related health behaviours, knowledge, and attitudes in postmenopausal women with osteoporosis.

Material and Methods: Fifty postmenopausal women currently prescribed anti-osteoporosis medications were randomised to 24 weeks of automated osteoporosis education content (video/audio/text) on medication, nutrition, and exercise (including 3 sessions/week home-based strength, balance and impact exercise) broadcast via a supplied Amazon Alexa Echo Show device located in their home (Alexa), or monthly educational emails (control). The primary outcome was the 12-month anti-osteoporosis medication possession ratio (MPR; total days of osteoporosis medication supply from baseline/365 days), determined via Pharmaceutical Benefits Scheme data. Secondary outcomes included 6-month changes in accelerometer-determined moderate-to-vigorous physical activity (MVPA) and sedentary behaviour, dietary calcium intakes (via 3-day food records), and scores for the Modified Falls Efficacy Scale, Osteoporosis Knowledge Assessment Tool, and Adherence Evaluation of Osteoporosis Treatment Questionnaire. The Alexa group also completed the System Usability Scale (SUS) at 6 months to explore the acceptability of the DVA program.

Results: Forty-eight (96%) women (mean±SD age 64.3±6.1 years) completed follow-up (24 Alexa group; 24 control group). Alexa group participants engaged with 57±18 of 72 (mean adherence=80%) prescribed education and exercise sessions with no adverse events and reported mean SUS scores of 77.8±13.3 (scores ≥68 indicate acceptable usability). The 12-month anti-osteoporosis MPR did not significantly differ between groups but was higher for Alexa compared with control (93.7±22.8% vs 83.3±31.6%; P>0.223). Over 24 weeks, MVPA time significantly increased (+17.9±28.8 mins/day P=0.008) and sedentary time significantly decreased (-40.2±71.7 mins/day; P=0.016) for Alexa only, but these changes did not differ compared with controls (both P>0.05). Calcium intakes similarly increased in Alexa and decreased in controls (+84±372 vs -91±393 mg/day) with no significant group differences (P=0.127), whereas scores for falls efficacy (P=0.032) and osteoporosis knowledge (P=0.038) significantly increased for Alexa compared with controls. Changes in attitudes to osteoporosis medication adherence did not differ between groups (P=0.114) but scores improved within the Alexa group only (baseline 18.7±3.3 vs follow-up 19.9±2.0; P=0.041).

Conclusions: This pilot 24-week digital voice assistant-delivered multifaceted exercise and education intervention achieved excellent adherence, safety, and acceptability for postmenopausal women with osteoporosis. Larger trials are needed to confirm its effectiveness for improving osteoporosis-related health behaviours, knowledge and attitudes, and clinical outcomes such as bone mineral density.

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Early life predictors of bone health

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While commonly chronic conditions such as osteoporosis are associated with geriatric populations, there is a growing body of research that focus on these conditions in the context of a “paediatric disease with geriatric consequences”. Maximising bone mass accrual in childhood and early a key strategy in reducing the risk of osteoporosis in later life. This presentation will focus on early life predictors of bone health using research examining associations between maternal dysglycaemia during pregnancy and offspring bone health as a focal example. Adults and children with diabetes mellitus have poorer bone health and greater likelihood of fracture compared to healthy populations. Thus, it is plausible that the glycaemic environment in utero may impact offspring bone development. Studies examining bone outcomes in the offspring of mothers with gestational diabetes are conflicting and limited, and no study has examined these outcomes beyond infancy. Using data from the Vitamin D in Pregnancy study we examined associations with maternal gestational dysglycaemia and offspring bone health in childhood in children aged 8-11 years of age. We found that children of mothers who were dysglycaemic during pregnancy had a 2-fold increased risk of childhood fracture. There was no overall association was detected with maternal glycaemic status during pregnancy and child BMD. There was, however, evidence of a sexually dimorphic association, whereby mothers who were dysglycaemic during pregnancy had boys with higher bone density. Together, in boys, these associations mirror that of individuals of Type 2 diabetes, whereby despite an increase in bone density there is an incongruent risk of fracture.

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Mapping chemistry and metabolism across the musculoskeletal system at the nanoscale

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Publish consent withheld

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Measures of physical function are associated with increased fracture and mortality risk

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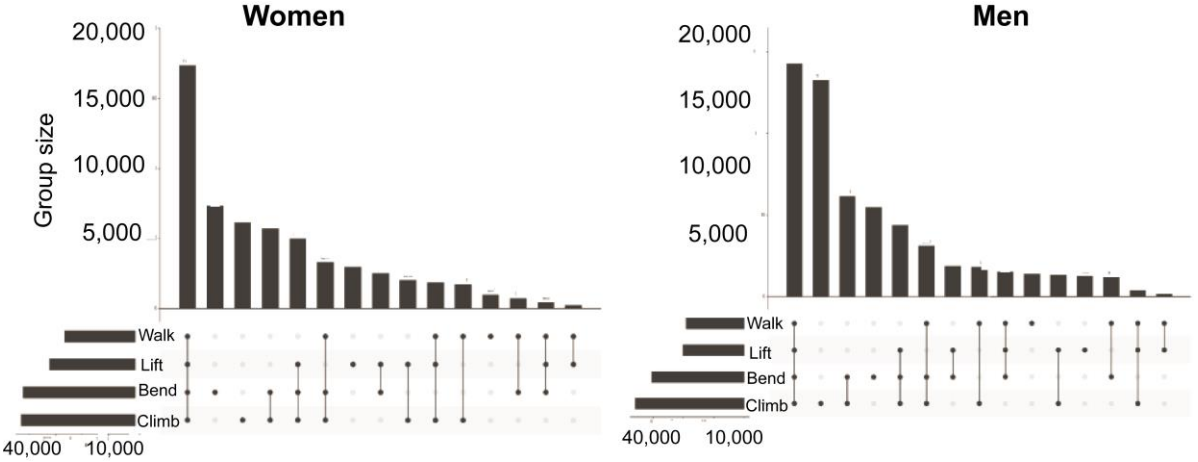
Poor physical performance as measured by common tests increase the risk of fracture. However, those unable to perform these tests have the highest risk, suggesting that qualitative measures might be useful. This study investigated the association between self-assessed physical function and fracture risk.

45 and Up is a population-based cohort of 116,001 people with baseline questionnaire data linked to hospital, emergency, and mortality datasets. Four self-reported measures of physical function (PF), walking, lifting, climbing stairs and bending were analysed. Fractures were identified from hospital admission and emergency presentations and mortality from vital statistics data over 5 years.

Association of PF and fracture risk was determined using cause-specific Cox models; a competing risk approach which produces simultaneous estimates of fracture and mortality. The models were adjusted for fracture risk factors and stratified by age (< 75, ≥ 75).

Approximately 20% of participants reported limitation in all PF domains (Figure). There were 7190 fractures and 4958 deaths in women and 4267 fractures and 7845 deaths in men. All PF domains were associated with increased risk of fracture and mortality. Those who reported a limitation in all 4 PFs had the highest risk. In those < 75, the hazard ratio was 1.73 (1.58 – 1.89) for fracture and 1.21 (1.12 – 1.32) for death in women and 1.64 (1.45 – 1.86) and 1.28 (1.19 – 1.37) in men. In those ≥ 75, the respective hazard ratios for fracture and death were 2.16 (1.88 – 2.47) and 1.46 (1.34 – 1.60) in women and 2.44 (2.09 – 2.84) and 1.50 (1.40 – 1.58) in men.

PF limitation is common and is associated with increased fracture and mortality risk. This study suggests that simple self-assessments may be a useful tool to select candidates for further investigation.



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Associations between ultra-distal forearm bone mineral density and incident fracture in women

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Publish consent withheld

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What a knock-out! Recombinant adeno-associated viruses for generation of post-natal bone knockouts

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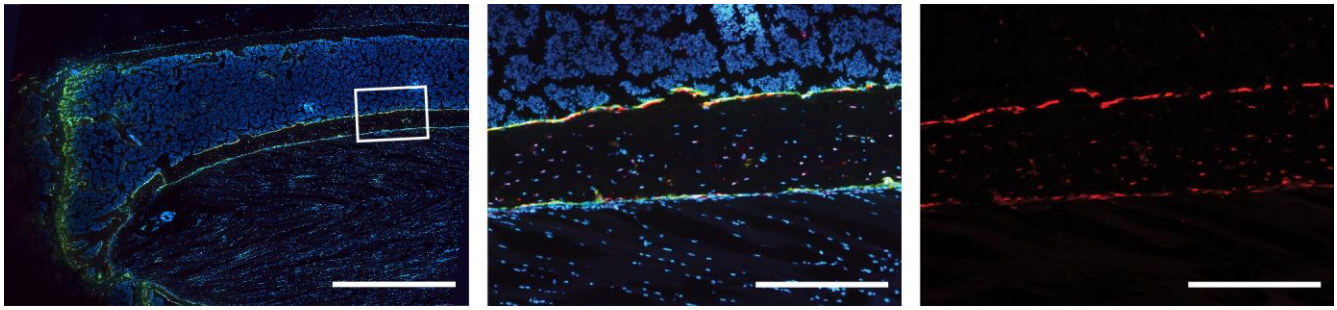
Background: Generating conditional gene knockout mice using traditional technologies can be challenging and costly. Such models are of high utility in bone research as many global gene knockouts can be embryonic lethal or yield confounding phenotypes. Our group engineered a bone-targeted recombinant adeno-associated viral vector (AAV8-Sp7-Cre) and speculated it enable bone-selective gene deletion without a need for crossbreeding with Cre-strains. This study aimed to show proof-of-principle in a mouse possessing a conditional allele for the Sclerostin (*Sost*) gene, which is a critical factor in the negative regulation of bone mass.

Methods: 8-week-old *Sost*$^{flox/flox}$ were systemically injected with AAV8-Sp7-Cre (5×10^{11} vg/mouse) or saline controls. After 6 weeks, detailed bone analysis was performed via microCT, biomechanical testing, and bone tissue histology. *Ai9* fluorescent Cre-reporter mice were dosed in parallel to verify the specificity and longevity of gene editing.

Results: MicroCT analysis of *Sost*$^{flox/flox}$:AAV-Cre mice confirmed a functional effect on bone mass, with an increase of 22% in the bone volume of the vertebrae ($p<0.01$), translating to a 17% increase in compressive strength ($p<0.01$). Significant alterations were also seen in the bone microarchitecture and were associated with a +25% increase in the mineral apposition rate. Immunohistochemistry for sclerostin protein and analysis of AAV8-Sp7-Cre mediated recombination in an Ai9 fluorescent reporter mouse model showed specificity and efficiency in osteoblasts and later osteocytes.



Discussion: *Sost*$^{flox/flox}$:AAV-Cre mice showed a high bone mass phenotype and increased bone anabolism consistent with prior reports of *Sost* null mice. This technology represents a streamlined and versatile approach to generate conditional bone knockout mice that could be applied to a range of floxed mouse strains. Our future research aims to develop AAV8-Sp7-CRISPR gene editing vectors able to disrupt or repair wild-type alleles.

Strengthening Bone with Evidence-Based Exercise Therapy in the Real World: 3D Hip Data from The Bone Clinic

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Purpose

High intensity resistance and impact training (HiRIT) improves bone, muscle and function in older women and men with low bone mass in clinical trials. Translation of HiRIT into effective therapy for osteoporosis was daunting due to the need for supervision, comorbid conditions, and a lack of Medicare reimbursement, prompting the establishment of a research clinic designed for safe delivery and data monitoring. Positive BMD and functional outcomes have previously been reported. Curiously, the outcomes least responsive to heavy weight-bearing loading are FN and total hip BMD suggesting the bone response to loading at the hip is structural not densitometric. The current report describes 3D hip analysis outcomes following 12 months of HiRIT at The Bone Clinic.

Methods

Clinic clients undergo comprehensive testing for biometrics, function, falls, fracture, and bone outcomes. Some undergo 3D hip analysis for indices of proximal femur strength. Twice-weekly supervised HiRIT is undertaken on a voluntary client basis. Adherence and injuries are recorded. Clients with >30% HiRIT adherence were included in the current analyses. In the absence of a control group in this clinical sample, outcomes were examined using repeated measures GLM comparing baseline and follow-up visit, adjusting for adherence.

Results

We report outcomes from 106 Clinic clients (62.0±7.4yrs, 161.4±12.2cm, 62.9±16.2kg, average adherence 63.1±24.8%). Improvements were observed in trochanteric trabecular volume ($P<0.025$) along with trochanteric cross-sectional moment of inertia (CSMI) ($P<0.026$) and section modulus (Z) ($P<0.023$), with a trend for increased total trabecular volume and total volume, despite a loss in femoral neck cross sectional area ($P<0.026$), and Z ($P<0.017$).

Conclusions

12 months of ‘real-world’ supervised HiRIT increased proximal femur trabecular volume which enhanced parameters of bone strength at the hip in individuals at risk of fracture. Long term monitoring of this unique translational dataset has confirmed the efficacy and appeal of exercise intervention for osteoporosis.

Revealing bone remodelling dynamics with longitudinal imaging

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Introduction

Combining image registration and *in vivo* micro-computed tomography (microCT) imaging has allowed quantitative assessment of bone remodelling at local sites in mouse models. Previous longitudinal studies have primarily been conducted using a two- to four-week scan interval [1]. However, the turnover rate of murine trabecular bone was reported to be as high as 4.9% per week [2]. To provide additional insights into bone remodelling activities, more frequent observations between those time points are needed. We hypothesise that bone remodelling rates in healthy mice change significantly from one week to the next. In this study, we aim to uncover these remodelling dynamics by combining longitudinal microCT imaging with image registration.

Method

Four healthy C57Bl/10 mice (n = 8 knees) were scanned weekly for 9 weeks using microCT (vivaCT80, Scanco Medical) at 10 µm voxel size. Three-dimensional image registration was performed to overlay follow-up scans with their baseline to define common volume of interest (VOI) for localised bone analysis. Dynamic bone morphometry, such as mineral apposition rate (MAR) and mineral resorption rate (MRR), was calculated based on the formed and resorbed bone extracted from the overlaid follow-up images (**Fig.1a**). Measurements of standard bone morphometry were also performed to monitor microstructural changes over time.

Results

Preliminary results from a fully processed knee are shown in **Fig.1**. Variance in bone remodelling rates, from 2.79 µm/day to 9.55 µm/day, are observed for MAR (**Fig.1b**), and from 2.57 µm/day to 9.38 µm/day for MRR (**Fig.1c**). Similar magnitudes of MAR and MRR for each time interval, representing similar thickness of formed and resorbed bone, match the constant trabecular thickness over time (**Fig.1d**).

Discussion and Conclusion

Preliminary results point toward changes in mice bone remodelling dynamics from one week to the other. Increasing temporal resolution can provide additional insights into these processes.

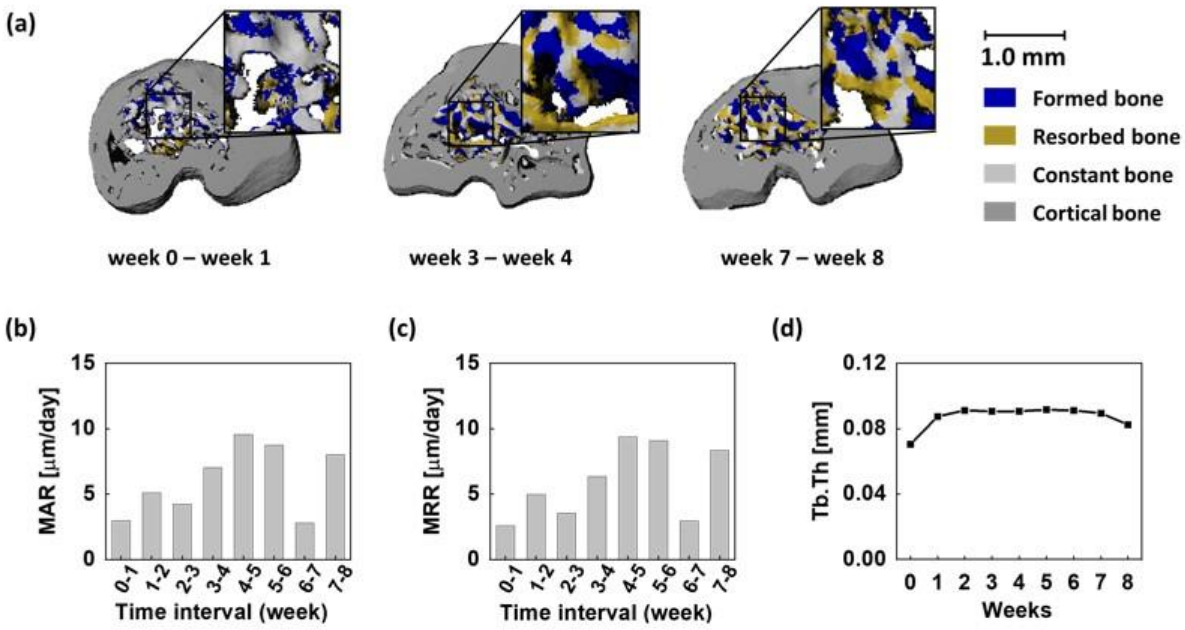

Figure 1. (a) Three-dimensional visualization of formation and resorption sites in the right knee tibial trabecular compartment of a single animal over time. A measurement acquired one week later was superimposed onto an earlier measurement of the same animal to visualize bone remodelling sites, followed by identification and extraction of formed and resorbed bone from the superimposed image. Bone formation is represented by blue, and bone resorption by yellow. Dynamic bone morphometry, including (b) mineral apposition rate (MAR), and (c) mineral resorption rate (MRR), within each time interval. (d) Changes in epiphyseal trabecular thickness (Tb.Th) over time.

1. Botter, S. M., et al., Arthritis and Rheumatism, 2011, 63(9), 2690–2699.
2. Jilka, R. L., et al., The Journals of Gerontology: Series A, 2013, 68(10), 1209–1217.

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Inflamed osteocytes directly induce inflammatory osteolysis through MYD88 signalling in bacterial bone infection.

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Osteomyelitis and periodontitis are bacterial diseases that impact the skeleton, leading to severe inflammatory osteolysis. While osteocytes are known to produce a wide range of inflammatory mediators in response to microbial pathogens and are the primary source of RANKL responsible for osteoclastogenesis, it remains unclear if osteocytes can function as inflammatory cells in conditions such as osteomyelitis and periodontitis. Additionally, the molecular mechanisms osteocytes utilize are not well understood. As osteocytes express toll-like receptor 2 (TLR2) and its signal transducer MYD88, we created *Dmp1-Cre*;*Myd88*$^{lsl/lsl}$(loxP-stop-loxP) mice, in which MYD88 function is restored predominantly in osteocytes. Injection of a TLR1/2 agonist Pam3CSK4 onto calvaria or induction of periodontitis by oral infection with *P.gingivalis*(*Pg*), one of the most common human bacteria causing periodontitis responsible for alveolar bone loss in a TLR2-dependent manner, resulted in significant osteolysis comparable to *Myd88*$^{+/+}$ mice. In both models, *Dmp1-Cre*;*Myd88*$^{lsl/lsl}$ mice exhibited increased *Rankl* expression and osteoclast induction in bone tissues. H&E staining showed considerable infiltration of inflammatory cells on the calvaria of Pam3CSK4-injected *Dmp1-Cre*;*Myd88*$^{lsl/lsl}$ mice. Expression levels of inflammatory cytokines, such as *Tnf* and *Il1b*, were increased in skin lesions overlying the calvaria of Pam3CSK4-injected *Dmp1-Cre*;*Myd88*$^{lsl/lsl}$ mice and in gingival tissues of *Pg*-infected *Dmp1-Cre*;*Myd88*$^{lsl/lsl}$ mice compared to *Myd88*$^{lsl/lsl}$ mice treated with Pam3CSK4 or *Pg*. Immunohistochemical staining and qPCR analysis of skin lesions revealed that calvarial lesions of Pam3-injected *Dmp1-Cre*;*Myd88*$^{lsl/lsl}$ mice contain large numbers of cells positive for F4/80 or Ly6G, a marker for macrophages and neutrophils, respectively. Inflamed osteocytes increased chemotaxis of neutrophils and macrophages in response to Pam3CSK4. Collectively, these results suggest that inflamed osteocytes directly contribute to inflammatory osteolysis through MYD88 signaling in bacterial bone infection. The osteocyte MYD88 pathway and osteocyte-derived inflammatory factors that regulate immune cell activation may be potential therapeutic targets for osteolysis caused by bacterial infection, as seen in osteomyelitis and periodontitis.

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Chemical digestion-assisted extracellular matrix profiling of differentiating osteoblasts

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Introduction: Quantification of extracellular matrix (ECM) proteins is challenging due to their insoluble nature, however, chemical digestion using Hydroxylamine (HA) has recently been introduced to overcome this problem. We evaluated the efficacy of HA for ECM solubilization in comparison to traditional chaotropic buffer and successfully established a chemical digestion-assisted protocol that provides a more in-depth ECM coverage. During osteoblastic differentiation, the proportion of the ECM is rich in proteoglycans and glycoproteins, and they were gradually replaced by fibrillar collagens, which then mineralized to form bone. Although the transcriptional regulation of osteoblasts is relatively well understood, the changes in the ECM during osteoblastic differentiation remain elusive.

Objective: The objective of this study was to present the compositional changes in the ECM proteome of differentiating osteoblasts using chemical digestion-assisted proteomics.

Methods: Mouse MC3T3-E1 cells were cultured in a differentiation medium to obtain osteoblasts-derived ECM. The cells were harvested by RIPA buffer after 1, 2, 3, and 4 weeks, and the ECM fraction of the cells was obtained by centrifugation. We employed the established chemical digestion-assisted protocol, which features serial protein extraction with Guanidine hydrochloride (Gnd-HCl) and Gnd-HCl+HA. Tryptic peptides were prepared from the entire protein extracts and analyzed by mass spectrometry. The obtained proteome list was curated with the Matrisome database.

Results: In the differentiating osteoblasts, the proportion of collagen in the matrisome increased each week from 71.4% to 87.6%, with the increasing type I collagen proportion from ~60% to 80% among collagens. The proportions of proteoglycans and glycoproteins were highest at 1 week of differentiation with 4.9% and 8.7%, respectively, and decreased thereafter.

Conclusion: Using chemical digestion-assisted proteomics, we present the changes in the ECM profile of differentiating osteoblasts, which partly illustrate the ECM maturation process involved in bone formation.

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Development of *in vitro* 3D osteocyte culture models in ECM-derived hydrogels

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Currently, most bone knowledge derived from *in vitro* models use cells cultured in 2D, due to inherent technical difficulties in establishing osteocyte cultures in 3D. Unlike other bone cells, osteocytes cannot faithfully be studied *in vitro* in the absence of their surrounding extracellular components, including collagen proteins and minerals. We propose to use extracellular matrix (ECM)-derived 3D hydrogels composed of tuneable gelatin methacrylamide (GelMA)[1], for the establishment and study of 3D osteocyte networks *in vitr*o. Gelatin is relevant as it chemically resembles native collagenous bone matrix, contains integrin attachment sites that facilitate interaction of the cytoskeleton and ECM, and is conducive to mineralization, as shown by us and others[2,3]. We hypothesised that combining GelMA and biomimetic culture would provide new avenues to control the formation and mineralisation of osteocyte 3D cultures *in vitro* and identify how ECM and culture cues support network formation and functions. We used either human primary osteoprogenitor cells (derived from knee/hip arthroplasty from Prof. Ross Crawford, QUT) or murine *DMP1*-GFP OCY454 cell lines (gift from Prof Natalie Sims, St Vincent's Institute) and encapsulated cells in 5% w/v GelMA (3D) and also cultured them in 2D. We assessed the use of growth medium (GM) or osteogenic medium (OM) up to 28 days with/without the use of a mineralisation medium (MM) boost for 3 days following cell encapsulation, known to enhance nanoscale biomineralisation[4], and compared normoxic/hypoxic (20%/2% O_2) culture conditions in 2D and 3D settings. Mineralisation was assessed by micro-computed tomography; osteocyte phenotype and branching by brightfield and confocal laser microscopy; and protein and gene expression by downstream analysis. Ongoing experiments preliminarily showed that supportive environmental cues such as MM and OM positively modulated mineralisation and osteocyte phenotype. Further optimisation of *in vitro* 3D osteocyte culture models will enable understanding the effects of ECM-cell interactions and addressing fundamental osteocyte biology questions.

1. Loessner, D. et al. Functionalization, preparation and use of cell-laden gelatin methacryloyl-based hydrogels as modular tissue culture platforms. Nat. Protoc. 11, 727–746 (2016).
2. Mestres, G. et al. Advantages of microfluidic systems for studying cell-biomaterial interactions-focus on bone regeneration applications Biomedical Materials. Biomed. Mater 17, 65027 (2022)
3. Bessot, A. et al. GelMA and Biomimetic Culture Allow the Engineering of Mineralized, Adipose, and Tumor Tissue Human Microenvironments for the Study of Advanced Prostate Cancer In Vitro and In Vivo. Adv. Healthc. Mater. 2201701 (2023) doi:10.1002/adhm.202201701
4. Thrivikraman, G. et al. Rapid fabrication of vascularized and innervated cell-laden bone models with biomimetic intrafibrillar collagen mineralization. Nat. Commun. 10, 3520 (2019)

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Associations between bone material strength index and FRAX scores

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Publish consent withheld

Fracture following kidney and simultaneous pancreas-kidney transplantation is predicted by DXA-derived bone mineral density and advanced hip analysis

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Patients with kidney failure have accelerated trabecular and cortical deterioration and elevated fracture risk that remains high following transplantation. However, BMD is less predictive of fracture in these patients. This study aimed to determine if the DXA-derived trabecular bone score (TBS) and advanced hip analysis (AHA) improved post-transplant fracture prediction.

Patients receiving kidney or simultaneous pancreas-kidney (SPK) transplants within two Australian centres were included. Baseline information included demographics, medications and laboratory data. A DXA provided BMD, TBS and AHA parameters; femoral neck, calcar and shaft cortical thickness (CTh), and the femoral neck buckling ratio (BR), an indicator of structural instability defined as radius/CTh. Patients received treatment to reduce post-transplant BMD loss using a risk algorithm.[(1)] Parameters with skewed distributions were log transformed, and hazard ratios were determined using Kaplan Meier and Cox proportional hazard models with multivariable adjustment.

Of 357 kidney and SPK transplant recipients, 289 (83%) received a kidney-only transplant. Mean age was 48±13 years, 62% were male, 20% had type 1 diabetes mellitus (T1DM) and median dialysis vintage was 29 months (IQR: 12, 60). There were 81 incident fractures, with median time to fracture or censoring 4.4 years (2.5, 5.5). Incident fracture was predicted by T1DM ($p<0.001$), former smoking ($p=0.017$), lower serum 25OHD ($p=0.045$), axial BMD ($p<0.01$), CTh (all sites $p\leq0.01$) and the BR ($p=0.004$; HR 1.83 (1.34, 2.49) for each log-unit increase), but not by the TBS. After multivariate adjustment, T1DM, 25OHD, smoking, prevalent fracture and hip BMD remained significant predictors. Using the BMD-based risk algorithm, inclusion of an interaction parameter for BR above or below the median improved the model fit (HR 2.36 (0.96, 5.80); $p=0.06$).

DXA-derived BMD, AHA cortical parameters and the BR predict incident fracture in kidney and SPK transplant recipients, but TBS does not. AHA by DXA may improve fracture risk assessment.

1. Elder G. Transplantation Feb. 2023

Global prevalence of osteoporosis in rheumatoid arthritis: Systematic review and meta-analysis

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Abstract

Background: Rheumatoid Arthritis (RA) patients have a higher risk of developing osteoporosis, which increases morbidity, mortality rates, and healthcare costs [1]. There is limited data on the prevalence of osteoporosis and associated risk factors in RA.

Objectives: Estimate the global prevalence of osteoporosis in RA patients, identify associated risk factors, and determine high-risk RA patients who require preventive osteoporosis treatment.

Methods: We conducted a search in several databases (MEDLINE, Scopus, ProQuest Central, Web of Science, EMBASE, CINAHL, and Google Scholar) to estimate the global prevalence of osteoporosis in RA populations. We also evaluated the influence of geographical location, prevalence methods, and diagnostic criteria on prevalence estimates from 1980 to 2023.

Results: We included 29 studies involving 31,473 RA patients with osteoporosis out of 130,989 RA populations. The global prevalence of osteoporosis in RA was estimated to be 21.5% (95% confidence interval 16.5-27.0), with a prediction interval of 1.4% - 56.1% (Figure 1). The point-prevalence of osteoporosis was 19.2% (95% CI 13.3-25.8), while the period-prevalence was 27.1% (95% CI 18.9-36.3). The highest pooled prevalence of osteoporosis was observed in Asia (30.0%; 95% CI 22.1-38.6), while Europe (13.4%; 95% CI 8.9-18.7) and North America (13.3%; 95% CI 11.0-15.9) had lower estimates. Factors influencing osteoporosis prevalence included continents, prevalence methods, and diagnostic osteoporosis criteria. The World Health Organization (WHO) osteoporosis criteria exhibited greater consistency in prevalence estimates in RA populations, regardless of demographic characteristics.

Conclusion: The estimated global point- and period-prevalence of osteoporosis in RA were 19.2% and 27.1%, respectively. Higher prevalence rates observed in Asia may be influenced by restricted healthcare access or variations in risk environments, highlighting the significance of the WHO criteria in predicting and guiding preventive treatment.

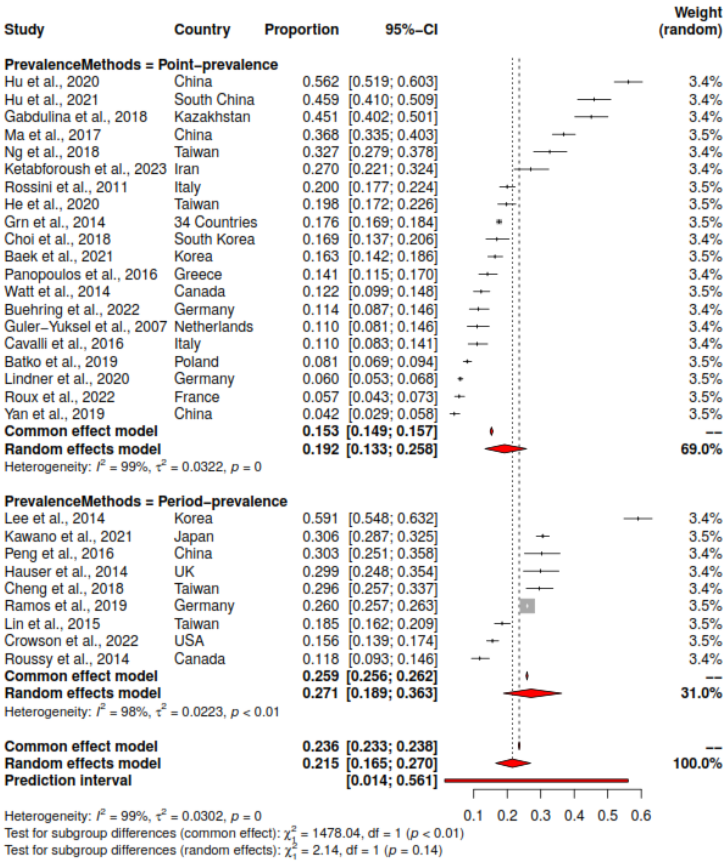

Figure 1: Global point- and period- prevalence of osteoporosis in Rheumatoid Arthritis populations and prediction interval between 1980- 2023.

1. Adami G, Saag KG. Osteoporosis Pathophysiology, Epidemiology, and Screening in Rheumatoid Arthritis. Curr. Rheumatol. Rep. 2019;21(7):34.

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Osteoporosis Development in Patients with Acute Kidney Injury

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Acute kidney injury (AKI) is an acute reduction in kidney function characterised by an increase of serum creatinine and reduction in urine production, and is a known risk factor for chronic kidney disease. Reduced kidney function can have adverse effects on bone. There is a well-established relationship between chronic kidney disease and poor bone health; however, the relationship between AKI and bone health has not been well defined. A retrospective cohort study was conducted to determine the association, if any, of AKI with development of osteoporosis using de-identified data from a regional health district in News South Wales, Australia. Data included hospital admissions for adults, 18 years and older, who presented to a hospital or pathology service between 2008 and 2017. Presence of CKD was confirmed using serum estimated glomerular filtration rate in accordance with the Kidney Disease: Improving Global Outcomes classification. Presence of osteoporosis was confirmed in accordance with the 10th edition of the Australian modification of the international classification of diseases. The study included 14,590 patients with AKI. Of those patients, 13.8% developed osteoporosis (n = 2,013). Preliminary chi-square analysis indicated a significant association between AKI and osteoporosis ($p < 0.001$). Osteoporosis is a debilitating disease that often results in fracture and a subsequent decrease in quality-of-life. Importantly, AKI is not currently considered a risk factor for osteoporosis. It is imperative that the actual risk AKI presents for the development of osteoporosis is determined, as this may impact clinical management of patients post-AKI.

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Fracture prevalence in adults with cystic fibrosis with end-stage lung disease post transplantation

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Background

Cystic fibrosis (CF) is associated with reduced bone mineral density and increased risk of fragility fractures. Adults with end-stage CF lung disease requiring lung transplantation (LT) would be expected to be at highest risk of osteoporotic fractures. Our institution implemented a protocol for zoledronic acid (ZA) infusion for all adults waitlisted for LT to prevent anticipated bone loss. The objective of this study was to compare fracture prevalence in adults with CF to those with non-CF aetiologies post-LT.

Methods

This was a single centre retrospective study of all adults who received LT at Alfred Health, Australia between January 2012 to December 2018 to evaluate prevalence of osteoporotic fracture. Data was retrieved from electronic medical records. LT recipients were divided into two groups based on CF status and compared using statistical methods with p-value <0.05 deemed significant.

Results

In total, 405 LT recipients were included in our audit, of whom 58 (14.3%) had CF. Of these adults with CF, 51.7% (30/58) were female and this was comparable to the non-CF group (p-value 0.09). In the non-CF group, 18% (n=63/347) osteoporotic fractures whilst no osteoporotic fractures occurred in our CF group post LT. Compared to non-CF aetiologies, adults with CF were significantly younger in age 34.2 ± 10.2 vs 58.8 ± 9.93 (p-value <0.0001). We also found no significant differences in pre- or post LT ZA infusion rates between the two groups.

Discussion

We found adults with CF experienced no osteoporotic fractures post-LT. We postulate our results may be attributed to young age, protocol-driven ZA with cumulative benefits from improved nutrition, aggressive vitamin D replacement and reduced inflammation due to enhanced anti-microbial management in adults with CF.

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Isolation and comparison of osteoblast lineage cells from periosteal, endocortical and intracortical bone surfaces

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Bone houses multiple niche microenvironments with different cellular compositions, including the bone marrow, and the endocortical, intracortical, and periosteal surfaces. This may underpin differences in osteoblast progenitor populations and their responses to therapy. Our aim was to isolate osteoblast-lineage cells from each microenvironment and identify phenotypic and transcriptional differences.

By dissection and serial digestion of long bones, we achieved simultaneous isolation of cells from bone marrow, endocortical, intracortical and periosteal surfaces from 6-12 week old mice, with osteoblast-related gene promoters driving fluorescent reporter expression (*OsxCherry.Col1a1GFP*). Cells were cultured for 7 days in regular αMEM media.

At days 6-7 in culture, bone marrow, endocortical, and intracortical cells grew sparsely, while periosteal cells grew as colonies. Very few, if any, bone marrow-derived cells expressed OsxCherry+. In contrast, endocortical, intracortical and periosteal derived cells contained >40% OsxCherry+ cells. Very few Col1a1GFP+ cells were observed in any culture in this short time frame. This suggests that isolation of cells close to bone surfaces yields a greater proportion of cells that can commit to the osteoblast lineage.

By qPCR, endocortical, intracortical and periosteal cells exhibited greater mRNA levels for osteoblast commitment (*Runx2*, *Sp7*) and differentiation genes (*Alpl*, *Col1a1*, *Bgalp*) than bone marrow stromal cells (BM). Endocortical and intracortical cells had lower adipogenesis-associated mRNAs (*PPARγ*, *AdipoQ*) compared to BM. Periosteal cells showed no difference in *PPARγ*, but greater *AdipoQ* mRNA transcripts than BM cells. This suggests stromal populations from endocortical and intracortical surfaces have stronger osteogenic potential but less capacity to form adipocytes compared to stromal cells from periosteum and BM.

We conclude that it is feasible to culture stromal cells from different long bone surfaces, and this reveals that stromal cells isolated from close to bone surfaces more readily commit to osteoblast differentiation than marrow cells, and differ in their potential to become adipocytes.

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Roquin1 maintains bone mass by repressing mitochondrial biogenesis in osteoclasts

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Publish consent withheld

High-intensity interval training mildly improves trabecular bone microarchitecture in adult male C57BL/6 mice

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Objective

Ageing is associated with a reduction in bone quality, and exercise, particularly high-intensity exercise, may improve bone quality. We tested the hypothesis that high-intensity interval training (HIIT) would improve bone microarchitecture in adult mice.

Methods

Adult mice (52-week-old male, C57BL/6) were randomly allocated to either sedentary (*n*=6) or high-intensity interval training (HIIT, *n*=8) groups. HIIT group underwent 4x4 minutes of treadmill running (0° incline, 85-90% individual maximum speed) 3 x per week for 6 weeks. Mice in the sedentary group were placed on a stationary treadmill for the same duration. Younger mice (12-week-old male, C57BL/6, n=7) served as a control group. Post-intervention, mice were euthanised; tibiae were then dissected, fixed in 4% paraformaldehyde, and analysed by micro-CT. Trabecular bone was assessed in the metaphysis, and cortical bone analysed slice-by-slice along the central 80% of the bone length.

Results

Adult mice exhibited significantly less trabecular bone mass than younger mice. There were also site-specific differences in the cortical bone structure with age, including greater cross-sectional area, lower normalised bone area, and lower cortical thickness. HIIT did not affect any cortical bone parameters; it also did not alter trabecular bone volume, thickness, or number, compared to sedentary mice. Trabecular separation was significantly higher (by 44%) in adult sedentary mice compared to young mice. In contrast, trabecular separation in the HIIT group was only 16% higher than in young mice, which was not statistically significant. The degree of anisotropy was also significantly lower in the HIIT group compared to both young and adult sedentary mice, indicating greater diversity in trabecular network directionality with HIIT.

Conclusions

Ageing is associated with significant changes to bone microarchitecture and HIIT may be used as a tool to attenuate the effect of ageing on trabecular bone, but not cortical bone, in adult male mice.

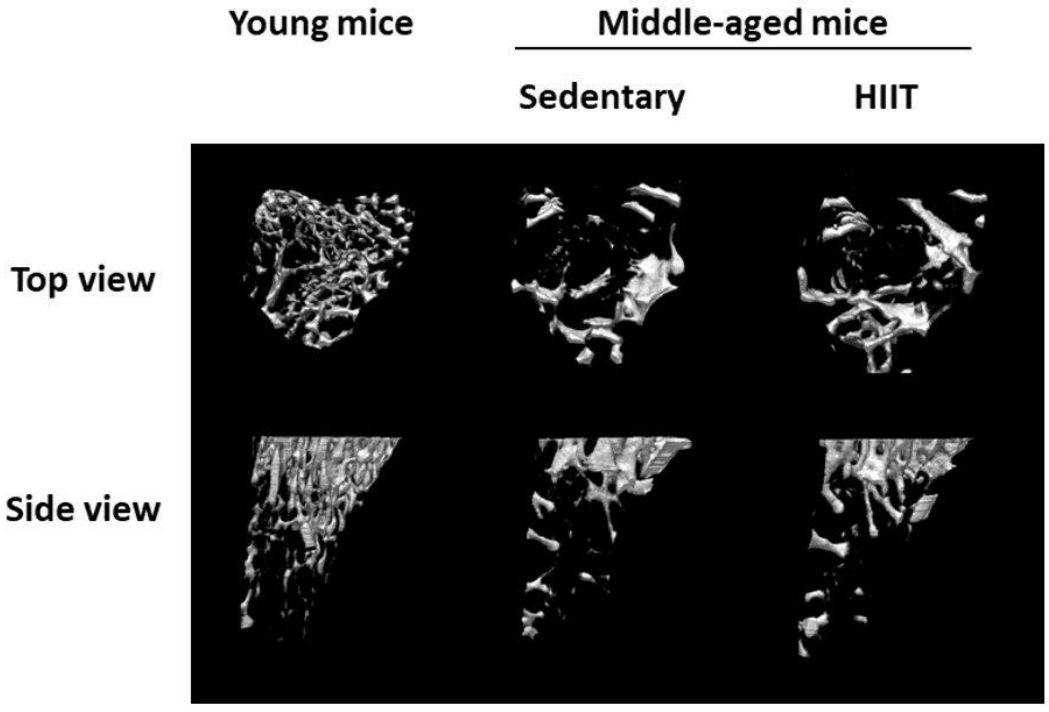

Fig 1. Representative images of trabecular bone from young (12-week-old) and adult (58-week-old) mice, including from both the sedentary and HIIT groups.

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Age-related normative values of bone microarchitecture parameters in older Japanese: the Bunkyo Health Study.

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Background Bone microarchitecture parameters have been focused on as an index of bone quality, which describes the bone structure and strength. However, normative values of bone microarchitecture parameters for older adults have been limited, and further accumulation of data is needed for clinical application. Therefore, we aimed to indicate normative values of the bone microarchitecture parameters of the lumbar spine (LS) and total hip (TH) in older Japanese men and women. **Method** This study was conducted using data from the "Bunkyo Health Study," a cohort study for 65-84 years older adults. We included in the analysis 1053 older adults (500 men and 553 women) for the LS and 1372 older adults (662 men and 710 women) for the TH. Trabecular bone score (TBS) at LS was evaluated using the textural analysis of the pixel gray-level in the LS DXA image. Trabecular volumetric BMD (vBMD), cortical vBMD, integral vBMD, cortical thickness, and cortical surface BMD at TH were assessed using DXA-based-3-dimensional modeling. The mean values and standard deviations were calculated for each parameter of LS and TH in every five-year-old group (age 65-69,70-74,75-79,80-84), and the median values were compared among the four groups using the Kruskal-Wallis test. The post-hoc test was performed the Bonferroni correction. **Results** TBS was significantly lower in the 65-69 and 70-74 age groups than in the 80-84 age group only in women, but not in men. In men, only total mean cortical thickness was significantly lower in 80-84 than in 65-69 and 70-74. In women, cortical vBMD cortical surface BMD, total mean cortical thickness were significantly lower in 80-84 than in 65-69 and 70-74. Trabecular vBMD and integral vBMD were significantly lower in 80-84 than other three groups. **Conclusion** Bone microarchitectures show different age-related changes from BMD by sex and site in elderly Japanese.

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Development and evaluation of an osteoporosis medication adherence intervention

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Introduction. Osteoporosis is undertreated despite effective pharmacotherapy due to poor patient persistence, adherence and limited medication management services.

Aims. To evaluate a medication management intervention for osteoporosis in community pharmacy.

Methods. Australian community pharmacists were trained to deliver an osteoporosis medication management intervention. The intervention was delivered to patients with diagnosed osteoporosis, and after 4 weeks and 12 months, the patients were followed up by the research team. Measures reported included pharmacists' competency in service delivery, pharmacists' and patients' perceptions of service, dispensing records of osteoporosis medications, changes in patients' self-reported adherence and beliefs about their osteoporosis medications.

Results. Five community pharmacies completed a total of 24 interventions over a 6-week trial period. 17 patients were available for follow up at 4 weeks, 14 were available at 12 months. Patients highly rated the intervention's service quality. Pharmacists reported that providing the intervention is worthwhile for patients and provides professional satisfaction. The main barriers to service delivery were time and workload, particularly relating to the COVID-19 pandemic. There were no significant changes in patients' self-reported adherence and beliefs about their osteoporosis medicines.

Discussion. An osteoporosis medication management intervention in community pharmacies can be feasible and acceptable for both patients and pharmacists.

Fracture falls frailty and sarcopenia predict hospitalisation risk in geriatric populations referred for ACAT assessment

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Introduction

This study aims to assess comorbid factors associated with hospitalisation in a cohort referred to the Aged Care Assessment Team (ACAT). Geriatric assessments improve outcomes in older patients across different conditions and settings. Frailty and sarcopenia in older patients lead to negative consequences such as falls, fractures social isolation, functional decline and hospitalisation.

Method

Quality Improvement project to assess utility and comparison of frailty assessments and risk factors for hospitalisation. Questionnaire included validated clinical assessment of risk factors for geriatric syndromes undertaken by ACAT Teams. Formal assessments included falls, mood (Geriatric Depression Scale - GDS5), Social isolation (Lubben Social Network Scale - LSNS 5), Quality of life, Cognitive assessment (Mini Cog), Sarcopenia (SARCF), Frailty (Clinical Frailty Scale - CFS, Reported Edmonton and (Frail Scale - REFS). Statistics (SPSS): Univariate comparison of proportions using Chi squared tests and logistic regression reported as odds ratio (OR).

Results

Total 990 ACAT assessments (76% community and 24% inpatient assessments). Female 65% and male 35%; mean age 84.5 (SD 3.7 years); age range 60 106 years; 96% aged 70 years and above. In the preceding 12 months 59% reported 1 or more falls, 67% were hospitalised (35% once, 15% twice and 17% three or more times); 35% reported a fracture since age 50 years (22.3% in the preceding 3 years).

Table 1. Association of baseline assessment for hospital admissions

Variable	N (%)	Chi square	Correlation coefficient	Odds Ratio (R 95% CI)	*p* value
Gender:					
Males	249 (37.5)	3.785	0.064	1.337 (1.008-1.773)	0.052
Females	415 (62.5)				
Depression (GDS-5)	883 (89.2)	29.574	0.185	2.327 (1.717-3.153)	<.0005*
Socially isolated (LSNS-6)	956 (96.6)	4.033	-0.067	0.750 (0.572-0.984)	0.045*
Pain	969 (97.9)	3.565	0.063	1.308 (0.999-1.713)	0.059
Falls	990 (100)	52.217	0.232	2.721 (2.072-3.574)	<.0005*
Fractures (Previous 3 years)	990 (100)	15.541	0.128	2.041 (1.435-2.902)	<.0005*
Cognitive impairment (Mini-Cog)	763 (77.1)	0.607	-0.031	0.876 (0.647-1.186)	0.436
Sarcopenia risk (SARC-F)	971 (98.1)	54.958	0.240	2.931 (2.200-3.903)	<.0005*
Frail (CFS)	851 (86.0)	11.805	0.121	1.971 (1.347-2.884	<.0005*
Frail (REFS)	693 (70.0)	102.622	0.389	6.928 (4.655-10.311)	<.0005*

Table 1 reports the main significant association ($p < 0.05$) with hospitalisation from highest to lowest ranking: REFS ($r = 0.389$), SARC F ($r = 0.240$), falls ($r = 0.232$), GDS ($r = 0.185$), fractures (0.128), CFS ($r = 0.121$) and LSNS 6 ($r = 0.067$)

Conclusion

Incorporating falls, fracture, frailty and sarcopenia assessment tools into an ACAT assessment adds value in predicting patients at high risk of admission.

1. 1. Cunha Ail et al. Frailty as a predictor of adverse outcomes in hospitalized older adults: a systematic review and meta analysis. Ageing Res Rev. 2019. 2. Ozturk ZA et al. Health related quality of life and fall risk associated with age related body composition changes; sarcopenia, obesity and sarcopenic obesity. Intern Med. J. 2018;48(8):973 81.

Quality of life in population-based women with comorbid arthritis and mood disorders

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Background: Separately, mental and musculoskeletal disorders are leading contributors to disability worldwide. We examined the quality of life (QoL) of women with arthritis, mood disorders, and the comorbidity of these.

Methods: This cross-sectional study analysed data from the Geelong Osteoporosis Study (n=840 women aged 28–95 years; 2011–2014). A history of arthritis was self-reported; lifetime mood disorders were assessed using the SCID-I/NP. QOL was measured using the WHOQOL-BREF (26-items; 5-point Likert scale); we calculated four domains: I) perceived physical health (pain & discomfort/energy & fatigue/sleep & rest/medication/mobility/activities of daily living/work capacity); II) psychological health (positive/negative feelings/self-esteem/cognition/body image/spirituality/religion); III) social health (relationships/sex/practical support); and IV) environmental health (financial resources/information/skills/recreation/leisure/home environment/access to health/social care/physical environment/transport). QoL domains were dichotomised using normative mean scores. Age-adjusted logistic regression models investigated associations between arthritis, mood disorders, and their comorbidity in relation to QOL domains.

Results:

A total of 18.7% (157/840), 26.3% (221/840), and 11.01% (93/840) of women had lifetime arthritis, mood disorders, or their comorbidity (both arthritis and mood disorders), respectively.

Compared to women with no history, the odds of lower physical health QoL were increased in women with arthritis (OR=2.06; 95%CI 1.34-3.16) and mood disorders (OR=3.08; 95%CI 2.12-4.47), but the highest odds belonged to the comorbidity group (OR=6.18 95%CI 3.57-10.69). Women with mood disorders had increased odds of both lower psychological (OR=3.10; 95%CI 2.16-4.44), and social (OR=2.33; 95%CI 1.64-3.31) health QoL with the comorbidity groups having similar odds (OR=2.91; 95%CI 1.73-4.88; OR=2.80; 95%CI 1.71-4.60). Arthritis was not significantly associated with psychological or social health QoL, whereas none of the three groups were associated with environmental health QoL.

Conclusion: Women with comorbid arthritis and mood disorders have disproportionately lower physical health QoL than women with no history.

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Association between lower gait speed or handgrip strength and their combination with depressive symptoms in community-dwelling older adults

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Objective: This study investigates the association between gait speed and handgrip strength, and their combination, with developing depressive symptoms in community-dwelling older adults using longitudinal data from the ASPirin in Reducing Events in the Elderly (ASPREE) study.

Methods: Participants were community-dwelling older adults in Australia and the USA, aged 70+ years (65+ years for USA minorities) and followed for a median (interquartile) of 3.9 (2.3) years. Baseline handgrip strength (measured in kilogram-force using a hydraulic hand dynamometer) and gait speed (time to walk 3 meters) based on quantiles taking the worst quantile, Q1 for grip and Q5 for gait were exposure variables. Depressive symptoms were measured annually using the modified Center for Epidemiological Studies Depression (CES-D 10) scale with a cutoff 10+. A total of 18,156 participants (55.8% females) included in the analysis after excluding participants with CESD-10≥10 at baseline. Cox regression was used to estimate Adjusted Hazard Ratios (AHR) with 95% Confidence Intervals (CI) were reported after adjusting for socio-demographics, BMI, comorbidity, polypharmacy and lifestyle factors.

Result: Depressive symptoms were significantly associated with weak handgrip (AHR 1.15, CI (1.07-1.22), slow gait (1.22; (1.14-1.31) and both weak grip and slow gait (1.33; (1.19-1.48). Each kilogram-force increase in grip strength or one meter-per-second slowing in gait speed was associated with a 5% (0.95; (0.93-0.97)) decrease and 7.5% (1.075; (1.05-1.10)) increase in depressive symptoms, respectively.

Conclusion: The development of depressive symptoms was associated with poorer hand grip strength and gait speed in graded pattern, and the combination of these physical measures predicted worsening depressive symptoms. Being physically strong may serve as a protective factor for depression in older adults. This highlights the link between physical and mental health of older adults and informs potential clinical as well as public health prevention strategies for depression, particularly for older adults with declining physical capacity.

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Commencement of osteoporosis therapy in patients presenting with neck of femur fracture at University Hospital Geelong

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Background: Osteoporosis is a chronic disease characterised by bone fragility due to an increase in bone resorption and decrease in bone formation. This leads to an increased risk of fracture which can lead to significant morbidity and mortality. University Hospital Geelong is a large tertiary centre, servicing a catchment area of over 500,000. With an aging population, the impact of osteoporosis and related fractures are significant.

Aim: To determine the percentage of patients appropriately commenced on osteoporosis therapy as per the recommended guidelines after presenting with neck of femur fractures over a 12-month period at University Hospital Geelong.

Method: A retrospective audit was undertaken of all patients who presented to University Hospital Geelong over a 12-month period with a neck of femur fracture. The primary outcome was the number of patients appropriately commenced on osteoporosis therapy prior to or on discharge. The secondary outcome was the number of these patients readmitted over the next 12-month period with subsequent fractures.

Results: 112 patients were admitted with neck of femur fractures treated with operative management over the 12-month period. The median age was 81 years old, with 69% being female. Only 53% (n=60) were commenced on osteoporosis therapy prior to or on discharge, with 87% of these patients being commenced on denosumab. 38% (n=20) of patients not commenced on therapy had a contraindication to current therapy. 6% (n=7) of patients were readmitted with an insufficiency fracture within 12 months, however this did not show a statistically significantly relationship with commencement of osteoporosis therapy.

Conclusion: A low percentage of patients admitted to University Hospital Geelong with a neck of femur fracture are commenced on osteoporosis therapy. This was not found to cause a statistically significant increase in readmission with fracture over the next 12 months.

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Improving Bone, Function, Falls and Fractures with Evidence-Based Exercise Therapy for Osteoporosis in the Real World

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Purpose

Clinical trials have shown that high intensity resistance and impact training (HiRIT) improves bone, muscle and function in older women and men with low bone mass, but the risk of fracture to frail skeletons, comorbid conditions, and lack of Medicare funding creates doubt that it is a practical therapy for osteoporosis. A dedicated research clinic was therefore established to examine effectiveness, safety and feasibility in a real-world clinical practice. The current report describes bone and functional outcomes along with falls and minimal trauma fractures following 12 months of HiRIT at The Bone Clinic.

Methods

Clinic clients undergo comprehensive testing for biometrics, BMD, muscle, function, falls and fractures at baseline and annually thereafter. Twice-weekly supervised HiRIT is undertaken on a voluntary client basis. Adherence and injuries are recorded. Clients with >30% HiRIT adherence were included in the current analyses. In the absence of a control group in this clinical sample, outcomes were examined using repeated measures GLM comparing baseline and follow-up visit, adjusting for adherence.

Results

Outcomes from 432 Clinic clients (62.1 ± 7.0 yrs, 162.4 ± 11.2 cm, 62.1 ± 12.6 kg, at baseline) with 70.1 ± 22.0% adherence to HiRIT are reported. Improvements were observed in LS BMD ($P = 0.002$), FN BMD ($P = 0.013$), FN area ($P = 0.024$) and percent fat ($P < 0.001$) along with functional reach, back extensor strength, tandem walk, timed up and go, sit to stand, and dietary calcium (all $P < 0.001$). Fewer falls and fractures were sustained ($P<0.001$).

Conclusions

12 months of real-world supervised HiRIT improved bone and function and reduced falls and fractures in individuals with low to very low bone mass. Dietary advice improved calcium consumption. Ongoing monitoring of this unique translational dataset continues to confirm the effectiveness and feasibility of targeted supervised exercise therapy for osteoporosis.

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Investigating Eph-ephrin communication within the neuro-osteo network utilising the avian embryo

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Publish consent withheld

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Generating cell-based models of FGFR3-related diseases

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Background: Fibroblast growth factor receptor 3 (FGFR3) is a critical factor in mammalian growth and development. Several well-defined mutations in FGFR3 are associated with human disease. Achondroplasia (ACH) or dwarfism is an autosomal dominant disease associated with short stature. The most common causative mutations for ACH are c.1138G>A and c.1138G>C, which make up >90% of all cases. Thanatophoric dysplasia type 1 and 2 (TDI/TDII) are also caused by mutations in FGFR3 but are more severe and result in neonatal death. All reported cases of TDII are caused by a c.1948A>G mutation. These common mutations represent ideal gene therapy targets they are base editable using emerging CRISPR technology.

Aim: Generate cell models featuring ACH and TDII mutations using CRISPR/Cas9 editing.

Methods: CRISPR gene editing strategies were designed using CHOPCHOP v3 and Integrated DNA Technology systems. Oligonucleotides (Sigma-Aldrich) encoding for specific sgRNAs were subcloned into CRISPR plasmids (Addgene) and long oligos were made incorporating c.1138G>A and c.1948A>G mutations. Cell pools and stable lines were generated using HEK293T cells (CellBank Australia). Sequencing of constructs and gDNA was performed by AGRF and analysed using the ICE CRISPR Analysis Tool (Synthego).

Results: Expression plasmids with subcloned FGFR3-specific sgRNAs were confirmed by sequencing. Both dsDNA cutting and dual nickase vectors were produced. CRISPR constructs were introduced by lipofection and used to create cell pools. No-long-oligo controls demonstrated a lack of HDR. Approaches with high HDR rates were then used to generate clonal cell lines and also confirmed by sequencing. CRISPR-BE constructs for therapeutic repair were designed and subcloned in parallel.

Discussion: Generation of these cell lines is an important first step towards validating future gene therapy approaches. While plasmid delivery is not practicable as a gene therapy treatment, the CRISPR-BE methodology can be adapted using adeno-associated viral vector (AAV) delivery systems targeting musculoskeletal tissues available in-house.

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Investigating early-onset osteoporosis in individuals with Down syndrome

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Publish consent withheld

1. 1. de Graaf, G., F. et al., Genet Med, 2017. 19(4): p. 439-447.
2. 2. Down Syndrome Statistics in Australia. 2011 19 June 2023.
3. 3. Sherman, K.M., et al., Low bone mass and impaired fracture healing in mouse models of Trisomy21 (Down syndrome). Bone, 2022. 162: p. 116471.
4. 4. Lee, Y., et al., Negative Feedback Inhibition of NFATc1 by DYRK1A Regulates Bone Homeostasis. Journal of Biological Chemistry, 2009. 284(48): p. 33343-33351.
5. 5. LaCombe, J.M. and R.J. Roper, Skeletal dynamics of Down syndrome: A developing perspective. Bone, 2020. 133: p. 115215.
6. 6. Fowler, T.W., et al., Low Bone Turnover and Low BMD in Down Syndrome: Effect of Intermittent PTH Treatment. PLOS ONE, 2012. 7(8): p. e42967.
7. 7. González-Agüero, A., et al., Bone mass in male and female children and adolescents with Down syndrome. Osteoporosis International, 2011. 22(7): p. 2151-2157.
8. 8. Garcia-Ramírez, M., et al., Down's Syndrome: Altered Chondrogenesis in Fetal Rib. Pediatric Research, 1998. 44(1): p. 93-98.
9. 9. Kjaer, M.S., et al., Hand development in trisomy 21. Am J Med Genet, 1998. 79(5): p. 337-42.

Bone health education in Australian PDHPE classrooms

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Introduction. Osteoporosis has been considered a paediatric disease with geriatric consequences. Fostering healthy bone behaviours during adolescence may reduce the incidence and disastrous outcomes of poor bone health in older age.

Aims. To develop and evaluate bone health educational materials for Australian PDHPE students from years 7-10.

Methods. A co-design approach was used to develop the modules, involving semi-structured stakeholder meetings with endocrinologists, academic pharmacists, PDHPE teachers, and students. The modules were implemented in 9 Australian high schools. A pre-post quiz was conducted to evaluate knowledge change. Interviews were conducted with students and teachers to guide widespread implementation in the high school curriculum. Thematic analysis was conducted using the Theory of Planned Behaviour.

Results. The co-design process resulted in 4 modules which were rated as highly acceptable to teachers and students. Average knowledge scores significantly improved from 81.25% at baseline to 87.50% ($p<0.001$) in tests taken immediately post-module delivery. Interviews after module delivery revealed high levels of satisfaction among students and teachers. Students expressed increased awareness of the importance of bone health “I realised that I need to be doing a little bit better and taking care of my bones in a more serious way”. Students indicated that they intended to undertake some preventive health behaviours, such as obtaining calcium in the diet but seemed only somewhat willing to regularly do weight-bearing exercise.

Discussion. A collaborative approach has resulted in highly engaging modules for high school students that improved knowledge and may result in healthy behaviours to be adopted to improve bone health.

A systematic review of parameters used for the assessment of subchondral bone in osteoarthritis (OA) with computed tomography (CT)

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Introduction

Bone is critically involved in OA pathology with features such as sclerosis and osteophytes appearing early in disease development [1]. The imaging modalities of choice for OA assessment are radiography and magnetic resonance imaging. However, there are significant advantages to CT imaging compared to these modalities. Primarily, CT is capable of high-resolution three-dimensional image reconstruction allowing standardised bone structure analysis. Therefore, the objective of this systematic review was to gain an overview of published CT parameters for the assessment of subchondral bone in OA, and current practices and standards.

Methods

Search strategies were run in Medline, Embase, and Cochrane Library databases (2010-January 2023) and results were independently screened by two reviewers. Pre-determined inclusion/ exclusion criteria deemed studies conducted with CT *in vivo/ex vivo* in human adults (>18 years) to assess subchondral bone in OA eligible. Data was extracted from included studies and analysed in a qualitative summary and formal narrative synthesis.

Results

Of all search results, 202 studies were deemed eligible. The CT modalities used were summarised in four groups: micro-/nano-CT, conventional clinical-type CT, quantitative CT, and cone-beam CT (Fig. 1a). Nine anatomical locations were found to be of interest for OA assessment (Fig. 1b). Six parameter categories were identified that combine measurements of related osseous features: microstructure, bone adaptation, gross morphology, mineralisation, joint space, and mechanical properties (Fig. 1c).

Conclusions

CT techniques are increasingly popular for OA assessment and clinically meaningful CT measurements as well as parameters with the potential to perform in the clinical field. Quantification is crucial for their sensitivity and reproducibility. Finally, consistent reporting and standardised measurement protocols enhance the value of parameters in future OA research and clinical practice.

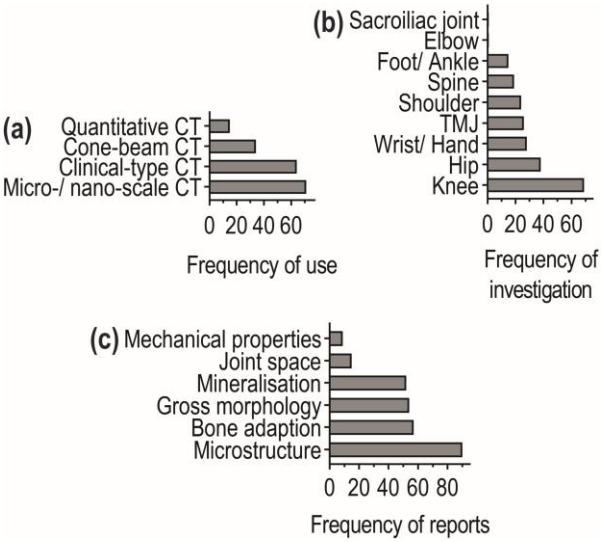

Figure 1 | Overview of a) use frequency of CT groups, b) investigation frequency of anatomical locations, and c) reporting frequency of parameter categories.

1. 1. Altman, R., et al., Arthritis & Rheumatism. 29(8):1039-1049, 1986.

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Association of frailty (J-CHS standard) with related factors among older adults living at home in Tokyo; A cross-sectional study

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Objectives: This study evaluated the association of frailty with related factors among community-dwelling Japanese older adults.

Methods and Study Design: This cross-sectional study in 152 older men and women aged 65 or above who are members of the Tokyo West Health Cooperative, from June to August 2021. We conducted a questionnaire survey and physical measurements. Frailty was evaluated using the Japanese version of the Cardiovascular Health Study (J-CHS) criteria as three groups (frailty, pre-frailty, non-frailty). Creating a binary variable of “frailty/pre-frailty” and “non-frailty” and using multiple logistic regression analysis to estimate odd ratio and adjust for confounders.

Results: Of the 152 participants 6 individuals (4.0%) and 75 individuals (49.3%) had frailty and pre-frailty. 71 individuals (46.7%) had non-frailty respectively. The results showed significant associations between frailty and related factors of presence of internet usage (OR=2.45, 95% confidence interval [CI]: 1.27-4.71)(p=0.007), Experience of psychological stress or acute illnesses in the past three months (OR=2.94, 95%CI: 1.21-7.11)(p=0.017). The results remained consistent even after adjusting for age, gender, and hypertension in the multiple logistic regression analysis. The factors associated with presence of internet usage (OR=2.20, 95%CI: 1.04-4.63)(p=0.038) and Experience of psychological stress or acute illnesses in the past three months (OR=2.80, 95%CI: 1.10-7.17)(p=0.031).

Conclusions: The study confirmed a significant association between non-internet usage and the experience of psychological stress or acute illnesses in the past three months, indicating a higher risk of frailty. These results suggest that limited social engagement may contribute to frailty.

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Bone mineral density and sedentary ageing: a cross-sectional analysis

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Introduction

Physical activity, particularly weight-bearing exercise and resistance training, are recommended for maintaining bone health during ageing. We investigated age-related patterns of bone mineral density (BMD) in association with non-specific physical activity levels.

Methods

Participants were 1021 women and 1397 men (aged 20-96y) from the Geelong Osteoporosis Study. Femoral neck BMD was measured using Lunar densitometers (DPX-L for women; DPX-L or Prodigy-Pro for men). Physical activity was self-reported using a 7-point mobility scale ranging from ‘very active’ to ‘unable to walk’ based on Metabolic Equivalent of Task values; descriptors for each intensity category were provided. For analyses, groups were collapsed into very-active, active and sedentary levels. Associations between physical activity and age were explored using sex-stratified multivariable regression models, in which cubic age adjustments were made for women, and linear adjustments were made for age for men and for weight for both sexes.

Results

For men, 305 (21.8%) were sedentary, 803 (57.5%) active and 289 (20.7%) very-active; for women numbers were 211 (20.7%), 542 (53.1%) and 268 (26.2%). For men, BMD increased with increasing levels of physical activity across all age groups (Figure). For women the pattern was less consistent. Regression modelling showed that for men, compared to the sedentary group, mean adjusted BMD was 2.2% greater for the active and 6.0% greater for the very-active group (both p<0.001). For women, mean adjusted BMD for the active group did not differ to the sedentary group (p=0.520), however, the very-active group had a mean adjusted BMD that was 3.7% greater than the sedentary group (p=0.004).

Discussion

For men, higher levels of general physical activity were dose-dependently associated with greater BMD at the hip, a weight-bearing site, across adulthood. The pattern for women was less clear; however, a very-active lifestyle was consistently associated with higher BMD in comparison to a sedentary lifestyle.

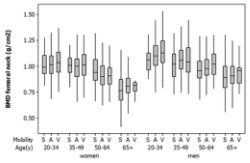

Figure: Boxplot showing summary values for femoral neck BMD by age and sex, according to physical activity level where S=sedentary, A=active and V=very-active.

The effect of prednisolone on lipocalin-2 and its forms in young males effects of exercise

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Table 1. The effects of acute aerobic exercise and prednisolone treatment on circulating LCN2

	Placebo Treatment				Prednisolone Treatment			
	Baseline	Post-ex.	1 h post-ex.	3 h post-ex.	Baseline	Post-ex.	1 h post-ex.	3 h post-ex.
LCN2 (ng/mL) Abcam	127 ± 26	164 ± 28*	No Data	131 ± 32*	131 ± 27#	188 ± 35#*	No Data	150 ± 35#*
hLCN2 (ng/mL)	128 ± 67	161 ± 67*	122 ± 48	136 ± 51	126 ± 44	144 ± 42*	121 ± 48	117 ± 55
C87A (ng/mL)	46 ± 30	66 ± 25*	42 ± 14	53 ± 15	42 ± 7.6	56 ± 16*	48 ± 17	48 ± 15
R81E (ng/mL)	49 ± 44	92 ± 64*	55 ± 49	71 ± 60	40 ± 32	81 ± 66*	46 ± 38	64 ± 55

Mean ± SD. * $p < 0.05$ compared to baseline; # $p < 0.05$ compared to placebo (main treatment effect).

Objectives

Elevated lipocalin-2 (LCN2) is associated with increased risk of cardio-metabolic disease. LCN2 has different forms including a polyaminated (hLCN2), secreted by osteoblasts, and non-polyaminated (C87A and R81E), secreted by adipocytes. Glucocorticoids negatively affect bone and energy metabolism while exercise improves energy metabolism. As such, both glucocorticoids and exercise potentially regulate circulating LCN2. We hypothesised that glucocorticoids would suppress LCN2 and its forms, at baseline and following exercise

Methods

In a double-blind, randomised crossover design, nine young, healthy males (aged 27.8 ± 4.9 years, BMI 24.4 ± 2.4 kg/m^2) completed 30 mins of high intensity aerobic exercise (4 sets x 4 mins at 90-95% HRR) after glucocorticoid (20 mg prednisolone) or placebo treatments. Blood was collected at baseline, immediately post-exercise, 1 h post-exercise (variant analyses only), and 3 h post-exercise. LCN2 was analysed using commercially available ELISA (LCN2 Abcam – primary outcome) and different forms of LCN2 (hLCN2, C87A and R81E) using in-house assays previously validated, secondary outcomes.

Results

LCN2 (Abcam) was elevated after prednisolone compared with placebo (main treatment effect of ~10%; $p = 0.015$). Prednisolone treatment had no effect on individual LCN2 forms (all $p > 0.53$). Regardless of treatment, or assay used, LCN2, C87A, R81E, and hLCN2 increased immediately after exercise (all $p < 0.033$). LCN2, but not the forms, remained elevated at 3 h post-ex ($p = 0.048$).

Conclusion

In contrast to our hypothesis, prednisolone had a limited effect on LCN2, however, both LCN2 and its forms are transiently increased by acute exercise in young healthy males, independent of assay used. The role of LCN2 and it forms in exercise and glucose metabolism warrant further investigation.

Cortical bone mineralization density distribution in paediatics – can peripheral QCT images act as a pain-free bone biopsy?

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"Bone mineralization density distribution" (BMDD) reports the bone mineral frequency distribution within a biopsy image. In addition to density, it is purported to describe bone heterogeneity, turnover, mineralization kinetics and average bone matrix age[1,2]. Unfortunately, bone biopsies are invasive, time consuming and are generally performed at trabecular bone sites.

Although BMDD is a type of image analysis, it has had limited uptake in other bone imaging techniques. This may be related to the lack of suitable image-analysis software and normative data.

Aims

To generate BMDD data using peripheral QCT (pQCT) images, describe BMDD in a normal paediatric population, and investigate if BMDD may act as a "screening" bone biopsy in clinical subjects.

Methods

pQCT images of the Radius 65% and Tibia 66% sites were analysed using ImageJ (Fiji v1.52q) and a modified "pQCT plugin"[3]. Cortical bone voxels were categorized into one of six density bins (280, 480, 710, 955, 1200 and 1700, as mg/cm^3) and bin frequencies calculated.

Normal bin frequencies (age 4-18 years, N≥316) were described by bone site, gender, and pubertal status.

Spinal cord injury (SCI) subjects, without prior exposure to anti-resorptive therapy, were used to demonstrate possible application.

Results

After minor modifications to the generic pQCT plugin, it was possible to produce BMDD data from pQCT images.

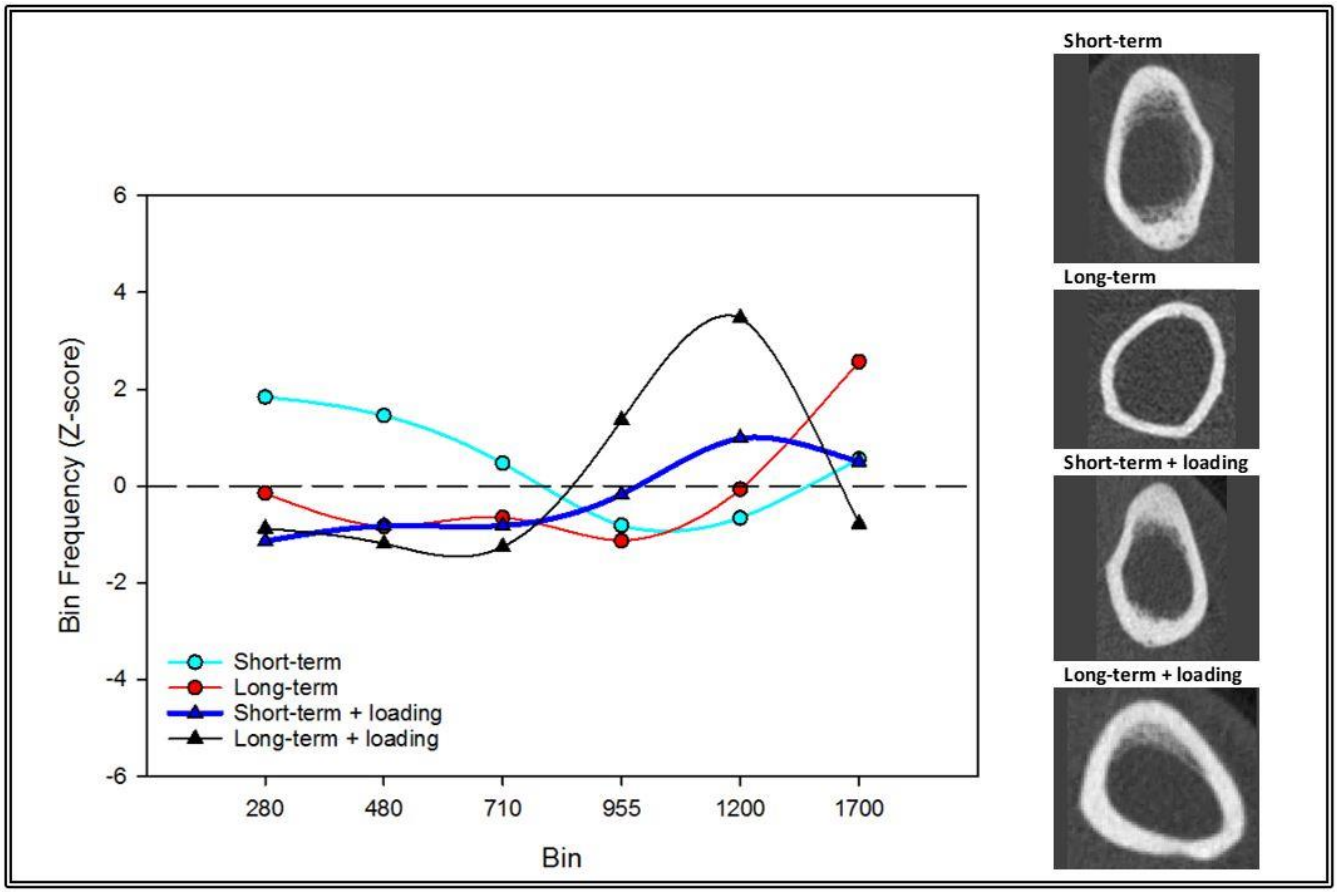
Unlike published histomorphometry data[4], gender and pubertal differences were observed in normal paediatric BMDD.

Figure 1 illustrates different BMDD in short-term non-loading (complete neurological status), and loading (incomplete neurological status) SCI subjects (both 14.9 years and 0.4 years follow-up).

Conclusions

Results suggest BMDD by pQCT might be used clinically to guide intervention selection. For example, the BMDD bin frequency data of the short term, non-loading SCI subject is suggestive of increased bone remodelling which may justify anti-resorptive therapy.

Figure 1. SCI Tibia images and BMDD Z-scores



1. Ruffoni et al (2007) Bone 40(5):1308-1319
2. Roschger et al (2008) Bone 42(3):456-466
3. Rantalainen et al (2011). Journal of musculoskeletal & neuronal interactions 11(3):243-248.
4. Fratzl-Zelman et al (2009) Bone 44(6):1043-1048

Quality of life (QoL) and transition care for patients with Osteogenesis imperfecta (OI) and X-linked hypophosphataemia (XLH)

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Background

OI and XLH are the two most common metabolic bone conditions seen in the Bone Health Clinic at the Royal Children's Hospital (RCH). The transition period for these patients represents a current challenge due to the lack of guidelines and standardised processes.

Aim

To evaluate the 1) QoL outcomes using a disease-specific questionnaire for patients with OI (1) and 2) Strengths and challenges of the current transition model.

Method

A cross sectional survey using questionnaires was given to OI and XLH patients of the RCH and staff involved in transition care.

Results

118 questionnaires were sent; 5 were returned to sender (incorrect address). 42 questionnaires were completed (33 patients with OI, 7 patients with XLH and 2 staff members). Patient characteristics are reported in Table 1.

In the OI cohort, increasing age correlated with lower "being careful" scores ($r=-0.23$, $R^2=0.0698$) and higher "pain" scores ($r=0.35$, $R^2=0.0688$). There was no correlation between QoL and the number of bisphosphonate doses received.

Themes surrounding the transition period for patients with OI and XLH included "unfamiliar", "frustrating", "no continuity of care" and "lack of understanding". Suggestions for improvements included "5-year plan", "valid referral", "education", "clear transition plans" and "consistency".

Following transition from the RCH, patients received care (patient-reported) from a private endocrinologist (9/17, 53%) or public hospital (2/17, 12%). 6 patients (35%) received no ongoing care. Referrals to adult care (patient-reported) were organised by the RCH (6/17, 35%), GP (4/17, 24%) and emergency department (2/17, 12%).

Conclusion

The transition period is a challenging and frustrating time for our patients, with over one third no longer receiving care. A prospective trial is required to evaluate outcomes following the implementation of consistent guidelines and/or a personalised transfer tool.

Table 1: Patient characteristics

	OI cohort n=33	XLH cohort n=7
Gender (n (%))		
Female	21 (64%)	6 (86%)
Male	12 (36%)	1 (14%)
Age (years, median (IQR))	23 (11-34)	10 (6-14)
Age at diagnosis (months, median (IQR))	6 (0-39)	9 (4-14)
Fracture history (n (%))		
Nil	0 (0%)	7 (100%)
One or more	33 (100%)	0 (0%)
Treatment (n (%))		
Bisphosphonate	31 (94%)	N/A
Burosumab	N/A	4 (57%)
Mobility (n (%))		
Independent	18 (55%)	6 (86%)
Crutches	2 (6%)	0
Walking frame	4 (12%)	1 (14%)
Wheelchair	9 (27%)	0

IQR, interquartile range; N/A, not applicable

1. Hill CL, Baird WO, Walters SJ. Quality of life in children and adolescents with Osteogenesis Imperfecta: a qualitative interview based study. Health Qual Life Outcomes. 2014;12:54.

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Bone Fragility is the Result of Bone Loss from Frugally Assembled Larger Bones

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Introduction Bone modelling and remodelling achieve paradoxical properties of bone strength yet lightness by assembling larger bones with relatively less material (thinner, porous cortices), and smaller bones with relatively more material (thicker, less porous cortices); features that accommodate loading and mobility during young adulthood. However, longevity is accompanied by remodelling imbalance. We therefore hypothesised that bone loss will deteriorate bone microarchitecture, compromising the already frugally assembled structure of larger bones.

Methods In 324 twin pairs aged 26-76 years, (364 premenopausal, pre-MP), 255 postmenopausal, post-MP) we used HRpQCT to measure distal radial cross-sectional area (CSA) and deterioration in microarchitecture (cortical porosity and trabecular density) captured by the Structural Fragility Score (SFS) and finite element analysis to estimate compressive strength. Associations are presented as correlation coefficients (SEM).

Results As shown in numbered rows (table): univariate associations (1) estimated strength increased in pre-MW but decreased in post-MW across age. (2) SFS was unchanged in pre-MW but increased in post-MW across age, (3) SFS was higher in pre-MW and post-MW with a larger CSA, but (4) larger CSA was associated with greater strength in pre-MW, not in post-MW despite (5) lower strength with higher SFS.

In a multivariate associations (6) larger CSA was now associated with greater strength in both pre-MW and post-MW independent of SFS and age, (7) higher SFS was associated lower strength independent of CSA and age and (8) age remained a predictor of strength independent of SFS and CSA in pre-MW, but not post-MW.

Conclusion Bone fragility is likely to be accounted for by microstructural deterioration due to loss of minimised mass assembled during growth and compromise of the biomechanical advantage of larger bone size. Bone microarchitecture deterioration is a pivotal determinant of bone fragility, is quantifiable noninvasively and can be prevented or reversed if detected early.

Trait associations Univariate	Premenopausal Women	P value	Postmenopausal women	P value
(1) Strength vs. Age	12.08 (3.96)	0.002	-16.26 (5.15)	0.002
(2) SFS vs. Age	- 0.20 (0.13)	NS	0.85 (0.17)	<0.001
(3) SFS vs. CSA	0.147 (0.02)	<0.001	0.123 (0.03)	<0.001
(4) Strength vs CSA	1.97 (0.60)	0.001	-0.46 (0.80)	NS
(5) Strength vs. SFS	-15.25 (1.3)	<0.001	-20.29 (1.5)	<0.001
Multivariate				
(6) Strength vs. CSA	5.72 (0.38)	<0.001	2.39(0.69)	0.001
(7) Strength vs. SFS	-24.1(1.40)	<0.001	-21.29 (1.47)	<0.001
(8) Strength vs. Age	5.19(2.23)	0.02	1.96(3.96)	NS

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Immunoengineering for periodontal tissue regeneration

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Periodontitis is an infection-induced inflammation, evidenced by an increase in inflammatory macrophage infiltration. Recent research has highlighted the role of plasma-activated medium (PAM) as a regulator of the innate immune system, where macrophages are the main effector cells. This study therefore aims to investigate the immunomodulatory effects of PAM on macrophages and its potential applications for periodontitis management. PAM was generated using an argon jet and applied to culture macrophages. Proinflammatory macrophage markers were significantly reduced after PAM stimulation, and this was correlated with the activation of autophagy via the Akt signalling pathway. Further investigations on the pro-regenerative effects of PAM-treated macrophages on periodontal ligament cells (PDLCs) revealed a significant increase in the expression of bone/cementum markers as well as mineralisation nodule formation. Our findings suggest that PAM is an excellent candidate for periodontal therapeutic applications.

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Three cases of vitamin D deficient osteomalacia probably associated with mild dysfunctional variants in the causative genes for vitamin D-dependent rickets

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【Objective】

Although severe vitamin D deficiency is considered to cause rickets and osteomalacia, these cases are extremely rare in the clinical setting. In the present study, whole genome sequencing (WGS) was performed in 3 patients with osteomalacia with severe dietary restriction and scarce sun exposure in adulthood, to explore the involvement of mild dysfunction in causative genes for vitamin D-dependent rickets (VDDR).

【Case1】

At age 25, she started strict diet therapy and UV protection for her dermatitis and 11 years later, general bone pain developed. Low serum Pi, Ca, 25OHD, and $1,25OH_2D$, high BAP and iPTH were observed. Bone scintigraphy revealed pseudofractures in the rib. WGS identified a heterozygous *CYP3A4* variant (c.554C>G; p.Thr185Ser). After cholecalciferol supplementation, blood Pi, Ca, BAP, and iPTH were normalized and pseudofractures disappeared.

【Case2】

A 24-year-old woman with eating disorder noticed stiffness and numbness in the fingers. Low Ca, 25OHD, and relatively low $1,25OH_2D$, high ALP and iPTH were identified. WGS disclosed a homozygous *CYP3A4* intronic variant (c.218+544 del$(CA)_4$). Low Ca and high ALP were improved by cholecalciferol supplementation.

【Case3】

She has avoided meat and milk from childhood, then, pain in the hip and knee appeared after adulthood. Low Ca, 25OHD, and high $1,25OH_2D$, ALP, and iPTH were presented. Bone scintigraphy revealed multiple pseudofractures. Some homozygous *VDR* large intronic variants were detected. Cholecalciferol supplementation resulted in normalization of Ca, ALP, and iPTH.

【Discussion】

Although functional analysis was not conducted, yet, the involvement of mild *CYP3A4* gain-of-function and *VDR* loss-of-function variants were suspected in 3 cases of vitamin D deficient osteomalacia. With these results, we hypothesize that the majority of patients with vitamin D deficient osteomalacia might harbor mild pathogenic variants in the causative genes for VDDR.

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Severe osteoporosis in a female with type 1 diabetes and pancreatic exocrinopathy with chronic malabsorption and vitamin D deficiency.

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The prevalence and severity of osteoporosis in patients with type 1 diabetes (T1DM) is increased compared to non-diabetic patients, attributed mainly to insulin deficiency and its positive effects on osteoblast proliferation. The association of T1DM with other autoimmune malabsorptive conditions, such as coeliac disease, predisposing to osteoporosis is not uncommon. However, the co-existence of pancreatic exocrinopathy and T1DM leading to osteoporosis is exceptionally rare. Here we present a case of a 29-year-old female with a 10-year history of suboptimally controlled T1DM presenting with significant weight loss and osteofragility fractures who was found to have chronic malabsorption, prolonged vitamin D deficiency and probable osteoporosis/osteomalacia due to severe pancreatic exocrinopathy.

The patient initially presented with an atraumatic left tibial fracture. The patient had suboptimally controlled T1DM with an elevated HbA1c (10.1-14.7%), proliferative retinopathy, polyneuropathy, nephropathy and autonomic neuropathy. Physical examination revealed reduced muscle strength and body composition confirmed very low skeletal muscle mass (19.2kg) but an elevated fat mass (25.5kg). She had a 4-year history of amenorrhea due to weight-related hypothalamic hypogonadism (low FSH <0.1mu/L and estradiol <70pmol/L). Spinal x-rays demonstrated osteofragility fractures. DXA confirmed a low peak bone mass/osteoporosis with significantly reduced total hip BMD of 0.60g/cm² (t-score of -3.4). Malabsorption was documented with low serum 25-vitamin D (50nmol/L), ferritin (8µmol/L), vitamin B12 (12pmol/L) and faecal elastase (42mcg/G). Coeliac serology was negative.

Therapy was initiated with oral cholecalciferol 5000IU daily, calcium citrate 500mg TDS and Creon pancreatic enzyme capsules 75,000IU TDS. Intensive diabetic management was achieved using a Medtronic-770G Smartguard insulin pump and dietary adjustments. The benefits of parenteral bisphosphonates were considered.

This case highlights the complex nature of osteofragility fractures in a young individual with T1DM and highlights the need for a comprehensive investigation of potential contributing factors. A multifactorial approach to management is vital to ensure overall long-term well-being.

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Incidence and prevalence of osteoporotic fracture in adult lung transplant recipients: a single centre audit

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Background

As life expectancy in lung transplant (LT) recipients improves, there is increasing vulnerability to morbidity associated with long-term, high-dose glucocorticoid immunosuppression, including osteoporotic fractures. For LT recipients at our institution, protocolised intravenous zoledronic acid infusion to minimise glucocorticoid-induced osteoporosis and fracture is offered. The objective of this study was to determine the incidence and prevalence of osteoporotic fracture and treatment-associated adverse events in our contemporary cohort.

Methods

We conducted a retrospective study of all adults who underwent LT from January 2012 to December 2018 and survived at least 6 months. Data relating to demographics, fractures and anti-resorptive therapies were obtained from electronic medical records. Primary outcomes were incidence and prevalence of osteoporotic fractures post-LT. Secondary outcomes included treatment-related adverse events.

Results

In total, 405 LT recipients (41% female, median age 59 years) were included, with a median follow up of 4.9 years (IQR 3.4-6.7). The cumulative incidence of osteoporotic fracture was 3.2%, 11.6% and 14.6% at 1, 3 and 5 years respectively. The prevalence of osteoporotic fractures in our study cohort was 21% (n = 86), with pre-LT osteoporotic fracture occurring in 9% (n=37) and post-LT osteoporotic fracture in 16% (n=63) of individuals. The median time to first osteoporotic fracture post-LT was 1.6 years (IQR 1.1 – 3.0 years). The most common major osteoporotic fracture site was the vertebrae. Anti-resorptive therapy was received by 47% pre- and 89% post-LT with treatment-related events uncommon. Two individuals sustained atypical femoral fractures during the study period.

Discussion

We found the incidence and prevalence of osteoporotic fracture post-LT in our cohort was lower compared to historical cohorts and in keeping with recently published contemporary cohorts. Protocol driven intravenous zoledronic acid infusions appear well tolerated in adults with end-stage lung disease and may prevent bone loss and associated fracture.

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Change in Z-score in young adults with Cystic Fibrosis on modulator therapies attending a large CF Centre.

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Background

Most people living with Cystic fibrosis (CF) in Australia are now adults >18 years of age. With significant advancements in therapies including access to cystic fibrosis transmembrane (CFTR) modulator therapies, life expectancy continues to improve. CF is a monogenic disorder affecting the CFTR protein, which is present in bone. Adults with CF are known to have lower bone mineral density and are at risk of fragility fractures. However, the impacts of CFTR modulator therapies on bone health in adults with CF remains unclear. The objective of this study was to explore initial bone changes associated with the introduction of Elexacaftor-Tezacaftor-Ivacaftor (ETI) in adults attending a large CF centre.

Methods

Our CF centre uses a dedicated database to record clinical parameters for their entire patient cohort. We accessed the database to identify all adults with CF on ETI. Those with data available on change in hip or spine Z-scores on subsequent dual Xray absorptiometry (DEXA) performed before and at least 6-months after initiation of ETI were included in the study.

Results

We identified in total 7 (4 females) who met our inclusion criteria. Average vitamin D level was 72.6nmol/L. 3/7 (42%) also had concurrent CF-related diabetes. Most recent hip Z-scores ranged between -2.5 to +1.1 and spine Z-scores ranged between -1.9 to +2.9. We found improvement in hip Z-score in 2/7 (28%) and spine Z-scores in 1/7 (24%). The remainder of the group had deterioration in their Z-scores at both the hip and spine.

Discussion

Less than half of subjects demonstrated an increment in z-scores within 6 months of ETI commencement. Longer term and mechanistic studies will be required to determine whether through improved nutrition, reduced inflammation,, or direct effects on CFTR function within bone, ETI results in improved bone health.

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Prevalence of diagnosable depression in patients awaiting orthopaedic specialist consultation: a cross-sectional analysis

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Objectives

To determine the prevalence and severity of depression and pain in individuals awaiting specialist orthopaedic consultation. Secondarily, to determine the relationship between pain and depression.

Methods

Cross-sectional analysis of individuals awaiting orthopaedic consultation at a public hospital in Melbourne, Australia. Relevant data were extracted from medical records and questionnaires. Descriptive statistics were used to summarise participant characteristics. Multiple linear regression analyses were used to establish the relationship between pain and depression.

Results

Participants: 986 adults (54.1 ± 15.7 years, 53.2% women) participated in the study. Osteoarthritis (OA) was present in 56% of the population. 34% of the entire population had moderate depression or greater, 19% of which met the criteria for major depressive disorder. Moderate-to-severe pain was present in 79% of individuals with OA and 55% of individuals with other musculoskeletal complaints. Pain was significantly associated with depression scores ($\beta = 0.84$, adjusted $R^2 = 0.13$, $P < 0.001$). This relationship remained significant after accounting for gender, age, education and employment status, OA status, number of joints affected and waiting time ($\beta = 0.91$, adjusted $R^2 = 0.19$, $P < 0.001$).

Conclusions

Depression affects one-third of individuals on an orthopaedic waitlist. A strong link between pain and depression in patients awaiting specialist orthopaedic consultation exists, indicating a need for an integrated approach in addressing pain management and depression to manage this complex and comorbid presentation.

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Prevalence of dementia in patients with chronic kidney disease and osteoporosis: a retrospective cohort analysis

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Recent advances in bone research have identified a possible causal link between low bone density and dementia. Similarly, poor kidney function has been associated with an increased risk of dementia. Given the established relationship between chronic kidney disease (CKD) and poor bone health, we sought to investigate the prevalence of dementia in patients with CKD, osteoporosis and both CKD and osteoporosis. We hypothesised that the odds of dementia would be greatest in patients with both CKD and osteoporosis. A retrospective cohort study was conducted using de-identified data from a regional health district in News South Wales, Australia. Data included hospital admissions for adults, 18 years and older, who presented to a local hospital or pathology service between 2008 and 2017. Presence of CKD was confirmed using serum estimated glomerular filtration rate in accordance with the Kidney Disease: Improving Global Outcomes classification. Presence of osteoporosis and dementia was confirmed in accordance with the 10th edition of the Australian modification of the international classification of diseases. There was a 110% increase to the odds of developing dementia for those with CKD alone, (OR = 2.10; 95% CI = 1.98-2.21), a 59% decrease to the odds for those with osteoporosis alone (OR = 0.59, 95% CI 0.51-0.69), and a 20% increase to the odds for those with CKD and osteoporosis (OR = 1.20, 95% CI 1.11-1.3). Interestingly, comorbid osteoporosis lowered the odds of developing dementia in patients with CKD compared to patients with CKD alone. There may be a protective effect of osteoporosis medications on dementia development in patients with CKD. Medication data was not available for this study; as such, it was unknown if patients with osteoporosis were receiving treatment. More research is needed to delineate the effect of untreated and treated osteoporosis on dementia risk in CKD patients.

BONEcheck: a digital tool for personalized bone health assessment

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Background and Aim: Accurate and early identification of individuals at high risk for fractures is important for the prevention of fractures. Existing fracture risk calculators face difficulties in conveying the risk to users in an effective manner. In this context, we introduce an innovative bone health assessment and prevention digital tool called 'BONEcheck'.

Methods: The development of BONEcheck utilised data from multiple cohort studies. BONEcheck has 3 modules: input data, risk estimates, and risk context. Input variables include age, gender, prior fracture, fall incidence, bone mineral density (BMD), comorbidities, and genetic variants. By utilising published methodologies, BONEcheck generates output related to the probability of fracture and outcomes. BONEcheck also includes a module pertaining to fracture prevention. The vocabulary utilised to convey risk estimation and management is tailored to individuals with a reading proficiency at level 8 or above.

Results: The tool is designed for men and women aged 50 years and older who either have or have not sustained a fracture. Based on the input variables, BONEcheck estimates the probability of any fragility and hip fracture within 5 years, subsequent fracture, skeletal age, and the time to reach osteoporosis (T-score<-2.5). The probability of fracture is shown in both numeric and human icon array formats. The risk is also presented in the context of treatment and management options based on Australian guidelines. Skeletal age was estimated as the sum of chronological age and years of life lost due to a fracture or exposure to risk factors that elevate mortality risk.

Conclusions: BONEcheck is a new system of algorithms that aims to provide not only fracture risk probability but also contextualize the efficacy of anti-fracture measures concerning the survival benefits. The tool can enable doctors and patients to engage in well-informed discussions and make decisions based on the patient's risk profile.

1. https://doi.org/10.1101/2023.05.10.23289825
2. BONEcheck is now accessible to users through multiple platforms. Users can access it directly from our website or download the app from the Apple Store or Google Play. Please click on the links below to start utilizing the BONEcheck tool: 1. Website: https://bonecheck.org/ 2. Apple Store: https://apps.apple.com/app/bonecheck/id6447424513 3. Google Play: https://play.google.com/store/apps/details?id=org.saigonmec.bonecheck 4. Auto access: https://onelink.to/8cjb7m

"Skeletal Age" for quantifying the association between bone mineral density and mortality

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Skeletal Age (SA) is conceptually defined as the age of an individual's skeleton resulting from a fragility fracture or exposure to risk factors for fracture. Low bone mineral density (BMD). a well-established fracture risk factor is in turn associated with an increased mortality risk. This study sought to quantify the association between BMD and mortality by using the SA metric.

This study was based on the online data from the Osteoporotic Fractures in Men Study (n= 5994) and the Study of Osteoporotic Fractures (n= 7960). BMD was measured by DXA. The incident fractures were radiologically ascertained, and deaths by reviewing death certificates. Cox's proportional hazard model was used to quantify the association between femoral neck BMD and mortality from which years of life loss (YLL) were estimated. SA was calculated as the sum of chronological age and YLL.

During a median follow-up of 12.4 years (IQR: 7.0, 17.2) and 9.3 years (4.4, 15.2), 1,085 men and 3,879 women had sustained a fracture, yielding the incidence rate of 16 fractures/1,000 person-years (95% CI: 15, 17), and 45 fractures/1,000 person-years (44, 46), respectively. There were 3,612 men and 5,665 women who died during a median follow-up of 14 years. Overall, individuals with a higher risk of fractures were associated with an increased risk of death and thus had higher SA. Specifically, a 70-year man with a medium- and high-risk profile were estimated to have a SA of 71.4 (~ 1.4 YLL), and 72.5 (~ 2.5 YLL) years old, respectively (Table).

These data indicate that low BMD is associated with an increased mortality risk, and this association is an indication of bone fragility which can be measured by the SA metric. By using this metric, medical professionals can better communicate the severity of bone fragility to patients, enhancing doctor-patient fracture risk communication.

Table. Skeletal age for a 70-year individual at risk of fractures

Risk profile	Man		Woman	
	5-year fracture risk*	Skeletal age (95% CI)	5-year fracture risk*	Skeletal age (95% CI)
Medium-risk profile: Femoral neck BMD T-score = -2.5; 1 fall during the last 12 months; 1 fracture since the age of 50	19%	**71.4** (70.9, 71.9)	21%	**71.8** (70.8, 72.7)
High-risk profile: Femoral neck BMD T-score = -3.0; 2 falls during the last 12 months; 2 fractures since the age of 50	46%	**72.5** (71.3, 73.5)	47%	**72.4** (71.3, 73.3)

**The 5-year fracture risk is calculated using the Garvan Fracture Risk Calculator.*

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Coronary artery calcification is associated with smaller increases in femoral neck bone mineral density in patients on anti-resorptive therapy

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Introduction

Anti-resorptive medications are first-line treatments for osteoporosis. Additionally, patients with osteoporosis are at high cardiovascular risk, partly due to vascular calcification, such as in the coronary vessels. It is uncertain if the presence of coronary artery calcification (CAC) effects bone mineral density (BMD) response to anti-resorptive treatment. We therefore assessed changes in BMD following initiation of anti-resorptive treatment for osteoporosis in patients with and without evidence of CAC.

Methods

Individuals dispensed at least one prescription for an anti-resorptive medication (bisphosphonates or denosumab) at Monash Health between 2009-2022 were identified. Unique record numbers for these individuals were then cross-matched against the cardiac CT imaging service at Monash Heart (HREC#73603). CAC was detected by CT coronary angiogram (CTCA). We included only those patients having a baseline BMD measurement within two years of CTCA. The annualised percentage change in femoral neck BMD was calculated and adjusted for age, sex, height, weight, and number of years on anti-resorptive treatment.

Results

106 individuals were identified of which 85 (women=70 [85%], median age=73 years [interquartile range 64-79 years]) had a follow-up BMD measurement including 19 with, and 66 without, evidence of CAC. Those with CAC were older (76 years versus 64 years, p<0.001). There were 70 bisphosphonate users and 15 denosumab users. Individuals with evidence of CAC experienced, on average, a 1.2% lower increase [(0.345% (0.343 to 0.348) versus -0.881% (-0.883 to -0.879), mean difference -1.226% (-1.493 to -0.959; p<0.05)] in annualised femoral neck BMD with anti-resorptive therapy after adjusting for important clinical risk factors.

Interpretation

These preliminary data suggest that CTCA-determined CAC may negatively impact femoral neck BMD increases with anti-resorptive therapy. Analysis of the full cohort is presently underway.

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Osteoporosis is associated with increased risk for cardiovascular disease mortality in community dwelling older women: the Perth Longitudinal Study of Ageing in Women

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Publish consent withheld

Associations Between Social Disadvantage and Bone, Muscle, and Physical Function Outcomes in Community-Dwelling Older Adults: The SEBA Study

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Objectives: Health equity describes a concept that everyone should have an equal opportunity to achieve good health. This study aimed to explore whether bone, muscle, and physical function outcomes differ by socioeconomic status (SES).

Methods: Data were collected from 300 older adults (mean age: 66.4 years; 62% female; 201 with osteopenia/osteoporosis). This included bone mineral density (BMD), handgrip strength and several physical function measures (gait speed, 5STS, SPPB). SES measures included education, income/employment, social isolation and health literacy (HL). HL was measured using the HL questionnaire (HLQ) comprising nine distinct scales: 1) Support from health providers; 2) Having sufficient information to self-manage health; 3) Actively managing health; 4) Social support for health; 5) Appraisal of health information; 6) Engagement with health providers; 7) Navigating the healthcare system; 8) Ability to find health information; and 9) Understanding health information.

Results: Mean scores were lower in six HLQ scales for participants with osteopenia/osteoporosis compared to those with normal BMD, however these were not statistically significant. Associations were analysed in operational measures of sarcopenia (not sarcopenia diagnosis due to low prevalence; n=14, 4.7%). Positive correlations were seen between handgrip strength and HLQ scales 4-7 ($p<0.05$); faster 5STS times were associated with higher scores in most HLQ scales (except scale 4; $p<0.05$); and positive correlations were reported between gait speed and majority of HLQ scales (except 1, 6 and 7; $p<0.05$). Higher SES levels were significantly correlated with improvements in all muscle and physical function outcomes (Table 1).

Conclusions: This is one of the first studies to provide insights into the important role of SES and HL in musculoskeletal health. These findings are particularly relevant for clinicians and policy makers seeking to implement and/or develop interventions to improve prevention of osteopenia/osteoporosis and sarcopenia. We suggest clinical attention be directed towards specific social groups.

Table 1: Adjusted logistic regression analyses (Odds Ratio, 95% CI)* modelling the associations between SES levels and muscle and physical function outcomes

	Completed vs not completed HS^	Highest income level (>$90,000) vs lowest level (<$40,000)^	Employed vs unemployed^	Highest SEIFA quintile vs lowest quintile^	Living with someone vs living alone^
Handgrip strength	**2.18 (0.01, 2.37)**	**4.79 (1.83, 7.75)**	**2.28 (0.08, 4.65)**	1.63 (-0.80, 4.06)	**4.86 (2.30, 7.43)**
Gait Speed	**0.16 (0.10 , 0.23)**	**0.12 (0.03. 0.21)**	**0.14 (0.07 , 0.22)**	0.04 (-0.03 , 0.12)	**0.11 (0.03 , 0.19)**
5STS	**-2.99 (-5.53, -0.47)**	**-4.46 (-7.89, -1.03)**	**-2.66 (-5.37, -0.06)**	**-3.10 (-5.92, -0.28)**	**-3.87 (-6.86, -0.89)**
SPPB	**0.56 (0.22 , 0.91)**	**1.00 (0.54, 1.46)**	**0.64 (0.28 , 1.01)**	**0.51 (0.13 , 0.89)**	**0.42 (0.01, 0.84)**

5STS=Five-repetition sit-to-stand test; HS=Highschool; SEIFA=Socio-Economic Indexes for Areas; SPPB=Short Physical Performance Battery

Bold values are significant ($p<0.05$)

*All analyses are adjusted for age and sex

^Reference group

Integrating Post-Fracture Care into the Primary Care Setting (interFRACT): Phase 1 Results of a Mixed-Methods Co-Design Study

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Objectives: To develop the interFRACT care pathway to enhance diagnosis/treatment of osteoporosis and improve initiation of fracture prevention strategies for older adults in the primary care (PC) setting.

Methods: This mixed-methods study uses an established co-design approach (Figure 1). Phase 1 involved developing a Stakeholder Advisory Committee (SAC) to guide study procedures and outcomes; interviews with 15 GPs and 20 consumers (older adults >50 years with osteoporosis and/or fragility fracture) to explore beliefs and attitudes toward osteoporosis and fractures; and a scoping review to investigate current approaches, needs and barriers to osteoporosis and post-fracture treatment within PC.

Results: The SAC was developed and includes 2 GPs; 1 geriatrician; 1 fracture liaison nurse; 1 exercise physiologist; 2 representatives from the Fragility Fracture Network and Musculoskeletal Australia; and 5 consumers. Analyses of interviews showed that most GPs gave low priority to osteoporosis and saw other medical conditions as more important; consumers also shared this view. Most GPs felt confident in managing osteoporosis, however consumers perceived their care as inadequate and felt GPs lacked knowledge in non-pharmacological treatments (e.g. exercise). Noted as the "acute-PC divide", GPs desired better collaboration with hospitals and commonly spoke about delayed or poor-quality communication. Both groups made suggestions of how osteoporosis and fracture management could be improved in PC. Ninety studies were included in the scoping review, which identified several key themes: Lack of GP knowledge; Low osteoporosis diagnosis and treatment Rates; Interventions to Improve Osteoporosis Diagnosis/Treatment; and Barriers to Osteoporosis Management.

Conclusions: These findings have allowed us to gain an understanding of consumer and GP experiences and needs within PC. Phase 2 will include a series of co-design workshops with the SAC to develop the interFRACT care pathway using phase 1 results, and a feasibility study with GPs to determine the usability/acceptability of the care pathway.

Figure 1: Phases and steps of co-design in the *Boyd et al.* framework

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Associations between sarcopenia and domains of quality of life in older adults

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Background: Sarcopenia is characterised by an accelerated loss of skeletal muscle mass and function. It is associated with numerous adverse health outcomes including an increased risk for falls, hospitalisation, and mortality. Studies examining the relationship between sarcopenia and aspects of QoL are scarce.

Methods: This cross-sectional study involved 339 women and 343 men (aged 60–96 years) from the Geelong Osteoporosis Study. Sarcopenia was defined according to the EWGSOP2 algorithm. Appendicular lean mass (ALM) was assessed using dual-energy X-ray absorptiometry. Handgrip strength (HGS) was used to assess muscle function and the timed up-and-go test was used to assess physical performance. The WHOQoL-BREF was used to assess quality of life. Associations between sarcopenia and WHOQoL-BREF domains (physical, psychological, social relationships and environment) were investigated using multivariable logistic regression while testing for potential confounding.

Results: Based on the EWGSOP2 algorithm, 57 participants (8.4%) had probable sarcopenia (low HGS strength), 12 (1.8%) had confirmed sarcopenia (low HGS and low ALM), and two were considered to have severe sarcopenia (low HGS, low ALM, and poor physical performance). The number of participants with confirmed sarcopenia was too small for meaningful analyses. However, associations between probable sarcopenia and QoL domains were assessed. In adjusted models, probable sarcopenia was associated with poor QoL in domains of physical health [OR 2.35 (95% CI 1.22-4.52) p = 0.010] and psychological [OR 2.22 (95% CI 1.19-4.12) p = 0.012]. No associations were detected between probable sarcopenia and the social relationships or environment domains.

Conclusion: Older men and women with low HGS were more likely to have poor physical- and psychological-related QoL. Our findings reinforce the importance of muscle function for good QoL. Interventions to prevent or manage sarcopenia among older adults may contribute to better QoL for this population.

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Bipolar disorder and markers of bone turnover: a case control study

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Introduction: Bipolar disorder (BD) is associated with significant psychological and physical comorbidity, yet little is known about bone health. Interestingly, there is an increasing body of evidence to suggest that lithium, commonly used in the treatment of BD, may possess bone protective properties. To explore this further, we aimed to determine whether BD is associated with serum markers of bone turnover.

Method: Men and women with a history of BD (cases; n=116) were recruited from public and private health care settings and a sample of age-and sex-matched controls (3:1 ratio), without BD, were drawn from the Geelong Osteoporosis Study (n=348). BD was identified using a semi-structured clinical interview (SCID-I/NP). Blood samples were obtained and the bone resorption marker, C-telopeptide (CTx) and formation marker, type 1 procollagen amino-terminal-propeptide (PINP) were measured. Anthropometry and socio-economic status (SES) were determined and information on medication use and lifestyle was obtained via questionnaire. Multiple linear regression was used to determine the association between BD and bone turnover markers (CTx and PINP).

Results: Cases were heavier, more likely to smoke, be less active, have lower SES, were more likely to take bone active medications; otherwise the groups were similar. Adjusted mean CTx values were 5.7% lower [15.5 (95% CI 13.6–17.3) vs 16.4 (95% CI 14.6–18.2) pg/ml, p=0.03] and PINP values were 7.3% higher [6.3 (95% CI 5.6–6.9) vs 5.8 (95% CI 5.1–6.5) pg/ml, p=0.008] among the cases compared to controls. These relationships were independent of age, sex, weight, smoking, activity, alcohol and calcium consumption, SES and bone active medications. Following the removal of lithium users (n=47), these relationships were attenuated [CTx, p = 0.05; P1NP, p=0.09].

Conclusion: Our data suggests that lithium contributes to the observed differences in bone turnover observed between those with and without BD.

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A qualitative assessment of healthcare professionals bone health knowledge and management of people with multiple sclerosis

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Background: People with multiple sclerosis (MS) have a higher prevalence of osteoporosis (27% vs 12.6%) and onset occurs at a younger age (41.5yrs±7.9) when compared to the general population (>50 years). MS-related osteoporosis and poor bone health are under-recognised and managed in people with MS, and if not managed well, it may reduce independence and impair quality of life.

Aim*:* This study aimed to assess the healthcare professionals who are part of the multidisciplinary care team for people with MS about their knowledge, current practices, barriers and enablers to bone health management in MS.

Methods: Participants were 30 healthcare professionals, including: neurologists (4), endocrinologists (8), MS nurses (9), general practitioners (5), and physiotherapists (4), recruited through network contacts. Participants were interviewed using a semi-structured script (mean: 26min, range: 17.8 – 33.1min). Interviews were undertaken and recorded using Zoom. Transcription was performed using Otter.ai and reviewed for accuracy by the interviewing researcher (LAJ). Transcripts were coded in NVivo Plus (v12) using framework analysis.

Results: Most participants with experience in managing people with MS acknowledged the higher risk of osteoporosis compared with the general population. Apart from the endocrinologists and nurses, many participants reported limited bone health knowledge in people with MS which hindered proper bone health management. MS-related priorities overshadowed bone health concerns. Many endocrinologists and half of the general practitioners found bone health management practices in MS insufficient, and some endocrinologists were uncertain of best practice for people with MS. Improved clinician and patient educational materials were recommended.

Conclusion: There is a clear need for greater proactive management of bone health in people with MS. This would be supported by guidelines for managing bone health in MS and development of educational resources for people with MS and healthcare professionals.

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Osteoporosis in Eating Disorders: A Clinical Update

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Eating disorders, especially anorexia nervosa (AN) but also eating disorders not otherwise specified and sometimes bulimia nervosa, are associated with low bone density, bone microarchitectural deterioration, and increased fracture risk.[1-7] Low bone density may not reverse readily following recovery from AN, and chronic deficits and increased fracture risk often persist many years after recovery.[1, 2, 7-9] Oestrogen deficiency is a key contributing factor to osteoporosis in women with AN.[10] Women with AN have been shown to have lower bone density in comparison to age-matched normal weight women with hypothalamic amenorrhoea and comparable duration of amenorrhea, prior oestrogen use, and age of menarche.[11] Low lean body mass and nutritional deficiencies are also important predictors of low bone density in women with AN.[11] Similarly, low lean body mass, nutritional deficiencies, and low testosterone levels are the key contributing factors to osteoporosis in men with AN.[12, 13] Nutrition therapy, psychological interventions, and avoidance of excessive exercise are crucial to improving bone health in people with eating disorders. Weight restoration results in the most robust improvement to bone mineral density (BMD) in people with osteoporosis secondary to AN.[10] At present, there is no consensus on the optimal pharmacological approach to treat osteoporosis in those living with eating disorders. In this clinical update, we will review the available data to date from the literature and discuss our own evidence-based practice.

MET-Call during Zoledronate Infusion: An Unusual Case of Allergy

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MET-Call during Zoledronate Infusion: An Unusual Case of Allergy

Clinical Case:

A 54-year-old woman presented post fragility fracture of left distal tibia and fibula sustained in October 2021. Her past medical history is significant for metacarpal fracture after falling from standing height in 2018, recurrent renal calculi, asthma, endometriosis, and multiples allergies including corn, grapes, iodine, maize, oranges, NSAIDs and penicillins. Her medications include Fluticasone/Salmeterol inhaler, Vitamin D3, Calcium carbonate 1200mg daily, and promethazine as required. There was no oral glucocorticoid exposure. She was post-menopausal at age 53, there was no family history of osteoporosis. Bone densitometry revealed generalised osteoporosis (LS: T-score -3.7, FN: -4.3, TH: -4.2). Her secondary osteoporosis screen was remarkable for mild persistent hypercalcaemia. Fractional excretion of calcium has ruled out familial hypocalciuric hypercalcaemia (FHH) (Table). After informed consent, she proceeded to receive zoledronate infusion 5 mg over 30 minutes as per local protocol in October 2022. Seven minutes into the infusion, patient started experiencing shortness of breath, itchy throat and generalised urticarial rash over face, upper back and upper chest. This required a MET response to be initiated. There was no lip or tongue swelling and her observations revealed blood pressure of 165/110mmHg, heart rate 104 beats/minute, oxygen saturation of 98% on room air. There was no wheeze on auscultation.

Zoledronic infusion was promptly ceased. Intravenous hydrocortisone 50mg and oral promethazine 10mg was administered with relief in symptoms. Estimated dose of 1.16mg of zoledronic acid was administered prior to cessation. Patient was subsequently discharged home.

Subsequent pathology revealed a normal Tryptase level of 5.9 ug/L, ruling out possibility of mastocytosis. Her bone turnover markers taken 8 months post partial zoledronate infusion was incompletely suppressed.

She sustained further fragility left distal radius fracture after falling on outstretched hands in March 2023. At the time of writing, she is being investigated by the Immunology team given potential allergens in other anti-resorptive agents and is being considered for romosozumab.

Table:

Investigations	**October 2021 (Post Tibial Fracture)**	**October 2022 (Pre zoledronic acid)**	**June 2023 (Post zoledronic acid and Radius fracture)**
Hb (g/L)	141		142
eGFR	>90	74	75
Corrected calcium (mmol/L)	2.56	2.71	2.76
Phosphate (mmol/L)	1.11	1.10	1.21
ALP (units/L	117	127	110
TSH (mU/L)	2.24		1.85
PTH (pmol/L)	7.1		5.1
C Telopeptide (ng/L)	812	445	1272
P1NP (ug/L)	84	93	172
Vitamin D	81	91	96
Urinary calcium (mmol/d)		8.8	
Urinary Creatinine (mmol/d)		8.3	
Fractional Excretion of Calcium		3%	

Discussion:

Bisphosphonates are widely used for treatment of osteoporosis in postmenopausal women. Zoledronic acid, a Nitrogen-containing bisphosphonate is a potent inhibitor of osteoclasts [1]. Its use has well recognized beneficial effect on bone mineral density and reduction in fracture risk for vertebral and non-vertebral fractures [2].Its use is generally well tolerated, acute phase reactions including chills, fever, influenza-like symptoms, night sweats, rigors and shivering, diffuse musculoskeletal pain, gastrointestinal effects, and eye inflammation can occur in up to 40% of patients and are self-limiting [3].

Whilst mechanism of acute phase response is not well established, direct activation of γΔT cells, leading to activation of immune system and production of inflammatory cytokines such as TNF-α, INF-γ and IL-6 has been implicated [4]. Anaphylaxis and acute urticaria post zoledronate is rare and thought to be IgE mediated [5] . The IgE level of this patient was 94 kunits/L (N= <114 kunits/L) done in July 2023.

Our case is unique and presents a management dilemma. It is unclear if the reaction is secondary to zoledronic acid, bisphosphonates as a class or its excipients. All preparations of zoledronic acid commonly contain mannitol as an excipient. Mannitol is a naturally occurring sugar in many plants, fruits and vegetables. Common indications include reduction of raised intracranial pressure and as an inhalational irritant in bronchial provocation tests. It is also used widely as an excipient in variety

of medications. Mannitol has been implicated in anaphylaxis to intravenous paracetamol via IgE mediated hypersensitivity reaction [6].

Unfortunately, most of antiresorptive agents have mannitol as an excipient, which severely limits treatment option for our patient. Denosumab has sorbitol, which due to structural similarity to mannitol also poses a risk of further adverse allergic reactions[7]. Romosozumab would be a suitable alternative in the short term as there are no interfering excipients listed. An alternative would be to co-administer short course of glucocorticoids at the time of zoledronate infusion prophylactically, which has reduced acute phase reactions in cystic fibrosis patients [8]. Patients with multiple drug allergies present a challenging barrier to patient care. Our patient illustrates a management dilemma with significant limitation in treatment options for her severe osteoporosis exacerbated by patient anxiety and reluctance to trial new therapy.

Take home messages:

- Allergy to excipients in anti-resorptive agents is exceptionally rare. This might be influenced due to under recognition of this effect as most excipients are not listed on drug labels.
- Non-anaphylactic drug reactions can sometimes be mitigated with co-administration of glucocorticoids and anti-histamines with the support of immunology department.
- Subjects with multiple drug allergy/ intolerance syndrome are challenging to manage due to anxiety to accept new therapies, multi-disciplinary approach involving immunologist can assist.

Broken bones, miserable moans, and unfortunate unknowns

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Case-Summary

A 62-year-old female presented to a new specialist for review of profound skeletal fragility with numerous fractures despite 5 years of potent antiresorptive and osteoanabolic treatment. She was initially diagnosed with severe osteoporosis at age 58, when she presented with 12-months of persistent lumbar, hip, and bilateral foot pains associated with prominent myalgias. Whole body bone scan with SPECT CT demonstrated several minimal trauma vertebral, rib, pelvic and metatarsal fractures. Initial BMD demonstrated a T-score of -3.3 at the lumbar spine (0.779 g/cm^2) and -2.9 at the femoral neck (0.655 g/cm^2). She was an ex-smoker with a 40 pack-year history and had a BMI of 14.6 kg/m^2. There were no syndromic features, nor any history of early onset skeletal fragility or family history of minimal trauma fractures. There were no other significant risk factors for osteoporosis.

Investigation for secondary causes of skeletal fragility included a normal FBC, tryptase, TFT, PTH, HbA1c, corrected calcium and renal function. ALP was 133 U/L (N 30-115). Other LFTs were normal and 25-hydroxyvitamin D was 68 nmol/L. Myeloma assessment, inflammatory & autoimmune markers and 24-hour urinary free cortisol were normal.

Initial treatment comprised optimising dietary calcium, vitamin D supplementation and denosumab 60 mg 6-monthly. However, over the next 12-months her myalgias continued and resulted in progressive disability. Repeat bone scan demonstrated new focal lesions in the left clavicle, left rib cage, bilateral ulnar and right radius, with progressive uptake in bilateral femoral heads. Oncologist review and staging CT of the chest, abdomen, and pelvis, ^{18}FDG-PET, bilateral mammography, pap smear, skin check and colonoscopy did not reveal any signs of malignancy. Bilateral hip MRI demonstrated a 12-mm lesion in the right acetabulum of indeterminate appearance, lacking FDG or scintigraphic uptake. She was subsequently treated with 18-months of teriparatide and then continued regular denosumab. Despite an improvement in BMD with a lumbar spine T-score of -1.7 (+0.22 g/cm^2, +28%) and -2.4 at the hip (+0.065 g/cm^2, +10%) within 4 years, she continued to experience numerous minimal/no trauma fractures, one of which required a right dynamic hip screw.

Upon referral to an endocrinologist, it was noted she had an acquired and persistent moderate to severe hypophosphatemia (~0.40 mmol/L) over the last 5 years, with a normal serum phosphate 10 years prior. Renal phosphate wasting was demonstrated by a markedly reduced tubular maximum reabsorption of phosphate to glomerular filtration rate (TmP/GFR) of 0.29 (N >0.84) without evidence of a proximal tubulopathy with normal urine glucose and plasma bicarbonate. 1,25 dihydroxyvitamin D was 74 (N 50-190), and IGF-1 and iron studies were normal. FGF23 was elevated at 310 ng/L (N 23.2 - 95.4). A subsequent ^{68}Ga-DOTA PET/CT demonstrated an avid focus in the right acetabulum, corresponding to a 13mm well-circumscribed lucent and stable lesion on CT, present on previous MRI studies. A diagnosis of tumour-induced osteomalacia (TIO) due to a presumed benign FGF-23 producing phosphaturic mesenchymal tumour was made.

She was commenced on phosphate and calcitriol with rapid improvements in muscle strength and reduced myalgias. Serum phosphate increased from 0.37 to 0.78 mmol/L (N 0.8-1.5) within 4 weeks. Currently, she is awaiting an orthopaedic opinion regarding potential resection of the lesion, which may be complicated due to existing metalware in the affected joint. Burosumab via compassionate access is being arranged if definitive treatment is not viable.

Tumour-Induced-Osteomalacia

TIO is a rare acquired paraneoplastic syndrome characterised by overproduction of FGF23 by ectopic phosphaturic mesenchymal tumours of the mixed connective tissue type.(1) FGF23 is produced by osteocytes and is the principal regulator of phosphate homeostasis. It acts at the proximal renal tubule to reduce expression of the sodium phosphate cotransporters NaPi-2a/2c causing decreased tubular phosphate reabsorption. FGF23 also inhibits expression of 1-alpha-hydroxylase in the proximal tubules, leading to reduced concentration of calcitriol and subsequent decreased intestinal phosphate and calcium absorption.(2) The resulting hypophosphatemia, phosphaturia, and low or inappropriately normal concentration of calcitriol, lead to muscle weakness, bone pain, osteomalacia, and ultimately fragility fractures.(3) The occult nature, small size, slow growth, and often obscure anatomical location of an underlying tumour leads to an average delay from symptom onset to diagnosis of 2.9 ± 2.3 years, resulting in skeletal deformities and severe disability.(4)

Once TIO is suspected on biochemical and clinical abnormalities, successful tumour localisation is best performed utilising whole-body ^{68}Ga-DOTA PET/CT imaging, which demonstrates the highest sensitivity and specificity.(5) After successful detection of neoplastic lesions with functional imaging, precise localisation is performed using anatomical imaging typically involving CT or MRI. Complete tumour resection is the only curative treatment for TIO. This results in prompt reversal of the biochemical abnormalities over days and remineralisation of affected bone over 12 months.(6) In cases of incompletely resected tumours, adjuvant radiotherapy has been successfully used in a few patients(7). If tumour localisation or resection is not possible, conventional medical treatment is with oral phosphate and calcitriol supplementation. The aim is to increase serum phosphate to the lower limit of the age-appropriate normal range, normalise ALP, and maintain PTH within the normal range. However, sustained adherence to this therapy is often poor due to treatment burden and gastrointestinal side effects. Major long-term complications include nephrocalcinosis, nephrolithiasis, and secondary/tertiary hyperparathyroidism.(8)

Burosumab is a novel humanised monoclonal antibody against FGF23. It has demonstrated efficacy in two small trials and corrects the biochemical and radiological abnormalities, and physical symptoms associated with osteomalacia in TIO.(9, 10) It is currently PBS listed for X-linked hypophosphataemia, but not for TIO, making access difficult.

Take-Home-Points

- In a patient with multiple/unusual minimal trauma fractures despite potent osteoporosis therapy, it is imperative to consider rarer causes.
- Persistently low serum phosphate concentration in a patient with fragility fractures should prompt investigation for osteomalacia, FGF-23 excess, and renal phosphate wasting.
- The diagnosis of tumour-induced osteomalacia is often delayed by several years resulting in significant skeletal deformities and severe disability.
- ^{68}Ga-DOTA PET/CT is the most sensitive and specific imaging modality to detect mesenchymal tumours hypersecreting FGF23.
- Tumour resection affords curative treatment, however burosumab has demonstrated effectiveness in small trials and avoids the problems with phosphate and calcitriol supplementation.

1. Folpe AL, Fanburg-Smith JC, Billings SD, Bisceglia M, Bertoni F, Cho JY, et al. Most osteomalacia-associated mesenchymal tumors are a single histopathologic entity: an analysis of 32 cases and a comprehensive review of the literature. Am J Surg Pathol. 2004;28(1):1-30.
2. Shimada T, Hasegawa H, Yamazaki Y, Muto T, Hino R, Takeuchi Y, et al. FGF-23 is a potent regulator of vitamin D metabolism and phosphate homeostasis. J Bone Miner Res. 2004;19(3):429-35.
3. Jonsson KB, Zahradnik R, Larsson T, White KE, Sugimoto T, Imanishi Y, et al. Fibroblast growth factor 23 in oncogenic osteomalacia and X-linked hypophosphatemia. N Engl J Med. 2003;348(17):1656-63.
4. Florenzano P, Hartley IR, Jimenez M, Roszko K, Gafni RI, Collins MT. Tumor-Induced Osteomalacia. Calcif Tissue Int. 2021;108(1):128-42.
5. Zhang J, Zhu Z, Zhong D, Dang Y, Xing H, Du Y, et al. 68Ga DOTATATE PET/CT is an Accurate Imaging Modality in the Detection of Culprit Tumors Causing Osteomalacia. Clin Nucl Med. 2015;40(8):642-6.
6. Chong WH, Andreopoulou P, Chen CC, Reynolds J, Guthrie L, Kelly M, et al. Tumor localization and biochemical response to cure in tumor-induced osteomalacia. J Bone Miner Res. 2013;28(6):1386-98.
7. Mishra SK, Kuchay MS, Sen IB, Garg A, Baijal SS, Mithal A. Successful Management Of Tumor-Induced Osteomalacia with Radiofrequency Ablation: A Case Series. JBMR Plus. 2019;3(7):e10178.
8. Huang QL, Feig DS, Blackstein ME. Development of tertiary hyperparathyroidism after phosphate supplementation in oncogenic osteomalacia. J Endocrinol Invest. 2000;23(4):263-7.
9. Jan de Beur SM, Miller PD, Weber TJ, Peacock M, Insogna K, Kumar R, et al. Burosumab for the Treatment of Tumor-Induced Osteomalacia. J Bone Miner Res. 2021;36(4):627-35.
10. Imanishi Y, Ito N, Rhee Y, Takeuchi Y, Shin CS, Takahashi Y, et al. Interim Analysis of a Phase 2 Open-Label Trial Assessing Burosumab Efficacy and Safety in Patients With Tumor-Induced Osteomalacia. J Bone Miner Res. 2021;36(2):262-70.

FEVR and fractures: *CTNNB1* mutation as a cause of autosomal dominant osteoporosis-pseudoglioma-like syndrome

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Clinical case: A 27-year-old woman with known familial exudative vitreoretinopathy (FEVR) presented to the metabolic bone clinic for further evaluation of recurrent fragility fractures occurring since childhood including a wrist fracture at age 7, a hip fracture at age 10 and numerous vertebral fractures in pre-adolescence. Bone mineral density (BMD) performed at the spine (L1-4) at age 9 was 0.47 g/cm^2 (Z-score -2.6). Her most recent BMD at age 26 showed a nadir BMD at the left neck of femur of 0.68g/m^2 (Z-score -2.5). Her height is below the 1st centile. Her 45-year-old mother, also affected by FEVR, shares striking similarities including distinct facies, dental hypoplasia, thin hair, mild intellectual impairment, and height below the 1st centile. She had fractures of the humerus, scapula and clavicle after a fall from standing height at age 36. Her most recent BMD at age 44 shows density at the spine of 0.56 g/cm2 (T-score -5.2, Z-score -4.3) and right neck of femur 0.51g/cm2 (T-score -4.0, Z-score -3.0).

The combination of osteoporosis and exudative vitreoretinopathy strongly suggested osteoporosis pseudoglioma syndrome (OPPG), however both mother and daughter were negative for *LRP5* mutation. Targeted exome sequencing revealed a missense variant in *CTNNB1* (c.1723G>A; pGly575Arg), resulting in a single amino acid change in a highly conserved glycine residue in exon 11 of the ß-catenin protein; it was absent from controls (frequency of 0 in gnomAD database) and predicted to be deleterious by a number of *in silico* tools, and classified as likely pathogenic. Segregation testing in the mother revealed the same variant. Mutations in *CTNNB1* are a known cause of autosomal dominant FEVR. However, although the ophthalmological phenotype is well described, no skeletal phenotype within this patient population has previously been reported.

Mutations in *LRP5* are the established cause of OPPG, a hitherto autosomal recessive condition characterised by abnormal development of peripheral retinal vasculature leading to sight-threatening complications of ischaemia, neovascularization, retinal traction, detachment, exudation and dysplasia and corneal opacity (1). Classically, OPPG causes low bone mass and frequent fractures but *LRP5* mutations may also cause FEVR without skeletal abnormalities, including autosomal-dominant forms (2,3). Similarly, *CTNNB1* mutations can cause a range of ophthalmological conditions including FEVR, optic nerve atrophy, refractive errors and strabismus (1,4) including in an 11-year-old patient with the same p.Gly575Arg mutation seen in the family we are reporting (5). Other reported conditions associated with variants of *CTNNB1* include behavioural abnormalities including autism, developmental delay and intellectual disability with a wide variability in severity observed (6). In one family affected by FEVR with a *CTNNB1* mutation the unaffected mother was found to be a carrier for the mutant allele. As such, it appears that penetrance and expressivity is highly variable (4).

Both LRP5 and ß-catenin are widely expressed and form part of the canonical Wnt signalling pathway which is critical for normal skeletal development. Wnt signalling potentiates mesenchymal stem cells towards osteochondral differentiation and subsequently, to an osteoblast lineage cell fate (9). In a mouse model, Hill et al. demonstrated that ß-catenin stabilisation and knockout resulted in osteopetrotic and osteopaenic phenotypes respectively (9). In mature bone, Wnt binds to cell-surface Frizzled (Fzd) receptor and the LRP5/6 coreceptor on osteoblasts, resulting in canonical amplification of ß-catenin and a pro-anabolic milieu by promoting osteoblast and osteocyte survival and inhibiting osteoclast differentiation (10).

The association of *CTNNB1* gene changes and bone disease in humans has not been clearly described. In a meta-analysis of genome-wide association studies, a single-nucleotide polymorphism (SNP) on chromosome 3, upstream of the *CTNNB1* gene was identified as one of twenty statistically significant SNPs for low BMD at the femoral neck (7). Pertussa et al. demonstrated a similar association of low spine BMD with this SNP in a cohort of Spanish women (8).

Despite compelling evidence for an effect of *CTNNB1* haploinsufficiency on bone, to our knowledge, this is the first report to highlight the bone phenotype in patients with FEVR due to a *CTNNB1* mutation, and the first report of an autosomal dominant cause of an OPPG-like syndrome.

Take Home Messages:

- *CTNNB1* encodes ß-catenin, a downstream mediator of Wnt signalling and an important pathway in maintaining normal bone mass.
- Clinical presentation of FEVR and osteoporosis should raise suspicion for a mutation within the Wnt signalling pathway.
- Further description of bone-related disease in patients with *CTNNB1* mutations are needed to clarify fracture risk and guide potential therapeutic intervention.
- Coordinated subspecialist care throughout the lifespan is required to provide timely diagnosis and optimal management in patients with complex genetic conditions.

1. 1. Panagiotou ES, Sanjurjo Soriano C, Poulter JA, Lord EC, Dzulova D, Kondo H, et al. Defects in the Cell Signaling Mediator β-Catenin Cause the Retinal Vascular Condition FEVR. The American Journal of Human Genetics. 2017 Jun;100(6):960–8.
2. 2. Toomes C, Bottomley HM, Jackson RM, Towns KV, Scott S, Mackey DA, et al. Mutations in LRP5 or FZD4 Underlie the Common Familial Exudative Vitreoretinopathy Locus on Chromosome 11q. The American Journal of Human Genetics. 2004 Apr;74(4):721–30.
3. 3. Tao T, Xu N, Li J, Li H, Qu J, Yin H, et al. Ocular Features and Mutation Spectrum of Patients With Familial Exudative Vitreoretinopathy. Invest Ophthalmol Vis Sci. 2021 Dec 3;62(15):4.
4. 4. Huang L, Lu J, Wang Y, Sun L, Ding X. Familial Exudative Vitreoretinopathy and Systemic Abnormalities in Patients With CTNNB1 Mutations. Invest Ophthalmol Vis Sci. 2023 Feb 15;64(2):18.
5. 5. Rossetti LZ, Bekheirnia MR, Lewis AM, Mefford HC, Golden-Grant K, Tarczy-Hornoch K, et al. Missense variants in CTNNB1 can be associated with vitreoretinopathy—Seven new cases of CTNNB1 -associated neurodevelopmental disorder including a previously unreported retinal phenotype. Mol Genet Genomic Med [Internet]. 2021 Jan [cited 2023 Jul 25];9(1). Available from: https://onlinelibrary.wiley.com/doi/10.1002/mgg3.1542
6. 6. Miroševič Š, Khandelwal S, Sušjan P, Žakelj N, Gosar D, Forstnerič V, et al. Correlation between Phenotype and Genotype in CTNNB1 Syndrome: A Systematic Review of the Literature. IJMS. 2022 Oct 19;23(20):12564.
7. 7. the Genetic Factors for Osteoporosis (GEFOS) Consortium. Twenty bone-mineral-density loci identified by large-scale meta-analysis of genome-wide association studies. Nat Genet. 2009 Nov;41(11):1199–206.
8. 8. Pertusa C, Ruzo SP, Panach L, Mifsut D, Tarín JJ, Cano A, et al. Polymorphisms in Genes Involved in Osteoblast Differentiation and Function Are Associated with Anthropometric Phenotypes in Spanish Women. Genes. 2021 Dec 17;12(12):2012.
9. 9. Hill TP, Später D, Taketo MM, Birchmeier W, Hartmann C. Canonical Wnt/β-Catenin Signaling Prevents Osteoblasts from Differentiating into Chondrocytes. Developmental Cell. 2005 May;8(5):727–38.
10. 10. Glass DA, Bialek P, Ahn JD, Starbuck M, Patel MS, Clevers H, et al. Canonical Wnt Signaling in Differentiated Osteoblasts Controls Osteoclast Differentiation. Developmental Cell. 2005 May;8(5):751–64.

Severe hypocalcaemia postoperatively from a laryngectomy, total thyroidectomy and total parathyroidectomy requiring Teriparatide

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Summary

A 71-year-old gentleman underwent a salvage total laryngectomy and bilateral selective neck dissection for recurrent glottic squamous cell carcinoma that included a total thyroidectomy and total parathyroidectomy. Post-operatively complicated by severe hypoparathyroidism and hypocalcaemia, which required intravenous and oral calcium replacement and Teriparatide on a short-term basis to maintain normocalcaemia. Pre-operatively, his corrected calcium level was 2.55mmol/L (RR 2.10-2.60), ionised calcium level 1.21mmol/L (RR 1.15-1.30), and an intact PTH level 5.2pmol/L (RR 1.6-7.2). Renal function and other electrolytes were normal. Post-operatively, the corrected calcium level remained intact at 2.45mmol/L, however the PTH level was undetectable at <0.4pmol/L with an ionised calcium level of 0.91mmol/L but no symptoms of hypocalcaemia. He required 4.4mmol/L of calcium gluconate TDS a day intravenously as well as oral calcium carbonate 1200mg TDS, and 0.25mcg calcitriol for replacement of calcium for a total of 19 days whilst an inpatient, and oral replacement was continued on discharge. Due to the persistently low hypocalcaemia Teriparatide 20mcg BD SC was commenced and then weaned on discharge.

Brief Outline of Literature

Acute hypocalcaemia is considered a medical emergency and requires rapid treatment with a combination of calcium and vitamin D supplementation, with severe cases requiring intravenous calcium supplementation.[1,-3] A novel approach to the treatment of severe hypocalcaemia and hypoparathyroidism not responsive to conventional therapy is the use of Teriparatide. The studies, although small and limited, have shown that it is efficacious in improving hypocalcaemia and reducing the supplementation required to maintain normocalcaemia post-operatively.[4-7]

Long-term management of hypoparathyroidism involves aiming for a serum calcium level towards the low normal reference range to avoid symptoms, associated complications, and to preserve bone health. Part of therapy is to prevent hypercalciuria, and to reduce the total amount of calcium supplementation required, hydrochlorothiazide can be added to the daily regimen.[1,3] A long-term risk with oral calcium supplementation is the potential for renal calculi, and there is also the added component of pill burden. For patients with difficult to control or recalcitrant hypocalcaemia/hypoparathyroidism, teriparatide is an option for long-term management beyond a few weeks.[8-9]

1. Lechner, M., Moghul, G., Chandrasekharan, D., Ashraf, S., Emanuel, O., Magos, T., et al. (2023). Preoperative and postoperative optimisation of patients undergoing thyroid surgery: a multicentre qualify improvement project at Barts Health NHS Trust. BMJ Open Quality 12; 1-7.
2. Chen, Z., Zhao, Q., Du, J., Wang, Y., Han, R., Xu, C., et al. (2021). Risk factors for postoperative hypocalcaemia after thyroidectomy: A systematic review and meta-analysis. J Int Med Res 49(3); 1-21.
3. Orloff, LA., Wiseman, SM., Bernet, VJ., Faheylll, TJ., Shaha, AR., Shindo, ML., et al. (2018). American Thyroid Association Statement on Postoperative Hypoparathyroidism: Diagnosis, Prevention, and Management in Adults. Thyroid 28(7); 830-841.
4. Shah, M., Bancos, I., & Thompson, GB. (2015). Teriparatide Therapy and Reduced Postoperative Hospitilization for Postsurgical Hypoparathyroidism. JAMA Otolaryngol Head Neck Surg 141(9); 822-829.
5. Cheng, ML., & Gupta, V. (2012). Teriparatide – Indications beyond osteoporosis. Indian J Endocrinol Metab 16(3); 343-348.
6. Andrysiak-Mamos, Elzbieta, Zochowska, E., Kazmierczyk-Puchalska, A., Popow, M., Kaczmarska-Turek, D., Pachucki, J., et al. (2016). Treatment of severe life-threatening hypocalcaemia with recombinant human teriparatide in patients with postoperative hypoparathyroidism – a case series. Endokrynol Polska 67(4); 403-412.
7. Marcucci, G., Masi, L., Cianferotti, L., Giusti, F., Fossi, C., Parri, S., et al. (2021). Chronic hypoparathyroidism and treatment with teriparatide. Endocrine 72(1); 249-259.
8. Cusano, NE., Rubin, MR., McMahon, DJ., Zhang, C., Ives, R., Tulley, A., (2013). Therapy of Hypoparathyroidism with PTH(1-84): A Prospective Four-Year Investigation of Efficacy and Safety. JCEM 98; 137-144.
9. Upreti, V., Somani, S., & Kotwal, N. (2017). Efficacy of Teriparatide in Patients with Hypoparathyroidism: A Prospective, Open-label Study. Indian J Endocrinol Metab 21(3); 415-418.

Bone tumours with giant cell proliferation on histology in primary hyperparathyroidism: Are they always brown tumours?

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Brown tumours can be difficult to differentiate from giant cell tumours of bone (GCTB) as both tumours consist of mononuclear stromal cells with large multinucleated giant cells on histology (1). Calcium and parathyroid hormone (PTH) levels can be useful indicators of the underlying pathology but can occasionally be misleading.

A 71-year-old female was incidentally found to have an elevated adjusted serum calcium of 2.59mmol/L with a PTH level of 14pmol/L (1.6-6.9pmol/L) in 2017 and reviewed by endocrinology. She had a background of Parkinson's disease diagnosed in 2018 and a patella fracture following a fall at home in 2010 requiring surgery. A hysterectomy and bilateral salpingo-oophorectomy for severe endometriosis was performed at age 34 with subsequent hormone replacement therapy for less than ten years. In 2018 her plasma 25-hydroxyvitamin D was 47nmol/L (50-150nmol/L), and was adequate on repeat testing after supplementation, normal liver function tests and 24-hour urine calcium creatinine ratio of 0.018. She sustained a plain film proven fracture of her left distal radius and ulna in 2019 after falling from a standing height, which was reduced and casted. Despite this, she suffered ongoing left wrist deformity and swelling.

DEXA bone density scan in February 2020 showed a left forearm T-score of -3.4, left hip T score -0.9, L1-L4 T score of 0.0, Garvan score of 11% ten-year hip fracture risk and 39% of any osteoporotic fracture risk. Nuclear medicine parathyroid uptake scan in October 2020 revealed uptake consistent with a right superior parathyroid adenoma. Neck ultrasound was non-diagnostic for an adenoma. She was referred to the endocrine surgeons in April 2021 and underwent a minimal access focused right superior parathyroidectomy for primary hyperparathyroidism in July 2022. Histology was consistent with a parathyroid adenoma weighing 0.69g. Repeat calcium in January 2023 was 2.34 mmol/L with PTH remaining mildly elevated at 7.1pmol/L. She was commenced on alendronate from January 2022 and switched to zoledronic acid in August 2022.

She re-presented one month after her parathyroidectomy in August 2022 due to acute on chronic pain and deformity of her left wrist having suffered a minor wrist injury 2 months prior. X-ray showed a lucent lesion in the distal meta-diaphysis of the radius that was not present on x-rays in 2019 or on follow up x-rays in 2020. MRI of her left wrist showed a multiloculated mixed cystic and solid lesion with areas of cortical thinning and destruction with surrounding periosteal oedema. Ultrasound-guided biopsy of the left distal radius in September 2022 showed cancellous bone composed almost entirely of tightly packed giant cells with osteoclast erosion of trabecular bone. The patient was given a presumptive diagnosis of brown tumour related to her recent surgically treated primary hyperparathyroidism.

She underwent left distal radius excision, fusion, and allograft of the tumour in February 2023 due to worsening left wrist swelling. Initial histology was again suggestive of a brown tumour. However, a GCTB was subsequently diagnosed, with stromal tumour cells showing strong nuclear positivity for histone H3.3 G34W on immunohistochemistry with breach of the bone cortex and tumour involving periosteum. The recommendation of the sarcoma MDM was for surgical revision and further imaging. Whole body turbo stir MRI in March 2023 showed generalised increased marrow trabecular signal with no discrete geographic lesion to suggest further destructive osseous lesions.

This is an unusual case that demonstrates the presumed coincidental diagnosis of primary hyperparathyroidism with a GCTB rather than the expected diagnosis of brown tumour. Brown tumours are well known to be associated with primary hyperparathyroidism, and solitary small brown tumours often regress post-parathyroidectomy.

GCTB represent 4-5% of primary bone tumours with a peak onset of 20-39 years and generally occur after skeletal maturity (2). There is a significantly higher incidence in Asian populations, with Paget's disease thought to be a risk factor for their development (3). They commonly involve both the metaphysis and the epiphysis (4).

GCTB are locally aggressive but rarely metastasize. H3F3A gene mutation is detected in neoplastic stromal cells in as many as 95% of giant cell tumours (1). Immunohistochemistry shows the presence of mutant histone variant H3.3 G34W which is absent in brown tumours, and the expression of H3.3 G34V and H3.3 G34R have also been reported (1). H3.3G34W was found by Amary et al. in 2017 to be a sensitive and specific marker to differentiate GCTB from its mimics, particularly brown tumours. It is incorporated into chromatin and can be associated with epigenetic alteration on DNA methylation, chromatin accessibility and histone modification (5). RANK ligand is highly expressed by the stromal cells within GCTB which stimulates recruitment of osteoclastic cells from normal monocytic preosteoclast cells (6). The osteoclastic giant cells then absorb bone via a cathepsin K and matrix metalloproteinase 13 mediated process resulting in osteolysis (7).

Treatment ranges from intralesional options such as curettage to en bloc resection for extraosseous extension with or without reconstruction. The postoperative recurrence rates have been reported to be 10-65% (8). Denosumab use both in the neoadjuvant setting and where surgery is contraindicated can be helpful with a reduction in H3.3 G34W positive tumour cells and a decrease in osteoclastic giant cells accompanied by matrix and osteoid formation (9). Radiation therapy, arterial embolization and radiofrequency ablation should also be considered if surgery is contraindicated.

Take home messages:

- Brown tumors and GCTB are types of osteoclastomas that can be difficult to differentiate from each other with similar clinical, radiological and histology findings.
- Confirming a tissue diagnosis with immunohistochemistry to differentiate these bone tumours from one another is particularly important in cases with atypical clinical and radiological findings for brown tumours, even in the setting of primary hyperparathyroidism, as treatment is significantly different for both tumours.
- 3 G34W immunohistochemistry is a sensitive and highly specific marker to facilitate the diagnosis of GCTB.
- Ensuring complete resection of GCTB is important to prevent the risk of future local recurrence, and rarely metastases.
- Denosumab has been shown to reduce H3.3 G34W positive tumour cells and osteoclastic giant cells as RANK ligand is highly expressed by stromal cells within GCTB

1. 1. Amelio JM, Rockberg J, Hernandez RK, Sobocki P, Stryker S, Bach BA, Engellau J, Liede A. Population-based study of giant cell tumor of bone in Sweden (1983-2011). Cancer Epidemiol. 2016 Jun;42:82-9.
2. 2. Hosseinzadeh S, De Jesus O. Giant Cell Tumor. 2023 Feb 12. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2023 Jan–
3. 3. Campanacci M, Baldini N, Boriani S, Sudanese A. Giant-cell tumor of bone. J Bone Joint Surg Am. 1987 Jan;69(1)
4. 4. Yamamoto H et al. Diagnostic utility of histone H3.3 G34W, G34R, and G34V mutant-specific antibodies for giant cell tumors of bone. Hum Pathol. 2018 Mar;73:41-50
5. 5. Lutsik P et al. Globally altered epigenetic landscape and delayed osteogenic differentiation in H3.3-G34W-mutant giant cell tumor of bone. Nat Commun. 2020 Oct 27;11(1):5414.
6. 6. Lau YS, Sabokbar A, Gibbons CL, Giele H, Athanasou N. Phenotypic and molecular studies of giant-cell tumors of bone and soft tissue. Hum Pathol. 2005 Sep;36(9):945-54. doi: 10.1016/j.humpath.2005.07.005. PMID: 16153456.
7. 7. Lindeman JH, Hanemaaijer R, Mulder A, Dijkstra PD, Szuhai K, Bromme D, Verheijen JH, Hogendoorn PC. Cathepsin K is the principal protease in giant cell tumor of bone. Am J Pathol. 2004 Aug;165(2):593-600
8. 8. Hu P, Zhao L, Zhang H, Yu X, Wang Z, Ye Z, Wu S, Guo S, Zhang G, Wang J, Ning X, Hu Y, Zhang Y. Recurrence Rates and Risk Factors for Primary Giant Cell Tumors around the Knee: A Multicentre Retrospective Study in China. Sci Rep. 2016 Nov 9;6:36332
9. 9. Lüke J, von Baer A, Schreiber J, Lübbehüsen C, Breining T, Mellert K, Marienfeld R, Schultheiss M, Möller P, Barth TFE. H3F3A mutation in giant cell tumour of the bone is detected by immunohistochemistry using a monoclonal antibody against the G34W mutated site of the histone H3.3 variant. Histopathology. 2017 Jul;71(1):125-133

Management of osteoporosis and fracture risk in a 30-year-old 6 months post-partum woman with adult-onset hypophosphatasia (HPP)

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Introduction: HPP is caused by a missense mutation in the ALPL gene, which is inherited in either an autosomal recessive or dominant pattern, and results in loss or decreased activity of the TNSALP (Tissue Non-Specific Alkaline Phosphatase) enzyme[1]. It is rare, with a prevalence of 1 in 100000 births[2], often resulting in a diagnostic delay.

Case presentation: A 30-year-old lady was referred to the Endocrine department by clinical genetics after being sent for DNA analysis following lethal skeletal dysplasia in two pregnancies. This confirmed carrier ALPL mutation status c.931G>A.

She had a mild phenotype hypophosphatasia with known dental enamel hypoplasia. Her husband also had an ALPL gene mutation (c 650 T > C variant).

She had a previous history of a wrist fracture as a child (fall from standing height) and shin pains with running but no fractures. She also has a history of knee pain with effusion, in keeping with a patellofemoral syndrome rather than hypophosphatasia. She had one serving of dairy daily and enjoyed walking. She never required steroids and had regular menstrual cycles since menarche at 14.

Medications included Vitamin D 1000 units daily and a pregnancy multivitamin.

Her family history includes osteoporosis in her mother at age 28 who sustained a coccygeal fracture during pregnancy and was treated with zoledronic acid infusions annually though was later found to carry the same ALPL mutation. Her grandfather had osteoporosis aged 40-50. Her grandmother had dental issues at the age of 20 requiring full dentures.

On examination, there were no clinical manifestations besides dental enamel hypoplasia.

On presentation in 2019 she had low alkaline phosphatase at 25 IU/ L (30-100 RR) with a repeat level of 30 IU/L in 2020. She had an elevated pyridoxal-5, -phosphate (PLP) in 2019 of 145 nmol/L (35-110). PLP is a substrate of ALP and due to the ALP mutation, the substrate accumulates. Another substrate, phosphoethanolamine is excreted in the urine, however, her levels were normal. In keeping with the low bone turnover state her CTx1 was low at 101 ng/ L (150-800) but her DPD and DPX cross-links were normal. Vitamin D level was normal. Her Bone Mineral Density (BMD) in mid 2020 demonstrated a femoral neck T score of -1.8 SD, Z score of -1.8 SD and a lumbar spine T score of 0.1 SD, Z-score - 0.2 SD.

She was advised to do weight-bearing exercises and incorporate 3 servings of dairy into her diet. There were no additional recommendations for pregnancy planning. She was advised that if she does experience a fracture or reduction in BMD she should avoid bisphosphonates as this can increase the fracture risk and that teriparatide could be considered outside of pregnancy. She was recommended for review in 12 months with BMD.

She subsequently had a natural pregnancy with no evidence of skeletal dysplasia and was reviewed six months post-partum at which point she was breastfeeding. There was no further fracture history though she did have chronic lower back pain post-delivery. ALP was at the lower end of normal at 43 U/L with a vitamin D of 72 nmol/ L. She had evidence of worsening BMD in December 2022 with a Z-score of -2.8 in her total right femur (T-score of -2.4). Lumbar spine Z score was -0.5 with a T score of -0.5.

She was recommended to cease breastfeeding immediately and for review in 9 months with repeat BMD and if worsening, to consider teriparatide. She has since had a CT pelvis to investigate the longstanding coccygeal pain post-delivery, which suggests an undisplaced cortical fracture of the first coccygeal segment. This now poses the dilemma of how best to manage her osteoporosis.

Literature:

There is conflicting evidence on the effect of lactation on BMD, with multiple cross-sectional and cohort studies suggesting a negative association between duration lactation and BMD[3]. Additional studies suggest a positive or no association between lactation and BMD[3] and hence the management of osteoporosis in breastfeeding women with HPP is unclear.

As the skeletal disease of HPP results from the extracellular accumulation of the TNSALP substrate inorganic pyrophosphate (PPi) and its inhibitory effect on mineralization, HPP patients or carriers have adverse effects from bisphosphonates.[4] Bisphosphonates are analogues of PPi and can suppress bone turnover but also deactivate TNSALP.[4]

Currently, evidence is limited to several case reports which demonstrate atypical femoral fractures during bisphosphonate exposure in adults with hypophosphatasia[4].

Evidence for teriparatide is limited. Treatment with teriparatide to increase osteoblast production of ALP has been reported to date in a total of 10 adult patients. However, its effects on BMD have been variable[5]. Teriparatide reportedly improved pain, mobility, and fracture repair in two sisters with HPP ages 56 and 64 years and can be considered as a treatment option[6].

There is a single case report of romosozumab use in an 81-year-old with hypophosphatasia and osteoporosis. Though ALP remained the same, after 1 year of Romosuzumab therapy, BMD improved by 21%, and 10% at the lumbar spine and total hip, respectively, suggesting benefit[7].

There is promising evidence for asfotase alfa, an enzyme replacement therapy developed to treat HPP. A multi-centre randomised open-label study evaluating the efficacy and safety of asfotase alfa in patients aged 13-66 years with HPP demonstrated improvement in both PLP and PPi within subjects after 6 months and over 5 years of treatment with asfotase alfa ($P < 0.05$). There was also improvement in gross motor function, muscle strength and reported functional disability over 5 years of treatment however no improvement in mean BMD. [8]

Take-home:

1/ If you note a low ALP and features of osteoporosis, consider HPP.

2/ Bisphosphonates are associated with an increased risk of atypical femoral fractures in HPP.

3/ There is conflicting data to suggest lactation increases bone turnover.

4/ There is limited evidence for teriparatide, romosozumab and asfotase alfa in the management of osteoporosis in HPP.

1. Galeano-Valle F, Vengoechea J, Galindo RJ. A rare mutation in hypophosphatasia: a case report of adult form and review of the literature. Arch Endocrinol Metab. 2019 Feb;63(1):89-93. doi: 10.20945/2359-3997000000108. PMID: 30864637; PMCID: PMC10118836.
2. Nunes ME. Hypophosphatasia. 2007 Nov 20 [Updated 2023 Mar 30]. In: Adam MP, Mirzaa GM, Pagon RA, et al., editors. GeneReviews® [Internet]. Seattle (WA): University of Washington, Seattle; 1993-2023. Available from: https://www.ncbi.nlm.nih.gov/books/NBK1150/
3. Salari P, Abdollahi M. The influence of pregnancy and lactation on maternal bone health: a systematic review. J Family Reprod Health. 2014 Dec;8(4):135-48. PMID: 25530765; PMCID: PMC4266784.
4. Sutton RA, Mumm S, Coburn SP, Ericson KL, Whyte MP. "Atypical femoral fractures" during bisphosphonate exposure in adult hypophosphatasia. J Bone Miner Res. 2012 May;27(5):987-94. doi: 10.1002/jbmr.1565. PMID: 22322541.
5. Camacho PM, Mazhari AM, Wilczynski C, Kadanoff R, Mumm S, Whyte MP. ADULT HYPOPHOSPHATASIA TREATED WITH TERIPARATIDE: REPORT OF 2 PATIENTS AND REVIEW OF THE LITERATURE. Endocr Pract. 2016 Aug;22(8):941-50. doi: 10.4158/EP15890.OR. Epub 2016 Apr 4. PMID: 27042741.
6. Camilla Schalin-Jäntti and others, Parathyroid Hormone Treatment Improves Pain and Fracture Healing in Adult Hypophosphatasia, The Journal of Clinical Endocrinology & Metabolism, Volume 95, Issue 12, 1 December 2010, Pages 5174–5179, https://doi.org/10.1210/jc.2010-1168
7. Khanjee N, Maalouf NM. Romosozumab-aqqg in the Treatment of Osteoporosis in a Patient With Hypophosphatasia. J Endocr Soc. 2022 Oct 27;6(12):bvac159. doi: 10.1210/jendso/bvac159. PMID: 36381012; PMCID: PMC9646966.
8. Kishnani PS, Rockman-Greenberg C, Rauch F, Bhatti MT, Moseley S, Denker AE, Watsky E, Whyte MP. Five-year efficacy and safety of asfotase alfa therapy for adults and adolescents with hypophosphatasia. Bone. 2019 Apr;121:149-162. doi: 10.1016/j.bone.2018.12.011. Epub 2018 Dec 18. PMID: 30576866.

Rare cause of osteoporosis secondary to systemic mastocytosis

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Introduction

Systemic mastocytosis (SM) is a clonal disorder of mast cells and is a rare cause of osteoporosis. The prevalence of osteoporosis is about 20-28% in patients with indolent systemic mastocytosis (ISM) (1)(2) with the spine being the predominating site involved (3). We report two cases of osteoporosis secondary to ISM presenting with vertebral fractures.

Cases

A 47-year-old Pakeha male farmer presented following low-trauma multi-level vertebral fractures. DEXA bone mineral density (BMD) scan confirmed osteopenia of L1-L4 spine (T-score -2.3 SD and Z-score of -2.6 SD). His past medical history included asthma, ex-cigarette smoker, current cannabis use, moderate alcohol intake and previous carpal tunnel release. He had been diagnosed with cutaneous mastocytosis 4 years prior however, was not on treatment for this. Medications included asthma inhalers and proton-pump inhibitor for reflux. Extensive investigation did not reveal an underlying endocrine cause of fragility fracture. The diagnosis of indolent systemic mastocytosis was confirmed with elevated tryptase 26.1 ug/L (RI 0-10), bone marrow aspirate confirmed the presence of an aberrant population of mast cells and cKIT D816V mutation detected on molecular studies. He was started on vitamin D supplementation and bisphosphonates with a plan to repeat BMD after 2 years. He takes antihistamine medication for symptom relief and remains on surveillance for progression of systemic mastocytosis.

A 72-year-old New Zealand European female presented following low trauma L2 vertebral compression fracture and BMD T score -2.9 SD of the spine. Menopause was at age 50, followed by hormone replacement therapy (HRT) until age 68. She kept active and was a lifelong non-smoker. She had a long-standing rash on her thigh, a biopsy done 10 years prior suggested cutaneous mastocytosis. Other past medical history includes a platelet function disorder, previous cholecystectomy, hysterectomy and right total knee joint replacement. Investigations excluded underlying endocrine causes of osteoporosis but found she had an elevated tryptase (28.4ug/L {RI 0-10}) and positive cKIT D816V mutation. She is currently awaiting a bone marrow biopsy with the working diagnosis of indolent systemic mastocytosis. Current management is with Vitamin D replacement and bisphosphonates.

Conclusion

These cases highlight ISM as a rare but important cause of secondary osteoporosis. The diagnosis should be considered when common secondary causes of osteoporosis are excluded or in patients with features of cutaneous mastocytosis and elevated tryptase.

take home points

- Systemic mastocytosis is a rare but recognised cause of osteoporosis, often involving the vertebra.

- Osteoporotic fractures may be the first clinical presentation of the condition, sometimes in addition to cutaneous mastocytosis rash.

- When there is clinical suspicion, initial investigations include tryptase levels and cKIT D816V mutation molecular studies, in addition to Haematology input.

- Osteoporosis treatment involves treating underlying haemtological condition if indicated, in addition to usual osteoporosis treatment, in our cases, bisphosphonates were prescribed.

References

1. Rossini, M *et al*. Bone mineral density, bone turnover markers and fractures in patients with indolent systemic mastocytosis. Bone, Volume 49, Issue 4, 2011, Pages 880-885, ISSN 8756- 282. https://doi.org/10.1016/j.bone.2011.07.004.
2. Van der Veer, E *et al*. High prevalence of fractures and osteoporosis in patients with indolent systemic mastocytosis. Allergy. 2012 Mar;67(3):431-8. doi: 10.1111/j.1398-9995.2011.02780.x.
3. Rossini, M *et al*. Bone involvement and osteoporosis in mastocytosis. Immunol Allergy Clin North Am. 2014 May;34(2):383-96. doi: 10.1016/j.iac.2014.01.011.

1. Rossini, M et al. Bone mineral density, bone turnover markers and fractures in patients with indolent systemic mastocytosis. Bone, Volume 49, Issue 4, 2011, Pages 880-885, ISSN 8756- 282. https://doi.org/10.1016/j.bone.2011.07.004.
2. Van der Veer, E et al. High prevalence of fractures and osteoporosis in patients with indolent systemic mastocytosis. Allergy. 2012 Mar;67(3):431-8. doi: 10.1111/j.1398-9995.2011.02780.x.
3. Rossini, M et al. Bone involvement and osteoporosis in mastocytosis. Immunol Allergy Clin North Am. 2014 May;34(2):383-96. doi: 10.1016/j.iac.2014.01.011.

Bone microarchitecture and disorganized bone tissue in a young woman with pycnodysostosis and an atypical femur fracture: A case report

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BACKGROUND: Pycnodystosis (PYCD) is a rare autosomal recessive lysosomal storage disorder, involving a loss of function mutation of the gene encoding cathepsin K (CTSK). The loss of function of this osteoclastic lysosomal protease reduces bone resorption, increases bone mineral density (BMD), but impairs the structure and material properties of bone. This case report demonstrates that bone fragility in a young woman with PYCD, presenting with an atypical femur fracture (AFF), was not explained by changes in bone density but by microarchitectural defects, disorganized bone and microcracks.

CLINICAL CASE:

A 24-year-old woman with PYCD presented with an AFF in 2020 following a long history of prior minimal trauma fractures (MTF). PYCD was diagnosed in childhood and as an adult this patient displayed several signs and symptoms, including, but not limited to: short stature;

dystrophic nails; short fingers; prominent forehead; prognathism, and; a significant history of 10 peripheral MTFs. Her first MTF occurred at 6-years-of-age. No other medical conditions or significant family history were identified. Her parents were consanguineous.

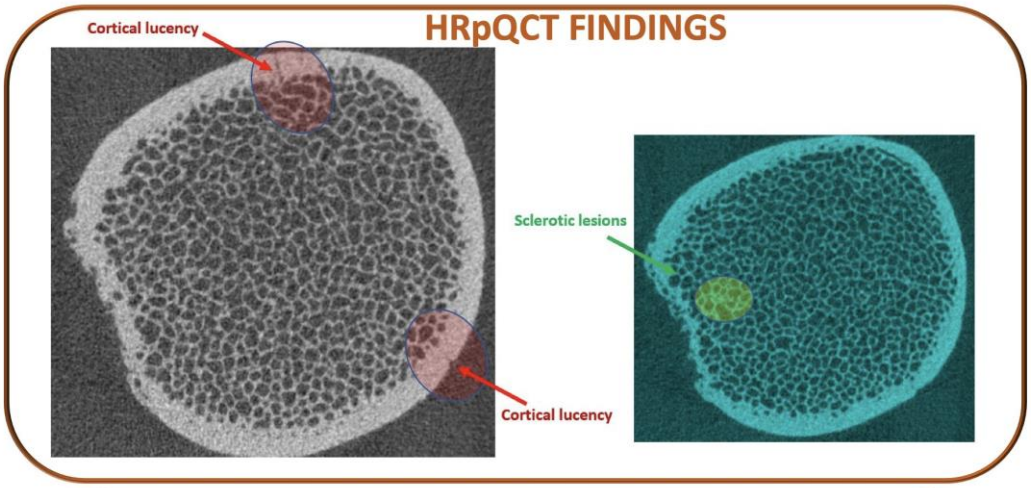
At the time of the AFF, the patient was treatment naïve. Three years prior she gave birth via Caesarean-section and was able to breastfeed her baby for 12 months. Following the AFF, her vitamin D was slightly reduced (45 nmol/L), while P1NP and CTX were high, at 124 ug/L and 518 ng/L, respectively. Other pathology results were unremarkable.

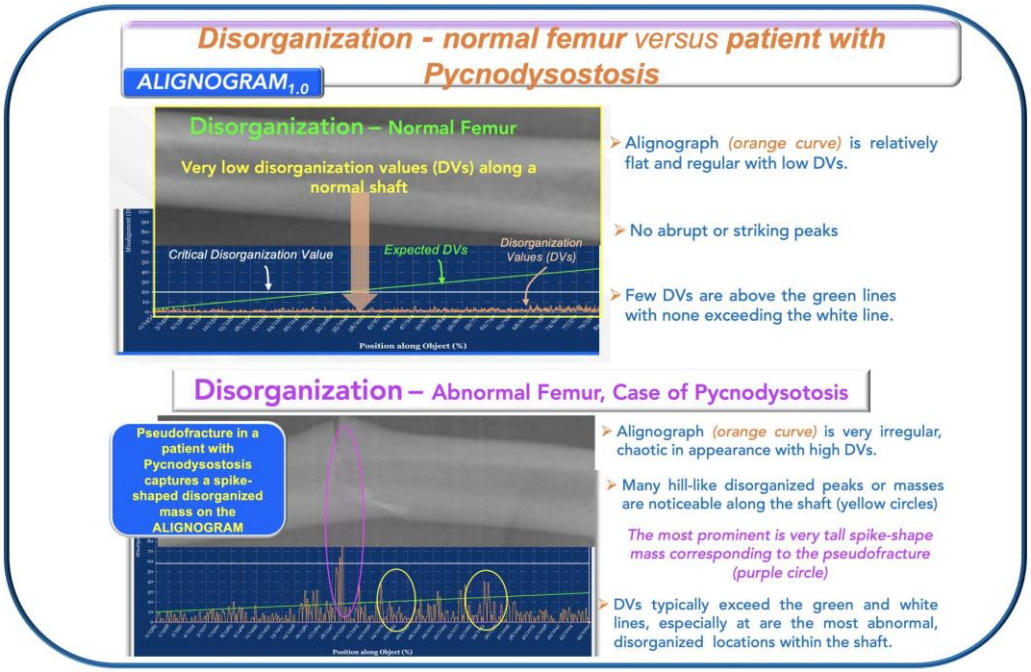
In the year following the AFF, BMD was high at the spine (T-score = +3.3), total hip (+5.4); and femoral neck (+6.7). High-resolution peripheral quantitative computed tomography (HR-pQCT) of the distal tibia detected unexpected cortical lucencies and sclerotic lesions in the trabecular compartment of the distal tibia. Both total and trabecular volumetric BMD (vBMD) were high and trabecular numbers were increased in both the distal radius and tibia. Disorganization analysis using a novel software (ALIGNOGRAM) showed chaotically disorganized femoral shaft with spike shape masses at locations corresponding to a pseudofracture and sclerotic lesions (Fig1).

CLINICAL CONCLUSION

These imaging techniques detected disorganized bone and microarchitectural defects in a treatment naïve patient with PYCD and an AFF. The suppression of bone resorption in PYCD allows the accumulation of microdamage and disorganized bone that produces bone fragility. Antiresorptive therapy should be avoided.

FIGURE


HRpQCT FINDINGS
Cortical lucency
Sclerotic lesions
Cortical lucency


Disorganization - normal femur versus patient with Pycnodysostosis
ALIGNOGRAM1.0
Disorganization – Normal Femur
Very low disorganization values (DVs) along a normal shaft
Critical Disorganization Value
Expected DVs
Disorganization Values (DVs)
Position along Object (%)
Alignograph (orange curve) is relatively flat and regular with low DVs.
No abrupt or striking peaks
Few DVs are above the green lines with none exceeding the white line.
Disorganization – Abnormal Femur, Case of Pycnodysotosis
Pseudofracture in a patient with Pycnodysostosis captures a spike-shaped disorganized mass on the ALIGNOGRAM
Position along Object (%)
Alignograph (orange curve) is very irregular, chaotic in appearance with high DVs.
Many hill-like disorganized peaks or masses are noticeable along the shaft (yellow circles)
The most prominent is very tall spike-shape mass corresponding to the pseudofracture (purple circle)
DVs typically exceed the green and white lines, especially at are the most abnormal, disorganized locations within the shaft.

Atypical femur fracture… What to do AFFTER Teriparatide?

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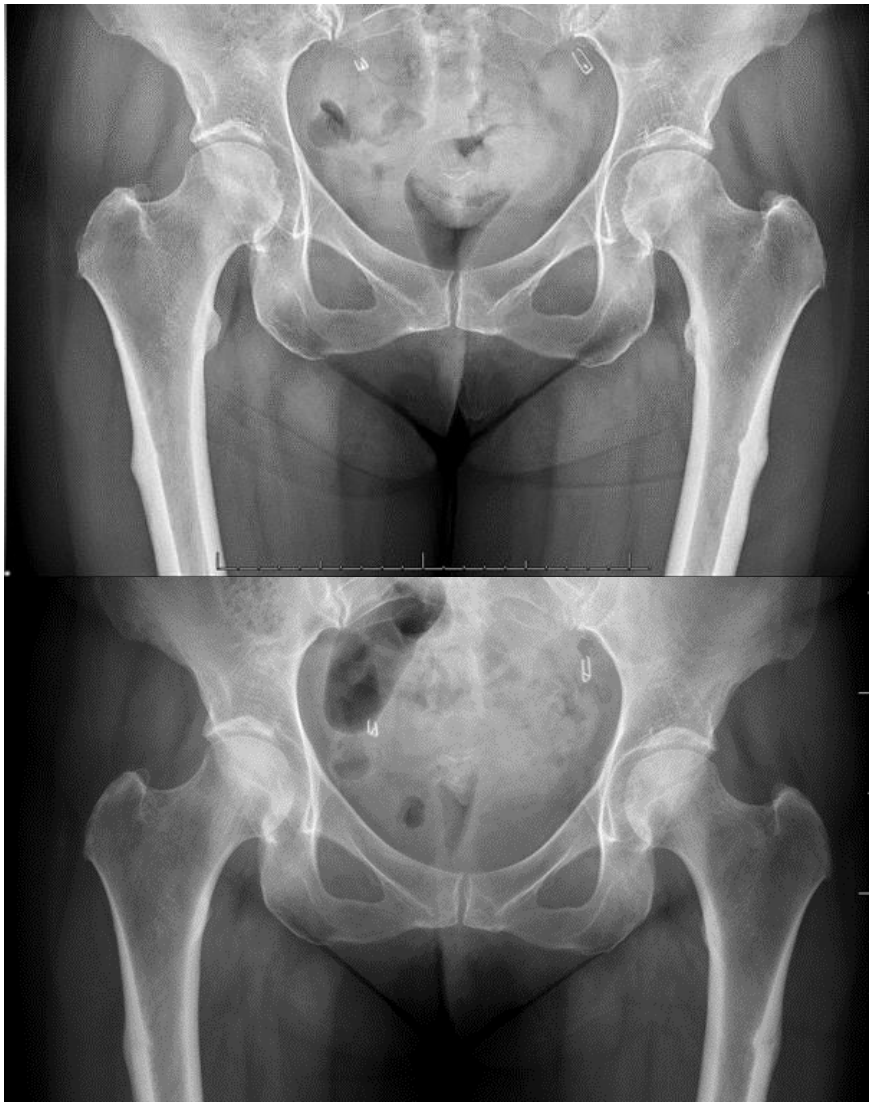

Atypical femur fracture… What to do AFFTER Teriparatide?

We present the case of Ms JA, a 73-year-old lady who developed bilateral atypical femur fractures that were treated conservatively with Teriparatide.

JA is originally from the UK and lives between Sydney and Southeast England. She lives with her husband, is a non-smoker, drinks 20g of alcohol per day and is generally fit and well for her age.

In addition to osteoporosis, her medical history includes a meningioma that was resected in 2012.

This was complicated by growth hormone deficiency which is treated with daily Somatropin 0.2 mg subcutaneous at night. She is also treated for hypertension, hypercholesterolemia and glaucoma. Her other medications include Ramipril 10mg, Pravastatin 10mg and vitamin D 2000 units daily.

In terms of her long-standing osteoporosis, JA had a minimal trauma distal radius fracture in 2007 and again in 2015, and compression fracture of T7 in 2010.

She commenced treatment with alendronate in 2009. At the time her spine T-score was -3.2 and total hip T-score was -1.5. In 2012 due to issues with gastrointestinal tolerability, she was changed to Denosumab 60mg six monthly injections.

In 2019, after 11 years of continuous anti-resorptive treatment, she developed bilateral thigh pain on walking. Her symptoms worsened progressively over the next 12-18 months with walking becoming difficult due to pain.

In October 2020 when living in the UK, X-ray demonstrated bilateral femoral sub trochanteric cortical thickening with a subtle fracture line on the left.

She was subsequently diagnosed with having bilateral atypical femur fractures (AFFs) and Denosumab was ceased with her final dose being in April 2020.

Fig 1. X-ray October 2020

JA declined surgical management with prophylactic intramedullary nail insertion. She was managed by a Rheumatologist and Teriparatide and Strontium ranelate were considered. She did not meet NICE criteria for Teriparatide, nor was her treating team successful in gaining approval for special access funding. Strontium ranelate was deemed too high risk in terms of cardiovascular risk in the setting JA's history of hypertension and hyperlipidaemia.

Consequently, she did not receive any immediate treatment, but her pain improved. By 12 months after her last Denosumab dose, she was walking up to 10,000 steps per day but still experienced a dull ache following more intense physical activity.

She returned to Australia and plain film from February 2021 (fig 2) demonstrated ongoing cortical thickening of the lateral cortex of both femurs, but fracture lines had improved.

Fig 2. X-rays February 2021 and ten months post Denosumab cessation.

March 2021 she was reviewed by the St Vincent's bone and calcium clinic.

DEXA scan demonstrated only slight reduction in bone density since cessation of Denosumab and spine T-score was -2.2 and hips scores ranged from -1.0 to -1.4.

As there was clinical and radiological improvement of the AFFS and bone density was relatively stable, conservative management with ongoing surveillance continued.

In May she was admitted with back pain and imaging demonstrated further vertebral compression fracture of T9.

Due to her high risk of further fractures, treatment was reinstated.

Romosuzomab was discussed but due to the paucity of data in atypical femur fractures, daily Teriparatide 20mcg injections were commenced in June 2021.

She returned back to the UK and hypercalcaemia was noted on routine bloods in August 2021 up to 2.90mmol/L. Medical advice in the UK was to decrease the Teriparatide to 20mcg second daily and cease her calcium supplement.

JA has continued on second daily Teriparatide and serum calcium has normalised and CTX and P1NP remain elevated consistent with Teriparatide treatment.

A CT scan from June 2022 demonstrated complete healing of the prior atypical femur fractures. Her bone density continued to decrease despite the Teriparatide which can be attributed to cessation of Denosumab and that Teriparatide was only alternate days dosing. Discussion: This case raises several complex clinical issues, including the nature, incidence and management of atypical femur fractures, specifically conservative management, and the use of Teriparatide rather than prophylactic intramedullary nails.

A JCEM review of case reports and cohort studies supports that Teriparatide may assist healing post atypical femur fracture, especially if surgically managed but the efficacy in non-surgically managed AFFs is less clear [i].

The authors recognise the reported data is not sufficient for an evidence-based recommendation of the use of teriparatide to accelerate healing of AFF. A high-quality RCT on the use of teriparatide (or abaloparatide) in healing of AFFs would aid clinical decision making, however of yet, such a study has not been completed.

This case also raises the very challenging clinical question of ongoing osteoporosis management following AFF for patients who remain at high risk of osteoporotic fractures.

Continuation of anti-resorptive treatment is associated with increased risk of contralateral AFF, however patients remain at high risk of further osteoporotic fracture.

Teriparatide is often recommended after atypical femur fracture and decreases risk of fragility fracture, however currently there are no guidelines for consolidation anti-resorptive treatment and expert opinion is recommended[ii]. Currently there is minimal data reviewing Romosozumab post AFF but this will likely change in coming years.

Managing side effects of Teriparatide, including hypercalcaemia is also raised. Although this is a usually a mild side-effect that does not impact treatment, there have been rare cases of severe hypercalcaemia causing acute renal failure[iii]. Finally, and quite specific to this case, is if her growth hormone deficiency, even if treated, contributed to her osteoporosis and development of atypical femur fractures. References:

[i] van de Laarschot, Denise M., et al. "Medical management of patients after atypical femur fractures: a systematic review and recommendations from the European Calcified Tissue Society." *The Journal of Clinical Endocrinology & Metabolism* 105.5 (2020): 1682-1699.

[ii] Bégin, Marie-Josée, et al. "Fracture risk following an atypical femoral fracture." *Journal of Bone and Mineral Research* 37.1 (2022): 87-94.

[iii] Karatoprak, Cumali, et al. "Severe hypercalcemia due to teriparatide." *Indian journal of pharmacology* 44.2 (2012): 270.

Fragile Foundations: A Genetic Cause of Osteoporosis

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Case

A 24-year-old female presented with more than 30 fractures throughout her childhood and adult life. Her risk factors for osteoporosis included long-term prednisolone use, minimal calcium intake, low vitamin D and oligomenorrhoea.

Her past medical history was significant for chronic constipation identified in early infancy. She previously had a cecostomy tube, and multiple electronic stimulations with no benefit. She currently managed with two litres of colonLYTLEY daily via a nasogastric tube.

Other past medical history included chronic eczema, on high dose prednisolone (25-50mg daily) for 6 years, and she had lost contact with dermatology. She was obese, with a BMI of 56, with limited response to very low energy diets or phentermine, and financial restrictions prohibited commencement of a GLP-1 analogue.

At presentation, her medications included prednisolone 25mg daily, colonLYTLEY 2L daily, topiramate 50mg BD, and an implanon in-situ. She was a non-smoker, with no family history of osteoporosis.

Her obese BMI precluded a DXA attainment at the spine and hip, but wrist bone density was normal. Initial management included dietary optimisation and restoration of vitamin D levels. Given her significant fracture history and risk factors, zoledronic acid was administered, but was complicated by significant extravasation. This resulted in early cessation of the infusion, and later cellulitis. Bone turnover markers pre- and post-infusion showed a mild reduction, indicating some degree of bone suppression from the partial zoledronic acid dose.

Consent for genetic testing was taken, and showed an autosomal dominant heterozygous mutation in the LRP5 gene, a likely de novo pathogenic mutation. After expert discussion, an application was made for compassionate access romosozumab for further management of her bone health.

Re-engagement and review by dermatology led to reduction in prednisolone to 7.5mg daily, and bariatric review allowed commencement of hospital funded semaglutide for weight-loss.

Her relevant investigations at presentation are listed below:

Na	138
K	4.5
Mg	0.80
Adj Ca	2.54
PO4	1.36
eGFR	>90
Creat	60
Vitamin D	17
TSH	1.94
HbA1c	5.2

CLINICAL EXOME SCREENING

Clinical Notes: Colonic dysmotility / neuronal intestinal dysplasia and osteoporosis with 33#.

Phenotype (HPO): **HP:0000939** Osteoporosis

HP:0002579 Gastrointestinal dysmotility

Test Performed: Gastrointestinal neuromuscular disease v1.15
Osteogenesis Imperfecta v0.65

Results: **A likely pathogenic variant was identified in LRP5.**

Variants related to phenotype:

Gene Name	Variant in HGVS Nomenclature	Exon Location	Zygosity	Inheri-tance	Disease	ACMG Classification
LRP5	Chr11: NC_000011.10:g.68386605 delG NM_002335.4:c.1307delG p.(Gly436Alafs*7)	6	Hetero-zygous	AD	LRP5-related osteoporosis	Likely Pathogenic

Legend: AD = Autosomal Dominant.

Interpretation:
This genetic result is consistent with a diagnosis of osteoporosis. No medically significant variants were identified in the clinical context of gastrointestinal neuromuscular disease. Lack of finding does not exclude a genetic diagnosis. Not all types of variants can be reliably detected using this assay (see Limitations for details). The results should be reviewed in the context of the patient's clinical presentation and progression.

Bone Density Scan GE Lunar
-Right forearm BMD is 0.909grams/cm^2
-Z-score +0.2, T-score +0.2

Thoracolumbar x-ray
-no fractures

Discussion

The Wnt signalling pathway plays a critical role in bone metabolism [1,2,3]. The Wnt ligand binds to LRP5/6 and Fzd receptors on the cell surface, inducing an intracellular signalling cascade causing increased beta-catenin in the nucleus [3,4]. Beta-catenin promotes the progression of mesenchymal stem cells from osteoblastic precursor cells into mature osteoblasts, while suppressing differentiation into adipogenic and chondrogenic lineages [1,2,4,5]. (See Figure 1).

A mutation in the LRP5 gene causes a defect in the Wnt signalling cascade, resulting in decreased osteoblasts, and thus decreased bone formation [3]. Osteoporosis-pseudoglioma syndrome (OPPG) is a rare autosomal recessive disorder of severe juvenile osteoporosis and congenital blindness, due to biallelic mutations in the LRP5 gene [3].

Heterozygous LRP5 loss-of-function mutations cause juvenile onset osteoporosis and familial exudative vitreoretinopathy. Although a less severe phenotype than OPPG, it still results in increased fractures from childhood, and patients are at risk of blindness due to premature arrest of the retinal vasculature [5].

Treatment for patients with LRP5 mutations is not well established, limited to case reports and series.

Bisphosphonates appear safe and effective in this cohort [6]. A case series in children with OPPG demonstrates improvement in bone density in 4 patients treated with oral bisphosphonates over a 1.5-6-year period [6]. They had increases in Z-scores and decreased fractures, with 3 patients having normalisation of bone density.

Denosumab is uncommonly used due to the young age of patients, although theoretically will have similar effects to bisphosphonates. A case report of a 19-year-old with OPPG showed increased bone density of 26.4% in the lumbar spine after 12-months of denosumab therapy [7].

Teriparatide is a PTH analogue which stimulates osteoblastic function [8]. Theoretically, osteoanabolic therapy may be more successful than anti-resorptive therapy in patients with LRP5 mutations, given the principal defect is reduced bone formation. Again, the research is limited to case studies. A 19-year-old with OPPG completed 24-months of teriparatide therapy, after 6 years of bisphosphonate treatment. He had an ensuing 10% increase in bone density, with no fractures on therapy [9].

Romosozumab is a humanised monoclonal sclerostin-neutralising antibody that binds to and inhibits sclerostin. Sclerostin is a Wnt antagonists, binding to LRP5/6, thus resulting in inhibition of bone formation [6]. It has been proposed that romosozumab may be less effective in patients with LRP5 mutations, whereby sclerostin already does not effectively bind the receptor.

However, a study found dual LRP5 and sclerostin knock-out mice had stronger bones than mice solely lacking the LRP5 gene. The LRP5 deficient mice were then treated with anti-sclerostin therapy for 3-weeks, and showed improvement in bone density [10]. The hypothesis being without LRP5, the anabolic effects of sclerostin depletion occur via other receptors (such as LRP4/6).

Novel therapies for osteoporosis include antibodies targeting the LRP6 receptor, resulting in stimulation of the Wnt signaling pathway, and anti-DKK therapy (DKK being another Wnt antagonist). A study in mice with and without multiple myeloma showed improvements in trabecular bone volume after LRP6 antibody treatment. The combination treatment of LRP6 antibodies and anti-DKK therapy more than doubled trabecular bone volume [11]. This is a promising area for patients with LRP5 mutations, as they are likely more reliant on LRP6. By increasing the activity of the Wnt pathway via LRP6, these patients may have significant gains in bone density.

Take Home Messages

- The Wnt pathway plays a critical role in bone remodelling, and is already targeted for anti-osteoporosis therapy (romosozumab)
- The optimal therapy for patients with LRP5 mutations causing osteoporosis is yet to be determined, currently most evidence for safety and efficacy lies with bisphosphonates
- Romosozumab may be beneficial in these patients given alternate receptors involved in the Wnt signaling pathway
- Novel therapies such as LRP6 antibodies may be particularly useful in this cohort
- Other osteoporosis risk factors including long-term steroid use, and suboptimal vitamin D levels are significant contributors to this patient's osteoporosis, and thus should be managed as a priority

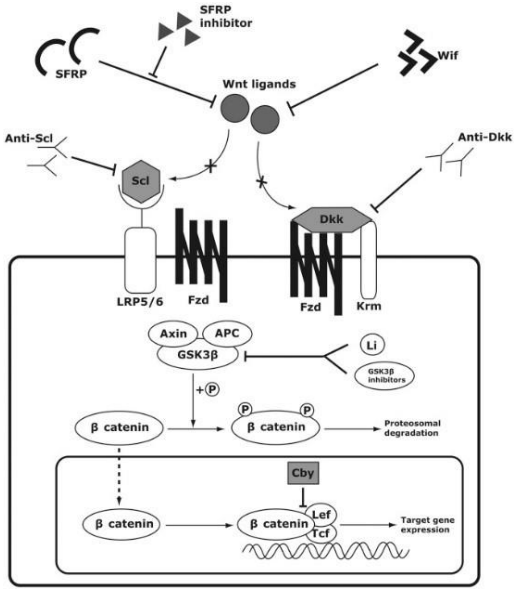

Figure 1 The Wnt signalling pathway, showing potential therapeutic targets [1].

1. Kim JH, Liu X, Wang J, Chen X, Zhang H, Kim SH et al. Wnt signaling in bone formation and its therapeutic potential for bone diseases. Ther Adv Musculoskelet Dis. 2013 Feb;5(1):13-31.
2. Houschyar KS, Tapking C, Borrelli MR, Popp D, Duscher D, Maan ZN et al. Wnt Pathway in Bone Repair and Regeneration - What Do We Know So Far. Front Cell Dev Biol. 2019 Jan 7;6:170. doi: 10.3389/fcell.2018.00170.
3. Stürznickel J, Rolvien T, Delsmann A, Butscheidt S, Barvencik F, Mundlos S et al. Clinical Phenotype and Relevance of LRP5 and LRP6 Variants in Patients With Early-Onset Osteoporosis (EOOP). J Bone Miner Res. 2021 Feb;36(2):271-282. doi: 10.1002/jbmr.4197. Epub 2020 Nov 12.
4. Baron R, Kneissel M. WNT signaling in bone homeostasis and disease: from human mutations to treatments. Nat Med. 2013;19:179-192.
5. Collet C, Ostertag A, Ricquebourg M, Delecourt M, Tueur G, Isidor B et al. Primary Osteoporosis in Young Adults: Genetic Basis and Identification of Novel Variants in Causal Genes. JBMR Plus. 2017 Nov 6;2(1):12-21. doi: 10.1002/jbm4.10020.
6. Streeten EA, McBride D, Puffenberger E, Hoffman ME, Pollin TI, Donnelly P et al. Osteoporosis-pseudoglioma syndrome: description of 9 new cases and beneficial response to bisphosphonates. Bone. 2008 Sep;43(3):584-90.
7. Miyazawa S, Nakamura Y, Kosho T, Kawame H, Narumi S, Hasegawa T et al. Efficacy of denosumab therapy for osteoporosis-pseudoglioma syndrome with osteoporosis: a case report. Modern Rheumatology Case Reports. 2019;3(1):45-48.
8. Canalis E, Giustina A, Bilezikian JP. Mechanisms of anabolic therapies for osteoporosis. N Engl J Med. 2007 Aug 30;357(9):905-16. doi: 10.1056/NEJMra067395.
9. Arantes HP, Barros ER, Kunii I, Bilezikian JP, Lazaretti-Castro M. Teriparatide increases bone mineral density in a man with osteoporosis pseudoglioma. J Bone Miner Res. 2011 Dec;26(12):2823-6.
10. Kedlaya R, Veera S, Horan DJ, Moss RE, Ayturk UM, Jacobsen CM et al. Sclerostin inhibition reverses skeletal fragility in an Lrp5-deficient mouse model of OPPG syndrome. Sci Transl Med. 2013 Nov 13;5(211):158. doi: 10.1126/scitranslmed.3006627.
11. Simic MK, Mohanty ST, Xiao Y, Cheng TL, Taylor VE, Charlat O et al. Multi-Targeting DKK1 and LRP6 Prevents Bone Loss and Improves Fracture Resistance in Multiple Myeloma. J Bone Miner Res. 2023 Jun;38(6):814-828.

A Case Report of SH3BP2-Related Autosomal Dominant Cherubism in an Adult Treated with Denosumab

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A 58 year-old man with a childhood history of a right-sided mandibular lesion noted growth of a left mandibular lesion and corresponding mandibular nerve paresthaesia without dental pain. Past history was notable for ocular shingles and dyslipidaemia. He has had no fractures.

At age 5y, he was diagnosed with a right-sided mandibular lesion for which he had multiple childhood surgeries. There was no history of developmental delay. In his 20s, a biopsy confirmed a giant cell granuloma of the right mandible and he underwent enucleation and debulking to his right maxilla and orbit. This was followed by a right hemi-mandible resection and iliac crest bone graft in his 30s. He had multiple adult teeth that never developed and required implants.

There was a family history for dental problems on his paternal side in an autosomal dominant inheritance pattern. His father had significant dental issues without clear bone lesions, his sister also had mild dental issues. Two paternal cousins had giant cell lesions, a nephew had deformed nasal passages as a child and a grand-nephew was diagnosed with dental issues secondary to cherubism.

More recently, he was referred to the oral maxillofacial surgical unit for workup of the left mandibular lesion. OPG and CT revealed an expansile lucent lesion of the left hemi-mandible with internal osseous septation, cortical thickening and a lack of periosteal reaction. Subsequent biopsy revealed numerous osteoclast-like giant cells in the stroma and no evidence of malignancy. He was referred to the endocrinology unit for consideration of a trial of medical therapy with the intention to avoid major facial reconstructive surgery.

Further investigations revealed elevated bone-turnover markers, with otherwise normal biochemistry. Myeloma screening was unremarkable (Table 1). DXA showed osteopaenia of the lumbar spine (Table 2). Genetic testing revealed a heterozygous pathogenic missense mutation in Exon 9 of the SH3BP2 gene (SH3BP2 NM_001122681.2: c.1253C>G; p.(Pro418Arg), Exon 9 missense) which causes autosomal dominant cherubism.

Given the high risk of recurrence post-surgical resection due to his genetic predisposition, a multi-disciplinary team consensus decision was made to treat with denosumab 60mg 6monthly - the involvement of the mandible likely predisposes him to antiresorptive associated osteonecrosis of the jaw (ONJ), however the alternative was major surgical intervention. He also commenced colecalciferol 1000IU daily.

6 months after starting denosumab, there was notable improvement in his mandibular nerve paresthaesia. Whilst on anti-resorptive therapy, he lost a tooth and the socket healed well without issue. Bone-turnover markers are now suppressed (Table 1). Repeat OPG and CT shows a clear improvement in the left hemi-mandible lesion which is less expansile and has increased internal bone matrix.

Cherubism

Cherubism is a rare autosomal dominant condition resulting in giant cell lesions of the jaw. This presents with progressive swelling of the face and fullness of the cheeks due to expansion of the underlying bony structures that are eventually replaced by fibrous tissue. Dental structures are commonly affected and tooth eruption is impacted. Maxillary bone changes also lead to stretching of the skin of the cheeks creating the appearance of an upturned eye. Onset of clinical or radiographical findings are during childhood and generally regress by adulthood but persist occasionally. This condition has a male predominance (56%); females typically have reduced penetrance and a less severe phenotype.

The majority of cherubism cases (93.5%) are due to a mutation of Exon 9 of the *SH3BP2* (SH3-domain binding protein 2) gene. Mutations are generally familial in nature, however sporadic cases do occur. A gain of function mutation is typical and modulates osteoclastogenesis. When RANKL binds RANK expressed on osteoclast progenitor cells, this induces osteoclast formation via activation of transcription factor NFATc1. Over-expression of SH3BP2 has been shown to increase NFATc1 and also TRAP activation, leading to the osteoclastic bone lesions of cherubism.

Primary treatment for severe cases is surgical resection of granulomas and fibrous tissue. Orthodontic treatment is also required to avoid permanent dental issues. There is limited evidence for medical treatment of cherubism and current literature is exclusively confined to the paediatric population. A recent systematic review identified 5 cases treated with denosumab. All reported positive outcomes including increased bone density and reduced expansion of bony lesions. There were no reports of ONJ. In adults, there are no case reports of cherubism managed with medical therapy. Other medical treatments of cherubism had variable outcomes, including includes bisphosphonates, calcitonin, tacrolimus, TNF-alpha inhibitors and imatinib.

Key Points

1. Cherubism is predominantly inherited in an autosomal dominant pattern and caused by a mutation in exon 9 of the *SH3BP2* This has downstream effects on osteoclastogenesis resulting in giant-cell lesions of the jaw.
2. In severe phenotypes, primary treatment is surgical resection and jaw reconstruction, with orthodontic intervention to preserve dental health.
3. There have been promising case reports of treatment with denosumab. Other medical therapies that have been trialled include bisphosphonates, calcitonin, tacrolimus, TNF-alpha inhibitors and imatinib. Most target osteoclast proliferation and function as the mechanism of action.

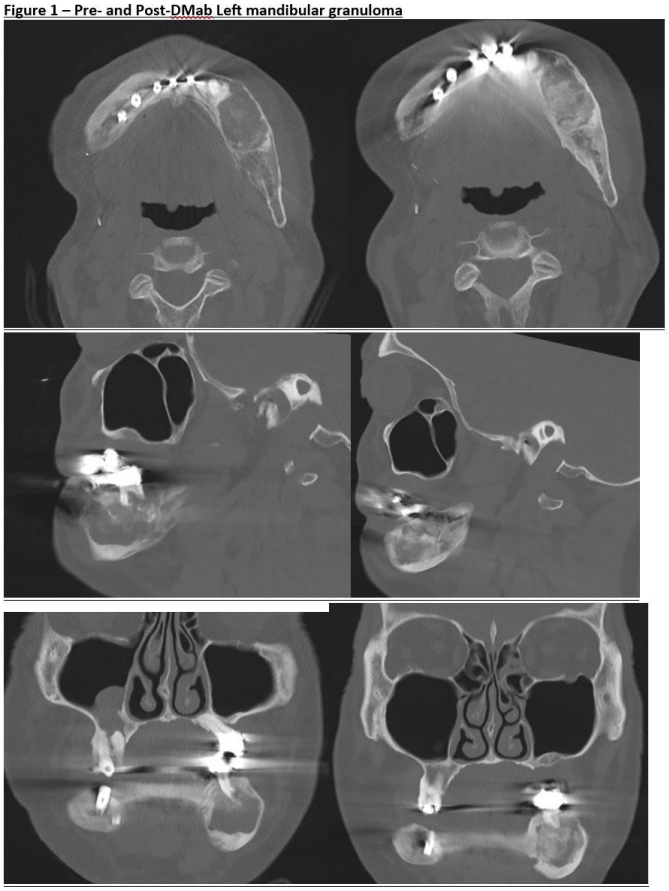
Table 1 – Pre- and Post-DMab Biochemistry

Investigation	Pre-DMab	Post-DMab	Reference Range
C-telopeptide	**656**	**<70**	100 – 600 ng/L
P1NP	**84**	**15**	15 – 80 ug/L
ALP	119		30 – 100 U/L
CorrCa	2.49	2.37	2.15 – 2.55 mmol/L
Phosphate	0.94	0.99	0.8 – 1.5 mmol/L
PTH	8.4		1.7 – 10.0 pmol/L
Vitamin D	97	87	50 – 250 nmol/L
Magnesium	0.79	0.90	0.70 – 1.10 mmol/L
eGFR	>90	>90	
Protein Electrophoresis	No paraprotein detected		
Kappa FLC	12.4		3.3 – 19.4 mg/L
Lambda FLC	7.9		5.7 – 26.3 mg/L
K/L FLC ratio	1.57		0.26 – 1.65

Table 2 – DXA Pre-DMab

Region	T-score	Z-score
AP spine (L1-L4)	-1.1	-0.4
Femoral neck (right)	-0.5	0.3
Total hip (right)	-0.2	0.3

Figure 1 – Pre- and Post-DMab Left mandibular granuloma



1. William A. Jones; Familial Multilocular Cystic Disease of the Jaws1. The American Journal of Cancer 1 April 1933; 17 (4): 946–950
2. Faircloth WJ Jr, Edwards RC, Farhood VW. Cherubism involving a mother and daughter: case reports and review of the literature. J Oral Maxillofac Surg. 1991;49(5):535-542.
3. Li CY, Yu SF. A novel mutation in the SH3BP2 gene causes cherubism: case report. BMC Med Genet. 2006;7:84.
4. Reichenberger EJ, Levine MA, Olsen BR, Papadaki ME, Lietman SA. The role of SH3BP2 in the pathophysiology of cherubism. Orphanet J Rare Dis. 2012;7 Suppl 1(Suppl 1):S5.
5. Chrcanovic BR, Guimarães LM, Gomes CC, Gomez RS. Cherubism: a systematic literature review of clinical and molecular aspects. Int J Oral Maxillofac Surg. 2021;50(1):43-53.
6. Ahmad M. AlAli, Hussain Dashti, Yahya Al-Yahya, Hamad Ali. A report of two atypical genetic cases of cherubism: Reduced penetrance and sporadic occurrence. Journal of Oral and Maxillofacial Surgery, Medicine, and Pathology. 2021;33(2):234-238.
7. Lietman SA, Yin L, Levine MA. SH3BP2 mutations potentiate osteoclastogenesis via PLCγ. J Orthop Res. 2010;28(11):1425-1430.
8. Takayanagi H, Kim S, Koga T, et al. Induction and activation of the transcription factor NFATc1 (NFAT2) integrate RANKL signaling in terminal differentiation of osteoclasts. Dev Cell. 2002;3(6):889-901. doi:10.1016/s1534-5807(02)00369-6
9. Lietman SA, Yin L, Levine MA: SH3BP2 is an activator of NFAT activity and osteoclastogenesis. Biochem Biophys Res Commun. 2008, 371: 644-648.
10. Cailleaux PE, Porporatti AL, Cohen-Solal M, Kadlub N, Coudert AE. Pharmacological management of cherubism: A systematic review. Front Endocrinol (Lausanne). 2023;14:1104025.
11. Papadaki ME, Lietman SA, Levine MA, Olsen BR, Kaban LB, Reichenberger EJ. Cherubism: best clinical practice. Orphanet J Rare Dis. 2012;7 Suppl 1(Suppl 1):S6.

Concurrent denosumab and parenteral iron therapy precipitating severe hypocalcaemia and hypophopshataemia: a case report

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Background

Parental iron and denosumab are commonly prescribed drugs within inpatient and community settings. There is increasing awareness of possible drug-drug interactions and risk of FGF23 mediated hypophosphataemia[4], which can be severe and life-threatening[4,5]. Close monitoring and prompt treatment of electrolyte disturbance is required, whilst avoiding significant delays in denosumab treatment.

Case

A 76-year-old man presented to the Emergency Department with lethargy and mixed respiratory failure. He was found to have severe hypocalcaemia (1.72mmol/L; reference range [RR]: 2.15-2.65), hypophosphataemia (<0.16mmol/L; RR: 0.75-1.50) and QT prolongation (525ms) on electrocardiogram. He had normal creatinine (86micromol/L; RR: 60-110), hyperparathyroidism (36.9pmol/L; RR: 2.0-8.5) and sufficient 25-OH vitamin D levels (67nmol/L; RR: >50).

Four weeks prior, he had received his second dose of denosumab 60mg for a diagnosis of osteoporosis (hip T-score -2.6; spine T-score -1.6). Two weeks later he was also administered intravenous iron polymaltose (Figure 1) for chronic iron deficiency anaemia secondary to gastrointestinal angioectasiae, requiring frequent intravenous iron infusions every 3 months for the last 12 months.

The patient required urgent treatment with non-invasive ventilation, cardiac telemetry, and intravenous calcium gluconate and phosphate replacement. Oral calcitriol, calcium carbonate and phosphate were commenced once his electrolytes stabilised, and gradually weaned following discharge

Despite multiple treatments with argon plasma coagulation of angioectatic lesions, he required further iron infusions with iron polymaltose and ferric carboxymaltose. Each was complicated by transient hypophosphataemia without hypocalcaemia, requiring calcitriol and phosphate replacement (nadir phosphate levels 0.68mmol/L and 0.43mmol/L, respectively). Subsequent investigations at the time of recurrent hypophosphataemia demonstrated hyperparathyroidism ((24.7pmol/L), normal 25-OH vitamin D (102nmol/L), and an inappropriately high fractional excretion of phosphate (36%; RR: 10-20%). Fibroblast growth factor 23 (FGF23) levels were not available at the time of the hypophosphataemia.

A diagnosis of recurrent iron infusion-related (FGF23-mediated) hypophosphataemia, and a subsequent denosumab and iron infusion interaction causing a presentation with profound hypocalcaemia and hypophosphataemia was made. Denosumab was ceased. Unfortunately, persistent iron deficiency anaemia necessitated further parental iron therapy. Iron sucrose has since been administered on multiple occasions without calcium or phosphate disturbance.

Discussion

According to Pharmaceutical Benefits Scheme (PBS) data, parenteral iron administration has rapidly increased from ~70,000 prescriptions in 2014 to more than 160,000 in 2016, with ferric carboxymaltose most frequently prescribed following its PBS listing[1]. With increasing indications[1], parenteral iron is prescribed by a range of clinicians, including general practitioners, gastroenterologists, nephrologists, haematologists and internal medicine physicians[1]. Denosumab is now the most frequently administered osteoporosis therapy, rising from 36.5% to 76.1% of prescribed osteoporosis treatments between 2014-2018[2]. Denosumab induced hypocalcaemia is well recognised, in dialysis and advanced chronic kidney disease patients, and in the setting of vitamin D deficiency. Recent reports have highlighted the problem of FGF23 mediated hypophosphataemia secondary to iron infusions, and iron infusion and denosumab drug-drug interactions are now starting to be reported[3-6].

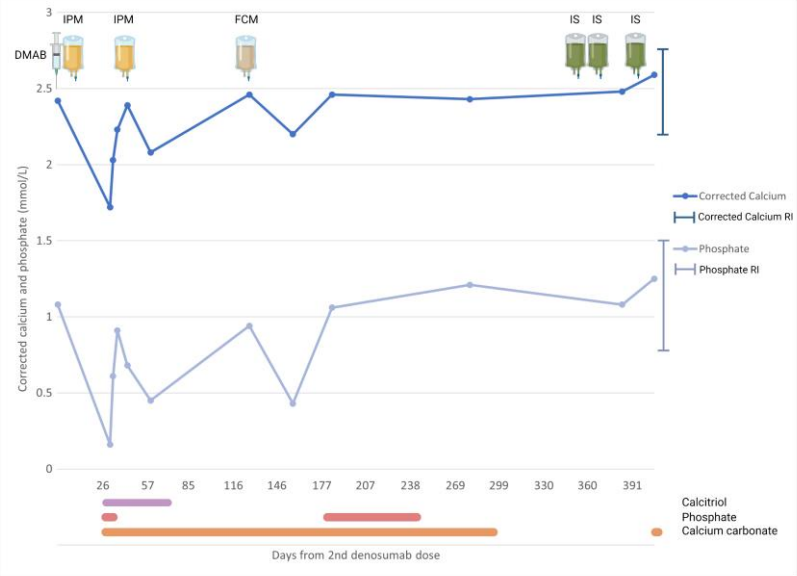
The mechanism of hypocalcaemia and hypophosphataemia with intravenous iron and denosumab has been proposed, relating to regulation via PTH, 1,25-(OH)2 vitamin D and FGF23. Denosumab inhibits osteoclastic bone resorption, transiently reducing plasma calcium concentration and resulting in elevated PTH levels[7]. The subsequent hyperparathyroidism decreases renal phosphate reabsorption and promotes phosphaturia. Iron infusions inhibit cleavage and inactivation of FGF23[8]. High levels of FGF23 decrease renal phosphate reabsorption and inhibit renal 1α-hydroxylation of 25-OH vitamin D to activated 1,25-(OH)2 vitamin D, thereby impairing intestinal absorption of calcium and phosphate[4]. Recurrent iron infusion-related hypophosphataemia has been associated with osteomalacia. Co-administration of iron infusions and denosumab may compound the phosphaturia. Additionally, FGF23 mediated impairment of 1,25-(OH)2 vitamin D activation blunts the physiological response to hypocalcaemia induced by denosumab, further contributing to both hypocalcaemia and hypophosphataemia. Acute hypophosphataemia and hypocalcaemia can cause tetany, cardiorespiratory failure, seizures and coma in severe cases[7,8].

A strategy to safely administer iron infusions in patients on denosumab is necessary, due to risk of rebound bone loss and vertebral fractures with delay of denosumab. Although hypophosphataemia was observed in our case with both iron polymaltose and ferric carboxymaltose, hypophosphataemia was not observed upon challenging with iron sucrose. This may be due to the waning effect of denosumab, or may support the proposal that different iron preparations may have varying risk of hypophosphataemia[9,10]. From meta-analysis data, ferric carboxymaltose was associated with a significantly higher risk of hypophosphataemia than iron isomaltose (risk ratio 7.90, 95% confidence interval 2.10-28.0) and iron sucrose (risk ratio 9.40, 95% confidence interval 2.30-33.0)[9]. Electrolyte disturbance occurred in cases at 8-26 days post denosumab[3-6], so reasonable monitoring could include at 7, 14 and 28 days and not thereafter if there are no abnormalities. Studies to determine the frequency of this serious drug interaction, and to assess which iron preparations reliably reduce the risk of hypophosphatemia with denosumab are required.

Learning points

1. Denosumab and parenteral iron replacement have increasingly been used after PBS listing and are prescribed across multiple specialties and settings.
2. Although denosumab induced hypocalcaemia and iron infusion related hypophosphataemia are well described, this case highlights that co-prescription increases the risk of life-threatening electrolyte derangement requiring hospital admission and parenteral therapy.
3. Clinicians should be alert to the potential drug interaction between denosumab and iron infusions, in order to develop strategies to prevent, monitor and mitigate the electrolyte abnormalities.
4. Alternative iron preparations may be associated with a reduced risk, however further research to determine the frequency of the interaction and reliability of different parenteral iron formations in reducing risk of hypophosphataemia is required.

Figure 1 – Timeline of serum correct calcium and phosphate in relation to denosumab, iron infusions (iron polymaltose, ferric carboxymaltose, iron isomaltose)



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1. 1. Department of Health. Ferric carboxymaltose: 24 month predicted versus actual analysis. Canberra: Department of Health; 2017. Available from: https://www.pbs.gov.au/info/industry/listing/participants/public-release-docs/dusc-public-release-documents-by-condition (accessed Mar 2023).
2. 2. Department of Health. Denosumab for osteoporosis: utilisation analysis using PBS data. Canberra: Department of Health; 2020. Available from: https://www.pbs.gov.au/info/industry/listing/participants/public-release-docs/dusc-public-release-documents-by-condition (accessed Mar 2023).
3. 3. Cohen A, Chacko B. Severe hypocalcaemia following denosumab and iron infusion. Nephrology. 2022;27(9):781-2. Doi:https://doi.org/10.1111/nep.14078.
4. 4. Smyth B, Ong S. Severe hypocalcaemia and hypophosphataemia following intravenous iron and denosumab: a novel drug interaction. Intern Med J. 2016 Mar;46(3):360-3. doi: 10.1111/imj.13001. PMID: 26968599.
5. 5. Fang W, McMahon LP, Bloom S, Garg M. Symptomatic severe hypophosphatemia after intravenous ferric carboxymaltose. JGH Open. 2019;3(5):438-40. Doi:10.1002/jgh3.12150.
6. 6. Tai R, Mouchaileh N, Ting C. Hypocalcaemia and hypophosphataemia following denosumab and IV ferric carboxymaltose in an older patient with normal renal function. Journal of Pharmacy Practice and Research. 2022;52(1):49-52. Doi:https://doi.org/10.1002/jppr.1791.
7. 7. Megapanou E, Florentin M, Milionis H, Elisaf M, Liamis G. Drug-Induced Hypophosphatemia: Current Insights. Drug Saf. 2020 Mar;43(3):197-210. doi: 10.1007/s40264-019-00888-1. PMID: 31776845.
8. 8. Coppolino G, Nicotera R, Cernaro V, Calimeri S, Leonardi G, Cosentino S, Comi A, Donato C, Lucia CM, Provenzano M, Michael A, Andreucci M. Iron Infusion and Induced Hypophosphatemia: The Role of Fibroblast Growth Factor-23. Ther Apher Dial. 2020 Jun;24(3):258-264. doi: 10.1111/1744-9987.13435. Epub 2019 Oct 25. PMID: 31483921.
9. 9. Bellos I, Frountzas M, Pergialiotis V. Comparative Risk of Hypophosphatemia Following the Administration of Intravenous Iron Formulations: A Network Meta-Analysis. Transfus Med Rev. 2020 Jul;34(3):188-194. doi: 10.1016/j.tmrv.2020.07.002. Epub 2020 Jul 23. PMID: 32819760.
10. 10. Schaefer B, Tobiasch M, Viveiros A, Tilg H, Kennedy NA, Wolf M, Zoller H. Hypophosphataemia after treatment of iron deficiency with intravenous ferric carboxymaltose or iron isomaltoside-a systematic review and meta-analysis. Br J Clin Pharmacol. 2021 May;87(5):2256-2273. doi: 10.1111/bcp.14643. Epub 2020 Dec 7. PMID: 33188534; PMCID: PMC8247006.